PREVALENCE AND RISK FACTORS OF BRUCELLOSIS IN COMMERCIAL CATTLE FARMS IN LUSAKA PROVINCE

BY

CHIMANA HENRY MWELWA

A thesis submitted to the University of Zambia in fulfilment of the requirements of the degree of Master of Science in Public Health

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DISEASE CONTROL DEPARTMENT

©MAY 2012

DECLARATION

I, **Chimana Henry Mwelwa** do hereby declare that the contents of the dissertation being submitted herein are my original work and they have not been previously submitted to any university for the award of a degree or any other qualification.

Signature--------------------------------------- Date--

CERTIFICATE OF APPROVAL

This dissertation submitted by **CHIMANA HENRY MWELWA**, is approved as fulfilling the requirements for the award of the degree of **MASTER OF SCIENCE IN PUBLIC HEALTH** of the University of Zambia.

Dr B. M. Hang'ombe	------------------------------	--------------------------
Supervisor	Signature	Date
	------------------------------	--------------------------
Examiner	Signature	Date
	------------------------------	--------------------------
Examiner	Signature	Date
	------------------------------	--------------------------
Examiner	Signature	Date
	------------------------------	--------------------------
Chairman (Board of Examiners)	Signature	Date

ABSTRACT

A cross-sectional study was conducted in Lusaka Province of Zambia to estimate brucellosis seroprevalence and identify associated risk factors. This study was done between January 2007 and February 2008 in commercial cattle farms in 3 districts of Lusaka Province (Kafue, Chongwe and Lusaka districts) and one district in Central Province (Chibombo). Sera were collected from animals randomly selected from sampling herds and at the same time data such as sex, age and parity were also recorded.

The collected sera were screened for *Brucella* antibodies using the Rose Bengal test (RBT) followed by confirmation on Competitive Enzyme Linked immunosorbent Assay (C-ELISA). Results were interpreted both in series and parallel. The risk factor identification was done using a pre-tested questionnaire which was administered simultaneously with the blood sampling. The factors assessed included types of cattle breeds, farm ownership, care responsibilities, marketing, feeding practices, herd size, stock density, disease control and proximity to other herds. Prevalence estimates, including the 95% confidence interval, were determined for each district and also for Lusaka province as a whole. The Fisher's exact test was used to test for associations between *Brucella* positivity on the farm and the hypothesised risk factors.

A total of 897 serum samples from Lusaka province (n=849) and Chibombo district (n=48) were screened. The estimated overall seroprevalence in Lusaka province was 9.4% (95% CI: 5.0% to 13.6%) while that for Chibombo district was 18.7% (95% CI: 7.5% to 29.9%) based on serial interpretation. The overall herd level prevalence for Lusaka Province was 40.6% (95% CI: 16.4% to 67.9 %) while that for Chibombo was 100%. The prevalences according to sex were oxen 0%; cows 8.1% (95% CI: 4.6% to 11.6%) and bulls 12.5% (95% CI: 3.8% to 21.1%). Prevalences according to age groups were 1 to 4 years 10.7% (95% CI: 4.9% to 16.9%); 4.5 to 5 years 4.2 % (95% CI: 0.0 to 7.8%); 5.5 to 7 years 6% (95% CI: 2.0% to 9.9%) and more than 7 years 9.9% (95% CI: 2.3% to 17.5%). At the animal level, seroprevalence varied according to sex with bulls having slightly higher odds of being seropositive (OR=1.7) compared to females, according to age groups, with the age category 1 to 4 years recording the highest seroprevalence. Chongwe district had a significantly higher prevalence ($p < 0.001$) than all the other districts in Lusaka province.

A univariate analysis of risk factors showed that the method of acquiring a farm was the most important risk factor. However, other factor such as source of animals was significant at 95% confidence level. The study therefore highlights the need for effective control measures to be put in place to reduce the observed brucellosis prevalences.

DEDICATION

I dedicate this work to my late father, Mr Simon Mwelwa Chimana for his insistence to see me attain higher education and achieve better things. My late maternal uncle, Mr Obino Mulima Musongo for urging me to be self dependent and be a family asset. My late elder brother, Mr Lucien Chungu Chimana and late young sister, Mrs Agnes Mwelwa Katowando for the encouragements, they were giving me to push on to higher heights and be a better person. My wife, Catherine Nyawa Chimana and my children, Maria Lubilo Mwamba Chimana and Charles Mumanga Chimana who have been understanding me through this very demanding academic life. Finally God almighty for good health.

ACKNOWLEDGEMENTS

I am indeed grateful to the University of Zambia through the Staff Development Office for providing finance assistance which enabled me complete the whole research work with fewer problems. I am really grateful to the Samora Machel School of Veterinary Medicine of the University of Zambia for availing me the chance to conduct this research in Disease Control Department.

I am sincerely thankful to my supervisors, Prof. Kenny L. Samui and Dr Bernard M. Hang'ombe for the patience they had and for helping me through the proposal formulation up to the write up of this dissertation. I also acknowledge them for the numerous corrections they made to the dissertation so that it could reach acceptable standards. I am greatly humbled by Dr John B. Muma and Dr Musso Munyeme for the initial partial financing of this work, conceptualising, guidance and corrections during the proposal and dissertation write ups. I also thank them for providing materials, reagents and kits in the Public Health laboratory which I used during the research work at the School of Veterinary Medicine. To them I am greatly indebted.

I am grateful to Dr Andrew M. Phiri and Dr Joyce Siwila-Saasa for their critical and analytical approach during the write up of this dissertation. I am most grateful to the following members of staff who had some inputs, Mr Max Masuku, Mrs Carol Chisenga, Ms Georgina Chifumpa (late), Mrs Manda Chitambo and Mr Ladslav Moonga. I would

like to acknowledge the contributions made by the drivers who made it possible for me to get to various farms. The farmers are also acknowledged for allowing me access to their animals.

Last but not the least, my sincere thanks to all the members of staff who have been or acted as Heads of Disease Control department during this period: Prof. Andrew Nambota, Prof. Girjah S. Pandey, Prof. Aaron S. Mweene and Dr Michello Syakalima. They were all very supportive and encouraged me to soldier on.

TABLE OF CONTENTS

CONTENT	PAGE
Declaration	i
Certificate of approval	ii
Abstract	iii
Dedications	iv
Acknowledgements	v
List of tables	x
List of figures	xi
List of abbreviations	xii
CHAPTER ONE: INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	9
2.0.0 General overview of brucellosis	9
2.1.0 Distribution of *Brucella* infections	11
2.2.0 Host range of brucellosis	13
2.2.1 Animal brucellosis	13
2.2.2 Human brucellosis	14
2.3.0 Pathogenesis of brucellosis in animals	15
2.3.1 Pathogenesis of brucellosis in man	18
2.4.0 Risk factors associated with brucellosis transmission	19

2.5.0 Clinical manifestations of brucellosis 21
2.5.1 Brucellosis manifestation in animals 21
2.5.2 Brucellosis manifestation in human 22
2.6.0 Pathology of brucellosis 23
2.6.1 Pathology of brucellosis in animals 23
2.6.2 Pathology of brucellosis in humans 24
2.7.0 Diagnostic methods of brucellosis 25
2.8.0 Brucellosis control 30
2.8.1 Control of brucellosis in animals 30
2.8.2 Control of brucellosis in humans 31

CHAPTER THREE: MATERIALS AND METHODS 33
3.0.0 Study sites 33
3.1.0 Study design 33
3.1.1 Reference population / Sampling frame 33
3.1.2 Sample size calculations 35
3.1.3 Sampling techniques 36
3.2.0 Laboratory analysis 38
3.2.1 Rose Bengal test 38
3.2.2 Competitive ELISA 39
3.3.0 Questionnaire survey for risk factors 41
3.4.0 Statistical analysis 41

CHAPTER FOUR: RESULTS 42

4.0.0 Descriptive results 42

4.1.0 Risk factor analysis 45

CHAPTER FIVE: DISCUSSIONS 49

CHAPTER SIX: RECOMMENDATIONS 57

CHAPTER SEVEN: CONCLUSIONS 59

REFERENCES 61

APPENDICES 81

Appendix 1.0: Serological tests 81

Appendix 1.1: Rose Bengal Plate Test 81

Appendix 1.2: Competitive ELISA 81

Appendix 2.0: Questionnaire 87

LIST OF TABLES

TABLE		PAGE
Table 1	Sampling activities in Lusaka Province/Chibombo District	42
Table 2	RBT and C - ELISA and estimated district prevalence	43
Table 3	Herd level district prevalence of brucellosis in Lusaka Province	44
Table 4	Individual prevalence of brucellosis	45
Table 5	Risk factor analysis	46

LIST OF FIGURES

CAPTION **PAGE**

Figure 1	Gram stain smear – negative coccobacilli	10
Figure 2	*Brucella abortus* colonies	10
Figure 3	Map of Lusaka Province showing district boundaries	34
Figure 4a	Blood sample collection from cattle by jugular vein	37
Figure 4b	Drawing blood using the sterile vaccutainer tube	37
Figure 5	Rose Bengal plate test showing positive and negative results	38
Figure 6	Microscopic views of positive and negative RBT results	39
Figure 7	C – ELISA test plate showing positive and negative results	40

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organisation
WHO	World Health Organisation
OIE	Organisationn for Animal Health
C - ELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
FPA	Fluorescent Polarisation Assay
GDP	Gross Domestic Produc
ZADL	Zambia Agricultural Development Limited
MZCC	Mediterranean Zoonoses Control Centre
MAFF	Ministry of Agricultural Food and Fisheries
SGOT	Serum glutamate oxaloacetate transaminase
RBT	Rose Bengal Test
CFT	Complement Fixation Test
SAT	Serum Agglutination Test
SLPS	Smooth Lipopolysaccharide
RFLP	Restriction Fragment Length Polymorphism
ZNFU	Zambia National Farmers Union
CDC	Center for Disease Control
APHS	American Public Health Society
GDP	Gross Domestic Product
DNEI	Diseases of National Economic Importance

CHAPTER ONE

INTRODUCTION

Brucellosis is an infectious bacterial disease that primarily infect livestock but also humans (Pappas *et al.,* 2006; Muma *et al*., 2006). The aetiological agent of brucellosis is a bacterium of the genus *Brucella* and the species are *B. melitensis, B. abortus, B. suis, B. ovis, B. canis, B. neotome, B. microfti* and recently isolated from marine animals *B. maris* (Cloeckaert *et al*., 2001; 2003). The disease is of both socio-economic and public health importance especially in developing countries (Anonymous, 1986). It remains a major zoonotic disease with a worldwide distribution (Corbel, 1997; Olsen and Staff, 2005). However some countries such as the United States of America, United Kingdom, Australia and Japan have eradicated it in livestock rendering the human populations free from the disease (Anon., 1986). The disease affects a wide range of animals including ruminants in which it is characterised by abortion (Corbel, 1997; Cloeckaert *et al*., 2001; 2003). Brucellosis in cattle is also characterised by stillbirths or weak calves.

The mode of transmission among animals is through exposure of mucous membranes, inhalation of aerosols or direct contact with infected materials (Anon., 1986; Bishop *et al*., 1994). Humans contract brucellosis from animals through ingestion of contaminated, unboiled or unpasteurised milk and by direct contact with infected animals, animal carcasses and aborted materials (Anon., 1986; Al-Shamay *et al*., 2000; Omer *et al*., 2000). Human-to-human transmission by tissue transplantation or sexual contact has been reported (Mantur *et al.,* 1996). Human brucellosis is the most common zoonosis in the

world accounting for more than 500 000 reported cases annually (Anon., 1997; Pappas, 2006; Rust, 2006). *Brucella* infections in humans are characterised by septicaemia, undulating fever, fatigue, night sweats and myalgia (Elzer, 1998).

Prognosis and clinical manifestations of the disease depend on the infecting *Brucella* species and the host animal involved (Corbel *et al*., 1997). In terms of milk production the estimated loss due to *Brucella* infections is in the range of 20 to 25% of estimated annual output (Alton, 1990; Benjamin, 1992).

The control of livestock brucellosis in some developed countries has resulted in significant economic gains as well as a reduction in human cases (Anon., 1997). However the disease remains a major problem in the Mediterranean and surrounding regions. In recent times, the disease has re-emerged in Malta and Oman indicating the difficulties in its eradication (Amato, 1995).

In most African countries including Zambia, brucellosis is still a major disease of both socio-economic and public health importance (Munang'andu, 2000; Anon., 2000). As a result of endemic *Brucella* infections in animals, millions of people are at risk of the disease because of unsafe methods of food preparation, lifestyles that bring them in direct contact with infected animals, consumption of contaminated foods and occupational contact (Anon., 1997).

Most commercial beef enterprises in Lusaka Province source their animals from two major cattle producing provinces namely Southern and Western Provinces. In case of dairy enterprises, most of the animals come from Southern province (Ahmadu *et al*., 1999). Together with Eastern and Central Provinces, these provinces comprise about 90% of cattle in the traditional sector (Perry *et al*., 1984). According to 2006 estimates, the total cattle population in Zambia was estimated at 2,904,880 (Anon., 2006), with 84% and 16% of them coming from the traditional and commercial sectors, respectively.

Zambia's livestock industry is divided into the commercial and traditional sectors. It is an important socio-economic activity and contributes significantly to the National Gross Domestic Product (GDP). The commercial livestock is predominantly reared in Lusaka, Southern and Central Provinces. A large proportion of traditional livestock is reared in Western, Southern, Central and Eastern Provinces. The country's total livestock population is currently estimated at 2,904,880 cattle, 956 304 goats, 101 191 sheep of which about 84%, 96% and 64%, respectively is found in the traditional sector (Anon., 2007). This sector has low productivity as a result of numerous production and management problems (Anon., 2006).

The traditional livestock sector is dominated by the local people who raise cattle on communal land which is generally administered by the traditional chiefs. Under this extensive grazing system the predominant breed types are the zebu and sanga. Livestock keeping is the major occupation providing income, food and social security (Perry *et al.,* 1984).

Although there is a high potential for increased livestock production in this sector, factors such as limited grazing capacity, water shortages (in some areas especially during dry seasons), heavy disease burdens, abortion, stillbirths and low fertility affect production drastically. Some of the major diseases that affect livestock production in the traditional sector are brucellosis, contagious bovine pleural pneumonia (CBPP), bovine viral diarrhoea (BVD), infectious bovine rhinotrachietis (IBR), theileriosis and swine fever.

Before 1991, the Zambian Government used to provide free veterinary services to the traditional cattle farmers. Following privatisation of the veterinary service delivery in 1991 the free services that this sector was accustomed to ended, resulting in the occurrence of many epizootics of diseases such as anthrax, east coast fever (ECF), CBPP, tuberculosis, foot and mouth disease, brucellosis, and African swine fever. Furthermore diseases that were hitherto confined to certain areas of the country spread to other parts of the country. The increases in livestock disease occurrence and recurrent drought conditions have impacted severely on this sector.

The low productivity in the traditional cattle sector has also negatively impacted on the commercial sector. This is because the traditional cattle sector is the nursery for some of the commercial farmers who buy their stock from traditional farmers, fatten them in their feedlots and then sell to slaughterhouses. Average calving rates in the traditional sector are estimated to be between 40% and 55% while those of the commercial sector are between 55% and 75%, (Anon., 2004b). In terms of milk production the annual output in the traditional sector is 42 million litres compared to the commercial sector with about 80

million litres (Anon., 2004b). Therefore there could be a major socio-economic and public health impact in this sector resulting from livestock diseases.

The commercial livestock sector is dominated by farmers using exotic breeds supplemented with local ones. The sector comprises beef and dairy farms with a small number of farmers also keeping goats, sheep and pigs. The farms are on titled land and mostly located along the line of rail to facilitate easy transportation of inputs and products. They are essentially operated as private business enterprises and prominently feature high production efficiency. Before 1991 during the era of Government–run parastatal organisations, a large proportion of the commercial livestock sector (dairy farms and beef ranches) was run by the state through Zambia Agricultural Development Limited (ZADL). The Cold Storage Board of Zambia controlled the beef market through the state owned farms and chains of slaughterhouses. Similarly, the Government prominently ran the dairy industry through the Dairy Produce Board of Zambia. The two organisations also set the standards for meat and milk quality. After privatisation of the state owned commercial cattle farms there was proliferation of private owned ones. The direct effect of such privatisation was the elimination of the meat and milk quality control systems. Currently this role has been taken over by a few private firms such as Parmalat, Finta and Diamondale, and this sometimes disadvantages small scale farmers. This resulted from the lack of an effective government mechanism for food quality control. The advent of privatisation paved way for the growth of small private meat and milk enterprises whose motive is to maximise on profits while paying little attention to the quality of their products. All this culminated in the government's failure to effectively

control diseases of socio-economic and public health importance due to limited capacity within its institutions. A good example is that there is very little or no law enforcement to ensure that private beef/dairy farms are free of bovine brucellosis or tuberculosis. The control of such diseases is currently the farmer's own responsibility. All these limitations in the livestock sector, coupled with the failure to control livestock diseases, have had a negative effect on the country's ability to gain access to foreign markets which have stringent sanitary mandates.

Although there has been no systemic study, to investigate the contribution of brucellosis to livestock production in Zambia, some serological studies indicate wide distribution and high prevalence of the disease especially in the traditional sector (D' Cruz, 1987; Muma *et al*., 2006). There are also some suggestions that brucellosis has contributed to the decline of wildlife populations in Zambia (Matsukawa and Yoshima, 1995; Pandey *et al*., 1999). The disease is widespread and endemic in more densely populated cattle raising areas. Southern and Western Provinces have reported an increase in *Brucella* infections in wildlife, which share grazing ground with domestic animals (Pandey *et al*., 1999; Munang'andu, 2000). Brucellosis in Zambia's occupationally exposed people (abattoir workers, butchers, veterinarians, farm workers and shepherds) has been estimated at about 1% (Kadohira *et al*., 1996). However, this estimate may be inconclusive due to the small sample size (n= 20) involved in the study. Although cattle *Brucella* sero-prevalence in the traditional sector in Zambia generally ranges between 6-28% based on Rose Bengal Test (RBT), Competitive Enzyme Linked ImmunoSorbent Assay (C-ELISA) and Fluorescent Polarisation Assay (FPA) (Pandey *et al.,* 1999; Munang'andu, 2000; Muma

et al., 2006) there are no specific reports on the disease prevalence and associated risk factors in cattle in the urban and peri-urban commercial farms of Lusaka Province. Commercial beef and dairy farms in Lusaka Province are mainly located around the urban/peri-urban areas along the line of rail. Commercial cattle farms in the Province consist of private, institutional and co-operative farms. Foreign investors rearing exotic and local breeds mostly run large commercial cattle farms while most of the small-scale farmers are run by local investors. Institutional farms are those being run by government institutions like Universities, Colleges, Research and Training institutes. Co-operative farmers generally consist of rural people who rear animals initially donated by non governmental organisations (NGOs), especially for milk for commercial purposes as a way of empowering such people. Under the co-operative scheme, milk, is collected, and taken to a central collection point in readiness for transportation to processing factories. A close look at dairying in Lusaka Province will indicate that the number of such enterprises is growing and constitute a fair share of dairy industry.

Under the current Government livestock development policy (Anon., 2005) the small scale dairy sub sector is viewed as the driving force of dairy development in the country. This as well as public health concerns call for a change in the traditional perception of a commercial dairy farmer. As such, any person that produces milk mainly for commercial purposes must be regarded as a commercial dairy farmer regardless of the scale of his/ her level of production. This is also important so that poor quality milk especially with regard to brucellosis and tuberculosis contamination, whether from a few dairy cows or hundreds of them, may not be allowed for public consumption.

With this background, this study was initiated to look at the epidemiology of *Brucella* infections in commercial cattle raised within Lusaka Province. In order to comprehensively undertake this study, the following specific objectives were to:

a) Determine the prevalence of *Brucellosis* on commercial cattle farms in Lusaka Province.

b) Identify risk factors associated with *Brucella* infections in commercial cattle in Lusaka Province.

Study justifications

Recent studies in rural areas of Zambia have shown that brucellosis is endemic in the livestock-wildlife interface areas of the Blue Lagoon (herd level prevalence between 14.1% and 46.2%) and Lochinvar (herd level prevalence between 28.1% and 74.0%) game management areas (Muma *et al*., 2006). The results of studies in human populations around these interface areas showed a 5.0% serological positivity to *Brucella* species antigens (Muma *et al*., 2008). However, not much has been done in determining the prevalence and risk factors of *Brucella* infections in human and commercial cattle populations in Zambia which may be significant in drawing up policies for zoonoses control in Zambia. In addition most cattle in areas known to have brucellosis are usually imported into Lusaka where there is a very good market.

CHAPTER TWO

LITERATURE REVIEW

2.0.0 General overview of Brucellosis

In 1860, Marston provided the first modern clinical description of brucellosis and named it Mediterranean gastric remittent fever, while Bruce and Carrauna–Seciuna of Malta in 1887 demonstrated the aetiological role of *Brucella melitensis* (Ross *et al*., 1994; Madkour, 2001; Rust, 2006). This organism was first isolated from the brain of a goat in 1897 by Hughes who published a classic description of this illness (Amato *et al*., 1995). His term “undulant fever” became the most widely accepted clinical description until “brucellosis” became the most commonly used name. In 1924, Lemaire first isolated *Brucella melitensis* from the spinal fluid of a goat (Rust, 2006).

Brucellosis is caused by a group of gram-negative cocco-bacilli belonging to the genus *Brucella* (Fig.1). These bacteria are essentially pathogens of cattle, goats, sheep and pigs (Coghlan, 1995). *Brucella* species are aerobic with the exception of *Brucella abortus*, which requires 5-10% carbon dioxide for growth (Alton *et al*., 1988). All *Brucella* strains grow well in media enriched with animal serum and glucose at an optimum temperature of 37°C (Alton *et al*., 1988). *Brucella* occurs singly, in groups or short chains and is non-motile, non-capsulated and non-sporing (Anon., 1997). On solid medium they are smooth, moist, translucent and glistering colonies which may take several days to appear

(Fig.2). The organisms tend to mutate phenotypically forming rough colonies (Anon., 1997).

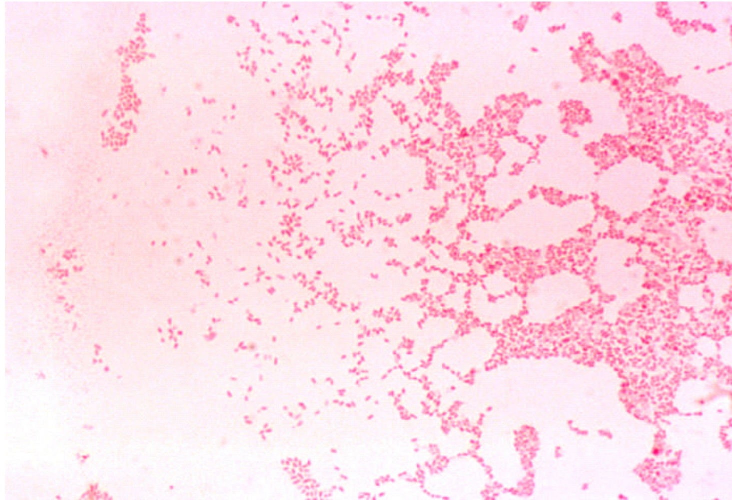

Figure 1: A gram-stain of *Brucella abortus* showing the Gram-negative coccobacillary shape. Source: Centers for Disease Control, Public Health Image Library number 1937. (Anon., 2002)

Figure 2: Colony appearance of *Brucell*a *abortus*. It grows slowly, even on rich media to give pinpoint, translucent colonies with a smooth surface. Source: CDC/Courtesy of Larry Stauffer, Oregon State Public Health Laboratory, Public Health Image Library number 1902. (Anon., 2002).

There are six classical *Brucella* species, which differ from one another in their choice of animal hosts. Other differences observed include biochemical characteristics, culture appearance and the amount or number of the main antigens they posses (Stack and Macmillan, 2000). The major species are *B. abortus* which infects cattle; *B. melitensis* affecting goats and sheep; *B. suis* affecting pigs; *B. canis* which infects dogs; *B. ovis* which infects sheep and *B. neotome* which infects desert rats. *B. microfti* has been isolated from soil and mice (Corbel, 1997; Cloeckaert *et al*., 2002). The host parasite relationship for the major species is not absolute, and both man and domestic animals are susceptible to infection by strains of all the eight species (Bernard *et al*., 1982). The isolation of a distinctive *Brucella* strain tentatively named *B. maris* from marine animals in the United Kingdom, Australia and the United States extends the ecologic range of the genus and its scope as a zoonosis (Ross *et al*., 1994; Cloeckaert *et al.,* 2001). *B. maris* has been divided into two subspecies namely *B. cetaceae* from otter/seal and *B. pinnipediae* of the whale/porpoise (Cloeckaert *et al*., 2001, 2003).

2.1.0 Distribution of *Brucella* infections

Brucellosis has been recognised as an endemic illness in almost all Mediterranean countries, India, China, Southern Africa and much of Central and South America (Pappas, 2006; Rust, 2006). Sporadic outbreaks occur in many other parts of the World, including North America. Pasteurisation of cow and goat milk has considerably reduced the incidence of the disease in humans especially in the developed world (Anon., 1986). Europe and countries such as Austria, Denmark, Estonia, Finland, Hungary, Iceland, Luxembourg, Moldavia, Netherlands, Sweden, Switzerland and the United Kingdom

have been declared brucellosis-free (Anon., 1986). However, the disease is widespread in countries such as the Czech Republic, Latvia, Lithuania, Russia, Bulgaria, Croatia, Slovakia, Slovenia and Yugoslavia where they may not be pasteurising the milk for the belief that, this process interferes with flavour and taste in the milk (Anon., 1986).

Similarly in some African countries such as Algeria, Eritrea, Congo (Brazzaville), Guinea, Ghana, Tanzania, Democratic Republic of Congo and all the way through Zimbabwe to South Africa, higher prevalences are still being reported (Amato, 1995). However in Kenya, lower seroprevalence of brucellosis was reported in commercial herds as compared to other regions of Africa as a result of improved management systems (Hussein *et al*., 1978).

In Zambia, brucellosis was first reported in 1915 (Tuchili, 1988). Since then, there has been a steady increase in sero-prevalence levels, especially in cattle and wild ruminants (Munang'andu, 2000). There are also reports of the disease in sheep, goat, and humans (Tuchili, 1988; Pandey *et al*., 1999; Muma *et al*., 2008). Several species of wildlife such as Kafue lechwe (*Kobus leche kafuensis*), African buffalo (*Syncerus caffer*), Hippopotamus (*Hippopotamus amphibious*), Zebra (*Equus burchellii*), Eland (*Tautotragus roryx*) and Impala (*Aepyceros melampus melampus*) have serologically tested positive for brucellosis (Rottcher, 1987; Bishop *et al.,* 1994).

2.2.0 Host range of Brucellosis

2.2.1 Animal brucellosis

Brucella infections are widely distributed in domesticated animals especially in the developing World (Corbel, 1997; Godfroid, 2002). Cattle infections are commonly caused by *Brucella abortus* (Corbel, 1997). In cases where cattle come in contact with infected pigs or goats, *Brucella suis* and *Brucella melitensis* infections may take place (Corbel, 1997; Godfroid, 2002). However the two strains usually cause less severe disease in cattle. Infection is most commonly through ingestion, contact with foetal and placental contents while *Brucella abortus* can also be transmitted through coitus (Foster and Smith, 2008). Young cows are less susceptible compared to mature or older animals which tend to be sexually active since brucellosis is considered to be more of a sexually transmitted disease among animals (Richey and Harrell, 1997; Parker, 2007). Unborn calves are usually aborted at about seven months and in case of birth, they are weak and die shortly afterwards (Corbel, 1997). In terms of milk production, a severe drop is experienced as a result of infection in the herd (Bishop *et al*., 1994; Bandara and Mahipale, 2002). There are large swellings in the joints of limbs called hygromas in infected cows (Geering *et al*., 1995; Huebner, 1998; Anon., 2002). Brucellosis mainly affects sexual organs with serious results of endometritis and epididymitis (Huebner, 1998; Bandara and Mahipale, 2002). Bulls may exhibit sterility and orchitis. The infected herd may also exhibit disabilities such as discospondylitis, bursitis or arthritis (McDermott *et al*., 1994; Traboulsi *et al*., 2007).

Pigs are affected most commonly by *B. suis* (Godfroid, 2002; Pappas *et al*., 2005). However, pigs may also be affected by *B abortus* in cases where they come in contact with infected cattle (Stuart *et al.,* 1987). Sexual contact and ingestion may be the modes of transmission (Godfroid, 2002). Abortion and other reproductive disorders may occur in sows. In boars, orchitis occurs and less commonly arthritis, spondylitis or abscesses in various organs may occur (Pappas *et al*., 2005).

In sheep and goats, *B. melitensis* is the classical species affecting females of both animal species (Thimm, 1982; Diaz-Aparicho *et al*., 1994). In cases where infected cattle or pigs come in contact with small ruminants, infections of *B. abortus* and *B. suis* can occur (Stuart *et al.,* 1987). *B. melitensis* infections are acquired primarily by ingestion (Alton, 1990). Abortion and mastitis usually occur in infected goats (Corbel, 1997).

Dog brucellosis is most commonly caused by *B. canis* (Foster and Smith, 2008). However infections by *B. abortus, B. suis* and *B. melitensis* may occur occasionally when dogs eat placentas from infected farm animals. The disease is most commonly transmitted sexually and bitches abort at 40 to 60 days of gestation (Foster and Smith, 2008). In stud dogs, epididymitis, orchitis and scrotal dermatitis, which sometimes progresses to complete scrotal necrosis, may occur.

2.2.2 Human brucellosis

Human brucellosis is normally associated with the consumption of milk and other animal products contaminated with *Brucella* organisms from infected animals primarily

ruminants such as cattle and goats (CDC, 2000a; 2000b). The people at risk are usually laboratory workers, veterinarians, farm, and slaughter house workers (Young, 1995; Wafa *et al*., 2006). Vaccines such as *Brucella abortus* strain 19 can cause disease in humans through accidental injections (Berkerlman, 2003; Ashford *et al*., 2004). The symptoms are inconsistent fever, sweating, weakness, anaemia, headaches, depression and body pains (Roushan *et al.,* 2006). These symptoms are similar to those associated with many other febrile diseases (Colmenero *et al*., 1996; Morata *et al*., 1999; Pappas *et al*., 2003). However in brucellosis, there is some exceptional emphasis on muscular pain and sweating (Anon., 2007). The duration of the disease varies from a few weeks to months or even years (Sauret-Vilissova*,* 2002). Septicaemia appears in the first stage of the disease and leads to the classic triad of undulant fevers, sweating usually with characteristic wet hay smell, migratory arthralgia and myalgia (Anon., 2007; Burnade, 2007). Moreover, the incidence of paravertebral and/or epidural abscess which may imitate disk herniation, the neurological involvement and the high rate of important functional disabilities as well as cervical spondylitis constitute a very severe complication of the disease (Colmenero *et al*., 1996).

2.3.0 Pathogenesis of Brucellosis in animals

In animals, chronic *Brucella* infections have been associated with survival mechanisms, namely initial survival and dissemination of the organisms (Riley and Robertson, 1984). Primarily the virulent factor is the lipopolysaccharide (LPS) which protects the organism from complement-mediated lysis, enhancing intracellular survival (Rege *et al*., 2006). The dissemination is based on the inhibition of primary degranulation and oxidative

bursts in polymorphoneutrophils, thereby preventing phagolysosomal fusion (Riley and Robertson, 1984; Frenchick *et al*., 1985; Harmon *et al*., 1988). This is more likely to occur in cases where the initial antibiotic treatment of brucellosis was inadequate. Since chronic brucellosis does not develop in all untreated individuals, other host factors may be playing a role in susceptibility to chronic infection. For example, certain individuals may be more vulnerable than others to development of a chronic state of infection because they have lower than average immunocompetence specific for *Brucella* species. (Frenchick *et al*., 1985; Betram *et al*., 1986; Canning *et al*., 1986; Harmon *et al*., 1988; Latimer *et al*., 1992). These individuals may also have greater-than-average failure of T-cell sensitisation or T-cell mediation of macrophage activation. Other factors such as HIV/AIDS, malaria, tuberculosis, sex, physiological state and stress may also predispose the individual to *Brucella* infection (Scrimgeour *et al*., 1999; 2003; Rust, 2006; Rege *et al*., 2006).

Humoral immune mechanisms may participate in the control of acute infection, although the nature of that participation is not yet clearly understood (Cloeckaert *et al*., 2001; Munoz *et al.,* 2005; Rust, 2006). The capacity of humoral immune mechanisms to influence the course of the infectious reaction is likely to be limited because of the intracellular response in the liver and bone marrow achieved by *Brucella* organisms (Seema *et al.,* 2001). Nonetheless, the level of immunoglobulin M (IgM) antibodies begin to rise at the end of the first week following infection and usually peaks at approximately one month, when immunoglobulin G (IgG) antibodies begin to appear. The level of IgG antibodies declines in the ensuing months, while IgM antibody titres remain elevated for

years. Immunoglobulin A (IgA) antibodies are elaborated late and also may persist for very long intervals (Rust, 2006; Munoz *et al*., 2005). The two scenarios of the antibodies and transaminate enzymes indicate the involvement of the liver and bone marrow in an acute phase. This phase is where macrophages mediate control of the infection, without specific activation. However, after the first 2 weeks of infection, sensitised T lymphocytes specifically activate the macrophage response and this considerably reduces the survival rate of *Brucella* organisms in the liver and spleen of most infected individuals (McCullough and Paulson, 1998; Rust, 2006). Due to the high levels of antibodies, at this stage there is a classic positivity on the Rose Bengal test. There is melitococcemia which is the presence of *Brucella* in blood, which if untreated, gives rise to focalizations of infection leading to a chronic stage (Rienzo, 1948; Villafane *et al*., 1948). These focalizations usually occur in bones and joints and may result in spondylodiscitis of lumbar spine accompanied by sacroilitis which is very characteristic of the disease. In animals the incubation period takes about 30 to 60 days. When infection occurs in pregnant animals, the initial lesion is in the wall of the uterus and later it spreads to other parts of the organ. There is an association between the production of erythritol and the rate of proliferation of *Brucella* organisms (Nicoletti, 1980; Radostits *et al*., 1994). This leads to severe ulcerative endometritis of the intercotyledonary spaces affecting the allantoic chorion, foetal fluids, placental cotyledons and destruction of villi (Radostits *et al*., 1994). Following bacteraemia, there is localisation in the cow's gravid uterus resulting in placentitis, which enhances production of prostaglandins curtailing the corpus luteum, and then abortion occurs (Roushan *et al*., 2006; Woods and Jan, 2005). In cases where the animal is not pregnant, there is localisation in the udder resulting in

interstitial mastitis and involvement of the mammary glands that may cause the organisms to be excreted in milk for months or even years where the animal becomes a carrier (Cokca, 1999; Mdegela *et al*., 2005; Akay *et al*., 2007).

2.3.1 Pathogenesis of brucellosis in man

Brucellosis in human is transmitted through ingestion of contaminated unpasteurised milk or other animal food products such as improperly cooked meat (Rust, 2006). From the gastrointestinal tract, the bacteria pass through the mucosa into the blood stream and the circulatory system. However, transmission may occur through cuts, abrasions, inhalation and direct contact with mucous membranes (Rust, 2006). The organism quickly becomes an intracellular pathogen, colonizing the lymphatic system (i.e. lymph nodes, spleen, and bone marrow) as well as the liver (Enright, 1990). The bacteria seek cells that are capable of providing the nutrient erythritol, hence their predilection towards genital tracts of animals (Enright, 1990; Rust, 2006). Reticular endothelial cells, particularly the macrophages are also preferred in animals and are the chief site of the infection in humans (McDermott *et al*., 2002; McGill *et al*., 2002; Rust, 2006). The organisms often enter macrophages using host micro-filaments, where they are protected from the various defence mechanisms of the immune system (Finlay and Falkow, 1989). The protection mechanism involves the capacity of the internalised bacteria to evade the phagosome – lysosome fusion pathway. In advanced stages, in men, frequently there may be orchitis (Rienzo, 1948; Villafane *et al*., 1948).

2.4.0 Risk factors associated with brucellosis transmission

The natural reservoirs of *Brucella* include terrestrial mammals such as domestic animals, wildlife and humans (Bishop *et al*., 1994). It has also been reported that transmission of *Brucella* species from these natural reservoirs depends on factors such as herd size, density of animal populations in a given locality, presence of host animals, management style, animal movement dynamics, husbandry systems, type/breed of animals and ecological scenario of farms (Cloeckaert *et al*., 2001). Animal to animal transmission is mainly through mucous membranes after contact with infected materials or aerosols and by sexual contact (Bishop *et al*., 1994). However, new *Brucella* strains have recently been isolated from aquatic animals, indicating an increase in the modes of transmission of the organism as this could be a risk factor to consumers, hunters and researchers (Cloeckaert *et al*., 2001).

In most parts of Africa, a combination of various factors such as climate, altitude (ecological factors), herd size, number of people owning animals in one herd and husbandry methods contribute tremendously to the prevalence and transmission of brucellosis (Thimm, 1982). The high (16 to 25%) and very high (over 25 %) seroprevalence rates reported in rainfall regions of West and Central Africa respectively, are surrounded by moderately (11 to 20 %) affected areas in the wet and dry savannah regions (Thimm, 1982). In commercial dairy farms of Eritrea, a prevalence of 35.9% (Omer *et al*., 2000) has been reported in conformity with other regions with similar management systems such as Nigeria (Rikin, 1988), Sudan (McDermott *et al.,*1987) and Tanzania (Msanga *et al.*, 1986).

Brucellosis due to *B. melitensis* has emerged as an important problem in cattle in some southern European countries and Israel (Amato, 1995). The major contributing factor to the problem is the rather ineffective *Brucella abortus* vaccine being used instead of *Brucella melitensis*, which is yet to be evaluated in cattle as there seem to be no cross protection between the two species (Vito *et al*., 2001; Uzureau *et al*., 2007).

In the temperate climate zones of North America and the surrounding areas, brucellosis tends to be harboured by wild animals such as buffalo (*Bubalus bubalus*), bison (*Bison bison*) and reindeer (*Rangifer tarandus*). During winter, these wild animals tend to wonder close to commercial cattle farms in search of food. This potential interaction between cattle and wildlife poses a risk for possible spread of infection (Rust, 2006).

The United States of America has remained essentially free from the disease for some years although there have been some incidental cases resulting from relaxation of surveillance standards and increasing exchange of animals that may harbour *Brucella* organisms (Tanoue and Euge, 2003; Keyes *et al*., 2004; Hollet, 2006). For the rest of the Americas the prevalence is still high in some parts of central and mainly southern American countries, including Dominican Republic, Nicaragua, El Salvador, Bolivia, Brazil, Colombia and Ecuador. as a result of the use of unspecific vaccines (Xin, 1986; Garin and Benkirane, 1995; Anon., 1997; Ko and Splitter, 2003; Meeusen *et al*., 2007). In these countries, the problem of brucellosis is reportedly due to the fact that the *B. abortus* vaccines being used do not effectively protect against *B. suis* in cattle (Xin, 1986;

Garin and Benkirane, 1995; Anon., 1997; Ko and Splitter, 2003; Meeusen *et al*., 2007). The vaccine for *B. suis* has not yet been evaluated for use in cattle. This implies that *B. suis* is emerging as a public health/socio-economic problem in these countries especially Brazil and Colombia, where it causes infection in cattle but without abortion (Lopez, 1989).

Countries like Japan, Malaysia, Cyprus, Singapore, Bahrain and Philippines are brucellosis-free while other countries such as Sri Lanka, Mongolia, South Korea, China, Iraq and Indonesia have reported incidences of brucellosis in recent years (Anon., 1986). For example in 1995 Sri Lanka reported 6 human cases of brucellosis, South Korea had 14 human cases, China had 391 human cases, and Iraq had 7 human cases while Indonesia had 71 human cases (Corbel, 1997). In Oceania, countries such as New Zealand, Australia, Cooks Island and New Caledonia have eradicated the disease, except for Samoa (Rust, 2006).

2.5.0 Clinical Manifestations of brucellosis

2.5.1 Animal manifestation

In animals the disease is characterised by abortion, premature birth, dead or weak calves as well as loss in milk production (Benjamin and Annobil, 1992; Alton, 1990). *B. abortus* is the major pathogen for cattle while *B. suis* and *B. melitensis* can also cause infection in cattle as a result of sharing pasture and other facilities with infected pigs, goats or sheep (Hosie *et al*., 1985; Kiel and Khan, 1989). One third of the infected animals usually abort

at six months or later and there is sterility or infertility of either the male or female. When the organism is localised in the bull's testicles and other genital organs, one or both testicles may become enlarged resulting in decreased libido and infertility. Sometimes there is testicular atrophy as a result of adhesions and fibrosis. Cattle may present with hygromas and arthritis while seminal vesculitis and ompholitis are common in bulls (Musa *et al*., 1990; Geering *et al.,* 1995).

2.5.2 Human manifestation

In humans, *B. melitensis* is the most pathogenic and invasive species followed by *B. suis* and *B. abortus* (Anon., 1986; Alton *et al.,* 1988; 1990; Bricker and Halling 1994; Chomel *et al*., 1994). The symptoms take one to three weeks to manifest but sometimes can take several months. Patients usually manifest septicaemia, prolonged undulating fever, chills, profuse sweating and high temperatures (Falagas and Bliziotis, 2006). In advanced cases, there is encephalitis, meningitis, peripheral neuritis, spondylitis, suppurative arthritis and vegetative endocarditis (Woodruff *et al*., 1978; Young., 1995; Agarwal *et al*., 2000). Patient temperatures vary, ranging from normal (about 37ºC) in the morning to 40ºC in the afternoon. The most common symptoms are general malaise, insomnia, arthralgia, headache, anorexia, constipation and sexual impotence. Depression, nervousness and irritation present evidence of a marked effect on the nervous system. Other symptoms include enlarged peripheral lymph nodes, jaundice are indicative of hepatomegaly and spleenomegaly (Tsolia *et al*., 2002; Cobbaert *et al*., 2007; Mantur *et al*., 2007; Zribi *et al*., 2008). The duration of the disease varies from weeks or months to several years. The duration has been reduced to some extent by improved supportive treatment which has

also resulted in a reduction in the incidence of relapses. Some of the treatment strategies include a combination of injectable deoxymicin (12.5 %), streptomycin and rifampicin for 6 months. Some Tuberculosis first line drugs can also be used to treat brucellosis and these are ethambutol, pyrazinamide, rifampicin and streptomycin (Mantur *et al*., 2007).

2.6.0 Pathology of Brucellosis

2.6.1 Pathology of Brucellosis in animals

In animals following infection, the organism is found in blood (bacteremic phase). There is focalisation in a variety of tissues resulting in pathological changes in lymph nodes and in the female reproductive tract. These pathological changes affect a great deal of the female/male reproductive tract, udder, mammary gland and the lymphoidal tissues (Nicoletti, 1980; Radostits *et al*., 1994). Furthermore, there is a general development of granulomatous inflammatory response which is often found within lymphoid tissues and other organs of the reticuloendothelial system (Enright, 1990).

To a less extent focalisation of bacteria and white blood cells occurs in the bone marrow, bones, renal cortex and synovial membranes (Bracewell and Corbel, 1980; Jubb *et al*., 1985). Similarly, there is formation of granulomatous lesions composed primarily of macrophages, lymphocytes, plasma cells, variable numbers of neutrophils and generally, fibrosis or necrosis are rarely observed (Theon and Enright, 1986). Localised nonsuppurative interstitial nephritis is observed where the lesions are composed of

infiltrates of macrophages, large numbers of lymphocytes and plasma cells confined to the renal cortex (Jubb *et al*., 1985).

Necrotising orchitis in infected bulls is usually observed. The damage is irreversible, most cases are acute and one or both testicles may be affected (Madkour, 2001). This involves swelling of the scrotum and considerable inflammation of the tunics. Tunic spaces become distended, releasing hemorrhagic and fibrinopurulent exudates (Madkour, 2001). The testicular parenchyma shows the presence of necrotic foci which become progressively larger and finally become exudate – filled cavities enclosed by a fibrous wall (Bracewell and Corbel, 1980).

There is more frequent occurrence of epididymitis than orchitis in domestic animals as a result of *Brucella* infections. This may be due to spermatic granulomas as a result of abnormalities of the duct during development or trauma and infection (Jubb *et al.,* 1985). During infection there are lesions in the epididymis accompanied by large palpable swellings in the tail parts (Jubb *et al.,* 1985). There is unilateral or bilateral distribution of lesions causing blockade of the duct system, oedema and testicular degeneration (Jubb *et al.,* 1985). In severe epididymitis there is involvement of the prostrate glands which result in the atrophy of the testicles (Jubb *et al.,* 1985).

2.6.2 Pathology of Brucellosis in humans

In humans there are a number of manifestations that follow the colonization of the lymphatic system and the liver (Alton *et al*., 1988; Rust*,* 2006). This is an acute stage of

illness, patient's usually manifest prolonged undulating fever and malaise. The liver, spleen and other reticuloendothelial organs may develop characteristic pathological changes that include infiltration with epitheloid cells, foreign bodies and Langhans cells (Jensen *et al*., 1998; Rust, 2006). The blood picture presents leucopoenia and anaemia. There is elevation in the levels of serum enzymes, aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT) in the blood (McCullough and Paulson, 1998). In summary, the liver is the organ where granulomas are readily found and most likely the spleen and other organs may have lesions which together could be confused with tubercular granulomas (Spink, 1948). There is also necrotic inflammatory lesions and liver cirrhosis in humans (Costero, 1946).

2.7.0 Diagnostic methods of Brucellosis

The routine diagnostic laboratory tests for *Brucella* performed on suspected milk samples start with the Milk Ring Test, Californian Mastitis Test (CMT), Somatic Cell Counts and bacterial culture whose positive results might give positive confirmatory results for the disease. The overall laboratory diagnostic regime techniques for *Brucella* are bacteriological, serological, phenotypic grouping and molecular based methods (Nielsen, 1996). The bacteriological method includes culturing of samples such as aborted foetal stomach contents, milk, blood, lymph nodes and vaginal discharges from suspected cases for isolation and identification of the infecting *Brucella* organisms (Quinn *et al*., 1999; Anon., 2004a). The isolated organisms are further tested using molecular based tests. This sequence of confirmatory procedures is referred to as the "gold standard" method of identifying *Brucella* (Keid *et al*., 2007). The media that can be used for isolation of

Brucella is tryptocase soy agar (TSA) and selective *Brucella* agar enriched with 5% bovine serum or *Brucella* supplements for both media. Other media are *Brucella* broth, 5% Horse blood *Brucella* agar, modified Thayer-Martin's and Farrell's media (Anon., 2004a; Keid *et al*., 2007). The use of modified Thayer-Martin's and Farrell's media in duplicate increases the isolation sensitivity (Alton *et al*., 1988; Romero *et al*., 1995a; Gall and Nielsen, 2004). Among all the suspected *Brucella* samples, blood and foetal stomach contents give some of the best results in terms of isolation sensitivity as long as they are first cultured in enrichment broths and then sub cultured on enriched solid media (Alton *et al*., 1988; Bridgewater, 1989; Meyer *et al*., 2008). The culture media are then incubated in an atmosphere of 5 to 10% CO_2 at 37ºC for a period of 7 to 14 days depending on when the colonies start to appear (Alton *et al.*, 1988; Bridgewater, 1989; Meyer *et al*., 2008). It should be noted that samples such as milk, foetal stomach contents, and tissues are negatively affected by storage conditions from collection to processing, temperature, levels of contamination, availability of facilities and professional training (Macmillan *et al*., 1990; Romeo *et al*., 1995b; Bricker, 2002b; Anon., 2004a).

The serological methods for the diagnosis of *Brucella* include Rose Bengal Plate Test, Serum Agglutination Test, Complement Fixation Test, Fluorescent Polarisation Assay and Enzyme Linked Immuno-Sorbent Assay (Nielsen *et al*., 1996; Greiner and Gardner, 2000a; 2000b; Gall and Nielsen, 2004; Thrusfield, 2005). The Rose Bengal Plate Test (RBT) is a screening test with high sensitivity (90%) but low specificity (75%). As such it does not discriminate between S19 vaccinations and natural infections (Nielsen *et al*.,

1995; 1996). The procedure of RBT is that pink dyed *Brucella* antigens are added to equal amounts of test sera, mixed using an applicator stick and incubated at room temperature for 4 minutes. If *Brucella* antibodies are present in the sera, agglutination takes place. This is evidenced by formation of small aggregates on the slide for a positive test result.

The Serum Agglutination Test (SAT) is used to detect antibodies of IgM, IgA and IgG isotypes directed against the surface molecules (Alton *et al*., 1988; Chappel, 1989; Baum *et al*., 1995). The test is performed by preparing doubling dilutions of the serum in phenol saline in round–bottomed tubes and then adding equal volumes of standard antigen. The tubes are gently shaken to mix and incubated at 37ºC overnight. Standards representing different levels of agglutination are read by comparing the opacity, and the titers are then converted to international units (Dohoo *et al*., 1986; Alton *et al.,* 1988; Chappel, 1989; Baum *et al*., 1995). The SAT is more effective in vaccinated animals but is limited in that it does not discriminate among the relevant antibody isotypes (Nielsen *et al*., 1996).

The Complement Fixation Test (CFT) is used as a confirmatory test for *Brucella* species with high specificity (100%) but lower sensitivity (89.9%) (Nielsen *et al*., 1996). However its major limitations are the cumbersome procedures of inactivation of non-specific complements. Other limitations are the time consuming technical procedures and possible existence of residual anti complementary sera.

The Enzyme-linked immunosorbent assay (ELISA) in general detects antibodies that could have been missed by the RBT, SAT or CFT (Van Aert *et al*., 1984) while Indirect ELISA with its high specificity (98.9%) takes care of the limitations experienced in SAT which are cumbersome. Competitive ELISA (C-ELISA) is the best tool in that it has fewer stages compared to the other three diagnostic tests mentioned above. It is quite adaptive to reagents/automations and can discriminate vaccinated from infected cattle at the level of at least 85% specificity (Dohoo *et al*., 1986; Uzal *et al*., 1995). The principle for *Brucella* antibody C–ELISA is that serum samples are subjected to *B. abortus* smooth lipopolysaccharide (SLPS) coated wells on microtiter plates together with a mouse monoclonal antibody (mAb) specific for an epitope on the O–polysaccharide portion of the SLPS antigen. The microplates are washed after incubation and goat anti–mouse IgG antibody with horseradish peroxide is added which binds to any mAbs bound to the SLPS on the micro plate. If anti–*Brucella* natural antibodies are present in the test serum (positive) they would compete with the mAb for the epitope sites and thus inhibits it from binding to the O–polysaccharide portion of SLPS. In case the anti–*Brucella* natural antibodies were absent in the test serum (negative), the mAb would bind to the O–polysaccharide epitope of SLPS antigen. When the conjugate is added it binds specifically to this mAb. Unbound materials are removed by rinsing before the addition of substrate solution. Colour development is due to the conversion of substrate by the conjugate. Sera from strain 19 vaccinated cattle do not compete with mAbs because of their specificity and lower affinity. The optical density is measured by a micro plate photometer at 450 nm based on the amount of light transmitted after passing through the

solution. This is directly proportional to the concentration of bound antibodies in the solution.

Molecular based techniques have replaced phenotypic characterisation which classifies *Brucella* into biotypes according to the biological and physiological characteristics. These techniques comprise Polymerase Chain Reaction (PCR) using primers from 16S rRNA sequence of *Brucella abortus* and Restriction Fragment Length Polymorphism (RFLP) (Sifuentes *et al*., 1997; Bricker, 2002a; 2002b; Navara, 2003). Primers are universal and standardised so that they can be applied across all the molecular tests where *Brucella* is suspected. A variety of target sequences have been chosen for *Brucella* DNA amplification (Bricker and Halling, 1995; Rijipens *et al*., 1996).The most frequently used genes for amplification are omp2, 16S rRNA, IS711 targeting the erythritol and protective genes (Debeaumont *et al*., 2005). The IS711/alkB based primers have a high sensitivity and species specificity (Doust *et al*., 2007). These two distinct primer sequences targets the alkB and IS711 genes (Newby *et al*., 2003). The 157 bp fragments are amplified with *Brucella arbortus* primers derived from alkB genetic elements and IS711gene (Bally *et al.,* 1992). The primers have the following sequences respectively: 5'-CCATTGAAGTCTGGCGAGC-3' and 5'-CGATGCGAGAAAACATTGACCG-3'.

PCR amplifications are carried out as described by Nielsen *et al* (1996) and the PCR products are confirmed by RFLP technique using specific endonuclease enzyme. The PCR is more specific up to species level. These are some of the recent advances in microbiological techniques to counter the limitations in culture and serological methods.

Though expensive to run these methods are efficient in detecting *Brucella* organisms (Nielsen *et al.,* 1996; Diallo and Thiaucourt, 1997).

2.8.0 Brucellosis control

2.8.1 Control of Brucellosis in animals

There are a number of approaches in the brucellosis control and eradication programmes which include vaccination of animals, surveillance, testing, quarantine and culling (Godfroid, 1992; Madkour, 2001). In some countries like the United Kingdom, the United States of America and Canada, animal vaccinations, surveillance, testing (serological and molecular based) and slaughter methods have essentially freed them from the disease for some years although there have been some incidental cases as a result of relaxation of the above mentioned control methods. The other factor is the increasing exchange of animals that may be harbouring *Brucella* organisms (Hosie *et al*., 1985; Kiel and Khan, 1989). Animal vaccination in endemic areas has been the most effective control method. An attenuated vaccine strain that induce a T- cell mediated immune response grants a more improved immunity than killed vaccines (Tizard, 2000). In many countries, S19 vaccine was the only *Brucella* vaccine used for the control programmes until recently, RB-51 vaccine has been introduced on the market which is also a live vaccine derived from rough strain of *B. abortus*. Standard brucellosis serological tests do not detect antibodies stimulated by RB-51 hence avoiding the problem of detecting brucellosis-vaccinated animals testing positive (Uzal *et al*., 2000; Ramirez *et al*., 2002). However, S19 is still the most effective vaccine used to control

brucellosis. The attenuated strain is a live vaccine that ignites the immune response of the vaccinated animal to resist *Brucella* infection by producing antibodies against the attacking organisms and getting rid of the dead organisms by phagocytes. These antibodies produced against the disease disappear from the systemic circulation in a few months although life long immunity has been suggested so that the animal retains the resistance to disease for years (Tizard, 2000). In developing countries the S19 vaccine is still in use as it is easily produced. The disadvantage in its use has been the tendency to stimulate systemic clinical signs such as anorexia, drop in milk yield, oedema at the injection site, listlessness and high fever. Other signs may include abortion in pregnant cows, orchitis in bulls and febrile disease in humans (Tizard, 2000).

2.8.2 Control of Brucellosis in humans

Prevention of human brucellosis depends on the capacity to control the disease in animals. Moreover in humans, vaccinations with both killed and attenuated live vaccines have been attempted. However, the risk of using live vaccines and the painful oily adjuvants of killed vaccines makes the research in finding suitable vaccines an on going activity in several countries (Madkour, 2001). Therefore as such, vaccination of cattle, goats and sheep is the effective method to reduce the prevalence of brucellosis in both livestock and humans (Madkour, 2001).

In Zambia there have been no notable Brucella control programs at national level. Although the commercial dairy sector has made it mandatory for all its members to control brucellosis, not all farmers have adhered to these regulations. This is because of

the tendency to avoid costs of vaccinating animals and factors associated with vaccination (Tizard, 2000). Members of co-operative societies who include small scale dairy farmers are also under obligation to vaccinate their animals using locally produced S19 vaccine as a pre-condition for the sale of their milk to the processors but, the majority do not meet these conditions. The control of stock movement is one of the methods generally used to control diseases such as brucellosis in Zambia. This is because screening and testing of animals is done along with the exercise of stock movement (Anon., 2004a).

Surveillance is one of the preventive measures, but may not capture all *Brucella* cases as some may go unnoticed. Testing and culling may help in screening and confirming suspected cases at the same time getting rid *of Brucella* positive animals, while in some cases cross reactions give false positive reactions resulting into culling wrong animals. Quarantine of suspected or animals in transit may give chance for screening the animals to prevent transmission of brucellosis. Therefore prevention of brucellosis is accomplished by official calfhood vaccination of heifer calves. Furthermore, no treatment is used in animals as the economic value of an animal is out weighted if treatment is applied. A combination of two or more antibiotics like doxycycline, streptomycin, rifampicin and gentamycin are used in human beings. In case of animals, the amount of drugs needed for the exercise outweigh the economic value of an animal. Granted this economic and opportunity cost involved, most farmers don’t have the capacity to continue with the treatment because it is time consuming and very expensive. Therefore, vaccination of young animals has proved to be the best preventive measure.

CHAPTER FOUR

RESULTS

4.0.0 Descriptive results

A total number of 50 farms (from Lusaka, Chongwe and Kafue districts) comprising 849 cattle were investigated during the study period between January 2007 and February 2008. In addition 5 farms comprising 48 cattle in Chibombo District of Central Province were sampled for comparison. The latter is an important nursery for cattle farms in Lusaka Province. Details of targeted and actual number of herds and cattle from each district are shown in Table 1 below.

Table 1: Details of sampling activities in Lusaka province and Chibombo district commercial cattle farms during the study period.

Province	Study area (District)	Number of farms targeted	Targeted number of cattle	No. of Farms sampled	Actual number of cattle sampled	Median herd size
Lusaka	Kafue	19	190	25	427	66
	Chongwe	19	190	19	359	58
	Lusaka	19	190	6	63	25
	Total	57	570	50	849	50
Central	Chibombo	10	100	5	48	15
Overall	Total	67	670	55	897	41

Of the 427 cattle sampled from Kafue district, 33 (7.73%, 95% CI: 5.5 to 10.8) were positive on RBT out of which 1 (0.2%, CI: 0 to 1.5) was confirmed positive on C-ELISA (Table 2). Of the 359 cattle sampled from Chongwe district, 79 (22.0%, CI: 17.9 to 26.7) were positive on RBT and 10 (2.7%, CI: 1.42 to 5.23) were confirmed positive on C-ELISA. 63 cattle were sampled from Lusaka district out of which 4 (6.4%, CI: 2.1 to 16.3) were positive on RBT but were all negative on C-ELISA. From the total 849 cattle sampled from Lusaka Province, 116 (13.7%) were positive on RBT out of which 11 (1.3%, CI: 0.07 to 2.4) were positive on C-ELISA. In Chibombo district which was included for comparisons and close proximity to Lusaka Province 48 cattle were sampled and 12 (25.0%, CI: 14.1 to 39.9) were positive on RBT out of which 3 (6.3%, CI: 1.6 to 18.2) were confirmed positive on C-ELISA. The estimated overall seroprevalence in Lusaka Province was 9.4%, (CI: 5 to 13.6 %) based on serial interpretation. Seroprevalence in the Chibombo district, which is more rural, was even higher than all, the sampled districts in Lusaka Province (Table 2). The confidence intervals, serial and parallel interpretations of the results are also depicted in Table 2.

Table 2: Test results on RBT and C-ELISA with the estimated district prevalence at 95% Confidence Interval by serial and parallel analysis.

Study area (District)	Serum samples tested on RBT	Proportion (%) positive on RBT	Proportion (%) positive on C-ELISA	Serial interpretation (%)	Parallel interpretation (%)
Kafue	427	33 (7.7)	1 (3.0)	2.9 (0 to 6.6)	8.2 (0 to 15)
Chongwe	359	79 (22.0)	10 (2.8)	12.8 (8.2 to 91.7)	22.8 (14.1 to 31.5)
Lusaka	63	4 (6.3)	0 (0)	3.1 (0 to 6.5)	6.3 (0 to 13)
Total	849	116 (13.7)	11 (1.3)	9.4	14.2

		(7.8 to 19.5)	(0.07 to 2.38)	(5 to 13.6)	(8.2 to 20.1)
Chibombo	48	12 (25.0)	3 (6.3)	18.7 (7.5 to 29.9)	25 (10.6 to 39.3)

At herd level, sero-prevalences according to district are depicted in Table 3. A total number of 50 herds were sampled in Lusaka Province. Kafue district had 25 herds sampled of which 5 herds tested positive representing 20% (95% CI: 3 to 36 %) prevalence. Chongwe district sampled 19 herds and 13 (68.4%, 95% CI: 47.6 to 89.1 %) herds tested positive, representing 68.4% (95% CI: 47.6 to 89.1%) prevalence. In Lusaka district 6 herds were sampled of which 2 (33.3%, 95% CI: 17 to 63.3%) tested positive. Therefore from the total of 50 herds sampled in Lusaka Province, 20 tested positive. In Chibombo district all the 5 herds sampled were positive representing 100% prevalence.

Table 3: Herd level prevalence of Brucellosis in Lusaka Province according to district and in Chibombo district.

District	Number of Herds Tested	Number of Herds Positive	Prevalence (%)	95% Confidence Interval
Kafue	25	5	20	(3 to 36.1)
Chongwe	19	13	68.4	(47.6 to 89.1)
Lusaka	6	2	33.3	(17.6 to 63.3)
Overall Lusaka Province	50	20	40.6	(16.4 to 67.9)
Chibombo	5	5	100	-

Additional to individual and herd level sero–prevalences, serial and parallel interpretations of the RBT and C-ELISA results were used to estimate prevalence levels

by sex and age group. This perhaps provides an insight into the possible contributions of these variables to the overall individual level prevalences observed above.

The prevalences according to sex were oxen (n=10) 0%; cows (n=791) 8.1% (CI: 4.6 to 11.6%) and bulls (n=96) 12.5% (CI: 3.8 to 21.1%). Prevalences according to age group were 1 to 4 years (n= 357) 10.7% (CI: 4.9 to 16.9 %); 4.5 to 5 years (n= 152) 4.2 % (CI: 0.0 to 7.8%); 5.5 to 7 year (n=178) 6 % (CI: 2.0 to 9.9 %) and more than 7 years (n=162) 9.9 % (CI: 2.3 to 17.5%). The results of the estimations are depicted in Table 4.

Table 4: Individual level prevalence of brucellosis in Lusaka province and Chibombo district according to sex and age group during the period January 2007 to February 2008.

Variable	Category	Number Tested	Number Positive	Prevalence (%)	95 % Confidence Interval
SEX	Oxen	10	0	0	-
	Cows	791	64	8.1	(4.6 to 11.6)
	Bulls	96	12	12.5	(3.8 to 21.1)
AGE	1 to 4 years	357	38	10.7	(4.9 to 16.94)
	4.5 to 5 years	152	6	4.2	(0.0 to 7.8)
	5.5 to 7 years	178	10	6	(2.0 to 9.9)
	> 7 years	162	16	9.9	(2.3 to 17.5)

4.1.0 Risk factor analysis.

Risk factors associated with *Brucella* infections were compared using the Fisher's exact test. Each variable and its response categories, prevalence and P-values are shown in Table 5. The tests are based on serial interpretation.

Table 5: Management related risk factors/predictor variables for prevalence of brucellosis in commercial cattle herds in Lusaka Province (January 2007 to February 2008).

Variable	Responses	Negative (%)	Positive (%)	Total	*P* Value
Acquisition of property	Bought	20 (55.6)	16 (44.4)	36	
	Heritage	5 (31.3)	11 (68.8)	16	0.085
	Others	0 (0.0)	3 (100.0)	3	
Hire out bull	No	2 (28.6)	5 (71.4)	7	
	Yes	8 (16.7)	35 (81.3)	43	0.141
	Occasional	1 (100.0)	0 (0.0)	1	
Source of stock	Animal market	8 (47.1)	9 (52.9)	17	
	Any farm	5 (16.7)	25 (83)	30	0.013
	Donation/NGOs	1 (14.3)	6 (85.7)	7	
	No records	1 (100.0)	0 (0.0)	1	
Manure disposal	Dried collected later	8 (20.0)	32 (80.0)	40	
	Drain sewerage	3 (37.5)	5 (62.5)	8	0.068
	Dried & Drain Sewerage	3 (60.0)	2 (40.0)	5	
	Others	1 (50.0)	1 (50.0)	2	

Member of a cooperative	No Yes	10 (33.3) 5 (20.0)	20 (66.7) 20 (80.0)	30 25	0.164

For the method of "acquisition of property" risk variable, the responses categories were "bought" referring to farmers who had bought their cattle from markets such as fellow farmers, institutions or cooperatives. Of these, 16 out of 36 farms were sero-positive for brucellosis. "Heritage" was the acquisition of farms through succession and 11 out of 16 cattle herds under this category were sero-positive. "Others sources" meant farms with animals that had been acquired mainly through donations and all the 3 farms were sero-positive.

The variable of "hiring out a bull" had "Yes" or "No" and "Occassional"responses which entailed the practice of a farmer hiring out the bull to other farmers. For the seven farmers who did not hire out their bulls, five had brucellosis positive bulls whereas among those who hired out (n=43), 35 had sero-positive bulls. The response, "Occasional" described those farmers who occasionally hired out the bull and had no (0) sero-positive bull.

The variable "Source of stock" had "animal market" which implied sourcing stock from established farms such as fellow farmers, institutions and cooperatives. Of the 17 cattle herds under this category, 9 were sero-positives. The variable, "Any farm" under this category, meant those farmers who bought animals from anywhere else and of the 30 under this category, 25 herds were sero-positives. The response, "Donation/ NGOs" represented farmers who sourced animals through funding from Non Governmental

Organisations and had 6 of the 7 herds sero-positives. “No records” referred to farmers sourcing animals from elsewhere and had no sero-positives animals.

The “Manure disposal” variable referred to the methods of getting rid of manure which had four categories of disposal or responses (Table 5). The results show that this method of manure disposal not was statistically associated with herd *Brucella* positivity (P=0.068) at 95% confidence level. The variable, “Member of a cooperative” had two responses, “No” representing those farmers who were not members comprising of 30 herds in this category, 20 herds were sero-positives. Those who were members of any cooperative were in the “Yes” category of which 20 herds were sero-positive out of the total 25. Thus from the above, source of stock was the only significant risk factor identified at 95% confidence interval.

CHAPTER FIVE

DISCUSSION

This study was carried out to estimate the sero-prevalence of brucellosis and determine the risk factors associated with the disease's occurrence in commercial cattle farms in Lusaka Province and Chibombo district. For the sake of this study a commercial cattle farmer was regarded as any person who keeps/rears cattle to produce milk/beef mainly for sale regardless of his or her scale of production in this study. This meant that even people owning only a few dairy and beef cattle were regarded as commercial farmers and therefore were included in the study. This was important as it greatly minimised the probability of missing a possible risk factor.

Prior to this study being undertaken in Lusaka Province, there was a perception that there is little or no brucellosis in commercial cattle farms in Zambia (Kadohira *et al*., 1996). The results of this study indicate an individual cattle sero-prevalence of 9.4% (Table 2) and a herd level sero-prevalence of 40.6% on the average (Table 3), thus contradicting this perception and perhaps laying a foundation for enhanced control efforts against the disease on these farms. Currently, Government is in a dilemma on how to effect its "test and slaughter" policy against brucellosis in the traditional sector but this should not be the case with regard to the commercial sector. This study identified "source of stock" as a risk factor that is significantly associated with brucellosis occurrence in the commercial dairy and beef–sector. This could provide a guide for would-be small scale dairy producers on the possible prevention of brucellosis. To the best of our knowledge this is

the first time such a study on brucellosis has been carried out in Lusaka Province commercial cattle farms.

The projected total number of herds that were available for sampling in Lusaka Province was 76. However, only 50 herds could be sampled due to a number of factors such as resource availability and time, and the brucellosis vaccination status of animals observed in some areas which led to the dropping out of some farms. The author tried to compensate for this when chances allowed by sampling more farms in Kafue and Chongwe districts. This resulted in a total sample of 849 cattle from the 3 districts of Lusaka Province instead of the projected 760.

Areas close to Lusaka Province such as Chibombo District in Central Province constitute an important cattle nursery for Lusaka Province and an important source of milk/beef for Lusaka Urban District. Thus, cattle from this district can act as a source of infection to cattle in both the Province and the District. In this regard and for comparison purpose, a sample of 5 herds comprising 48 heads of cattle was drawn from Chibombo District. Although the sample sizes was low in comparison to the districts in Lusaka Province, the resulting individual animal and herd level sero-prevalences of 18.7% and 100% respectively were relatively high.

As indicated in Table 2 and in agreement with Nielsen *et al.* (1996), a general observation in all the four study areas with regard to individual and overall positivity is that RBT tended to show high sensitivity while C-ELISA exhibited high specificity.

Because of the high sensitivity, it is possible that the RBT positivity (13.7 %) included some animals that might have been acquired from outside and therefore possibly vaccinated as calves but without any proper records (Nielsen, 2002). Furthermore, cross reactions with smooth lipopolysaccharides (SLPS) of organisms such as *Yersinia enterocolitica* 09, *Escherichia coli* 0157: H7, *Campylobacter* and *Salmonella* antibodies (Murioz *et al*., 2004; Mainar-Jaime *et al*., 2005), could have added to false positives. While this could be true for RBT, it should not be the case with C-ELISA (Gardner *et al*., 2000; Godfroid *et al*., 2002). Hence the two tests were suitably and correctly used in this study as screening and confirmatory tests respectively.

In this study the RBT and C-ELISA results were interpreted both in series and parallel (Table 2) according to OIE (2002) guidelines. The interpretation in series testing means that only those animals which were positive both on RBT and C-ELISA were classified positive where as in the parallel testing animals reacting positive either RBT or C-ELISA are classified as positive (Gall and Nielsen, 2004). This study, being an epidemiological one and because of the implications of the "test and slaughter" policy currently being effected in the commercial dairy sub-sector with regard to brucellosis, series interpretation of total prevalence was preferred.

Previous studies on brucellosis in the traditional sector in Zambia have estimated its probable individual prevalence at 16% (CI: 14 to 28%) (Muma *et al*., 2006). *Brucella* prevalence of 9.4% (CI: 5 to 13.6%) in commercial cattle reported in this study is significantly lower ($P < 0.05$). The difference in prevalence rates between the two sectors

could be due to the fact that most commercial farmers vaccinate their animals or take precautions when purchasing their replacement stock. Other factors may be management of the livestock within restricted boundaries and improved feeding regimen. The sero-prevalence reported in the current study is lower than the sero-prevalence reported in high rainfall regions of West (16% to 25%) and Central (over 25%) Africa (Thimm, 1982). May be, the climate in high rainfall regions allows the organism to survive longer and then contamination is in the environment for a much longer time. Our environment is dry, meaning the bacteria could be having a shorter period of survival as a result of hot weather. In commercial dairy farms of other regions such as Tanzania (Msanga *et al*., 1986), Sudan (McDermott *et al.*, 1987), Nigeria (Rikin, 1988) and Eritrea (Omer, 2000), an average sero-prevalence of about 35.9% has been reported which is considerably higher than that obtained in this study (9.4%). This has good implications for Zambia with regard to a possible milk trade within the COMESA region as this shows better quality milk.

At individual animal level, more positives were recorded among bulls compared to other sex categories (Table 4). This could be attributed to sourcing of bulls which is often done without screening for brucellosis or having records thereof (Gray and Martin, 1980). This presents a big problem especially that the practice of hiring out of bulls was found to be common. The major cause of all this being the exposure factor where the risk is higher in bulls. For instance, one bull services up to 30 cows in a contiguous herd. Therefore multiple exposures are a big problem with bulls per time. Outsourcing of bulls, taking out

or bringing in a bull from one or a different herd where one bull can be used to serve up to more than 10 independent herds.

Some variations in infection levels according to age group were observed (Table 4). The prevalence was highest (10.7%) among animals aged 1 to 4 years followed by those above 7 years (9.9%), the age group of 4.5 to 5 years had the lowest prevalence level of 4.2%. This pattern of positivity is difficult to explain or interpret as it does not seem to follow the norm where the prevalence is expected to be low in the 1 to 4 years age group and to rise with age even if the positive cattle were later vaccinated (Kadohira *et al*., 1997). Thus for this aspect of the study, further work is suggested.

At herd level, Chongwe district had the highest prevalence of 68.4% (CI: 49.2 to 90.7%) followed by Lusaka, 33.3% (CI: 4.3 to 84.3%) and Kafue 20% (CI: 3 to 36.1%). With regard to Chongwe and Lusaka districts, this could be attributed to the fact that the former had a larger cattle population and therefore a bigger sample size than the later. Another reason for lower prevalence in Lusaka district could be the easier access to information and veterinary services on brucellosis for farmers in the district. Although Kafue district had the largest cattle population and sample size, the prevalence was the lowest. This could be due to the fact that in terms of preventive measures, commercial cattle farmers in Kafue district seemed to be quite ahead of those in the other districts as evidenced by many animals being excluded from the study based on their brucellosis vaccination status. Chibombo district in Central Province which was included in the study for comparison had the highest herd level prevalence (100%). However, the fact

that all the 5 herds sampled were positive could also imply a very high prevalence, thus supporting the assertion of its being a potential source of brucellosis for Lusaka Province. On average, the overall brucellosis herd level prevalence in Lusaka province commercial cattle farms in this study was 40.6% (CI: 16.4 to 67.9%).

In addition to sero-prevalence, this study was also carried out to determine the risk factors associated with disease occurrence in commercial cattle farms in Lusaka Province and its vicinity. Among the hypothesized risk factors subjected to analysis (Table 5), the study found significant associations between herd level brucellosis prevalence and sourcing of animals on the farm (P=0.013). In the “acquisition of farms” variable, as witnessed by author during interviews, the significance of “heritage (68.7% positivity)” could be attributed to the fact that in many cases where farms were inherited, the person who took over seemed to have little or no knowledge of animal husbandry while in the case of “purchase”, although still not significant as a risk factor, the lower positivity (44.4%) compared to the other categories may imply that the farmers might have been practising preventive measures such as vaccination.

In the “Sourcing of animals” variable, this study’s *Brucella* positivity results at herd level were 85.7% for those acquired through donations by NGOs, 83.3% for those where stock had been purchased from any farm and 52.9% for those where stock had been purchased from an animal market (Table 5). Thus sourcing of stock from clean herds is very important (Thimm, 1982). The tendency when animals are being donated could be that there is no particular attention paid to attributes such as animal health, history and disease

screening before delivery as evidenced for such animals during interviews with the farmers. The animals could have been bought from any farm and donated to a cooperative, organization or an individual. This observation can significantly increase the risk of Brucellosis in animals. Preventive measures that include vaccination, screening (test and slaughter) through monitoring and surveillance, closed herd systems by using artificial insemination and no bull importation policy are strictly supposed to be noted. These are records that are supposed to be known for animals kept in a closed population.

With regard to the methods of manure disposal, herd *Brucella* positivity was 80% on farms that dried and collected the manure later and 62.5% for those that used a sewerage drainage system. This is in agreement with findings by Nicoletti (1980) and Anon. (1986) of *Brucella* organisms can survive in the afterbirth, aborted foetus and water in manure left to dry on the pasture for a considerable number of days, hence contaminating the soils and water sources. This finding is also in agreement with Omer (2000) who reported an association between *Brucella* antibodies in dairy cattle herds and disposal of manure through sewage in some regions of Africa.

The system of hiring out a bull had *Brucella* sero-positivity at 81.4% for farmers practising it while those not engaged in it had *Brucella* sero-positivity at 71.4% herd level positivity hence not significantly different. The farmers hiring-out bulls had a higher prevalence because this led to multiple herd contact and therefore a higher chance of contracting the disease especially with regard to *Brucella abortus* which can be sexually transmitted (Foster and Smith, 2008). These results are similar to findings of a study in

the traditional sector (Muma *et al*., 2006) where farmers who were hiring-in bulls had a higher herd-level *Brucella* sero-positivity (73.0%) than those using their own bulls (57.5%).

In this study the farmers who were members of a cooperative had 80% herd level positivity compared to 66.7 % for those who were not. It seems that being a member of a cooperative predisposes the herd to a higher risk of *Brucella* infections although in this study the association was not significant (p=0.164). A farmer who is a member of a cooperative renders his animals to mix with other herds at dipping sites and vaccination campaigns thus increasing the risk of *Brucella* infections (Muma *et al*., 2006). When artificial insemination is used in most cooperatives and some of the large scale set ups, there may be no sanitisation of equipments as it was observed in this study. Furthermore there was no change of AI equipments between animals as well as no sanitisation between animals. This results in passing of infection from one cow to the other. Since *Brucella* organisms are known to survive in disseminated body fluids, it should be stressed that aseptic techniques must be applied when performing AI to avoid transmission through this method. In case only one insemination gun is being used, it should be disinfected adequately in a suitable disinfectant for at least 10 minutes. Thereafter rinsed in clean water and then used in between cows. The handlers should also be disinfecting and washing all the protective clothing's in between cows. I

CHAPTER SIX

RECOMMENDATIONS

1. There must be enhanced control efforts against the disease on these farms according to both nationally and internationally laid down procedures for commercial dairy and beef cattle enterprises without regard to their size. The following control efforts are recommended:

a. Mandatory vaccination of cattle against brucellosis.

b. Screening (tests (RBT & Competitive ELISA) and slaughter).

c. Monitoring by local Veterinarians through periodic surveillance as per standard procedures.

d. Closed herd management system (use of AI).

e. Strict herd health system (use of pedigree bulls).

f. Enhance efforts to mitigate disease transmission from wildlife especially traditional herds.

g. Establishment of a brucellosis national control scheme through beef/dairy marketing companies to screen animals for brucellosis every six months.

2. The sourcing of animals on farms should be cautiously done especially when buying from small scale farmers for fattening.

3. Animals being transferred from surrounding districts of Lusaka Province for breeding purposes should first be screened for brucellosis using RBT and C-ELISA tests.

4. More work is recommended to explain the pattern of brucellosis positivity according to age groups observed in this study.

CHAPTER SEVEN

CONCLUSION

1. This is the first study to estimate the sero-prevalence of brucellosis in commercial cattle farms in Lusaka Province. This study has indicated an average individual sero-prevalence of 9.4 % and a herd level sero-prevalence of 40.6 % by C-ELISA.

2. The results of this study indicate the presence of brucellosis in commercial cattle farms as opposed to the earlier perception that brucellosis was only prevalent in traditional cattle.

3. The study has exposed the need for change in the current definition of a commercial cattle farmer due to the high public health risk involved. In this study, a commercial farmer was regarded as any person who keeps/rears cattle to produce beef/milk mainly for sale regardless of his or her scale of production.

4. Chibombo district in Central Province which was included in the study for comparison purposes had the highest herd level prevalence (100 %).

5. The presence of brucellosis in Chibombo district could imply that districts neighbouring Lusaka Province could indeed be acting as sources of brucellosis for the Province.

6. The study found significant associations between herd level brucellosis prevalence and source of animals on the farm.

7. This study therefore lays a foundation for enhanced control effort against brucellosis.

REFERENCES

Agarwal, N., A. Joshi, N. Jain, B. V. Sharma and D.K. Kochar. (2000). Human Brucellosis: A Grossly Under diagnosed Disease in India (A prospective, study of 75 cases). Department of Medicine, SP Medical College, Bikaner.

Ahmadu, B., M. S. Sikazwe, P. Sakala and G. S. Pandey. (1999). Sero-prevalence of Bovine Brucellosis in cattle at Lusaka abattoirs. Bulletin of Animal Health and Production in Africa, 47:119 – 121.

Akay, H., S. Girgin, C. A. Ozmen, I. Kilic and H. Sakarya. (2007). Department of Radiology and General Surgery, University of Dide Faculty of Medicine, Diyashkir Government Hospital, Diyarbakir, Turkey. Acta Chir belg, 107: 320-322.

Al-Shamahy, H. A., C. J. M. Whitty and S. G. Wright. (2000). Risk factors for human *Brucellosis* in Yemen: A case control study. Epidemiology of infections, 125: 309 – 313.

Alton, G. G., L. M. Jone, R. D. Angus and J. M. Verger. (1988). Bacteriological methods, in laboratory techniques in brucellosis, 2nd Ed. World Health Organisation, Geneva, Page 11-64.

Alton, G. G., (1990). *Brucella melitensis*. In:“Animal brucellosis“ (Nielsen,K., J.R. Duncan eds).CRC Press. Boston, Page 383-409.

Amato Garcia, A. J. (1995). The return of Brucellosis. Maltese Journal, 7: 7 – 8.

Anonymous (1986). Joint FAO/WHO Expert committee on Brucellosis: Sixth report, Technical Report series 740. In Sixth report (Geneva, Swizerland, WHO).

Anonymous (1997). WHO meeting on development of new/improved animal brucellosis vaccines.Mediterranean Zoonosis Control Centre, Information Circular No. 44 December.

Anonymous (2000). Office International Des Epizootics: Annual report http//www.oie.int.eng/normes.

Anonymous (2002). Bovine Brucellosis Chapter 2.3.1. Office International Des Epizootics: Paris, France,

Anonymous (2004a). Manual of Diagnostic tests and vaccines for terrestial animals. Office International Des Epizootics: Paris, France, Page 409-438.

Anonymous (2004b). Annual Report for 2003 (Lusaka, Zambia, Ministry of Agriculture Food and Fisheries, Department of Veterinary Services).

Anonymous (2005). Government of Zambia: Fifth National Development Plan 2006 to 2010.

Anonymous (2006). Annual report of the department of research and specialist services. Government Printers, Lusaka Zambia; Lusaka, Zambia.

Ashford, D., J. D. Pietra, J. Lingappa, C. Woods, H. Noll, B. Neville, R. Weyaint, S. Bragg, R. Speigel and J. Tappero. (2004). Adverse events in humans associated with accidental exposure to the livestock brucellosis vaccine RB51.Vaccine, 22: 3435-3439.

Baily, G. G., J. B. Krahn, B. S. Drasar and N. G. Stoker. (1992). Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. Journal of Tropical Medical Hygiene, 95: 271-275.

Bandara, A. B. and M. B. Mahipale. (2002). Incidence of brucellosis in Sri Lanka: An overview. Veterinary Microbiology, 90: 197-207.

Baum, M., O. Zaimir, R. Bergman, E. Katz, Z. Beider, A. Cohen and M. Banai. (1995). Comparative Evaluation of Micro agglutination Test and Serum Agglutination Test as supplementary Diagnostic Methods for Brucellosis. Journal of Clinical Microbiology, 33: 2166-2170.

Benjamin, B. and S. H. Annobil. (1992). Childhood brucellosis in South-western Saudi Arabia: a five-year experience. Journal of Tropical Paediatrics, 38: 167 – 72.

Berkelman, R. N. (2003). Human illness Associated with use of Veterinary Vaccines. Clinical Infectious Diseases, 37:407-414.

Bernard, T. M. (1982). Brucellosis distribution in man, domestic and wild animals Berlin Springer – Verlag Berlin, Page 13 – 26.

Betram, T., P. Canning and J. Rotto. (1986). Preferential inhabitation of primary granule release from bovine neutrophils by a *Brucella abortus* extract. Infectious Immunology. 63:3297 – 3301.

Bishop, G. C., P. Bostoman and P. Herrs. (1994). Bovine Brucellosis. In: infectious diseases of livestock with special reference to Southern Africa. Vol. 2 (Coetzer, G.R and Tustin, R.C., Eds), Oxford University Press Cape Town.

Bracewell, C. D. and M. J. Corbel. (1980). An association between arthritis and persistent serological reactions to *Brucella abortus* in cattle from apparently brucellosis-free herds. Veterinary Record, 106: 99.

Bricker, B. J and B. M. Halling. (1994). Differention of *Brucella abortus* by 1, 2, and 4 *Brucela melitensis, Brucella ovis* and *Brucella suis* by 1 by PCR. Journal of Clincal Microbiology, 32: 2660.

Bricker, B. J and S. M. Halling. (1995). Enhancement of the *Brucella* AMOS PCR assay for differentiation of *Brucella abortus* vaccine strains S19 and RB51. Journal of Clinical Microbiology, 33: 1640-1642.

Bricker, B. J. (2002a). Diagnostic Strategies used for the Identification of *Brucella.* Veterinary Microbiology, 90: 433-434.

Bricker, B. J. (2002b). PCR as a diagnostic tool for brucellosis. Veterinary Microbiology, 90: 435 – 46.

Bridgewater, D. R. (1989). Epidemiological report, Western regional epidemiologist U.S. Dept. Agric. Animal Plant Health Inspection service.

Canning, P. C., J. A. Roth and B. L Deyoe. (1986). Release of 5 – guanosine monophosphate and adenine by *Brucella abortus* and their role in the intracellular survival of the bacteria. Journal of Infectious Diseases, 154:464-470.

Cannon, R. M. and R. T. Roe. (1982). Livestock disease surveys. A field manual for veterinarians. Bureau of Range Science, Department of Primary Industry. Canberra: Australian Government Publishing Service.

Cannon, R. M. (2001). Sense and Sensitivity designing surveys based on an imperfect test. Preventive Veterinary Medicine, 49: 141-163.

Centers for Disease Control and Prevention. (2000a). Suspected brucellosis case prompts investigation of possible bioterrorism – related activity: New Hampshire and Massachusetts, 1999. MMWR: Morbidity and Mortality Weekly Report, 49: 509 – 512.

Centers for Disease Control and Prevention. (2000b). Biological and Chemical terrorism: strategic plan preparedness and response. MMWR: Morbidity and Mortality Weekly Report, 49: 1 – 4.

Chappel, R. J. (1989). Diagnosis of bovine brucellosis: principles, practice and problems. Surveillance, 16: 3 – 6.

Cloeckaert, A., J. M. Verger, M. Grayen, J. Y. Paquet, B. Garin-Bastufi, G. Foster and J. Godfroid. (2001). Classification of *Brucella spp* isolated from marine mammals by DNA polymorphism at the *Omp2* locus. Microbes and infection, 3:729 – 738.

Cloeckaert, A., J. Y. Vizcaino Nipaguet, R. A. Bowden and P. H. Elzer. (2002). Major outer membrane proteins of *Brucella* spp therefore past, present and future. Veterinary Microbiology, 90: 229-247.

Clockaert, A., M. Grayon, O. Grepinet and K. S. Boume Dine. (2003). Classification of *Brucella* strains Isolated from marine mammals by infrequent restriction site-PCR and development of specific PCR identification tests. Infectious Immunology, 75:11.

Cobbaert, K., A. Pieters, M. Devinck, M. Devos, I. Goethals and H. Mielants. (2007). *Brucella* spondylidicitis: Case report. Acta Cline Belg., 5: 304 – 307.

Coghlan, J. D. (1995). *Brucella.* In: Greenwood D., Slack R.C.B, Peuther J.F. (eds) Medical Microbiology. A microbiology guide to Microbial infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control. Page 325 – 328.

Cokca, F., A. Azap and O. Meco. (1999). Bilateral mammary abscess due to *Brucella melitensis.* Scandinavian Journal of Infectious Diseases, 2:318 – 319.

Colmenero, J.D, J. M. Reguera, F. Martos, D. Sanchez-de Mora, M. Delgadon, M. Causse, A. Martin–Farfan and C. Juarez. (1996). Complications associated with *Brucella melitensis* infra a study of 530 cases. Medicine (Baltimore) 75:195 – 211.

Corbel, M. J. (1997). Brucellosis an overview. Emerging Infections, 3: 213-221.

Costero, I. (1946). Tratado de Anatomia Ratologica, Edit. Attante, Mexico. Page 49 – 52

D'cruz, S. (1987). Zambia: Brucellosis in cattle and small ruminants: Diagnosis, Control and vaccination. Technique series office of international Epizootics, 6:74 – 79.

Debeaumont, C., P. A. Falconnet and M. Maurin. (2005). Real-time PCR for detection of *Brucella* spp. DNA in human serum samples. European Journal of Clinical Microbiology of Infectious Diseases, 24: 842-845.

Diaz–Aparicho, E., C. Marin, B. Alonso–Urmeneta and V. Arogon. (1994). Evaluation of serological tests for diagnosis of *Brucella melitensis* infection of goats. Journal of Clinical Microbiology, 32:1159 – 1165.

Diallo, A and F. Thiaucourt. (1997). Application of Molecular Techniques to disease diagnosis. An overview in proceedings: Symposium on Diagnosis and control of

livestock diseases using molecular and related techniques, April 7 – 11 Vienna (Austria); Page 115 – 135

Dohoo, I. R., F. Wright, G. M. Ruckerbauers, B. S. Samagh, F. J. Robertson, and L. B. Forbes. (1986). Comparis on of five serological tests for bovine brucellosis. Canadian Journal of Veterinary Research, 50: 485 – 493.

Doust, S. R. H., Z. Ahmadi, A. Ahamdi, M. Hajia, M. Izadi and A. M. Mobarez. (2007). Detection of *Brucella abortus* by alkB and IS711 based primers. Journal of Research in Medical Sciences, 12:62-67.

Elzer, P. H. (1998). Brucellosis: review, update and vaccination program in US. Journal of Korean Society for Veterinary Science, 38: 61 – 69.

Elzer, P. H., M. D. Edmonds, D. S. Hagius, J. V. Walker, J. M. Gilsderf and S. D. Davis. (1998). Safety of *Brucella arbortus* strain RB51 in Bison. Journal of Wildlife Diseases, 34:825 – 829.

Enright, F. M. (1990). The pathogenesis and pathology of *Brucella* infections in domestic animals. Research in Veterinary Science, 60: 48-50.

Esuruoso, G. O and F. O. Ayanwale. (1980). Bovine brucellosis in Lagos state of Nigeria. Bulletin of Animal Health and Production in Africa, 28:11-15.

Falade, S., J. K. Nwufoln and L. Y. Nmezi. (1981). Brucellosis investigation in selected herds in Oyo state, Nigeria. Bulletin of Animal Health and Product in Africa, 29: 197-201.

Falagas, M. E. and I. A. Bliziotis. (2006). Quinolones for Treatment of Human Brucellosis: Critical Review of the Evidence from Microbiological and Clinical Studies. Antimicrobial Agents and Chemotherapy, 50: 22-33.

Fenchick, P. J. R., J. F. Markham and A. H. Cochrane. (1985). Inhibition of phagosontlysosome fusion in macrophages by soluble extracts of virulent *Brucella abortus.* American Journal Veterinary Research, 46:332-335.

Finlay, B. B and S. Falkow. (1989). Common themes in Microbial Pathogenicity. Microbiology Reveiws, 53: 210 – 230.

Foster, R and M. Smith. (2008). Brucellosis (*Brucella canis*) and Abortion in Dogs. Biology Work group-Citizedium. www.peteducation.com

Gall, D and K. Nielsen. (2004). Serological diagnosis of bovine brucellosis: a review of test performance and cost comparison. Review of Science Technological Office of International Epizootics, 23: 989-1002.

Gardner, I. A., H. Strynn, P. Lind and M. T. Collins. (2000). Conditional dependence between tests affects the diagnosis and surveillance of animal diseases. Preventive Veterinary Medicine, 45: 107-122.

Garin Bostuji, B and A. Benkirane, (Eds). (1995). FAO/WHO/OIE round table on the use of Rev.1 vaccine in small ruminants and cattle. CNEVA Alfort, France Page 21-22.

Gray, M. D and S. W. Martin. (1980). An evaluation of screening programs for the detection of brucellosis in dairy herds. Canadian Journal of Comparative Medicine, 44: 52-60.

Geering, W., A. J. Forman and M. J. Nunn. (1995). Exotic Diseases of Animals, Aust Gov. Publishing service, Canberra. Australia, Page 301 – 306.

Ghirotti, M., G. Semproni, D. D. De Meneghi, F. N. Mungaba, D. Nannini, G. Calzetta and G. Paganico. (1991). Sero-prevalences of selected cattle diseases in the Kafue flats of Zambia. Veterinary Research and Communications, 15. 25-36.

Godfroid, J., (1992). Diagnosis of bovine brucellosis for its eradication. Annals in Medicine in Veterinary, 136: 429 – 434.

Godfroid, J. (2002). Brucellosis in wildlife. Reveiw of Science Technological Office of International Epizootics, 21: 277-286.

Godfroid, J., C. Salgerman and V. Wellemansa. (2002). How to substantiate eradication of bovine brucellosis when a specific serological reaction occurs in the course of brucellosis testing. Veterinary Microbiology, 90:461-477.

Greiner, M. and L. A. Gardner. (2000a). Application of diagnostics in Veterinary epidemiological studies. Preventive Veterinary Medicine, 45: 43-59.

Greiner, M and L. A. Gardner. (2000b). Epidemiologic issues in the validation of Veterinary diagnostic tests. Preventive Veterinary Medicine, 45: 3 – 22.

Harmon, B. C., L. C. Adams and M. Frey. (1988). Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. American Journal Veterinary Research, 49: 1092 – 1097.

Hollet, R. B. (2006). Canine brucellosis: Out breaks and Compliance. Theriogenology, 66: 575 – 587.

Hosie, B. D., O. M. Al-Bakri and R. J. Futter. (1985). Survey of Brucellosis on goats and sheep in Yemen Arab Republic. Tropical Animal Health and Production, 17:93-99.

Huebner, R. A. (1998). Brucellosis in cattle, Merck Veterinary Manual, National Publishing Inc. Eighth edition, 1998, Philadelphia.

Hussein, A. S., S. S. Signh and H. Hoji. (1978). A survey of bovine brucellosis in the Southern parts of Somalia Democratic Republic. Bulletin in Animal Health and Production in Africa, 26: 150 –153.

Jensen, A. E., M. V. Palmer and M. G. Stevens. (1998). Evaluation of serological responses, lymphocyte proliferative responses, and clearance from lymphatic organs after

vaccination of bison with *Brucella abortus* strain RB51. American Journal of Veterinary Research, 59: 410-415.

Jordan, D. (1995). Herdacc: a programme for calculating heard level (aggregate) sensitivity and specificity (Guelph, O N, Canada, N 1 G 2 W1, Department of population Medicine, University of Guelph).

Jubb, K. V. F., P. C. Kennedy and N. Palmer. (1985). Pathology of Domestic Animals, Vol.3, Academic Press, Orlando, FL, Page 345 - 349

Kadohira, M., J. J. McDermott, M. M. Sheukri and M. A. Thornburn. (1996). Assessing infections at multiple levels of aggregations. Preventive Veterinary Medicine, 29:161 – 177.

Kadohira, M., J. J. McDermoth, M. M. Sheukri and M. N. Kyule. (1997). Variations in the prevalence of antibody to *Brucella* infection in cattle by far, area and district in Kenya. Epidemiology and infection, 118:35-41

Keid, L. B., R. M. Soares, S. A. Vasconcellos, D. P. Chiecao, J. R. Megid, V. R. Salgado and L. J. Richtzenhain. (2007). A polymerase chain reaction for the detection of *Brucella canis* in semen of naturally infected dogs. Theriogenology, 67: 1203 – 1210.

Keyes, D. C., J. L. Burstein, R. B. Schwartz and R. E. Swienton (eds). (2004). Medical Response to Terrorism: Preparedness and clinical practice. Edition 2nd, revision. Publishers Lippincett Williams and Wilkins.

Kiel, F. W and M. Y. Khan. (1989). Brucellosis in Saudi Arabia. Social Science Medicine, 29: 999 – 1001

Ko, J and G. A. Splitter. (2003). Molecular host pathogen interaction in brucellosis: current understands future approaches to vaccine development for mice and humans. Clinical Microbiology Reviews, 16: 65-78.

Latimer, E. J., N. Simmers, R. M. Srirangamatthan, I. T. Roop, G. G. Schuring and S. M Boyle. (1992). *Brucella abortus* difficient in Copper/Zinc superoxide dismutase is virulent in BALB/c mice. Microbiology and Pathogenesis, 12:105 – 113.

Lopez, M. A. (1989). *Brucellosis* in Latin America. Young E.J., Corble M.H. Editors. Brucellosis; Clinical and Laboratory aspects. Boca Raton: CRC Press Inc.

MacMillan, A., I. Greiser–Wilde, V. Moening and L. Mathias. (1990). A competitive enzyme immuno assay for brucellosis diagnosis. Dtsch.tierarztl, Wochenschr, 97: 83 – 85.

Madkour, M. M. (2001). Madkour's brucellosis. Barlin: Berlin and Heiderberg: springer – Verlay 306.

Mainar-Jaime, R. C., R. M. Munoz, M. J. de Miguel, M. J. Grillo, C. M. Marin, I. Moriyan and J. M. Blasco. (2005). Specificity dependence between serological tests for diagnosing bovine brucellosis in *Brucella* free farms showing false positive serological reactions due to *Yersinia enterocalitica* 0:9. The Canadian Veterinary Journal, 46: 913-916.

Mantur, B. G., S. S. Mangalgi and B. Mulimani. (1996). *Brucella melitensis* a sexually *transmissible* agent. Lancet, 347: 1763.

Mantur, B. G., S. K. Amarnath and R. S. Slinde. (2007). Review of clinical and laboratory features of human brucellosis. Indian Journal of Medical Microbiology, 25: 188-202.

Matsukawa, K and M. Yoshima. (1995). Orchiepidymitis due to *Brucella* in Kafue Lechwe. In proceedings: The Effect of enlargement of domestic animal pastures on the wildlife in Zambia, Lusaka, Zambia, Page 121 – 125.

McCullough, D. R and L. R. Paulson. (1998). Brucellosis in the greater Yellowstone Area. National Academy Press. National Academy of Sciences.

McDermott, J. J., K. A. Deing, E. L. Jayatileke and M. A. Jack. (1987). Prevalence estimates and age, sex and breed associations for brucellosis and contagious bovine pluro pneumonia. Preventive Veterinary Medicine, 5: 111-123.

McDermott, M., B. O'Connell, T. E. Mulvinill and E. C. Sweeing. (1994). Chronic *Brucella* infection of the supra-patellae bursa with sinus formation. Journal of Clinical Pathology, 47: 764 – 766.

McGill, P. E and G. O. Oyoo. (2002). Rheumatic disorders in Sub-Saharan Africa. East Africa Medical Journal, 79: 214 – 6.

Mdegela, R. H., E. Karimuribo, L. J. Kusiluka, B. Kabula, A. Manjurano, A. M. Kapaga and D. M. Kambarage. (2005). Mastitis in small holder dairy and pastoral cattle herds in the urban and peri-urban areas of the Dodoma municipality in Central Tanzania. Livestock Research for Rural Development Report, 17: 11.

Meeusen, E. N., J. Walker, A. Peters, P. P. Pastoret and G. Jungersen. (2007). Current status of Veterinary Vaccines. Clinical Microbiology Review, 2: 489-510.

Meyer, K. F., B. Eddie, L. Veazie, I. M. Stevens, B. Stewart and J. C. Gelger. (2008). The heterogenous infection chains as occupational diseases. Intern, Arch. Occup and Env. Health, 5: 1934

Morata, P., M. I. Queip-Ortuno, J. M. Reguera, M. A. Garcia-Ordonez, C. Pichardo and C. J. De Dios. (1999). Post treatment follow-up of brucellosis by PCR Assay. Journal of Clinical Microbiology, 37: 4163 – 4166.

Msanga, J. F., D. J. A. Mukangi and R. Tugaraza. (1986). Bovine brucellosis in the Lake Zone of Tanzania: The present situation. Bulletin Animal Health and Production in Africa, 34:230-234.

Muma, J. B., E. Skjerve, K. L. Samui, V. Siamudala, J. Oloya, G. Matope, M. Munyeme, C. Mubita and M. K. Omer. (2006). Prevalence of antibodies to *Brucella spp*. in Traditional Cattle and Goats Reared in Livestock-wildlife interface Areas of Zambia. Tropical Animal health and Production, 38: 138 – 206.

Muma, J. B., K. L. Samui, A. Lund, K. Nielsen, H. Chimana and E. Skjerve. (2008). Brucellosis in Rural Communities in Zambia and Factors Associated with increased anti-Brucella spp. Antibody presence. Journal of Science and Technology, 12: 9-18.

Munang'andu, H. M. (2000). A review of the veterinary diseases in the Kafue flats with Emphasis on the proposed conservation area in Mazabuka District, Zambia submitted to World Wide Fund for Nature. Central Veterinary Research Institute, Lusaka, Zambia.

Munoz, P. M., C. M. Marin, D. Monreal, D. Genzalez, B. Garin–Bastyi, R. Diaz, R. C. Mainar Jaine, I. Moriyon and J. M Balsco. (2005). Efficacy of several Serological Tests and Antigens for Diagnosis of Bovine Brucellosis in the Presence of false positive serological results due to *Yersinia enterocolilica* 0:9. Clinical and Vaccine Immunology, 12: 141 – 151.

Murioz, P., C. M. Marin and D. Monreal. (2004). Efficacy of several serological tests and antigens for diagnosis of cattle brucellosis in the presence of false positive serological results to *Yersinia enterocoliltila* 0:9. Diagnostic Laboratory Immunology, 12: 141-151.

Musa, M. T., K. L. Jahans and M. E. Fadalla. (1990). Clinical manifestation of brucellosis in cattle of the Southern Darfour Province, Western Sudan. Journal of Comparative Pathology, 103: 95-99.

Navara, E. (2003). Comparison of three different PCR, methods for detection of *Brucella spp* in human blood samples. FEMS Immunology Medical Microbiology, 41: 144 – 148.

Newby, D. T., T. L. Hadfield and F. F. Roberto. (2003). Real-time PCR detection of *Brucella abortus*: A comparative study of SYBR green I, 5'-exonuclease, and hybridization probe assays. Applied Environmental Microbiology, 69: 4753-4759.

Nicoletti, P. (1980). The epidemiology of bovine brucellosis, In: Advances of Veterinary Sciences and Comparative Medicine. Academic Press, London.

Nielsen, K and J. R. Duncan. (1990). Animal Brucellosis., CRC Press, Inc., Page 203 – 206.

Nielsen, K. H. (1996). Comparison of enzyme immunoassays for the diagnosis of bovine brucellosis. Preventive Veterinary Medicine, 26: 17-32.

Nielsen, K. H., L. D. Gall, S. Balsevicus, J. Bosse, P. Nicolette and W. Kelly. (1995). Comparison of enzyme immuno assasy for the diagnosis of bovine brucellosis. Preventive Veterinary Medicine, 26: 17-32.

Nielsen, K., D. Gall, M. Jolley, G. Leishman, S. Balsevicius, P. Smith, P. Nicoletti and F. Thomas. (1996). A homogeneous fluorescence polarization assay for detection of antibody to *Brucella abortus*. Journal of Immunological Methods, 195: 161 –168.

Olsen, S. C and W. S. Staff. (2005). Essential role of vaccines in brucellosis control and eradication programs for livestock. Expert Review of Vaccines, 4: 915-928.

Omer, M. K., T. Assefow, E. Skjerve, T. Tekleghiorghis and Z. Woldehiwet. (2000). Prevalence of antibodies to *Brucella* species and risk factors related to high risk occupational groups in Eritrea. In: PhD Thesis, Norwegian School of Veterinary Science, Oslo, Norway.

Pandey, G. S., K. Kobayashi, Y. Nomura, A. Nambota, H. K. Mwima and A. K. Suzuki. (1999). Studies on sero-prevalence of *Brucellosis* of Kafue Lechwe (*Kobus Lechwe Kafuensis*) in Zambia. Indian Veterinary Journal, 76: 275-278.

Pappas, G., M. Bosilkovski, N. Akritidis, M. Mastora, H. Ketena and E. Tsianos. (2003). Brucellosis and the Respiratory system. Clinical Infectious Diseases, 37: 95-99.

Pappas, G., N. Arkritidis, M. Bosilkouski and E. Tsiano. (2005). Brucellosis, Medical Progress. New England Journal of Medicine, 352: 2325-2336.

Pappas, G., P. Panagopoulou, L. Christou and N. Akritidis. (2006). *Brucella* as a biological weapon. Cell Molecular Life Sciences, 63: 2229-2236.

Parker, R. (2007). Diseases affecting Reproduction in Beef Cattle. College of Agriculture and Home Economics New Mexico State University. USA.

Perry, B. D., B. Mwanaumo, H. F. Schels, E. Eicher and M. R. Zaman. (1984). A study of Health and Productivity of Traditionally Managed Cattle in Zambia. Preventive Veterinary Medicine, 2: 633 –653.

Quinn, P. J., M. E. Carter, B. Markey and G. R. Carter. (1999). Clinical Veterinary Microbiology., Edinburgh: Mosby International limited, Page 261 – 267.

Radostits, O. M., D. C. Blood, C. C. Gay and J. H. Arundel. (1994). Diseases caused by *Brucella* spp, Veterinary Medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses. Bailliere Tindal, London. Page 787 – 813.

Ramirez, M., M. E. Hamdy and M. Amin. (2002). Serologic response and time to eradication in herds with brucellosis vaccinated with strain 19 or strain RB-51. Arch. Med.Vet, 34: 143-151.

Rege, J. E. O., A. M. Nyamu and D. Sendalo (eds). (2006). The role of Biotechnology in animal agriculture to address poverty in Africa; opportunities and challenges. Proceedings of the 4th All Africa conference on Animal Agriculture and the 31st Annual meeting of Tanzania Society for Animal Production Arusha, Tanzania.

Richey, E. J and D. C. Harrell. (1997). *Brucella abortus* Disease (Brucellosis) in Beef cattle, UF/IFAS.

Rienzo, S. (1948). 1stInteramerican Congress on Brucellosis, 583-603 (Ediciones del Hosp). Gen, Secretaria de Salubridad y Asistencia, Mexico.

Rijipens, N. P., G. Jannes, M. Van Asbroeck, R. Rossau and L. M. Herman. (1996). Direct detection of *Brucella* spp. in raw milk by PCR and reverse hybridization with 16S-23S rRNAspacer probes. Applied Environmental Microbiology, 62:1683-1688.

Riley, D. K and D. C. Robertson. (1984). Ingestion and intercellular survival of *Brucella abortus* in human and bovine polymorphonuclear leucocytes. Infectious Immunology, 46: 224 – 230.

Rikin, E. U. (1988). Brucellosis of cattle in Nigeria. Proposals for a control program under intensive and extensive husbandry systems. Acta Vet Scand., 84: 94 – 7.

Romero, C. (1995a). Evaluation of PCR and Indirect Enzyme Linked ImmunoSorbent Assay on with samples for diagnosis of brucellosis in dairy cattle. Journal of Clinical Microbiology, 33: 615-7.

Romeo, C. (1995b). Specific detection of Brucella DNA by PCR. Journal of Clinical Microbiology, 33: 615-17.

Roushan, M. R. H., M. Mohraz, M. Hajiahmadi, A. Ramzani and A. A. Valayati. (2006). Efficacy of gentamycin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in Humans. Clinical Infectious Diseases, 42: 1075-80.

Ross, H. M., G. Foster, R. J. Reid, K. L. Jabans and A. P. Macmillan. (1994). *Brucella* species infection in sea mammals. Veterinary Records, 132: 359.

Rottcher, D. (1987). Final Report, Veterinary wildlife, Department of Veterinary and Tsetse Control Service, Zambia.

Rust, R. S. (2006). Brucellosis. In: Shah A.K., F. Talavera, D, Parma, F.P, Thomas, S.R. Benbadis and N. Lorenzo, (Eds). eMedicine specialisties, website: http//medscape.com.

Sauret, J. M and N. Vilissova. (2002). Human Brucellosis. Journal of American Board of Family Practice, 15: 401-405.

Scrimgeouur, E. M., F. R. Melita and A. J. Suleiman. (1999). Infectious and tropical diseases in Oman a review. American Journal of Tropical Med and Hygiene, 61: 920-925.

Scrimgeour, E. M., S. I. N. AL Ismaily, J. M. Rolain, S. H. S. Al-dhnalsy, H. S. EL-Khatim and D. Ravoutt. (2003). Q fever in Human and livestock populations in Oman. Annals New York Academic Sciences, 990:221-225.

Seema, E., D. McMurray and T. A. Fichil. (2001). Interruption of the Cyd B Locus in *Brucella abortus* Attenuates Intracellular survival and virulence in the Mouse Model of Infection, Journal of Bacteriology, 183:2454-2462.

Sifuentes-Rincon, A. M., A. Revol and H. A. Barrera-Saldana. (1997). Detection and differentiation of the six *Brucella* species by polymerase chain reaction. Molecular Medicine, 3:734 – 9.

Stack, J. A and A. P. Macmillan. (2000). Identification and Biotyping of *Brucella* spp. Clinical and Vaccine Immunology, 146:166-167.

Stuart, F. A., M. Corbel and R. A.Brewer. (1987). Experimental *Brucella abortus* infections. Veterinary Microbiology, 14: 365-379.

Spink, W. W. (1948). Pathogenesis of human brucellosis with respect to prevention and treatment. Annals Internal Medicine, 29: 238–258.

Tanoue, L. T and J. Mark Euge. (2003). A 43 year old Man with Fever and night sweats. Case records of the Massachusetts General Hospital. USA.

Thimm, B. M. (1982). Brucellosis distribution in man, domestic and wild animals. CD. Theon and J.H. Stelle (eds). Berlin: Berline and Heidelbergy: Springer Verlag. Page 55.

Thoen, C. O and F. M. Enright. (1986). *Brucella,* in Pathogenesis of Bacterial Infections in Animals, Gyles C.L and Thoen C.O., (eds)., Iowa State University Press, Ames.

Thrusfield, M. (2005). Veterinary Epidemiology 3rd Edition. Blackwell Science Ltd, Oxford. Page 303 – 330.

Tizard, I. R., (2000).Veterinary immunology: An introduction. 6th Edition, Philadephia, Pennsylviania 19106: W.B Saunders Company. Page 238-265.

Traboulsi, R., I. Uttman and S. S. Kani. (2007). Prepatellar *Brucella melitensis* bursits: Case report and literature review. Clinical Rheumatology, 11:1941-1942.

Tsolia, M., S. Drakonaki, A. Messaritaki, T. Farmakakis, M. Kostaki, H. Tsapra and F. Karpathias. (2002). Clinical Features, Complications and Treatment outcome of Childhood brucellosis in Central Greece. Journal of infection, 44: 257 – 262.

Tuchili, M. L. (1988). In Proceedings of the international conference on epidemiology, control and prevention of rabies and brucellosis in Eastern and Southern African countries, Gaborone November, 23-25, Page 121-123.

Uzal, F. A., A. E. Carrasco, S. Echaide, K. Nielsen and C. A. Robles. (1995). Evaluation of an Indirect ELISA for the diagnostic of bovine brucellosis. Journal of Veterinary Diagnosis, 7: 473 – 75.

Uzal, F. A., L. Samartino, G. Schuring, A. Carrasco, K. Nielsen, F. Cabrera and R. Taddeo. (2000). Effect of vaccination with *Brucella abortus* strain RB: 51 on heifers and pregnant cattle. Veterinary Research Communications, 24: 143-151.

Uzureau, S., M. Godfroid, C. D. Changes, J. Lemaire, X. D. Belle and J. J. Leteson. (2007). Mutations of the quorum and sensing dependent regulator Vjbr lead to drastic surface modifications in *Brucella melitensis*. Journal of Bacteriology, 189: 6035 – 6047.

Van Aert, A., P. Brioen, P. Dekeyser, A. Uytter halgen, R. J. Sijens and A. Boeye. (1984). A Comparative study of ELISA and other methods for the detection of *Brucella* antibodies in bovine sera. Veterinary Microbiology, 10: 13 – 21.

Villafane–Lastra, T., R. Bergogao and J. F. Garcia. (1948). 1st Inter-American Congress on Brucellosis, 441-63 (Ediciones del Hospital General. Secretarial de salubridady. Asistencia, Mexical).

Woods, C and B. Jan (eds). (2005). USAMRHD's Medical Management of Biological casualties Handbook, 6th Ed, U.S Army Medical Institute of Infectious Diseases, Fort Detrick, Maryland, 53.

Woodruff, A. W., S. Bell and J. Wright. (1978). A synopsis of Infectious and Tropical Diseases,website: http//journals.cambridge.org.

Xin, X. (1986). Orally administrable brucellosis vaccine: *Brucella suis* strain 2. Vaccine, 4: 212 – 216.

Young, E. H. (1995). An Overview of human brucellosis. Clinical Infectious Disease, 21: 283-290.

Zribi, M., A. Masmoudi, A. Tiouiri and H. Fenolril. (2008). Clinical manifestations, Complications and treatment of brucellosis, 45 patients study. Pathologie biologie, 57:349-352.

APPENDICES

1.0 SEROLOGICAL TESTS

1.1 Rose Bengal Test

MATERIALS

- RBT antigen.
- Controls - Positive and Negative sera.
- Agglutination glass slides.

TECHNIQUE

Principle:

Pinkish dyed *Brucella* antigens are added to equal amounts of test sera, mixed using orange sticks and incubated. If specific antibodies are present in the sera, agglutination takes place. This is shown by formation of small aggregates on the slide for a positive test result. No agglutination means negative results. The test does not distinguish animals that are vaccinated from those that are infected and there is like hood of cross reactions by gram – negative bacteria such as *Campylobacter* and *Yersinia*.

Test Procedure

1. Bring the serum samples and antigen to room temperature (18–22ºC ± 4ºC), only sufficient antigen for the day′s tests should be removed from the refrigerator.

2. Place 25–30 µl of each serum sample in the wells on the agglutination large glass slides.

3. Gently shake the antigen bottle and place an equal volume of antigen near each serum spot.

4. Immediately, mix the serum and antigen thoroughly (using a clean a clean orange stick for each test to produce a circular or oval zone approximately 2cm in diameter.

5. Agitate the mixture for 4 minutes at ambient temperature on a rocker.

6. Read for agglutination immediately after 4 minute period is completed. Any visible reaction is considered to be positive. A control serum that gives a minimum positive reaction should be tested before each day's tests are begun to verify the sensitivity of test conditions.

1.2 Competitive ELISA (C – ELISA)

MATERIALS

- *Brucella abortus* S – LPS antigen non – infectious coated microtitreplates (sealed and stored dry).
- Lyophilized mAb.
- HRP Conjugate (goat anti – mouse IgG horse – radish peroxidase) Ready to use.
- PBS – Tween Solution 20 x concentrate.
- Sample Dilution Buffer.
- Substrate Solution – (tetramethyl-benzidine in substrate buffer containing H_2O_2) – **STORED IN THE DARK**.
- Stop Solution – Contains sulphuric acid – CORROSIVE.
- Positive Control Serum – 0.05% merthiolate.
- Negative Control Serum – 0.05% merthiolate.
- Weak Control Serum – 0.05% merthiolate.
- Precision pipettes (range from 5 to 200 µl).

- Disposable pipette tips.
- Distilled water.
- Wash bottle.
- 1 container: 1 to 2 liters for PBS – Tween.
- Microplate photometer.

Serum specimen

Used 5 µl of fresh, refrigerated or previously frozen serum for testing.

Reagents preparation

- **PBS – Tween Buffer :**

Dilute the PBS – Tween solution 20 x concentrate 1/20 in distilled water.

Prepare 500 ml per plate by adding 25 ml PBST solution to 475 ml distilled water and mix thoroughly.

There should be no crystal precipitation in the bottle. If crystals are seen, warm and shake well.

- **mAb Solution:**

Reconstitute freeze dried mAb with 6 ml Sample Dilution buffer.

Add the buffer carefully into the bottle

Prepare immediately before use.

Mix gently – do not use vortex mixer.

TECHNIQUE

Principle:

In *Brucella* – Antibody C – Elisa, serum samples are subjected to *Brucella abortus* smooth lipopolysaccharides (SLPS) coated wells on microtiter plates together with a mouse monoclonal antibody (mAb) specific for an epitope on the O – polysaccharide portion of the S – LPS antigen. The micro plates are washed after incubation and goat anti – mouse IgG antibody with horseradish peroxide is added which binds to any mAb's bound to the SLPS on the micro plate. When anti – *Brucella* natural antibodies are present in the test serum (positive) they compete with the mAb for the epitope sites and inhibit the mAb binding to the O – polysaccharide portion of S – LPS . In case of anti – *Brucella* natural antibodies are absent in the test serum (negative), the mAb binds to the O – polysaccharide epitope of S – LPS antigen. When the conjugate is added it binds specifically to mAb. Unbound materials are removed by rinsing before the addition of substrate solution. Colour development is due to the conversion of substrate by the conjugate. Sera from strain 19 vaccinated cattle do not compete with mAb because of their specificity and lower affinity. The optical density is measured by a microplate photometer at 450 nm.

Test Procedure

1. Bring all test serum samples, positive, negative controls and reagents to room temperature.
2. Add 45 µl of Sample Dilution Buffer into each well that will be used for serum samples and serum controls.

3. Add 5 µl of Serum Controls. Positive, Weak Positive and Negative, into each of the appropriate wells, respectively. Run each control in duplicate.

4. Add 5 µl of Sample Dilution Buffer into two appropriate wells (designated as Conjugate Control, Cc).

5. Add 5 µl of test sample to each of the appropriate wells. For confirmation purposes, run each sample in duplicates.

6. Add 50 µl of mAb – Solution into all wells used for controls and samples. Do this step within or less than 10 minutes.

7. Seal the plate and mix the reagents thoroughly by shaking the plate for 5 minutes on a shaker.

8. Incubate at room temperature (18 – 25ºC) for 30 minutes.

9. Rinse the plates 4 times with PBS – Tween Buffer, fill up the wells at each rinse, empty the plate and tap hard to remove all remains of fluids.

10. Add 100 µl of Conjugate solution into each well. Seal the plate and incubate at room temperature 18 to 25ºC for 30 minutes.

11. Repeat step 9.

12. Add 100 µl of Substrate Solution to each well and incubate for 10 minutes at room temperature 18 to 25ºC. Start timing after filling the first well.

13. Stop the reaction by adding 50 µl of Stop Solution to each well and mix thoroughly. Follow the same order as the Substrate Solution was added in step 12.

14. Measure the optical density (OD) of the controls and samples at 450 nm in a micro plate photometer (use air as a blank). The ODs should be measured within 15 minutes after addition of Stop Solution to prevent fluctuation in OD values.

2.0 QUESTIONNAIRE (RISK FACTORS)

A copy of the questionnaire was given to the owner, family member or caretaker of the farm as blood sample collection commenced and collected later after sampling was complete. The questionnaire was structured as shown below and comprised some thirty questions representing various hypothesized risk factors.

A SURVEY TO DETERMINE THE PREVALENCE AND RISK FACTORS OF BRUCELLA INFECTIONS IN COMMERCIAL HERDS IN LUSAKA PROVINCE.

Department of Disease Control, School of Veterinary Medicine, University of Zambia box 32379 Lusaka Zambia, Tele-fax 292737

SECTION ONE: IDENTIFICATION

Day of visit:……………………………………………………………………………

Farm Sampling No:……………………………………………………………………

Farm location:…………………………………………………………………………..

Zone:	Herd/Farm:

Owner's name and Address:

……………………………..

....

Year of establishment:…………………………..

Name of person interviewed:……………………...

Relation of the person interviewed:

1. Owner ☐ **2. Family member** ☐ **3. Care taker** ☐

Name of interviewer:

………………………...

Q1.

i. What type of animals do you have on this farm?

Cattle	Sheep	Goats	Pigs	Others (Specify)

Give a breakdown of herd structure

Cattle	Number	Small Ruminants	Number
1. Number of cows		Doers	
2. Number of bulls		Bucks	
3. Number of heifers >1 yr		Females > 8 Months	
4. Number of female calves <1yr		Female kids <8 months	
5. Number of males calves <1 yr		Male kids <8 months	

ii. How did you acquire this property?

0. Bought……………………………………………………☐

1. Heritage……………………………………………………☐

2. Others specify……………………………………………………

iii. When did you acquire this property?……………………………………

vi. Are you a member of any farmer's animal-health organisation/cooperative?

0. Yes……………………………………………………☐

1. No……………………………………………………☐

FARM OWNERSHIP AND CARE

Q2. How many people own the animals on this farm?

0. One…………………………………………………………………… ☐

1. Two…………………………………………………………………… ☐

2. Other specify ……………………………………………………………

Q3. What type of animal breeds do you have on this farm?

Cattle	**Breed type**	**Small Ruminants**	**Breed type**
1. Exotic		1. Exotic	
2. Local		2. Local	
3. Mixed		3. Mixed	

Q4. What kind of breeding methods do you use on this farm?

0. Artificial insemination……………………………………………… ☐

1. Natural methods……………………………………………………… ☐

2. Both 1 and 2...………………………………………………………… ☐

Q5. If you use bulls, where do you get your bulls?

0. Use own bull…………………………………………………………… ☐

1. Use hired bulls………………………………………………………… ☐

Q6. What is the source of your stock?

0. Animal market………………………………………………………… ☐

1. Any farm……………………………………………………………… ☐

2. Others specify……………………………...

Q7. Have you brought any animal onto your farm during the past 3 years?

0. Yes…………………………...……............... ☐

1. No………………………….. ☐

Q8. If you brought animals on the farm, which sex were they?

0. Male…………………………... ☐

1. Female………………………... ☐

2. Male and Female……………………………... ☐

If yes specify the numbers.………………...

Q9. Do you hire out your bull/buck to other farmers for breeding?

0. **Y**es …………………………... ☐

1. No……………………….. ☐

If yes specify……………………………...

Q10. Do you hire other people's bulls/buck for breeding your animals?

0. Yes…………………………... ☐

1. No…………………………... ☐

If yes specify……………………………...

Q11. If there are farms near by, how far is the closet farm from yours?

Specify in meters…………………………………………………………………………

Q12. Where do your animals give birth?

0. In the same pens where they sleep……… ………………………………………… ☐

1. In separate maternity pens…………………………………………………………… ☐

2. On pasture………………………………………………………………………………… ☐

Q13. How do you get rid of manure from the kraal/animal houses

0. Left to dry and collected later………………………………………………………… ☐

1. Drained through sewerage………………………………………………………………… ☐

2. Others specify…………………………………………………………………………………

Q14. Who is primarily responsible for looking after the animals?

0. Owner/ family member…………………………………………………………………… ☐

1. Hired caretaker…………………………………………………………………………… ☐

2. Both 1 and 2……………………………………………………………………………… ☐

3. Others (specify)………………………………………………………………………………

Q15. Do you keep any written records for the animals?

0. Yes ……………………………………………………………………………………… ☐

1. No…………………………………………………………………………………………… ☐

Q16. Do you receive any veterinary supportive services?

0. Yes………………...☐

1. No……………………..…………☐

If yes specify……………………………………………………………………………..

SECTION TWO: FEEDING

Q17. How do you feed your animal?

0. Pasture plus grain supplements all year……………………...............................☐

1. Mainly concentrate feeding……………………...☐

2. Pasture plus grain supplement occasionally……………………......................☐

3. Tethering (zero grazing)……………………..☐

4. Free grazing/browsing………………………...☐

Q18. If you practice grazing what type of grazing is it?

0. Own pastures…………………...☐

1. Only communal pastures………………...☐

2. Own and communal pastures……………………..☐

ANIMAL MOVEMENT PATTERNS

Q19. Do you graze your animal in one place whole year round or you change with seasons? (Transhumance)

0. Yes .... ☐

1. No.. ☐

If yes specify where you take the animals?...

Q20. Do you hire animals to fertilize your fields?

0 Yes .... ☐

1. No.. ☐

Q21. Do you use oxen for transport purpose?

0. Yes .... ☐

1. No.. ☐

MARKETING

Q22. Have you sold any animal in the last 12 months?

0. Yes .... ☐

1. No.. ☐

If yes specify..

Q23. If you sold, where did the buyers come from?

0. Within the neighbourhood……………………........................……………..☐

1. Within town……………………….......................................…..................☐

2. Within the province………………………...........................……………...☐

3. Outside the province……..………………….........................……………☐

4. Both within and outside the province……………………...................…...…...☐

5. Others …………………………...☐

Q24. Do you sell your milk directly to consumers?

0. Yes…………………...☐

1. No………………..…...........................☐

Q25. If yes where do you sell your milk?

1. Within the neighbourhood………………………...☐

2. Within town………………………..☐

3. Within the province……………..…...……………..☐

4. Outside the province……………………....................................……………..☐

5. Both within and outside the province……………………………....……………..☐

6. Others …………………………...……………..☐

DISEASE AND DISEASE CONTROL

Q26. Has any animal aborted in the last three years on this farm?

0. Yes………………………... ☐

1 No…………………………...………..... ☐

If yeas, give the identity of the animals……………………………….........................

Q27. If any animal aborted, which part of pregnancy was it?

0. First three months ..……………………...………… ☐

1. Middle of pregnancy ……………………..………….. ☐

2. Towards the end of pregnancy…..………………….. ☐

Q28. Do you have infertile animals or repeat breeders on this farm?

0. Yes………………………..………… ☐

1. No……………………………... ☐

If yes, give the identity of the animals……………………………….........................

Q29. Have you heard of a disease called brucellosis?

0. Yes ……..……….. ☐

1. No………………….. ☐

Q30. Since you don't vaccinate, why?

0. Not necessary…….. ☐

1. Not aware of the disease…... ☐

2. It is expensive….. ☐

3. Others………... ☐

Q31. How often do you conduct disease preventive measures such as dipping?

0. None……………………..…………... ☐

1. Regular (at least once per year)………….. ☐

2. Irregular (once after many years)... ☐

Q32. Do you assist your animals when they are giving birth?

0. Yes………………...…………. ☐

1. No………………….. ☐