

**RELATIONSHIP OF HUMAN AND ANIMAL TUBERCULOSIS IN THE
INTERFACE AREAS OF THE NATIONAL PARKS OF THE KAFUE BASIN IN
ZAMBIA**

BY

MUSSO MUNYEME

THESIS
M. SC
MUN
2005

*A dissertation submitted to the University of Zambia in partial fulfillment of the
requirements for the award of the degree of Master of Science in Veterinary Public
Health*

**THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
DEPARTMENT OF DISEASE CONTROL
LUSAKA**

©2005



DECLARATION

I, **Musso Munyeme**, do hereby declare that this dissertation represents my own work and that it has never been submitted before for the award of a degree or any other qualification at this or indeed any other university.

Signature:
Date..... 06/02/06

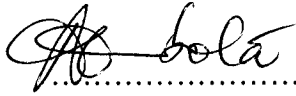
DEDICATIONS

At every stage of man's life, one is bound to receive corrective measures though not always willingly. As a little boy, my grandparents and parents did that. As I grew up, I was exposed to the school system where teachers had a strong social presence and every parent called upon them to correct their children more so with the rod and I was no exception. Later on family members, friends, workmates and lecturers continued in their various ways to apply corrective measures in my life. So to all these wonderful people I dedicate this work.

APPROVAL

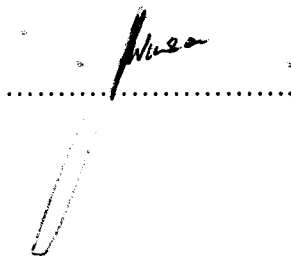
This dissertation of **Musso Munyeme** is approved as fulfilling the requirements for the award of the Degree of Master of Science in Veterinary Public Health of the University of Zambia.

(Supervisor)

.....

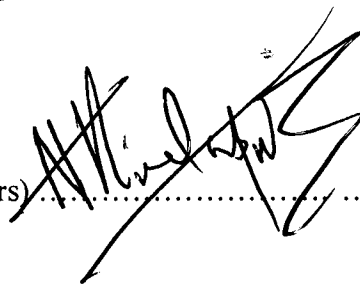
06/02/06
.....
Date

(Internal Examiner)

.....

08/02/06
.....
Date

(Chairperson, Board of Examiners)

.....

08/02/06
.....
Date

TABLE OF CONTENTS

Title	i
Declaration	ii
Dedication	iii
Approval	iv
Table of contents	v
List of figures	xi
List of tables	xv
List of symbols	xvii
List of Abbreviations	xvii
Acknowledgements	xx
Abstract	xxiii

CHAPTER ONE: INTRODUCTION	1
----------------------------------	----------

CHAPTER TWO: LITERATURE REVIEW	5
---------------------------------------	----------

2.1	Background	5
2.1.1	A general brief history of tuberculosis	5
2.1.2	The Zambian Situation	8
2.2	Aetiology	9
2.2.1	Classification	11
2.3	Transmission and Infection	12

2.4	Pathogenesis	14
2.5	Distribution and epidemiology	16
2.5.1	Tuberculosis distribution and epidemiology at the global level	16
2.5.2	Tuberculosis distribution and epidemiology in Zambia	18
2.5.3	Zoonotic tuberculosis	18
2.6	Public health importance of tuberculosis	20
2.6.1	Tuberculosis and its fatal alliances	21
2.7	Implications of Zoonotic Tuberculosis	22
2.8	Importance of Tuberculosis in Zambia	27
2.9	Diagnostic Tools	30
2.9.1	Bacteriology and Biochemical Means with their limitations	31
2.9.2	Pathogen Characterization	32
2.9.2.1	Polymerase Chain reaction (PCR) technique.	34
2.9.2.2	Characterization using Spoligotyping Technique	37
2.10	Treatment and prevention	38
2.10.1	Applications in animal populations	38
2.10.2	Applications in human populations	39

CHAPTER THREE: MATERIALS AND METHODS

3.1	Study Areas	41
3.1.1	Background of the study areas	43

3.2	Study Design	46
3.3	Study subjects	47
3.4	Mid cervical comparative tuberculin tests (MCT)	49
3.4.1	Baseline studies	49
3.4.2	Sampling procedure	50
3.4.3	Sample size determination	50
3.4.4	Selection criteria in cattle populations	51
3.4.5	Tuberculinisation of Cattle in the Field	52
3.4.6	Questionnaire Survey (Cattle) population	54
3.5	Human Communities	55
3.5.1	Questionnaire Survey	55
3.5.2	Collection of human sputum samples.	56
3.5.2.1	Inclusion criteria for patients giving sputum	57
3.5.2.2	Exclusion criteria for patients giving sputum	57
3.5.2.3	Sputum sample collection guidelines	58
3.5.2.4	Direct smear examination using Auramnie and Ziehl-Neelsen staining	59
3.5.2.5	Decontamination of sputum and culturing	60
3.6	Abattoir surveys	60
3.7	Wildlife sampling	62
3.8	Culturing of decontaminated and homogenized tissue sediments	64
3.8.1	Pre-treatment of Samples	64

2.8.2	Ziehl-Neelsen staining	65
3.8.4	Direct microscopic smear examination	65
3.9	Preparation of Mycobacterium lysates from colonies for DNA extraction.	66
3.10	Spoligotyping technique (Kamerbeek et al., (1997)	67
3.11	Data analysis	68

CHAPTER FOUR: RESULTS 70

4.1	Descriptive results on sampling characteristics in cattle	70
4.2	Prevalence of bovine tuberculosis in cattle	71
4.2.1	Prevalence of bovine tuberculosis at individual animal level	71
4.2.1.1	Overall prevalence at individual animal level	71
4.2.1.2	Individual animal prevalence in Lochinvar	71
4.2.1.3	Individual animal prevalence in Blue Lagoon	71
4.2.1.4	Individual animal prevalence in Kazungula District	71
4.2.2	Prevalence of bovine tuberculosis at herd level	72
4.2.2.1	Overall prevalence at herd level	72
4.2.2.2	Herd level prevalence in Lochinvar	72
4.2.2.3	Herd level prevalence in Blue Lagoon	72
4.2.2.4	Herd level prevalence in Kazungula District	72

4.3	Risk factors associated with the prevalence of bovine tuberculosis	73
4.3.1	The effects of geographical area on tuberculosis prevalence	73
4.3.2	The effects of grazing systems on tuberculosis prevalence	74
4.3.3	Effects of herd size on tuberculosis prevalence	79
4.3.4	The effects of age on tuberculosis prevalence	81
4.3.5	The effects of sex on tuberculosis prevalence	83
4.4	Bovine tuberculosis based on abattoir survey	84
4.4.1	Frequency of organ condemnation suspected to be tuberculous on gross postmortem inspection	87
4.4.2	Tuberculosis prevalence based on gross abattoir results	87
4.4.3	Microscopic examination of tissue homogenates	87
4.4.4	Tuberculosis prevalence from culture results	88
4.5	Tuberculosis survey in wildlife	89
4.5.1	Gross pathological results in Lechwe	90
4.5.2	Microscopic examination of tissue homogenate and culture results.	92
4.6	Tuberculosis survey in human communities of the study areas	94
4.6.1	General sampling characteristics for sputum sample examination	94
4.6.2	Direct Microscopy Examination Results	95
4.6.3	Culture growth and Isolation	96
4.7	Results from the questionnaire survey	97

4.8	Risk factor assessment for tuberculosis in the study Areas	100
4.9	Spoligotyping of <i>Mycobacterium</i> isolates from humans and animals.	106
4.9.1	Spoligotyping results of animal isolates	106
4.9.2	Spoligotyping results of human isolates	110

CHAPTER FIVE: DISCUSSION **112**

5.1	Prevalence of tuberculosis in cattle	112
5.2	Risk factors associated with tuberculosis prevalence in the studied areas	114
5.3	Observations from abattoir survey	119
5.4	Tuberculosis survey in the wildlife.	121
5.5	Risk Factors associated with Tuberculosis in humans	124
5.6	Molecular Typing of Animal and Human isolates	127

CHAPTER SIX: CONCLUSION AND **130**

RECOMMENDATIONS

REFERENCES **136**

APPENDIX **156**

LIST OF FIGURES

Figure 3.1	Map of Zambia with all the three sampling sites of Blue lagoon and Lochinvar depicted in the interface areas and Kazungula District.	42
Figure 3.2	Main study areas of Lochinvar and Blue lagoon in the Kafue Basin Interface areas in relation to the Game Management areas of the Kafue Flats and the main surrounding towns from which cattle for abattoir survey were originating	45
Figure 3.3	Cattle of the Kafue Basin in the interface areas of National Parks	48
Figure 3.4	Cattle in the Villages of the Kazungula District of Zambia outside the interface area of National Parks	48
Figure 3.5	Baseline data collection with the local Senior Veterinary Assistant checking a list of cattle farmers in the Sikaunzwe area of Kazungula District.	49
Figure 3.6	In the Interface areas, no crush pens are available, animals were cast down and before reading of skin thickness and inoculating the tuberculin	52
Figure 3.7	Shaved area on the neck region with inoculated sites having formed nodules	53
Figure 3.8	Conducting a questionnaire survey in the human communities of the study areas to ascertain risk factors of TB in both humans and animals.	56

Figure 3.9	Detailed postmortem examination of every organ from all hunter harvested wild animals in the Game Management Areas of the Kafue Basin National Parks	63
Figure 4.1	The prevalence of Bovine tuberculosis by area at individual animal and herd level.	73
Figure 4.2	Village resident herds were more accessible and exposed to easy handling facilities and were kept in small sizeable numbers	75
Figure 4.3	Transhumance and interface herds are kept in vast open plains without handling facilities and graze freely with access to wildlife grazing areas.	77
Figure 4.4	Prevalence of bovine tuberculosis according to grazing systems from comparative tuberculin test results.	78
Figure 4.5	Comparative tuberculin results of tuberculosis prevalence according to herd sizes using categorical classification.	79
Figure 4.6	The prevalence of Tuberculosis according to herd size using a Lowess Smoother scatter plot based on the comparative tuberculin test results.	80
Figure 4.7	The prevalence of tuberculosis according to age using a Lowess scatter plot incorporating a trend line depicting the effect of age on herd tuberculosis positivity	81
Figure 4.8	Tuberculosis prevalence according to age groups on	83

categorical analysis depicting variations that occur in different age groups.

Figure 4.9	Prevalence of tuberculosis in male ($n = 279$) and female ($n = 665$) animals from the comparative tuberculin test.	84
Figure 4.10	A whole carcass condemned after meat inspection at an abattoir in Monze District of Zambia due to suspected tuberculosis. Extensive milliary and granulomatous lesions were observed on almost every body organ and tissue on this animal (Courtesy Dr. Phaniel Nyimba District Veterinary Officer-D.V.O, Monze District).	85
Figure 4.11	ZN-stained smear of tissue homogenate from cattle. The slender, beaded, red-staining (ZN-positive) rods were noted with very few clumps on most of the cattle slides (X10X100 Magnification).	88
Figure 4.12	The lung tissue from the lechwe sampled in the Lochinvar National Park GMA showing extensive granuloma formation.	90
Figure 4.13	Heart of a ten year old male Lechwe antelope with extensive granulomas with cut section exposing the yellow caseous materials	91
Figure 4.14	ZN-stained smear of tissue homogenate from the lechwe which had extensive tubercles of the lungs. The slender, beaded, red-staining (ZN-positive) rods are seen to form	93

clumps against a methylene blue background (X10X100 Magnification).

- | | | |
|-------------|---|-----|
| Figure 4.15 | ZN-stained smear of a human sputum sample The slender, beaded, red-staining (ZN-positive) rods characteristic of <i>Mycobacterium tuberculosis</i> are much thinner and slender compared to those isolated from lechwe and cattle tissue homogenates as presented against a methylene blue background (X10X100 Magnification). | 95 |
| Figure 4.16 | Receiver operated characteristic (ROC) curve showing the sensitivity and specificity of the logistic regression model indicating its good predictive ability. | 105 |
| Figure 4.17 | Spoligotype dendrogram of <i>M. bovis</i> (C9 to C41) and <i>M. tuberculosis</i> (S1 to S30) isolates studied. The presented patterns were generated using the Bionumerics version 3.0 computer software (Applied Maths, St. Maartens-Latem, Belgium). The spoligotypes were compared using the band-based Dice coefficient and clustering determined by the unweighted pair group algorithm with arithmetic averages (UPMGA) method. Designation of spacers from left to right is 1 to 43. The dotted lines for S24 indicate uncertain bands. The isolates obtained from cattle and humans are indicated | 107 |

LIST OF TABLES

Table 3.1	Interpretation Table for Acid Fast Bacilli seen on Zhiel Nielsen and Auramine stains.	66
Table 4.1	Tuberculosis results based on the comparative test on cattle population in all the three study areas at individual animal as well as at herd level sampling.	70
Table 4.2	Detailed results on tuberculosis prevalence of Bovine tuberculosis at individual animal level in all the three areas of study	74
Table 4.3	Individual reactor animals by grazing systems in all the three study areas.	76
Table 4.4	Variations in the mean herd sizes according to Grazing Systems recorded.	76
Table 4.5	Details on Bovine tuberculosis prevalence according to grazing systems based on avian reactors, bovine reactors and comparative results.	78
Table 4.6	Details on the prevalence of bovine tuberculosis on avian, bovine and comparative tests according to different age groups.	82
Table 4.7	Number of animals received from each of the district for abattoir survey	86
Table 4.8	Summary of animals slaughtered and organ condemnation suspected to have TB lesions on gross meat inspection at the Kembe cold storage abattoir.	86

Table 4.9	Tuberculosis culture positive samples and their areas of origin after being cultured on Lowenstein Jensen media.	89
Table 4.10	Results from gross postmortem, microscopic and culture examinations	94
Table 4.11	Results of human sputum sample smears on Auramine and ZN stain by District	96
Table 4.12	Growth pattern of Mycobacteria cultures according to weeks	97
Table 4.13	Crude descriptions of risk factors for TB herd prevalence status in traditional cattle in Zambia.	101
Table 4.14	Some associations between presence of a reactor animal and variables in the study areas.	103
Table 4.15	Final multivariate multiple logistic regression analysis of herd level TB positivity in cattle found in the three main study areas	104
Table 4.16	Distribution of <i>Mycobacterium bovis</i> isolates from cattle by spoligotype and District.	108
Table 4.17	Distribution of 30 <i>Mycobacterium tuberculosis</i> spoligotypes by District.	111

LIST OF SYMBOLS

=	Equal to
>	Greater than
<	Less than
χ^2	Chi-square
°	Degree reading
%	Percentage
+/+Ve	Positive
-/-Ve	Negative

LIST OF ABBREVIATIONS

AD	Sterilized Distilled Water
AFB	Acid Fast Bacilli
bp	Base pair
CDL	Chest Disease Laboratories
CI	Confidence Interval
df	Degrees of freedom
DNA	Deoxyribonucleic acid
DOTS	Directly Observed Treatment Short Course
DR	Direct repeat region
GMAs	Game Management Areas
HH	Household

IFH	Interface Herds
ITM	Institute of Tropical Medicine
KMnO ₄	Potassium permanganate
L-J	Lowenstein Jensen media
MCT	Mid Cervical Comparative Tuberculin Test
MDR-TB	Multi-drug resistant tuberculosis
ml	milliliter
mm	millimeter
<i>n</i>	Study population (sample size)
<i>N</i>	Reference population
NaOH	Sodium Hydroxide
OR	Odds ratio
PCR	Polymerase chain reaction
PPD	Purified protein derivative
ppm	Parts per million
RFLP	Restriction fragment length polymorphism
ROC	Receiver operating Characteristic curve
RR	Risk ratio
SDS	Sodium dodecyl sulfate
SPSS	Statistical programme for social sciences
<i>Spp.</i>	Species
<i>Taq</i>	<i>Thermus aquaticus</i> -bacterium
TB	Tuberculosis
TH	Transhumance Herds

UPMGA	Unweighted pair group algorithm with arithmetic averages
μl	microliter
VRH	Village Resident Herds
WHO	World Health organization
z	Confidence level
ZN	Ziehl-Nielsen Stain

ACKNOWLEDGEMENTS

This research study was funded by the Flemish Inter University Council (VLIR) Project under the Belgium Inter University Cooperation at the University of Zambia (UNZA-VLIR IUC programme) for which I will always be grateful. I am also grateful to the University of Zambia for awarding me the Staff Development Fellowship in the School of Veterinary Medicine, Disease Control Department. I owe a great deal of thanks to the members of staff in the School of Veterinary Medicine, in particular the Dean, Dr. A. Nambota, who also was my local supervisor for his guidance and the smooth facilitation of my studies. I would also like to thank staff from the Directorate of Research and Postgraduate Studies (DRPGS) of the University of Zambia for their various help in obtaining information and keeping me updated.

I wish to acknowledge with deep gratitude Dr. I. K. Phiri, the Zambian project leader VLIR-UNZA IUC Veterinary Project for his support and guidance. Thank you for your support and encouragement to do this masters.

I am very grateful to my external supervisor Dr. Lean Rigouts for ably assisting me to learn molecular typing techniques especially spoligotyping in her laboratory at the Institute of Tropical Medicine (ITM) Antwerp in Belgium. I am deeply indebted and appreciative of the support Isidore Chola Shamputa and other members of staff at ITM gave me in the processing of DNA samples.

I would like to thank Prof. Pierre Dorny without whom this adventure would never had begun. Thank you for your support and guidance, you have been very instrumental at every stage of my study in ensuring that every struggle I made was worthwhile.

I would also like to thank Dr. John Bwalya Muma for all his help. John even though you were not officially one of my supervisors I appreciate all of your efforts more than I can express. I cannot imagine being at this point in writing my thesis if it had not been for your help, support, guidance and confidence you instilled in me. You showed just how great you are when my project was moribund. To you I owe many thanks.

I owe a great deal of thanks to the staff at the Chest Disease Laboratory (CDL), in particular Mr. Mweemba Muvimi and Ms. Ruth Tembwe for all the support and providing their laboratory space for my work. I am also highly indebted to my “field colleagues” whom I shared with various difficulties, none insurmountable, but challenging none the less. These are Mr. C Mubita, I Nyambe, B.Chulu, S. Mateyo, B. Phiri, Mr. H. Chimana, Dr. V. Siamudaala , Dr. H. Munangandu and many others.

I greatly appreciate the help and friendly cooperation I got from the Central Board of Health (CBOH), Monze and Namwala District Health Management Boards, including the rural health centers in these Districts and all the members of staff whom I would have liked to mention individually wasn't it for space. All the District Veterinary Officers in Mumbwa, Namwala, Monze and Livingstone/Kazungula District are highly appreciated together with the Veterinary Assistants.

I owe a great deal of thanks to all farmers who participated in the survey. Zambia wildlife Authority (ZAWA) and its staff are highly appreciated for the warm cooperation. I am greatly indebted to the patients, who participated in the survey, without your input, this investigation would have been meaningless. Dr. Evans Mwengwe and Namatama Imakumbili are thanked on behalf of CARE international. Kembe Cold Storage abattoir and its members of staff are highly acknowledged for allowing us carry out the abattoir survey on their premises.

I also appreciate VLIR's facilitation of a conference in Lusaka Zambia under the multi-disciplinary projects where I presented a paper in 2004.

I am deeply and sincerely appreciative to my family for their support despite my "neglect" of family matters which the MSc studies precluded me during the prolonged absences in the field.

There are many other people that I have had the joy of working with though I can't mention all by name.

Last but not the least I want to thank God almighty for the good health and care through out the study period.

ABSTRACT

Tuberculosis is a disease of worldwide public health and economic importance. In Zambia the disease has been consistently reported in the Kafue Basin without a clear understanding of transmission dynamics in the livestock-wildlife interface.

The main objective of this study was to determine the relationship between human and animal tuberculosis in the livestock-wildlife interface. In order to determine TB prevalence in cattle, a cross-sectional study was conducted using the mid-cervical comparative tuberculin test in the Kafue Basin (Lochinvar and Blue Lagoon) and a control area outside the interface areas (Kazungula District). Farmers were interviewed using a pre-tested questionnaire in order to determine TB risk factors. Tissue samples were collected from abattoirs and hunter harvested wildlife animals for isolation of TB. Sputum samples were also collected from health centers for TB isolation from humans. All cultures from these species were subjected to DNA analysis using spoligotyping to determine the *Mycobacterium* species causing disease in these hosts. Questionnaire results were used to build a model on risk factors for cattle TB in the study areas.

A total of 944 cattle from 111 herds were subjected to the mid-cervical comparative tuberculin test. Overall at individual animal level, the prevalence of TB in all the study areas was found to be 5.50%. In Lochinvar and Blue lagoon, the individual cattle prevalence was 5.35% and 7.34% respectively. However, in the control area (Kazungula) the individual animal prevalence was lower at 0.57%. At herd level, the

overall TB prevalence in cattle was herds 24.32% and a trend similar to individual cattle results was observed. These results, suggest an existence of foci of TB infection in the Kafue Basin of Zambia.

Tuberculosis prevalence was observed to vary according to the type of grazing system ($P = 0.000$) with interface herds recording higher prevalence (11.6%) than transhumance and village herds. Herds of cattle that practiced interface grazing system were eighteen times more likely to be TB positive than those that practiced village grazing system (Odds ratio = 18.07; CI 95% = 4.3-81.0; $p=0.000$). These results based on the grazing system suggest that TB prevalence increased with increasing level of contact with wildlife.

From the questionnaire results, logistic regression analysis revealed that herd size and grazing system were the two most significant risk factors influencing TB herd status.

From the abattoir survey, gross postmortem examinations revealed a 13% organ condemnation. However, only 6.62% of the overall condemnations had characteristic TB colonies on culture. The abattoir study provided evidence of the respiratory tract as a primary route for the establishment of tuberculosis infection in cattle as 87.64% of condemned organs were lungs.

In the wild animal survey, gross postmortem examination indicated a prevalence of 43% in the lechwe. Based on these findings, TB may appear to be probably maintained

principally by the lechwe may be due to its abundance or owing to the small sample sizes obtained from the other wildlife species.

Based on the questionnaire results, no statistical association was found between keeping cattle and having a TB patient in the household (Relative risk = 1.14; CI = 0.64-1.94); $p = 0.83$). Further, DNA analysis of animal and human isolates indicated that there was no sharing of infection. From the DNA results, all animal isolates were *M. bovis* while all human isolates were *M. tuberculosis*. These results may indicate species adaptability in certain hosts. However, animals may still be able to transmit TB to humans, albeit at a low prevalence.

Based on the generated TB model, in terms of TB control in the interface areas, the most critical factors to consider will be herd size and the grazing system.

CHAPTER ONE

INTRODUCTION

Tuberculosis (TB), one of the most widespread infectious diseases, is a worldwide threat to human health and has emerged as a disease with a devastating effect upon global public health. It is one of the most leading causes of death from a single infectious agent, killing about three million people every year (Cosivi *et al.*, 1998 and Sudre *et al.*, 1992).

Tuberculosis is an infectious, contagious and communicable granulomatous disease caused by slow growing acid-fast bacilli of the family *Mycobacteriaceae* and it manifests itself by the formation of granulomas especially in the lungs and lymph nodes (O'Reilly & Daborn 1995). Although commonly defined as a chronic, debilitating disease, TB occasionally assumes an acute, rapidly progressive course (Anonymous 1998). The disease affects practically all species of vertebrae, and it is a major disease of man, domestic animals as well as wildlife in many regions of the world, Zambia included (Cosivi *et al.*, 1998). Tuberculosis contains a single genus, *Mycobacterium*, but over fifty different closely related species (Holt, J.G., *et al.*, 1994). Of all these species, *Mycobacterium tuberculosis* and *Mycobacterium bovis* share microbiological and biochemical characteristics with closely related organisms (*M. microti*, *M. africanum*, and *M. canetti*), and are referred to as the *Mycobacterium tuberculosis* complex (Holt, J.G., *et al.*, 1994).

Infection with tubercle bacilli is usually followed by a slow disease progression in which clinical signs are mostly limited to the advanced stages (Francis J, 1958). Excretion of infectious particles, which leads to transmission of the disease to other animals, appears to be dose-dependent and can commence relatively shortly after infection (Neill *et al.*, 1988).

Acha and Szyfres (1987) stated that *Mycobacterium tuberculosis* is the most common cause of human tuberculosis, but an unknown proportion of cases are due to *Mycobacterium bovis*. The extent of bovine tuberculosis is not known in Zambia and it is furthermore disregarded as a source of human infection, however, a number of recent reports indicate that it's still a problem in humans, domestic and wild animals (Cook *et al.*, 1996 Cosivi *et al.*, 1998 Pandey *et al.*, 1998 and Sitima 1997). These reports have not been able to establish any causal relationship in the identified hosts. Apart from documenting mere presence of the disease, no direct correlation between *Mycobacterium* infection in cattle, wildlife and the disease in human population has been established. The extent of the risk factors hasn't been established in both humans and animals in the identified high-risk areas of Zambia.

In Zambia, Pandey (1998), Stafford (1991) and Kraus *et al.*, (1986) all report of diagnosing tuberculosis in the Kafue lechwe that has been described as the reservoir of a non-treatable source of TB. Pandey (1998) postulated that the disease could be transmitted through contamination of pastures and soils as reported in the Badger in the United Kingdom and Possums in New Zealand. This poses a very significant problem in the Kafue Basin of Zambia as cattle share pastures and water with the lechwe for the

most part of the year. In these interface areas, like in many other parts of the pastoralists communities, cattle ownership is a symbol of status as well as wealth. It forms a very complex social system of mutual obligation within families as cattle are also used as a medium of exchange in place of money. Thus apart from milk and meat products there is a lot of exchange of cattle between families and villages. This implies that cattle is neither kept in closed nor in stable populations due to these movements within and between herds and kraals. In the same areas, cattle share dipping facilities, watering points and grazing grounds. All these factors increase the risk of the disease being easily spread from an infected herd.

The transhumance kind of nomadic grazing practices have entailed livestock and wildlife fighting for little available grazing land in and around these National Parks. Human presence and encroachment in these areas has further contributed to the reduction of available grazing land outside the National parks (Sheppe, 1985). The actual impact of tuberculosis on the human and animal population has not been investigated. Studies on the disease have generally been limited to animal postmortem diagnosis and mere reporting of such findings. Little is therefore known of the disease in the context of its impact, extent, the risks it poses to the humans in the interface areas and possible transmission patterns to livestock.

Dillmann (1976), suggested that due to the constant contact of livestock and wildlife in the Kafue Basin, tuberculosis might have had been introduced into the lechwe from cattle and in turn the lechwe may have introduced the disease to other wild animals and

to un infected cattle. Wildlife is reported to have remained as the untreatable reservoir host (Gallagher et al., 1972).

This existing pool of tuberculosis in the wildlife population coupled with the presence of the Human Immuno Deficiency Virus (HIV) in the human population makes the situation to be conducive for zoonotic tuberculosis to flourish. This might mean the prevalence of tuberculosis being higher and the probable incidence of activated and latent disease within these hosts will likely be high too.

Previous investigations on zoonotic tuberculosis had focused on reporting the mere presence of the disease in animals and humans without establishing any possible associations in the purportedly high risk interface areas of the Kafue Basin. This study was therefore designed to provide more information on tuberculosis and the association that exists in wildlife, cattle and the human population. Specific objectives included:

1. Determining the prevalence of tuberculosis in cattle and wildlife.
2. Assessing risk factors associated with tuberculosis in cattle and humans.
3. Isolating *Mycobacterium* species from wildlife, cattle and humans.
4. Determining the level of relatedness of the isolates from these hosts.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

In the Southern, Western, Central and Eastern regions of Zambia, cattle ownership is a symbol of status and wealth. In the Central-Southern parts of Zambia, certain areas have been identified as 'hot spots' for bovine tuberculosis (Sitima 1997, Cook *et al.*, 1996 and Pandey *et al.*, 1998), which is one of the major zoonotic diseases and animal health constraint for both domesticated and wild animals. Despite the prevalence of bovine TB being reported as high in certain parts of Zambia, it is not clear how much human TB is derived from cattle or vice versa. It is likely that despite the lack of data, cattle may be a significant source of infection for man (Acha & Szyfres, 1987). Conversely, man is becoming an increasing risk to cattle, more to the fact that the HIV pandemic has caused a dramatic increase in the number of "open" TB cases (Collins & Grange, 1983).

2.1.1 A general brief history of tuberculosis

The origin of *Mycobacterium tuberculosis* is not known, but genetic concordance suggests that human infections of *M. tuberculosis* could have evolved from *Mycobacterium bovis* sometime after the domestication of cattle (Patrick, 1997). The earliest account of TB infection in man dates back to the Neolithic period. This period is also trademarked by the first domestication of cattle, suggesting that *M. tuberculosis* evolved from a mutant of *M. bovis* (Metcalf, 1991). However, recent molecular

biological studies involving DNA typing show that *M tuberculosis* has been present for a long time (Donoghue *et al.*, 2001). Donoghue *et al.*, (2001) obtained samples from 18th century mummies and were able to generate spoligo patterns that resemble the present *M. tuberculosis*. Similarly, Rothschild *et al.*, (2001) successfully applied spoligotyping to a 17,000-year-old skeletal specimen of an extinct North American bison and the pattern revealed that the respective bacteria were probably not related to *M. bovis* or *M. microti*, but best fitted *M. africanum* and *M tuberculosis* patterns.

Despite this scientific battle on the origin of TB, this disease is the most prevalent and widespread disease experienced by human kind over such an extensive period of time. It is now apparent that human TB infection predates the first written records thereof and has occurred for several millennia.

During the industrial revolution in Europe with its crowded cities, tuberculosis became epidemic and was referred to as the 'great white plague' (Sudre *et al* 1992; Bloom & Murray, 1992). In ancient times, the picture of shriveled and wasted people from tuberculosis was called *phthisis* (Greek to "waste away"). With time, TB became the number one killer and it was referred to as the "captain of all these men of death" and accounted for 25% of all human deaths in the first decade of the nineteenth century (Sudre *et al* 1992). The tuberculin test was introduced in 1907 and 80% of the United States human population were found positive for TB (Walker, 1996).

Around the early 1900s, several factors in industrialized nations began reducing the human TB. In Britain, the link between human and bovine TB was confirmed by the

Royal Commission of Tuberculosis in 1911 (Francis, 1959). Early in 1882, Robert Koch first discovered and described the causative agent of tuberculosis. He further described the tuberculin test, which proved to be a valuable diagnostic tool for TB detection. Therefore in European and North American countries, TB in cattle was successfully controlled by the tuberculin test and slaughter schemes (Pritchard, 1988). These measures, together with pasteurization of milk led to a reduction in bovine borne human TB. Myers (1941) described this phase as “man’s greatest victory over tuberculosis”. In 1947, the drug Streptomycin became available with Isoniazid following in 1952. During this same period, most industrialized nations used the tuberculin test in the test and slaughter elimination programs in animal TB control (Collins and Grange 1983). These measures drastically reduced the incidence of tuberculosis in both cattle and humans. During the 1950s, TB rates dropped by 75% and TB hospitals (commonly known as sanatoriums) began closing their doors for lack of patients (Sudre *et al* 1992).

However, in 1983, for the first time after a number of decades, the decline in human TB stopped (Dankner *et al.*, 2000). Many nations including the industrialized world started recording increases in the incidence and prevalence of tuberculosis (Daborn & Grange, 1993). The increase has persisted through the 1990s to present time. Once thought to be nearing eradication in developed countries, tuberculosis is undergoing a dramatic resurgence throughout the world. The emergence of significant levels of multiple-drug-resistant (MDR) tuberculosis isolates further complicated treatment, underscoring the need to develop new strategies for prevention and treatment of tuberculosis beyond currently available antimicrobial therapies (Bloom and Murray 1992). It has been claimed by most authors that the sharp increase in the incidence and prevalence of TB

which started in the mid-1980s, was fuelled by the human immunodeficiency virus epidemic which stirred a lot of active disease in individuals and communities (McKenna *et al.*, 1995, McCray *et al.*, 1997).

2.1.2 The Zambian situation

The first mention of tuberculosis in Zambia (then Northern Rhodesia) was in 1912 in the records of the Northern Rhodesian Medical Department, which describes the disease as “not uncommon in villages” (*Anonymous* 1913, 1914). In 1947, the Veterinary Department diagnosed TB in cows at Nega Nega, Kabwe and Mazabuka (*Anonymous* 1948). The Veterinary annual report of 1956, highlighted a number of areas where TB was diagnosed mostly in Abercorn (now Mbala), Mazabuka, Monze and Kalomo (*Anonymous* 1957). The abattoir compilation done by the Veterinary Department in the same report, showed the following findings; 1.6% of all cattle slaughtered at Lusaka abattoir had tuberculosis lesions; 2% at Livingstone abattoir; 5.2% at Mazabuka and 16.8% were coming from Namwala (*Anonymous* 1957).

In 1956, a Veterinarian reported of a sick Lechwe (*Kobus leche kafuensis*) on the Kafue flats of Lochinvar. Two Lechwe from Lochinvar were slaughtered and on examination at the Central Research Station, extreme TB lesions were found (*Anonymous* 1957). Gallagher *et al.*, 1972, report of having had cropped a sizeable number of Lechwe between 1967 and 1970 at Lochinvar, where they found the percentage of those with gross TB lesions to have had been 14%. An abattoir was built in Lochinvar in 1969 and by 1972, lesions of TB were found in 36% of all the Lechwe shot at randomly by various hunters (Gallagher *et al.*, 1972).

which started in the mid-1980s, was fuelled by the human immunodeficiency virus epidemic which stirred a lot of active disease in individuals and communities (McKenna *et al.*, 1995, McCray *et al.*, 1997).

2.1.2 *The Zambian situation*

The first mention of tuberculosis in Zambia (then Northern Rhodesia) was in 1912 in the records of the Northern Rhodesian Medical Department, which describes the disease as “not uncommon in villages” (*Anonymous* 1913, 1914). In 1947, the Veterinary Department diagnosed TB in cows at Nega Nega, Kabwe and Mazabuka (*Anonymous* 1948). The Veterinary annual report of 1956, highlighted a number of areas where TB was diagnosed mostly in Abercorn (now Mbala), Mazabuka, Monze and Kalomo (*Anonymous* 1957). The abattoir compilation done by the Veterinary Department in the same report, showed the following findings; 1.6% of all cattle slaughtered at Lusaka abattoir had tuberculosis lesions; 2% at Livingstone abattoir; 5.2% at Mazabuka and 16.8% were coming from Namwala (*Anonymous* 1957).

In 1956, a Veterinarian reported of a sick Lechwe (*Kobus leche kafuensis*) on the Kafue flats of Lochinvar. Two Lechwe from Lochinvar were slaughtered and on examination at the Central Research Station, extreme TB lesions were found (*Anonymous* 1957). Gallagher *et al.*, 1972, report of having had cropped a sizeable number of Lechwe between 1967 and 1970 at Lochinvar, where they found the percentage of those with gross TB lesions to have had been 14%. An abattoir was built in Lochinvar in 1969 and by 1972, lesions of TB were found in 36% of all the Lechwe shot at randomly by various hunters (Gallagher *et al.*, 1972).

2.2 Aetiology

Tuberculosis is caused by infection with certain species of acid-fast bacteria of the genus *Mycobacterium*. Robert Koch first demonstrated the first conclusive evidence that tuberculosis is an infectious disease in 1882, when he isolated and cultured *Mycobacterium tuberculosis* from crushed tubercles. Tuberculosis contains only a single genus (the genus *Mycobacterium*), but has over fifty species (Holt *et al.*, 1994). *Mycobacterium* is a genus of nonsporing, nonmotile Gram-positive bacteria. Generally, they are straight or curved rods, although coccobacillary, filamentous, and branched forms may occur (McFadden 1992). They are distinguished by the large amounts of lipids present in their cell walls, mostly in the form of branched, long chain fatty acids, the mycolic acids. Consequently, they form hydrophobic colonies on solid media and are difficult to stain by conventional stains such as Gram's. However, once stained, they are resistant to decolouration by acid-alcohol, which is the basis for the Ziehl-Neelsen method, and their identification property of acid-fastness (Murray *et al.*, 1990). The *Mycobacteria* are divisible into a few obligate pathogens and a much larger number of free-living environmental saprophytes that exist and multiply on dead and decaying matter (Grange *et al.*, 1990 and Nel 1981). Some of the saprophytic *Mycobacterium* may cause opportunistic infections in animals and humans but others may cause actual disease (Grange and Yates, 1986). Of the obligate pathogens, the most important includes: *Mycobacterium tuberculosis*, the predominant cause of tuberculosis (TB) in humans; *Mycobacterium africanum*, a rarer cause of TB in humans that was first isolated in equatorial Africa; and *Mycobacterium microti*, which occurs in voles and certain other rodents.

Of the approximately fifty species, the term tubercle bacillus is usually synonymous with *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *M. bovis* and *M. tuberculosis* are so closely related that some authors regard them as the mammalian strains and can only be differentiated from culture and biochemical tests and not from clinical presentation of the disease. *M. bovis*, which is closely related to *M. tuberculosis* causes tuberculosis mainly in cattle but also affects other species including humans (Collins *et al* 1994). *M. tuberculosis* causes disease in humans but has also been shown to be passed to cattle (Collins & Grange, 1983). Infection of humans by either strain cannot be differentiated clinically as the presentation of the disease is clinically indistinguishable (Griffith, 1941) and can only be differentiated by isolating and typing the strains. *M. tuberculosis* is susceptible to pyrazinamide and theiophene-2-carboxylate and can reduce nitrate, while growth of *M. bovis* is enhanced by the addition of Pyruvate but suppressed by Glycerol.

A group of tuberculosis species from the *Mycobacterium* genus makes up what is called as the *Mycobacterium tuberculosis* complex. This complex consists of species known as tubercle bacilli and cause tuberculosis in humans and animals (Collins, *et al.*, 1994). They consist of *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. avium* and *M. canetti*.

2.2.1 Classification

There are a number of classifications used to describe and classify the *Mycobacterium* genus, but according to recent and latest edition of the Bergy's Manual of Determinative Bacteriology (Holt *et al.*, 1994), the genus is classified into two groups:

- The slow growing species of *Mycobacterium*. There are about nineteen species classified under this group.
- The rapid growing species and subspecies of *Mycobacterium* that include 33 subspecies.

In terms of full classification, the bacteria can be classified as follows:

Kingdom:	Bacteria
Phylum:	Actinobacteria
Order:	Actinomycetales
Family:	Mycobacteriaceae
Genus :	<i>Mycobacterium</i>
Species:	<i>Mycobacterium bovis</i> , <i>M. tuberculosis</i> , <i>M. avium</i> etc.

The other bacteria under this genus, which don't cause tubercular disease in either humans or animals, are classified as the atypical bacilli. Runyon (1959) grouped the atypical mycobacteria on the basis of pigmentation, colonial morphology and growth rate. Collins and Grange (1993) classified 5 types of mycobacteria commonly associated with TB in man and animals, based on cultural and other properties. Those of human

origin were called classical human, Asian, Africa I or Africa II. Those from cattle were called classical bovine.

2.3 Transmission and infection

Inhalation of tuberculosis infected aerosols has been described as the most common route of infection in cattle and has been estimated to account for about 80 to 90% of infections (Morris *et al.*, 1994 and Riley *et al.*, 1959). A small proportion of infection has been shown to be congenital and sexually transmitted (Timoney *et al.*, 1988). In calves, infected milk is a very important source of infection (Liebana *et al.*, 1996). Pandey (1998) postulated that the disease in free-range grazing animals could be transmitted through contamination of pastures and soils as reported in Badgers in the United Kingdom and Possums in New Zealand. When transmission occurs by ingestion, the source of infection is usually infected milk, feed and water contaminated by discharges, and faeces (Clifton-Hadley *et al.*, 1993).

Human infection of *M. bovis* is usually of cattle origin, however, other animals are also known to be infected with *M. bovis* and remain as a potential source of human infection. Person to person transmission of infection is however possible (Bouvet *et al.*, 1993). Human populations are more susceptible to develop disease when *M. bovis* or *M. tuberculosis* is transmitted through inhalation than ingestion (Cook *et al.*, 1996).

Ultimately, the airborne transmission through inhalation of contaminated aerosol droplet nuclei containing one to three bacilli is the predominant route of infection together with

the alimentary route, through the ingestion of the bacilli in faeces, urine, genital discharges, milk from infected mammary glands, or contaminated feed or water.

Tuberculosis in cattle is most commonly transmitted by inhalation or ingestion, and these routes of infection determine the resulting lesions (Radostits *et al.*, 1994). Following inhalation, the *M. bovis* lodges in the terminal bronchi and, if infection is successfully established, then typically disseminates to the regional lymph node. Both components-the initial focus and the reactive lymph node-are known as the primary complex (Dungworth 1993). Following ingestion, a similar process occurs in the pharynx or the intestine.

The pathogenicity for cattle of the human type of the tubercle bacilli has been generally regarded as lower than that of the bovine type (Liebana *et al.*, 1996). Infection of cattle by *M. tuberculosis* is usually self-limiting and is associated with small lesions of less clinical significance (Radostits *et al.*, 1994). It is possible for cattle with no gross lesions to excrete *M. tuberculosis* in milk just as they excrete *M. bovis* (Schmitt *et al.*, 1997). However, several cases of cattle infection with human type bacilli have been reported (Kleeberg 1975). The bacilli may escape through the blood stream from the original lesion to lodge into new locations where they form new tubercles. When such systematically translocated bacilli lodge in the mammary glands, it creates a serious zoonotic problem for milking cows. Active tubercles can contaminate milk meant for calves and humans (Carlton and McGavin 1995). Due to the chronicity of the disease, this process can occur for a long period.

2.4 Pathogenesis

When contracted through the aerosol route, the bacteria gain access to the alveoli where they are engulfed by alveolar macrophages, which presumably are equipped with multiple microbicidal mechanisms, including phagolysosome fusion and a respiratory burst, to rid the host of infecting micro organisms (Sibike and Reynolds 1990). The alimentary route allows the bacteria to deposit along its tract such as in the pharyngeal or intestinal mucous membranes. In previously unexposed animals, local multiplication occurs as macrophages on the scene ingest the organisms (Radostits *et al.*, 1994). To establish infection successfully, the tubercle bacillus must survive its encounter with these macrophages and ultimately gain access to the lymphatic or the bloodstream (Canetti 1965). Haematogenous dissemination may produce milliary tuberculosis: multifocal tubercle formation throughout an organ.

In the lymphatics, proliferation and inflammatory responses continue. After the first week, cell mediated immune reactions begin to modify the host response from essentially a foreign body reaction to a reaction characteristic of infectious granulomas. Their efficacy in doing so depends on the adequacy of the host's immune response and the virulence of the bacteria (Rich 1951).

The lipid components in the bacteria are implicated in the pathogenesis. Mycosides, phospho-, and sulfolipids apparently protect the tubercle bacilli against phagocytic killing. Other lipids elicit the production of granulomas and the tubercular proteins stimulate cell mediated allergic responses, which is a central factor of tuberculosis. Epithelioid and giant cells appear among the macrophages and these seem not to be

effective phagocytes. At the centre of the lesion, caseous necrosis develops. This may proceed to calcification or liquefaction. At the periphery of the lesion are unaltered macrophages mixed with lymphocytes. Fibrocytes appear, and a fibrous layer eventually invests the lesion, which is called a tubercle. Tubercles may enlarge, coalesce and eventually occupy sizable portions of organs. Such tubercles consist mostly of caseous material. Clinical signs then depend on the type of organ(s) affected by these necrotic lesions. With progression, generalized TB develops, manifested by weakness, debility, and eventual death (Radostits *et al.*, 1994).

The above pathogenesis described is typical of both human and bovine tuberculosis caused by mammalian tubercle bacilli. It has a chronic characteristic and the lesion is called productive or proliferative. Occasionally an acute exudative process takes place, marked by predominantly neutrophilic responses and fluid effusion (Anonymous 1998). It is thought to be favoured by such factors as: a large infecting dose; focally delivered; high virulence of the infecting strain; constitutional predisposition of the host; loose tissue architecture as in the lung, serous membranes, or meninges; and a high degree of tuberculous allergy. One such acute process is tuberculous pneumonia, which may cause extensive necrosis and can be rapidly fatal ("galloping consumption") or can resolve almost completely, or even subside into the chronic pattern (Anonymous 1998).

Once cell-mediated reactivity is established, subsequent reinfection follows a different course: antigen-specific T lymphocytes and activated macrophages promptly converge on the infection site, and prevent lymphatic spread. Reinfection tuberculosis is most often endogenous; that is, it results from reactivation of previously dormant foci.

Antigen-specific T lymphocyte responses, however, also mediate allergic cytotoxic reactions and cause extensive tissue destruction, which is characteristic of progressive tuberculosis, while lymphatic dissemination is limited by the immune response. Tissue damage facilitates bacterial spread by contiguous extension or erosion of bronchi, blood vessels, or viscera introducing infection to new areas. Wherever microorganisms lodge, the allergic (as well as the immune) reaction will be repeated with cumulative consequences.

2.5 Distribution and epidemiology

2.5.1 Tuberculosis distribution and epidemiology at the global level

Tuberculosis is a worldwide problem. The World Health Organization (WHO) has declared the new resurgence of TB a global emergency. Its prevalence varies in different countries and it is low in developed countries yet high in developing ones (Carlton and McGavin 1995). The World Health Organization estimated the tuberculosis incidence and deaths for 1990 to 1999 to be eighty-eight (88) million and thirty (30) million, respectively, with most cases occurring in developing countries (Cosivi *et al.*, 1998). Currently, it is estimated that one-third of the world's population is infected with tuberculosis (Enarson & Murray, 1996). This translates to one out of every three people in the world (about two billion people) are estimated to be infected and it remains the most common infectious cause of death among adults, killing 2 to 3 million people each year (Cosivi *et al.*, 1998). However, only 5 to 10% of the infected individuals go on to develop primary or reactivation tuberculosis, suggesting that host factors contribute to the pathogenicity of the disease. In several regions of the world, there is an alarming rise of incidence, linked to the increasing devastating impact of HIV epidemics and

deficiencies of current tuberculosis control programs (Dankner *et al.*, 1993). The worldwide development of transport and migration contributes to globalize those threats (Bloom and Murray 1992).

It has been shown that if current trends continue, 10.2 million new TB cases are expected in 2005, with Africa having more cases than any other region in the world (WHO, 2001).

Most authors still believe that the global incidence of tuberculosis is greatly underestimated. In 1995, 3.3 million cases of TB were reported to the Global Tuberculosis Programme of WHO, whereas a more likely number is 8.8 million (Cosivi *et al.*, 1998). Of the reported cases, 62% occurred in the Southeast Asian and Western Pacific regions, 16% in sub-Saharan Africa, and 7% to 8% in each of the regions of the Americas, Eastern Mediterranean, and Europe. However, many countries, especially those with few resources, are unable to report all TB cases because of difficulties in identifying suspected cases, establishing a diagnosis, and recording and reporting cases.

In 1995, a total of 3.3 million cases of TB were reported to the Global Tuberculosis Programme of the WHO of which 17% were attributed to sub-Saharan Africa and it was estimated that by the year 2000, there would be a total of 10.2 million new cases of which 81% were to be attributed to sub-Saharan Africa (Dolin *et al.*, 1994). However, the total number of new cases was expected to double by the year 2000 (Sudre *et al.*, 1992).

2.5.2 *Tuberculosis distribution and epidemiology in Zambia*

In Zambia, the incidence of tuberculosis has increased from around 100/100,000 in 1984 to over 300/100,000 in 1993 (*Anonymous 1994*) in the human population. At the same time, Elliot *et al.*, (1993) reported that by 1989, of the total number of patients who were receiving antituberculosis therapy in Zambia, 73% of them were seropositive for HIV. Duncan *et al.*, (1995) reported that in Zambia, most of the adult population is infected with tuberculosis with reactivation of the latent infection as an important mechanism especially with the dual infection of HIV. However, very little information is available as to ascertain how many of the observed human tuberculosis cases can be attributed to infections with *Mycobacterium bovis*.

2.5.3 *Zoonotic tuberculosis*

Bovine tuberculosis (caused by *M. bovis*) is a significant zoonosis in many parts of the world and is commonly known as Zoonotic Tuberculosis. *Mycobacterium bovis* has a very wide host range and a complex epidemiological pattern involving interactions between man, domestic animals and wildlife.

Although it is well documented that Mycobacterium tuberculosis is the most common cause of human TB, unknown proportions of cases are due to Mycobacterium bovis (Acha & Szyfres, 1987). Because of the various species of *Mycobacterium* that can cause tubercular disease, it is mainly documented that members of the *Mycobacterium tuberculosis* complex cause tuberculosis. *Although isolates from man and cattle have* regarded as separate species, they are closely related in terms of their biochemical

istics (Tsukamura, 1976, Karlsson & Lessel, 1970) and DNA profiles (Imaeda,

1985). Isolates with the characteristics of bovine strains have been isolated from all forms of TB in humans, causing disease clinically indistinguishable from that due to human strains of *M. tuberculosis* (Griffith, 1941). Human infections with bovine strains of the *M. tuberculosis* complex have been transferred to cattle (Baldwin, 1968) and transmission from man to man has been reported in the presence (Bouvet *et al.*, 1993) and absence (Collins & Grange, 1983) of concurrent HIV infection. Exposure of cattle to human strains of the *M. tuberculosis* complex has resulted in both a transient sensitivity to tuberculin (Lesslie, 1960) and to clinical infection (Steniwo, 1938).

Lesslie *et al.*, (1972) reported of having established a direct correlation between the prevalence of tuberculosis in cattle and the prevalence of tuberculin reactors among thirteen-year-old school children. They further found out that a substantial proportion of tuberculin reactors among the children were from bovine sources and the majority of the children in this category came from cattle keeping areas. The high figures of Zoonotic Tuberculosis in areas with high cattle population suggest the existence of a relationship between keeping cattle and getting the *M. bovis* organism. Similarly, Cook *et al.*, (1996), in an epidemiological study in Zambia, showed an association between tuberculin-positive cattle and human tuberculosis. The study further found out that households that reported a human tuberculosis case within the previous twelve months were likely to own herds containing tuberculin positive cattle. Daborn *et al.*, (1997) reports that in their investigation in Tanzania, seven of the nineteen lymph node biopsies from suspected extra pulmonary tuberculosis patients were infected with *M. tuberculosis* and four with *M. bovis*. Mposhy *et al.*, (1983) stated that in North Kivu (Zaire), the frequency of human tuberculosis of animal origin is increasing with records at one time showing that

out of fifty-eight hospitalized patients, forty-nine were cattle herdsmen. In this same study, the researchers were able to isolate *M. bovis* from gastric secretions in two of five patients with pulmonary tuberculosis (PTB). They further found that the prevalence of the disease in the local cattle was approximately 8% by tuberculin testing and isolation of *M. bovis*.

2.6 Public health importance of tuberculosis

Dolin *et al.*, (1994), estimated that TB morbidity and mortality will reach 88 million and 30 million, respectively with 70% of the new cases occurring in patients 15 to 59 years of age, the most economically productive segment of the population. Such a trend will be seen with a dual infection with HIV. However, some countries have high prevalence depending on different regions. Sonnenberg *et al.*, (2001) reports that the prevalence of tuberculosis in South Africa among gold miners was found to be 1,536/100,000 which is thought to be perhaps higher than in any setting today.

Blancou *et al.*, (1971) in their survey that was carried out in Madagascar found that the percentage of tuberculosis condemnations in the slaughter houses for the whole island was 21% of the 1,465,000 cattle examined. This high figure was reported to have had a significant public health implication to abattoir workers apart from the economic losses incurred from condemning such carcasses.

Prevalence rates obtained in local cattle from which human tuberculosis of bovine origin has been determined are correspondingly higher too. Waddington (1965), reported of tuberculosis prevalence greater than 6.6% among native cattle in some areas of Kenya,

but in Malawi they had some areas recording 50% prevalence. Additionally, they also discovered corresponding higher human cases of tuberculosis in areas with high bovine cases, although no characterization was done to determine which strain was in humans.

2.6.1 *Tuberculosis and its fatal alliances*

Duncan *et al.*, (1995) reported that in Zambia, a proportion of the economically productive adult populations are infected with tuberculosis with reactivation of the latent infection as an important mechanism especially with the dual infection of HIV. They further stated that individuals with HIV-induced immune suppression appear to be particularly susceptible to tuberculosis infection even at moderate stages of viral infection. It has been shown that HIV-infected persons are at marked risk for developing both primary and reactivation tuberculosis (Faussett *et al.*, 1995, Daley *et al.*, 1992). Without the effects of the HIV virus, the TB infection is mostly contained and only progresses in late stages of life when the balance is tipped in favour of the mycobacterium due to senility and waning immune response. However, in people infected with HIV, TB progresses rapidly at any age of infection, and these people disseminate TB to those in contact with them (whether the in contacts are HIV-infected or not) (Darborn and Grange 1993).

Most recently, the incidence of TB has undergone a dramatic resurgence, even in developed countries due to the occurrence of TB in AIDS patients and the development of multi-drug resistant (MDR) tuberculosis (Daborn & Grange 1993). Additionally, tuberculosis is one of the very few HIV / AIDS-related diseases that is transmissible to other human beings, whether HIV positive or not. Hence, this disease and its

transmission require special consideration. *Mycobacterium bovis* infection has been reported in several patients with AIDS in San Diego, California (Dankner *et al.*, 1993) and a multidrug-resistant strain of *M. bovis* has been reported to have had spread between patients in an AIDS ward in France (Bouvet *et al.*, 1993). As a result of the HIV epidemic, the crude incidence rate of TB was expected to increase in sub-Saharan Africa from 191 cases per 100,000 in 1990 to 293 in 2000. However, the total number of new cases was to double by the year 2000. While demographic factors, such as population growth and changes in population structure, will largely account for the expected increase in TB incidence worldwide, the HIV epidemic in sub-Saharan Africa will have a greater role than demographic factors (Cosivi *et al.*, 1998).

Apart from the co-infection with HIV, other studies have shown the exacerbation of other pathogens due to the presence of TB. Rodriguez *et al.*, (1999) found that tuberculosis had a significant and potential influence over the immune response and susceptibility of other pathogens especially chronic helminthes. They further indicated the massive and strong co-infection which exists when tuberculous lymphadenitis and lymphatic filariasis occurs.

2.7 Implications of Zoonotic tuberculosis

The public health importance of animal TB was recognized early by the WHO, which in its 1950 report of the Expert Committee on Tuberculosis (WHO Expert Committee on Tuberculosis on the Fifth Session; 1950) stated: " The committee recognizes the seriousness of human infection with bovine tuberculosis in countries where the disease in cattle is prevalent. There is a danger of transmission of infection by direct contact

between diseased cattle and farm workers and their families, as well as from infected food products.” Since then, TB in animals has been controlled and almost eliminated in several industrialized countries but in very few developing countries.

Raviglione *et al.*, (1995) showed that the situation is worse now than it was before especially in developing countries such as Zambia. The report stated that TB is the most frequent opportunistic disease associated with HIV infection. In most African countries, Zambia included the HIV seroprevalence rates greater than 60% have been found in TB patients.

Reports in Zambia show an annual risk progression rate of infected persons with both pathogens (HIV and TB) to active TB to be over 15% depending on their level of immunosuppression (Anonymous 1998).

Another public health risk in most rural and urban health care centers is the misdiagnosis, as they do not have the resources and equipment to diagnose between species of the *M. tuberculosis* complex and the disease even caused by *M. bovis* is merely reported as TB.

Walshe *et al.*, (1991) stated that Africans boil their milk and that the souring process destroys *M. bovis*. Other sources (Barrera & de Kantor [1987]) as well as Sitima (1997) strongly contradict these statements. Even the reports from the World Health Organisation (WHO) of 1994 working group on zoonotic tuberculosis, showed that *M. bovis* was isolated from seven (2.9%) of 241 samples of raw milk in Ethiopia. This

indicates another potentially public health hazard of contracting the disease from infected food products.

Idrisu and Schnurrenberger (1997) in Nigeria as well as Nafeh *et al.*, (1992) in Egypt, showed that both *M. bovis* and *M. tuberculosis* were found in milk. Thus serious public health implications of potentially contaminated milk and milk products should not be underestimated especially in countries like Zambia where traditional methods of milk processing entails usage of non-heat treatment methods.

Sitima (1997) showed evidence that traditional ways of processing sour milk does not eliminate *M. bovis* even at the time of consumption. He further found out that traditional cattle owners in Southern Province consume an estimated 90% of the total milk produced as sour or fresh and mostly ignore basic standards of hygiene and quality further increasing the risk of contracting the disease from milk.

It is well known, that *M. tuberculosis* will be largely responsible for the new TB cases and deaths, but an unknown, and potentially important, proportion will be caused by *M. bovis*. The direct correlation between *M. bovis* infection in cattle and the disease in the human population has been well documented in industrialized countries. Whereas little information is available from developing countries (Collins and Grange 1983), risk factors for *M. bovis* in both animals and humans are present in the tropics, Zambia inclusive.

Available information reviewed clearly shows the extent and magnitude of this zoonotic problem. Studies in Argentina (Barrera & De Kantor, 1987) and the Southeast of England (Yates & Grange 1993) have reported incidences of between 0.4 and 6% respectively of the total cases of human TB to be due to zoonotic tuberculosis. According to data from the Pan American Health Organization and the World Health Organization, seven thousand (7,000) new cases of tuberculosis in humans caused by *M. bovis* arise in South America each year (PAHO/WHO, 1991) and some authors have suggested the incidence could be eight times higher (Ritacco & De Kantor, 1992). In Africa, Zambia included, the zoonotic importance of *M. bovis* infection may further be exacerbated by closer contact with animals, consumption of nonpasteurized milk, and the ever increasing incidence of the human immunodeficiency virus infection (Daborn & Grange 1993). Similar studies by van der Hoeden (1964), had at that time reported the different levels of the incidences of bovine tuberculosis among humans in different European countries to be 4% France, 5% Netherlands and 5% Germany. Barrera and de Kantor (1987), report from a national study in Argentina during 1982 to 1984 that 36 (0.47%) of 7,672 *Mycobacteria* cultured from sputum samples were from *M. bovis*. However, Sequeira *et al.*, (1990) reported that during their study from 1984 to 1989, *M. bovis* caused 0.7% to 6.2% of Tuberculosis cases in the Santa Fe Province. This is the province of Argentina where most of the dairy cattle industry is concentrated. The high figures of Zoonotic Tuberculosis in areas with high cattle population confirm the existence of a relationship between keeping cattle and getting the *M. bovis* organism. Such findings tie up with Gervois *et al.*, (1972), who estimated that the proportion of human cases due to *M. bovis* accounted for 3.1% of all forms of tuberculosis: 2.1% of pulmonary forms and 9.4% of the extra pulmonary form. Other studies by Idrisu and

Schnurrenberger (1977) reported in their study in Nigeria that one in every ten of *Mycobacteria* they isolated from sputum positive cultures was actually *M. bovis*. The proportion thus indicates 10% of the culture isolates which still is a high figure. Similarly, Idigbe *et al.*, (1986), report of isolating *M. bovis* from sputum samples of patients with pulmonary tuberculosis in Nigeria. They report that of the 102 tuberculosis complex isolates 4 (3.9%) were for *M. bovis*. Such differences from the same country may be due to a number of reasons but in most regions it has come out clear that the presence of cattle in an area raises the chances of having a human population with Zoonotic Tuberculosis. Such findings tie up with Elsabban *et al.*, (1992) who carried out a study in Egypt on bovine tuberculosis and its extent of spread as a source of infection to man and animals. In this study, they found out that the proportions of tuberculosis patients of sputum positive infected with *M. bovis*, recorded during three observations varied from 0.4. %, 6.4% and 5.4%. In the same country in Egypt, Nafeh *et al.*, (1992), showed that nine of the twenty randomly selected patients with tuberculosis peritonitis were infected with *M. bovis* and the remaining with *M. tuberculosis*.

Sharma *et al.*, (1988), carried out a prevalence study of bovine tuberculosis in cattle of India. In their study, the prevalence of TB was highest in exotic breeds (26%), followed by crossbreeds with 9.7% and median breeds had 7% prevalence rate. Another finding was that the prevalence increased with age, from 1.7% in cattle between 6 months to 1 year of age to 22.3% in those over 5 years of age. These findings tie up with Lall (1969) whose study in India found a prevalence rate of TB in cattle to range from 1.6% - 16%. All these findings by various researchers allude to the fact that tuberculosis is a problem and there is enough literature which supports the zoonotic potential of the disease.

It is evident that TB is one of the greatest threats to human health since the bubonic plague and it still poses direct and indirect threats to domesticated, wild animals and humans. In Zambia currently, the contribution of zoonotic tuberculosis to reported increased national TB figures is unknown owing to the incapability of most laboratories to differentiate the species of the *M. tuberculosis* complex. However, from the reviewed literature, there are indications that human tuberculosis due to *M. bovis* does exist and is mostly transmitted from cattle to man and may cause non-pulmonary primary infections. In addition, the evidence of man to man spread of *M. bovis* is very limited although transmission from man to cattle is well documented. The relatively low incidence of development of open (infectious) pulmonary tuberculosis due to *M. bovis* in man is almost certainly due to immunological factors which would be abrogated in HIV / AIDS. Thus there is every reason to be seriously concerned that the HIV pandemic will result in an increase of human tuberculosis due to *M. bovis* and a greater degree of transmission of infection to other human beings and to animals could well occur.

2.8 Importance of tuberculosis in Zambia

Under the World Health Organization (WHO), Zambia is ranked in Group three on the grounds of prevalence and incidence of human tuberculosis. This group consists of countries where tuberculosis is a major problem and the decline path for the situation has never been recorded. In Zambia, the incidence of tuberculosis has been on an increase. The incidence of tuberculosis in Zambia has doubled in less than 10 years, from 1984 to 1993 (Anonymous 1998) in human populations. At the same time, Elliot *et*

al., (1993) reported that by 1989, of the total number of patients who were receiving antituberculosis therapy in Zambia, 73% of them were seropositive for HIV.

Although statistical figures show significant levels of tuberculosis infection among human populations in Zambia, very little information is available as to ascertain how many of the observed human tuberculosis cases can be attributed to infections with *Mycobacterium bovis*. Sitima (1997), Cook *et al.*, (1996) and Pandey *et al.*, (1998) reported that there is a relationship between rearing animals and acquiring tuberculosis infection in Zambia. The authors however, have not been conclusive on establishing any causal relationships as only general surveys were conducted without specific species differentiation. Statistical figures show that by 1988/9, Southern province of Zambia had the highest number of cattle in the traditional sector totaling 866,378 animals followed by Western province with 709, 564, while Central had 322,732, Lusaka had 37,647 and Luapula province had only 10,031 animals (Anonymous 2000) During the same years (1988/9), the Ministry of Health reported the following incidences of tuberculosis in the same areas; Southern province registered 2,385 new cases, Western had 1,867 new cases while Central, Lusaka and Luapula Provinces had lower figures of 703, 891, and 704 respectively although the population sizes of all these provinces vary. Usually it would be expected that human tuberculosis prevalence would be higher in highly populated areas although the above statistics show that this is not the case. This translates to other factors being more significant than just human populations and settlements. It is therefore imperative to establish these causal factors and their role in disease transmission and propagation. Such information, if correct, has serious public health implications in the cattle rearing areas of Zambia especially in the light of the current

HIV/AIDS epidemic. This above scenario has also been observed in a survey study by Buve & Foster (1993). These researchers found a high prevalence of tuberculosis in humans and HIV in the Monze District of Zambia. They found that 10% of adults in the district were HIV positive and up to 70% of TB patients were concurrently HIV positive. In contrast with statistics from the capital city, which despite being crowded Elliott *et al.*, (1990) found that in Lusaka only 60% of the TB patients were found concurrently to be HIV positive reflecting a lower percentage than in Monze district.

Studies in cattle that were conducted by Pandey *et al.*, (1996) in the Monze district of Zambia reported an estimated prevalence of tuberculosis in cattle of 7.4%. Another compounding factor in certain animal rearing populations in Zambia is the presence of tuberculosis in wild animals. Gallagher *et al.*, (1972) reported the isolation of *Mycobacterium bovis* from the Lechwe (*Kobus lechwe Kafuensis*) in the Lochinvar National Park (LNP) of Zambia. In this park, wild and domesticated animals share pastures at certain times of the year enhancing the spread of the disease between these hosts. Wild animal tuberculosis represents a permanent reservoir of infection and poses a serious threat to control and elimination programs.

Epidemiological surveys that have been conducted in Zambia have been able to show a relationship between rearing animals and acquiring tuberculosis infection (Cook *et al.*, 1996, Sitima 1997, Pandey *et al.*, 1998). Currently, it is not known how many of the observed human tuberculosis cases can be attributed to infections with *M. bovis*. Certain high-risk areas have been identified to have multiple hosts for zoonotic tuberculosis in Zambia. One such area is the Lochinvar National Park (LNP). Bovine tuberculosis has

constantly been reported in the Kafue lechwe (*Kobus leche Kafuensis*), a medium sized semi-aquatic antelope inhabiting the Kafue Flats of Southern Province of Zambia. Due to the difficulties in applying any meaningful and practical control measures in wildlife, the occurrence of disease in these animals pose a grave risk of remaining permanent reservoir hosts. In Zambia, the Kafue flats are part of the country's biggest pastoral lands where cattle graze in the same plains as the Lechwe. This also involves the presence of human settlements in the interface areas of the National Parks. All these factors complement in the existence and perpetuation of the disease as well as making it a serious public health risk to the communities within the park (Cosiyl et al., 1995, Grange et al., 1994 and Daborn et al., 1997). In Zambia as in many of other African and developing countries, in most rural populations only a fraction of the population has access to health services. This may mean even a more incomplete picture of the situation as it may be coupled with none or poor recording systems of most rural health cares. The assessment of Zoonotic Tuberculosis in high risk suspected human contacts with a reported prevalence in both cattle and wildlife requires the understanding of all the transmission dynamics and the existing risks on the ground.

2.9 Diagnostic Tools

In Developing countries like Zambia, the zoonotic implications of bovine tuberculosis will require the correct diagnosis and more importantly the correct identification of species within the *Mycobacterium tuberculosis* complex. It is also important to determine whether the strain causing disease in one particular host specie is identical to that causing disease in other species or even in cases of the same specie. Such kind of technology in DNA fingerprinting also does assist in determining whether transmission

could have occurred within susceptible species apart from determining the level of genetic relatedness. DNA fingerprinting methods can also be applied in tracking sources of infection in complex epidemiologies such as that of tuberculosis. Additionally, risk factors for transmission of tuberculosis can be determined thus enhancing strategies for control measures of the disease.

2.9.1 Bacteriology and biochemical means with their limitations

Gillespie *et al.*, (1986) reported their findings on the propensity of the members of the *Mycobacterium tuberculosis* complex to change growth characteristics. Since traditionally, the members of the complex are characterized on the basis of a set of tests of microbiological and biochemical phenotypes, differentiation and drug susceptibility testing of these organisms may be complicated by the slow growth in culture and the reported propensity to change their growth characteristics. Species identification may sometimes be difficult with multiple-drug-resistant organisms.

Gutierrez *et al.*, (1999) through the use of traditional identification methods of microbiological and phenotypic biochemical characteristics demonstrated that earlier they had described a multidrug-resistant strain of tuberculosis to be due to *Mycobacterium bovis* outbreak which genetic typing confirmed to be due to *Mycobacterium tuberculosis* instead

Habib and Warring (1996) stated that the similarity in the clinical presentation and treatment of tuberculous diseases has meant that many laboratories do not fully identify or correctly isolate the species of *Mycobacterium*. However, it is important to correctly

identify these isolates so that epidemiological investigations can detect potential reservoirs that may be a risk to public health.

Kataria (1969) indicated the resistance of *Mycobacterium bovis* to pyrazinamide, a drug commonly used to treat tuberculosis. The identification of such strains helps in the reduction of MDR tuberculosis through the application of appropriate medicaments.

Many laboratories now use molecular techniques for the identification of *M. tuberculosis* complex isolates. In Zambia, many rural areas where cattle are kept there is a high probability of the community being exposed to *M. bovis* infection. In the proposed study area of Locinvar National Park interface, there is a considerable movement of cattle and wild animals between herds. It is in these same areas that Sitima (1997), and Pandey *et al.*, (1998) reported high prevalence of tuberculosis in humans and cattle as well as in wild animals particularly the Lechwe. In recent years, the development of DNA fingerprinting of *M. tuberculosis* complex isolates has proven very useful both in epidemiological and diagnostic capabilities of investigating tuberculosis from different host species.

2.9.2 Pathogen characterization

DNA fingerprinting has been used by many scientists for epidemiological studies of diseases caused by the *Mycobacterium tuberculosis* complex. The most commonly used genetic marker is the insertion sequence IS6110, (van Embdan *et al* 1993 & Thierry *et al* 1990). IS6110 restriction fragment length polymorphism (RFLP) typing is the most widely applied and standardized molecular typing method. It is based on the detection of

the repetitive sequence IS6110 that can be found in most isolates of the *M. tuberculosis* complex.

To generate IS6110 RFLP patterns, a well-grown *M. tuberculosis* complex isolate is required. DNA is extracted, purified and digested with the restriction enzyme *PvuII*. This restriction enzyme cleaves the IS6110 element at a single site. The *PvuII* restriction fragments are separated overnight on an agarose gel and subsequently transferred to a DNA membrane. IS6110 containing restriction fragments are visualized by adding a peroxidase labeled probe with a DNA sequence complementary to the right-hand part of the IS6110 sequence in a hybridization buffer onto the DNA membrane. The hybridizing restriction fragments are detected by a chemiluminescence reaction initiated by two substrates and the RFLP patterns are detected on a light sensitive film. The most critical aspects of the RFLP procedure (the restriction enzyme, the probe and the markers) have been standardised to facilitate the inter-laboratory comparability of IS6110 RFLP patterns. This has facilitated accurate computer-assisted comparisons of RFLP patterns, but this is beyond the scope of this work.

Whipple *et al.*, (1997) used restriction fragment length polymorphism (RFLP) in investigating outbreaks of tuberculosis in deer, elk and coyotes in the United States. Szewzyk *et al.*, (1995) used the method in deer in Sweden while van Soolingen *et al.*, (1994) used various genetic makers to differentiate *M. bovis* strains from animals and humans in The Netherlands.

However, this sequence (IS6110) is present in only one or two copies in most *M. bovis* species, it is thus of limited value for typing this species (van Soolingen *et al* 1994 & D. Cousins *et al* 1998), as it is insufficiently discriminative. The DNA of strains with less than five copies of IS6110 is therefore digested by the enzyme *AluI*, and after electrophoresis and southern blotting, subjected to a polymorphic GC-rich sequence (PGRS) probe (van Soolingen *et al.*, 1993). Other markers such as the direct repeat (DR) have been used to type *M. bovis*, and a relatively new polymerase chain reaction (PCR) based method known as "Spoligotyping" has shown promise in typing strains of *M. bovis* that have a single copy of IS6110.

Cousins *et al.*, (1998), van Soolingen *et al.*, (1994) analysed the use of different genetic markers such as RFLP analysis with probes derived from the insertion element IS6110, for the differentiation of *M. tuberculosis* complex strains and it has proved to be one of the most useful method. Cousins *et al.*, (1998) however, said compared to *Mycobacterium tuberculosis*, most isolates of *Mycobacterium bovis* contain relatively few copies of IS6110. Nevertheless, other authors, van Soolingen *et al.*, (1994), Liébana *et al.*, (1997) have shown proof of how useful RFLP has been for strain differentiation of the *Mycobacterium tuberculosis* complex.

2.9.2.1 Polymerase Chain reaction (PCR) technique

Saiki *et al.*, (1985) defined PCR as being an in-vitro method of producing large amounts of specific DNA fragment of defined length and sequence from small amounts of a complex template. Developed at the Cetus Corporation in 1984, the PCR permits the

scientists to produce a billion copies of a fragment DNA from a single copy in a few hours.

The PCR takes advantage of an enzyme that uses a defined segment in a strand of DNA as a template for assembling a complimentary strand. In early PCR applications, the enzyme used was the *Klenov* fragment of *Escherichia coli* DNA polymerase 1, but this heat-labile enzyme had to be added in each subsequent cycle because the enzyme was inactivated during each denaturation step. The *Klenov* fragment has since been replaced by a more thermal stable DNA polymerase of *Thermus aquaticus* (*Taq*), a development that has permitted automation of the procedure because all reaction components can be combined at the beginning of the test reaction. Another advantage of *Taq* is that it has improved the specificity, yield, sensitivity and length of the target DNA that can be amplified (Saiki *et al.*, 1988).

White *et al.*, (1989) described PCR as a test based on the enzymatic amplification of a DNA fragment that is flanked by two oligonucleotide primers that hybridise to opposite strands of the target sequence. The primers are oriented with their 3' ends pointing towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complimentary sequences and extension of the annealed primers with a DNA polymerase results in the amplification of the segment defined by the 5' ends of the PCR primers.

The PCR reaction requires three steps:

1. **Denaturation** of the target double DNA.
2. **Annealing** of primers.
3. **Primer extensions**.

Denaturation is achieved by heating the DNA sample to around 95°C for five minutes. This process unwinds the DNA double helix. Annealing the DNA is cooled to between 37 and 60°C in the presence of an excess of two DNA primers, which are short oligonucleotides (in the region of 18-25 nucleotides long). Extension usually takes place at 72°C in which the heat resistant DNA polymerase (*Taq* polymerase from the bacterium *Thermus aquaticus*) extends the primers to make complementary DNA strands from each single stranded template. Mullis *et al.*, (1987) described a cycle as taking 2-5 minutes usually and is repeated at least 20-40 times.

The PCR reaction mixture contains buffers, nucleotides (dATP, dCTP, dGTP and dTTP), Primers, *Taq* DNA polymerase and target DNA from the sample to be analysed. The first step of denaturation separates the complimentary strands of DNA held together in the duplex by hydrogen bonds. Heating the reaction between 95-100°C for a minute is sufficient. In the second step, the primers are annealed or attached to the dissociated DNA strands. Each primer is complimentary to one of the original DNA strands to either the left (5') or right (3') side of the sequence of interest. The primers are present in such vast molar excess that they are more likely to anneal to the dissociated strands than the strands are to anneal to each other. Once annealing has occurred all in the presence of the *Taq* DNA polymerase, the number of strands essentially doubles at the end of each cycle. Approximately after 30 cycles, a single copy of DNA can be increased up to one

million copies. Visualisation of the amplified DNA can be done using gel electrophoresis and ethidium bromide staining.

2.9.2.2 Characterization using Spoligotyping Technique

Kamerbeek *et al.*, (1997) developed a more recent, faster and less labour intensive method than RFLP called Spacer oligonucleotide typing (Spoligotyping). Spoligotyping is a Polymerase Chain Reaction (PCR) based method that detects DNA polymorphism in the spacer sequences of the direct repeat (DR) region. This *M. tuberculosis* complex-specific genomic region contains 36bp DRs, interspersed by unique DNA spacers of 35-41bp in length. The numbers of DRs per strain and there by the presence of particular spacer sequences differ significantly between strains. The spacer sequences of the isolates being typed are amplified and labeled by PCR and are superimposed on a series of known spacer sequences attached to a membrane. The pattern of hybridization will depend on the number of membrane-bound spacer sequences attached to a membrane. Kamerbeek *et al.*, (1997) stated that the membranes presently in use have 37 different spacer sequences from *M. tuberculosis* H37Rv and 6 spacer sequences from *M. bovis* BCG. Thus a total of 43 oligonucleotides are used, based on the DNA sequence of spacers in *M. tuberculosis* strain H37Rv and BCG vaccine strain P3. These oligonucleotides are covalently bound to a Biotin C membrane in parallel lines. The denatured PCR products are allowed to hybridize perpendicular to the oligo lines in the reversed line blot hybridization. The hybridization is detected by adding streptavidine conjugate and substrate, which results in a chemiluminescence reaction that is detected on film. The spoligotypes identified by different laboratories can be readily compared as

spoligotyping detects the presence or absence of specific spacer sequences and does not require measurement of fragment size.

Nevertheless, Cousins *et al.*, (1998) and Roring *et al.*, (1998) showed that spoligotyping does not give the same degree of differentiation between strains as RFLP typing. However, certain groups of strains have shown highly unique spoligotypes e.g. caprine strains of *M. bovis* from Spain, (Aranaz *et al.*, 1996) and the Beijing strains of *M. tuberculosis* (van Soolingen *et al.*, 1995).

Additionally, Kamerbeek *et al.*, (1997), showed that Spoligotyping is the only method which can allow simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* complex bacteria without the need of culturing this slow growing organism. The authors also stated that different strains of *Mycobacterium* isolates would show different hybridization patterns. Those isolates from the same area and relatively closed stable populations will have a likelihood of showing similar spoligotypes thus allowing for the tracing of possible transmission patterns and hence the effecting of adequate control measures.

2.10 Treatment and prevention

2.10.1 Applications in animal populations

Treating cattle with drugs has been tried but is of no practical use. The severity of infection can be reduced but prevalence is little changed. In these species, the cost is extremely prohibitive.

In terms of control, the majority of African countries have had little or no success in controlling bovine TB. However, most developed countries eradicated bovine TB in cattle by the use of the test and slaughter method. The tuberculin test has been the single most important tool in the test and slaughter schemes for the eradication of bovine TB. This type of scheme has failed in Africa, as all reactors to the test should be culled and owners compensated. Further still, herd tests have to be repeated to detect latent infections. Once herds are tested and certified TB free, no contact is allowed with those not tested. In most African countries, communal grazing is practiced further compounding the application of such a control scheme. All this requires a high level of organization from government services and a lot of cooperation from animal owners. However, in Zambia, just like in many other African countries, both are deficient at this point in time.

2.10.2 Applications in human populations

In the human populations, intervention strategies to combat TB commenced soon after the discovery of the bacilli by Robert Koch. The first significant historical intervention strategy against TB was the sanatoriums movement, initiated by Hermann Brehmer in Europe in 1854, and later established by Dr. Edward Livingston Trudeau in America (Bloom & Murray 1992). This intervention method entailed the isolation of the infected patient in sanatoriums, providing the patient with bed, rest and an adequate, nutritional diet, and simultaneously isolating the source of infection from the general population. It was not until the discovery of rifampicin in 1943 (the foundation of the current TB therapy regimen), that significant progress in the management of TB has become evident.

The BCG vaccine is available as an anti-TB vaccine for humans. The discovery of an avirulent bovine tubercle bacillus variant by Albert Calmette and Camille Guérin in 1908 created the foundation for the BCG vaccine. It has remained as the most extensively utilized vaccine in the world (Bloom & Murray, 1992).

In terms of treatment, apart from streptomycin, a cocktail of four drugs – ethambutal hydrochloride, isoniazid, pyrazinamid and rifampicin have been and are still widely used in the treatment of TB patients in Zambia and other parts of the world. A six to eight month regimen is recommended for the success of this treatment approach, which is divided into two phases:

☞ *Induction Phase:* All the four drugs are taken daily for 2 months.

☞ *Continuation Phase:* Isoniazid and rifampicin is taken daily or twice weekly for a minimum of 4 months.

This treatment regimen is effective against active TB infection, within two months of treatment resulting in an almost complete reduction in infectiousness.

However, some patients do abandon their treatment especially in the second phase. Such patients may end up with recurring TB and multi-drug resistant TB (MDR-TB) cases. To ensure patient compliance, the WHO formulated the Directly Observed Treatment Short Course (DOTS) programme, which is today considered to be the most effective strategy in controlling the TB epidemic (WHO, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study areas

The study was carried out in three areas that formed up the three sampling strata according to regions. Of the three study areas, two were in the livestock keeping areas of the interface areas of the Game Management Areas (GMAs) of the Kafue Basin. Kazungula District of Southern Province, which formed the third sampling strata by region and was both outside the National Parks and GMAs, was added as a control area (Figure 3.1). The Kafue Basin encompasses the Lochinvar National Park in the south and Blue Lagoon National Park in the north. The interface areas of the Kafue Basin National Parks are endowed with wildlife, which easily interacts with livestock from neighboring communities and transhumant cattle from distant areas. Based on previous reports of TB in both lechwe and cattle, and the interaction that exist between them in the Kafue Basin interface area, it was thus selected as the study area. Additionally, the presence of human settlements around these Parks made the areas easily accessible to conduct such type of research. In the interface area, cattle populations come in close contact with wild animals particularly the lechwe (*Kobus leche Kafuensis*). In dry months (April to October), the level of contact is very high as both livestock and wildlife virtually share grazing grounds. The control area of Kazungula District has a different environmental setting with predominantly savannah woodlands, mostly the *Mopane* species and the *Acacia* species interspacing the dry veld. Limited grazing land is also available along the Zambezi River to which some cattle seasonally trek. In this area, no

large mammals are found and cattle rarely have any significant contact with wild animals. Kazungula was included for comparative purposes due to its different ecological and environmental setting from the interface areas of the Kafue Basin.

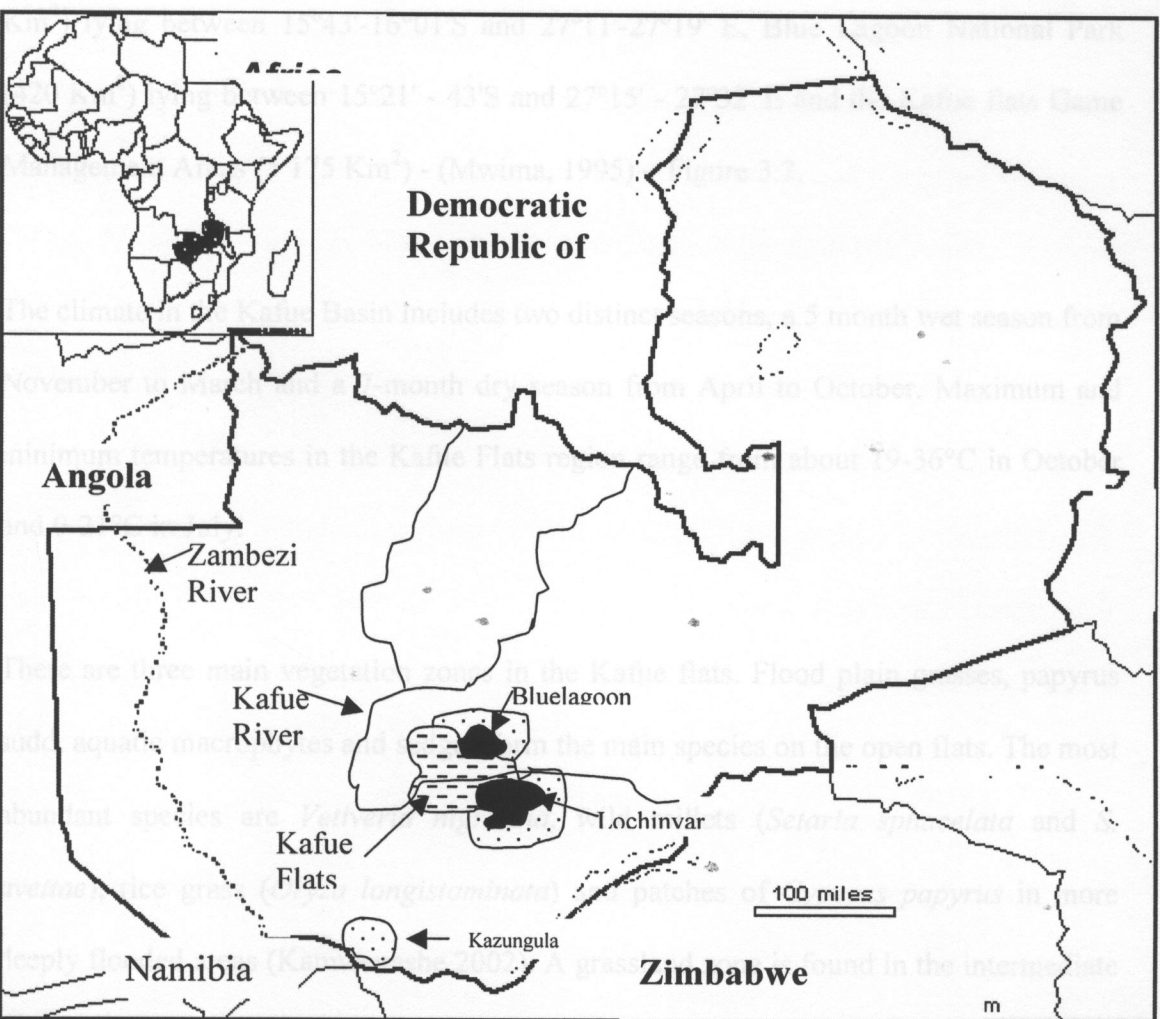


Figure 3.1 Map of Zambia with all the three sampling sites of Blue lagoon and Lochinvar depicted in the interface areas and Kazungula District.

3.1.1 Background of the study areas

The Kafue Basin is a floodplain along the Kafue River of about 6,000 km² located in the South-Central region of Zambia (Sheppe, 1985)-(Figure 3.1). It lies between the lines of latitude 26°-28°E and 15°20'-15°55' S. It is comprised of Lochinvar National Park (410 Km²) lying between 15°43'-16°01'S and 27°11'-27°19' E, Blue Lagoon National Park (420 Km²) lying between 15°21' - 43'S and 27°15' - 27°32' E and the Kafue flats Game Management Areas (5 175 Km²) - (Mwima, 1995) – Figure 3.2.

The climate in the Kafue Basin includes two distinct seasons, a 5 month wet season from November to March and a 7-month dry season from April to October. Maximum and minimum temperatures in the Kafue Flats region range from about 19-36°C in October and 0-21°C in July.

There are three main vegetation zones in the Kafue flats. Flood plain grasses, papyrus sudd, aquatic macrophytes and sedges form the main species on the open flats. The most abundant species are *Vetiveria nigritana*, wild millets (*Setaria sphacelata* and *S. avettae*), rice grass (*Oryza longistaminata*) and patches of *Cyperus papyrus* in more deeply flooded areas (Kamweneshe 2002). A grassland zone is found in the intermediate termitaria. The third vegetation zone is found in the upper margins of the plain boundaries. This zone consists of scattered woodland, palm savanna, miombo, *Acacia* spp.-*Combretum* spp (*munga woodland*) and *Colophospermum Mopane* woodlands (Kamweneshe 2002).

Riparian vegetation and forest occur along some of the tributary watercourses in the upper zones. A foreign invasive plant from Mexico, the *Mimosa pigra* is rapidly spreading along Chunga lagoon of the Lochinvar National Park and is claiming huge tracts of rich grazing grounds.

The Kafue lechwe is the predominant wildlife species of the Kafue flats (Sheppe, 1985) and is confined to a relatively small area, particularly on and around Lochinvar and Blue Lagoon National Parks. The Kafue lechwe population on the flats has steadily declined over the years from the estimated 80, 000 in 1975 to 41 000 in 1982 (Kamweneshe 2002). Poaching, infectious diseases and grazing pressures from livestock are the major factors incriminated for the decline in wildlife population (Kapungwe, 1993). Communities living in (Interface Herds) and around the GMAs usually let their livestock graze liberally sometimes deep inside the National parks where they freely mingle with wild animals. In addition, transhumant cattle from distant areas also come to graze in the GMAs.

The control area of Kazungula District lies in the most Southern part of the country, located by coordinates 17°05'S to 17°45'S and 25°38'E to 25°59'E. The area has similar climatic conditions to those of the Kafue Basin with two distinct seasons, a 5 month wet season from November to March and a 7-month dry season from April to October. Maximum and minimum temperatures in the Kazungula District range from about 21-38°C in October and 0-19°C in July. The vegetation is mostly the savannah woodland, miombo, and the abundant *Colophospermum Mopane* woodlands interspersed with the

dry veld savannah grasslands. Animals reared in the Kazungula District have no contact with wild animals as they are outside the interface areas.

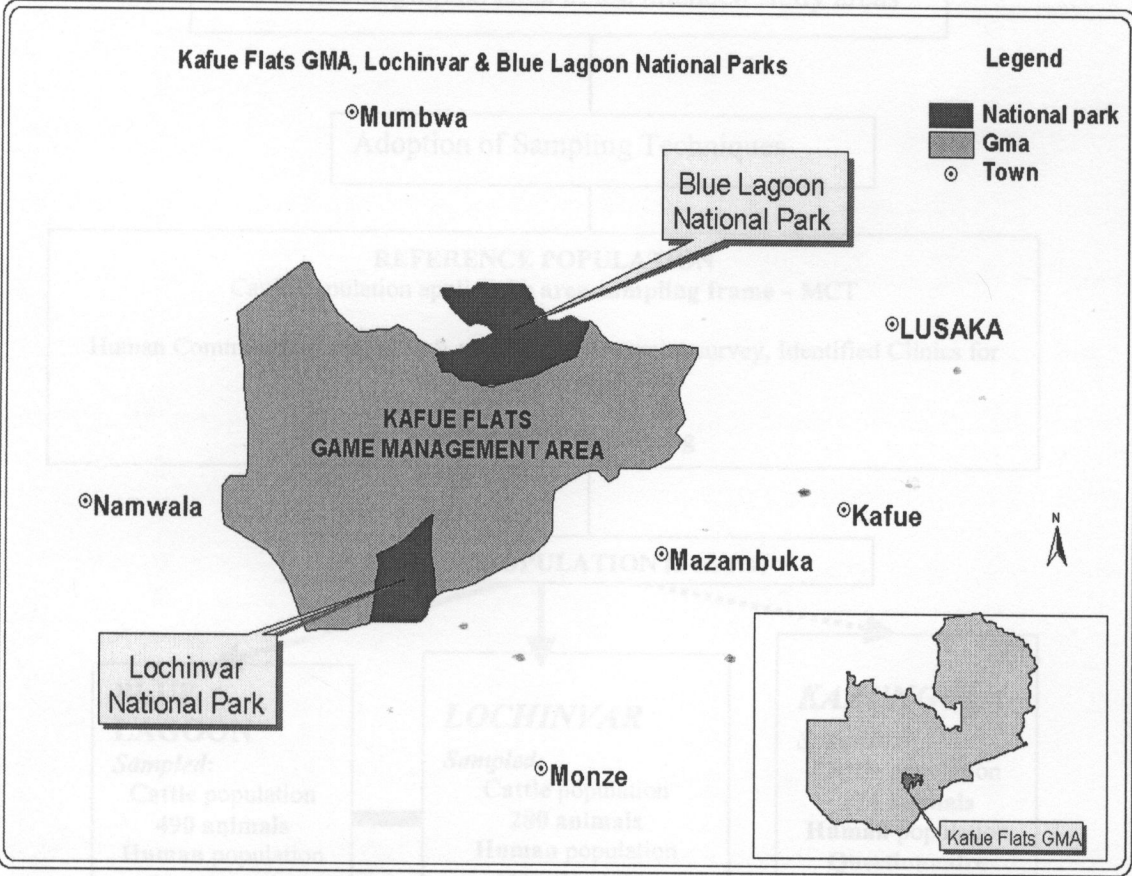
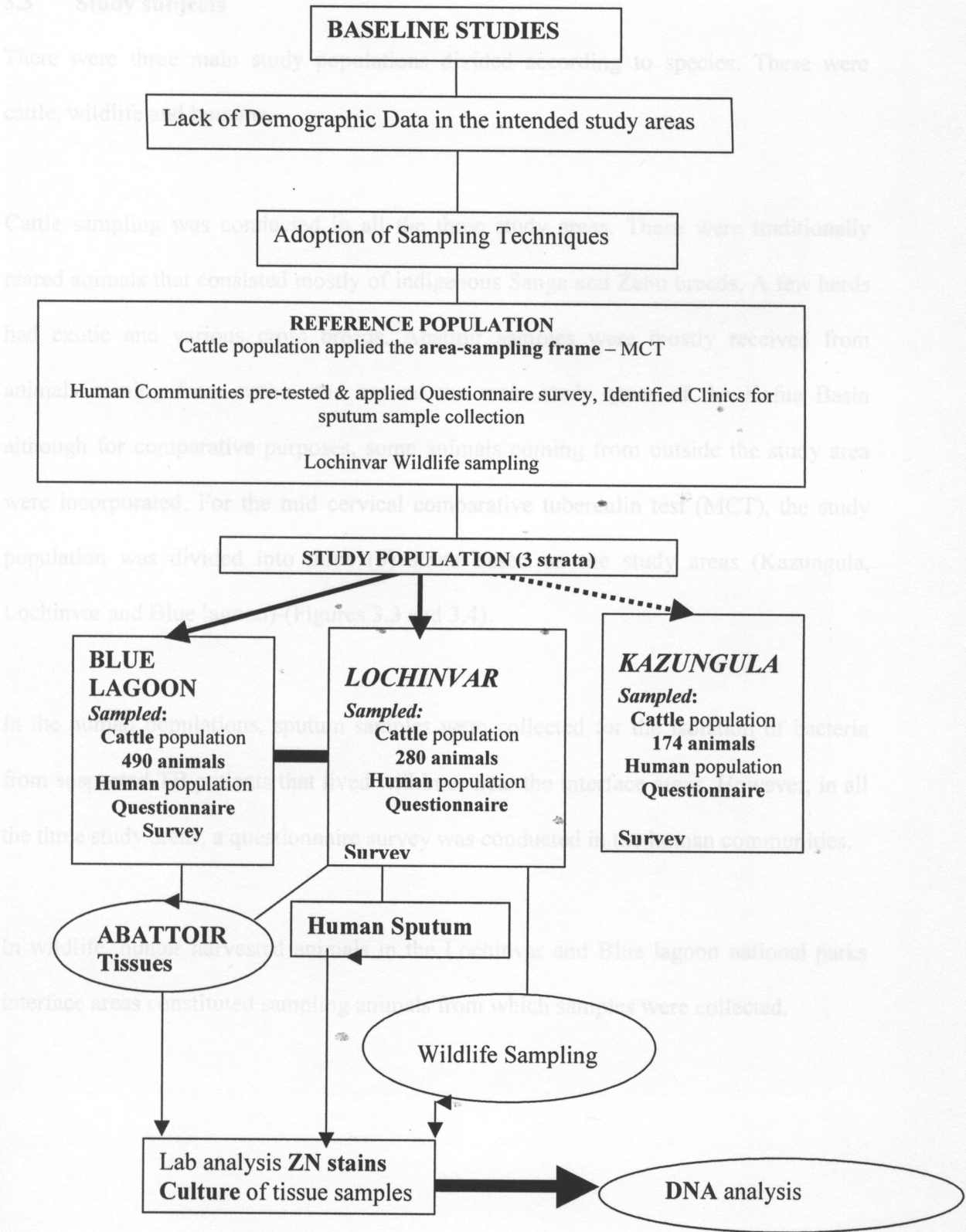


Figure 3.2 Main study areas of Lochinvar and Blue lagoon in the Kafue Basin Interface areas in relation to the Game Management areas of the Kafue Flats and the main surrounding towns from which cattle for abattoir survey were originating (Adapted from milner-2003).

3.2 Study design

3.3 Study subjects



3.3 Study subjects

There were three main study populations divided according to species. These were cattle, wildlife and humans.

Cattle sampling was conducted in all the three study areas. These were traditionally reared animals that consisted mostly of indigenous Sanga and Zebu breeds. A few herds had exotic and various cross breeds. Abattoir samples were mostly received from animals coming from within and around the main study areas of the Kafue Basin although for comparative purposes, some animals coming from outside the study area were incorporated. For the mid cervical comparative tuberculin test (MCT), the study population was divided into three (3) strata based on the study areas (Kazungula, Lochinvar and Blue lagoon)-(Figure 3.3 and 3.4).

In the human populations, sputum samples were collected for the isolation of bacteria from suspected TB patients that lived within or near the interface areas. However, in all the three study areas, a questionnaire survey was conducted in the human communities.

In wildlife, hunter harvested animals in the Lochinvar and Blue lagoon national parks interface areas constituted sampling animals from which samples were collected.



Figure 3.3 Cattle of the Kafue Basin in the interface areas of National Parks.



Figure 3.4 Cattle in the Villages of the Kazungula District of Zambia outside the interface area of National Parks.

3.4 Mid cervical comparative tuberculin tests (MCT)

3.4.1 *Baseline studies*

This stage involved the collection of the vital information on the cattle population in all the study areas before the actual field study started (Figure 3.5). Due to lack of comprehensive information on cattle farmers, in the study areas, a sampling frame was constructed with the help of the local Veterinary assistants and farmers (cattle owners) to come up with a list of cattle owners and the approximate numbers of animals owned by each farmer in the area (Figure 3.5).



Figure 3.5 Baseline data collection with the local Senior Veterinary Assistant checking a list of cattle farmers in the Sikaunzwe area of Kazungula District.

3.4.2. Sampling procedure

A cross sectional study design was applied in this study. The first stage involved obtaining the entire or the estimated cattle population in the area of study (Reference population). Then a study population (n) was picked from the reference population after subjecting the reference population to selection criteria. From this study population, which consisted of village clusters, the individual herds were randomly picked after calculating the sample size from the baseline information (Dohoo *et al.*, 2003). A herd was taken as a primary sampling unit (PSU) while individual animals were secondary sampling units (SSU). These were the herds that were recruited for Mid-Cervical Tuberculin test (MCT). For the determination of the prevalence of tuberculosis in cattle, a MCT test was done.

If a herd was tuberculinised in the morning, the reading was done 72 hours at the same time the inoculation was done. In each herd, at least minimums of ten percent of randomly selected animals were included for tuberculin testing.

3.4.3 Sample size determination

For the purpose of this study, and assuming low heterogeneity between herds, we used detection power ($1-\beta$) 90% and level of significance (α) =95% at an estimated TB herd prevalence of 50 %($p=0.5$) Then we applied the formula to estimate a sample proportion with a desired precision as described by Dohoo *et al.*, 2003.

$$n = \frac{Z_{\alpha}^2 pq}{L^2}$$

n = number of herds

z = confidence level

L = the precision of the estimate (allowable error) equal to ½ the confidence interval

P = a priori estimate of the proportion (estimated prevalence of TB)

q = 1-p

The required Z value at 95% CI was $Z_{\alpha} = Z_{0.05} = 1.96$. Applying the values in the sample size calculation formula, and correcting it for a finite population, it was then calculated that in Lochinvar at least 30 herds were to be sampled. Likewise, in Blue Lagoon, a total of 43 herds were to be sampled while in Kazungula area, a total of 20 herds was to be sampled.

3.4.4 Selection criteria in cattle populations

For the application of the MCT test, sampling was done at both the individual animal and herd levels.

On selection of individual animals, all those that were below 12 months, were excluded from the study. At herd level, all those herds that had a history of having had some animals tuberculinised before were also eliminated.

In cases where herds were exposed to similar risk factors (e.g. small herds in a village), though belonging to many individuals, such herds were taken as a single independent unit and one person within the cattle owners was assigned as the owner. This was done to eliminate sampling bias.

3.4.5 Tuberculinisation of cattle in the field

Animals were cast down using physical restraint by the aid of a soft but strong intertwined cotton and sisal rope (Figure 3.6). An ear tag was applied for the purpose of identification, and those with names, the name was also recorded. A lateral side of the neck was then exposed by pulling the horns either to the right or to the left depending on which side was easy to expose (Figure 3.6). Then two sites of approximately 8 cm in diameter (about 16 cm²) in the mid neck area and approximately 10 to 12 cm apart, one in front and behind, were shaved using a Gillette® razor blade (Figure 3.7).



Figure 3.6

In the Interface areas, no crush pens are available, animals were cast down and before reading of skin thickness and inoculating the tuberculin.

The initial skin thicknesses at the two sites were then measured using a vernier calliper. The measurements were recorded on a record sheet. A single comparative tuberculin test (SCTT) was carried out using the McLintock[®] Tuberculin Testing equipment to inject 0.1ml of avian (containing 2,500 international Units) tuberculin (Lelystad[®]) onto the cranial site and 0.1ml of mammalian-bovine (containing 5,000 Tuberculin units) tuberculin (Lelystad[®]) on the caudal site (Figure 3.7). Avian and bovine purified protein derivative (PPD) antigens (The Central Veterinary Institute, Lelystad, The Netherlands) were used. After inoculation, a small nodule formed on both sites (Figure3.7).

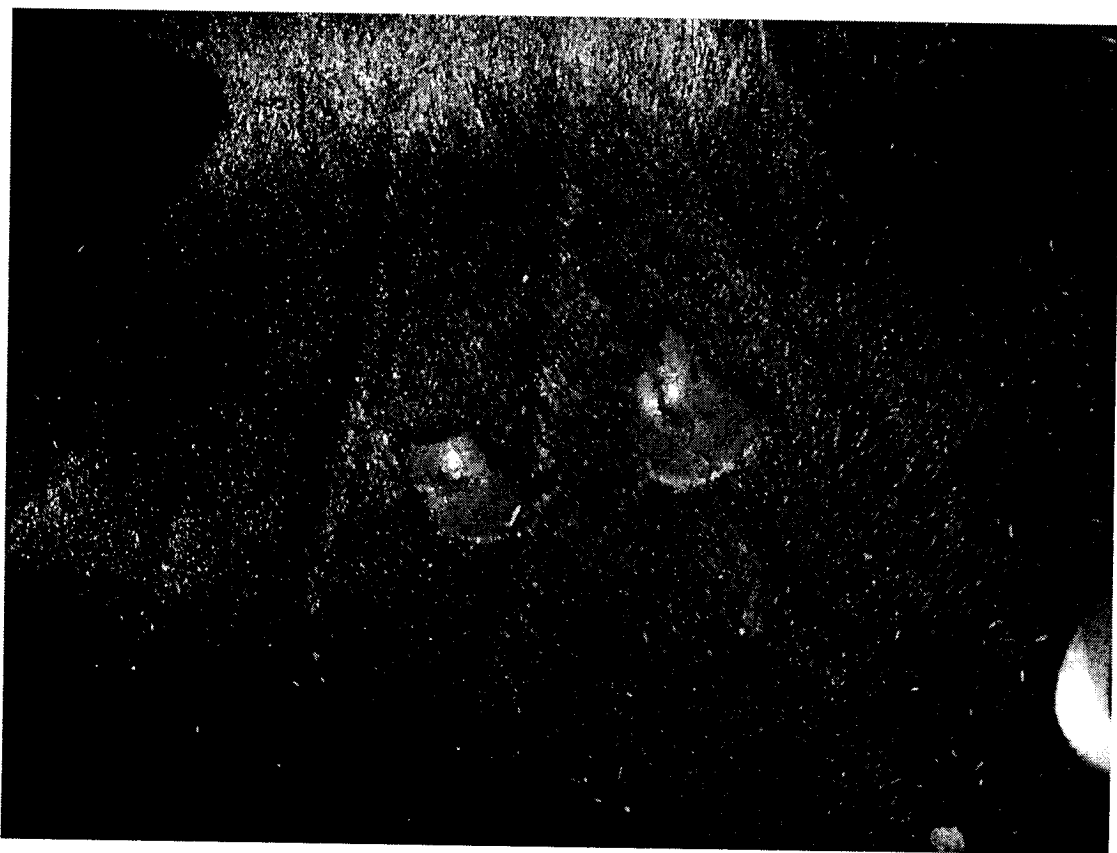


Figure 3.7 Shaved area on the neck region with inoculated sites having formed nodules.



After seventy-two hours post inoculation, each animal was restrained and the same person who took the previous readings took a second measurement of the skin thickness. The skin thickness at the two sites of inoculation were measured and entered on a record sheet in the appropriate column. A strict standard level of interpretation was used to classify reactors according to the OIE manual (2001). Negative reactors were indicated by increases in skin thickness of less than 2 mm. Inconclusive reactors were indicated by skin thickness increases of between 2 mm and 4 mm. A positive reaction was indicated by an increase in skin thickness of more than 4 mm. A negative reactor was identified when there was a negative reaction to bovine tuberculin, or a positive or inconclusive reaction to bovine tuberculin that was equal to, or less than a positive or inconclusive reaction in avian test and also when negative to both (OIE Manual 2001).

An animal was classified inconclusive if a reactor was identified by a positive or inconclusive reaction to bovine tuberculin that is 1 – 4mm greater than the reaction to avian tuberculin. A positive reactor was identified by a positive reaction in bovine tuberculin that was 4mm greater than the reaction to avian tuberculin.

At herd level, a herd was classified as positive on MCT test if at least one animal reacted positive to the test.

3.4.6 Questionnaire survey (cattle) population

In designing the questionnaire, a practicable balance had to be struck between including the widest possible range of potential factors influencing tuberculosis incidence in cattle

and the need to be concise and easy to use. All cattle herds recruited for tuberculinisations were automatically qualified for a questionnaire survey, unless where the owners declined consent for interviews. These same farmers whose cattle herds were included for tuberculinisation survey are the same ones that were included in the human questionnaire survey using one questionnaire but with a cattle component and human component. Cattle information pertaining to location, management, movements, grazing patterns, presence and contact with wildlife were some of the questions that were asked.

3.5 Human communities

3.5.1 *Questionnaire survey*

A cross-section questionnaire survey to identify potential risk factors for TB was conducted alongside animal tuberculinisation. From each household whose animals were tested for TB, a pre-tested close-ended questionnaire was administered (Appendix 4). On each household that represented an independent unit, data on a wide range of potential risk factors to TB were collected (Figure 3.8).

Information collected included bio-data, eating habits, contact with animals, history of TB in a household etc (Appendix 4). Data was collected relating to 12 month prior to the survey. The 12-month period was chosen to cover the period most relevant to disease transmission, and to avoid collecting unnecessary (and possibly avoid bias associated with incorrectly recalled) data from previous years.

One hundred and eleven (111) randomly selected rural households in the Kafue Basin and the Kazungula interface areas were recruited for the questionnaire survey. Eighty-

seven (87) of these accepted to be interviewed and presented animals for tuberculinisation. Twenty-four (24) other households couldn't be interviewed due to various constraints and resource limitations but presented their animals for tuberculinisation.

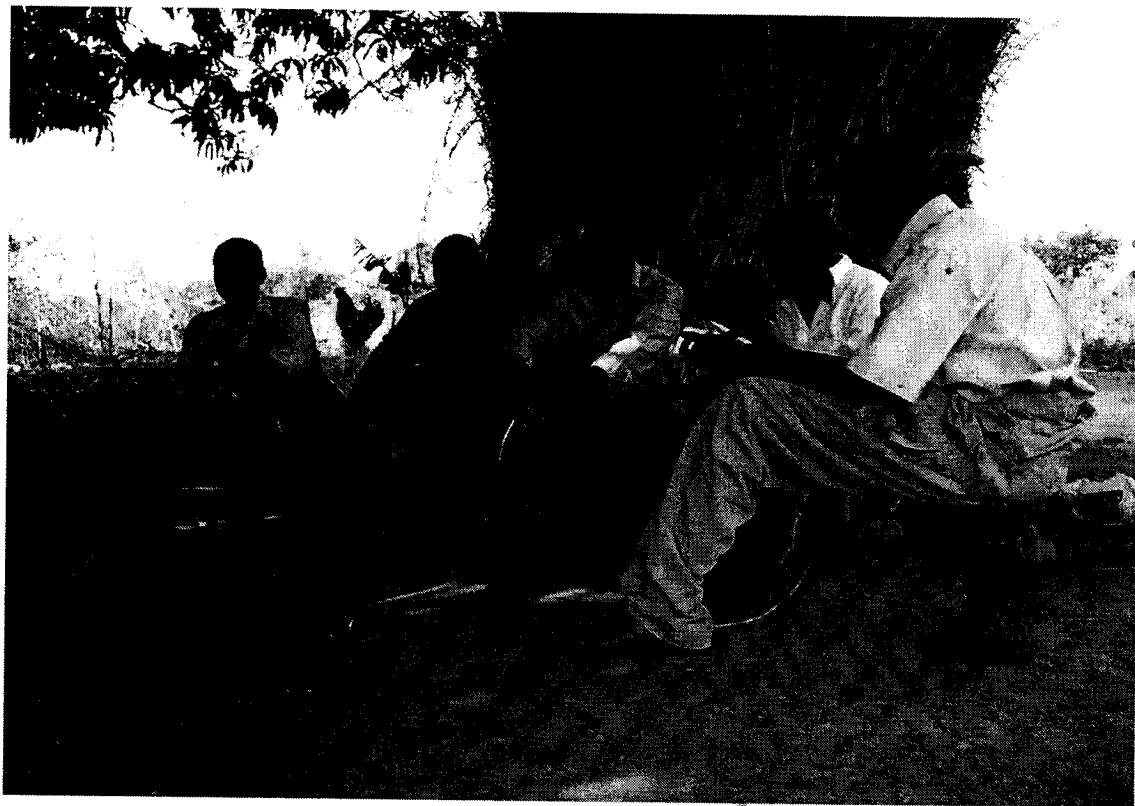


Figure 3.8 Conducting a questionnaire survey in the human communities of the study areas to ascertain risk factors of TB in both humans and animals.

3.5.2 Collection of human sputum samples

The human sputum samples were collected in health centers found in the Interface areas of the Kafue Basin National Parks for the purpose of isolating *mycobacteria*. The dissemination of information as well as the handling of submitted TB sputum samples was done by the local health personnel.

3.5.2.1 Inclusion criteria for patients giving sputum

Only new patients who had not yet been started on anti-tuberculosis treatment, but on acid fast staining for bacilli (AFB) staining, were found positive were considered for inclusion in the study. These involved both hospitalized and outpatients. In addition, patients who had earlier been on TB treatment but have relapsed (resistance?) and upon subjecting them to AFB were still positive were also included in the study.

Sputum collection was purposely done with priority being given to those patients coming from deep in the interface areas of the National Parks of the Kafue Basin.

3.5.2.2 Exclusion criteria for patients giving sputum

All hospitalized and outpatients who were already on TB therapy were not eligible for inclusion in the study. New patients either hospitalized or not, who were AFB positive, but were not residents from the areas under study i.e. came from outside the study areas as visitors and fell sick whilst there, were not eligible for inclusion. Further, new patients who were AFB positive but felt not obliged to be part of the study were also not included together with those who felt like withdrawing. Exclusion from the study was without any obligations on both sides. Cases such as prisoners coming from confined places were not eligible for sampling and were out rightly excluded. Since sputum sample collection was a purposive sampling, no sampling frame was used.

3.5.2.3 Sputum sample collection guidelines

All the patients participating in the study had to follow the instructions for collecting the sputum samples. The best specimens were obtained from the first deep expectorated sputum in the morning after preliminary clearing of saliva from the mouth and throat. Patients were encouraged to collect a series of 3 to 5, single, early-morning samples in the 50 ml plastic centrifuge tubes that were provided. A volume of 5 to 10 ml was adequate for each sample, although there was no advantage in collecting a larger volume.

All the specimens were kept refrigerated at all times. However, in areas where no refrigeration facilities were available or where electricity was unreliable, an equal volume of 1% Cetylpyridinium Chloride ($C_{21}H_{38}NCl \cdot H_2O$, FW358.0) was added to the sputum sample as transport media and this allowed samples to be kept at room temperature. All samples were collected within one week of submission at the health center.

At the health centers, the outsides of plastic tubes were wiped with appropriate disinfectant (1000 ppm sodium hypochlorite). The containers were labeled with the patient's name and date of collection. Other patient details were recorded on a data form.

Of all the eligible candidates for sputum submission, only 42 cases were included in the study. These were the patients with suspected TB positive cases who were selected after being subjected to the inclusion and exclusion criteria. These patients did not differ much in terms of ethnicity, demographic and socio-economic status as they mostly came

from the pastoralist communities of either the interface or near the interface areas (*ccf exclusion and inclusion criteria*).

3.5.2.4 Direct smear examination using Auramnie and Ziehl-Neelsen staining

At the Chest Disease Laboratories (CDL), all the sputum samples had to be examined using the direct smear examination.

Method using Auramine staining

A few drops of the sputum were collected from the 50 ml falcon centrifuge tube using a rough end of a stirring stick broken into two halves. The smear was made on a slide, then air-dried and heat fixed on a warm hot plate. The smears were placed on the staining rack, without touching each other and were then flooded with Auramine stain for 15 minutes without heating. The slides were then rinsed with distilled water and drained off the excess before decolorizing with 3% Acid Alcohol for 2 minutes. The slides were again rinsed with distilled water and drained. The smears were then counter stained with potassium permanganate (KMnO_4) for 2 minutes. The staining time with KMnO_4 is critical because counter staining for longer time may quench the florescence of the AFB. The slides were again rinsed with distilled water and drained. They were allowed to air-dry without blotting. The smears were examined as soon as possible after staining. Interpretation of results was according to Table 3.1. The same slides were again stained with Zhiel Neelsen stain according to Section 3.8.2 and examined according to Table 3.1 for interpretation.

3.5.2.5 Decontamination of sputum and culturing

The purpose of this process is to homogenize the specimen and kill the undesirable microorganisms other than acid-fast bacilli in the specimen.

3.5.2.6 Identification of *Mycobacterium* species

The identification of *Mycobacterium bovis* in the laboratory was based on culture characteristics, colony morphology, preferential growth on either pyruvate or glycerol enriched Lowenstein Jensen media and the use of biochemical tests (nitrate and niacin reductase tests).

Method

An equal volume of digester (1N NaOH) was added to the sputum sample in the 50 ml falcon tube left at room temperature for 15 minutes. The product was centrifuged for 15 minutes, and the supernatant decanted. The sediment was resuspended in 20 mls of distilled water and again centrifuged for 10 minute at a minimum of 3000 x g. Then the supernatant was decanted. The Sediment was resuspended in sterile AD and then part of the final sediment was inoculated on solid media (Lowenstein-Jensen) and incubated at 37°C. The remaining amount was mixed with an equal volume of 10% Glycerol and stored at -20°C. The culture growths were read every week and recorded.

3.6 Abattoir surveys

Abattoir studies were conducted for the purpose of collecting cattle tissues suspected of being tuberculous from animals coming from the study areas. Being a purposive study, no sampling frame was constructed. Since the target was to collect samples on the dates

when animals from the main study areas were being slaughtered, Tuesdays and Thursdays were the two days of sample collection. These were the particular days that mostly the animals from the study areas were slaughtered having been received a day or two earlier.

The animals once received were allowed twenty-four hours of rest before slaughter. On the morning of slaughter, vital information was recorded and this information was identified with the specimens. Individual animal level factors which included, sex, age and breed were it was available were recorded. Herd level factors such as Provinces, Districts, Villages or farms of origin and the date of slaughter, with any other relevant information such as herd size, disease history etc were also included and recorded. Owners and abattoir workers were able to give the sex but had difficulties to recall or give the ages of the animals. Although a few came outside the study areas, the majority of the animals sampled came from around the areas near the Kafue Basin National Parks.

Once an animal was slaughtered, a carcass was followed up to the examination line where standard post mortem examination procedures for tuberculosis in cattle were performed according to Gracey (1987). Suspected TB organs such as lungs and other visceral organs, and lymph nodes from the mediastinal, retropharyngeal, bronchial and mesenteric regions were collected. The specimens were then sectioned in two parts. One half was placed in a cooler box with ice packs at around 4 to 8°C and the other half was used to make impression smears.

All the abattoir samples were only allowed a week in the refrigerator, before they were processed.

3.7 Wildlife sampling

From earlier reports on the prevalence of tuberculosis in the lechwe, it was found that the estimated prevalence was about 30% (Gallagher *et al.*, 1972). Thus the smallest sample size of animals that could be sampled to give us at least this prevalence was 10. From this number it was expected that at least 3 of the animals would be positive for tuberculosis. There was no proper sample size estimation done due to the difficulty in obtaining a large sample size required to be statistically amenable. The animals were obtained from the hunting quota for research purposes. Additionally hunter harvested lechwe where the owners agreed to sample were also included in the study. Post-mortem was conducted and data on lechwe in the study area were collected after sacrificing the animals that were allocated for the hunting season. All the animals had their lung tissues with draining lymph nodes collected together with any visceral organ that had suspicious TB lesions. The mediastinal, medial retro pharyngeal and submandibular lymph nodes were specifically collected for use in TB determination. These organs and tissues were sliced using surgical scalpels and visually inspected for lesions or abnormalities suggestive of TB (i.e. pale caseogranulomas, gritty texture present on incision, purulent material, yellow discoloration and softening present on incision) - (Schmitt *et al* 1997)-(Figure 3.9). Suspected infected organs were collected and chilled for subsequent microbiological evaluation.



Figure 3.9 Detailed postmortem examination of every organ from all hunter harvested wild animals in the Game Management Areas of the Kafue Basin National Parks.

All chilled portions of suspected TB infected lymph nodes and other tissue organs were decontaminated using the modified Petroff's procedure (Petroff 1915). Smears were then made for acid fast bacilli (AFB) examination and the rest of the decontaminated material was stored at -20°C after inoculating some on Lowenstein Jensen media slants.

3.8 Culturing of decontaminated and homogenized tissue sediments

All the sample organs that were collected from wild animals and from abattoir surveys were subjected to the Petroff's decontamination procedure (Petroff 1915). A detailed account on the preparation of Lowenstein Jensen (L-J) media used in this study is given in the appendix section (Appendix 3).

3.8.1 Pre-treatment of samples

The purpose of pre-treatment was to homogenize the specimen and to kill the undesirable microorganisms other than acid fast bacilli in the specimen. Care was taken during this phase, especially with preservation of *M. bovis*, which may also be affected by alkaline treatment. Since acid fast bacilli are only relatively resistant to alkaline treatment. High concentration and prolonged exposure to sodium hydroxide inhibits the growth of *mycobacteria*.

The method used was that by Carter & Chengappa (1995), with slight modifications.

Fatty tissues were trimmed off from TB suspected tissues. At least 3 to 6 g of the trimmed tissue was measured into a mortar. Then 10ml of 4% NaOH was added to the mortar. About 3 to 6 g of sterile sea sand was added to tissue in the mortar. The tissue was then ground in a sterile mortar with sterile sand. The product was transferred to a McCartney bottles where it was centrifuged at low speed for 10 minutes and afterwards

decanted the supernatant fluid. Then 20 mls of sterile water was added to the sediment and mixed vigorously by shaking to a uniform homogenate. Then the contents were centrifuged at low speed for 20 minutes and decanted the supernatant fluid. Finally the sediment was inoculated on Lowenstein-Jensen slants with one set going on Glycerol, the other on Pyruvate. The slants were incubated at 37°C for up to eight to twelve weeks. A few drops of the decontaminated tissue samples were also spread on a slide for staining together with the impression smears made earlier on. All the cultures were being examined once a week for a minimum of eight weeks.

3.8.2 Ziehl-Neelsen staining

All the slide preparates of smears were fixed by heating them with the blue cone of the flame after air-drying. All slides were covered with newly filtered carbolfuchsin (working solution). Then the slides were carefully heat to steaming with a flame of a Bunsen burner but not allowing boiling. They were allowed to stand for 5 minutes without further heating. Then the slides were washed in running water. Acid-alcohol was spread on the slides to decolourise them to a faint pink colour. This was done several times. The decolourizer was applied until no more colour came out. This procedure was mostly accomplished within 2 minutes. Counterstaining was done by Loeffler's methylene blue (working solution) for 20-30 sec. Then the slides were washed with water dried and examined under oil emersion objective lenses.

3.8.4 Direct microscopic smear examination

All the stained slides were subjected to a direct microscopic examination under oil immersion. Interpretation was based on the recommendations stipulated by OIE (2001)

tabulations as well as the ‘American Thoracic Society’ scale from Lenneth adapted at the Chest Disease Laboratories (CDL)-Lusaka Zambia. The interpretation scales involved examination of 200 fields (Table 3.1).

On all animal samples, the Ziehl-Neelsen staining procedure was used because of its high sensitivity. It has been shown that approximately 1AFB/field is equal to 10,000AFB/ml on ZN staining (Burdash N.M *et al.*, 1975 & Kommareddi S *et al* 1984).

Table 3.1 Interpretation Table for Acid Fast Bacilli seen on Zhiel Nielsen and Auramine stains.

Number of AFB	Interpretation
0	Negative
1-2/200Fields	Number of AFB seen was Written
1-9/100Fields	1+
1-9/10Fields	2+
>9/Field	3+

3.9 Preparation of mycobacterium lysates from colonies for DNA extraction.

At least two loops of culture colonies were suspended in a minimum of 250 μ l sterilized distilled water in Eppendorf tubes. Then the cells were heat Killed by incubation at 80°C for 1 hour.

3.10 Spoligotyping technique (Kamerbeek *et al.*, (1997))

The primers used to amplify the whole DR region by PCR were DRa and DRb [DRa (GGTTTTGGGTCTGACGAC, 59 biotinylated) and DRb (CCGAGAGGGGACGGAAAC)]. About 10 ng of genomic mycobacterium DNA lysates from cultured cells was used as a target for spoligotyping. Fifty microliters of the following reaction mixture were used for the PCR: *Tth* buffer (5 mM Tris z HCl, 5 mM KCl, 0.7 mM MgCl₂, pH 9.0), each deoxynucleosidetriphosphate at 200 mM, 20 pmol each of primers DRa and DRb, 10 ng of DNA, and 0.5 U of *Tth* polymerase (HT Biotechnology Ltd., Cambridge, United Kingdom). The mixture was heated for 3 min at 96°C and subjected to 20 cycles of 1 min at 96°C, 1 min at 55°C, and 30 s at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR locus. These oligonucleotides were covalently bound to a membrane as previously described (Kamerbeek *et al.*, 1997). The oligonucleotides were applied to the membrane in parallel by using a miniblotted system (MN45; Immunetics, Cambridge, Mass.). After a short incubation, the membrane was inactivated by using 100 mM NaOH and washed in 23 SSPE (13SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA [pH 7.7]; Gibco BRLLife Technologies, Inc.) supplemented with 0.1% sodium dodecyl sulfate (SDS; Sigma). For hybridization, 20 ml of the amplified PCR product was diluted in 150 ml of 23 SSPE–0.1% SDS and heat denatured. The diluted samples (130 ml) were pipetted into the parallel channels in such a way that the channels of the miniblotted apparatus were perpendicular to the rows of oligonucleotides deposited previously. Hybridization

was done for 60 min at 60°C. After hybridization, the membrane was washed twice in 250 ml of 23 SSPE–0.5% SDS for 10 min each time at 60°C and then incubated in 1:4,000-diluted streptavidin-peroxidase conjugate (Boehringer) for 45 to 60 min at 42°C. The membrane was washed twice, for 10 min each time, in 250 ml of 23 SSPE–0.5% SDS at 42°C and rinsed with 250 ml of 23 SSPE for 5 min at room temperature. Detection of hybridization signals was optimized by chemiluminescent ECL (Amersham) detection liquid, followed by exposure to X-ray film (Hyperfilm ECL; Amersham) in accordance with the instructions of the manufacturer. For repeated use of membranes, the membranes were stripped by being washed two times for 30 min each time in 1% SDS at 80°C and then incubated for 15 min in 20 nM EDTA (pH 8) at room temperature. Membranes were sealed in plastic and stored at 4°C until further use.

3.11 Data analysis

Data on various individual animal and herd attributes, including the Mid Cervical Comparative Tuberculin (MCT) test data, were stored and established in the Microsoft Excel® database. The questionnaire survey results were entered and established in the SPSS® database. The variables hypothesized to be associated with TB transmission were included in the questionnaire analyses.

Analysis was done separately for the data on MCT in Microsoft Excel®. Cross tabulations of data with binary interactions was done in SPSS®. The chi-square test and in some cases the Fisher's exact test were used to test the significance of association between the dependent and the response variables. Two-way contingency table analyses

were also used for univariate analysis of data. The effects of area, age, sex and herd sizes on MCT reaction were assessed.

Final handling for the MCT database from Microsoft Excel[®] was transferred to STATA intercooled Version SE 8.2 (Stata Corp. College Station, TX).

Similarly, data from abattoir survey wildlife, and human sampling, were also established in Microsoft Excel[®]. Final data analysis was done in STATA intercooled Version SE 8.2 (Stata Corp. College Station, TX). MCT analysis was done at both individual animal and herd level. At the individual animal level, the outcome was whether or not an animal was deemed a reactor of a tuberculin test (that is if the difference in skin test after 72 hours was more than 4mm). At herd level, it was whether or not a herd had at least one animal reacting positive.

At both levels, the data were analyzed in two stages. In the first stage, the univariate analysis, of possible association between each of the possible risk factors and occurrence of TB was examined for each factor individually. Associations between the independent variables and the outcome variables were tested using unadjusted odds ratios for categorical factors and these were evaluated for statistical significance using a Chi-square test for independence. In multivariable analysis, factors with the outcome ($P < 0.25$) at the initial screening were evaluated using unconditional multiple logistic regression.

CHAPTER FOUR

RESULTS

4.1 Descriptive results on sampling characteristics in cattle

A total number of 944 cattle from 111 randomly selected herds in the 3 study areas that made up the 3 sampling strata (Kazungula, Blue lagoon and Lochinvar), were subjected to the mid cervical-comparative tuberculin (MCT) test. The numbers sampled were grouped according to the two levels of sampling, at individual animal level as well as at herd level which were the secondary and primary sampling units respectively (Table 4.1).

Table 4.1 Tuberculosis results based on the comparative test on cattle population in all the three study areas at individual animal as well as at herd level sampling.

<i>Area</i>	<i>Individual Animal Level</i>			<i>Herd level</i>		
	<i>n</i>	Positive	%	<i>n</i>	Positive	%
Lochinvar	280	15	5.4	34	12	35.3
Blue	490	36	7.3	54	14	25.9
Lagoon						
Kazungula	174	1	0.6	23	1	4.5
Totals	944	52	5.5	111	27	24.3

4.2 Prevalence of bovine tuberculosis in cattle

4.2.1 Prevalence of bovine tuberculosis at individual animal level

4.2.1.1 Overall prevalence at individual animal level

At the individual animal level, out of the total of 944 animals sampled, 52 animals reacted positive to the tuberculin test in all the three study areas representing an overall prevalence of 5.50% (95% CI = 3.87 – 7.15%).

4.2.1.2 Individual animal prevalence in Lochinvar

In Lochinvar, a total of 280 cattle were subjected to the MCT test. Of this number, 15 animals were positive representing 5.35% (95% CI = 2.8-7.9%) individual cattle prevalence.

4.2.1.3 Individual animal prevalence in Blue Lagoon

In Blue Lagoon, a total of 490 animals were tested. Out of these, 36 animals tested positive giving an individual animal prevalence of 7.34% (95% CI = 4.6-10.1%).

4.2.1.4 Individual animal prevalence in Kazungula District

In Kazungula District, 174 animals were tested and only one (1) animal was found positive, indicating a 0.57% (95% CI = 0.54 – 1.70%) individual animal prevalence.

4.2.2 Prevalence of bovine tuberculosis at herd level

4.2.2.1 Overall prevalence at herd level

Out of the total of 111 herds of cattle sampled from all the three sampled areas, 27 herds contained at least one positive animal representing an overall herd prevalence of 24.32% (95% CI = 16.53 – 54.71).

4.2.2.2 Herd level prevalence in Lochinvar

In Lochinvar, a total of 34 herds of cattle were subjected to the tuberculin test. Of this number, 12 herds contained at least one positive animal giving a herd level prevalence of 35.29% (95% CI = 21.8 – 64.1%).

4.2.2.3 Herd level prevalence in Blue Lagoon

In Blue Lagoon, a total of 54 herds of cattle were tested. Out of these, 14 herds contained at least one positive animal, giving a herd level prevalence of 25.93% (95% CI = 19.6% -33.8 %).

4.2.2.4 Herd level prevalence in Kazungula District

In Kazungula District, 23 cattle herds were tested with only one herd (1) containing one animal that reacted positive, representing a herd prevalence level of a 4.35% (95% CI = 2.1 – 8.73%).

4.3 Risk factors associated with the prevalence of bovine tuberculosis

4.3.1 The effects of geographical area on tuberculosis prevalence

There was an overall significant difference in TB prevalence among the three study areas ($P=0.001$). A significant difference in prevalence between Kazungula and Blue Lagoon existed ($P=0.0008$). Similarly, a significant difference was observed between Kazungula and Lochinvar ($P=0.0072$). There was no significant difference between Lochinvar and Blue Lagoon ($p=0.286$). The individual animal prevalence and herd prevalence in Kazungula District were far much lower than those in Lochinvar and Blue lagoon (Figure 4.1).

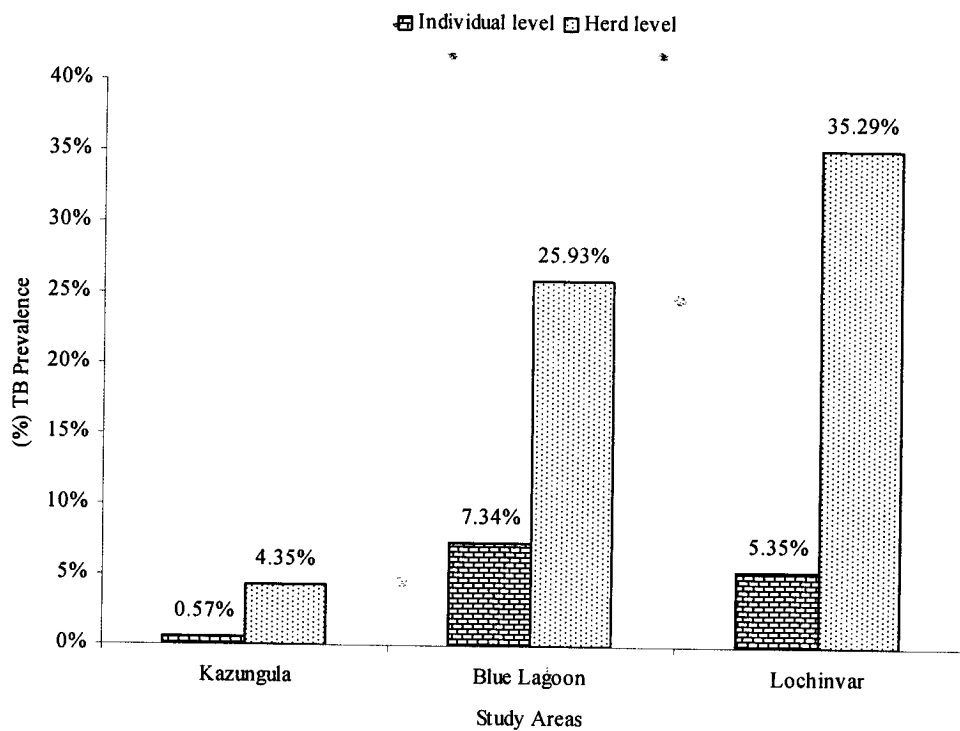


Figure 4.1 The prevalence of Bovine tuberculosis by area at individual animal and herd level.

Blue Lagoon had significantly higher individual animal prevalence while Lochinvar had significantly higher herd level prevalence (Figure 4.1). In all the study areas, herd level prevalence was higher than individual animal prevalence (Figure 4.1). The prevalence of bovine only reactors was significantly higher in all the three study areas compared to the avian and the comparative tests (Table 4.2).

Table 4.2 Detailed results on tuberculosis prevalence of Bovine tuberculosis at individual animal level in all the three areas studied

Area	Avian reactors		Bovine reactors		MCT-Results	
	%		%		%	
	<i>Prev.</i>	<i>Std.err</i>	<i>Prev.</i>	<i>Std.err</i>	<i>Prev.</i>	<i>Std.err</i>
Lochinvar	5.71	1.86	12.85	2.90	5.35	1.30
B/Lagoon	5.91	1.42	18.77	2.25	7.34	1.37
Kazungula	6.89	3.08	4.59	2.34	0.57	0.56
Overall by test type	6.03	1.08	14.40	1.55	5.50	0.83

4.3.2 The effects of grazing systems on tuberculosis prevalence

Village resident herds constituted the greater majority of the animals sampled at 42.16%, followed by transhumance herds at 31.36% (Figure 4.2). The 250 animals from the interface herds represented only 26.48% of the total animals sampled in the entire study. The highest prevalence of all TB reactor animals was recorded from the animals that were practising the interface grazing system (Figure 4.3).

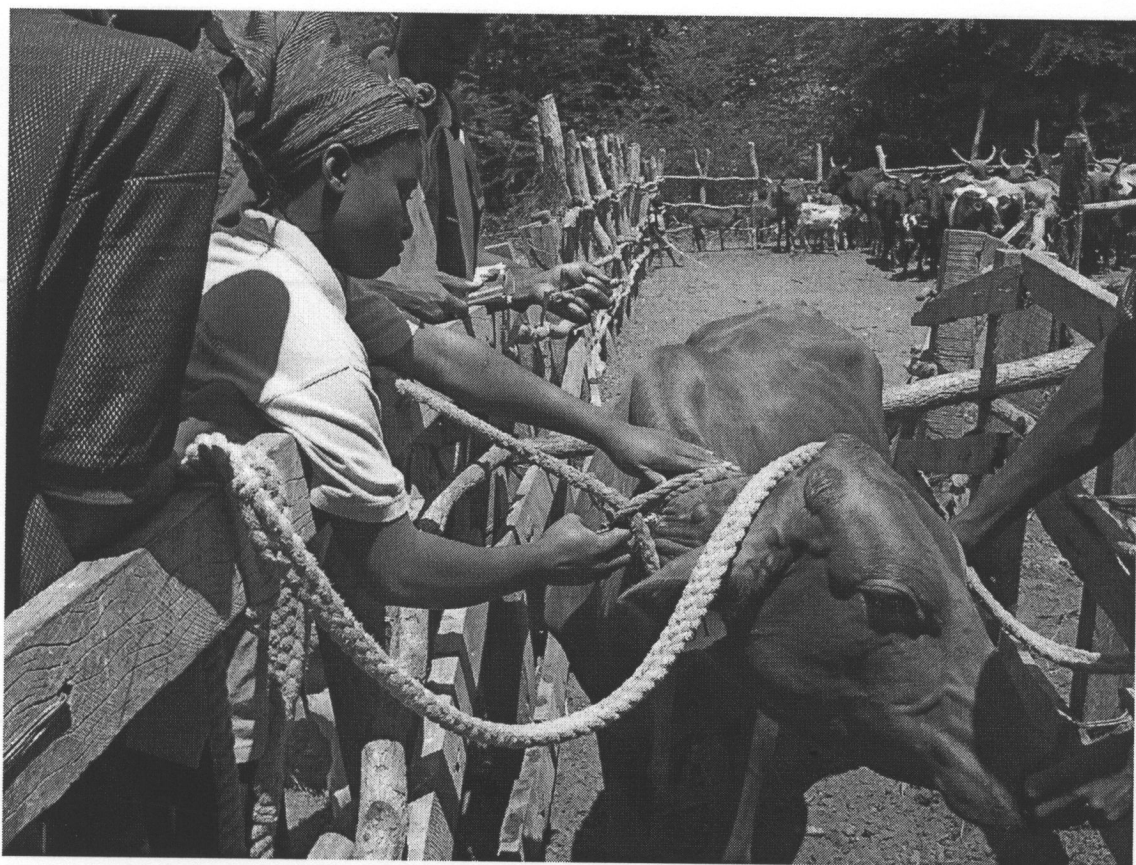


Figure 4.2 Village resident herds were more accessible and exposed to easy handling facilities and were kept in small sizeable numbers.

Blue lagoon and Lochinvar had animals in all the three grazing systems, while Kazungula District had animals in two grazing systems only, i.e. in the village resident herds and the transhumance herds with none in the interface areas. There were only three herds practicing transhumance-grazing system in the Kazungula District. The other 20 herds practiced village resident type of grazing system. Thus in Kazungula District, 87 % of the herds were village resident with only 13% being transhumance herds. In Lochinvar, 61% of the animals sampled came from the transhumance herds, while in the Blue lagoon, the transhumance herds made up 26% of the animals sampled. Under the interface herds, 27% and 35% of the sampled animals came from Lochinvar and Blue

lagoon, respectively. Only 12% of the animals came from village resident herds in Lochinvar while 39% of animals were village resident herds in Blue lagoon.

Table 4.3 Individual reactor animals by grazing systems in all the three study areas.

Areas	Grazing Strategies (Systems)			
	Village Resident		Transhumant	Interface
	<i>n</i>	Herds	Herds	Herds
Lochinvar	15	2	2	7
Kazungula	1	1	0	0
Blue Lagoon	36	5	9	22

From a total of 52 positive reactors, the majority were recorded from the Interface herds (Table 4.3). Comparatively more animals came from the village resident herds, despite this grazing system recording the least number of reactors (Table 4.3).

Table 4.4 Variations in the mean herd sizes according to Grazing Systems recorded.

Grazing Pattern	Mean Herd Size	95% Confidence Interval
Village Resident Herds	61	41-81
Transhumance Herds	33	25-41
Interface Herds	144	82-207

Herd sizes and their means varied significantly in all the three grazing strategies with those in the interface areas having larger herds (Table 4.4).

There was a significant statistical difference in the TB prevalence among the three grazing systems ($P=0.000$)-(Figure 4.4). TB prevalence was significantly different between village resident and transhumance herds ($P=0.026$). Highly significant differences in TB prevalence were noted between village resident herds and interface herds ($P=0.0000$). There was a clear significant difference in TB levels between transhumance and interface herds ($P=0.0052$).



Figure 4.3 Transhumance and interface herds are kept in vast open plains without handling facilities and graze freely with access to wildlife grazing areas.

The individual animals found in the interface areas showed a markedly higher bovine only reactor on the tuberculin test than the other two grazing systems (Table 4.5). Village resident herds showed higher avian only reactors than both transhumance and interface herds (Table 4.5).

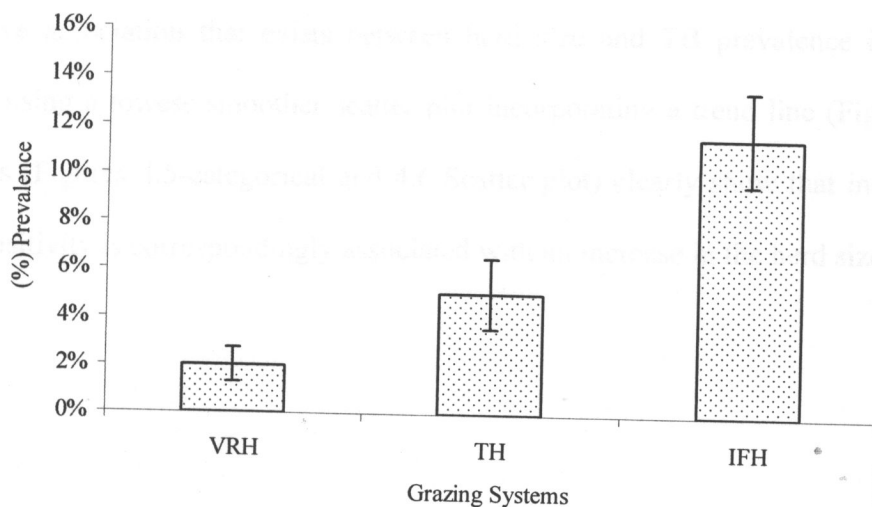


Figure 4.4 Prevalence of bovine tuberculosis according to grazing systems from comparative tuberculin test results. (Key- VRH=Village Resident Herds, TH=Transhumance Herds; IFH=Interface Herds)

Table 4.5 Details on Bovine tuberculosis prevalence according to grazing systems based on avian reactors, bovine reactors and comparative results.

Grazing Systems	Avian Reactors %		Bovine Reactors %		Comparative Results %	
	Prevalence	Std.err	Prevalence	Std.err	Prevalence	Std.err
VRH	7.03	2.0	8.29	1.74	2.01	0.72
TH	4.73	1.16	14.19	2.52	5.06	1.45
IFH	6.00	1.84	24.4	3.69	11.6	1.92
Prevalence	6.03	1.08	14.40	1.55	5.50	0.83

Key- VRH=Village Resident Herds, TH=Transhumance Herds; IFH=Interface Herds

4.3.3 Effects of herd size on tuberculosis prevalence

There was a positive association between herd size and herd level TB status (Figure 4.5). The positive association that exists between herd size and TB prevalence is further confirmed using a lowess smoother scatter plot incorporating a trend line (Figure 4.6). Both charts (Figures 4.5-categorical and 4.6-Scatter plot) clearly show that increase in herd TB positivity is correspondingly associated with an increase in the herd size.

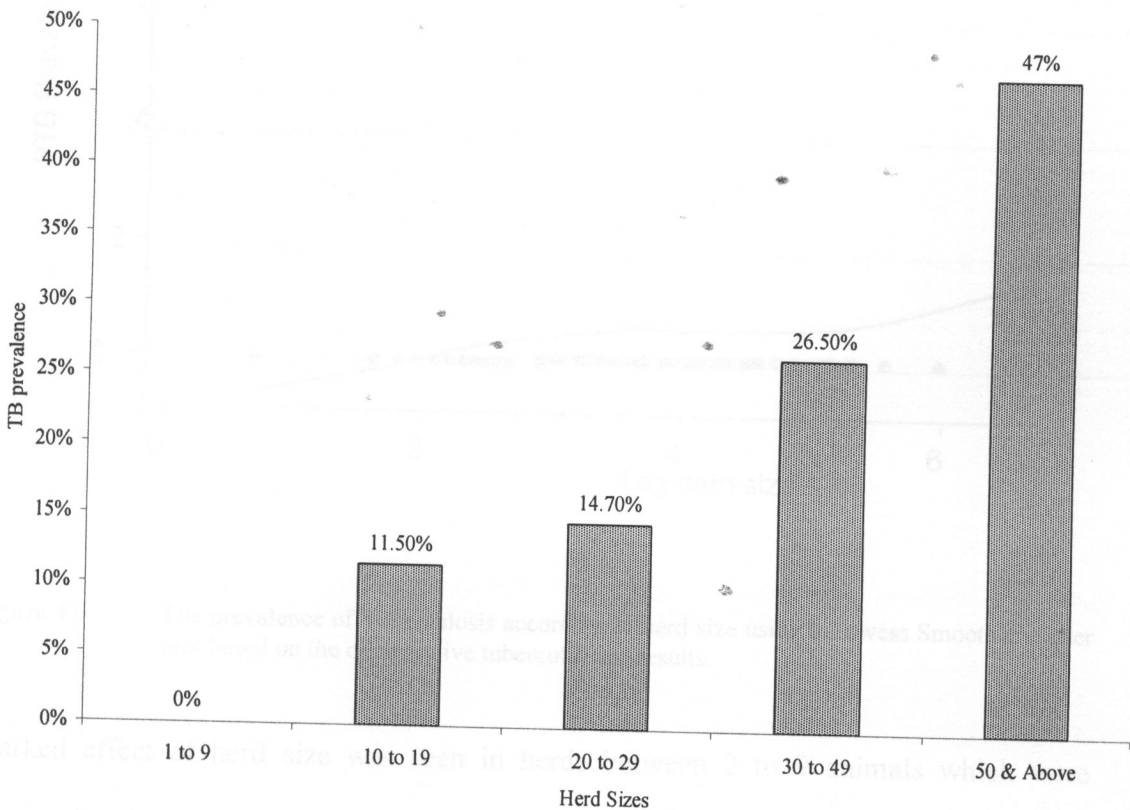


Figure 4.5 Comparative tuberculin results of tuberculosis prevalence according to herd sizes using categorical classification.

The effect of herd size on tuberculosis prevalence was markedly noted in the herds that were found in the Kafue Basin areas (Lochinvar and Blue Lagoon), where the likelihood

of getting at least one positive animal correspondingly increased with the increase in the size of the herd.

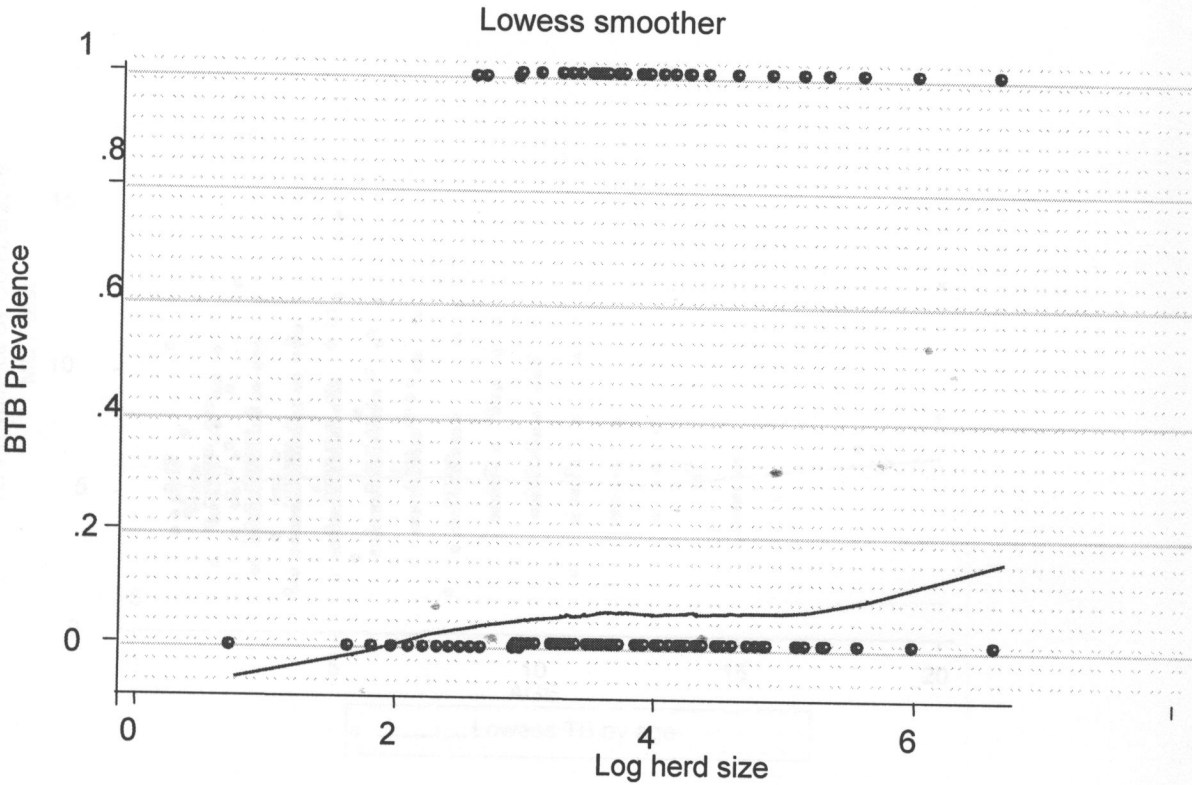


Figure 4.6 The prevalence of Tuberculosis according to herd size using a Lowess Smoother scatter plot based on the comparative tuberculin test results.

Marked effect of herd size was seen in herds between 2 to 9 animals which were observed to have no reactor animals on MCT. Herds above 50 animals were markedly observed to have the greater majority of reactor animals (Figures 4.5 and 4.6).

4.3.4 The effects of age on tuberculosis prevalence

On overall statistical analysis, age was found not to be significantly associated with TB positivity ($P= 0.4273$)-(Figure 4.7).

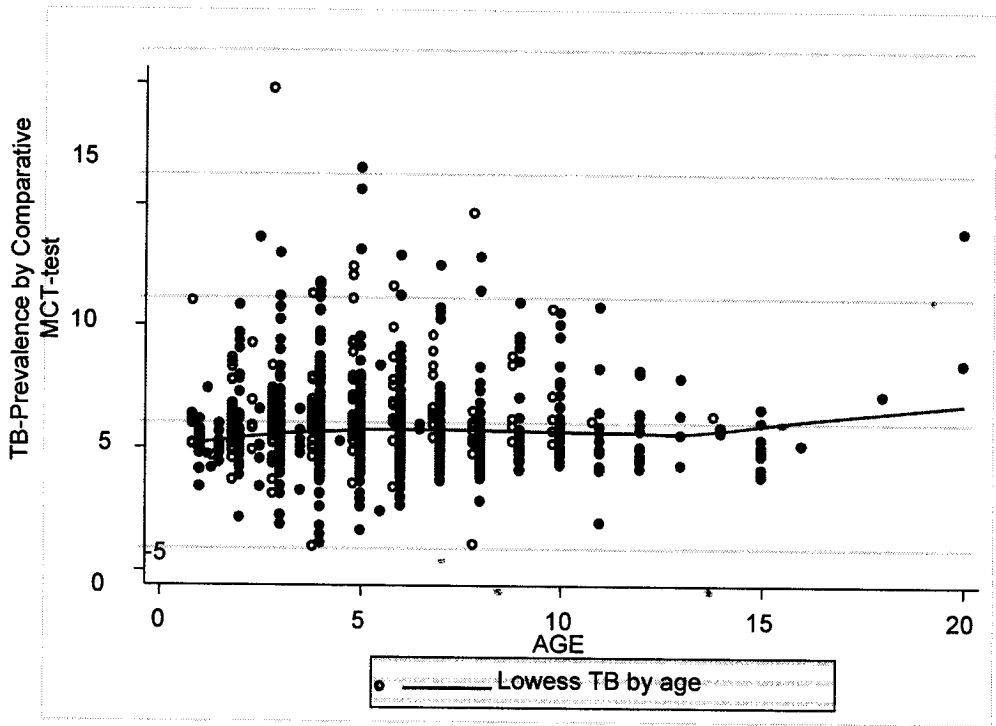


Figure 4.7 The prevalence of tuberculosis according to age using a lowess scatter plot incorporating a trend line depicting the effect of age on herd tuberculosis positivity.

Figure 4.7 indicates that the degree of skin reaction (Bovine –Avian difference) on the MCT test is very minimal and the trend line dips on certain sections in the chart indicating a negative correlation in prevalence.

The same data was applied for categorical analysis of age and its effect on TB prevalence. When the ages of the animals were categorized according to age groups, a

weak association was observed between age and TB positivity. This association was seen to vary according to different age ranges (Table 4.6 & Figure 4.8).

Table 4.6 Details on the prevalence of bovine tuberculosis on avian, bovine and comparative tests according to different age groups.

Variable	Avian Reactors		Bovine Reactors		Comparative		
	%		%		Results		
					%		
	Prevalence	Std.err	Prevalence	Std.err	Prevalence	Std.err	
Age	1-3yrs	4.96	1.31	11.06	2.10	4.96	1.34
	3-5yrs	6.14	1.58	16.18	2.38	5.82	1.38
	5-7yrs	7.88	2.75	14.78	3.59	3.94	1.75
	>7yrs	5.32	1.72	15.97	3.26	7.69	1.76
Overall							
Prevalence	6.03	1.08	14.40	1.55	5.50	0.83	

A general increase was observed in TB prevalence in the age ranges from 12 months through 36 months up to 60 months. There after, there was a slight decrease in prevalence in animals of the age ranges between 60 to 84 months old. A relatively significant rise was then observed with animals above 84 months old (Figure 4.8).

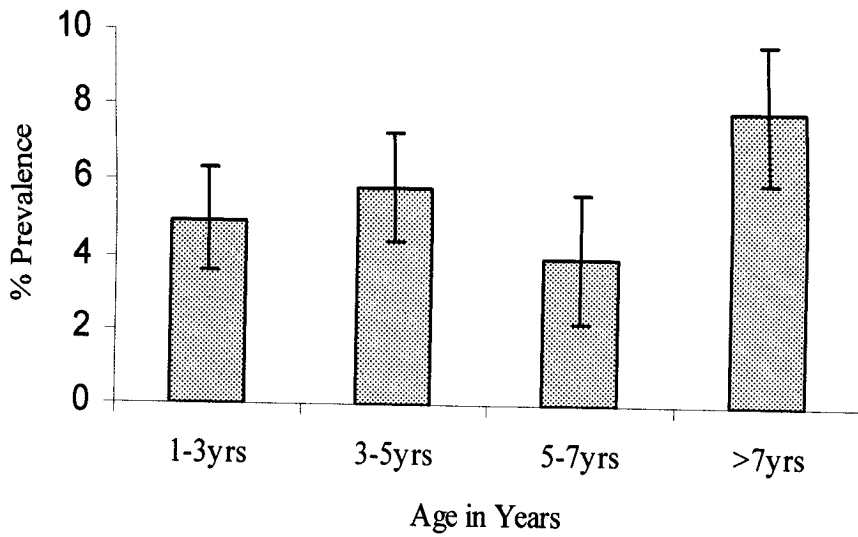


Figure 4.8 Tuberculosis prevalence according to age groups on categorical analysis depicting variations that occur in different age groups.

4.3.5 *The effects of sex on tuberculosis prevalence*

Sex was not a predilection for TB positivity in the study areas as no statistical association was observed on the overall prevalence ($P = 0.7103$) (Figure 4.9).

The prevalence according to sex was proportionately higher in males than females when avian only and bovine only reactors were considered. On the overall comparative results, no statistical association was seen between sex and TB prevalence.

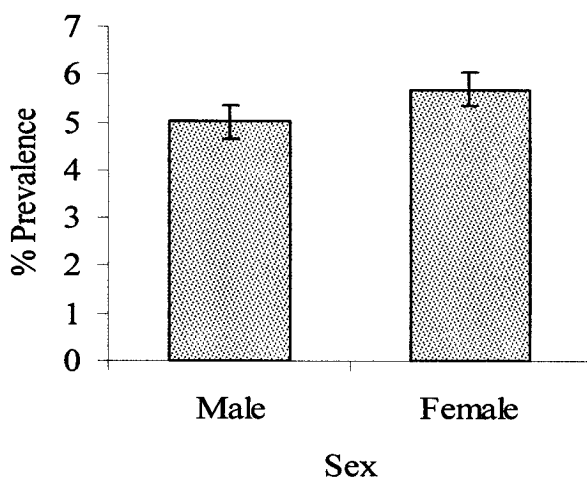


Figure 4.9 Prevalence of tuberculosis in male ($n = 279$) and female ($n = 665$) animals from the comparative tuberculin test.

4.4 Bovine tuberculosis based on abattoir survey

Animals for the abattoir study were received from six districts within and around the primary study areas. A total number of 695 animals were examined through the slaughter line during the four months of abattoir survey (Figure 4.10).



Figure 4.10 A whole carcass condemned after meat inspection at an abattoir in Monze District of Zambia due to suspected tuberculosis. Extensive milliary and granulomatous lesions were observed on almost every body organ and tissue on this animal (Courtesy of Dr. Phaniel Nyimba, District Veterinary Officer, Monze District-2003).

Of all the six districts from which cattle were received at the abattoir, Namwala District contributed the highest number of animals (34.24%)-(Table 4.7). Similarly, the district had the highest number of animals with organ condemnation suspected to be due to tuberculosis. Mazabuka and Choma Districts supplied relatively lower numbers of animals to the abattoir compared to the other Districts (table 4.7).

Table 4.7 Number of animals received from each of the district for abattoir survey.

Sampling periods	Origin of cattle received at the abattoir for study					
	Namwala	Monze	Mumbwa	Lusaka	Mazabuka	Choma
Totals	238	158	139	96	36	28
% totals	34.24%	22.73%	20%	13.81%	5.18%	4.03%

Out of 695 animals sampled, a total of 89 organs were condemned as being suspected to have lesions due to tuberculosis (Table 4.8).

Table 4.8 Summary of animals slaughtered and organ condemnation suspected to have TB lesions on gross meat inspection at the Kembe Cold Storage Abattoir.

Frequency of Sampling	Number slaughtered per sampling (n)	Suspected TB Lesions on Gross-PM
First sampling	93	12
Second sampling	108	14
Third sampling	138	21
Fourth sampling	120	18
Fifth sampling	86	10
Sixth sampling	57	6
Seventh sampling	99	8
Totals	695	89

4.4.1 Frequency of organ condemnation suspected to be tuberculous on gross postmortem inspection

Out of the 89 condemned organs, 78 (87.64%) consisted of lung tissue and their draining lymph nodes. Almost all the lung tissue condemned had similar nodular caseous lesions in their accessory lymph nodes, especially in the broncho-mediastinal lymph nodes. Other organs which were condemned of suspected TB lesions were: the Kidneys ($n=4$), representing 4.5% of the condemned organs, the pleura and gut ($n=3$); (3.37% condemnation rate), liver ($n=2$); (2.25% condemnation rate) and heart ($n=1$). One case involved the entire visceral organs being condemned because it had lesions of millary tuberculosis of between 3-4mm in diameter that virtually affected every other visceral organ in the body. During the 4 month abattoir study period, there was no whole carcass condemnation to suspected tuberculosis.

4.4.2 Tuberculosis prevalence based on gross abattoir results

Of the total number of 695 cattle that were inspected for tuberculoid and tuberculosis lesions on slaughter at the Kembe Cold Storage abattoir in Lusaka, a total of 89 organs and tissues were suspected to have had TB lesions and were condemned. This represented a prevalence of 13% based on the observed gross pathological lesions.

4.4.3 Microscopic examination of tissue homogenates

All the 89 organs suspected to be TB positive after decontamination had part of the sediment smeared on the slides for examination. Only 31 slides had visible acid fast staining bacilli out of the total number of 89 slides prepared and examined (Figure 4.11).

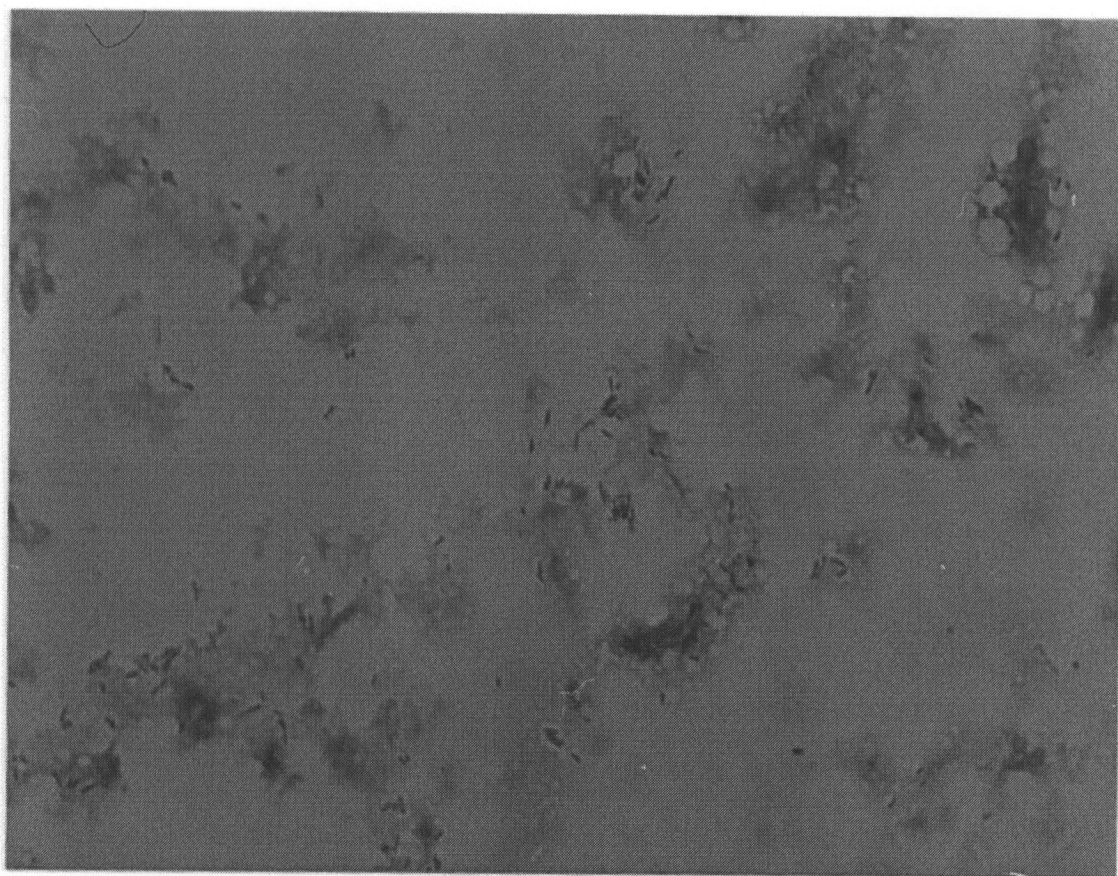


Figure 4.11 ZN-stained smear of tissue homogenate from cattle. The slender, beaded, red-staining (ZN-positive) rods were noted with very few clumps on most of the cattle slides (X 10 X 100 Magnification).

4.4.4 Tuberculosis prevalence from culture results

All the 89 condemned organs were decontaminated and inoculated on Lowenstein Jensen media. Of this number, 46 slants had characteristic TB colonies by the end of the eighth week (Table 4.9). This represented an overall 6.62% prevalence of positive TB on culture media from the 695 animals which passed through the slaughter line. In the other 43 culture slants, nothing grew while others had just heavy contamination and rotting of media. Even on repeat inoculation no growth was observed.

Table 4.9 TB culture positive samples and their areas of origin after being cultured on Lowenstein Jensen media.

Area of Origin	Positive TB Cultures	Percent % Positives
Namwala	18	39.13
Monze	7	15.22
Mumbwa	9	19.56
Lusaka	7	15.22
Mazabuka	3	6.52
Choma	2	4.35
Actual totals	46	100

4.5 Tuberculosis survey in wildlife

This study included a total number of 25 hunter-harvested wildlife species between mid October to late December of 2003. Of the wildlife species sampled, there were lechwe (*Kobus leche Kafuensis*)-(n=14), wildebeest (*Connchaetes taurinus*)-(n=5), Buffalo (*Syncerus caffer*) (n=2), Zebra (*Equus burchellii*)-(n=2) and Impala (*Aepycerus melampu*)-(n=2).

Out of the 25 wildlife species sampled, gross pathologic lesions suggestive of TB were observed only in 6 lechwe and 1 impala (Appendix Two). Thus a total of 7 wild animals (28%) had suggestive TB lesions. Out of this total, the lechwe represented 24% while the impala 4% of gross TB suspected animals.

4.5.1 Gross pathological results in Lechwe

There were six (6) lechwe that were suspected to have TB lesions on gross examination out of the 14 that were harvested. Taking into account the lechwe population only, this represented a crude prevalence value for tuberculosis infection at 43%.

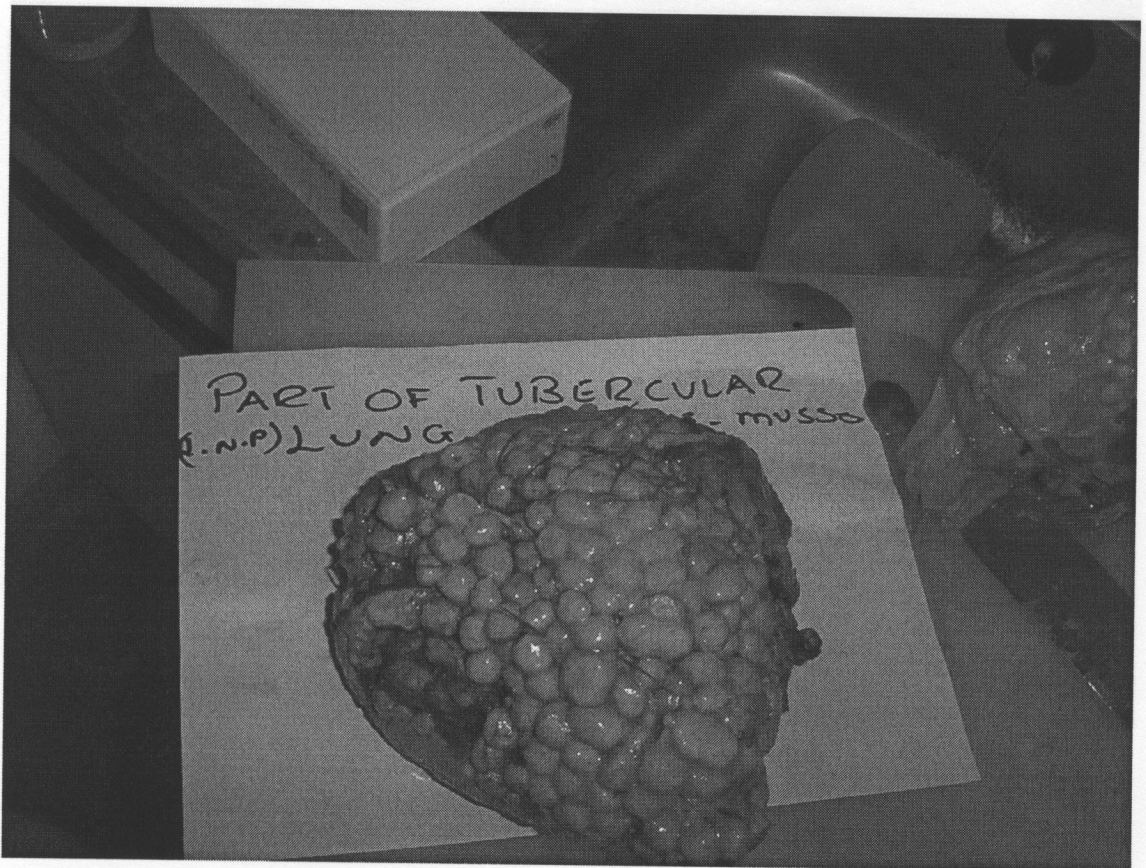


Figure 4.12 The lung tissue from the lechwe sampled in the Lochinvar National Park GMA showing extensive granuloma formation.

Three (3) adult male lechwe had suggestive TB lesions localized to lungs. One of the three was severely affected involving the lungs and the heart in the form of very clear calcified nodular granulomas (Figures 4.12 and 4.13). Their accessory draining lymph

nodes especially those of the mediastinal origin had several small necrotic lesions measuring 1 to 2mm. Some lymph nodes were hardened and gritty in texture due to calcification while others were tremendously enlarged and softened with liquefaction.

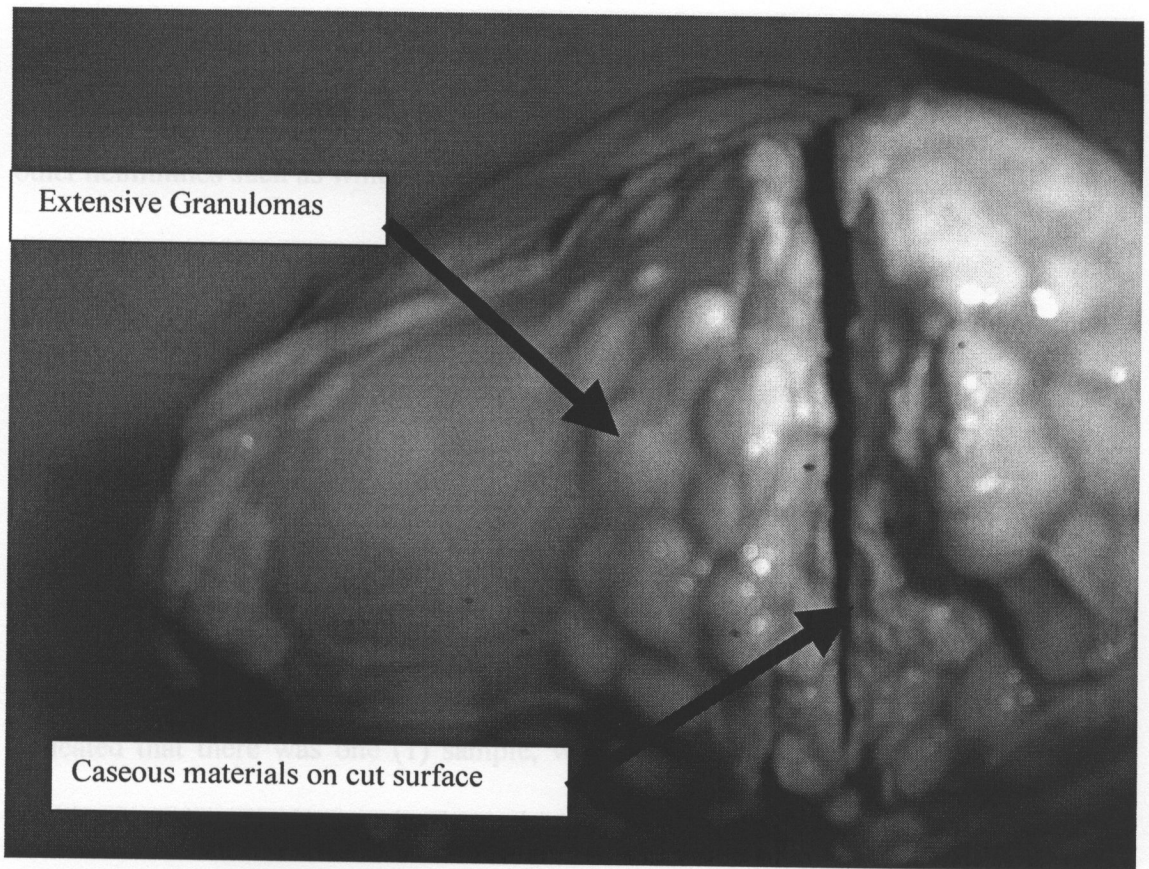


Figure 4.13 Heart of a ten year old male Lechwe antelope with extensive granulomas with cut section exposing the yellow caseous materials

One (1) female adult had soft diffuse granulomatous inflammatory lesions on certain areas of its lung lobes. The other animals had their lesions more prominent in the bronchio-mediastinal lymph nodes while the rest had no visible lesions, either in the lungs or other organs.

The youngest lechwe in males found to have gross TB lesions in the lymph nodes was approximately 6 years old. The other three (3) adult males were about 9 years ($n=2$) and 10 years ($n=1$). These were the lechwe with frank lung caseation and calcified lesions. The other two (2) adult females had lesions only in the lymph nodes, apart from the one with liquefied lung lesions. It was difficult to determine on gross examination all the tubercular lesions in some other organs like liver due to the heavy burden of flukes and other helminthes such as which cause caseation in these organs too.

4.5.2 Microscopic examination of tissue homogenate and culture results.

Lung tissues collected with their accessory lymph nodes from all the 25 wild animals were examined.

In all the other wildlife species, none had microscopic reading showing AFBs on Ziehl Nielsen (ZN) stain. However, in the lechwe, an extra sample from the homogenate indicated that there was one (1) sample, which had no visible gross lesions, but was positive on microscopic examination using the ZN stain (Figure 4.14). On culture, two (2) more samples from the lechwe had characteristic growth of TB on Lowenstein Jensen media. Thus there were a total of eight (8) lechwe on culture compared to six (6) on gross examination. This implies two lechwe were negative on gross postmortem examination, but positive on culture (Table 4.10).

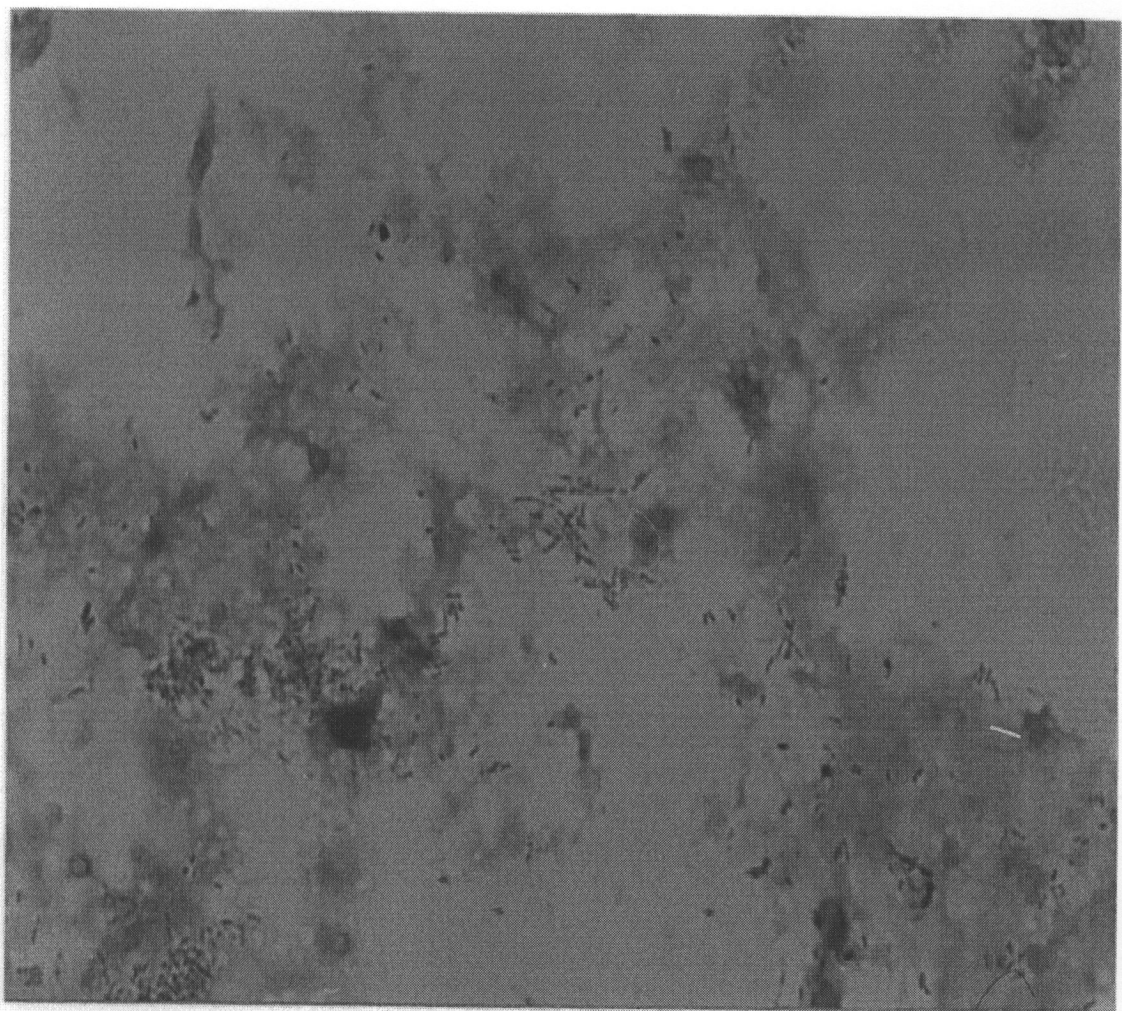


Figure 4.14 ZN-stained smear of tissue homogenate from the lechwe which had extensive tubercles of the lungs. The slender, beaded, red-staining (ZN-positive) rods are seen to form clumps against a methylene blue background (X 10 X 100 Magnification).

From Table 4.10, it is clear that the culture results yielded 8 positives representing 32% crude prevalence when considered from the overall total number of animals sampled, but 57% if only lechwe are considered in the sample frame.

Table 4.10 Results from gross postmortem, microscopic and culture examinations.

Animal Species	No. Sampled <i>n</i>	Methodology		
		<i>PM</i>	<i>on ZN</i>	<i>Culture</i>
Lechwe	14	6 ^a	7	8
Wildebeest	5	0	0	0
Buffalo	2	0	0	0
Zebra	2	0	0	0
Impala	2	1 ^b	0	0
Totals	25	7	7	8

^a There were two No - visible lesions on gross PM examination

^b No AFB seen and no growth occurred on culture.

4.6 Tuberculosis survey in human communities of the study areas

4.6.1 General sampling characteristics for sputum sample examination

Out of the 42 patients sampled, 24 were from the Monze District, while 18 were from Namwala District. The mean age was found to be 37 years (95% CI= 33 to 41 years). The youngest patient was 18 years while the oldest patient was 68 years old. Out of the 42 patients, four couldn't recall their ages but were estimated to be above 55years. These were left as the 4 missing variables on age analysis. Thus only 38 patients knew their real ages. Of this number, 21 were males while 17 were females. Overall, there were a total of 22 males and 20 females sampled.

4.6.2 Direct microscopy examination results

Out of the 22 male cases examined, 5 (22.73%) were negative while 16 (72.73%) were positive with 1 (4.55%) showing no acid fast bacilli on ZN stain (Figure 4.15). The females had 12 out of 20 (60%) being positive while 5 out of 20 (25%) were negative with 3 (15%) having no acid fast bacilli seen.

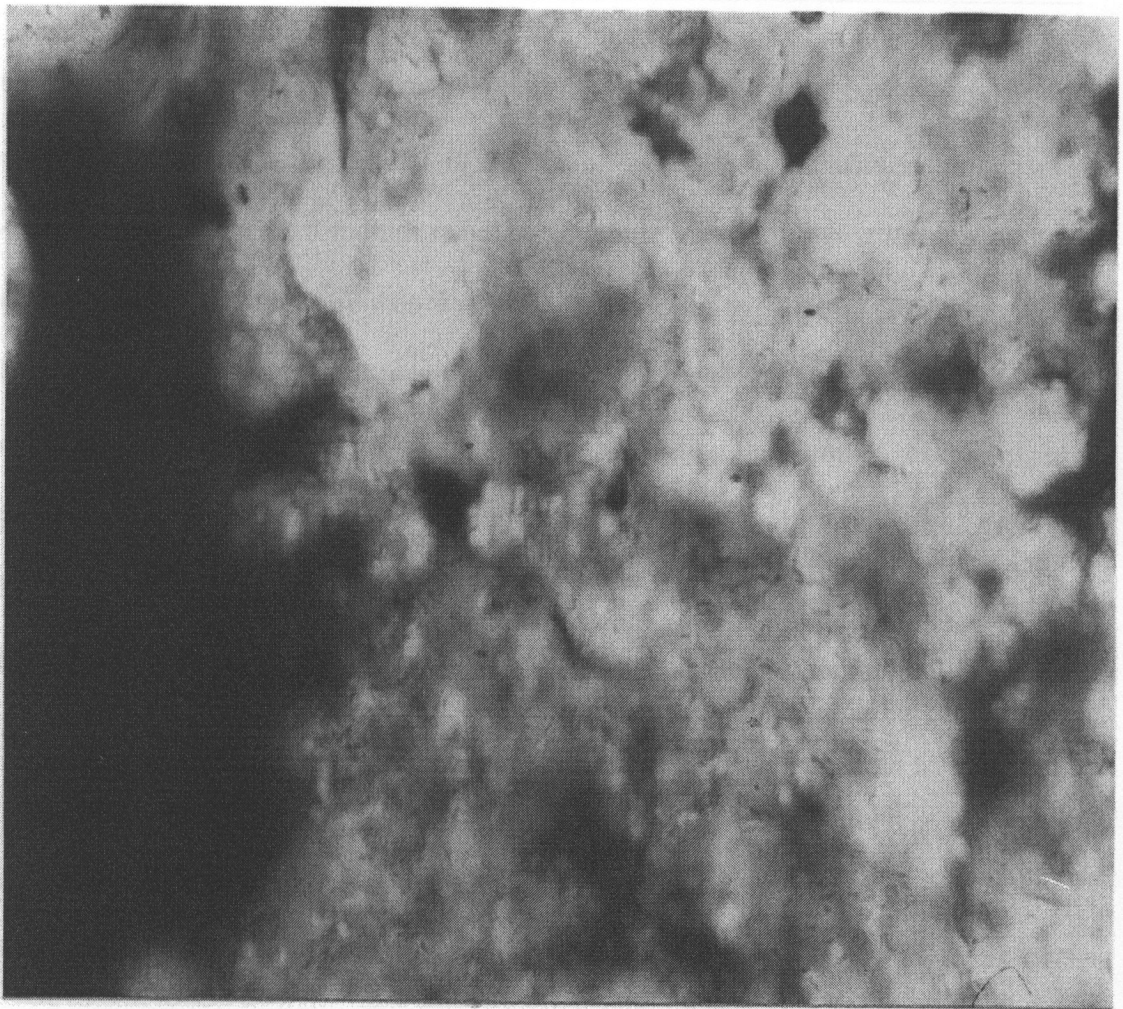


Figure 4.15 ZN-stained smear of a human sputum sample. The slender, beaded, red-staining (ZN-positive) rods characteristic of *Mycobacterium tuberculosis* are much thinner and slender compared to those isolated from lechwe and cattle tissue homogenates as presented against a methylene blue background (X10X100 Magnification).

Table 4.11 Results of human sputum sample smears on Auramine and ZN stain by District

Area	n	Positive	%	Inconclusive	%
Monze	24	16	66.67%	5	20.8%
Namwala	18	15	83.3%	1	5.56%
Totals	42	31	73.8%	6	14.3%

Of the 42 samples from suspected TB patients, 31 were positive on direct microscopic examination, representing a total of 73.8% of the sputum samples submitted (Table 4.11). Of this number, 10 sputum samples, under the smear interpretation schedule, were classified as 3+ (that is more than 9 AFB per field / refer to Table 3.7 for interpretation). Four (4) of these were from Monze, while 6 were from Namwala. Although Monze had more samples, Namwala had more patients with sputum samples classified as 3+ positives than Monze. Samples classified under 2+ were only 4 with 2 from each District. Those classified under 1+ were also 4, with 3 from Monze and 1 from Namwala. The other 13 samples were classified under various numbers of bacilli (from 1 bacillus seen to at least 7 bacilli seen).

4.6.3 Culture growth and Isolation

The rate of culture growth was characterized according to weeks in terms of fast growers, moderate and slow growers. The results in table 4.12 depict the growth rates tabulated in percentages of culture growth per week of observation.

Table 4.12 Growth pattern of *Mycobacteria* cultures according to weeks

Period of growth in weeks	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Monze	3	12	22	22	23	23
Areas Namwala	3	11	15	15	15	15
% Totals	6(14.3%)	23(54.8%)	37(88.1%)	37(88.1%)	38(90.5%)	38(90.5%)

There were 6 (14.3%) fast growing *Mycobacteria* by the end of the first week of inoculation. The greatest growth was observed by the end of the third week ($n=37$), representing a growth rate of 88.1%. This growth rate was observed to be the same even in the 4th week. By the fifth week, 90.5% of the cultures had grown and these were maintained through the sixth to the seventh week. Out of the 42 cultures, one slant had growth on Pyruvate enriched media only, with no growth on Glycerol enriched media. There was also one slant with yellow pigmentation on Glycerol were it was seen to have grown. Another important observation was that almost all the other slants had growths on both Glycerol and Pyruvate enriched media.

4.7 Results from the questionnaire survey

Out of the one hundred and eleven (111) randomly selected rural households in the Kafue Basin interface areas and the Kazungula District recruited for the questionnaire survey and tuberculinisation we managed to interview a total of eighty seven (87) households. Other households could not be interviewed because of time constraints, and

resource availability. However, of the 87 households interviewed, Blue lagoon had a higher response ($n = 43$)-(49.4%) followed by Lochinvar ($n = 25$)-(28.7%) and lastly Kazungula ($n = 19$)-(21.8%). The other 24 households however, had their animals available for tuberculinisation. In all these areas, 63.2% of the persons interviewed were heads of the households while interviews with other family members represented 35.6% of the respondents. Out of all the interviewed households, 86 of 87 (98.9%) were male-headed households with only 1 of 87 (1.1%) household being female headed and she happened to be a village head-woman.

Household (HH) size ranged from 2 to 39 persons with sixteen (16) households containing more than 20 persons per household. The average number of persons per HH was 14 (*std.dev* ± 8). In total, the 87 households contained 1,214 people. All the households owned cattle but only 12 households (40%) had their animals testing positive on MCT. Of the 87 HH, 79 HH, representing 90.8% of the HH, managed their cattle under the communal systems with some owning paddocks but yet allowing their animals to graze communally at certain times of the year.

Thirty households (34.5% of respondents) reported having had a family member receiving TB treatment during the preceeding 12 months. Blue lagoon had 20 HH (66.7%), Lochinvar 3 HH (10.0%) and Kazungula 7HH (23.3%) responding to having had a TB patient. In Blue lagoon, there was one HH that had three members of one family receiving TB treatment. One HH in Blue lagoon had a 19-year-old girl receiving TB treatment. Sixteen homes had patients of the ages ranging from between 21 to 30 years being affected with TB. Three households had patients between the ages of 41 to

50 years while 4 homes had patients over 50 years old. Thus the median affected age was between 21 to 30 years.

On the knowledge of TB in cattle, 53 of 87 (60.9%) of the respondents knew of the disease in cattle but 34 of 87 (39.1%) had no knowledge of the disease. About sixty four percent of the HH responded to having had all their family members vaccinated with BCG, while 25 HH (28.7%) responded to having had received no BCG vaccine with 6 (6.9%) of the HH not knowing whether they or their family members were vaccinated.

From the questionnaire survey, a number of possible routes for TB transmission from cattle to humans were investigated. The results showed that all the respondents had been in contact with cattle in one way or another with 55 of 87 (63.2%) respondents having had contact through milking of cows. There were 65 of 87 (74.7%) of the HH that claimed to boil their fresh milk before consumption and mostly in such cases it was only for making tea. However, 22 of 87 (25.3%) of the HH consumed raw unboiled milk directly from the cows. The greater majority of the respondents consumed fresh milk (97.7%). All the 87 HH processed sour milk from raw fresh milk without heat treatment with 68 of 87 (78.2%) of the HH consuming raw sour milk on a daily basis. Of the 87 HH, 84 (96.6%) obtained milk from their own animals with 3HH (3.4%) obtaining milk from neighbouring villages. Knowledge of respondents on human tuberculosis showed that 53 of 87 (60.9%) of the respondents knew of the existence of human TB, while 34 of 87 (39.1%) had no knowledge of the disease.

4.8 Risk factor assessment for tuberculosis in the study areas

On univariate analysis of independent variables at herd level, a number of factors were observed to have had independent effects on TB herd status (Table 4.13).

Of these variables, the most significant were area, contact with wildlife, moving to the plains, herd size and sharing of grazing grounds with wild animals (grazing system).

Grazing cattle in the interface areas (Interface herds/IFH) increased the odds of herd tuberculosis positivity by approximately 18 times compared to grazing animals within Villages (Village Resident herds/VRH) - (Odds ratio = 18.67; CI at 95%, = 4.3-81.0); $p = 0.000$). Equally herds that practiced transhumance (TH) were approximately twice more likely to test positive for TB than VRH (Odds ratio = 2.0; CI = 0.6 – 5.9); $p = 0.210$).

There was a significant difference in TB prevalence within the three study areas of Lochinvar, Blue Lagoon and Kazungula District (Fisher's exact $p = 0.003$). Moving animals to the plains was noted to be significantly associated with TB positivity ($\chi^2 = 9.95$; $p = 0.0016$). Similarly, contact with wildlife was seen to have a significant effect on finding a herd positive on tuberculinisation ($\chi^2 = 10.21$; $p = 0.0014$). Animals that were grazed in areas frequented by wildlife only in certain times of the year without much contact were seen to be at moderate risk of reacting positive compared to those known to have actual contact through out the year ($\chi^2 = 5.31$; $p = 0.0212$).

Table 4.13 Crude descriptions of risk factors for TB herd prevalence status in traditional cattle in Zambia.

Risk Factor	Levels	Number of herds tested	Percentage with TB reactors
Area	Kazungula	19	21.84
	Lochinvar	25	28.74
	Blue lagoon	43	49.43
Grazing type	1: Village herds	40	19.54
	2: Transhumant herds	30	34.48
	3: Interface herds	17	45.98
Herd Size	0: small herds 5 – 40	44	13.6
	1: large herds 40 - 400	43	60.5
Moving to the plains	0: No	41	47.13
	1: Yes	46	52.87
Contact with wild animals	0: Yes	44	50.57
	1: No	43	49.43
Share water &	0: Yes	44	50.57
Grazing with wild animals	1: No	43	49.43

Note: Variables with fisher’s exact $p \leq 0.25$ were identified as risk factors for inclusion in the multivariable model

On risk ratio univariate analysis, animals that moved to the plains were approximately two times more likely to test positive for TB than those that didn’t (Relative risk = 2.67; CI = 1.42-5.29); $p = 0.0016$), just like those that had contact with wildlife (Relative risk

= 2.50; CI = 1.36-4.76); $p = 0.0014$) were also twice more likely to be TB positive than those without contact with wildlife.

From univariate analysis of results, it was clear that animals which moved to the plains in search of grazing land were approximately thirty eight times more likely to have had contact with wild animals than those that didn't (Relative risk=38.3; CI =9.4 – 207.8); $p = 0.000$). Factors such as coughing in animals were found not to be statistically associated with herd level TB positivity (Relative risk = 1.01; CI = 0.59-1.76); $p = 1.000$). There was no statistical association in TB positivity for cattle herds that were kept by households that had a known human TB case in the preceding 12 months (Relative risk = 1.14; CI = 0.64-1.94); $p = 0.83$).

Variables considered to be significant on univariate analysis were assessed via a multivariable logistic regression model. All the main variables with their interaction-variables were included as predictors in the model, as each variable or its interaction could affect the odds of an animal being positive for TB. All the variables and their interactions were previously analyzed using contingency tables (table 4.14), and those that were statistically significant ($p < 0.05$) on univariate analyses were included in the full model (Appendix one).

Table 4.14 Some associations between presence of a reactor animal and variables in the study areas.

Risk Factor		MCT-Results		Risk Ratio		Chi Square		
		+	-	Value	95% CI	χ^2	df.	P
Cattle go to plains	Y	24	22	2.67	1.42 - 5.29	9.95	1	0.0016
	N	8	33					
Cattle contact wildlife	Y	23	20	2.50	1.36 - 4.76	10.21	1	0.0014
	N	9	35					
Coughing Seen in cattle	Y	17	29	1.01	0.59 - 1.76	0.000	1	1.000
	N	15	26					
Lungs condemned	Y	15	14	1.77	1.024 - 2.891	3.27	1	0.07
	N	17	41					
Human with TB last 12 months	Y	12	18	1.14	0.64 - 1.94	0.047	1	0.83
	N	20	37					
Variable		Wildlife contact		Risk Ratio				
Moving to the plains	Y	43	3	38.3	9.4 – 207.8	68.28	1	0.000
	N	1	40					

Y = YES, N = NO.

The grazing system was strongly influenced by herd size. Large herds tended to stay in the plains or were transhumant and these were found to be 7 times more likely to be TB positive than village resident herds (Odds ratio = 7.32; CI = 1.23 – 43.56); p = 0.029) in the multivariable logistic analysis.

Table 4.15 Final multivariate multiple logistic regression analysis of herd level TB positivity in cattle found in the three main study areas.

Risk Factor	SE	Odds ratio	P	95% CI
Herd Size	0.01	1.03	0.001	1.01-1.05
TH Vs VRH	2.92	4.30	0.031	1.14-16.24
IFH Vs VRH	6.66	7.32	0.029	1.23-43.56

Not significant ($p>0.05$), Likelihood ratio = -36.72
Number of observations in the model = 87
Hosmer – Lemeshow χ^2 (8) = 3.87, Prob > χ^2 = 0.358

The two variables (herd size and grazing systems) were found to be highly significant. They were observed to be associated with increased prevalence in the earlier analyses than all the other variables including interaction variables ($p>0.05$). The model was tested for the goodness of fit using the Hosmer-Lemeshow statistic. The same test was used to test the model’s sensitivity and specificity at different probability cut points.

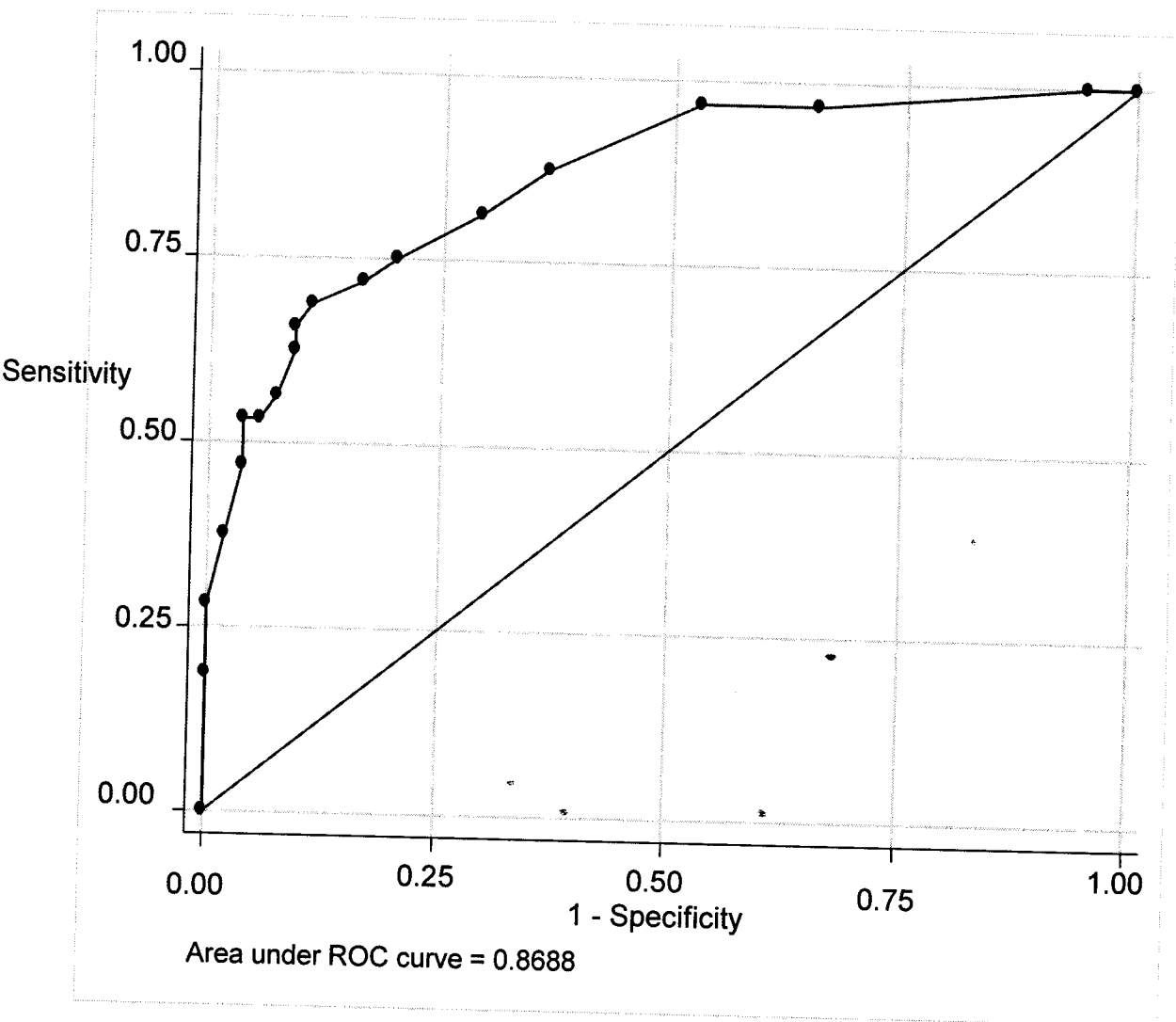


Figure 4.16 Receiver operated characteristic (ROC) curve showing the sensitivity and specificity of the logistic regression model indicating its good predictive ability.

From Figure 4.16, the Receiver Operating Characteristic (ROC) curve is seen to extend reasonably well into the upper left-hand corner of the graph and the area under the curve is 0.8688. Both of these characteristics of the model indicate its good predictive ability.

Appendix one, the generated causal model for TB positivity in cattle herds indicate that increase in herd size precedes movement of cattle into plains which is also preceded with contact with wild life. Appendix one indicates that the model has no causal

feedback loops hence it is recursive. The total effect of all the paths in this model is the increased probability of finding positive TB cattle herds.

4.9 Spoligotyping of *Mycobacterium* isolates from humans and animals.

4.9.1 Spoligotyping results of animal isolates

A total of 54 DNA *Mycobacterium species* from animals (cattle and lechwe) were submitted for DNA fingerprinting (spoligotyping). The results were only obtained from 31 cattle (67.4%) and none from lechwe isolates. The remaining samples contained too much Löwenstein-Jensen medium which may have inhibited the PCR reactions owing to the presence of a lot of proteins from one of its constituents (eggs). An attempt to remove most of the inhibitors by subjecting the respective samples to a DNA extraction method described by Boom et al, 1989 did not yield successful results. These latter samples were therefore excluded from further analysis.

Ten (10) different spoligotypes were obtained from the 31 cattle isolates submitted for DNA analysis. Figure 4.17 represents the spoligotypes obtained from this study while table 4.16 demonstrates their distribution in each main District.

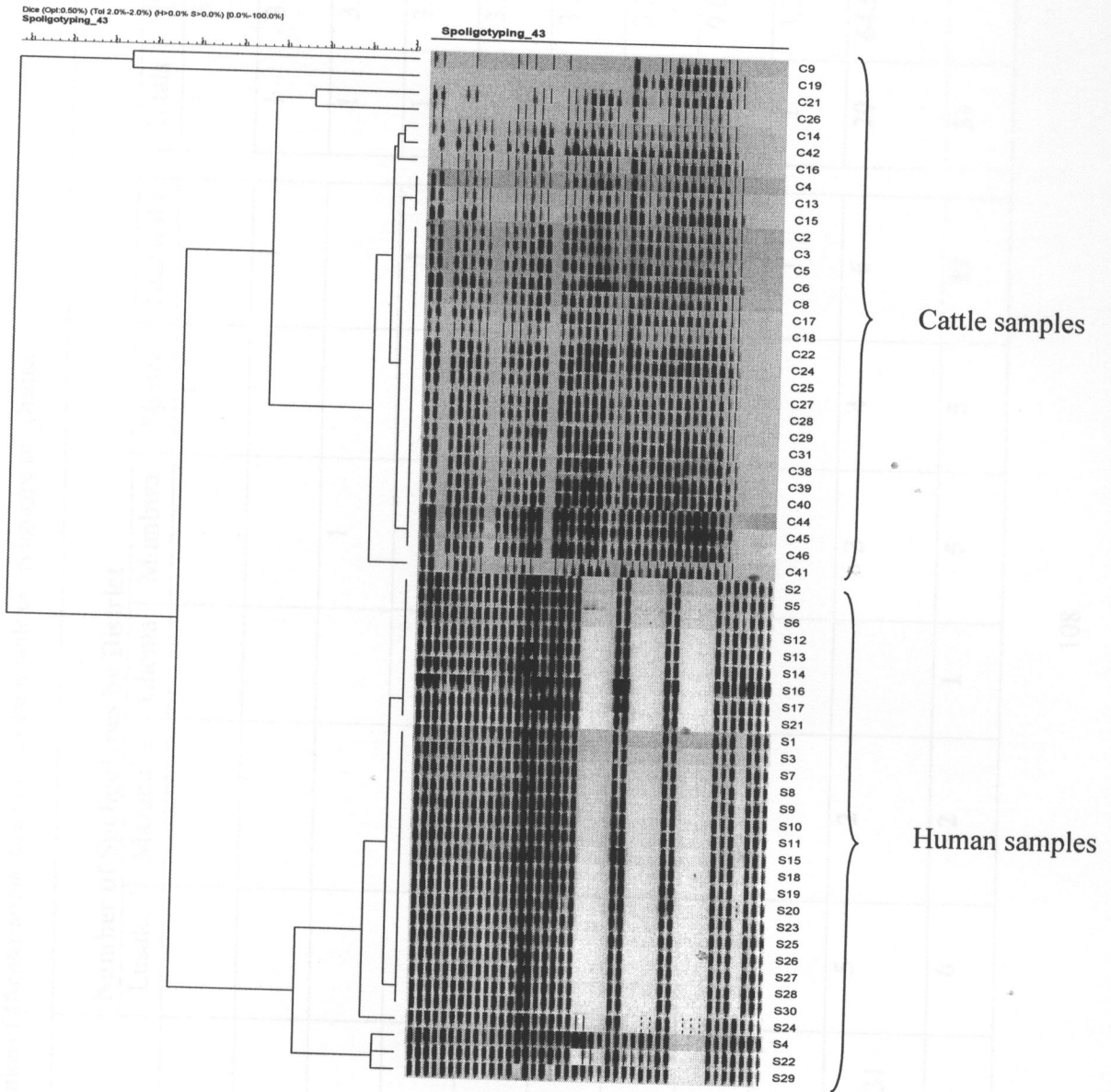


Figure 4.17 Spoligotype dendrogram of *M. bovis* (C9 to C41) and *M. tuberculosis* (S1 to S30) isolates studied. The presented patterns were generated using the Bionumerics version 3.0 computer software (Applied Maths, St. Maartens-Latem, Belgium). The spoligotypes were compared using the band-based Dice coefficient and clustering determined by the unweighted pair group algorithm with arithmetic averages (UPMGA) method. Designation of spacers from left to right is 1 to 43. The dotted lines for S24 indicate uncertain bands. The isolates obtained from cattle and humans are indicated.

Table 4.16 Distribution of *Mycobacterium bovis* isolates from cattle by spoligotype and District.

Isolates	Number of Spoligotypes by District							Totals	(%)	Comment
	Lusaka	Mazabuka	Choma	Mumbwa	Monze	Namwala				
C9					1		1	3.23	Monze C9	
C19						1	1	3.23	Namwala C19	
C21				1			1	3.23	Mumbwa C21	
C26						1	1	3.23	Namwala C26	
C14	1							3.23	Lusaka C14	
C42						1	1	3.23	Namwala C42	
C16						1	1	3.23	Namwala C16	
C4,C13,C15			1	1		1	3	9.66	Cluster (2)	
C41						1	1	3.23	Namwala C41	
C2,C3,C5,C6,C8,C17,C18,C22,C24,C25,C27,C28,C29,C31,C38,C39,C40,C44,C45,C46	5	2		3	4	6	20	64.50	Predominant Cluster (1)	
Total number of isolates per each District	6	2	1	5	5	12	31			

All the animal Spoligotypes in this study showed the absence of the spacers 3, 9, 16 and spacers 39 to 43, which is a characteristic feature of the majority of *M. bovis* strains.

As shown in Figure 4.17, DNA fingerprinting results showed that 64.5% (20/31) of the isolates tested belonged to one spoligotype. This spoligotype was found in all Districts that presented their animals at the abattoir during the study period except for Choma District.

Out of the 10 spoligotypes identified, Namwala District had a total of seven, five of which (spoligotype C19, C26, C42, C16 and C41) were present predominantly in Namwala District (Figures 4.17 and table 4.16).

One spoligotype (C19) from Namwala District, lacked spacers 1 to 24 and spacers 35-43, leaving it with only 14 spacers. Four other spoligotypes (C26, C42, C41 and C16), were present principally in Namwala District with only spoligotype C9 from Monze District being closely related to C19, one of the spoligotypes from Namwala District. Spoligotype C21 from Mumbwa was also found to be closely related to one of the Spoligotypes only found in Namwala District (C19). Further still, C21 from Mumbwa District was very closely related to C26 from Namwala District. On the other hand C14, which was restricted to Lusaka District, was found to be closely associated to C42 from Namwala District. One spoligotype was found in three Districts, these were C4 from Namwala, C13 from Choma and C15 from Mumbwa (table 4.16 & Figure 4.17).

4.9.2 *Spoligotyping results of human isolates*

Out of the 46 *Mycobacterium spp.* isolates submitted for DNA fingerprinting, results were available from 65.2% (30/46). As depicted in Table 4.17 and Figure 4.17 six (6) different spoligotypes were obtained. The most consistent and common feature with the human isolates under this study was the absence of spacers 33 to 36. This pattern was observed in all the isolates apart from S24, which had uncertain bands on spacers 34 to 36. There was a predominant spoligotype in this study that was not geographically restricted. Out of the 30 isolates with available results, the predominant spoligotype clustered a total of 17 isolates (56.68%)-(Table 4.17 & Figure 4.17). A second smaller cluster was observed and this was geographically restricted to Monze District and it comprised 9 isolates (30%). Three of the four different isolates (S4, S22 and S24) were geographically restricted to Monze District while the fourth isolate (S29) was from Namwala District.

Table 4.17 Distribution of 30 *Mycobacterium tuberculosis* spoligotypes by District.

Areas of Study	Spoligotype										Total number of isolates
	1	2	3	4	5	6	6	5	4	3	
Monze	S1,S3,S7,S8,S9,S10,S11,S1	S2,S5,S6,S12,S13,S14,S16,S17,S21	S4	S22		S24					24
5	S18,S19,S20,S23										
Namwala	S25,S26,S27,S28,S30				S29						6
Total number of Isolate per group	17	9	1	1	1	1	1				30
Percentages	56.68	30	3.33	3.33	3.33	3.33	3.33				100

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of tuberculosis in cattle

The prevalence of tuberculosis was high in the study areas of the Kafue Basin with Lochinvar and Blue Lagoon recording prevalence levels of 5.35% and 7.34% respectively. However, the control area of Kazungula had a very low prevalence of 0.57%. Statistically, significant differences in TB prevalence ($P=0.0001$) in the three study areas were recorded. Both Blue lagoon and Lochinvar had higher individual and herd prevalence levels than Kazungula District. While significant differences were seen between Kazungula District against both Lochinvar and Blue lagoon, however, there was no significant differences observed between Lochinvar and Blue lagoon ($P = 0.286$). Our current prevalence findings are consistent with previous reports by Cook *et al.*, (1996) and Sitima (1997). Cook *et al.*, (1996), found a TB prevalence of 7.4% in cattle that grazed on the eastern side of the Kafue Basin from the Monze District. Sitima (1997) found a TB prevalence of 12.8% in cattle that grazed the western part of the Kafue Basin from Namwala District. These results also confirm the findings of a similar survey during the same period by Pandey *et al.*, (1996), who established that cattle which went to graze in the Kafue Basin of the Monze District had a relatively higher prevalence of 7.4%. Overall, our results from this study and those done by earlier researchers indicate a persistent high prevalence of TB in the Kafue Basin. However, our study incorporated a control from which a very low prevalence of less than a percentage was recorded in the Kazungula District.

The findings of tuberculosis in and around the Kafue Basin have been demonstrated by earlier researchers who diagnosed the disease in cattle and the lechwe (wildlife) populations (Gallagher *et al.*, 1972, Dillmann 1976, Stafford 1991 and Pandey *et al* 1998). All these researchers recognized the likelihood of the pivotal role the lechwe was to serve as a source of a non-treatable reservoir host of the disease to cattle due to the interaction which was observed between these two species of animals. A similar scenario existing in the Kafue Basin was analyzed by O'Connor *et al.*, (1993) in Ireland, who concluded that the dominant factor contributing to the occurrence of reactors to the tuberculin test was the location of a herd in an area with a high level of tuberculosis. They suggested that the increased risk to a herd in such an area could be due to contact with infected cattle in neighbouring herds, or due to the presence of some common reservoir of disease in the area, such as infected badgers. This scenario may not be different with the Kafue basin of Zambia, where the lechwe populations are seen to interact with cattle and could explain the high prevalence of TB in Blue Lagoon and Lochinvar compared to Kazungula District.

One of the short comings of the tuberculin test is that cattle go through a period of desensitization before and after calving and as many as 30% may give false negative reactors ,returning to a positive status 4-6 weeks later (Radostitis 2000). Such a handicap may have the possibility of introducing classification bias as animals are likely to be misclassified. However, after screening through the data, only 15 (1.6%) animals of the 944 had given birth within the last 2 months prior to the survey and 3 of these cows reacted positive to the tuberculin test. However, it is unlikely that this was a major problem as the sensitivity at animal level of the comparative cervical tuberculin test is

believed to range from 80 to 98% (Griffin *et al.*, 1996). Further still, only 1.6% of the animals fall under this category.

5.2 Risk factors associated with tuberculosis prevalence in the studied areas.

Grazing systems, just like cattle management systems or enterprises, were observed to have had a profound influence on the prevalence of TB ($P=0.000$). Other studies have been able to show that management systems are significant risk factors for TB transmission (Collins *et al.*, 1994). Marangon *et al.*, 1998 reported that the type of cattle enterprise becomes a risk factor when the likelihood of cattle movement in that enterprise is high. However, with regards to grazing systems (pastoralists), there is little published information. From our findings, TB prevalence was significantly different between village resident herds and transhumance herds ($P=0.026$), and highly significant against interface herds ($P=0.0000$). These results suggest that as the proximity between cattle and lechwe is reduced, the probability of finding at least one animal reacting positive to TB increases. The only plausible explanation for such an observation is that there could be a difference in the exposure of cattle to agents capable of stimulating a positive reaction, including TB infection, or differences in response of such exposed cattle. It is evident that the odds of cattle TB positivity increased significantly with reduced proximity to the lechwe grazing grounds, as cattle that lived almost permanently in the plains were 500 times more likely to be in contact with wild animals than those that grazed within villages ($OR = 573, P = 0.000$). Our abattoir results showed that *Namwala District had the highest prevalence on both gross examination and on culture positive (18/46; 39.13%). This is the District that is centrally located right inside the Kafue plains with almost all its entire cattle population being found in the Kafue flats.*

This scenario adds to the suspicion of lechwe proximity and having TB positive reactor cattle in the herd. In a similar study conducted in interface areas of the Kafue Basin GMAs it was observed that TB positivity was influenced by the proximity to the lechwe antelopes (Cook *et al.*, 1996), although this potential association merits further investigation due to the unavailability of more detailed information especially in relation to the risks posed by infected lechwe. However, from this study, it was apparent that the mere movement of animals into the plains was more likely to bring them into contact with wild animals ($\chi^2 = 68.28$; $p = 0.000$).

On univariate analysis, herd size was found to be an important risk factor for the occurrence of TB in cattle. Our results agree with recent studies conducted in Africa and elsewhere in the world that were able to demonstrate that herd size influenced the occurrence of TB (Amen *et al.*, 2003, Asseged *et al.*, 2000, Cook *et al.*, 1996, and Kazwala 1996). However, Shirima *et al.*, (2003) demonstrated that herd size was not related to the distribution of bovine and avian reactors in any management system. Nevertheless, Griffin *et al.*, (1996), observed in their study in Ireland that herd size was an important risk factor for the occurrence of TB in the herds that they studied. Our findings and those of other authors may be due to the fact that the risk of an individual animal introducing tuberculosis infection into a negative herd may increase with increased herd size, and the lateral spread of infection within the herd may also be favoured. From our hypothesized causal diagram that was generated from the multiple logistic regression model it is clear that transhumance and interface herds are a function of increased herd size (increased herd size demands more grazing area), which entails animals moving into the plains and raising the risk of wildlife contact, hence the

increased chances of exposure to sources of TB. From the biology of infectious disease causation, it is likely that the probability of tuberculosis occurring in a herd increases as a function of increase in size (that is, the more animals that are at risk, the greater the likelihood that at least one of them will acquire infection).

On univariate analysis, the overall results showed that age of an animal was found not to be related to the distribution of bovine reactors. But this was in contrast to the observation made by Cook *et al.*, (1996) and Asseged *et al.*, (2000) that the risk of a positive result also increased as age increased, up to 6 years and thereafter diminished. Our findings in this study deviate from the findings by Cook *et al.*, (1996) and Asseged *et al.*, (2000) due to different reasons. Our findings on age may be due to the low prevalence of the disease in our study. Additionally, differences in the sample sizes from our 3 sampling areas coupled with marked age differences from each of the study strata may have had an effect on the final trend of the results. However, in our study, at area level, it was observed that Blue Lagoon which had older animals (mean age range = 5 to 7 years), had a higher prevalence than both Kazungula (mean age range = 3.5 to 7 years) and Lochinvar (mean age range = 3 to 5 years). Further still, the results of Blue lagoon when analyzed independently of the other two areas, age was found to be associated with a positive TB reactor confirming Cook *et al.*, (1996) 's findings. However, the overall results from the 3 areas indicate a lack of association between TB positivity and age. Radostitis *et al.*, (1994), explained that as animals get older, the chance of being infected is higher because of sufficient exposure time. However, in areas where the active disease is high this exposure time may be shortened thereby negating the age factor as the variable of importance in herd TB positivity. Further more, in our study, the overall

results included an area of very low TB prevalence (Kazungula District with 0.57%) coupled with Blue lagoon which had a prevalence of 7.34% as the highest. The marked differences obtaining in the three study areas may have had an overall unknown bias which may have reduced any difference in age related prevalence. The basic study design itself clearly restricted sampling of animals to those that were at least above 12 months old, thus foregoing calves which are deemed to be less likely positive at tuberculin tests and this finding ties up with what Griffin *et al.*, (1996) found. Studies conducted by other scientists show that it takes apparently 30 to 50 days for the immune response of cattle to react to TB infection (Francis 1947). Thus, those young animals that were exposed to similar challenges as adult animals may have had an equal chance of reacting to the tuberculin test more so taking into account the inclusion criteria that was used for this study. In the same vein, a close look at our results of age against TB prevalence, when age is taken as a categorical factor, it was observed that the prevalence varied according to the different age ranges. Decrease in prevalence was seen in animals between the age ranges of 60 to 84 months old, but a significant rise was seen in those above 84 months old. This particular finding is almost as close to what Asseged *et al.*, (2000) found in their studies.

The overall results from our study showed that sex of an animal was not a predilection for TB positivity ($P= 0.7103$). We could not demonstrate sex as having an effect on TB positivity as did Kazwala *et al.*, (2001) who in their study were able to demonstrate that there were significant differences between male and female cattle ($P<0.05$). This disagreement may be due to the female to male ratios observed in our study. In Kazungula District, males only accounted for 18% of the animals sampled while the

highest percentage of males sampled was in Blue lagoon but still this only accounted for 32%, while female animals ranged from 68% to 82% of the sampled animals in all the regions. This paucity in the number of male animals may have had unknown bias and a contributory effect on the overall bearing of the results. However, our findings are in agreement with Coleman (1989) who found no overall significant difference in the prevalence of infection between males and females. Despite our male to female ratio imbalance in this study, based on the biology of infectious diseases, these results suggest that the disease process operates similarly in either male or female. Differences in prevalence according to sex would have been significant if either sex were exposed to different risk factors for TB infection and development. However, in this study, both sexes were exposed to the same risk factors for infection and development of the disease hence the likelihood of our present findings.

On multiple logistic regression analysis, only two factors came out to be significantly related with TB positivity namely herd size and grazing systems. From the receiver operated curve (ROC) analysis, the area under the curve was 0.8688 which showed the greater discriminatory power of the test. This means that our model too predicts the outcome of the involved variables in TB positivity 86.88% correct. The use of ROC curves for evaluation of diagnostic tests has been discussed and advocated by numerous authors including Erdreich and Lee (1981), Swets (1988), and Fletcher, *et al.* (1988). In our study, the ROC analysis has been able to clearly demonstrate that TB positivity in *the studied areas was directly related to the type of grazing strategy and the size of the herd*. Thus the epidemiological performance characteristics of our MCT test was 86.9%.

5.3 Observations from abattoir survey

From this study, TB related condemnations accounted for 13% of all organs condemned on meat inspection, although on culture growth, only 6.62% of the organs were found to be BTB positive. When the areas of origin of animals were taken into consideration, the Kafue Basin had the highest condemnations. Districts that were outside the interface areas, as well as outside the Kafue Basin, were observed to have relatively lower organ condemnation rates of 6.52% and 4.35% for Mazabuka and Choma, respectively. It can thus be inferred that area variation in TB prevalence could be as a result of cattle which is reared in different areas that is likely to be exposed to different risk factors for TB infection.

Other studies done at the same abattoir with animals coming from almost the same Districts, by Pandey and Sharma, (1987), found a prevalence of bovine tuberculosis of 7.46%. However, a study conducted by Sitima (1996) exclusively in the Namwala district found a prevalence of bovine tuberculosis of 12.8%. This figure is still higher than most figures of TB in other areas of Zambia. Cook et al., (1996) found a TB prevalence of cattle surveyed in the Monze district of Zambia to be at 7.4% which is lower than that of Namwala District. Current studies including ours are still consistent with earlier reports of cattle TB in Zambia. The Veterinary Department abattoir records of as early as 1957 showed that 1.6% of all the cattle slaughtered cattle from Lusaka had tuberculosis lesions; 2% from Livingstone; 5.2% from Mazabuka and 16.8% were coming from Namwala District (Anonymous 1957).

In this study, tuberculoid and tuberculosis lesions were most often detected in the lungs and accessory lymph nodes (78/89; 87.64%). This high frequency of lung condemnation confirms the high incidence of the pulmonary form of this disease. This agrees with what Cadmus *et al.*, (2004) and Radostitis *et al.*, (1994) found. Our findings suggest that the major route of TB transmission is through the respiratory tract by inhalation of infectious aerosols. Similar observations were made by Gallagher (1998) and Grange and Yates (1994), who reported that tuberculosis in cattle was principally a pulmonary disease and that cattle excrete the tubercle bacilli by the aerogenous route with only 1% of the excretion being through milk. However, this is in contrast with Dvorska *et al.*, (2004) who in their abattoir survey in Czech Republic found a low frequency of tuberculosis lesions in the lungs. The observed differences in the predilection site of the infectious agents may be due to a number of factors ranging from environmental, type of TB strain, management system and the general health of the animals.

The major weakness with abattoir studies is the sampling bias that is difficult to control by the researcher. A lot of male animals were slaughtered than female animals. Although age was not easy to obtain, most of the animals brought for slaughter were quite old. Further still, some districts supplied more animals than others hence the differences in the proportions per district of study. However, the abattoir study clearly supported the tuberculin results in suggesting the existence of a focus of tuberculosis infection in the Kafue Basin area as evidenced by the ever high TB condemnation rates from Namwala District through out the years.

5.4 Tuberculosis survey in the wildlife

In Zambia, one of the earliest reports of TB in lechwe was in 1956 when a veterinarian reported of sick lechwe on the Kafue flats near Lochinvar (Veterinary Department Records, Northern Rhodesia 1957). Extensive tuberculosis lesions were found in this sick lechwe. In 1967 small scale cropping of lechwe at Lochinvar revealed a 14% incidence of tuberculosis. In 1969 an abattoir was built in Lochinvar. This meant that all hunter harvested animals had to be taken to central place for screening. As a result of this exercise, in 1972 lesions of tuberculosis were found in 36% of the hunter-harvested lechwe screened at this abattoir. However, no culture was done to ascertain which species of mycobacterium were involved.. Parallel studies in cattle during similar periods (Anon. 1969), noted that the incidence of TB in Zambia coincided closely with the locality of the lechwe population and that lechwe were capable of acting as carriers of the disease and findings were later confirmed by Cook *et al.*, 1996, Sitima 1997, and Pandey *et al.*, 1998. In contrast to the Kafue lechwe, no TB cases were diagnosed in the Black lechwe (*Kobus leche sithemani*) despite inhabiting similar environment, (Anon. 1965). Grimsdell and Bell (1975) concluded that TB was not a problem with the Black lechwe because of the absence of contact with cattle. This tie up with what Bakunzi *et al.*, (1995) found in Molopo South Africa. In their study they recorded a prevalence of bovine TB of 0.062%. They attributed this finding to the absence of mingling of wildlife with cattle. The lack of reports of TB in the Black lechwe, prompted Dillmann, (1976) to suggest that tuberculosis may have been introduced into the kafue lechwe through contact with cattle. This in turn resulted in the kafue lechwe becoming reservoir of TB infection. Our studies, confirms this finding. In this study, the effect of contact with

wildlife and TB positivity in the cattle population was clearly elucidated in univariate analysis of risk factors in cattle herds. Notably, cattle grazing in the interface areas (Interface herds/IFH) increased the odds of herd tuberculosis positivity by approximately 18 times compared to those grazing within villages (Village Resident herds/VRH) - (Odds ratio = 18.67; CI at 95%, = 4.3-81.0); $p = 0.000$). Whereas herds that practiced transhumance (TH) were only approximately twice more likely to test positive for TB than VRH (Odds ratio = 2.0; at 95%CI = 0.6 – 5.9); $p = 0.210$). These results indicate a positive correlation of having a TB positive animal with reduced proximity to the lechwe grazing areas. IFH are permanently in constant interaction with lechwe grazing grounds and have been found to be herds that have higher TB prevalence values than other grazing systems. Findings from this study suggest that contact with wild animals, mainly the lechwe, increased the risk of a herd being positive {i.e. from the results, contact with wildlife was seen to have a significant effect on finding a herd positive on tuberculinisation ($\chi^2 = 10.21$; $p = 0.0014$)}. Marrying cattle findings with the observed levels of tuberculosis in the lechwe of 6 (43 %) out of 14 lechwe may seem that the disease comes from areas of high concentration to low concentration. That is more likely from lechwe to cattle than the other way round. The fact that of all the wildlife species screened, only the lechwe was positive, is evidence enough to deduce that it is susceptible species to the disease. However the buffalo is equally susceptible but the existence of the lechwe in large groups standing at a total of 42,119, with huge herds of close to 2,000 animals in the Kafue basin may assist in the spread of the disease quite easily (Kamweneshe 2002). Other animals that were screened were found in small groups in the park compared to the lechwe. Behavioral interactions also facilitate wildlife/livestock disease transmission and may explain, in part, differences in disease

levels in different wildlife hosts. From our own observations, of all the wildlife species found in the Kafue Basin, the lechwe easily graze close to livestock (*personal com. Lochinvar community 2003/04*). The high prevalence of the disease in the lechwe could be due to overcrowding on shrinking grazing land due to the *Mimosa pigra* weed and the human-livestock encroachment. Concentration of animals at watering points has been shown to favour TB transmission (Andrews & Johnston, 1988). Although the numbers of lechwe sampled was low, the findings in this study are consistent with findings in other studies (Cook *et al.*, 1996, Gallagher *et al.*, 1972, Stafford 1991). Other factors which might influence TB transmission among the lechwe may be the social herd structure as these animals are found in huge herds making it easy for disease transmission as TB is contagious. Other related factors such as behavioural patterns, diminishing grazing grounds due to human and livestock encroachment all promote overcrowding and inevitably increasing the risk of TB transmission. Another factor that easily promotes infection is the susceptibility of the host to the agent. The presence of TB in the lechwe may mean the existence of a permanent and untreatable reservoir host of TB. Since the levels of livestock and wildlife interaction in the Kafue basin is high, cattle from this area may serve as a link in the transmission of TB infection to other herds. Those cattle herds without contact with wildlife but come in contact with these cattle from the Kafue Basin and also those enroute to abattoirs may become infected without primary contact with wildlife. Similar observations have been made in England and Ireland where a link was found between reinfection of cattle herds and the presence of infected colonies of badgers (*Meles meles*), as well as in New Zealand where the brushtail possums (*Trichosurus vulpecula*) were found to play a role in the epidemiology of TB in cattle (Chessman *et al.*, (1989) and Morris & Pfeiffer (1994).

5.5 Risk factors associated with tuberculosis in humans

An important finding of the study with regards to risk factors associated with TB in human communities was that there was no statistical association between keeping cattle and having a known human TB case in the preceding 12 months ($\chi^2 = 0.047$; $df = 1$; $p = 0.83$). These results agree with recent studies on the relationship between cattle TB and their owners by Ameni *et al.*, (2003) who in their study in Ethiopia found no statistical association between reactor cattle in the herd and human tuberculosis patients in the households.

Another striking feature of the TB cases is the average age range of confirmed human cases under treatment. It was found that the most affected age group from the questionnaire survey was between 21 to 30 years which is the most productive age group followed by ages of between 31 to 40. In a similar vein, patients that participated in the provision of sputum samples, had a mean age of 37 years (95% CI= 33 to 41 years). This is similar to Ameni *et al.*, (2003) in Ethiopia, who found that the most affected age range with TB in humans ranged from 16 to 45 years, a result which agrees with our findings. These results suggest an involvement of a dual infection with HIV as another more threatening risk factor. This alliance between TB and HIV is one of the most lethal combinations by disease known to mankind, each multiplying the impact of the other. TB generally results in an increase of HIV replication and elevated viral load (Berker *et al.*, 2000), whereas patients infected with HIV are at increased risk of reactivation and primary TB (Wood *et al.*, 2000). Approximately 50% of HIV seropositive individuals are co-infected with TB in the developing world with TB leading the cause of death in

AIDS patients (Gazzard; 2001). Since the acquisition of infection is often far removed from development of the disease and involves different physiologic mechanisms, the risk factors for infection are quite different from the risk factors for development of the disease following infection (Comstock G.W., 1975). Any condition modifying the balance established in the body between the tubercle bacilli and the host's immune defenses can have an impact on the risk of developing the disease, particularly so with HIV in humans. Similarly in humans and other animals, any factor influencing the risk of infection and or the risk of breakdown after infection has a possible effect on the incidence of tuberculosis. (Lienhardt. 2001).

The risks of getting TB through milk were assessed. In all the three study areas, the majority of the respondents (74.7%) indicated that milk was boiled before consumption. However, 25.3% of the respondents indicated consuming milk unboiled and preferred it directly from cows. All the households that were interviewed were found to process sour milk from unboiled milk. Walshe *et al.*, (1991) stated that the souring process of milk destroys *M. bovis*. If this assertion is true, then the chances of getting TB through sour milk is reduced. However, similar studies done by Barrera & de Kantor 1987 and Sitima 1997 strongly contradict this statement. Even the reports from the World Health Organization (WHO) of 1994 working group on zoonotic tuberculosis, showed that *M. bovis* was isolated from seven (2.9%) of 241 samples of sour milk in Ethiopia. Sitima (1997) further found out that traditional ways of processing sour milk in Zambia does not eliminate *M. bovis* even at the time of consumption. This indicates another potentially public health hazard of contracting the disease from sour milk as it is processed without heat treatment. However, the risk of transmission of *M. bovis* to

humans through the consumption of contaminated milk and eventual establishment of infection depends on the right permutation of numerous factors. Foremost, *M. bovis* must be present in the udder of the cow at the time of milking. This occurs only when the disease is at an advanced stage and has disseminated throughout the animals body. However, this is probably a rare occurrence even where tuberculosis is still present. As evidenced in our abattoir survey, none of the cows had TB lesions on the udders. Even at the time when TB was prevalent in European dairy cows, only 1% of udders of slaughtered tuberculous animals had visceral lesions (O'Reilly and Darbon 1995). In another study by Kazwala *et al.*, (1998) in Tanzania, of the 850 milk samples, *Mycobacterium bovis* was isolated and confirmed in only 2 milk specimens representing less than 1% of the cows as able to excrete the tubercle through milk. Secondly, infection with *M. bovis* bacteria is dose dependant and generally a large inoculum is required to establish infection (O'Reilly and Darborn 1995). Usually, a smaller infecting dose of tubercle bacilli often results in a protective immunity which in itself is a function of a variety of the body's physiological condition. Finally, the establishment of infection in the consumers depends on their immune status.

Apart from the consumption patterns of sour milk, the poor understanding of tuberculosis in cattle was another risk that was noted. Only 60.9% of the respondents knew that cattle can transmit tuberculosis to humans while 39.1% had no knowledge about the possibility of cattle transmitting TB. Although the majority of the respondents knew of the need to have their children immunized with BCG against tuberculosis, 6.9% of the respondents had no knowledge and had never heard of BCG vaccination due to remoteness of some of the rural areas where the study was conducted.

5.6 Molecular Typing of Animal and Human isolates

A total of 16 different genotypes were obtained from 61 isolates of cattle ($n = 31$) and humans ($n = 30$). Of these, 10 genotypes were from cattle while 6 from humans. Despite the high degree of homogeneity observed in the spoligotypes for both humans and cattle, in each of the species, some cluster of spoligotypes were dominant and these strains were found in most of the study areas. Similar findings like those of our study were made by Valway *et al.*, (1998), who explained that such clustering of dominant strains may be due to the strain's exceptional virulence. According to Kamerbeek *et al.*, (1997), the *M. bovis* isolates are usually characterized (for all known isolates) by the lack of the five 3' end spacers of the spoligotyping pattern, while *M. tuberculosis* isolates typically show an absence of spacers 33 to 36. One of the key observations from our present study was that all the cattle isolates were *M. bovis* while all the human isolates were *M. tuberculosis*. Both species of *Mycobacterium* showed host species specificity and adaptability. Thus these results suggest lack of evidence to support the notion of interspecies transmission of TB between animals and humans. These results however, differ with those of Kazwala *et al.*, (2001) who were able to indicate that 16% of the isolates were *M. bovis* from the humans found in pastoralist communities of Northern and Southern Tanzania. The diagnostic methods used in our study differ with those employed in Tanzania. Whereas we used sputum, they used biopsy samples from cervical lymph nodes. Further still, our small sample size has no statistical power to discount *M. bovis* as the agent for human TB as well as *M. tuberculosis* as an agent for animal TB. It is thus imperative that our results must be interpreted with great caution taking into consideration the various factors that would have lead to such findings. All

the human patients who were incorporated in the study had to be handled by qualified health personal at the clinics near the study areas following our criteria, but a number of factors were identified which would have had an effect on the overall bearing of the results. Mainly, due to the great distance that the TB patients had to walk to the clinics, poor adherence to medication and loss of follow-up were common. Such lapses may even mean those patients who were previously cured of TB which may have been caused by *M. bovis* strains may come back to the clinics with more resistant *M. tuberculosis* due to lack of adherence to the treatment protocol. Another aspect to consider may be the predilection site for *M. bovis* infection in humans may be different. Kazwala et al., (2001) found that significantly higher isolation rates ($P < 0.05$) of *M. bovis* strains recovered from humans were extra-pulmonary (26.8%) than pulmonary tuberculosis samples (4.3%). However, all our samples were from pulmonary origin which may explain our present results, coupled with a small sample size. Similarly, Anderson and May (1991) explained that due to species adaptability of infectious agents, there is a probability that *M. tuberculosis* in humans may have an advantage over *M. bovis* in its ability to be transmitted and cause disease. With the advent of HIV in human communities, reactivation of the dormant tubercles is likely to occur looking at the average age of the patients in the study. With the high unsatisfactory compliance to TB treatment exhibited by the people in the study areas, the likelihood of the development of drug resistant mycobacterial strains is high. Furthermore, HIV positive individuals contracting MDR-TB experience a much more rapid progression from diagnosis to death, with a known case-fatality rate of approximately 80% (Bloom & Murray, 1992). Regardless of a stringent selection criteria, it was virtually impossible for

the medical personal to screen only non-HIV free patients due to ethical considerations to which the study and the officials are bound.

With regards finding only *M. bovis* in the cattle population, it is a clear indication of how well adapted *M. bovis* is in cattle. It can be inferred from these genotyping results that *M. bovis* is “animal specific” while *M. tuberculosis* is “human specific” although other studies have been able to allude to the infection of man by *M. bovis* (Collins & Grange 1987 and O'Reilly & Daborn 1995). However, our present study was unable to confirm the relationship between interspecies transmission of TB between animals and humans. Usually according to the biology of infectious diseases, infection is established when those strains of an infectious agent are virulent enough to establish infection. If the type of *M. bovis* strains in the study areas were not virulent enough to infect humans, then there is a slim chance of getting positive results from such a small sample size. Therefore to enhance the plausibility of such findings, further studies are required to obtain more information.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

The main thrust of this study was to generate information on the levels of tuberculosis with regards to the association that exists between the disease in animals and humans in the purported high risk areas of the Kafue Basin of Zambia, more so the genetic relatedness of the strains. This is the first comprehensive study of its kind employing among other methods the molecular typing techniques to assess possible risk factors and the hypothesized causal relationship between animal (zoonotic tuberculosis) and human tuberculosis. Determining the prevalence levels of tuberculosis in livestock, humans and wildlife involves several approaches with important practical implications. Observational studies can detect disease and estimate prevalence and other parameters reasonably easily. However, tuberculosis is a disease with multiple hosts and the objectives of this study could only be adequately answered by the application of conventional observational studies with the combination of modeling and the application of molecular epidemiology. Observational studies have shown that TB infection in the Kafue Basin, especially in the lechwe can be highly prevalent. The level of infection varied across host species under study from an overall 5.50% in cattle to 43% in lechwe. Observational studies further demonstrated that the prevalence of cattle TB reduced with increasing proximity from the lechwe grazing areas. Modeling of the variables for TB prevalence showed that herd size and grazing systems through multivariable logistic regression were the dominant and most critical risk factors for herd TB positivity. Both variables are predictors for animal movement into the plains and consequent wild life interaction. From the model, it is clear that if TB was to be controlled in cattle of the

interface areas, then factors to be strictly considered will be the reduction in herd sizes and the changes in the type of grazing strategies.

It was anticipated that the study of strains from animal and human sources might help uncover associations that have been purported to occur between animal and human tuberculosis. In addition, the associations between genotype and host specificity was clearly elaborated through spoligotyping. Our results are broadly consistent with those previous reports which suggest that tuberculosis strains are more host specific adapted though this is cautiously concluded in this study considering the low number of strains used. Therefore a study employing genotyping should in future consider the use of large sample sizes.

Conclusively, the tuberculosis 'hotspot' of the Kafue Basin was associated with high prevalence values than the control area of Kazungula District, supporting the notion of an existence of a focus of TB infection in the animals of the Kafue Basin. No statistical association was found between having a human TB case and households that had TB positive reactor cattle on tuberculinisation. Strikingly, all animal DNA strains were found to be *Mycobacterium bovis* while all human strains were *Mycobacterium tuberculosis*. From the descriptive results obtained on the disease, it is quite clear that though individual animal prevalence may seem low in some areas, herd prevalence values have been relatively high in all the areas studied more so in the Kafue Basin.

Of extreme concern has been the capacity of the study, to show another potentially dangerous scenario of which the lechwe (wild animals) were found to harbor the disease

at relatively high prevalence. Wildlife tuberculosis doesn't only pose a threat to livestock and humans living in the interface areas of these National Parks, but the threat of spillover effect and "a break in species barrier" is a high possibility given the wide host range nature of the disease. The Buffalos which are so closely related to cattle may be considered the species in which a threat in the waiting is just at the door step. Insights in the disease among the lechwe population as the wild animal host suggests the existence of a non-treatable reservoir host of infection.

This study was able to show that in the Kafue Basin areas, close co-existence of domestic and wild animals exist. The lack of clear boundaries, fences and restrictions, have meant the owners of domestic animals extending their grazing fields deep into the wildlife sanctuary. Humans herding these animals spend almost all their time tending these animals in the vast plains. This also was seen to pose another threat to humans if animals were tuberculous or vice versa. Both the herdsman and the animals may serve as a route of transmission of TB from deep in the plains to the villages, as they always come in close proximity with wildlife once in the plains. This wildlife-livestock interface is therefore of crucial importance in the control and management of TB in the Kafue Basin especially in areas bordered by human communities.

Within the cattle rearing human communities, the preference of unpasteurized milk or a lack of access to pasteurized milk was frequently encountered. This further worsened the risk posed by *M. bovis* in milk and milk products.

The age ranges at which patients were receiving TB treatment may be linked to the soaring HIV/AIDS epidemic. The widespread immune suppression caused by HIV further increases the risk of developing clinical TB after such an individual is exposed to “open” cases of tuberculosis.

Overall, the results of our study suggest an existence of increased exposure to tuberculosis in the livestock of the Kafue Basin owing to either the high prevalence of the disease in lechwe or the existence of high levels of the disease within the cattle populations. Factors contributing to the optimal transmission of TB in cattle of the interface areas have been identified. It also appears that no real association exists between human TB and coming from a pastoralist community, although it is commonly thought that the population at high risk of contracting zoonotic TB are pastoralist ones. However, zoonotic TB does exist and these results are just but part of the findings that may need further investigations. It is also important to note from the results, that in human TB cases, a serious risk factor is at play fuelling TB cases at such age groups.

Recommendations

The only way to increase sensitization in countries such as Zambia where the compliance levels to most rules and laws are extremely low, is to institute measures that will have profound effects on the would be offenders. In terms of TB control in livestock, all animals that will be bought by abattoirs, butcheries and other traders should be requested to have had passed a tuberculin test within a 12 month period prior to the date of sale. Such a measure will reduce on the possibility of TB infection from

such sources either to abattoir workers or to the consumers. In the same vein, farmers will be forced to eliminate TB positive animals and thus develop clean herds. Similarly, all milk traders should be ordered to buy milk from herds with certified free TB status.

It is clear from the model that TB cases were high in Interface Herds. Thus the most important practical issues to consider in controlling this disease in these areas will be reducing the contact that these livestock have with the lechwe. This can be done by first reducing the herd sizes to manageable sizes that can be maintained within villages and those which are relatively larger, can be grazed within the transhumance system without need of being permanently stationed in the plains.

On the wildlife aspect specifically the lechwe, the control of TB was seen to increase with age. So to reduce active disease, a law can be passed to increase on the harvest of adult lechwe. This law should be accompanied by another which should compel all hunter harvested lechwe to be screened at an abattoir which should also be set in the lechwe habiting areas of the Kafue Basin. Alternatively, the lechwe grazing areas have to be extended by extending the present park boundaries to most of the fertile lands into the plains where GMAs extend but without human settlements.

Direction for further research in zoonotic tuberculosis

Although the study provided adequate insights of disease status in all the studied hosts and areas of concerns, while keeping the scope of study within its limits, it is clear that TB cases in livestock could still be found in areas without lechwe populations albeit at low levels. At such lower prevalence in areas outside the Kafue Basin, large sample

sizes will be required to adequately detect the disease and to have enough statistical power to make inferences. Further studies will need to be conducted at a much wider and larger scale to confidently ascertain if the geographical pattern of tuberculosis disease in Zambia's livestock is multi-focal or concentrically located within the Kafue basin.

The investigation in the zoonotic nature of *M. bovis* could be refined to include the use of cervical lymph node biopsy studies in the human population coupled with an increased sample size.

There is additional work to be completed on the model with respect to human tuberculosis although the model has provided useful insights in terms of what control strategies to adopt in cattle TB control.

Once these areas have been addressed, then there is scope to draw strong scientific conclusions on the relationship of human and animal tuberculosis.

References

- 1 **Acha P. N. and Szyfres B** (1987). Zoonotic tuberculosis. In: Zoonoses and communicable diseases common to man and animals. 2nd edition. Washington: Pan American Health Organization/World Health Organization: Scientific Publication No 503.
- 2 **Ameni G., Regassa A., Kassa T., Medlina G.,** (2001). Survey on bovine tuberculosis in cattle and its public health implications to cattle raising families in Wolaita Soddo, Southern Ethiopia. *Ethiopian Journal of Animal Production* Vol1, pp55-62.
- 3 **Ameni G., Ameni K. and Tibbo M.** (2003). Bovine tuberculosis: Prevalence and Risk Factor Assessment in Cattle and Cattle Owners in Wuchale Jida District, Central Ethiopia, *The Journal of Applied Research in Veterinary Med.* Vol 1, pp1-4.
- 4 **Andrews, L.G., and Johnston, J.H.** (1988). Epidemiology of bovine tuberculosis in Northern Australia. *Acta Veterinaria Scandinavica* Supplement 84, pp139-141.
- 5 **Anonymous** (1913/14). Tuberculosis disease in Rhodesia and other African territories. Annual Report for the year 1913/14.
- 6 **Anonymous** (1948). Annual Report of the Department of Veterinary and Tsetse Control Services. Ministry of Agriculture and Rural development, Rhodesia, 32pp.
- 7 **Anonymous** (1957). Report to the government of the Federation of Rhodesia and Nyasaland on the control of parasite disease in Livestock. 47pp.
- 8 **Anonymous** (2002). Tuberculosis. In: Standard Treatment Guidelines, Essential Medicines List and Essential Laboratory Supplies List for Zambia 1st Ed (CBOH), pp 43-50.

- 9 **Aranaz A., Liébena E., Mateos A., Dominguez L., and Cousins D., (1998)** Restriction fragment length polymorphism (RFLP) and Spacer oligonucleotide typing ("Spoligotyping"): a comparative analysis of fingerprinting strategies for *Mycobacterium bovis*. Journal of Vet Microbiology Vol. 61 pp311-324.

- 10 **Aranaz A., E. Liébana, A. Mateos, L. Dominguez, D. Vidal, M. Domingo, O. Gonzalez, E.F. Rodriguez-ferri, A.E. Bunschoten, J.D.A. van Embden and D. Cousins (1996);** Spacer Oligonucleotide typing of *Mycobacterium bovis* strain from cattle and other animals: a tool for studying epidemiology of tuberculosis. J. Clin Microbiol vol 34 pp 2734-2740.

- 11 **Asseged, B., Lubke-Becker, A., Lemma, E., Taddele, K., Britton, S., (2000).** Bovine Tuberculosis: A cross sectional and epidemiological study in and around Addis Ababa. Bull Animal Health Prod Africa Vol 48; pp71-80.

- 12 **Bakunzi, F. R., Zyambo, G.C.N and Morris S. (1995).** Bovine Tuberculosis Survey in Molopo District of the North West Province. South Africa Veterinary Journal 66 (1) pp 28-29.

- 13 **Barrera L., de Kantor IN. (1987)** Nontuberculous mycobacteria and *Mycobacterium bovis* as a cause of human disease in Argentina. Tropical and Geographical Medicine; 39 pp 222-227.

- 14 **Baldwin, J.H. (1968):** Pulmonary bovine tuberculosis an owner and his dairy herd. Cornell Veterinarian vol 58 pp 81-87.

- 15 **Blancou, J., Rorhbach, C., Perdrix, A., Choquel, P., Rosner, G., (1971).** Bovine tuberculosis in Madagascar. Rev. Elev. Med. Pays Trop. 24 (4) pp 505-517.

- 16 **Bloom, B. R. & C. J. L. Murray** (1992): Tuberculosis: commentary on a re-emergent killer. *Science* 257, pp1055-64
- 17 **Bouvet E., Casalino E., Mendoza-Sassi G., Lariven S., Vallee E., & Pernet M.,** (1993) A nosocomial outbreak of multidrug-resistant *Mycobacterium bovis* among HIV-infected patients. A case control study. *AIDS*; 7; pp 1453-1460.
- 18 **Buve, A. and Foster, S.D** (1993): HIV infection in a rural district in Zambia: prevalence and Socio-economic factors associated with seropositivity. Poster no. PO-D28-4231, IXth international conference on AIDS. Berlin 6-11 June 1993.
- 19 **Cadmus, S.I.B., Astanda, N.N., Oni, S.O., Akang, E.E.U.,** (2004). Bovine Tuberculosis in one Cattle Herd in Ibadan in Nigeria. *Vet Med-Czech*49 (11) pp406-412.
- 20 **Canetti, G., N. Rist, and J. Grosse** (1963). Mesure de la sensibilite du bacille tuberculeux aux drogues antibacillaires pour la methode des proportions. *Tubercle* 27: pp 217-272.
- 21 **Carlton, W. and McGavin, M.D.** (1995). Thomson's Special Veterinary Pathology. Mosby St. Louis, USA, pp 63-64 & 152.
- 22 **Chessman C.L., Wilesmith J.W and Stuart F.A.** (1989). Tuberculosis-the disease and its epidemiology in the badger: a review. *Epidemiology and infection* 103: pp113-125.
- 23 **Clifton-Hadley, R. S., Wilesmith, J. W., & Stuart, F. A.** (1993). *Mycobacterium bovis* in the European badger (*Meles meles*): Epidemiological findings in tuberculous badgers from a naturally infected population. *Epidemiology and Infection*, 111(9), pp 9-19.

- 24 **Coleman, J. D.** (1989). Distribution, prevalence, and epidemiology of bovine tuberculosis in Brushtail possums, *Trichosurus vulpecula*, in the Hohonu Range, New Zealand. *Australian Wildlife Research*, 15, pp 651-663.
- 25 **Collins C.H., Grange J.M** (1983): A review. The bovine tubercle bacillus. *Journal of Applied Bacteriology*. Vol 55 pp 13-29.
- 26 **Collins, D.M and Stephens, D.M.,** (1991): Identification of insertion sequence IS1081 in the *Mycobacterium bovis* FEMS Lett. 83 pp 11-16.
- 27 **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. *Veterinary Record*, 134, pp 681-682.
- 28 **Comstock, G.W.** (1975). Frost revisited: the modern epidemiology of tuberculosis. *Am J Epidemiol*. 5. pp 363-368.
- 29 **Cook A.J.C., Tuchili L.M, Buve A, Foster SD, Godfrey-Faussett p, Pandey GS, McAdam K.P.W.J.** (1996) Human and bovine tuberculosis in the Monze district of Zambia-a cross-sectional study. *Br Vet J*; V 152 pp37-46.
- 30 **Cosivi O, Grange J.M, Daborn C.J Raviglione M.C, Fujikura T, Cousins D, Robinson R.A, Huchzermeyer H.F.A.K, de Kantor and Meslin F-X.** (1998) Zoonotic Tuberculosis due to *Mycobacterium bovis* in developing countries. *J Emerging Infectious Diseases* vol. 4 No 1 pp 59-70.
- 31 **Cosivi O, Meslin F-X, Daborn C.J, Grange J.M.** (1995) The epidemiology of *Mycobacterium bovis* infection in animals and humans, with particular reference to Africa. *Scientific and Technical Review*; 14:pp733-746.

- 32 **Cousins D.V., Skuce R.A., Kazwala R.R., and van Embden J.D.A.,** (1998) Towards standardised approach to DNA fingerprinting of *Mycobacterium bovis* Int. J. Tuberc. Lung Dis Vol. 2 pp471-478.
- 33 **Cousins, D.V., Francis B.R., Gow. B.L., Collins D.M. McGlashan. C.H., Gregory .A, and Mackenzie.** (1990). Tuberculosis in captive seals: bacteriological studies on the isolate belonging to *Mycobacterium tuberculosis* complex. Res. Vet. Sci. Vol. 48: pp196-200.
- 34 **Daborn, C.J., and Grange, J.M.** (1993). HIV/AIDS and its implications for the control of animal tuberculosis. British Veterinary Journal Vol. 149 pp405-417.
- 35 **Daborn C.J, Grange J.M, Kazwala R.R.** (1997) The bovine tuberculosis cycle-an African perspective. J Appl Bacteriol [Symposium Supplement]; V81: pp27s-32s.
- 36 **Daley C.L., P.M. Small, G.F. Schecter, G.K Schoolnik, R.A. McAdam, W.R Jacobs and P.C Hopewell.** (1992): An outbreak of tuberculosis with accerelated progression among persons infected with the human immunodeficiency virus. An analysis using restriction fragment length polymorphism (RFLP) N. Engl. J. Med vol 326: pp231-235.
- 37 **Dankner, W.M., Waecker N.J., Essey E.M., Mosef K., Thompson M., and Davis C.E.,** (1993). *Mycobacterium bovis* infection in San Diego: a clinicoepidemiological study of 73 patients and a historical review of a forgotten pathogen. Medicine (Baltimore) Vol. 72 pp11-37.
- 38 **Dillmann J.S** (1976) Compiled Final Report. Veterinary Wildlife Research section, Department of Veterinary Sciences, Ministry of Agriculture, Lusaka Zambia. 395 pp.

- 39 **Dohoo, I.R., Martin, W and Stryhn, H.** (2003): Veterinary epidemiologic research. Atlantic Veterinary College Inc., University of Prince Edward Island, Prince Edward Island, Canada, 706pp.
- 40 **Dolin P.J., M.C. Raviglione, and A. Kochi** (1994): Global tuberculosis incidence and mortality during 1990-2000. Bull. W.H.O vol 72 pp 213-220.
- 41 **Donoghue H.D., Fletcher H, Pap I, Spigelman M.** :(2001) Molecular studies of Mycobacterium tuberculosis DNA from naturally mummified 18th century Hungarians. Abstracts 22nd Annual congress of the European Society of Mycobacteriology-Berlin 2001.
- 42 **Dungworth, D.L.,** (1993). The respiratory system. In pathology of domestic animals, Vol. 2ed. K.V.F. Jubb, P.C. Kennedy, and N. Palmer, 4th ed. San Diego: Academic, pp 539-698.
- 43 **Dvorska, L., Maltova, M., Bartos, I., Parmova, I., Bartl, p., Svastova, P., Bull, T.J., Pavlik,** (2004). Study of Mycobacterium avium complex strains isolated from cattle in the Czech Republic between 1996 and 2000. Vet Microbiol. (99) pp239-250.
- 44 **Erdreich, L. S., & Lee, E. L.** (1981). The use of relative operating characteristic analysis in epidemiology. American Journal of Epidemiology, 114(5), pp 649-662.
- 45 **Elasbaban M. S, Lofty O., Awad W M., Soufi H. S., Mikhail D. G and Hermen A. M.,** (1992). Bovine tuberculosis and its extent of spread as a source of infection to man and animals in the Arab Republic of Egypt. In: proceedings of the International Union against Tuberculosis and Lung Disease Conference on Animal Tuberculosis in Africa and the Middle East: April 28-30; Cairo Egypt. Paris: The Union: pp 198-211.

- 46 Elliot, A.M., Halwiindi, B., Hayes, R.J., Luo, N., Tembo, G., Machiels, L., Bem, C., Steenbergen, G., Pobee, J.O.M., & McAdam, P.W.J. (1993). The impact of HIV on presentation and diagnosis of TB in a cohort study in Zambia. *Journal of tropical Medicine and Hygiene*, 96, pp1-11.
- 47 Elliot, A.M., Luo, N., Tembo, G. (1990). Impact of HIV on tuberculosis in Zambia: a cross-sectional study. *British Medical Journal* 301 pp412-415.
- 48 Enarson, D.A and J.F. Murray: (1996) *Global epidemiology of tuberculosis*. Little Brown and Co., Boston, Mass.
- 49 Fletcher, R. H., Fletcher, S. W., & Wagner, E. H. (1988). *Clinical epidemiology - the essentials*. Williams and Wilkins, Baltimore p 457.
- 50 Frothingham, R., Hills H.G., and Wilson K.H., (1994). Extensive DNA sequence conservation throughout the *Mycobacterium*.
- 51 Francis, J. (1947). *Bovine Tuberculosis, including a contrast with Human tuberculosis*. London: Staple press.
- 52 Francis, j. (1950). Control of Infection with Bovine Tubercle Bacillus. *Lancet*, 258, pp34-39.
- 53 Francis J. (1959). The work of the British Royal Commission on Tuberculosis 1901-1911. *Tubercle* 40 pp124-132.
- 54 Gallagher, J., MacAdam, I., Sayer, J. & van Lavieren, L.P. (1972). Pulmonary tuberculosis in free-living Lechwe antelope in Zambia. *Tropical Animal Health and Production* 4 pp204 – 213.

- 55 **Gallagher, J., Monies, R., Gavier-Widen, M., and Rule, B.,** (1998). Role of infected, non-diseased badgers in the pathogenesis of tuberculosis in the badger. *Veterinary Records* Vol 135. pp 82-85.
- 56 **Gazzard, B.,** (2001). Tuberculosis, HIV and the Developing World. *Journal of Clinical Medicine*. Vol1, pp 62-68.
- 57 **Gervois M., Vaillant J. M., Fontain J. F., Laroche G., and Dubois G.** (1972). Epidemiologie de infection humaine par *Mycobacterium bovis*. *Archivio monaldi per la fisiologia e le malattie dell' apparato respiratorio* 27 pp 294-317.
- 58 **Gillespie, J., L. L. Barton, and E. W. Rypka.** (1986). Phenotypic changes in mycobacterium grown in oxygen-limited conditions. *Journal of Med. Microbiol* vol 21 pp251-255.
- 59 **Godfrey-Faussett P, Quigley M, Ayles H, Mwiinga A, Hosp M, Porter J.** (1999) Screening people living with HIV before tuberculosis preventative therapy. *AIDS*. Oct 22; 13(15): pp 2190-2191.
- 60 **Gracey, J. F.** (1992) *Meat Hygiene* 9th ed. Bailliere Tindall W.B Saunders.
- 61 **Grange, J.M., and Yates M.D.,** (1994) Zoonotic aspects of *Mycobacterium bovis* infection. *Vet Microbiol*. 40 pp137-151.
- 62 **Grimsdell J.J and Bell R. H** (1975). Black lechwe research project, final report. Published by National Council for Scientific Research, Lusaka, Zambia, 175 pp.
- 63 **Griffith A.S** (1941). The problem of the virulence of the tubercle bacillus. *Tubercle* vol 22 pp33-39.

- 64 **Gutierrez, M.C., Vincent, D., Albert .J., Bizet, O., Gaillot, L., Lebron, C., Le-pendeven, M.P., Le-Pennec, D. Mathieu,C., Offredo, B., and A.C. Pierre** (1998) Molecular fingerprinting of *Mycobacterium tuberculosis* and risk factors for tuberculosis transmission in Paris, France and surrounding areas. J Clin Microbiol Vol 36 pp486-492.
- 65 **Habib N.I., and F.C.J Warring** (1996). A fatal case of infection due to *Mycobacterium bovis*. Am. Rev. Respir. Dis vol 93 pp 804-810.
- 66 **Holt, G. J., Kreig, N. R., Sneath, P.H., Staley, T. J and Williams, S.T** (1994). Bergey's Manual of Determinative Bacteriology, Baltimore, Williams and Wilkins. Co. Ltd., USA, pp597-603.
- 67 **Houde C, Dery P** (1988). *Mycobacterium bovis* sepsis in an infant with human immunodeficiency virus infection. Padiatric Infect. Dis Journal: vol 7 pp810-812.
- 68 **Idigbe E.O, Anyiwo C.E, Oñwujekwe D.I** (1986). Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. Journal of Tropical Medicine and hygiene; 89 pp 143-148.
- 69 **Idrisu A. and Schnurrenberger P** (1977). Public health significance of bovine tuberculosis in four northern states of Nigeria; a mycobactereiology study. Nigeria Medical Journal vol 7 pp 384-387.
- 70 **Imaeda T.,** (1955) Deoxyribonucleic acid (DNA) relatedness among selected strains of *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium bovis BCG*, *Mycobacterium microti* and *Mycobacterium africanum*. Int. J. Syst. Bacteriol vol pp 147-150.
- 71 **Kataria Y.P.** (1969) observations on human infection with *Mycobacterium bovis*. Tubercle Vol 50 pp14-21.

- 72 **Kamerbeek J., Schouls L., Kolk A., M.van Agterveld. D. van Soolingen., S. Kuijper., A. Bunschoten. H. Molhuizen., R. Shaw., M.Goyal, and J van Embden** (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J clin. Microbiol Vol. 35 pp 907-914.
- 73 **Kamweneshe, B., Beilfuss, R., Simukonda, C.,** (2002). Population and Distribution of Kafue Lechwe and other Large Mammals on the Kafue Flats, Zambia. Report to WWF Partners for Wetlands.
- 74 **Kapungwe, E.M.,** (1993). Management and Utilization of Wildlife resources in Forestry areas of Zambia. Ministry of Environment and Natural resources. Workshop Proceedings. Lusaka.
- 75 **Karlson A.G. and Lessel E.F:** (1970), *Mycobacterium bovis* nom. Nov. International journal of systemic bacteriology vol. 20 pp 273-282.
- 76 **Kazwala, R.R., Daborn, C.J., Kusiluka, L.J.M., Jiwa, S.F.H., Sharp, J.M., Kambarage, D.M.,** (1998). Isolation of *Mycobacterium* species from raw milk of pastoral cattle of Southern Highlands of Tanzania. Tropical Animal Health and Production Journal. Vol 30 No.4, pp 233-239.
- 77 **Kazwala, R.R., Daborn, C., Sharp, J.M., Kambarage, D., Jiwa, S. and Mbembeti,** (2001). Isolation of *Mycobacterium bovis* from human cases of cervical adenitis in Tanzania: a cause for concern? International Journal of Tuberculosis and Lung Diseases, Vol 5, pp 87-91
- 78 **Kleeberg, H. H.** (1975). Tuberculosis and other Mycobacterioses in Disease Transmitted from Animals to Man. 6th Edition. Spring field, Illinois, USA. pp 303 – 360.

- 79 **Kremer, K., van Soolingen .D., Frothingham., R., Haas W.H., Hermans P.W.M., Martin, C., Palittaponyarnpim P., Plikaytis, B.B, W. Riley., L Yakrus, M.A., Musser J.M., & van Embden, J.D.A** (1999): Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: Interlaboratory study of discriminatory power & reproducibility *J. Clin. Microbiol* Vol 37, pp 2607-2618.
- 80 **Kraus H., Rottcher D., Weiss K., Danner K. and Hubschle O. J.,** (1986). Wildlife as a potential source of infection in domestic animals – studies on game in Zambia. *Animal Research and Development* 24: pp 41-58.
- 81 **Lesslie I.W.** (1960). Tuberculosis in attested herds caused by the human type tubercle bacillus. *Veterinary Record* vol 72. pp 218-224.
- 82 **Lesslie I.W., Mayus, K., & Stewart C.J.,** (1972): The prevalence of bovine type tuberculosis infection in man in the English rural population: *Tubercle* 53, pp 198-204.
- 83 **Liébana E.A., Aranaz L., Dominguez A., A. Mateos. O. Gonzalez-Llamazares., E.F. Rodriguez-Ferri., M. Domingo., D. Vidal., and D. Cousins** (1997). The insertion element IS6110 is a useful tool for DNA fingerprinting of *Mycobacterium bovis* isolates from cattle and goats in Spain. *J vet. Microbiol* vol. 54 pp 223-233.
- 84 **Liébana, E., Aranaz A., Francis, B., & Cousins D** (1996) Assessment of genetic markers for differentiation within the *Mycobacterium tuberculosis* complex. *J Clin. Microbiol* vol 34 pp 933-938.
- 85 **Lienhardt C.** (2001). From exposure to disease: the role of environmental factors in susceptibility to and development of tuberculosis, *Epidemiol Rev*, 23: pp 288-301.

- 86 **Marangon, S., Martin, M., Dalla Pozza, M., Neto, F.,** (1998). A case control study on bovine tuberculosis in the Veneto Region (Italy). *Journal of Prev. Vet Med* 34 (2-3): pp87-95.
- 87 **Metcalf, C.,** (1991). "A History of Tuberculosis". In Coovadia, H.M and Benatar, S.R.A. *Centaury of Tuberculosis: South African Perspectives*. Cape Town: Oxford University Press.
- 88 **McCray E., Weinbaum C.M., Baden C.R., Onorato I.M** (1997). The epidemiology of tuberculosis in the United States. *Clin. Chest Med*. Vol 16 pp 99-113.
- 89 **McFadden, J.,** (1992). Mycobacteria. In *Encyclopedia of microbiology*, Vol. 3, ed. J. Lederberg. San Diego: Academic pp 203-215.
- 90 **McKenna, M. T., M. Hutton, G. Cauthen & I. M. Onorato** (1996): The association between occupation and tuberculosis. *Amer J Resp Crit Care Med* 154 pp 587-593
- 91 **Morris, R. S., & Pfeiffer, D. U.** (1994). Directions and issues in bovine tuberculosis epidemiology and control in New Zealand. *New Zealand Veterinary Journal*, 43(7), pp 256-265.p
- 92 **Mposhy M, Binemo-Madi C, Mudakikwa B.** (1983); Influence of bovine tuberculosis on the health of the human population of North kivu (Zaire) *Review Elevage of de Medicine Veterinarie des Tropicaux* 36, 1 pp15-18.
- 93 **Myers, J.A.** (1941) *Man's Greatest Victory over Tuberculosis*. Springfield, Illinois, USA: Charles C Thomas Pubs.

- 94 **Mullis, K.B and Faloona, F.A** (1987). Specific synthesis of DNA *in –vitro* via a polymerase-catalyzed chain reaction. *Methods in Enzymology* 155: pp 335-350.
- 95 **Mwima, H.K.** (1995). Wildlife research and management in Zambia with special reference to some protected areas where wild and domestic animals co-exist. In *Proceedings of the Symposium. “The effects of enlargement of Domestic Animal Pasture on the Wildlife in Zambia”*, pp44-64.
- 96 **Nafeh M. A., Medhart A., Hameed AG., Ahmed Y. A., Rashwan N. M., Strickland G. T.,** (1992) Tuberculosis peritonitis in Egypt: the value of laparoscopy in diagnosis. *Am J Trop Med Hyg* 47 pp 470-477.
- 97 **Nel, E.E** (1981). *Mycobacterium avium* Intracellular Complex Serovars Isolated in South Africa from Humans, Swine and the Environment. *Reviews of Infectious Diseases*, 3 p 1013.
- 98 **Neill, S.D., Hanna, S., O’Brien, J.J., McCracken, R.M.,** (1988). Excretion of *Mycobacterium bovis* by experimentally infected cattle. *Veterinary Records*. Vol 24 pp 340-343.
- 99 **O’Connor, R., Conway, A., Murphy, M.,** (1993). Study of Socio-economic Impediments to Bovine Tuberculosis Eradication. *Eradication of Animal Disease Board, Department of Agriculture, Food and Forestry, Ireland*, pp 102-112.
- 100 **O’Reilly, L. M., & Daborn, C. J.** (1995). The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tubercle and Lung Disease*, 76 (supplement 1), pp 1-39.

- 101 **Pandey G.S. and Sharma R.N.** (1987). Survey of Bovine Pulmonary Diseases at Lusaka Abattoir in Zambia. Bulletin of Animal Health Production in Africa, 35, pp336-338.
- 102 **Pandey, G.S.** (1998). Studies of infectious diseases of Kafue lechwe (*Kobus leche Kafuensis*) with partivular reference to tuberculosis in Zambia. PhD Thesis. Azabu University.
- 103 **Pearson, M.L J.A., Janet, T.R., Frieda, J., Crawford, T., Davis B.J., S.W Dooley & W.R. Jarvis** (1992). Nosocomial Transmission of multidrug resistant *Mycobacterium tuberculosis*. Ann. Intern. Med vol 117 pp 191-196.
- 104 **Pritchard D.G** (1988): A Century of bovine tuberculosis 1888-1988: conquest and controversy. Journal of comparative pathology. Vol 99 p357-397.
- 105 **Radostits, O. M., Blood, D. C., & Gay, C. C.** (1994). Veterinary Medicine, Eighth Edition, Balliere Tindall, London.
- 106 **Raviglione M.C, Snider D.E, Kochi A.** (1995). Global epidemiology of tuberculosis. Journal of American Madical Association Vol. 273 pp 220-226.
- 107 **Rich, A. R.** (1951). The Pathogenesis of Tuberculosis. Charles C. Thomas, Springfield.
- 108 **. Riley, R. L., C. L. Mills, W. Nyka, N. Weinstock, P. B. Storey, L. K. Sultan, M. C. Riley, & W. F. Wells** (1959): Aerial dissemination of pulmonary tuberculosis: a two year study of contagion in a tuberculosis ward. Amer J Hyg 70, 185-96.

- 109 **Rittaco, V., B. López, I.N. De Kantor, L.Barrera, F. Errico, and A. Nader.** (1991) Reciprocal cellular and humoral immune responses in bovine tuberculosis. *Res. Vet. Sci* 50: pp 365-367.
- 110 **Ritacco, v. & de kantor,** (1992). Zoonotic Tuberculosis in Latin America. [Letter; comment] *J. Clin. Microbiol.* Vol 30: pp 3299-300.
- 111 **Rodriguez, L. C., V. K. Diwan & J. G** (1993). Wheeler: Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *Int J Epidemiol* 22, 1154-8
- 112 **Roring S., M. S. Hughes, L. A Beck, R.A Skuce, and S.D Neil.** (1998): Rapid diagnosis and strain differentiation of *Mycobacterium bovis* in radiometric culture by spoligotyping *Vet. Microbio.* Vol 61 pp 71-80.
- 113 **Rothschild B.M. Martin L.D, Lev G, Bercovier H, Bar-gal G.k, Greenblatt C, Donoghue H, Spigelman M, Brittain D** (2001): *Mycobacterium tuberculosis* complex DNA from an extinct bison dated 17,000 years before the present. *Clin. Infect. Dis*; vol 33 pp 305-311.
- 114 **Saiki, R.K., Scharf, S., Faloona, F., Mullis, K.B., Horn, G.T., Erlich, H.A., and Arnheim, N.** (1985) Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. *Science* 230 pp 1350-1354.
- 115 **Saiki, R.K., Gelfand, D.H., Scaffel, S., Scharf, S.J., Higuchi, R., Hoon, G.T., Mullis, K.B., and Erlich,H.A.** (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: pp 487-496.
- 116 **Samper, S., C.Martín, A.Pinedo, A.Rivero,J. Blazquero, D.van Soolingen, and J.D.A van Embden.** (1997). Transmission between HIV-infected patients of

multidrug-resistant tuberculosis caused by *Mycobacterium bovis*. AIDS vol 11 pp 1237-1242.

117 **Sequeira de Latini M.D. Latini O.A., Lopez M.L., Cecconi J.O** (1990) Tuberculosis bovina en seres humanus. A. parte: Periodo 1977-1989. Revista Argentina del torax 1990: 51 pp 13-17.

118 **Schmitt S. M., Fitzgerald S. D., Cooley T. M., Bruning-Fann C. S., Sullivan L., Berry D., Carlson T., Minnis .R. B., Payeur J. B., and Sikarskie J.** (1997) Bovine tuberculosis in free-ranging white-tailed deer from Michigan. Journal of Wildlife Diseases 33: pp 749-758.

119 **Sheppe, W.A.,** (1985). Effects of human activities on Zambia's Kafue Flats ecosystems. Environmental Conservation, Vol. 12, No. 1, pp. 49-57.

120 **Shirima, G.M., Kazwala, R.R., Kambarage, D.M** (2003). Prevalence of bovine tuberculosis in cattle in different farming systems in the eastern zone of Tanzania. Preventive Veterinary Medicine. 57 (3) pp 167-172.

121 **Skuce, R.A., D.Brittain, M.S. Hughes, L.-A. Beck and S.D.Neil.** (1994). Genomic fingerprinting of *Mycobacterium bovis* from cattle by restriction fragment length polymorphism analysis. J. clin. Microbiol. Vol 32 pp 2387-2392.

122 **Sitima A. C** (1997). Variability of *Mycobacterium bovis* in Traditionally Processed Sour Milk and The Prevalence of Bovine Tuberculosis in Namwala District of Zambia. Unpublished MSc Thesis. University of Zambia Lusaka.

- 123 **Stafford** (1991). A Review of Diseases of parasites of the Kafue Lechwe (*Kobus leche Kafuensis*). Journal of Wildlife Diseases 27 (4), pp 661-667.
- 124 **Steniwo R.** (1938): Differentiation by tuberculin testing of infection in cattle due to the human, bovine and avian types of tubercle bacilli. Veterinary Records vol 50 pp 633-637.
- 125 **Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P** (2001) HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. Lancet. Nov 17; 358 (9294):pp 1687-1693.
- 126 **Szewzyk R., S.B. Svenson S.E. Hoffmer, G. Bölske, H Wahlström, L. Englund, A. Engvall and G. Källerus** (1995) Molecular epidemiological studies of Mycobacterium bovis infection in humans and animals in Sweden. J. Clin. Microbiol. Vol, 33 pp 3183-3185.
- 127 **Sudre P., G. Ten Dam, and A. Kochi** (1992) Tuberculosis: a global overview of the situation today. Bull. W.H.O vol 70 pp 149-159.
- 128 **Swets, J. A.** (1988). Measuring the accuracy of diagnostic systems. Science, 220, pp 1285-1293.
- 129 **The Merck Veterinary Manual** (1998). 8th edition Tuberculosis, pp 489-495. Merck & Co.Inc. New jersey USA.
- 130 **Thierry D., M.D. Cere, K.D. Eisenach. J. T. Crawford, J.H. Bates, B. Gicquel, and J.L. Guesdu** (1990), IS6110, an IS-like element of Mycobacterium tuberculosis complex. Nucleic Acids. Res. 18 p188.

- 131 **Timoney, J. F., J. H. Gillespie, F. W. Scott, and J. E. Barlough, (1988).** The genus *Mycobacterium*. In Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th Ed. Ithaca, N.Y.: Comstock, pp. 270-281
- 132 **Tsukamura M. (1976):** Numerical classification of slowly growing Mycobacteria. International Journal of Systemic Bacteriology vol 26 pp 409-420.
- 133 **Valway, S. E., Sanchez, M. P. C., Shinnick, T. F., Orme, I., Agerton, T., Hoy, D., Jones, J. S., Westmoreland, H., & Onorato, I. M. (1998).** An outbreak involving extensive transmission of a virulent strain of *Mycobacterium tuberculosis* N. Engl. J. Med. Vol 338, pp 633-639.
- 134 **Van Embden, J.D.A., M.D. Cave, J.T. Crawford, J.W. Dale, K.D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R.McAdam, T.M. Shinnick, and P.M. Small. (1993)** Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. J. clin. Microbiol. Vol 32 pp 406-409.
- 135 **Van Der Hoeden J. (1964)** Tuberculosis in Zoonoses p9-49 Elsevier publication, Co, Amsterdam, London, NewYork.
- 136 **Van Soolingen, D., P. E. W. de Haas, J. Haagsma, T.Eger, P. W. M. Hermans, V. Ritacco, A. Alito, and J. D. A. van Embden (1994).** Use of various genetic markers in differentiation of *Mycobacterium bovis* strains from animals and humans and for studying epidemiology of bovine tuberculosis. J. Clin. Microbiol. Vol. 32 pp 2425-2433.
- 137 **Van Soolingen, D., Hermans .P.W.M., de Haas, P.E.W., & van Embden, J.D.A (1992)** Insertion element IS1081 – associated restriction fragment length polymorphism in *Mycobacterium tuberculosis* complex species: a reliable tool to recognize *Mycobacterium bovis* BCG. J Clin. Microbiol. Vol 30 pp 1772-1777.

- 138 **Walker, E.** (1996). Tuberculosis persists in US livestock. *J.A.V.M.A.* 209: pp 1529-1530
- 139 **Walshe M.J, Grindle J, Nelly A, Bachmann M.** (1991) Dairy development in sub-Saharan Africa. Washington DC: World Bank Technical Paper No 135.
- 140 **White, T.J., Arnheim, N., and Erlich, H.A** (1989) The Polymerase Chain Reaction (PCR) *Trends in Genetics* 5: pp 185-189.
- 141 **Whipple D.L., P Ryan Clarke, J.L Jaruagin and J.B payear.** (1997) Restriction fragment length polymorphism analysis of *Mycobacterium bovis* isolates from captive and free – ranging animals. *J. Vet. Diagn. Invest.* Vol 9 pp 381-386.
- 142 **Wood, R., Maartens, G., Hudson, C.P.,** (2000). Diagnosing HIV-associated Tuberculosis: reducing the costs and diagnostic delays. *Int. J. Tuberc. Lung Disease* Vol4. pp 240-245.
- 143 **World Health Organization** (1951). Expert Committee on Tuberculosis, report on the fifth session: 1950 Sep 11-16; Geneva, Switzerland: The organization 1951. Technical Report series 32.
- 144 **World Health Organization** (1994). Report of the WHO working Group on Zoonotic Tuberculosis (*mycobacterium bovis*), with the participation of FAO; 1994 June 14 Mainz, Germany. Geneva: The organization: 1994 document WHO/CDS/VPH/94.137.
- 145 **World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO)/Office International des Epizooties (OIE)** (2001). Report on Consultation on Animal Tuberculosis Vaccines. WHO, Veterinary Public Health Unit, Geneva. WHO/CDS/VPH/94.138.

- 146 **Yates M.D, Pozniak A, and Grange J.M** (1993). Isolation of mycobacterium from patients seropositive for human immunodeficiency virus (HIV) in southeast England: 1984-92. *Thorax*; 48: pp 990-995.

Appendix Four Tuberculosis Questionnaire



**A CROSS-SECTIONAL SURVEY ON HUMAN AND ANIMAL
TUBERCULOSIS TO DETERMINE THE RISK FACTORS ASSOCIATED
WITH THE DISEASE**

*Department of Disease Control, School of veterinary Medicine, University of Zambia, P.O Box 32379,
Lusaka Zambia, Tele-fax 292737*

SURVEY ON ANIMAL TUBERCULOSIS

SECTION ONE: IDENTIFICATION

Date:.....

Village / Farm Sampling No:.....

Area / Location:.....

Owner's name:.....

Address:.....

Name of the person interviewed:.....

Relation of the person interviewed:

1. Owner

2. Family member

3. Caretaker

Name of interviewer:.....

Q 1.

Breakdown of Herd structure:

Cattle and age category	Number
Female calves < (less than) 1 year	
Male calves < (less than) 1 year	
Male cattle between 2-3 years	
Female cattle between 2-3 years	
Female cattle over 4 years	
Male cattle over 4 years	

Q 2.

Who is primarily responsible for looking after the animals?

0. Owner / family member.....

1. Hired caretaker.....

2. Both 0 and 1.....

3. Others specify.....

Q 3.

Do you receive any Veterinary services?

0. No..... ☐
1. Yes..... ☐

SECTION TWO: CATTLE FEEDING PATTERNS, WILDLIFE CONTACT & MOVEMENTS

Q 4.

What type of feeding / grazing system do you practice?

0. Grazing from own fields / paddocks..... ☐
1. Grazing from communal pastures..... ☐
2. Communal and own pasture grazing..... ☐

Q 5.

Do you move your animals to the plains on certain times of the year for search of grazing land?

0. No..... ☐
1. Yes..... ☐

Q 6.

If yes, do they come in contact with wild animals in the plains?

0. No..... ☐
1. Yes..... ☐

Q 7.

Where do your animals drink water?

☐

- 0. Own watering points..... ☐
- 1. Shared / Communal watering points..... ☐
- 2. Own and communal watering points..... ☐

Q 8.

Do your animals share drinking water with wild animals?

- 0. No..... ☐
- 1. Yes..... ☐

Q 9.

Have you sold any animals in the last twelve months? *

- 0. No..... ☐
- 1. Yes..... ☐

Q 10.

If yes, where did you sell your animals, or where did the buyers come from?

- 0. Within the neighbouring villages..... ☐
- 1. Within the town..... ☐
- 2. Within the province..... ☐
- 3. Outside the province..... ☐
- 4. Others Specify..... ☐

Q 11.

Do you sell your milk?

☐

0. Within the neighbourhood..... ☐
1. Within the town..... ☐
2. Within the province..... ☐
3. Outside the province..... ☐
4. Others specify..... ☐

SECTION THREE: KNOWLEDGE OF THE DISEASE

Q 12.

Did any animal die in your herd in the last 12 months?

0. No..... ☐
1. Yes..... ☐

Q 13.

Were there any coughing animals in your herd in the last 12 months?

0. No..... ☐
1. Yes..... ☐

Q 14.

Do you have very thin and emaciated (wasted away) animals in your herd?

0. No..... ☐
1. Yes..... ☐

Q 15.

Did any animal showing the signs in Q13 & 14 above die?

- 0. No.....☐
- 1. Yes.....☐

Q16

Have you ever thrown away lungs or part of lungs due to nodular (hard growths) on them or at an Abattoir has any of your animal(s) been condemned for suspected TB?

- 0. No.....☐
- 1. Yes.....☐

SURVEY ON HUMAN TUBERCULOSIS

SECTION ONE: IDENTIFICATION

Date:.....
Area;.....Sampling No:.....
Name:.....Sex.....Age.....
Number in the household:.....

Q 1.

Occupation

- 0. Farmer.....☐
- 1. Shepherd.....☐

2. Others Specify..... ☐

SECTION TWO: OWNERSHIP & CONTACT WITH ANIMALS AND THEIR PRODUCTS

Q 2.

Do you keep cattle?

0. No..... ☐

1. Yes..... ☐

Q 3.

Have you been in contact with animals recently?

0. No..... ☐

1. Yes..... ☐

Q 4.

If yes, what type of contact was it?

0. Herding the animals..... ☐

1. Milking the animals..... ☐

2. Others specify..... ☐

Q 5.

Do you handle animal products?

0. No..... ☐

1. Yes..... ☐

2. If yes, specify..... ☐

Q 6.

Do you drink fresh milk?

0. No.....☐

1. Yes.....☐

Q 7.

If you do, how often do you drink fresh milk?

0. At least every day.....☐

1. At least every week.....☐

2. At least every month.....☐

3. Others specify.....☐

Q 8.

Do you boil your milk before drinking?

0. No.....☐

1. Yes.....☐

Q 9.

Do you process sour milk?

0. No.....☐

1. Yes.....☐

Q 10.

If yes, do you process sour milk from boiled milk?

- 0. No..... ☐
- 1. Yes..... ☐

Q 11.

Do you drink sour milk?

- 0. At least every day..... ☐
- 1. At least every week..... ☐
- 2. At least every month..... ☐
- 3. Never..... ☐
- 4. Other specify..... ☐

Q 12.

What is your main source of milk?

- 0. Own animals..... ☐
- 1. Others, i.e. neighbors..... ☐
- 2. Others specify..... ☐

SECTION THREE: KNOWLEDGE OF THE DISEASE

Q 13.

Have you ever heard of a disease called tuberculosis (TB)?

- 0. No..... ☐
- ☐

1. Yes.....

Q. 14.

Has any member of your family received treatment for tuberculosis (TB) in the last 12 months?

0. No..... ☐

1. Yes..... ☐

Q 15.

If yes, at what approximate age did they receive the TB treatment?

Specify..... ☐

Q16.

Are all members of your household vaccinated with BCG?

0. No..... ☐

1. Yes..... ☐

2. Don't know..... ☐

Tuberculin test Data entry form for mid cervical tuberculin test recording.

TUBERCULIN TEST DATA ENTRY FORM

OWNER _____

HERD NUMBER

VILLAGE

DATE FIRST VISIT _____

DATE NEXT VISIT DUE

[illegible]