PREVALENCE AND KNOWLEDGE OF BRUCELLOSIS IN DAIRY CATTLE IN MAKUYU DIVISION, MURANG’A COUNTY, KENYA

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156/CE/11170/07

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (IMMUNOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

AUGUST 2014
DECLARATION
This thesis is my original work and has not been presented for degree or other awards in any other university

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SUPERVISORS’ APPROVAL
We confirm that the work reported on this thesis was carried out by the candidate under our supervision as university supervisors

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Signature…………………………………… Date……………………
Dr. Eric Mwangi
Department of Zoological Sciences
DEDICATION

This thesis is dedicated to my lovely husband, Mwangi Kuria. He inspires me to be courageous, ardent and resilient with the vicissitudes of life.
ACKNOWLEDGEMENTS

Glory is to my almighty God for his abundant grace and faithfulness in all my academic achievements.

I am grateful to my supervisors Dr. Michael Gicheru and Dr. Eric Mwangi both from Kenyatta University and Dr. Peter Mbatha from Kabete Veterinary Laboratories for making my study a success. My gratitude also goes to the Director, Kabete Veterinary Laboratories for providing a conducive place to carry out my laboratory work.

I wish to record much appreciation to the District Veterinary Officer Dr. Jane Njeri for permitting me to collect samples in Makuyu Division, Veterinary Personnel Esther Njoki for assisting me in collection of samples and the dairy farmers for responding to my questionnaires.

Finally, my deep and sincere thanks goes to my lovely husband Willie Mwangi and sons Mike Mwangi and Nick Mwangi for their unrelenting support and understanding which gave me fresh impetus to ardently complete this course.

Lastly, thanks to my friends, Department of Zoological Sciences and the entire staff of Kenyatta University for their assistance and encouragement throughout my study.
**TABLE OF CONTENTS**

Title Page ........................................................................................................... i
Declaration ....................................................................................................... ii
Dedication ......................................................................................................... iii
Acknowledgements ........................................................................................ iv
Table of contents ............................................................................................. v
List of tables ..................................................................................................... ix
List of figures .................................................................................................... x
Acronyms and Abbreviations ........................................................................... xi
Abstract ........................................................................................................... xiv

**CHAPTER ONE: INTRODUCTION**

1.1 Background of information ......................................................................... 1
1.2 Problem statement ....................................................................................... 4
1.3 Study justification ......................................................................................... 5
1.4 Research question ....................................................................................... 6
1.5 Objectives .................................................................................................... 6
1.5.1 General objective .................................................................................... 6
1.5.2 Specific objectives .................................................................................. 6
1.6 Hypotheses .................................................................................................. 7

**CHAPTER TWO: LITERATURE REVIEW**

2.1 Brief history of *Brucella* species ............................................................... 8
2.2 General characteristics of *Brucella* organisms ......................................... 8
2.2.1 Resistance and survival of *Brucella* .................................................... 10
2.2.2 Cellular and colonial morphology and staining characteristics of *Brucella*........... 10
2.2.3 Growth requirements ............................................................................ 11
2.2.4 Biochemical characteristics .................................................................. 12
2.2.5 The Brucella cell wall molecular structure and its antigenic composition ............... 13
2.2.6 Lipopolysaccharides (LPS) composition and activity ........................................ 14
2.2.7 Brucella Proteins ................................................................................................. 18
2.3 Epidemiology of Brucellosis .................................................................................. 21
2.3.1 Transmission of Brucellosis in human ................................................................. 21
2.3.2 Brucellosis in human ........................................................................................... 23
2.3.3 Transmission of Brucellosis in animals ................................................................. 24
2.3.4 Brucellosis in animals ......................................................................................... 25
2.4 Clinical signs of Brucellosis .................................................................................... 30
2.5 Host immunity to infections by Brucella ................................................................. 31
2.6 Diagnosis of Brucellosis ......................................................................................... 33
2.6.1 Bacteriological tests .............................................................................................. 33
2.6.2 Immunological tests ............................................................................................. 34
2.6.2.1 Rose Bengal Plate Test ................................................................................... 34
2.6.2.2 Complement Fixation Test ............................................................................. 34
2.6.2.3 Milk Ring Test .................................................................................................. 35
2.6.2.4 Anigen Rapid Bovine Brucella Antibody Test .................................................. 37
2.6.2.5 Enzyme Linked Immunoabsorbent Assay (ELISA) .......................................... 38
2.7 Diagnosis in human ................................................................................................. 40
2.8 Diagnosis in animals ............................................................................................... 41
2.9 Control of Brucellosis ............................................................................................. 46
2.9.1 Control of Brucellosis in human ......................................................................... 46
2.9.2 Control of Brucellosis in animals .................................................................... 47

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area ............................................................................................................... 53
3.2 Sample size determination .................................................................................... 53
3.3 Inclusion and exclusion criteria ............................................................................ 54
3.4 Study design .......................................................................................................... 54
3.5 Data collection instrument ................................................................. 55
3.5.1 Questionnaires .............................................................................. 55
3.5.2 Milk and blood sampling ................................................................. 55
3.6 Laboratory analysis ........................................................................... 56
3.6.1 Rose Bengal Plate Test .................................................................. 56
3.6.2 Milk Ring Test ................................................................................ 56
3.7 Data analysis ...................................................................................... 57

CHAPTER FOUR: RESULTS
4.1 Demography of the farmers ................................................................. 58
4.2 Number of animals, breeds and farming practices ................................. 59
4.3 Knowledge of Brucellosis among dairy farmers ..................................... 60
4.4 Knowledge about the spread of brucellosis to human beings .............. 63
4.5 Control of Brucellosis ........................................................................ 65
4.6 Veterinary services ............................................................................ 66
4.7 Prevalence of Brucellosis in Makuyu Division ..................................... 68
4.8 Association between the prevalence and the sites (locations) .............. 69
4.9 Association of the type of farming practices ....................................... 69

CHAPTER FIVE: DISCUSSION
5.1 Introduction ....................................................................................... 71
5.2 Prevalence of *Brucella arbutus* in Makuyu Division ............................ 72
5.3 Knowledge level of brucellosis among dairy farmers in Makuyu Division .... 75
5.4 Accessibility of veterinary services to dairy farmers ............................. 77

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS
6.1 Conclusion ......................................................................................... 78
6.2 Recommendations ............................................................................. 78
REFERENCES .......................................................................................... 80
APPENDICES .................................................................................................................90
Appendix I: Study area .................................................................................................90
Appendix II: Questionnaire to dairy farmer .................................................................91
Appendix III: Authority to sample dairy cattle in Makuyu Division .......................96
LIST OF TABLES

Table 1: *Brucella* species and their host ................................................................. 9
Table 2: Farmers knowledge on the spread of brucellosis to human beings............... 64
Table 3: Accessibility of veterinary services to dairy farmers in Makuyu Division......... 67
Table 4: Test results of Brucellosis as diagnosed in milk and blood samples .............. 69
LIST OF FIGURES

Figure 4.1: Education levels of the respondents ................................................................. 59
Figure 4.2: Respondent farmer’s knowledge status on existence of brucellosis ............. 61
Figure 4.3: Farmer’s view on causes of brucellosis ............................................................ 61
Figure 4.4: Farmer’s of varying education levels views on causes of brucellosis .......... 62
Figure 4.5: Respondents’ knowledge on spread of brucellosis to human beings ........... 63
Figure 4.6: Respondents’ taking fermented milk .............................................................. 66
Figure 4.7: Respondents’ reports on outcome of treatment ............................................. 68
# ACRONYMS AND ABBREVIATIONS

<p>| Ab | Antibody |
| ART | Antigen Rapid <em>Brucella</em> Antibody Test |
| ASAL | Arid and Semi-Arid Land |
| CCF | Christian Children’s Fund |
| CFT | Complement Fixation Test |
| CFU | Colony Forming Unit |
| CMI | Cell Mediated Immunity |
| DAO | District Agricultural Office |
| DEO | District Education Office |
| DVO | District Veterinary Office |
| DVS | Department of Veterinary Services |
| FAO | Food Agricultural Organisation |
| IFA | Indirect Fluorescent Antibody |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IgA | Immunoglobulin Alpha heavy chain |
| IgG | Immunoglobulin Gamma heavy chain |
| IgG₁ | Immunoglobulin Gamma 1 heavy chain |
| IgG₂ | Immunoglobulin Gamma 2 heavy chain |
| IgM | Immunoglobulin Mu heavy chain |
| JKF | Jomo Kenyatta Foundation |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kdo</td>
<td>Keto-d-6-glutamic acid</td>
</tr>
<tr>
<td>KURET</td>
<td>Kenya, Uganda, Rwanda and Ethiopia Together</td>
</tr>
<tr>
<td>LPs</td>
<td>Lipopolysacharides</td>
</tr>
<tr>
<td>MAT</td>
<td>Monoclonal Antibody Test</td>
</tr>
<tr>
<td>2ME</td>
<td>2 Mercapto-ethanol</td>
</tr>
<tr>
<td>MR</td>
<td>Methyl Red</td>
</tr>
<tr>
<td>MRT</td>
<td>Milk Ring Test</td>
</tr>
<tr>
<td>MTTC</td>
<td>Murang’a Teachers Training College</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>OMP</td>
<td>Outer Membrane Proteins</td>
</tr>
<tr>
<td>Pg</td>
<td>Peptidoglycans</td>
</tr>
<tr>
<td>Ps</td>
<td>Polysacharides</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>RBPT</td>
<td>Rose Bengal Plate Test</td>
</tr>
<tr>
<td>RE</td>
<td>Reticuloendothelial</td>
</tr>
<tr>
<td>RIA</td>
<td>Radio Immunoassay</td>
</tr>
<tr>
<td>s99</td>
<td>Strain 99</td>
</tr>
<tr>
<td>SAT</td>
<td>Serum Agglutination Test</td>
</tr>
<tr>
<td>SAV</td>
<td>Spectrophotometric Absorbent Values</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package Social Sciences</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>VBD</td>
<td>Veronal Buffered Diluents</td>
</tr>
</tbody>
</table>
VO  Veterinary Officer

WHO  World Health Organization
ABSTRACT

Brucellosis is a cosmopolitan zoonotic disease that affects man, domestic animals and wildlife. The bacteria *Brucella abortus* is the principle cause of brucellosis in cattle. The incidence of the disease in humans, and which directly relates to that in other animals, is highly dependent on animal husbandry practices, the interaction between humans and animals, living standards, hygiene, food customs, and animal and human population density. Makuyu Division where the study was undertaken experiences hot and dry climate, the area has no permanent rivers and domestic water is obtained from boreholes. The standard of hygiene is low and most of the people are poor. In August 2009 six cases of retained placenta disease were reported to Makuyu veterinary Department among other diseases. This was confirmed to be due to brucellosis. The study was undertaken with the aim to determine prevalence of *Brucella abortus* in dairy cattle and assess knowledge of the disease among the dairy farmers in Makuyu Division. The study involved random sampling of dairy cattle in the division and a representative sample of two hundred and eight milk and blood samples were collected from the dairy cattle. The samples were screened at the Central Veterinary Laboratories, Kabete. Immunological tests Rose Bengal Plate Test and Milk Ring Test were used for *Brucella* antibody detection. A questionnaire was used to assess knowledge of brucellosis among the dairy farmers and determine utilization and accessibility to veterinary services. Data analysis involved descriptive statistics to obtain prevalence rate. The percentage prevalence of brucellosis in dairy cattle was 7.7%, knowledge of brucellosis was significantly related to the age and level of education of the farmers. The young and educated farmers were more knowledgeable on brucellosis than those who were old and not educated. The old and not educated were the majority and they had no idea on what causes brucellosis in cattle. I would recommend the Ministries of Health and Agriculture, Livestock and Fisheries use the results in targeting education and prevention programs to address the disease. Also the farmers be educated on methods of controlling brucellosis to reduce prevalence, use of artificial insemination to reduce venereal transmission of brucellosis during mating, herd health campaign with frequent screening for brucellosis to identify infected animals so that they can be slaughtered to eradicate the disease and food and occupational hygiene to prevent transmission of brucellosis to human beings.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Brucellosis is often called “undulant fever” or “Bang’s disease,” (Cloeckaert et al., 2003). The importance of this disease in humans dates back to 1887 when David Bruce first isolated the causative agent from livers of British soldiers dying in Malta (Young, 2009). In humans the onset of brucellosis is usually gradual, and the symptoms are not specific to the disease (Omer et al., 2000). Typically, patients complain of mild fever, sweating, weakness, aches and pains, enlarged lymph nodes, and weight loss (Cadmus et al., 2008). Even without treatment, most cases recover within 2 months, and only 15% will be symptomatic for more than 3 months (Pappal et al., 2004). In cattle the clinical signs are abortion, arthritic joints and retained afterbirth (Dricot et al., 2004).

Four varieties of the genus Brucella cause brucellosis in humans. DNA studies show that all members of the species fall into a single genus, Brucella melitensis, but traditionally, the different varieties were assigned species names depending largely on their preferred host: Brucella abortus invades cattle; Brucella canis, dogs; Brucella melitensis, goats and Brucella suis, pigs (Gumber et al., 2004). The Brucella organisms are small, aerobic, non-motile, Gram-negative rods with complex nutritional requirements in artificial media. The distinctions between the various strains are mainly useful epidemiologically and generally have little pathogenic significance.
(Harze, 2002). In human *Brucellae* enter the body through mucous membranes, skin abrasions or ingestion of unpasteurized milk (Hasanjani, 2006). In cattle, transmission occurs by ingestion of organisms in aborted fetuses, foetal membranes, vaginal discharges, contaminated feeds and water (Hong *et al.*, 2000). Also infected bulls transmit the infection to females during mating. Exposure to *Brucella* organisms is also likely to occur in utero or when calves born to healthy dams are fed on colostrum or milk from infected dams (Campell, 1994). The *Brucellae* are disseminated via the lymphatic and blood vessels to the heart, kidneys, and other parts of the body. The spleen enlarges in response to the infection.

*Brucella* organisms are not only resistant to phagocytic killing but also can grow intracellularly in phagocytes, where they are inaccessible to antibody and some antibiotics (Eisencheck *et al.*, 1995). Bone infection, osteomyelitis, is the most frequent serious complication. Brucellosis is typically a chronic infection of domestic animals involving the mammary glands and the uterus, thereby contaminating milk and causing abortions in the affected animals (Jennings *et al.*, 2007). Abortion is not a feature of human disease. Sixty percent of the cases of brucellosis occur in workers in the meat-packaging industry; less than 10% arise from ingesting raw milk or other unpasteurized dairy products (Young, 2009).
Only about 150 cases of human brucellosis are reported each year in the United States (Rizzo, 2005). In the United States, infections have been acquired by hunters from elk, moose, bison, caribou, and reindeer. About 20% of the Yellowstone Park bison herds are infected, and more than 1,000 have been killed when they wander outside the park because of fear that they will transmit the disease to cattle (Moreno et al., 2002). The most important control measures against brucellosis are pasteurization of dairy products and inspection of domestic animals for evidence of the disease. An attenuated vaccine effectively controls the disease in domestic animals (Seismenis, 1998).

In Kenya, prevalence of brucellosis is high in Narok and other pastoral areas. Many cases have been reported in the various annual reports of the Ministry of Agriculture and other literature (Muriuki et al., 1994). Serological tests for 10 years (1978-1987) at Veterinary Laboratories in Kabete, revealed that 17.08% cattle (N=16,273) 4% goats (N=2,851) and 7% sheep (N=1,374) were infected (Kagunya and Waiyaki, 1978).

Makuyu Division is a dry area which has no permanent rivers. Most of the inhabitants were squatters in coffee and sisal plantations during the colonial era. The people are poor and have low standards of living hence the Government provides them with relieve food (DAO, 2001). This area lacks clean water and therefore the standard of hygiene is low. Livestock diseases are common in Makuyu Division (DVO, 2009). Clinical
officers in Makuyu Health Centre refer about 5% of patients to Maragua or Thika Level Five Hospitals for treatment in suspicion that they are suffering from brucellosis (Dr. John Kamau, Makuyu Clinical Officer—personal communication). Therefore information on prevalence of brucellosis in dairy cattle in this division is important for better management and control of the disease.

1.2 Problem statement

In August 2009, about six cases of retained placenta disease which is a sign of brucellosis were reported to Makuyu veterinary Department among other livestock diseases (DVO, 2009). Families in Makuyu live in congested homes, the area is deficient of clean water, the standard of hygiene is low and contact with infected animals is common. The Makuyu Health Centre reportedly refers about 5% of patients every month to bigger hospitals on suspicion that they suffer from brucellosis (Hospital records). There are no facilities to confirm the suspected cases of brucellosis and there was no feedback from the bigger hospitals to confirm this diagnosis. The real assent of the problem is thus not known. In August 2009, a dairy farmer from Kagumo in Kandara District Murang’a County took milk samples to Kabete Veterinary Laboratories for diagnosis and all the six were positive on Milk Ring test (MRT). In the same year another dairy farmer from Kagaa in Makuyu Division took four milk samples which were also positive on MRT (DVS, 2009). This suggests likely there was high prevalence of Brucella organisms in Murang’a County. It is for this reason that this
study was undertaken with the aim of determining prevalence of brucellosis by establishing the reactor rate of randomly sampled dairy cattle in Makuyu Division.

1.3 Study justification

Although brucellosis can be found worldwide, it is more common in countries that do not have good standardized and effective public health and domestic animal health programs. The situation is worse in developing countries where there are low living standards and poor hygiene (Douglas and Bennet, 1999). In Kenya, the disease is endemic, whereby in some parts of the country the prevalence may be high. Kagumba and Nandokha (1978) reported a 10% prevalence of bovine brucellosis in extensive production systems in Nakuru, while Kodohira et al. (1996) reported a 2% apparent prevalence in the small holder system in Kiambu. In Dagoretti, Kang’ethe et al. (2007) reported antibody prevalence of 1.1% in dairy farming and 0.7% in non dairy farming households. Muriuki et al. (1994) reported antibody prevalence rate of 68% in cattle in Narok County. Many cases have been reported in the various annual reports of the Ministry of Livestock Development and other literature (Ndarathi and Waghela, 1991). Majority of the people in Makuyu Division live in congested homes and come into contact with possibly infected animals, contaminated milk and manure. Whole families may be infected through sharing shelter with animals (Zhan, 1996). Serological tests show that fluid such as serum, uterine discharge, vaginal mucus and milk or semen
plasma from suspected cattle may contain varying quantities of antibodies of IgM, IgG1, IgG2 and IgA directed against Brucella (Blood, 1995).

1.4 Research questions

i. What is the prevalence of Brucella abortus in dairy cattle in Makuyu Division?

ii. What is the knowledge level of dairy farmers on brucellosis in Makuyu Division?

iii. What is the accessibility of veterinary services among dairy farmers in Makuyu Division?

1.5 Objectives

1.5.1 General objective
To determine prevalence and knowledge level of brucellosis in dairy cattle in Makuyu Division, Murang’a County, Kenya.

1.5.2 Specific objectives

i. To determine prevalence of Brucella abortus in Makuyu Division, Murang’a County.

ii. To assess the knowledge level of brucellosis among the dairy farmers in Makuyu Division.
iii. To evaluate the accessibility of veterinary services to dairy farmers in Makuyu Division.

1.6 Hypotheses

i. Brucellosis is absent in Makuyu Division.

ii. People in Makuyu Division are deficient of knowledge about brucellosis.

iii. Veterinary services among the dairy farmers in Makuyu Division are minimal.
CHAPTER TWO: LITERATURE REVIEW

2.1 Brief history of Brucella species

*Brucella melitensis* was the first member of the genus *Brucella* to be isolated from livers of British soldiers dying from a fibrile disease in Malta (Bruce, 1887). Its source was found to have a link to milk of goats and sheep. This species is the most virulent for man among the *Brucella* species. *Brucella abortus* was isolated from cows showing infectious abortion (Bundle, 1998). It is pathogenic to cattle and is also infective to other domestic ruminants, pigs and man. *Brucella suis* whose natural host is the swine was isolated from fetuses of sows (Rizzo, 2005). *Brucella neotomae*, was encountered by Stoenner and Lackman from desert rats (Patricia et al., 2006). According to the same authors, *Brucella ovis* was reported from the genital tract of infected sheep while *Brucella canis* was described as a cause of wide spread abortions in dogs in USA (Cloeckaert et al., 2003). The disease caused by these organisms has been referred by various names. In man the names are brucellosis, undulant fever, malta fever and Mediterranean fever. In cattle, it is called infectious abortion, enzootic abortion and Bang’s disease among others (Randostits et al., 1994).

2.2 General characteristics of Brucella organisms

The genus *Brucella* consists of small non-motile, non-spore forming, gram-negative aerobic cocccobacilli. Each has specific biotypes that differ from one another on the
basis of slight biochemical activity, resistance to aniline dyes and phage. Many strains require supplemental carbon dioxide for growth especially on first isolation (Dricot et al., 2004). Each species has a predilection for a specific host but will also infect a wide range of other animals and humans (Table 1). *Brucella neotomae* has only been isolated once from desert rat in Ulta in the USA and whether it is pathogenic is not known for sure (Harze, 2002). All the pathogenic members of the genus lounge in the cells of the host’s reticulo-endothelial system (Cloeckaert et al., 2003).

**Table 1: Brucella species and their main hosts**

<table>
<thead>
<tr>
<th><em>Brucella</em> species</th>
<th>Main hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brucella abortus</em></td>
<td>Cattle</td>
</tr>
<tr>
<td><em>Brucella suis</em></td>
<td>Pigs</td>
</tr>
<tr>
<td><em>Brucella melitensis</em></td>
<td>Goats and sheep</td>
</tr>
<tr>
<td><em>Brucella cannis</em></td>
<td>Dogs</td>
</tr>
<tr>
<td><em>Brucella abortus, Brucella melitensis</em> and <em>Brucella suis</em></td>
<td>Man</td>
</tr>
</tbody>
</table>


2.2.1 Resistance and Survival of *Brucella*

*Brucella* organisms are destroyed by exposure to high temperature at 60°C for 10 minutes, exposure to phenol for at least 15 minutes or exposure to low temperatures such as at 0°c for a month. *Brucella abortus* and *Brucella melitensis* are rapidly destroyed in fermenting milk (Ettunge, 1995). *Brucella abortus* can however survive for 6 months in sealed culture tubes on agar. *Brucella melitensis* is reputed to survive for 6 days in urine, 6 weeks in dust, 10 weeks in water or soil and up to 8 months in moist manure (Capasso, 2002).

2.2.2 Cellular and colonial morphology and staining characteristics of *Brucella*

*Brucellae* may appear oval, coccal or diplococcal when about to divide (Watkinson, 1993). *Brucella melitensis* is considered more coccal than *Brucella abortus* with the latter being more capable of changing to bacillary forms especially in rich media like blood agar. On smear the cells appear singly, in pairs, short chains with end to end, in small groups or clusters (Spitter, 2003).

The short chains predominate when grown in liquid medium. All *Brucellae* have fairly constant morphological features. When viewed in reflected light, the colonies are smooth, intermediate, rough or mucoid. Smooth colonies are easily emulsifiable to form stable saline solution while rough forms are granular (Halling *et al.*, 2005). Ammonium
oxalate and violate greatly help to discern the different colonial forms, which in clear media appear raised with entire edges and transparent with a smooth shiny surface. They appear pale honey colour when viewed by penetrating light. The smooth forms are more pathogenic than the rough, and are encountered more frequently. The smooth species also have non-smooth variants that are antigenically different, less virulent and unstable. The change from smooth to rough involves genetic deletion and production of incomplete lipids (Hasanjani, 2006). The cell wall is responsible for the gram negativity and antigenic composition.

2.2.3 Growth requirements

*Brucellae* are slow growing fastidious organisms with complex nutritional requirements in artificial medium. Most strains require a rich medium with several amino acids such as thiamine and nicotinamide, calcium pantothenate and magnesium ions. They will not grow in anaerobic atmosphere but may require added carbon dioxide especially on primary isolation. Their optimum temperature for growth is 37°C; although they will grow in temperature ranges of 20-40°C. The most suitable pH is 6.6-7.4 (Capasso, 2002). Some species like *Brucella abortus* are metabolisers of mesoerythritol instead of glucose. This sugar alcohol which is abundant in pregnant uteri of some animals improves *in-vivo* growth (Jubb *et al.*, 1993).
In vitro growth is improved by addition of serum, blood or tissue extract. Serum dextrose broth and agar are ideal (McLean, 1992). The serum serves as a source of nutrients and neutralizes inhibitors present in peptone (Rizzo, 2005). Also essential for growth are iron and magnesium ions, which stimulate growth and have a regulatory role. In solid media, colonies are rarely visible in 48 hrs (Pappal et al., 2004). Only vigorous strains of Brucella abortus, Brucella suis and Brucella melitensis, grow on Macconkey’s medium, while all the rest are inhibited (Watkinson, 1993).

2.2.4 Biochemical characteristics of Brucella
Energy is yielded by an oxidative process through the pentose monophosphate pathway. The rate of oxidation is a characteristic of some strains (Juan, 2006). Brucellae are nitrate positive and are usually oxidase positive except for a few strains. They produce hydrogen sulphide at rates that are biotype specific. Brucella suis splits urea fastest, changing the colour of Christensen’s medium almost immediately upon inoculation. Brucella abortus especially the reference strain B544 takes longer than Brucella suis, with Brucella melitensis taking even longer. Brucella ovis has no urea’s activity at all. The rate of urea hydrolysis is a useful characteristic for speciation (Malhotra, 2004). Colmonero (1992) demonstrated that each Brucella species has a specific and definitive pattern of oxygen utilization on selected amino acids and carbohydrates as measured by Warburg apparatus and expressed as oxygen co-efficient (Franco et al., 2007). Brucella organisms do not produce acid from carbohydrate in conventional media except
probably *Brucella neotomae* (Robichaud and Libman, 2004). They do not lyse erythrocytes but turn blood agar medium green (Muriuki *et al.*, 1995).

### 2.2.5 The Brucella cell wall molecular structure and its antigenic composition

The cell wall is very important for maintaining the cell structure, for host attachment and colonization. It is the part of the cell that is in direct contact with the environment and to which the host responds. It is made up of an organized triple layered molecular organelle whose outermost part is a 9 Nanometer (nm) lipopolysaccharide-protein structure (Lapaque *et al.*, 2005). These cell wall layers of lipopolysaccharides (LPS) and proteins are linked to an electron dense inner layer of 3-5 nm. The dense part corresponds to the cross-linked muramic acid that contains peptidoglycans (Malay and Kocuba, 2006). The peptidoglycans are located just below the LPS and immediately above the periplastic space. The electron dense triple layered periplastic space is thought to harbour the enzymes that digest the cell wall in preparation for binary fission. The LPS covers most of the bacterial surface, whereby its side chain gives colonies their phenotypic smoothness. The phenotypic smoothness or roughness of the colonial forms of the organism is in turn related to the quantity of the LPS. Gas liquid chromatography shows that *Brucella canis* has a unique LPS profile (Maria *et al.*, 2007). The LPS, which are a diverse group of molecules, are made of the lipids bound to the polysaccharide (PS). The PS is the outermost part of the LPS, and so it is exposed to the exterior of the cell. These PS molecules are more prominent in *Brucella* than in
Entamoeba coli (Maria et al., 2007). Long chain fatty acid composition in the Brucella cell wall is significantly distinctive and is of value in identification, classification and taxonomy at the genus and sub-genus levels. It constitutes 17.5% of total cell wall weight. Among the LPS of Brucella melitensis is a unique lipid A that is linked to keto-d-6-glutamic acid (Kdo) at C-6. This lipid and its sister polysaccharides may be attached to the cytoplasmic membrane (Moreno et al., 1992). The cell wall accounts for 21% of the total smooth bacterium’s dry weight and 14% in the non smooth strains, since the latter lacks LPS in their walls. From this weight, about 6.2% in the rough LPS and 1.3% in the smooth cellular form is due to proteins. The proteins contribute much less to the quantity of the cell wall and are less prominent serologically. Their remnants during purification may be responsible for the mitogenic properties of the LPS.

2.2.6 Lipopolysaccharides (LPS) composition and activity

These are a group of complex molecules on the cell surface consisting of lipid A and polysaccharides (PS) that are also bound closely to protein moieties. Lipid A from different bacteria may have different biological activity among which is mitogenic activity (Zygmont et al., 1994). The LPS though of near similarity to PS is not identical to it. The LPS consists of five blocks of N formyl perosamine molecules. Four of these blocks have a molecular arrangement with \( \sim 1-3 \) linked residues. The residues are made of core oligosaccharides such as glucose, mannose, 2-amino-2, 6-dideoxy-D-glucose (glucosamine) and Kdo. The residues of Brucella share some activities with those of
enterobacteria and *Yersinia enterocolitica*, despite the latter having a distinctly different fatty acid composition (Malhotra *et al.*, 2004). This near identity for both is an attribute of PS, which consists of a side chain (‘O’ chain) that is exposed to the surface. This PS accounts for 1-9% of the cell’s dry weight. It bears the two major immuno-dominant antigens A and M in *Brucella abortus* and *Brucella melitensis* (Zhan, 1996). The ‘O’ chain in the rough *Brucella melitensis* is exposed intracellularly (Zaitsera *et al.*, 1995). Antigens A and M which are present in both smooth and non-smooth colonial forms of *Brucella* are responsible for the bacteria’s main serological activities. They account for the structural similarities of the smooth cell wall LPS and amino hydroxyl compounds (Vizcaino *et al.*, 1996). These antigens are carbohydrate in nature and are responsible for the cross reactivity between the two *Brucella* species. Antigen A is determinant in *Brucella abortus* and M in *Brucella melitensis* with each imparting its characteristics to the particular species. They are responsible for the immunological activities of both species and account for the cross-reactivity between the two (Aragon *et al.*, 1996).

The M antigen is a complex ‘O’ chain pentasaccharide homopolymer, made up of core oligosaccharides, that are made of glucose, mannose, 2-amino 4-6-dideoxy-D-glucose (glucosamine), amino dideoxy-D-glucose and Kdo. It has five N-formyl peosamine blocks in the ratio of 1:4 for amino dideoxy-D-glucose and Kdo. It has five N-formyl peosamine blocks in the ratio of 1:4 for 1-2 and 1-3 linked to the sugars 4, 6 dideoxy-4-formamido-D-mannose as shown by nuclear magnetic resonance (Ettunge, 1995). The one 1-2 and four 1-3 linked sugars are formyl derivatives. The M antigen also has
intermediate forms with varying ratios between $\alpha$ 1-2 linked and $\alpha$ 1-3 linked forms as occurs in *Brucella suis* biotype 3 (Capasso, 2002). Although the two species have both A and M antigens, the epitope densities for the two antigens in both species are variable. The $\alpha$ 1-2 linked components are on a tetrasaccharide segment which provides the structural basis for a common type A antigen determinant (Al-Sous, 2004). Apart from the cell wall antigens, all members of the genus possess characteristic intracellular ones too.

Antigen A is formamido homopolymer of $\alpha$ 1-3 linked 4,-6-dideoxy-4-formamido D-mannose residues as monomeric linear unbranched, repeating units that have functional isomerism. It is difficult to separate it from M (Harze, 2002). Both have extensive serological cross-reactivity based on polyclonal antisera due to their shared epitopic features involving $\alpha$ 1-2 linked tri and tetrasaccharides (Swai *et al*., 2005). These authors also suggested that quantities of the two antigens may be similar but their epitope densities may be different. There are common epitopes on A and M antigens that are responsible for the cross-reactivity between *Brucella abortus* and *Brucella melitensis* antisera. These epitopes are denoted by c and y. The formamido entities of both antigens are similar with rotational isomerism E and Z. Monoclonal antibody test (MAT) have shown that A and M are on the same molecule (Shirima, 2005).
The cross reactivity between A and M antigens of Brucella ‘O’ chain and those of other gram-negative bacteria is associated with the M amyl derivative at 4-amino 4-6-dideoxyl-D-mannose pyranose units (Pappal et al., 2004). Therefore, the difference between smooth Brucella abortus, Brucella suis and Brucella melitensis are quantitative and not qualitative. The ‘O’ chain of Brucella and that of Yersinia enterocolitica are identical, with the latter’s resembling those of Brucella abortus and Entamoeba coli 0:157. This similarity is due to the occurrence of the 2-O glysocylated residues of 4-acetamido 4-6-dideoxy α mannose pyranosyl units. The cross reactivity of Brucella and Yersinia is either due to tetrasaccharide determinants or their terminal oligosaccharide components that form part of the cross-reacting antigen polymers for A and M and which in turn are responsible for the cross-reactivity between Brucella abortus, Brucella melitensis and Yersinia enterocolitica 0:9 (Vizcaino et al., 1996).

The Antigen A of Yersinia is 10 times less reactive than that in Brucella (Young, 2009). This difference in the reactivity is because the antigen A of Yersinia lacks α 1-2 linkages. Antiserum to Brucella abortus removes LPS of Yersinia enterocolitica 0:9.

The Vibrio cholera ‘O’ chain also has quite a lot of resemblance to that of Brucella abortus though its LPS have quite a distinct fatty acid composition and structure. Between them, there are seven similar fatty acid components that account for 85% of the dry weight in both (Hasanjani, 2006). Both have lipid A as part of the LPS. Antigens A and M stimulate production of IgM while IgG2 production is stimulated by
protein antigens (Christopher, 2004). *Brucella abortus* and *Brucella melitensis* induce production of antibodies that react in immunoprecipitation test and also against the native hapten. It is thought that the native hapten is different but identical to the PS of the LPS. Its basic structure is identical to the PS in *Brucella melitensis*.

### 2.2.7 Brucella Proteins

These are the second most important cell wall constituents from the point of view of protection to the host. Those on the cell wall are referred to as the Outer Membrane Proteins (OMP). The proteins are less exposed to the surface than the LPS and so they are not accessible to host antibodies. As the organisms become rough, the OMP become more exposed to the surface (McDermott and Arimi, 2002). *Brucella* have three groups of major OMP and some minor ones, all being closely associated with peptidoglycans (Pg) that are in turn strongly associated with the cell wall but which can be unbound from each other by lysozymes (Cloeckaert *et al.*, 1992). The *Brucella* Pg has a composition like that of *Entamoeba coli* (Mclean, 1992). Each species also has a characteristic reproducible protein with unique qualitative and quantitative attributes (Zaitsera *et al.*, 1996). The chromatography pattern of the OMP revealed that all species of the genus have low or high quantities with minor qualitative and quantitative differences. The OMP are classified on the basis of molecular weight as; group 1 of 89-94 Kda, group 2 or porins of 35-40 Kda and group 3 of 25-30 Kda respectively (Sharma
and Adlakha, 1997). Group 1 is more exposed than the others (Cloekaert et al., 1992). The porins are trimmers linked to LPS. They occur in varying quantities in the cell and show a lot of homology within the smooth *Brucella* species and *Entamoeba coli*. The quantity in *Brucella ovis* and *Brucella suis* is lower than in *Brucella abortus* (Ahmed and Murier, 1995). Group 3 proteins are strongly bound to the peptidoglycans and have a lot of heterogeneity with group 2 and resemble OMP of *Entamoeba coli* on the basis of amino acid sequence. The total protein of group 2 and 3 for the smooth and rough *Brucella* are quantitatively equal. The two proteins form the cell wall matrix with its hydrophilic pores that allow passage of low molecular weight hydrophilic substances. Both have a common antigen; b (FAO, 2003).

Apart from the two matrix proteins there is also a 38 Kda protein bound to the peptidoglycan which also contributes to the matrix (Abbas and Aldeewan, 2009). Apart from the three OMP proteins, there are minor ones of 10, 16.5, 19 and 89-94 Kda among which are glycoproteins and lipoproteins. These minor proteins have little biological activity though probably different immunogens (Hill and Cook, 1994). One of the minor OMP is a 62 kda protein that was detected in pigs infected with *Brucella*. It is a homologue of a 65 Kda protein of *Mycobacterium leprae* and has also been recognized in *Yersinia entercolitlitica* 0:9. It may be a common cross-reactivity antigen as elucidated by immunoblot using *Brucella melitensis* antigen (Jennings et al., 2007).
Important antigenic components of OMP are denoted a, b, c, d, and e. Antigen d is the most predominant while b is most widespread within the 3 protein groups, having been demonstrated in *Brucella abortus*, *Brucella melitensis*, *Brucella ovis* and *Brucella canis*. Antigen a, is on group 2 and 3 only, while c, d and e are only in Group 3. These antigens may have relevance to the protective immunity as some of the organism’s proteins, especially the minor ones, have been traced in the plasma of infected animals even after bacteraemia has ceased (Gall and Nielsen, 2004). Antibodies against OMP are mainly targeted to group 2 and 3 and to a lesser extent on minor proteins of 55-62, 70-74 and 89-94 Kda. A protein denoted OMP 31 (31-34 Kda) is among the major ones in *Brucella melitensis* but minor in *Brucella abortus*. It has been said to be protective in mice. If indeed it is protective, then it may be a good candidate for development of subcellular vaccines against sheep and goat brucellosis due to both *Brucella melitensis* and *Brucella ovis* (Zugmunt *et al.*, 1994; Vizcaino *et al.*, 1996). It has shown some promise as a candidate for such vaccine development (Omer *et al.*, 2000).

Antibodies against it were found in cattle vaccinated with *Brucella abortus* s19. Its protection is derived from a 36 amino acid region (Abbas and Aldeewan, 2009). A cytoplasmic protein in *Brucella canis* and *Brucella abortus* is similar to the minor one stated above. Infected sheep produce antibodies against minor OMP and LPS molecules (Makita *et al.*, 2008). The reaction in the vaccinated ones was more intense in immunoblot based test than in infected ones (Staal and Kaguongo, 2003). If OMP are
protective, they would yield ideal vaccines since they lack the LPS to which serological results contain (Arimi et al., 2005). Immunity based on OMP is cell mediated which alone may not be adequate for protection.

2.3 Epidemiology of Brucellosis

2.3.1 Transmission of Brucellosis in human

Brucellosis is a health hazard to animal care takers, handlers, animal health professionals and veterinarians (Capasso, 2002). Man acquires brucellosis either directly or indirectly from infected animals, chiefly swine, goats, sheep and cattle. The most common way to be infected is by eating or drinking contaminated milk products. When cows, sheep and goats are infected, their milk is contaminated with the bacteria (Gall and Nielsen, 2004). If the milk is not pasteurized, these bacteria can be transmitted to persons who drink the milk or eat cheese made from it. Brucella organisms can enter into the body through skin wounds which is a problem mainly for persons working in slaughterhouses or meat packaging plants or for veterinarians. Handling new born animals or foetal membranes is a common direct method of transmission. Inhalation of Brucella organisms is not a common route of infection, but it can be a significant hazard for people in certain occupations, such as those working in laboratories where the organism is cultured (Makitat et al., 2008). Brucellosis in man is
rarely acquired by eating infected meat as cooking destroys the organisms. However, partially cooked goat meat has been incriminated as a source of human infection (Omer et al., 2000).

The human disease has been known in the Mediterranean region and the Middle East since biblical times. It has continued to be prevalent in the Near East and South America (Aragon et al., 1996). It is the cause of the third highest fevers in Cairo (Moreno et al., 1992). In some nations the disease has reached alarming proportions (Capasso, 2002). The main source of human infection in these places are cattle, sheep, goats and swine; with camels playing a minor role (Aragon et al., 1996). However, camels may be a major source of human epidemic in Kuwait and Mediterranean region (Omer et al., 2000). Man is infected by Brucella abortus, Brucella melitensis, Brucella suis and Brucella canis. Brucella melitensis is the most pathogenic, followed by Brucella suis, Brucella abortus and Brucella canis in that order, with cases due to the latter being reported since 1972 (Sharma and Adlakha, 1997). The incubation period is insidious and of variable duration possibly due to differences in the virulence among the various strains.
2.3.2 Brucellosis in human

The disease is acute and very rarely sub-acute, ranging from self-limiting fever to complicated and sometimes fatal disease. Persons of all ages are affected but more so those in the working stages of life. Patients will be seen to have abnormalities linked to reticulo-endothelial system with most of them manifesting with acute or chronic disease that is characterized by fever of 40°C, chills and profuse sweating often with malodour at night, headaches, lethargy, joint swellings and pains, anorexia and myalgia. The rare signs are weakness, easy fatigue, malaise, mental disorders and depression among others, depending on organs in which localization takes place (Young, 2009). Pain of the gluteal and lower lumbar are frequent clinical features (Sangori et al., 1996). The complications are mainly oesto-articular presenting as spondylitis and sacroillitis as shown by radiographic abnormalities mainly at the axial site. Sacroillitis is the commonest complication whereby some patients may require surgical treatment to relieve the pressure on the spinal cord (Abbas and Aldeewan, 2009). In infections involving the liver, the patient will have mild neutrophenia.

Most persons are exposed to low constant doses that might either sensitize them or give them immunity leading to serologically positive reactions (Patricia et al., 2006). Some patients may fail to develop serological reaction as shown in a sixteen year old girl who developed multiple subcutaneous absesses, ostemyelitis and severe colitis but was
serologically negative and was positively diagnosed on hemoculture. Serologically, she was positive only on lymphocyte proliferation assay (Staal and Kaguongo, 2003). Infection is acquired through skin abrasions, aerosols, mucosa of the gastrointestinal tract, and to a minor extent through the conjunctiva and the upper respiratory tract. Among affected communities the poor are most afflicted (Gall and Nielsen, 2004). Human patients on treatment with antibiotics may become serologically negative as the immune complexes dwindle upon such therapy.

2.3.3 Transmission of Brucellosis in animals

Transmission of *Brucella* is very likely to occur via the oral route because animals tend to lick aborted foetuses and genital discharge of the aborting animal. Exposure to *Brucella* organisms is also likely to occur in utero or when calves born to healthy dams are fed on colostrum or milk from infected dams (Abbas and Aldeewan, 2009). Brucellosis in bulls does not always result in infertility, although semen quality may be affected. Bulls that remain fertile and functionally active shed *Brucella* organism with the semen during the acute phase of the disease (Nammalwar et al., 2009). Dogs carry pieces of placenta or aborted foetuses from one place to another causing direct exposure. Contamination of a cowshed or pasture takes place when infected animals abort or have full term parturition. Mucus membranes of the eye are a possible portal of entry.
2.3.4 Brucellosis in animals

Almost all domestic animal species can be infected by *Brucella*. The establishment of disease depends on age, productive status, host resistance, infecting dose and virulence of the particular strain. The disease starts as septicaemia with relapsing bacteraemia and later localization to various organs. This tendency to localize is highest with *Brucella suis* (Blood, 1995). Young animals, though susceptible, retain infection up to puberty but they tend to shed the organisms only for a short period while in sexually mature animals the infection tends to persist indefinitely. Infection is acquired through the mucus membranes of the gastrointestinal and upper respiratory tracts, abraded skin, congenitally and by placental route in cattle and swine. In cattle, high doses of the pathogen are required to induce disease and so in most cases such as artificial insemination and through placental routes, focal infections do occur (Malay and Kocuba, 2006). From these portals of entry, the organisms drain into lymph nodes of head or the local ones, where they invade the lymphocytes leading to acute regional lymphadenitis. The organisms have a carbohydrate virulent factor on their cell walls that helps them to bind to B lymphocytes (Al-Sous, 2004).

From the lymph nodes the organisms are spread by haematogeneous route to the lymphoid organs from where they are then released to the blood stream leading to recurrent bacteramia or persistent chronic infection. The organisms then infect
macrophages in which they multiply and are spread to various organs. Multiplication in the lymphoid organs or tissues such as the liver, spleen and bone marrow leads to granuloma formation. The granulomas may later develop into abscesses. Virulent strains survive and multiply better in macrophages (Jubb et al., 1993). Infected lymph nodes are infiltrated by neutrophils and eosinophils leading to proliferation of the cellular components of the germinal centres. *Brucellae* have special affinity for the ungulate pregnant endometrium, foetal placenta, fluids and to a lesser extent testis in the male; possibly due to attraction by the little erythritol that these tissues contain.

In the pregnant uterus, the organisms are carried by blood to the periphery of the caruncles from where they spread to and invade foetal chorionic villi. Here they are phagocytosed by erythropagocytic trophoblasts of chorion leading to haematomas in foetal caruncle especially late in gestation. Localization in the uterus may lead to premature birth, still births arise or abortion if the lesions are severe. Calves so borne may be viable or not. About a third of infected animals abort (Jubb et al., 1993). It takes months before the slow spreading lesions lead to abortions that normally occur in the 7-8\textsuperscript{th} months of pregnancy in the bovine female. Less frequent in cattle, signs of spondylitis, arthritis and mastitis that are accompanied by nodular udder lesions are seen. Lesions remain for sometime after abortion or birth. Foetal membranes are thickened, necrotic with the cotyledonal spaces variously involved. Affected areas are thickened by gelatinous opaque fluid or toughened by coagulation of inflammatory
exudate. The necrotic cotyledons are soft yellow-grey and covered by a sticky odorless brown exudate. Their stroma is oedematous, with increased leucocytic mononuclear cells, while the epithelial cells are staffed with bacteria. The amniotic fluid may be viscid (Maria et al., 2007). Lesions in the bovine foetus include necrotic focal areas and granuloma in the lymph nodes, liver, spleen and kidneys. Calves born to infected mothers especially female ones are usually infected but the disease usually wanes in them before they reach puberty (Juan, 2006). The male calves may be less resistant to *Brucella abortus* than cattle.

Pigs acquire infection through alimentary system, *per vaginum* and through the broken skin. In this host, the disease is marked by low bacteraemia, arthritis and spondylitis. Infected swine manifest disease by storm abortions, under-developed piglets, stillbirth or weak neonates and a high incidence of embryonic death. Abortion occurs at 2-3 months. The incidence of placental retention is lower than in the bovine female. Infected swine may show lymphadenitis and bacteraemia for years with some sows shedding organisms all along. Localization occurs in many organs especially the genitalia, skeleton including vertebral synovial fluid, mammary glands, lymph nodes, spleen, liver, kidneys, bladder and even brain. The organism has a high affinity for the skeleton and joints where it leads to granuloma formation. The uterus may develop nodules or false plaques in the mucosa with thickening of the wall that may lead to
luminal structure or occlusion resulting in probable pyosalpinx and granuloma of the supporting ligament. The uterus may accumulate mononuclear cells or leukocytes enmeshed in strands of mucin or amorphous globes of mucus. Bacteria remain in granulomatous foci in the non-pregnant endometrium leading to necrosis and calcification. Articular lesions begin as synovitis, fibrinopurulent vertebral osteomyelitis especially in the lumbar region leading to paravertebral abscess. In males, the organism is shed in semen from the accessory genitalia and so coitus can transmit the disease. In equine, the disease may be marked by chronic bacteraemia that becomes intermittent with a tendency to recur during pregnancy. The disease may be accompanied by recurrent equine ophthalmitis.

The organisms localize at parturition in spleen, mammary gland, lymph nodes, pregnant uterus and lymphoid tissue. In the male they localize in the accessory sex glands. The most common manifestations in equine are bursitis and fistulas wither. Abortion and hygroma are rarely encountered signs (Lapaque et al., 2005). In camels *Brucella melitensis*, was isolated from milk and aborted fetuses, with cases being reported in Egypt, Saudi Arabia, Sudan, Kenya, Somali and Ethiopia (Capasso, 2002). *Brucella abortus* also affects domestic buffalos and yaks with signs similar to those of cows. It also infects camels, lama, hares in Europe and rats in carribous (Randostits et al., 1994). Fowls have been reported to harbor *Brucella abortus* organisms but show no symptoms
(Dricot et al., 2004). Brucella abortus affects sheep mainly through the oral route (Ettunge, 1995).

Infected flocks may show storm abortion, sterility and neonatal death in the dam and sterility in the ram; while some sheep may show no signs at all. Affected flocks may have many sero-reactors with few among them delivering under-developed fetuses. Ewes infected in the last quarter of gestation give birth to live lambs while those infected early may have abortion or weak neonates. Some shedding ewes give birth to live lambs that may be free of Brucellae. On post-mortem, the neonates show fibrinous deposits on the liver, heart and lungs with Brucella abortus being recovered from foetal stomach. The organism is recovered from milk of infected ewes (Hasanjani, 2006). In flocks infected by Brucella melitensis the organism is isolated for periods extending to two years which is no longer than the period in which Brucella abortus is recoverable from this species (Watkinson, 1993).

Goats may be culturally positive on second pregnancy with different flocks showing different after-births of abortion storms. Some flocks may show complete recovery (Halling et al., 2005). Canine brucellosis is characterized by abortion, embryonic death and infertility while males show testicular atrophy, epididymitis, prostatitis, scrotal dermatitis and infertility. Lymphadenitis and splenetic are infrequent signs. Dogs and cats when infected by Brucella abortus or Brucella melitensis manifest with fever,
emaciation, orchitis, anaestrous, arthritis and sometimes abortion (Franco et al., 2007). Also occurring are disk spondylitis, inter-vertebral disk infection and chronic lymphocytic endophthalmitis. This may be an immunologically mediated reaction (Harze, 2002). *Brucella abortus* can also infect dogs. From ten farms with infected cattle, dogs also yield the same organism. Infection occurs through ingestion of infected milk and foetal membranes or aborted foetuses (Christopher, 2004). Serological diagnosis in dogs is made difficult by extensive cross-reaction between rough cell envelope antigens of *Brucella canis* and heterospecific antibodies (Spitter, 2003). Seroconversion was not directly related to culture positive cases. Dogs may have the potential to infect cattle and could pose a danger for a longer duration of time though the risk of transmission is small (Jennings et al., 2007). The disease causes economic losses through abortions, infertility marked by prolonged calving intervals, retained placentas, weak unthrifty neonates that are prone to high mortality and loss of draught power due to lameness subsequent to hygromas (McDermott and Arimi 2002). It also causes economic losses due to restriction on international trade (Fensterbank, 1998).

### 2.4 Clinical signs of brucellosis in man

The onset of brucellosis in man is usually gradual and the patients complain of mild fever, sweating, weakness, aches, enlarged lymph nodes and weight loss. In animals the
signs are foul smelling vaginal discharge, abortion, arthritic joints and retained afterbirth.

2.5 Host Immunity to infections by *Brucella*

Animals infected by *Brucella* organisms accord themselves protection by production of humoral antibodies: IgG and IgM among others, and cell mediated immunity (CMI), or both. Vaccination also offers protection by production of humoral antibodies and probably by stimulating CMI. Cell mediated immunity is taken as the more effective arm of immunity (Stephens *et al.*, 1995). Some infected animals with very little or no antibodies do occur (Kangethe *et al.*, 2000). At the site of entry into the host the organism is destroyed by neutrophil bactericidal activity that is probably facilitated by peroxide and oxygen dependent mechanisms (Mclean, 1992).

The complement cascade has also been shown to mediate killing of *Brucella abortus*, though some strains may be resistant. This resistance is associated with pathogen’s virulence particularly the smooth forms, with the virulent strain being highly sensitive to serum mediated killing by complement (Eisencheck, 1995). Complement cascade is sometimes activated by antibody independent mechanism though not by alternative pathway. The *Brucellae* that survive killing invade and lounge the macrophages where they are either destroyed or are offered protection. Those that survive within
macrophages are transported through the circulatory system to various organs. In this way macrophages contribute both to immunity and pathogenesis (Maria et al., 2007).

Cells infected with *Brucella abortus* produce interferon, which activates plasma cells to transform to immunoglobulin producing cells. Complement fixing and haemagglutinating antibodies appear before agglutinins. Immunoglobulin M is the first one to be produced followed shortly by IgG$_1$ which rises and reaches peak by day 32 (Stephens et al., 1995). These and IgG$_2$ appear within a short time in infected animals and are later followed by IgE which has been detected in 76% of acute human cases using Indirect Fluorescent Antibody technique (IFA) and Radio Immunoassay (RIA) (Fensterbank, 1998). Secondary response of IgM is like in initial stimulus. Once production has reached peak, it stabilizes and the immunoglobulin persists for sometime before they start to disappear slowly. The peak of IgG$_1$ is three times that of IgM and persists longer in sero-reactors (Ahmad and Murier, 1995). Its level is related to the strength of the antigenic stimulus, thus serving as an indication of active disease, while its persistence is associated with progressive acute infection, chronic diseases or repeated infection.

Repetition of antigenic stimulus causes a more rapid, abundant and persistent production of IgG. When IgG disappears, it probably signifies an end of infection
(Malay and Kocuba, 2006). The distribution of IgG and IgM is different in chronically infected animals and vaccinated ones (Rijpends et al., 1995). Immunoglobulin M characterizes very early on persistent infection following vaccination. In vaccinated animals where only IgM may be produced, agglutinins appear earlier than in infected animals. They disappear fastest depending on the infecting dose, route of inoculation and animal species. In animals vaccinated with s19 at 8 month of age, the level of IgG is usually higher than that in animals vaccinated during adulthood (Bertu et al., 2010). These Immunoglobulins are responsible for serological reactions. Complement Fixation Test is a result of both IgG and IgM though the latter is measured more efficiently by SAT and RBPT than the former (Omer et al., 2000). In vaccinated animals RBPT is less reliable than both CFT and SAT (Seimenis, 1998). Swine do not have a similar immunological response as cattle (Makita et al., 2008).

2.6 Diagnosis of Brucellosis

2.6.1 Bacteriological tests

This involves microscopic examination of stained smears for a presumptive diagnosis. The sample is mixed with negative Gram stain by equal portion on a parafilm sheet. After drying for 15-30 minutes the specimen is examined by microscopy. The *Brucellae* stain red and are rod-shaped (Al-Sous, 2004). Other immunological tests should be used to confirm presence of *Brucella* antibodies.
2.6.2 Immunological tests

2.6.2.1 Rose Bengal Plate Test

Rose Bengal Plate Test (RBPT) is a qualitative screening test with a high sensitivity and specificity in which a positive reaction is an indication of a disease. It is cheap, rapid, sensitive and allows for testing of many samples in a short time (Davis et al., 1990). It misses very few infected animals whether early or later in the course of the disease. It is however, not adequate on its own since it has false reactions (Allen et al., 1998). False positives occur due to cross reaction with antibodies against other bacteria. The test is based on whole cell phenol inactivated *Brucella abortus* s99 stained with Rose Bengal dye and buffered at PH 3.65. The test is carried out by use of antigen and serum (Kundi, 2007). The antigens detect agglutinins to all smooth *Brucella* organisms. Antigens A and M of the smooth lipopolysaccharides are the important surface molecules in this test.

2.6.2.2 Complement Fixation Test

Complement Fixation Test (CFT) is a very useful supplemental test especially with sera that have low agglutination titre. It can be automated and used as a confirmatory test following RBPT screening. It is used on several herds and detects reactions before other tests. Its results are more specific than agglutination tests. It is highly recommended for diagnosis and it is also useful for differentiating natural infection from vaccination with
s19. In both cases, it relates best with clinical diseases as it classifies them as reactors or non-reactors (Young, 2009). The test results depend a lot on the accuracy of complement titration (Complement is the most fragile component of the test reagents). Complement Fixation Test failed to detect 9% of cattle positive on culture or aborting (Shirima, 2005). It is found to miss some infected animals in some herds when the disease is at chronic stages (Crasta et al., 2008). Reactions in CFT are based on large antigens with multivalent binding sites and IgG$_1$ as the major serum component (Swai et al., 2005). These authors observed that the immunoglobulins reactive in CFT are IgM and IgG$_1$ with IgM being more reactive. Immunoglobulin M is partially inactivated by heating to 60°C and so it may not be as important as IgG$_1$. Antibodies detectable by CFT start showing from day 10 post infection but as the disease advances to chronicity, its titres rise (Rijpens et al., 1995). Malhotra (2004) recommended that CFT titre of 25 units per millimeter (++ at 1/25) be taken as suspicious while higher titers be taken as positive.

2.6.2.3 Milk Ring Test

Milk Ring Test (MRT) is the best for screening dairy cattle and potentially infected herds for brucellosis (Kangethe et al., 2000). Milk, which is the test material, is tested by a heat-inactivated smooth *Brucella* antigen stained with hematoxylin although tetrazolium has also been used instead of hematoxylin (Ahmad and Murier, 1995). The resultant globular complexes combine with the fat in the cream to form a ring. The ring
formed in cow milk rises to the top while that of goat milk settles to the bottom of the tube. Attempts have been made to standardize the test and to estimate the titre but the idea has not gained much popularity (FAO, 2003). The results are affected by the sampling method that leads to scant or excess cream, heating of the milk above 110°C for 5 minutes and the duration of the milk storage. Storage of milk at 4°C will yield results for up to 2 weeks. False positive results may be obtained in fresh milk but this may disappear once the milk is chilled for a time.

Mastitic milk may also give false positive results due to presence of plasma proteins that leak into it (Bertu et al., 2010). Bacteria in the milk will affect results the same way. In milk, the antibody associated with agglutination is IgA, which is produced locally in the udder (Vanzini et al., 2001). Not all Brucella infected animals have udder infection. All those without udder infections will yield negative results at all times on Milk Ring Test. Milk Ring Test gives a good correlation with udder infection and animals so infected will shed the bacteria in milk for a long time (9-21 weeks) post partum (Zowghil et al., 1996). A high proportion of these animals may have Brucella isolated from the milk despite being MRT negative. From such animals, mechanical spread of the disease is very likely.
2.6.2.4 Anigen Rapid Bovine *Brucella* Antibody Test

The Anigen Rapid Bovine *Brucella* Ab Test Kit (ART) from South Korea (Lucero, 2005) is a chromatographic immunoassay for the qualitative detection of *Brucella abortus* antibody in whole blood plasma, serum and milk. The ART kit has a letter T and C as “Test Line” and “Control Line” on the surface of the kit. Both the “Test Line” and “Control Line” in the result window are not visible before applying any samples. The “Control Line” is used for procedural control. The control line should always appear if this procedure is performed properly and the test reagents of the control are working. A purple “Test Line” will be visible on the result window if there are enough *Brucella abortus* antibodies in the specimen.

The specifically selected *Brucella abortus* antigens are used in the test as both capture and detector materials. This enables the ART kit to identify *Brucella abortus* antibodies in specimen with high degree of accuracy. A colour band will appear in the left section of the result window to show that the test is working properly. This band is the “Control Line” (C) and the right section of the result window indicates the test results. If another colour band appears in the right section of the result window, this band is the “Test Line” (T). Presence of only one purple colour band within the result window indicates a negative result. Presence of two colour bands (“T” and “C”) within the result window, no matter which band appears first, indicates a positive result (Davis *et al.*, 1990).
2.6.2.5 Enzyme Linked Immunoabsorbent Assay (ELISA)

Enzyme Linked Immunoabsorbent Assay is a simple, specific enzyme based primary biding assay with a superior performance over conventional tests. It has high degree of accuracy and a wide range of modifications for diagnosis of brucellosis among other diseases. With appropriate modification it can be used to test sera from all animal species, while also increasing the effectiveness of the control programme. It is a rapid, sensitive and specific test that is useful for both mass screening and individual animal diagnosis. This test is also useful for identifying immunoglobulin profiles in serum and cerebrospinal fluid (Rafai, 2002). It is more recent of the serological tests and has been considered the method of choice for diagnosing brucellosis (Hegazy et al, 2009). It favourably deals with the shortcomings of CFT and RBPT and can be modified to use monoclonal antibody techniques and radioimmunoassay by raising antisera against IgG. Antisera so raised will agglutinate IgG\textsubscript{1} and IgG\textsubscript{2} but not IgM. Anti-light chain monoclonal antibody is conjugated to horseradish peroxidase for use as the conjugate.

Use of monoclonal antibodies and radioimmunoassay helps to circumvent the problems of standardization with polyclonal antibodies. The test is also useful in detecting *Brucella* antigens in plasma (Moreno et al., 2002). Antigens for use in ELISA are bacterial polysaccharides (PS), lipopolysaccharides (LPS) or ‘O’ chain of the LPS immobilized on polystyrene wells. ‘O’ chain antigen is used to discriminate between vaccinated and infected animals (Zygmunt et al., 1994). The results are read as optical
density and are expressed as Spectrophotometric Absorbance Values (SAV) based on the choice of a suitable threshold.

The choice of threshold is dependent on the prevalence of the disease, vaccination practice and the desired result. Serum with a high SAV is considered positive (Williams et al., 1991). Results are plotted as a graph where they appear as a continuous optical density curve unlike those for polyclonal serology which are dichotomous and therefore do not reflect small changes in titter, as they are based on double dilution. The sensitivity for ELISA, which normally stands at 95%, is equal for all the dilutions. This high sensitivity plus its high specificity of 99.7% makes the test results easy to analyze (Vizcaino et al., 1996). It is useful for mapping out problem areas, as its readings remain positive even when shedding of the organism has ceased (Davis et al., 1990). The test identifies the reacting immunoglobulins as IgM, IgA and IgE; thereby classifying the disease as acute or chronic (Mcdermott and Arimi, 2002). However, it has not been accepted to replace the conventional methods because it is more expensive and requires specialized laboratories (Cloeckaert et al., 1992). It has variously been shown to have a high correlation with RBPT in acute cases and that it produces positive results longer than CFT in chronic cases while detecting small changes in antibody levels (Nammalwar et al., 2009).
The sera of vaccinated animals do not react with ‘O’ chain of the PS. Post vaccine titer levels decline to very low but may remain positive for some time in a few animals. When using ‘O’ chain of the PS as antigen, ELISA shows a higher intensity of reaction in vaccinates than infected animals but fails to detect vaccinated, infected animals at very early stages (Aragon et al., 1996). Its results are affected by bacterial culture phase and sometimes plates may absorb antigen and affect the results. Titration should be done in duplicate and repeating sera with more than 5% variation between the two tests. The test reagents are fragile and liable to spoilage on freezing.

2.7 Diagnosis in human

Clinical diagnosis of human brucellosis presents a difficult challenge due to the protein nature of the disease, its multiple system involvement, divergent clinical manifestations and asymptomatic individuals (Moreno et al., 2002). Laboratory diagnosis is based on blood culture results, high or persistent serum antibodies or by measuring anti-protein humoral responses (Makita et al., 2008). Three Human blood samples taken in 24 hrs and cultured in 10% carbon dioxide showed a success rate of 17-84.5% (Young, 2009). In endocarditis, bacteraemia is continuous which increases the chances of recovering the organism from the blood. On serology, caution needs to be exercised as cross-reactivity occurs with cholera, tularaemia, and yersiniosis (Gumber et al., 2004). Culture is recommended where cross-reactivity is suspected.
2.8 Diagnosis in animals

Diagnosis of the disease in animals is by isolation of the causative bacteria or identification of bacterial antigens in plasma (Bundle et al., 1998). Material for culture from the dam are blood, milk, cotyledons, and foetal membranes, while from the foetus, suitable materials are stomach contents, lungs, liver, spleen and meconium (Jubb et al., 1993; Omer et al., 2000). From carcasses, lymph nodes are the material of choice. In males seminal vesicles and testicular material are useful as sources of Brucellae. Liquid medium is suitable for liquid material, especially using Casterneda’s method, and serum dextrose agar for solid ones. The organism can also be isolated by animal inoculation (Blood, 1995; Vanzini et al., 2001). In case the material is contaminated, selective media with antibiotic is preferred for primary culture. The isolates are identified on the basis of carbon dioxide requirement, acriflavine test, and colony staining with crystal violet and typing by biochemical reactions, dye resistance and phage susceptibility.

Serological methods of diagnosis are the most extensively used mode of diagnosis of brucellosis in both animals and humans. They have a lot of uncertainties but they are more successful than isolation of the pathogen that is definitive.

A wide range of tests are used in disease surveys, epidemiological studies and in eradication. None of the methods, however, is without its peculiar limitations. This necessitates use of more than one method to confirm diagnosis. In field testing, Rose Bengal Plate Test (RBPT) gives results more consistent with Complement Fixation Test
(CFT) (Shirima, 2005). Its results are most prolonged and persistent (McDermott and Arimi, 2002). Rose Bengal Plate Test can be carried out by persons with minimal training, especially where laboratory facilities are lacking (Malhotra, 2004). It is however, recommended that confirmation of diagnosis be sought in suitable laboratories (Omer et al., 2003). None of the tests or a combination of them can detect all cases of brucellosis and such results are only pointers to the disease state in the herd.

A combination of the tests minimizes the shortcoming of the individual test (Jelastopulu et al., 2008). Complement Fixation Test in conjunction with RBPT is considered most accurate tests. The two tests and Serum Agglutination Test (SAT) are used as basic operative techniques (Davis et al., 1990). Being indirect methods, serological tests do not identify the individual immunoglobulins involved in the reaction but only give an indication of the dominant ones. The uncertainties in serology based diagnosis is complicated by many factors such as low agglutinin levels, latency, very early infection, chronicity, prozone phenomenon, blocking antibodies and failure by the test to distinguish active infection, recovery, vaccination or cross reactivity.

Good serological methods should be able to classify animals as reactors or vaccinates. This is frequently difficult or uncertain. Non-specific reactions due to rough antigen suspensions do occur (Young, 2009). Positive results do not have much weight for single animals but are more significant on herd basis (Hegazy et al., 2009). The
methods in common use are Rose Bengal Plate Test (RBPT), Milk Ring Test (MRT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT). Other available tests are Card Test, Rivanol Test, Coombs Antiglobulin Test, 2-Mercapto-ethanol (ME) and Enzyme linked Immunoassays, among them Enzyme linked Immunoabsorbent Assay (ELISA) (Faye et al., 2005). These tests are an indirect indication of the presence of homologous antibodies (Cadmus et al., 2008). Some less important methods are indirect fluorescence assay and haemagglutination test (Gall and Nielsen, 2004). Some of the tests such as RBPT and MRT are good for herd screening as these can be carried out at the sampling point. The results so obtained can then be confirmed using a quantitative method. Rose Bengal Plate Test and CFT have also been found useful together as screening tests (Mosalagae et al., 2011).

Complement Fixation Test and ME have always been used as supplemental tests. The most used antigens for polyclonal antibody are *Brucella abortus* s99 or 1119-3. Complement Fixation Test may be negative due to progressive changes in concentration of serum antibodies, their antigenic structure, specific isotope composition and avidity and prozone phenomenon which is linked to the ratio of IgG1 to IgG2, all of which affect the serological results. These characteristics differ from animal to animal. The persistent reactions could be due to non-specific antigens and IgM (Bertu et al., 2010). Complement Fixation Test and SAT may persist at the rates of 0.5% to 1.5% in different animals, while RBPT may persist at 2%. Enzyme Linked Immunoabsorbent
Assay readings may remain positive long after shedding of organisms has ceased (Davis et al., 1990).

The screening test results are confirmed using complementary tests which are considered more reliable for confirming and verifying doubtful results so acquired (Nammalwar et al., 2009). They distinguish hetero-specific reactions while detecting incomplete antibodies. They are also useful for differentiating vaccinates from infected animals. These tests are of particular importance in problem herds. Problem herds are those with persistent reactors despite culling of all animals testing positive by MRT but which are negative on serology. Such animals require to be tested by serology and bacterial culture (Rafai, 2002). As control enters terminal stages, conventional tests are limited in their ability to detect all infected cattle in herds with persistent infections (Jelastopulu et al., 2008). Complement Fixation Test, used in conjunction with ME, is useful at differentiating vaccinates from infected animals (Hooton and Omore, 2007). None of these tests can confirm freedom from disease. The specificity and sensitivity to antibody assay for diagnosis of brucellosis are limited by the cross-reactions between Brucella antibodies and those against other bacteria. Some infected animals may fail to show clinical disease or develop antibodies; hence their failure to be detected. Nicoletti (1992) observed that it was impossible to detect all infected animals at any one time using whatever method or even on repeat examination. He suggested that a flock be
declared infected even if it has only one detectable reactor. Some tests are less efficient as regards some animal species. It was observed and is agreed that agglutinins appear earlier than complement fixing antibodies (Christopher, 2004) and they also disappear faster (Al-Sous, 2004). The level of antibodies is not influenced by stress on the infected animal (Dricot et al., 2004). At initial stages, SAT has a significantly higher titre, while CFT may be negative. However, negative cases on SAT when CFT is positive do occur, particularly in chronic illness or on recovery from the disease. There are changes in antibody titre, composition, antigenic specificity, isotype composition and avidity as disease progresses to chronicity. These characteristics vary from animal to animal (Halling et al., 2005). Complement Fixation Test titre may persist and remain positive for up to 8 months. Persistent reactions could be due to non-specific antigens reacting with IgM. Complement Fixation Test and ME detect IgG. 2-Mercapto-ethanol breaks down IgM. In RBPT, reaction to IgM is very sensitive (Juan, 2006). Precipitation reactions are due to ‘O’ chain of LPS. When serum becomes negative on SAT and CFT, it may signify recovery.

Immunoglobulin and their classes are quantifiable using Radioimmunoassay (RIA), ELISA and Immunofluorescence (Robichaud and Libman, 2004). Studies by Corbel (Spitter, 2003) have shown that s19 immunoglobulin which is basically IgM has no RBPT activity. The s7 or IgG are produced in infection and vaccination although serological responses vary with the stage of disease. Both IgG and IgM are detected by
SAT (Rizzo, 2005). In aborting ewes, SAT detected the highest number while diagnosing chronic brucellosis during clinical disease (Gall and Nielsen, 2004). Presence of IgG is likely to correlate with active or chronic infections (Nicoletti, 1992). These responses are directed against antigens A, M and the protein. A drop in their titre may indicate vaccinal titre (Bourg et al., 2007). No cross-reactions occur against protein antigens which are responsible for the humoral and delayed type hypersensitivity reactions. The commonly used antigens for polyclonal serological tests, *Brucella abortus* s99 and *Brucella melitensis* 1119-3 cannot diagnose brucellosis due to *Brucella canis* that is in rughos phase (Chain et al., 2006).

### 2.9 Control of brucellosis

#### 2.9.1 Control of Brucellosis in human

There are no human vaccines since it is difficult to ascertain quality. Such vaccines should have no adverse effect to human beings. Human disease is best controlled by effective control of disease in animals (Shirima, 2005). Most under-developed countries have no brucellosis control programmes due to lack of adequate information on disease situation, ineffective animal health education, poor animal husbandry practices and unavailability of financial resources (Michael et al., 1995). The most important control measures against human brucellosis are pasteurization of dairy products and inspection
of domestic animals for evidence of the disease. The use of goggles or face shield and rubber gloves helps protect veterinarians, butchers and slaughterhouse workers.

Treatment for brucellosis is by use of combinations of drugs which are tetracycline and streptomycin, doxycycline and rifampin. Treatment by tetracycline and streptomycin is the most reliable and effective (Dricot et al., 2004). Rifampin combined with cotrimoxazole has also shown quite a bit of success (Nammalwar et al., 2009). Treatment combinations involving rifampin are said to be less effective than those of tetracylines. Some of the shortcomings are: lower effectiveness and relapses, especially in severe cases (Vanzini et al., 2001). Cases with complications require more intensive treatment. When spondylitis is the complication, surgical intervention to relieve pressure on the spinal chord or to drain par vertebral abscesses may be essential (Bertu et al., 2010). Some of the treated patients may show a high frequency of relapses (Abbas and Aldeewan, 2009). The relapses are best diagnosed by hemoculture, specific symptoms such as prolonged fever, arthritis, orchitis and persistent serological titter after the treatment (Rafai, 2002).

2.9.2 Control of brucellosis in animals

Knowing that human brucellosis is an occupational hazard, being commonest in animal handlers and that man is a dead end host; its control should be through eradication of the disease in the source animal, though this is a difficult task. The aim in an eradication
programme is to eliminate the disease in the shortest possible time, using the most economical methods (Corbel, 1999). The mode of achieving this should be resource efficient, logistically feasible and locally sustainable (Douglas and Bennett, 1999). One common approach involves educating people on the proper herd management, extensive strategic use of vaccination programs and an enlightened application and interpretation of serological test results. Test and slaughter is effective and quick but it is expensive and sometimes impractical when the prevalence is high. It may also be unthinkable in some management set-ups (Eisencheck et al., 1995). It has however been noted that where all animals in an infected herd have been slaughtered, brucellosis does not recur. In areas of low prevalence, slaughtering the entire herd is the most effective strategy since it helps to contain or limit the excretion of the pathogen thereby halting its spread (Gumber et al., 2004). It cuts out the source of human infections and reduces the economic losses.

Vaccination is a vital tool in an eradication programme as it increases the number and proportion of resistant animals with a concomitant elimination of reactors. Any serological suspect can then be removed and slaughtered. Removal of infected cows prior to their abortion parturition reduces the exposure and consequently new infections and therefore increases the effectiveness of vaccination with Brucella abortus s19 (Young, 2009). Post parturient cows shed organisms and also give birth to calves that are nearly always infected. Some of these calves shed infection while others may retain
it as latent carriers up to the time of sexual maturity when they start shedding; thus leading to the breakdown in the vaccination programme (Cloeckaert et al., 2003). This means vaccination alone cannot lead to eradication because of continued contamination of the environment (Halling et al., 2005). The best way of preventing exposure to clean animals, is by vaccination followed by separation of calves from cows and backed by test and slaughter. It has however, been doubted whether removal of infected cows by frequent testing and slaughter is of much value as it has been argued that retention of reactor cattle did not decrease spread of brucellosis in vaccinated beef herds (Fernandes and Baldwin, 1995).

Two types of vaccines have found practical use in the control of animal brucellosis. The most extensively used ones are the live attenuated vaccines like *Brucella abortus* strain 19 and *Brucella melitensis* rev-1. Pappal et al. (2004) claim that a laboratory mutant of *Brucella abortus* strain 2309 code-named RB-51; a rough live vaccine is effective. Live vaccines have the best immunogenicity for prolonged periods of time where by a single dose may be adequate for life. However, they produce longstanding serological responses that interfere with diagnosis. This adverse effect is reduced by vaccination through the conjunctiva route (Jimenz et al., 1994). *Brucella abortus* s19 produces best results when it is used during calf-hood. It has been tried against infections by all *Brucella* species but more so against bovine brucellosis due to *Brucella abortus*. A
higher dose of s19 increases the serological activity and protection (Rizzo, 2005). Its efficacy is modified by the degree of vaccination cover, level of the risk of exposure, dose of infecting organism, colonial state of the vaccine seed, its viability and virulence of infecting organism (Al-Sous, 2004). In adults the normal dose of $10^9$ organisms is protective but may also be ineffective when administered by normal route and so a reduced dose is recommended (Lucero, 2005). Conjunctiva route of inoculation is more efficient than the subcutaneous one for vaccination (Jubb et al., 1993). *Brucella abortus* s19 vaccination of adult animals is risky because it may cause disease in some, especially those in late stages of pregnancy. Some calf-hood vaccinated animals were found infected at slaughter (Kang’ethe et al., 2007).

Strain 19 may also produce side effects such as epididymitis and epiphysitis in rams and may occasionally establish chronic infection in calves or cause persistent specific antibody titres. It is pathogenic to man (Lin and Ficht, 1995). Some resistant strain 19 mutant cell lines may appear in all vaccine cultures. These mutants are more pathogenic and may persist and cause disease as animals mature, with possible abortion, therefore leading to a breakdown in the control programme (Sangori et al., 1996). The s19 vaccine can be cleaned of these mutants by growing vaccine seed material in a minimal medium whose source of carbon is glycerol. The glycerol inhibits the growth of the mutants. This method of purification is simple and cheap (Capasso, 2002). Rev 1 has been found to be protective to sheep and goats against *Brucella Melitensis* and *Brucella*
**ovis** and is the best against ram epididymitis. It can be used in adults by applying a reduced dose of $10^4$ or $10^5$ Colony Forming Units (CFU) as a single dose about 1 month before service (Kodohira et al., 1996). The protection is dependent on the residual virulence of the vaccine strain and route of inoculation. The effective dose; $10^{5-7}$ CFU for rev 1 is less than the $10^9$ vaccine that was previously thought to be ideal. This reduced dose helps to cut on cost of production, and consequently that of controlling the disease. Rev-1 is more protective than *Brucella suis* strain 2 for sheep on challenge with virulent strain 53 H-38 (Maichomo, 1996). It however, evokes a strong serological response that interferes with diagnosis of infection due to *Brucella melitensis*; it is virulent to humans and may cause abortion in vaccinated animals. Use of conjunctiva route of inoculation interferes with serological diagnosis more than use of subcutaneous route. Early vaccination leads to low agglutination in CFT titres. In such cases residual antibody titres diminish rapidly and so sero-diagnosis is little affected when complementary diagnosis tests are used.

Vaccination of adult animals with reduced doses is nearly as effective and safe (Jennings et al., 2007). However, neither live vaccines nor the inactivated ones offer 100% protection. *Brucella abortus* strain RB-51 induces cell mediated immunity response that is associated with protection. It does not produce antibodies against LPS ‘0’ chain antigen or lipid A and so does not interfere with diagnosis using whole cell
antigens (Stephens et al., 1995). It is less virulent than strain 2308. However, resistance
to challenge is lower than that due to s19, probably because it is less persistent in the
body than s19 or due to lack of antibodies against s2308. Strain 19 vaccinated animals
had a higher proliferation of spleen cells when challenged with s2308. Protein fractions
of s2308 RB-51 were not protective in rams when challenged with Brucella ovis since
they do not induce CMI (Sangori et al., 1996). Brucella abortus stain RB-51 is not
protective at all against Brucella ovis infection (Hegazy et al., 2009). The failure of
protection is based on the fact that they have identical antigens that are extractable in
soluble fraction (Lapaque et al., 2005). Killed cell vaccines are Brucella abortus 45/20
and Brucella melitensis H-38 oil adjuvant vaccine. These have been tried in different
countries with low successes. Brucella suis biotype 2 has been developed and used in
China. It is administered to sheep in drinking water but it has not been accepted widely.
Fraction vaccines based on LPS, PS and proteins have been tried. LPS ones have been
shown to evoke antibody production but not CMI which is most important in protection
against intracellular pathogens (Kang’ethe et al., 2000).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The study area is Makuyu Division which is in Murang’a County, Central Kenya. It is an Arid and Semi-arid Land (ASAL) and is divided into 12 locations (Appendix I). Thika-Sagana highway forms the boundary to the East, Kenol-Murang’a highway to the West and River Thara to the North. The Murang’a-Ruiru railway line passes through the division on the West. The division is served by several dry weather roads. The main landmarks are Murang’a Teachers Training College at Gakungu location along Thika-Sagana road, Maranjau prison to the North and Kakuzi large scale farm to the South. Small scale farmers form a greater percentage of population and livestock farming is mainly on zero grazing units.

3.2 Sample size determination

According to August 2009 census (DVO, 2009) records at Makuyu livestock centre, there were 435 dairy cattle in Makuyu division. A representative sample size of number of dairy cattle was determined using the formula deduced by Yamane (1967).

\[ n = \frac{N}{1 + N(e)^2} \]
Where; $n = \text{Sample size, } N = \text{Population size, } e = \text{Level of precision at 95\% confidence interval.}$

$$Sample\ size = \frac{435}{1 + 435(0.5)^2} = 208.38$$

The sample size used in the study was 208 dairy cattle.

### 3.3 Inclusion and exclusion criteria

This study involved any lactating dairy cattle regardless of the breed and excluded non-lactating dairy cattle.

### 3.4 Study design

This cross sectional study involved multi-stage sampling of dairy cattle in Makuyu Division. The twelve locations in the division were numbered and using systematic sampling, every third location was selected hence third, sixth, ninth and twelfth locations. The locations were Kambiti, Kirimiri, Makuyu and Iganjo (Appendix III). In these locations homesteads are dispersed because the area is semi-arid. Each location was divided into thirteen clusters of homesteads using cluster random sampling. From each cluster of homesteads four lactating dairy cows were selected using simple random sampling. Each lactating dairy cattle in a cluster was assigned a number, and four numbers were picked from the total randomly (Appendix I). Therefore a total of 208
dairy cows were used in this study. The owner of the cow was also interviewed using a questionnaire.

3.5 Data collection instrument

3.5.1 Questionnaires

The types of questionnaires were structured non distinguished questionnaire in which questions were listed in a pre-arranged order and the respondent told about the purpose of collecting information. The types of questions included close ended questions in which the respondent selected answers from a fixed list of replies and chose any one of the multiple options given and open ended questions in which the respondent gave his or her opinion. The total number of questionnaires was 208 and about 90% of the dairy farmers responded.

3.5.2 Milk and blood sampling

With support of veterinary personnel ten millilitres of blood were drawn from jugular vein using a syringe with a 21-gauge needle. The blood was put into 10 ml vacutainer tubes to prevent coagulation. Ten millilitres of milk was also collected from each cow and stored in collection bottles containing boric acid as a preservative. Both samples one from milk and the one from blood were well labelled for identification. The samples were stored in Thika Level Five laboratory as collection continued, serum
prepared and transported to the Central Veterinary Investigations Laboratory at Kabete after a week for laboratory analysis

3.6 Laboratory analysis

3.6.1 Rose Bengal Plate Test

Serum was screened using Rose Bengal Plate Test (RBPT) carried out using the dyed *Brucella* antigen provided by the central veterinary laboratories, Ministry of Agriculture, Livestock and Fisheries. The tests were carried out according to Franco *et al.* (2007). Eight microlitres of test serum were placed in a white porcelain tile and an equal volume of the antigen was added. The contents were mixed with an applicator stick. The tile was then placed on an electric shaker, rocked for 4 minutes and checked for agglutination. Agglutination indicated a positive result. Known negative and positive sera were included in the test as controls (FAO, 2003).

3.6.2 Milk Ring Test

Milk was tested using Milk Ring Test (MRT) which is the best for screening dairy cattle and potentially infected herds for brucellosis (Jelastopulu *et al.*, 2008). Milk which is the test material was tested by a heat-inactivated smooth *Brucella* antigen stained with hematoxylin according to Gregory (1953). Eight microlitres of milk was put in a test tube and an equal volume of antigen added. The result was read after 1 hour and then
after 24 hours. Formation of a ring at the top of the tube indicated a positive result. Known negative and positive were included in the test as controls (FAO, 2003).

3.7 Data analysis

Descriptive statistics was used to analyze the data collected using questionnaires to show whether veterinary services were accessible to the dairy farmers. Social data to establish relationship between levels of education and knowledge were analysed using chi-square. Level of significance was $P < 0.05$. Prevalence of brucellosis was analysed by use of central tendencies particularly the percentages. Percentage prevalence of brucellosis in Makuyu division was calculated based on the number of positive animals detected using milk and serum samples.
CHAPTER FOUR: RESULTS

4.1 Demography of the farmers

This study established that most of dairy farmers in Makuyu Division above 40 years of age (76.4%). There were 13.5% dairy farmers between 31 and 40 years while the rest were below thirty years. Over half of the respondents were females (56.7%). Although most of the respondents were literate about 25% reported not to have had any formal education (Figure 4.1). About 60% had either attained primary or secondary school level of education. All the farmers also indicated that they did not have any other occupation besides their normal farming activities and this shows that their livelihood was based on farming.
4.2 Number of animals, breeds and farming practices

Most farmers in Makuyu division are small scale keeping less than ten animals. One hundred and fifty nine of the studied farmers (76.4%) fall under this category. Twenty eight farmers (13.5%) had 11-20 dairy animals, seven (3.4%) owned 21-30 dairy animals while fourteen (6.7%) had above 40 dairy animals. Friesian, Ayrshire and their classes with indigenous breeds were most common in Makuyu region. Majority (43.3%) of the farmers owned mixed breed, sixty two (29.8%) Ayrshire only while fifty six
(26.9%) owned Friesian breed of cattle. Eighty three (39.9%) of the farmers reported that they hire boys to either herd their animals in open field or in zero grazing set up. For the rest of the farmers (60%), the animals are taken care of by members of the household. In this area, one hundred and fifty three (73.6%) of the respondents practice zero grazing, forty one (19.7%) graze in an open field while fourteen (6.7%) of the farmers have paddocks in their farms where the animals are grazed.

4.3 Knowledge of brucellosis among dairy farmers

Majority of farmers (83.7%) had heard about brucellosis (Figure 4.2). These farmers said the following were the main symptoms of a cattle suffering from brucellosis: reduced production of milk, swollen mammary glands, blood stains in faecal matter and abortion which was said to be the main symptom of brucellosis in cattle. However, most of them (83.7%) had no idea on what causes this disease but a few of them (6.7%) thought brucellosis is caused by bacteria or virus while six (2.9%) of the respondents thought it is caused by fungi. These terms were translated using mother tongue for the farmers to understand (Figure 4.3).
Figure 4.2: Respondent farmer’s knowledge status on existence of brucellosis in Makuyu Division, Murang’a County

Figure 4.3: Farmers response on causes of brucellosis in cattle in Makuyu Division, Murang’a County
There was a significant relationship between level of education and knowledge on what causes brucellosis ($\chi^2 = 82.542; P < 0.05$). Farmers having secondary and college education were more knowledgeable about the causes of this disease (Figure 4.4). Most of the farmers (70.2%) were aware that this disease can infect human beings and hence they were cautious when handling suspected animals and their products. The rest of the farmers also practiced good hygiene in handling animals and their products such as boiling milk. Majority of the farmers (67.3%) said they had either been infected or seen a person suffering from brucellosis. The farmers strongly believe that this disease can be spread to human beings through raw milk as indicated by 63.5% of the respondents (Figure 4.5). According to the farmers, the known symptoms of brucellosis in human beings were; joint pain and body weakness, fever and headache, swollen legs, vomiting and backache.

Figure 4.4: Farmers of varying education levels’ views on causes of brucellosis in Makuyu Division, Muranga County
Knowledge of brucellosis was also significantly related to the age of the respondents ($\chi^2 = 35.800; P < 0.05$). All (100%) of the respondents in the age bracket of 26-30 years had heard of the disease while 75% of those in 31-40 years and 83% of those above 40 years had not heard of brucellosis.

**4.4 Knowledge about the spread of brucellosis to human beings**

Majority of the farmers (70.2%) were knowledgeable on the fact that this disease could be spread to human beings. Only 29.8% were not aware that it could be spread to human beings. This knowledge significantly varied in the education levels ($\chi^2 = 80.94$, $P = 0.0001$) and age of the farmers ($\chi^2 = 11.093$, $P = 0.004$) but was not significant in the gender ($\chi^2 = 0.302$, $P = 0.632$) of the farmers (Table 2).
Table 2: Farmers knowledge on the spread of brucellosis to human beings

<table>
<thead>
<tr>
<th>Respondents ages</th>
<th>Knowledgeable</th>
<th>Not knowledgeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 – 30</td>
<td>14 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>31 – 40</td>
<td>14 (50.0%)</td>
<td>14 (50%)</td>
</tr>
<tr>
<td>Above 40</td>
<td>111 (69.8%)</td>
<td>48 (30.2%)</td>
</tr>
<tr>
<td>Chi- value</td>
<td>11.093</td>
<td></td>
</tr>
<tr>
<td>P - value</td>
<td>0.004</td>
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</tbody>
</table>

**Gender**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Knowledgeable</th>
<th>Not knowledgeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>62 (74.7%)</td>
<td>21 (25.3%)</td>
</tr>
<tr>
<td>Females</td>
<td>84 (71.2%)</td>
<td>34 (28.8%)</td>
</tr>
<tr>
<td>Chi- value</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>P - value</td>
<td>0.632</td>
<td></td>
</tr>
</tbody>
</table>

**Education level of the farmer**

<table>
<thead>
<tr>
<th>Education level of the farmer</th>
<th>Knowledgeable</th>
<th>Not knowledgeable</th>
</tr>
</thead>
<tbody>
<tr>
<td>No education</td>
<td>14 (29.2%)</td>
<td>34 (70.8%)</td>
</tr>
<tr>
<td>Primary</td>
<td>56 (80.0%)</td>
<td>14 (20.0%)</td>
</tr>
<tr>
<td>Secondary</td>
<td>55 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>College/University</td>
<td>21 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Chi- value</td>
<td>80.94</td>
<td></td>
</tr>
<tr>
<td>P - value</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>
4.5 Control of brucellosis

The rate of abortion in cattle in the study area was apparently high as sixty three percent of the famers reported that it had occurred in their cattle. They were however not aware of what exactly caused the abortions. When abortion occurred, about one hundred and thirty nine (66.8%) of the farmers buried the carcasses while twenty seven (13.0%) gave the carcasses to dogs. Other hygiene practices include washing hand and udder before milking, using hot water when washing, using clean milking cans and observing good hygiene of the person milking. Some farmers also observe good storage of milk by keeping it in a cold store but most of them either store on a table or a cupboard in the kitchen.

Most of the farmers (73.1%) sell part of their raw milk to their neighbours, nearby shopping centres or to dairy firms. However, any milk meant for domestic consumption is boiled in two hundred and one (96.6%) of the households. Forty (19.2%) of the farmers indicated that they sometimes ferment milk for domestic consumption (Figure 4.6).
4.6 Veterinary services

Ninety one (43.8%) of the respondents knew there were veterinary personnel going around the Division once every month however, they also provided the services when requested by the farmers. Most of the farmers (86.5%) reported to have had their animals vaccinated at least once in a year. Seven (3.4%) of the farmers have their animals vaccinated once in a month while another seven (3.4%) get their animals vaccinated when sick. These farmers did not indicate the disease against which their animals were vaccinated. Fourteen (6.8%) of the farmers however, failed to indicate the frequency of the vaccination.
When the farmers request veterinary personnel to attend to their animals, one hundred and eighty seven (89.9%) got to know the disease and drug from the personnel while the rest (10.1%) did not enquire. In many cases (63.9%), the animals get healed after treatment (Table 3 and Figure 4.7). If the animal is suffering from brucellosis, the farmers suggested the following options; treat the animal, vaccinate all the animals, to prevent transmission, educate farmers through extension services and slaughter the sick animal.

Table 3: Accessibility of veterinary services to dairy farmers in Makuyu Division

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do you have a veterinary doctor in this location?</td>
<td>43.9% knew.</td>
</tr>
<tr>
<td>Does the veterinary doctor go visiting homes in the location?</td>
<td>Only upon a request.</td>
</tr>
<tr>
<td>When the veterinary doctor attends to your animal,</td>
<td>89.9% confirmed that the doctor informs them.</td>
</tr>
<tr>
<td>does he/she tell you the disease and drug used to treat the animal?</td>
<td></td>
</tr>
<tr>
<td>How often are your animals vaccinated?</td>
<td>86.5% do it once in a year.</td>
</tr>
</tbody>
</table>
4.7 Prevalence of brucellosis in Makuyu Division

The two methods used to test brucellosis gave different results. The Milk Ring Test detected 16 cases and RBPT detected four out of the 208 animals tested. This is a point prevalence of 7.7% and 1.9%. The point prevalence varied with the breed of animal. Overall brucellosis was more prevalent in Fresian (13.27%) than in Ayrshire (9.43%) as shown in table 4.
Table 4: Test results of brucellosis as diagnosed in milk and blood samples

<table>
<thead>
<tr>
<th>Methods</th>
<th>Fresian positive</th>
<th>Ayrshire Positive</th>
<th>Total percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Ring Test</td>
<td>10</td>
<td>6</td>
<td>7.7%</td>
</tr>
<tr>
<td>Rose Bengal Plate Test</td>
<td>4</td>
<td>0</td>
<td>1.9%</td>
</tr>
</tbody>
</table>

Two of the four animals that tested positive in blood samples also tested positive in milk samples.

4.8 Association between the prevalence and the sites

The findings showed that there was a significant association in the brucellosis prevalence to the various sites ($\chi^2 = 40.431$, $P = 0.0001$). Some of the farmers from Kambiti, Kirimiri and Makuyu stated that they had experienced sudden death of one of their calves in the recent times. In Kirimiri, none of the farmers had experienced sudden death of their calves.

4.9 Association of the type of farming practices

There was significant association in the prevalence of the disease to the farming practices carried out by the farmers ($\chi^2 = 9.142$, $P = 0.010$). Farmers who grazed heir
animals on a far field (82.9%) had experienced a sudden death of their calves. Those who practiced zero grazing (58.8%) and 50.0% of those who had their animals move only in the closed compound also experienced a sudden death of their calves.
CHAPTER FIVE: DISCUSSION

5.1 Introduction

Brucellosis is an infectious disease of non-human mammals that is contagious for man. The zoonotic disease mainly caused by *Brucella abortus* which infect cattle has severe hazards to human health (Hasanjani, 2006). In cattle the clinical signs are abortion, arthritic joints and retained after birth. It is transmitted by ingestion of *Brucella* in aborted foetuses, vaginal discharges, contaminated feeds and water and venereal transmission by infected bulls (Omer et al., 2000). Infected cows shed the organism in their milk for prolonged periods. In man transmission is mainly by ingestion of contaminated milk and milk products (Young, 2009). Clinical signs are mild fever, weakness, enlarged lymph nodes and weight loss. Application of serological diagnostic tests for brucellosis has been achieved in diverse areas using RBPT and MRT (Kagumba and Nandokha, 1978). These two tests and questionnaires were used to obtain information on *Brucella abortus* antibodies in dairy cattle and public knowledge of brucellosis among dairy farmers in Makuyu Division.

Most of the respondents in Makuyu Division were above 40 years of age and were mainly females. This is perhaps because most women in rural areas are household keepers taking care of children and livestock. The respondents indicated that they had no other occupation besides their normal farming activities hence their livelihood is based
on farming. Most of them are small scale and keep less than ten animals which are of cross breed. Majority practice zero grazing since their pieces of land are small while others graze in open fields where there is no settlement. Animals grazed in open fields feed on pasture contaminated by aborted foetuses which may have contributed to spread of brucellosis among the dairy cattle.

5.2 Prevalence of Brucella arbotus in Makuyu Division

The overall point prevalence of brucellosis as determined by both MRT and RBPT were 7.7% and 1.9% respectively. The Brucella organisms remain in blood for a few hours and are engulfed by polymorphonuclear leucocytes and monocytes. The organisms localize mainly in the lymphatic tissue, especially the supra-mammary lymph nodes, from where they are excreted via the mammary gland (Eisencheck et al., 1995). Cows remain carriers and shed the organism in their milk for prolonged periods (Abbas and Aldeewan, 2009). This explains why the point prevalence by MRT was higher than RBPT results.

Some of the farmers reported that when their cows abort they give the carcasses to dogs which could contaminate pasture and water contributing to increased spread of the disease. The percentage point prevalence of brucellosis in Makuyu Division based on milk samples was relatively high compared to that reported in some parts of the country. In Dagoretti, Kang’ethe et al. (2007) reported a prevalence of 1.1% in dairy
farming and 0.7% in other farming households using MRT. Kodohira et al. (1997) reported a 2% apparent prevalence in the small holder system in Kiambu. However, Kagumba and Nandhoka (1978) reported a prevalence of 10% bovine brucellosis in extensive production systems in Nakuru also using MRT. Low prevalence in the smallholder animals is likely to be explained by stall feeding that minimises contacts between dairy cattle and other domestic animals. Nevertheless, in the “cut and curry” feeding system of animals that is practiced by many smallholders can serve as a potential risk factor but this is likely to play a role when fodder is collected from areas used by indigenous traditional cattle which encroach the peri-urban and urban settings especially during the dry season (Malhotra, 2004).

Kang’the et al. (2000) reported an overall prevalence of brucellosis at consumer-level and informal market level as 3.9% and 2.4% using MRT. Informally traded raw milk from dairy co-peratives and milk outlets had the highest proportion of MRT positive samples and all these samples were from Narok District where extensively grazed pastoralist zebu herds predominate. Continued and uncontrolled movement of herds in semi arid and arid area such as Makuyu facilitates herd-to-herd transmission of infection through pastures and water contamination by aborting animals or following normal calving.
In Egypt, Samaha et al. (2008) reported varying prevalence of brucellosis in cattle in Benisuef (7.17%) and Monofia (7.14%) using MRT while using RBPT the overall prevalence was 3.52%. Variations in infection may be attributed to environmental factors and stress which may modulate susceptibility to infection. Increased prevalence can also be attributed to raising sheep and goats with cattle in villages and this occurs in Makuyu Division. Movement of infected sheep or goats can contaminate pastures and spread brucellosis to cattle.

Using RBPT, Junaidu et al. (2008) reported prevalence of 5.1% among nomadic cattle herds and 4.4% in settled cattle in Adamawa state in Nigeria. Movement of pastoral Fulani herdsmen and interaction of cattle with those of other Fulani herdsmen is a major factor in spreading brucellosis in Nigeria. High prevalence of brucellosis in pastoral management system may partly be attributed to long distance movement of cattle in search of pasture and water and co-mingling in communal grazing areas and at water points particularly during dry season. This is related to Makuyu Division where some of the farmers graze their cattle in open field and feed on pasture and water contaminated by other animals.

The overall brucellosis was more prevalent in Fresian crosses (13.27%) than in Ayrshire crosses (9.43%) and also no antibodies were detected in the blood in Ayrshire type of
breed. This may be probably because their CMI response is very strong hence the bacteria are cleared as soon as they enter into the circulatory system or they are resistant to *brucella abortus* infection. In Nigeria, Junaidu *et al.* (2008) reported the Sokoto Gudali breed to have the highest prevalence of brucellosis followed by Azuwarq, with Bunaji having the least. Genetic variation is an important factor in conferring resistance or tolerance of cattle breeds to a wide range of diseases, and the antibody response of animals classified as resistant to infection by *Brucella abortus* differed significantly from that of susceptible animals. Significant genetic variability in resistance/susceptibility to brucellosis has been detected in cattle and is associated with a 3’ untranslated polymorphism in the slc1a1 gene (Samaha *et al.*, 2008).

### 5.3 Knowledge level of brucellosis among dairy farmers in Makuyu Division

The young and educated farmers were more knowledgeable on brucellosis than those who were old and not educated. The old and not educated were the majority and they had no idea on what causes brucellosis. Poor disposal of aborted carcasses may have contributed to increased spread of the disease in Makuyu Division. This is related to Uganda where poor disposal systems for aborted materials are due to poor community knowledge about the zoonotic implications of the disease (Makita *et al.*, 2008). Therefore the farmers should be educated and the practise be discouraged.
The farmers were aware that brucellosis can infect human beings hence practiced good hygiene in handling suspect animals, boil milk before drinking and others bury the aborted carcasses. Boiling of raw milk (alone or in tea) achieves higher temperatures and duration than those attained during pasteurisation hence destroy all *Brucella* organisms (Young, 2009). Majority of the respondents were noncommittal on whether they ferment milk for domestic consumption. As the milk is not boiled before fermentation the home made fermented milk products could be a possible source of brucellosis infection to human beings (Zhan, 1996). This is related to Egypt where much of the milk from the traditional sector is consumed raw in form of naturally fermented milk as it applies to some quarters of urban centres (Jennings *et al.*, 2007). There is urgent need to formulate sensitisation programmes so as to raise the public awareness to farmers about the zoonotic risks associated with milk consumption.

Most of the farmers sell part of their milk to their neighbours, neighbouring shopping centres or dairy firms which is a potential route of spread of brucellosis outside the study area because milk for sale is not boiled. The risk of infection by milk-borne brucellosis is one reason why public health regulations discourage informal milk markets that sell unpasteurized milk. However, these regulations are not generally implemented in many developing countries such as Kenya where over 85% of marketed milk is not pasteurized and is sold through informal market pathways (Kangethe *et al.*, 2000). The informal milk market is difficult to eliminate because it provides social and
economic benefits to smallholder producers, small market agents and consumers in terms of higher farm gate prices, creation of employment and competitive consumer prices. Thus education, encourage buyers to boil milk on risk.

5.4 Accessibility of veterinary services to dairy farmers

Veterinary services are available and accessible to the dairy farmers in Makuyu thus minimizing spread of the disease. Majority of the farmers (73.5%) indicated that they request for veterinary personnel when their animals get sick and they are informed the disease and drug used to treat the animals. The farmers do not request for artificial insemination but use their bulls which may be carriers of the *Brucella* organisms hence transmit brucellosis to the cows during mating. In Kenya use of artificial insemination is encouraged as opposed to bulls to reduce prevalence of brucellosis and bulls used for semen collection are routinely tested for brucellosis.
CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

i) The point prevalence of brucellosis in Makuyu Division is estimated to be 7.7%. This shows that brucellosis occurs in Makuyu Division and therefore the human beings were exposed to the risk of the disease.

ii) Level of knowledge of brucellosis among dairy farmers is low. It is related to their education level and age. The young who are more educated are aware of the symptoms of the disease while the old and not educated are not. Most of the farmers are over 40 years of age and they have no idea on what causes the disease which may contribute to spread of brucellosis.

iii) Veterinary services are accessible to the farmers, sick cattle are treated and most are healed. Veterinary personnel also inform farmers of the disease the sick animals suffer which may reduce prevalence of brucellosis.

6.2 Recommendations

i) Public education on methods of controlling brucellosis to reduce prevalence of the disease in dairy cattle.

ii) Heard health campaign with frequent screening for brucellosis to identify infected animals so that they can be slaughtered to eradicate the disease.
iii) Use of artificial insemination as opposed to bulls to control venereal transmission of brucellosis.

iv) Future research on prevalence of brucellosis in human beings, goats and dogs.
REFERENCES


Bruce, D. (1887). A contribution to historical understanding of brucellosis in Chile. \textit{American Medical Association} \textbf{103}: 665-677.


District Education Office (DEO), Murang’a South, (2002). Needy students that require sponsorship in secondary schools in Makuyu Division. 6th May 2002.


APPENDICES

Appendix I: Study area; Map of Makuyu Division Murang’a County.

Sampled locations are shaded in black.
Appendix II: Questionnaire to dairy farmers

Section A: Background information

1. Date of interview……………………………………
2. Name of interviewee…………………………………
3. District………………………………………………
4. Division…………………….Farm code…………
5. Location………………………………………………
6. Respondent’s age (Yrs) 1. 0-18 ☐
   2. 1-25 ☐
   3. 26-30 ☐
   4. 31-40 ☐
   5. Above 40 ☐
7. Gender 1. Male ☐
           2. Female ☐
8. Education background
   1. Not educated ☐
   2. Primary level ☐
   3. Secondary level ☐
   4. College/university ☐
9. Occupation of the farmer, besides farming
   1. Teacher ☐
   2. Doctor/Nurse ☐
3. Retired civil servant □
4. None □

**Section B: Information on dairy cattle owned**

1. How many dairy animals do you have on this farm?

   1. 0 – 10 animals □
   2. 11 – 20 Animals □
   3. 21 – 30 animals □
   4. 31 – 40 Animals □
   5. Above 40 □

2. Which breed of dairy cattle do you own?
   a) Friesian □
   b) Ayrshire □
   c) Local breeds □
   d) Mixed breed □

3. Do you attend to the animals by yourself?
   a) Yes □
   b) No □

4. If No Who attends to the animals at home?
   a) Herd boy □
   b) Children □
   c) Spouse □
   d) Others (Specify).........

5. What farming practice do you carry out on your farm?
   a) Zero grazing □
   b) Animals are grazed in a far field □
   c) Animals move only in the enclosed compound. □
Section C: Knowledge of farmers on brucellosis

1. Have you heard of a disease called Brucellosis?
   a) Yes □  b) No □

2. If yes, what are the symptoms of the disease on an infected animal?
   ..................................................................................................
   .................................................................................
   .................................................................................

3. What do you think causes this disease brucellosis?
   a) Bacteria □  b) Virus □  c) Fungi □  d) No idea □

4. Are you aware that this disease (Brucellosis) can infect human beings?
   a) Yes □  b) No □

5. Have you seen/heard of any person in this location suffering from brucellosis?
   a) Yes □  b) No □

6. How can this disease be spread to human beings?
   a) Through raw milk □  b) uncooked Meat □  c) Cows Blood □
   d) Cow-dung □  e) Others (specify) .................................

7. What are the symptoms of a person infected by Brucellosis?
   ..................................................................................................
   .................................................................................
   .................................................................................
8. Have you experienced sudden abortion in your dairy animals?
   a) Yes ☐   b) No ☐

9. If yes, what did you do to the carcass of a dead calf?
   a) Buried ☐   b) Burnt ☐   d) Gave to dogs ☐

10. When using hands for milking, how do you ensure cleanliness of your milk?
    .............................................................
    ......................................................................

11. After milking, where do you keep the milk can with the milk before selling/consuming?
    ...........................................................................................................................
    ...........................................................................................................................

12. Do you consume all the milk produced in the family?
   a) Yes ☐   b) No ☐

13. How do you treat milk you produce before you consume it at home?
    a) Boiling ☐   b) Chilling ☐   c) Filtering ☐   d) No treatment ☐

14. How frequent do you take fermented milk in the home?
    a) Once in a week ☐   b) Twice in a week ☐   c) Once in a month ☐
    d) Others........................................
Section D: Veterinary services

1. Do you have a veterinary doctor in this location?
   a) Yes □    b) No □

2. Does the veterinary doctor go visiting homes in the location or he/she has to be called when needed?
   a) Has to be called when needed □    b) Goes round the Sub-location □

3. How often do you get your animals vaccinated?
   a) Once in a week □    b) Once in a month □    c) Once in a year □
   d) Only when the animals are not well □    e) Others. ........................

4. When you call a veterinary doctor to attend to your animals, does he/she always tell you the disease and drug used to treat your animals?
   a) Yes □    b) No □

5. Are the animals healed after the treatment?
   a) Yes □    b) No □    c) Others.....................

Please give us more information on veterinary services offered to your animals and how we can handle any animal with Brucellosis..........................................................
Appendix III: Authority to sample dairy cattle in Makuyu Division

MINISTRY OF LIVESTOCK DEVELOPMENT

The DVO,
MARAGWA DISTRICT

REF. AUTHORITY TO SAMPLE CATTLE IN MAKUYU DIVISION FOR BRUCELLOSIS TESTING (MSC PROJECT)

Mrs Rachel Mbaire Mwangi is an Msc student in Kenyatta University and has been allowed to sample cattle in Makuyu division for brucellosis diagnosis at CVL, Kabete. Please give her the necessary support.

Dr. P. M. Mbathe
For: Director of Veterinary Services