

**RESPONSES TO NATURAL TICK-BORNE INFECTIONS  
AMONG THE NKEDI ZEBU AND ANKOLE CATTLE IN  
AN AGRO-PASTORAL FARMING SYSTEM  
OF SOROTI DISTRICT**

**FREDRICK KABI (BVM) (MAK)**

**2004/HD17/2646**

**A DISSERTATION SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL  
FULFILMENT FOR THE AWARD OF MASTER OF VETERINARY  
PREVENTIVE MEDICINE DEGREE  
OF MAKERERE UNIVERSITY**

**2008**

## DECLARATION

I, Fredrick Kabi (BVM) (Mak), declare that this dissertation is my original work except where otherwise stated, and has never been submitted to any university or institution for the award of a degree or any other qualification.

Sign: F. Kabi Date: 25/11/08

This dissertation has been submitted for examination with the approval of the following supervisors:

1. Dr. Joseph W. Magona, PhD,

Principal Research Officer (PRO),

National Livestock Resources Research Institute (NaLIRRI),

P.O. BOX 96,

TORORO.

Sign: [Signature] Date: 22/12/2008

2. Associate Professor George W. Nasinyama, PhD,

Deputy Director, Research,

School of Graduate Studies,

Makerere University.

P.O. BOX 7062,

KAMPALA.

Sign: [Signature] Date: 29/11/08

## **DEDICATION**

This research is dedicated to my dear wife Catherine, my parents, Christopher and Esther, brothers and sisters. The Almighty God bless you.

© Fredrick Kabi, 2007

## TABLE OF CONTENTS

|                                              |          |
|----------------------------------------------|----------|
| DECLARATION.....                             | i        |
| DEDICATION.....                              | ii       |
| ACKNOWLEDGEMENT.....                         | vii      |
| LIST OF FIGURES.....                         | viii     |
| LIST OF TABLES.....                          | ix       |
| LIST OF ABBREVIATIONS AND ACRONYMS.....      | x        |
| ABSTRACT.....                                | xi       |
| <b>CHAPTER ONE.....</b>                      | <b>1</b> |
| <b>1.0 GENERAL INTRODUCTION.....</b>         | <b>1</b> |
| 1.1 Problem statement.....                   | 3        |
| 1.2 Overall objective.....                   | 5        |
| 1.3 Specific objectives.....                 | 5        |
| 1.4 Hypothesis.....                          | 5        |
| 1.5 Justification .....                      | 5        |
| <b>CHAPTER TWO.....</b>                      | <b>9</b> |
| <b>2.0 GENERAL LITERATURE REVIEW.....</b>    | <b>9</b> |
| 2.1 Ticks and their distribution.....        | 9        |
| 2.2 Responses of cattle breeds to ticks..... | 9        |
| 2.3 Ticks and tickborne diseases.....        | 11       |
| 2.3.1 East Coast Fever (ECF).....            | 12       |
| 2.3.1.1 The clinical signs of ECF.....       | 13       |
| 2.3.2 Bovine anaplasmosis.....               | 15       |

|                                                         |           |
|---------------------------------------------------------|-----------|
| 2.3.2.1. The clinical signs of bovine anaplasmosis..... | 16        |
| 2.3.3 Bovine babesiosis.....                            | 16        |
| 2.3.3.1 The clinical signs of bovine babesiosis.....    | 17        |
| 2.4. Control of ticks and tickborne diseases.....       | 18        |
| 2.4.1. Use of chemical acaricides.....                  | 18        |
| 2.4.2. Arsenic compounds .....                          | 18        |
| 2.4.3. Chlorinated hydrocompounds.....                  | 19        |
| 2.4.4. Organophosphorous compounds.....                 | 19        |
| 2.4.5. Carbamates.....                                  | 19        |
| 2.5. Application of acaricides.....                     | 19        |
| 2.5.1. Dipping.....                                     | 20        |
| 2.5.2. Sprays.....                                      | 20        |
| 2.5.3. Spot treatment or hand dressing.....             | 20        |
| 2.5.4. Other applications.....                          | 21        |
| 2.6. Safety measures.....                               | 21        |
| 2.7. Acaricide resistance by ticks.....                 | 22        |
| 2.8. Immunological control through vaccines.....        | 23        |
| 2.9. Host resistance to tick infestations.....          | 26        |
| <b>CHAPTER THREE.....</b>                               | <b>28</b> |
| <b>3.0 GENERAL MATERIALS AND METHODS.....</b>           | <b>28</b> |
| 3.1. The study area.....                                | 28        |
| 3.1.1. Selection of the study sites.....                | 31        |
| 3.1.2 Sample size determination.....                    | 33        |

|                                                                                                                                                        |           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 3.2 Collection of blood samples.....                                                                                                                   | 34        |
| 3.2 Data analysis.....                                                                                                                                 | 34        |
| <b>CHAPTER FOUR.....</b>                                                                                                                               | <b>36</b> |
| 4.0 The Sero-prevalences of tickborne infections among the Nkedi Zebu and Ankole<br>cattle in the Agro-pastoral farming system of Soroti district..... | 36        |
| Abstract.....                                                                                                                                          | 36        |
| 4.1 Introduction.....                                                                                                                                  | 36        |
| 4.2 Materials and methods.....                                                                                                                         | 38        |
| 4.2.1 Preparation of serum samples.....                                                                                                                | 38        |
| 4.2.2 Serological analysis.....                                                                                                                        | 38        |
| 4.2.3 Enzyme-linked immunosorbent assay (ELISA).....                                                                                                   | 38        |
| 4.2.3.1 Theileriosis.....                                                                                                                              | 38        |
| 4.2.3.2 Babesiosis.....                                                                                                                                | 40        |
| 4.2.3.3 Anaplasmosis.....                                                                                                                              | 41        |
| 4.3 Results.....                                                                                                                                       | 42        |
| 4.4 Discussion.....                                                                                                                                    | 49        |
| 4.5 Conclusions and recommendations.....                                                                                                               | 50        |
| <b>CHAPTER FIVE.....</b>                                                                                                                               | <b>51</b> |
| 5.0 Tick infestations among the Nkedi Zebu and Ankole cattle in the Agro-pastoral<br>Farming system of Soroti district.....                            | 51        |
| Abstract.....                                                                                                                                          | 51        |
| 5.1 Introduction.....                                                                                                                                  | 52        |
| 5.2 Materials and methods.....                                                                                                                         | 54        |
| 5.2.1 Determination of packed cell volumes.....                                                                                                        | 54        |

|                                                         |           |
|---------------------------------------------------------|-----------|
| 5.2.2 Tick counts.....                                  | 54        |
| 5.3 Results.....                                        | 54        |
| 5.4 Discussion.....                                     | 61        |
| 5.5 Conclusions and recommendations.....                | 62        |
| <b>CHAPTER SIX.....</b>                                 | <b>63</b> |
| <b>6.0 GENERAL DISCUSSION.....</b>                      | <b>63</b> |
| <b>CHAPTER SEVEN.....</b>                               | <b>66</b> |
| <b>7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS.....</b> | <b>66</b> |
| 7.1 General conclusions.....                            | 66        |
| 7.2. General recommendations.....                       | 67        |
| REFERENCES.....                                         | 68        |

## ACKNOWLEDGEMENT

Special acknowledgement to my supervisors, Dr. Joseph W Magona, Principle Research Officer and Head of Cattle disease research, National Livestock Resources Research Institute (NaLIRRI), Tororo and Assoc. Professor George W Nasinyama, Deputy Director, Research, School of Graduate Studies, Makerere University for the guidance, advice and never tiring supervision of my work.

Special thanks go to the Director, National Livestock Resources Research Institute (NaLIRRI), Tororo for granting me permission to study and the staff, Drs. DW Kakaire, H Kirunda, G Nsereko, R Kajobe, R Azabo and P Abila for provision of an enabling environment for my studies and this research work.

I am greatly indebted to Mrs. Teo Galwango, Mr. John Walubengo, Mr. Geoffrey Odyek, Mr. Joseph Muboli and Mr. Jackson Mawejje of National Livestock Resources Research Institute (NaLIRRI), Tororo for their technical assistance.

My sincere thanks further go to Dr. Eyudu and Mr. Donesius Okirror, Soroti district veterinary department for their assistance during collection of the data in the field. I am grateful to the farmers for the cooperation and permission to work on their cattle.

This study received financial support from the National Agricultural Research Organisation (NARO) through the ARTP II / World Bank project for which I am grateful.

## LIST OF FIGURES

|                                                                                                                                                                                       |    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Figure 3.1: An Ankole cow with a heifer calf.....                                                                                                                                     | 29 |
| Figure 3.2: The Nkedi cattle .....                                                                                                                                                    | 30 |
| Figure 3.3: A mixed herd of Nkedi Zebu and Ankole cattle.....                                                                                                                         | 30 |
| Figure 3.4: Map of Uganda and Soroti district, counties, subcounties and the<br>neighbouring districts (2006).....                                                                    | 35 |
| Figure 4.1: Mean antibody titres against <i>T. parva</i> , <i>B. bigemina</i> and <i>A. marginale</i> of the<br>Nkedi Zebu and Ankole cattle in Kasilo county, Soroti district.....   | 43 |
| Figure 4.2: Mean anti-body titres against <i>T. parva</i> in the different age groups of Nkedi<br>Zebu and Ankole cattle in Kasilo county, Soroti district.....                       | 45 |
| Figure 4.3: Mean antibody titres against <i>B. bigemina</i> in the different age groups of<br>Nkedi Zebu and Ankole cattle in Kasilo county Soroti district.....                      | 46 |
| Figure 4.4: Mean antibody titres against <i>A. marginale</i> in the different age groups of<br>Nkedi Zebu and Ankole cattle in Kasilo county Soroti district.....                     | 47 |
| Figure 4.5: Sero-prevalences of tickborne infections in the Nkedi Zebu and Ankole,<br>Kasilo county, Soroti district.....                                                             | 48 |
| Figure 5.1: Mean tick counts of <i>R. appendiculatus</i> , <i>B. decoloratus</i> and <i>A. variegatum</i> on<br>the Nkedi Zebu and Ankole cattle, Kasilo county, Soroti district..... | 56 |
| Figure 5.2: Mean tick counts of <i>R. appendiculatus</i> on the different age groups of Ankole<br>and Nkedi Zebu, Kasilo county, Soroti district.....                                 | 57 |
| Figure 5.3: Mean tick counts of <i>B. decoloratus</i> on the different age groups of Ankole and<br>Nkedi Zebu cattle, Kasilo county, Soroti district.....                             | 58 |
| Figure 5.4: Mean tick counts of <i>A. variegatum</i> in the different age groups of Ankole and<br>Nkedi Zebu, Kasilo county, Soroti district.....                                     | 59 |
| Figure 5.5: Mean PCV of the Ankole and Nkedi Zebu in the different age groups in<br>Kasilo county, Soroti district.....                                                               | 60 |
| Figure 5.6: Mean PCV of the Nkedi Zebu and Ankole cattle Kasilo county, Soroti<br>district.....                                                                                       | 61 |

## LIST OF TABLES

|                                                                                                                                                                                                                                             |    |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Table 3.1: Soroti district cattle population census, 2006.....                                                                                                                                                                              | 32 |
| Table 3.2: Distribution of cattle sampled in the different villages .....                                                                                                                                                                   | 34 |
| Table 4.1: Comparison of the mean antibody titres against <i>T. parva</i> , <i>B. bigemina</i> and <i>A. marginale</i> among the Nkedi Zebu (n = 103) and Ankole (n = 81) cattle, Kasilo county, Soroti.....                                | 42 |
| Table 4.2: Comparison of the mean antibody titres of <i>T. parva</i> , <i>B. bigemina</i> and <i>A. marginale</i> of the different age groups among the Nkedi Zebu (n= 103) and Ankole (n = 81) cattle, Kasilo county, Soroti district..... | 44 |
| Table 5.1: Comparison of the mean tick counts of the Nkedi Zebu (n=381) and Ankole (n=150) cattle among the different age groups in Kasilo county, Soroti .....                                                                             | 55 |
| Table 5.2: Comparison of the mean tick counts of <i>R. appendiculatus</i> , <i>B. decoloratus</i> and <i>A. variegatum</i> among the Nkedi Zebu (n = 381) and Ankole (n = 150) cattle, Kasilo county.....                                   | 55 |
| Table 5.3: Comparison of the mean PCV among the different age groups among the Nkedi Zebu (n = 381) and Ankole (n = 150) cattle, Kasilo county, Soroti .....                                                                                | 60 |

## LIST OF ABBREVIATIONS AND ACRONYMS

|         |                                                        |
|---------|--------------------------------------------------------|
| ARPT II | Agricultural Research and Training Programme II        |
| CAT     | capillary-agglutination test                           |
| CFT     | complement-fixation test                               |
| ° C     | Degree Celsius                                         |
| DNA     | Deoxyribonucleic acid                                  |
| ELISA   | Enzyme-linked immuno-sorbent assay                     |
| ECF     | East Coast Fever                                       |
| Hb      | Haemoglobin                                            |
| FAO     | Food and Agricultural Organisation                     |
| GDP     | Gross Domestic Product                                 |
| HCT     | Haematocrit centrifugation technique                   |
| HRP     | Horse raddish peroxidase                               |
| ibid    | as above                                               |
| IFAT    | immunoflorescent anti-body test                        |
| IgM     | Immunoglobulin M                                       |
| ILRI    | International Livestock Research Institute             |
| ITM     | Infection and Treatment Method                         |
| kD      | kilo Dalton                                            |
| LSRP    | Livestock System Research Programme                    |
| MAAIF   | Ministry of Agriculture, Animal industry and Fisheries |
| ml      | millilitre                                             |
| NARO    | National Agricultural Research Organisation            |
| NaLIRRI | National Livestock Resources Research Institute        |
| OD      | Optical density                                        |
| PAP     | Pastoral and Agro-pastoral                             |
| PBS     | Phosphate buffered saline                              |
| PBST    | Phosphate buffered saline-Tween                        |
| PCV     | Packed cell volume                                     |
| PP      | Percent positivity                                     |
| ®       | Registered trade mark                                  |
| RBC     | Red blood cells                                        |
| rpm     | revolutions per minute                                 |
| TBDs    | tick borne diseases                                    |
| TBM     | tick borne micro organisms                             |
| T&TBDs  | tick and tick borne diseases                           |
| µl      | micro-litre                                            |

## ABSTRACT

Ankole cattle have been recently introduced in eastern and northern Uganda, traditionally known for rearing Nkedi Zebu. This cross-sectional study was conducted to establish the responses to natural tick and tickborne infections in terms of mean tick counts, mean antibody titres and mean packed cell volumes among the Nkedi Zebu and Ankole cattle.

A total of 531 (381 Nkedi Zebu and 150 Ankole) randomly selected cattle were studied. The cattle came from six villages of Akumoi, Aswii, Osamito, Kamod, Opungure, and Okokoma in Soroti district. Packed cell volumes using Haematocrit centrifugation technique (HCT), tick counts of *Rhipicephalus appendiculatus*, *Boophilus decoloratus*, and *Amblyomma variegatum*, antibody titres using the antibody ELISA tests against *Theileria parva*, *Babesia bigemina*, and *Anaplasma marginale* were determined.

Results showed that the Ankole cattle aged 7-12 months had a significantly higher ( $p = 0.04$ ) mean tick counts of *R. appendiculatus* ( $24.2 \pm 3.2$ ) compared to the Nkedi Zebu ( $17.8 \pm 1.3$ ). The mean tick counts of *R. appendiculatus*, *B. decoloratus*, and *A. variegatum* on Ankole cattle ( $23.8 \pm 0.8$ ,  $9.6 \pm 0.6$ ,  $2.4 \pm 0.2$ ) were similar ( $p > 0.05$ ) to those of Nkedi Zebu ( $24.4 \pm 0.5$ ,  $10.4 \pm 0.34$ ,  $3.1 \pm 0.1$ ) respectively. The Ankole cattle had a significantly ( $p = 0.04$ ) lower antibody titre against *T. parva* infection ( $1.2 \pm 0.1$ ) compared to the Nkedi Zebu cattle ( $1.8 \pm 0.1$ ). Antibodies titres of Ankole cattle against *B. bigemina* and *A. marginale* infections ( $2.2 \pm 0.04$ ,  $0.05 \pm 0.05$ ) were similar ( $p > 0.05$ ) to those of Nkedi Zebu cattle ( $2.1 \pm 0.05$ ,  $0.06 \pm 0.05$ ) respectively. The sero-prevalences of ECF, babesiosis and anaplasmosis (100%, 100% and 58%) in Ankole cattle were not significantly ( $p > 0.05$ ) different from that of Nkedi Zebu cattle (99%, 100% and 57%) respectively.

The Nkedi Zebu are better primed to produce antibodies against *T. parva* and have better mechanisms to maintain a lower tick burden than Ankole cattle at weaning and as they are turned out to pastures. Therefore, extra tick control interventions are required for Ankole calves for them to thrive better in this region. In mixed herds both the Nkedi Zebu and Ankole calves require extra tick control interventions.

## CHAPTER ONE

### 1.0

### GENERAL INTRODUCTION

Ticks and tick-borne diseases (T&TBDs) cause the greatest constraints to the livestock industry in the tropics and the sub-tropics (Gray, 1985). An estimated 80% of the world's livestock live at risk of tick-borne diseases causing a loss of US\$ 13.9-18.7 billions annually (De Castro (1997).

In Africa, ticks and tick borne diseases are major constraints to the improvement of livestock health and productivity (Gray, 1985). These livestock diseases cause reduced growth and decreased milk yield coupled with high calf and adult mortalities under certain situations. Consequently, repeated attempts to improve animal productivity through introduction of improved exotic breeds have been thwarted by very high levels of susceptibility of these breeds to T&TBDs (Otim, 1997). The ticks of economic importance and the diseases they transmit in Africa are *Rhipicephalus* spp. for theileriosis, *Boophilus* spp. for anaplasmosis and babesiosis and *Amblyomma* spp. for theileriosis and cowdriosis (Okello-Onen *et al.*, 1996). Of these diseases, theileriosis is considered to be the most economically important due to the high mortalities caused and the productivity losses on recovery, the cost of control and the exclusion of taurine and taurine-cross cattle from endemic regions due to their high susceptibility (Young *et al.*, 1988). These diseases constitutes one of the major impediments to the development and improvement of the livestock industry in eleven countries in eastern, central, and southern Africa by causing significant mortality and production losses (Mukhebi *et al.*, 1992) in susceptible exotic and cross-bred cattle. Substantial losses have also been reported among the indigenous zebu and sanga cattle which have been raised in *theileria* free areas, in which the disease was introduced or when they are moved to endemic areas (Henning, 1932; Stobbs, 1966, Yeoman, 1966).

Livestock agriculture in Uganda is an integral part of the agricultural system contributing 7-9% of the total Gross Domestic Product (GDP) or 15-17% of the agricultural GDP (NARO/LSRP, 1999). Livestock resources offer a commendable tool in the poverty eradication programs because of the domestic and export market opportunities for

livestock and their products (NARO/LSRP, 1999). Several farmers in Uganda keep cattle, goats and sheep under either tethering, open grazing or communal grazing under a pastoral and agro-pastoral (PAP) farming systems. Their livelihoods are mainly dependent on livestock and this makes the pastoral and agro-pastoral (PAP) system important livestock producing systems and form the main source of milk and meat consumed in Uganda (Ocaido *et al.*, 2005). Among the major constraints to livestock productivity in pastoral and agro-pastoral areas in Uganda are ticks and tick-borne diseases (T&TBDs) (Ocaido *et al.*, 1996, Moran 1996). Keeping of disease tolerant breeds of cattle in areas of high disease challenge is an important disease control strategy. This strategy has not been widely encouraged in Uganda, consequently cattle breeds and types are selected for restocking without paying much attention to how they respond to diseases to match the degree of disease challenges in various areas that require restocking. This often creates a lot of production losses.

The national cattle herd in Uganda is at constant risk of tick borne diseases and the overall loss of the calf crop due to tick borne diseases is estimated to be 30% over the greater part of Uganda (Magona, 2004a). The cumulative mortality of 13.5% due to East Coast Fever (ECF) in the indigenous calves up to one year has been reported in a commercial cattle ranch in Uganda (Okello-Onen *et al.*, 1996). Of the deaths attributable to tick borne diseases, ECF is responsible for 79%, anaplasmosis 11%, and babesiosis 4.4% (Magona, 2004a).

Breed related susceptibility (resistance) to tick borne disease does exist and has been observed in several tropical areas. Studies done by Jacobsen (1983) in Zimbabwe found higher ECF induced mortality rate of 14% in crossbred calves than in the zebu calves (4.2%) and had a lower recovery rate (57%) in crossbred calves than in zebu calves (90%) in Zanzibar. In another study carried out in Rwanda, the mortality following experimental infection with *T. parva* was found to be higher in the crossbreds (64%), than Ankole cattle (32%) (Paling *et al.*, 1991). This difference in mortality was attributed to the genetic ability of the Ankole cattle to limit the explosive multiplication of *T. parva* microschizont (Paling *et al.*, 1991).

Breed susceptibility to bovine anaplasmosis has also been observed, with exotic breeds being more susceptible than the indigenous cattle breeds (Ajayi *et al.*, 1982). The clinical signs of anaplasmosis were found to be milder in Zebu cattle than the exotic crossbreds (Latif and Pegram, 1992; Latif *et al.*, 1995). To date, no studies have been conducted in Uganda to establish the differences in responses to major tickborne infections such as theileriosis, anaplasmosis and babesiosis among the indigenous Zebu and Ankole cattle and yet the indigenous cattle breeds have been observed to have tolerance to a range of endemic diseases. For example, the N'Dama cattle of West Africa have been reported to be trypanotolerant and possess endemic stability to tick-borne diseases (Kaufmann and Pfister, 1990; Kaufmann *et al.*, 1992; Mattioli *et al.*, 1995).

### **1.1 Problem statement**

Ticks and tick borne diseases constitute a major constraint to livestock productivity and they have considerable economic impact in the tropics and sub tropics (Jongejan and Uilenberg, 2004; Gray, 1985). These diseases, therefore, continue to impede livestock productivity in most parts of Uganda due to the existence of suitable climate and inadequate control of both ticks and other biting insects (Otim, 2000). Over 90% of the national cattle herd is at constant risk of TBDs with a sero-prevalence of 68.9-85% for ECF, 56.2-85% for anaplasmosis & 54.9-76.9% for babesiosis (Magona, 2004a). Their effects are diverse and include reduced growth and milk production, damaged hides, paralysis, and lesions of tick bites which are prone to secondary attacks from other parasites such as screw worm flies or infections by other pathogens such as dermatophilus (Sutherst, 1987). A study by Okello-Onen and others (1996), revealed significant losses in terms of calf mortality of up to 13.5% due to ECF in the indigenous calves on a commercial ranch up to one year old in Uganda. In this study, of the deaths attributable to TBDs, ECF was responsible for 79%, anaplasmosis 11% and babesiosis 4.4% (ibid). Recently, Rubaire-Akiiki and others (2004) described the prevalence of ticks and serum antibodies to tick-borne infections across different grazing systems and agro-ecological zones in Mbale and Sironko districts. The highest serum antibody prevalence (>60%) was recorded in the lowland Agro-ecological zone under the free range and tethering grazing systems. The lowest tick challenge and serum antibody levels (<50%)

were recorded in the midland and upland zones under a zero-grazing system. Otim and co-workers, (2004) in a study to establish the disease and vector constraints to cattle production in pastoral communities in Ssembabule district revealed a very high seroprevalence of ECF to be 93.3% in cattle. In this study *R. appendiculatus* was found to be the most abundant species with a mean tick count per head of cattle of  $3.7 \pm 0.28$ , followed by *R. evertsi* and *A. variegatum* with mean tick counts per head of cattle of  $0.51 \pm 0.3$  and  $0.24 \pm 0.012$ , respectively (Otim *et al.*, 2004). Control of tick-borne diseases in East Africa has proved difficult largely because of lack of epidemiological information (Norval *et al.*, 1992) and because control strategies commonly applied are not integrated in the production system (Perry, 1994). In the past the national tick control programme used to cost up to US \$ 10 million annually (Okello-Onen, 1995). The conventional control methods include the use of chemical acaricides although this, has had certain implicit drawbacks, such as the presence of residues in the milk and meat, the development of acaricide resistant tick strains (Willadsen and Kemp, 1988; Nolan, 1990), and the harmful, poisonous effects on the animals, human beings and the environment (García-García *et al.*, 2000). The development of new acaricides is a long and expensive process, which reinforces the need for alternative approaches to the control of tick infestations (Graf *et al.*, 2004). There is however, a continuous risk of development of resistance to successive acaricides produced and their ever increasing costs including civil unrest, illegal cattle movement and poor management has posed a major problem in the control ticks (Latif, 1984). A study by Norval *et al.* (1992) established that untreated indigenous cattle seldom or never become heavily infested with ticks and consequently suffer small or insignificant production losses as a result of ticks and tick-borne diseases. Alternative use of non-chemical tick control methods, such as the rational use of tick resistant hosts (Ali & de Castro, 1993; Fivaz *et al.*, 1992; Mathioli *et al.*, 1993; Latif, 1984) hasn't been widely encouraged.

## **1.2 Overall objective**

1. To determine the differences in response to tick-borne infections among the Nkedi Zebu and Ankole cattle under similar natural tick challenges.

## **1.3 Specific objectives**

1. To determine and compare the mean antibody titres of ECF (theileriosis), babesiosis and anaplasmosis among the Nkedi Zebu and Ankole cattle and within the different age groups in the agro-pastoral farming system of Soroti district.

2. To determine and compare the sero-prevalence of ECF (theileriosis), anaplasmosis and babesiosis among the Nkedi Zebu and Ankole cattle in the agro-pastoral farming system of Soroti district.

3. To determine and compare the mean tick counts of *R. appendiculatus*, *B. decoloratus*, and *A. variegatum* among the Nkedi Zebu and Ankole cattle and within the different age groups in the agro-pastoral farming system of Soroti district.

4. To determine and compare the mean packed cell volumes (PCV) among the Nkedi Zebu and Ankole cattle and within the different age groups in the agro-pastoral farming system of Soroti district.

## **1.4 Hypothesis**

There is no significant difference in response to natural tick challenges and tick-borne infections (theileriosis (ECF), babesiosis and anaplasmosis) among the Nkedi Zebu and Ankole breeds of cattle in the agro-pastoral farming system of Soroti district.

## **1.5 Justification**

Tick-borne protozoan infections are a major constraint to livestock development throughout the tropics (Gray, 1985). Worldwide, an estimated 600 million cattle are exposed to anaplasmosis and babesiosis, while 200 million cattle are exposed to theileriosis (Mukhebi *et al.*, 1992). In eastern, central and southern Africa, theileriosis (ECF), caused by *T. parva*, is considered to be the most significant tick borne disease of

cattle (Mukhebi *et al.*, 1992). Livestock, however, is regarded an important asset in the poverty eradication programs because of the domestic and export market opportunities for livestock and their products (NARO/LSRP, 1999).

A diagnostic survey using participatory rural appraisal methodologies by NARO/LSRP, (1999) ranked tick-borne diseases and helminthosis as the most important constraints to dairy production in Uganda. Ticks and tick-borne diseases (T&TBDs) are the major diseases of livestock in Pastoral and Agro-pastoral (PAP) farming areas in Uganda (Anon, 1997). A cumulative mortality of 13.5% due to ECF in the indigenous calves up to one year old has been reported in a cattle ranch in Uganda (Okello-Onen, 1995). In that study, the deaths attributable to tick borne diseases, ECF was responsible for 79%, anaplasmosis 11% and babesiosis 4.4% (Okello-Onen, 1995). TBDs of which East Coast Fever (ECF), anaplasmosis, babesiosis and cowdriosis are quite important, and are widespread throughout Uganda and yet lack seasonality (Otim, 2000). Tick-borne diseases (TBDs) are the main diseases which continue to impede livestock productivity in most parts of Uganda due to the existence of suitable climate for ticks and inadequate control methods of ticks (Otim *et al.*, 2000). This widespread incidence of these diseases, therefore, is one of the main constraints to increased production and productivity of cattle in Uganda. Ticks are the main vectors of these endemic diseases (Otim, 2000). These diseases deter fast growth of animals and cause mortality thereby leading to slow maturity and, therefore, low productivity of animals, with long intercalving periods which reduce the market value of animals and impair their multiplication for external and internal markets especially in areas that are undergoing restocking. (Magona, 2004). Livestock performance would be enhanced by identification and utilisation of cattle breeds with natural resistance to these major endemic diseases in areas of high disease challenges. Several studies have shown that different indigenous breeds of cattle have certain degrees of genetic resistance to both ticks and tick borne disease (Paling *et al.*, 1991; Norval *et al.*, 1992; Mattioli *et al.*, 1995; Lawrence *et al.*, 1996). Adoption of tick resistant breeds of cattle has been done in several countries, such as the Ethiopian Boran, Kenyan Boran (de Castro 1991), Zimbabwe Nkoni (Sanga) Norval *et al.*, 1988) and the Bonsmara in S. Africa (Scholtz *et al.*, 1991). The use of similar Ugandan breeds has not been considered and yet could be a cheaper alternative to the farmer.

Control of tick-borne diseases in East Africa has proved difficult largely because of lack of adequate epidemiological information (Norval *et al.*, 1992) and because control strategies commonly applied are not integrated in the production system (Perry 1994). There is need to identify which Ugandan cattle breeds have resistance to T&TBDs because the control of the endemic diseases could be selective, strategic and focused only on susceptible target cattle populations. This could reduce costs and environmental pollution associated with intensive acaricide use.

A recent study (Ocaido *et al.*, 2005) carried out in Soroti district, showed that farmers' knowledge of disease control using modern veterinary practices was very low as revealed by the low number of farmers (5.8%) who sought veterinary services to treat their animals, for instance the number of farmers practicing chemoprophylaxis against Trypanosomosis was only 5.2%, and 1.7% of the households used Berenil® and Samorin® for treatment and prophylaxis of trypanosomosis, respectively. A large number of farmers (54.4%) did not know the name of acaricide they were using. And yet the majority of farmers (91.1%) were not applying acaricides properly. This increases the risk of acaricide resistance and environmental pollution.

Studies have shown that acquired immunity against tick infestation can be induced by repeated tick infestation or by active immunization with either crude or purified native as well as recombinant antigens. This provides insights into the development of tick vaccines with reference to identification, purification and molecular cloning of candidate target antigens (Mulenga, *et al.*, 2000).

In Uganda the drive towards increased livestock production has compelled several farmers and other stakeholders in the livestock industry to take certain breeds of cattle, such as the Ankole, into areas, such as Soroti district, where they were not previously kept traditionally (Magona *et al.*, 2004b). The restocking, breeding and other livestock development programmes in the various districts of Uganda need to be guided by adequate knowledge on the responses of the various cattle breeds to the major endemic diseases such as the tick borne diseases. For example Rushingajiiki *et al.* (1986), observed that under a high level of trypanosome challenge in south-eastern Uganda, Nkedi Zebu cattle perform better than Zebu-Friesian crosses, which showed retarded

growth, poor bodily condition and were more susceptible to endemic diseases (Rushigajiiki *et al.*, 1986).

The aim of the present study therefore, was to compare the responses to natural tick and tick borne infections among the Nkedi Zebu and Ankole cattle in Soroti district, Kasilo county, in terms of the prevalence of tick borne infections as measured by the PCV, antibody titres and counts of the common and economically important ticks. Exploitation of breed resistance as a disease control strategy in areas of high diseases challenge would not only enhance production and productivity but also reduce the usage of drugs and minimize occurrence of drug resistance, make cattle treatment affordable to farmers and overall make disease control sustainable. Reducing on drug costs would make cattle keeping more profitable. Identification and keeping of diseases resistant cattle breeds would also satisfy drought power demands in areas of high disease challenges, there by reducing on the drudgery of cultivation of land by human labour. The results of this study will be useful to different stakeholders in planning of disease risk guided cattle restocking programmes which are on going in this region of the country and designing control strategies for ticks and tick borne diseases.

## CHAPTER TWO

### 2.0

### GENERAL LITERATURE REVIEW

#### 2.1 Ticks and their distribution

In cattle, the most important ixodid ticks and the diseases that they transmit include *R. appendiculatus* (ECF), *B. decoloratus* (Anaplasmosis and Babesiosis) and *A. variegatum* (Heartwater). The most common ixodid tick species in order of abundance observed in Uganda include; *R. appendiculatus*, *R. evertsi*, *A. variegatum* and *B. decoloratus* (Okello-Onen *et al.*, 1998). In many parts of Africa, several tick species occur on the same host, and each of them contributes to the observed effect which cannot be easily separated from the overall effects of tick borne disease(s) it transmits (Okello-Onen, 1995). Tick populations differ according to the climate and altitudes of given locations. A study out carried in Mbale and Sironko districts revealed that the highest tick challenges were recorded in the low lands, followed by the midland and the uplands, respectively (Rubaire-Akiiki *et al.*, 2004). Similar results were obtained in the Kenyan highland (Gitau *et al.*, 1997). Climatically, the lowlands are more suitable for *R. appendiculatus* than the midlands and uplands (Matthysse and Colbo 1987). Keeping of local indigenous breeds under the free-range grazing system is also known to increase the occurrence and spread of tick and tickborne diseases among the cattle herds (Rubaire-Akiiki *et al.*, 2004).

#### 2.2 Responses of cattle breeds to ticks

The location of a suitable host is a critical stage in the life history of a parasite (Belan and Bull, 1991). Ticks may locate their hosts either actively by moving towards distant stimuli or passively by waiting either on vegetation or within refuge sites until a host individual comes within reach (Waladde and Rice, 1982). However the rates at which ticks are picked up by a host animal is determined by both tick density and activity and by the availability of hosts (Sutherst *et al.*, 1987; Latif *et al.*, 1991). The ability of livestock to control their tick burden through the acquisition of natural resistance has been known for several decades (Johnston and Bancroft, 1918; Trager, 1939; Kelley, 1943; Bonsma, 1944; Macleod, 1975). Generally *B. indicus* breeds were reported to acquire natural resistance to tick infestation more effectively than those of *Bos taurus*

(Rick, 1962; Francis and Little, 1964; Utech *et al.*, 1978b; Newson and Chiera, 1986; Rechav, 1987). This is due to innate ability to mount immune response to components of the saliva of feeding ticks, mediated by antibodies, complement and T-cell-dependant mechanisms, associated with cutaneous hypersensitivity reactions at the attachment site with increased grooming activity (Branagan, 1973; Willadsen, 1980; Binta and Cunningham 1984; Fivaz and Norval, 1990) that may kill or debilitate the ticks (de Castro and Newson, 1993). The immunological response is often stronger towards species of which the host has had little or no evolutionary experience (Ribeiro, 1989). The use of cattle which are resistant to ticks has been adopted and incorporated into the integrated tick control strategies in Australia (Roberts, 1968 a, b, c; Wagland, 1978). Previous studies have suggested that cattle resistance to ticks is heritable (Johnson and Bancroft, 1918), although this heritability has been associated with crosses based on the zebu breed cattle (Bonsma, 1944; Heweston, 1968). Wharton *et al.*, (1970), observed that in a herd of Australian Illawarra Shorthorn (*B. taurus*) dairy cattle that acquired tick infestations naturally, heritability estimates were 39% from dam-calf correlation and 49% from full-sib correlations. Consequently, attempts have been made to breed tick-resistant cattle in Brazil (de Castro and Newson, 1993), Australia (Heweston, 1968) and South Africa (Bonsma, 1944) by balancing the desirable traits of *B. taurus* (high body weight gain and milk yield) and those of *B. indicus* (tick resistance, heat adoption, resistance to diseases and parasites). However this phenomenon has not been exploited much in Africa where multi-species infestations and several alternative hosts (wild and domestic) occur. Generally the effects of host resistance are expressed through a) an increase in the number of ticks which fail to complete their blood meal, b) a decrease in the mean weight of the engorged tick and c) an increase in the time needed by the ticks to complete the feeding process (Rechav *et al.*, 1991). Similar observations were made by Chiera *et al.* (1985) and Latif (1984) when they compared the weight of engorged ticks fed on resistant and susceptible host. In Uganda it was recognized that some animals showed greater resistance than others to all instars and species of tick (Kaiser *et al.*, 1982), and about 50% of the total tick population infesting herds were carried by 30% of cattle. The relative resistance to tick infestation of zebu cattle (*B. indicus*) all belonging to Tanganyika short horn Zebu were compared with crossbreds (*B. indicus* x *B. taurus*)

cattle. Parameters taken to distinguish between 'tick resistant' and 'tick susceptible' cattle were: tick counts on naturally exposed animals, serum complement levels and delayed skin hypersensitivity response to phytohaemagglutinin. Results showed that pure zebu cattle were less infested with ticks when compared to zebu-taurine crosses under identical field conditions. Zebu cattle also had significantly higher serum complement levels than crossbred cattle (Matoyelo *et al.*, 2002).

### **2.3 Ticks and tickborne diseases**

Ticks are well documented as efficient vectors of both human and animal diseases, and are known to transmit various micro-organisms including protozoa, rickettsiae, viruses and bacteria among vertebrate hosts (Uilenberg, 1981). None of these micro-organisms are known to cause harm to the arthropod hosts (tick) but all are pathogenic to the associated vertebrate hosts (Rehacek, 1965). In cattle, the most important ticks and the diseases that they transmit include *R. appendiculatus* (ECF), *B. decoloratus* (anaplasmosis and babesiosis) and *A. variegatum* (Heartwater). The most common tick species in order of abundance observed in Uganda include; *R. appendiculatus*, *R. evertsi evertsi*, *A. variegatum* and *B. decoloratus* (Okello-Onen *et al.*, 1998b). Studies from elsewhere in Africa indicate *B. decoloratus* as the most common tick species in South Africa (De Vos, 1979) and Cameroun (Ndi *et al.*, 1991). The tick population on cattle usually varies from year to year in many places, depending largely on climatic factors (Norval *et al.*, 1992; Perry and Young, 1995; Lawrence *et al.*, 1996; Billiouw *et al.*, 1999). A dry season adversely affects tick population and leads to low tick infestation and resistance of cattle to ticks is enhanced when there is continuous tick exposure (Kaiser *et al.*, 1982; Mattioli *et al.*, 1995; Lawrence *et al.*, 1996; Okello-Onen *et al.*, 2003).

Indigenous cattle have a certain degree of genetic resistance to both ticks and tick borne diseases (Norval *et al.*, 1992; Lawrence *et al.*, 1996). Resistance to tick attack and tick-borne micro-organisms (TBMs) varies among different breeds of cattle. To some extent, the magnitude of losses due to these parasites is related to the degree of breed resistance. Generally, zebu (*Bos indicus*) cattle possess a higher resistance to ticks and TBMs than European (*Bos taurus*) cattle. The host's immune system would appear to be the single most important factor that regulates this resistance. The main effector immune

mechanisms governing resistance against ticks and TBMs have been studied. The cellular immune response appears more effective and stable than humoral immunity in modulating resistance to ticks and TBMs (Mattioli *et al.*, 2000). Similarities between the immune mechanisms employed by trypanotolerant N'Dama cattle, when infected with trypanosomes, and those elicited by tick bites and TBMs seem to exist, particularly at the skin level in the early phases of parasitic invasion. Moreover, there is evidence that in the N'Dama breed, resistance against ticks *per se* also has a genetic basis (Mattioli *et al.*, 2000). Infection is maintained in a population by continuous exposure to ticks; cattle become infected as calves, when they acquire a degree of age related resistance or colostral immunity, which minimises the effects of diseases, and there after become immune (Norval *et al.*, 1992; Lawrence *et al.*, 1996). However, cattle of European breeds (*Bos taurus*) and their crosses, do not enjoy the same degree of resistance to T & TBDs as the indigenous cattle (Lawrence *et al.*, 1996).

### 2.3.1 East Coast Fever (ECF)

Bovine theileriosis is a protozoan disease caused by *T. parva*, which is transmitted by the three host ixodid tick *R. appendiculatus*. This disease occurs across a large area of east and central Africa, particularly Uganda, Kenya, Tanzania, Rwanda, Burundi, Zambia, Zaire and Malawi (Irvin and Cunningham, 1981). The causative agent of classical ECF is *T. parva*. Some previously recognized separate species and subspecies have been combined with *T. parva* as a result of recent studies on their DNA (Conrad *et al.*, 1989; Norval *et al.*, 1992). The life cycle of *T. parva* is complex in its tick and mammalian hosts (Norval *et al.*, 1992). The infectivity of *Theileria* infections in cattle was the basis of classification of *T. parva* into three separate species: *T. parva*, *T. lawrencei*, *T. bovis* (Barnett, 1968; Norval *et al.*, 1992). *T. lawrencei*, was distinguished from *T. parva* in being derived from Cape buffaloes (*Syncerus caffer*), causing self-limiting disease infections in cattle with no piroplasms and low schizonts numbers (Neitz, 1957, cited by Norval *et al.*, 1992), and from *T. parva* because it produces a carrier state after recovery and fewer parasites than *T. parva* (Neitz, 1957). *T. lawrencei* was later suggested to be synonymous with *T. parva* (Young *et al.*, 1973). The distinction of these parasites was then conveniently maintained by tri-nomenclature: *T. parva parva*, for classical ECF, *T.*

*parva lawrencei* for Corridor disease and *T. parva bovis* for January disease in Zimbabwe (Uilenberg, 1981).

The three stocks have been recently confirmed to be a single species by studies on cross immunity (Irvin and Cunningham, 1981), a carrier state (Maritim *et al.*, 1989), monoclonal antibody profile, protein analysis, DNA and RNA nucleotide sequence (Conrad *et al.*, 1989; Allsopp *et al.*, 1989), eliminating the tri-nomenclature. One justifiable *T. parva* type of infection is now recognised, producing either lawrencei type of infections (Buffalo-derived *T. parva* infection) of low schizonts and piroplasms numbers, accompanied by haemorrhagic anaemia or the parva type (cattle-derived *T. parva* infection) with large schizont numbers and parasitaemia, and intermediate syndromes such as January disease with few schizonts and no or very few piroplasms. Cattle in many endemic areas show a high degree of resistance to ECF; much of this is acquired as a result of repeated challenge, but there is good evidence that genetic selection of resistant stock can occur and there are instances where the progeny of particular sires or dams have shown high innate resistance to ECF (Irvin and Cunningham, 1981).

#### **2.3.1.1 The clinical signs of ECF**

The clinical signs of ECF vary according to the level of challenge and range from unapparent or mild to a severe and fatal disease. The prepatent period and severity of infection are directly proportional to the quantum of sporozoites injected by the ticks (Irvin and Cunningham, 1981). However there is a minimum prepatent period of about 5 to 15 days after attachment of infected ticks. This may be seen as a swelling of the draining lymph node, usually the parotid, for the ear is the preferred feeding site of the vector. This is followed by a generalized lymphadenopathy in which superficial subcutaneous lymph nodes such as the parotid, prescapular, and prefemoral lymph nodes, can easily be seen and palpated. Fever ensues and continues throughout the course of infection. This rise in temperature is rapid and is usually in excess of 103° F (39.5° C) but may reach 106° F (42° C). Anorexia develops, and loss of condition follows. Other clinical signs may include lacrimation, corneal opacity, nasal discharge, terminal dyspnea, and diarrhoea. Before death the animal is usually recumbent, the temperature

falls, and there is a severe dyspnea due to pulmonary oedema that is frequently seen as a frothy nasal discharge. Death usually occurs 18 to 30 days after infestation of susceptible cattle by infected ticks. Mortality in fully susceptible cattle can be nearly 100 percent. The severity and time course of the disease depend on, among other factors, the magnitude of the infected tick challenge, for ECF is a dose- dependent disease, and on the strain of parasites. Some stocks of parasites cause a chronic wasting disease. A fatal condition called "turning sickness" is associated with the blocking of brain capillaries by infected cells and results in neurological signs.

In recovered cattle, chronic disease problems can occur that result in stunted growth in calves and lack of productivity in adult cattle (Moll *et al.*, 1986). However, this syndrome tends to be in the minority of recovered clinical cases; in a majority of cases, asymptomatic carriers can be recognized with apparently little or no effect on their productivity (Moll *et al.*, 1986).

A frothy exudate is frequently seen around the nostrils of an ECF-infected animal. Signs of diarrhea, emaciation, and dehydration may be seen. Lymph nodes are greatly enlarged and may be hyperplastic, hemorrhagic, and edematous. In acute cases of ECF, lymph nodes are edematous and hyperemic but often become necrotic and shrunken in more chronic disease. Generally, muscles and fat appear normal but, depending on relative acuteness of infection, fat may become greatly depleted; serosal surfaces have petechial and ecchymotic hemorrhages, and serous fluids may be present in body cavities. Hemorrhages and ulceration may be seen throughout the gastrointestinal tract, particularly in the abomasum and small intestine, where necrosis of peyer's patches can be observed. Lymphoid cellular infiltrations appear in the liver and kidney as white foci that have been referred to as pseudoinfarcts. The most striking changes are seen in the lungs. In most cases of ECF, interlobular emphysema and severe pulmonary edema appear, the lungs are reddened and filled with fluid and the trachea and bronchi are filled with fluid and froth.

Morbidity and mortality depend on, among other factors, the magnitude of the infected tick challenge and susceptibility of the host and strain of parasite. ECF in susceptible

cattle, which are not indigenous to the enzootic area, is very severe with a mortality approaching 100%. Animals that recover are often unthrifty and sickly. Zebu cattle residing for many generations in endemic areas become infected (100% morbidity), but only a minor proportion succumb; however, many become carriers, and early infection with *T. parva* can affect their growth and productivity (Moll *et al.*, 1986).

### 2.3.2 Bovine anaplasmosis

Anaplasmosis formerly known as gall sickness refers to a disease of ruminants caused by obligate intraerythrocytic organism of the order Rickettsiales, family Anaplasmataceae, and genus *Anaplasma*. Cattle, sheep, goats, buffalo, and some wild ruminants can be infected with the erythrocytic *Anaplasma*. *A. centrale* causes a relatively "mild" form of bovine anaplasmosis in Africa (Ristic, 1968). Anaplasmosis occurs in tropical and subtropical regions worldwide (approximately 40° N to 32° S), including South and Central America, the USA, southern Europe, Africa, Asia, and Australia (Ristic, 1968).

The occurrence of anaplasmosis is determined by the distribution of its main vectors; *Boophilus* species, and *R. evertsi evertsi* (Jongejan *et al.*, 1988; Omuse, 1978). Detection of *A. marginale* has been observed to coincide with the peak of *B. decoloratus* infestations (Latif *et al.*, 1995). However, anaplasmosis has been transmitted by a variety of one-, two-, and three-host ticks (including *Boophilus* spp., *R. evertsi evertsi*) and biting flies. Life long carrier status of recovered animals and the presence of wild and domestic reservoirs of infection are known to determine the distribution the disease (Norval *et al.*, 1984; Potgieter and Stoltz, 1994; Urquhart *et al.*, 1996).

Parasitological prevalence of bovine anaplasmosis has been reported in Uganda to be 29-36% in Uganda (Ssenyonga *et al.*, 1991; Anon., 1996), in Kenya it has been reported to be 36-38.2% (Mulei and Rege, 1989; Latif *et al.*, 1995) and in Nigeria its has been reported to be 37.7% (Egbe-Nwiyi *et al.*, 1997). A sero-prevalence of bovine Anaplasmosis ranging from 81% to 94% was reported across different agro ecological zones in Kenya (Deem *et al.*, 1993) and from 14.7% to 38% in Zambia (Jongejan *et al.*, 1988). A carrier state occurs in cattle and the wild deer, which plays an essential role in the maintenance of this disease in nature (Ristic, 1968).

### 2.3.2.1 The clinical signs of bovine anaplasmosis

The incubation period/prepatent period seems to be indirectly related to the amount of parasitic mass used to infect the animals. Under laboratory conditions using needle induced infection with carrier blood, this period lasts 3 to 5 weeks (Ristic and Carson, 1977). The major clinical forms are mild, chronic, acute and peracute. The disease is mild in calves up to one year of age; acute but rarely fatal in cattle up to 2 years of age; acute and occasionally fatal in cattle up to 3 years of age; and often peracute and frequently fatal in cattle over 3 years of age (Ristic and Carson, 1977). The signs of acute anaplasmosis usually consist of anaemia, weakness, febrile reaction, constipation, icterus, inappetence, depression, dehydration, dyspnoea and abortion (Ristic and Carson, 1977). The peracute anaplasmosis constitutes the most severe and usually fatal form of the disease and occurs in pure bred stock or high producing milk cows, which succumb within a few hours of onset of infection (Ristic, 1968) in addition to anaemia, a severe drop in milk yield occurs in lactating cows, extensive salivation, very rapid respirations, often exhibits irrational behaviour and signs of nervousness ( Ajayi *et al.*, 1987; Potgieter and Stoltz, 1994; Egbe-Nwiyi *et al.*, 1997).

The gross pathological changes of anaplasmosis include icteric mucous membrane, enlargement of the liver, gallbladder, spleen and mainly the superficial lymph nodes. The liver and spleen maybe moderately congested, the carcass is jaundiced and gallbladder is grossly enlarged. The liver is suffused with bile and petechial haemorrhages on the heart muscles (epicardium and pericardium) (Losos, 1986; Egbe-Nwiyi *et al.*, 1997). The major haematological feature of anaplasmosis is anaemia, with a decreasing PCV, Hb and RBC to below normal (Egbe-Nwiyi *et al.*, 1997).

### 2.3.3 Bovine babesiosis

This is a tick-transmitted protozoan disease of cattle that is wide spread in tropical Africa, caused by several babesia species. These include; *B. bovis*, *B. bigemina*, *B. divergens*, and *B. major*, although *B. bovis* is considered the most important of all of them. *B. divergens* occurs more frequently in northern Europe and the United Kingdom, while *B. major* is found in several parts of the world, appears smaller and similar to *B. bigemina* (Ristic, 1981). Babes (1888), was the first to describe the parasite in blood of African

cattle showing haemoglobinuria. Presently babesiosis is considered to be one of the most important constraints of cattle production in most regions with tropical and semi-tropical climates (Ristic and Levy, 1979). Babesiosis is transmitted by ticks of the genus *Boophilus*. *B. bovis* is transmitted only by *B. micropilus* and *B. decoloratus* (De Vos, 1979; Norval *et al.*, 1983; Jongejan *et al.*, 1988).

#### **2.3.3.1 The clinical signs of bovine babesiosis**

The acute and chronic syndromes of babesiosis are common. The acute syndrome is characterised by high fever, anaemia, haemoglobinuria (redwater), jaundice, abortion and variable loss of weight (Ristic, 1981; Losos, 1986). The chronic syndrome is associated with anaemia and weight loss. If death does not occur, there is loss of weight, reduced milk yield, diarrhoea followed by constipation (Urquhart *et al.*, 1996; Losos, 1986). The main clinical signs observed in field cases of bovine babesiosis are; anaemia, pyrexia, haemoglobinuria and jaundice. These cases present with pale mucous membranes that maybe yellow-tinged and owners frequently report animals passing red urine (Omuse, 1978). Field cases of bovine babesiosis and Anaplasmosis usually present with very similar clinical signs, but the passage of red urine is only observed in cases of Babesiosis (Omuse, 1978). In cases of cerebral babesiosis of cattle caused by *B. bovis*, the terminal central nervous signs involving paddling limbs, ataxia and circling occurs (Losos, 1986). The major pathological features of babesiosis are anoxic anaemia and red blood cell destruction (Hildebrandt, 1981). Anoxia causes degeneration and swelling of endothelial cells, which impedes blood flow and further complicates the anoxic state of the organs. Damaged endothelium and hypoproteinaemia resulting from hepatic dysfunction leads to oedema (Hildebrandt, 1981). At necropsy, subcutaneous tissue and mucous membranes are pale, the liver is enlarged, gallbladder and urinary bladder are distended, the kidneys are congested, the lungs oedematous and there is massive splenomegally (Losos, 1986). In cases of cerebral babesiosis, marked parasitized erythrocytes are seen in the capillaries of the gray matter of the brain and the kidneys (Losos, 1986). Intravascular haemolysis is the most distinctive haematological feature in bovine babesiosis which is manifested by a decrease in PCV, red blood cell counts and haemoglobin values by over 50% (Losos, 1986). Reported prevalence of bovine babesiosis, as detected by presence of piroplasms

in giemsa stained smears in tropical African countries include; 16% in Uganda (Anon, 1996), 41.4% in central Kenya (Mulei and Rege, 1989) and western Kenya (Latif *et al.*, 1995).

## **2.4 Control of ticks and tickborne diseases**

Tick and tickborne diseases (T&TBDs) are controlled in various ways. These include mainly chemical and sometimes physical means as are presented below;-

### **2.4.1 Use of chemical acaricides**

Control of tick using acaricides is one of the methods for reducing tick-borne diseases (Spickett and Fivaz, 1992). A wide range of acaricides, including arsenical, chlorinated hydrocarbons, organophosphates, carbamates and synthetic pyrethroids are available for tick control on livestock. The effectiveness of an acaricide in the control of ticks depends not only on the activity of a product, but on the quality and quantity of active ingredient deposited on cattle or delivered internally (George, 2000). However chemical control is limited owing to the selection of acaricide resistant ticks; in addition, residues in meat and milk have raised public health problems. Intensive acaricide use also interferes with enzootic stability, rendering the more susceptible to the diseases (Varda *et al.*, 2007).

### **2.4.2 Arsenic compounds**

Arsenic compounds were the first effective method for controlling ticks and tick-borne diseases, and were used in many parts of the world before resistance to the chemical became a problem (George, 2000). Sodium arsenites have been used for many years in dip tanks to control ticks, especially ticks of the genus *Boophilus*. Arsenic dips were used successfully to eradicate *Boophilus* ticks from the southern United States of America. Unfortunately, arsenic compounds have a very short residual period (less than two days), and as such in most areas of the world *Boophilus* ticks have become resistant to arsenicals (Drummond, 1983).

### **2.4.3 Chlorinated hydrocompounds**

These are synthetic chlorinated hydrocarbon acaricides. Development of resistance to arsenicals in many species of ticks (Matthewson and Baker, 1975; Angus, 1996) resulted into replacement by chlorinated hydrocarbons (Graham and Hourrigan, 1977). Chlorinated hydrocarbon acaricides are very persistent and have been used extensively throughout the world for controlling ticks. Of particular interest are benzene hexachloride (toxaophene) (Drummond, 1983). Their mode of action is by interfering with nerve conduction of ticks (Solomon, 1983). However, these compounds have been withdrawn from market due to their high toxicity and long life span (Spickett, 1998).

### **2.4.4 Organophosphorous compounds**

Organophosphates were introduced as a replacement for the chlorinated hydrocarbons to which significant resistance had occurred (Shanahan and Hart, 1966). These are esters of phosphoric acid and have a wide range of activities against ticks at very low concentration in companion and livestock animals. However, their residual effectiveness is usually shorter than that of chlorinated hydrocarbons, and the risk of causing acute toxicity in livestock is greater (Drummond, 1983). Resistance in ticks was first recognized in 1963 and several tick species are now known to be resistant to organophosphorous compounds for example dichlorophenyls (Wharton, 1967).

### **2.4.5 Carbamates**

Carbamates are esters of carbamic acids and closely resemble the organophosphates (Spickett, 1998). They are a little more toxic than the organophosphates for mammals and are much more expensive.

## **2.5 Application of acaricides**

Various methods including dipping, spraying, ear tagging or pour on, have been used to apply chemicals to protect livestock against ticks. Direct application of acaricides to animals is the most popular method of controlling ticks on livestock (Drummond, 1983). Applications of acaricide to tick-infested cattle via dipping or spray pump is equally

effective under ideal conditions with proper handling of equipments without injuring animals and acaricide dilution of a product (George, 2000).

### **2.5.1 Dipping**

In this method, animals are immersed in a dip-tank containing solution of chemicals. By 1893 in Australia, Africa, and the United States the use of dip-tank to immerse tick-infested cattle in a variety of chemical agents was a component of the effort to control the ticks and tick-borne diseases affecting cattle (Mohler, 1906; Matthewson and Baker, 1975). A variety of acaricides including cottonseed oil, fish oil, crude petroleum, kerosene, creosote, tobacco extract, soap, and a combination of sulphur and kerosene were among the hundreds of possible acaricides tested for dipping (Mohler, 1906; Angus, 1996). In general, dip-tanks provide a highly effective method of treating animals with acaricides for tick control. However, their immobility, high initial cost of construction, and the cost of the acaricides may make dip-tanks impractical for many small ranching operations. Also, dip-tanks must be managed carefully so that the chemical are maintained at the proper concentration and the cattle are dipped properly (Drummond, 1983).

### **2.5.2 Sprays**

The application of liquid acaricides to an animal by means of spraying has been successfully practised for controlling ticks on animals (Barnett, 1961). Spraying equipment is highly portable, and only small amounts of acaricides need to be mixed for a single application. However, spraying is generally less efficient in controlling ticks than immersion in dip-tanks because of problems associated with applying the acaricides thoroughly on all parts of the animal body (Drummond, 1983).

### **2.5.3 Spot treatment or hand dressing**

There are predilection sites for certain tick species on part of the body which are not effectively treated by spray or dips. The inner parts of the ear, under part of the tail, the tail brush and the areas between the teats and the legs in cattle with large udder, are especially liable to escape treatment. Acaricides may be applied to these sites by hand

dressing (Barnett, 1961) or spot treatment. The application of insecticides with aerosols and in oils, smears, and dusts by hand to limited body areas is time-consuming and laborious, but in certain instances it may be more effective and economical (in terms of cost the of acaricides) than treating the entire animal (Drummond, 1983).

#### **2.5.4 Other applications**

Ear tags, neck bands, tail bands and pour-ons, particularly for the pyrethroids with long residual activity are other applications of acaricide. Mechanical applicators have been also developed (Duncan, 1991). In Kenya, an intra-ruminal ivermectin slow-release device provided 90 days protection against tick damage (Tatchell, 1992). Tick repellents used on livestock are limited (Mwase *et al.*, 1990). Ivermectin has been delivered orally in the case of *B. annulatus* on cattle as a single or double ruminal bolus and daily capsules to *B. microplus* infested cattle (Millar *et al.*, 2001). The control levels against standard engorging female ticks reached 99%. Despite this, the level of control of ticks on pastures grazed by treated cattle was insufficient to prevent cattle from becoming infested. Abdel-Shafy and Zayed (2002) examined the acaricide properties of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature and adult stages of *Hyalomma anatolicum excavatum*. Azadirachtin was applied at concentrations of 1.6% to 12.8% in water and applied to ticks in vitro for one minute. The ticks were examined up to 15 days post treatment for mortality and reduced viability. Abdel-Shafy and Zayed (2002) concluded that Neem can be used for tick control at economic concentrations of 1.6% to 3.2%. This study needs to be enlarged to test control of feeding ticks on cattle and possibly control of ticks by spraying the moulting and resting site of ticks in cattle pens.

#### **2.6 Safety measures**

Appropriate directions and precautions given on the label, should be adhered to while applying the acaricides. According to Drummond, (1983), these acaricides can be toxic to livestock and humans, creating residues in tissues of animals, and may be destructive to the environment if they are not used and handled in a safe and correct manner. The safe use of acaricides is essential to an efficient, well-run program for control of ticks. To

avoid accidents and misuse, it is necessary to continually review and employ safe use, precautions and procedures (Drummond, 1983). For safety reasons, it is important to prepare dipping and spray fluids correctly to ensure that a dose of the active ingredient, lethal for the tick, is administered to cattle safely and without exceeding the limits for milk and meat residues. Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle has been studied and found to lead to unexpected residues in meat and dairy products and as an environmental contaminant via cattle dung (Laffont *et al.*, 2001). Therefore, the route of potential contamination of parasiticides should be taken into account during product registration.

## **2.7 Acaricide resistance by ticks**

Acaricides resistance by ticks is an increasing problem and a real economic threat to livestock development and the allied industries. Unfortunately, the successive evolution of acaricides resistance to ticks in each chemical group with the concomitant reduction in the usefulness of a group of acaricides is a major reason for the diversity of acaricides. Whether a producer chooses a traditional method for treating cattle with an acaricide or uses a new method, he must recognize the benefits, limitations and potential problems with each application method and product. Simulation models and research were the basis of recommendations for tick control strategies advocating approaches that reduced reliance on acaricides. These recommendations for controlling ticks on cattle are in harmony with recommendations for reducing the rate of selection for acaricide resistance. There is a need to transfer knowledge about tick control and acaricide resistance mitigation strategies to cattle producers (George *et al.*, 2004).

Most stock owners depend completely on acaricides to control ticks, but do not have access to guidelines on how to make a profit from their tick control program or how to detect and resolve problems with resistance to acaricides (George, 2000). Resistance has led to instability and increased costs in areas where the one-host cattle ticks *B. microplus* and *B. decoloratus* have acquired resistance to a variety of toxic chemicals. The point has now been reached where such resistance must be expected in these ticks within five to 10 years of the introduction of any new type of acaricide, unless control practices are changed (Wharton, 1983). The evolution of tick resistance to acaricides has been a major

determinant of the need for new products (George, 2000). Recently, Fernandes (2001) showed that *B. microplus* ticks from Brazil were resistant to deltamethrin and cypermethrin, and that high levels of mortality were only possible at high concentrations of permethrin. Considering the frequency with which synthetic pyrethroid resistance has been reported in Brazil, and elsewhere, the claim of resistance is very likely to be correct. Sseruga *et al.* (2003) studied the serological evidence of exposure to tick fever organisms in young cattle on Queensland dairy farms. They concluded that, in tick-infested areas, vaccination is the most effective means of protecting cattle against tick fever. Resistance is usually recognized because of failure to obtain a satisfactory kill of the parasitic stages on treated animals. There is no doubt about resistance when cattle continue to be infested with large numbers of engorged ticks after frequent treatments. A survey of cattle tick control practices in S. Africa found that 35.7% of farmers using hand sprays have confirmed acaricide resistance compared with 25.8% and 23.9% of users of spray races and plunge-dips respectively (Spickett and Fivaz, 1992). Confirmation of resistance must be made by laboratory tests. The traditional test for diagnosing resistance to acaricides in single host ticks is the larval packet test, in which larvae are placed in envelopes impregnated with acaricide (Wharton and Roulston, 1970). Resistance in *B. microplus* populations in Australia and S. Africa to arsenic was observed about 50 years after use of the chemical began (Matthewson and Baker, 1975; Angus, 1996).

## **2.8 Immunological control through vaccines**

Several approaches have been used to actively immunize bovines against the cattle tick. The first attempts included the use of complex tick extracts (Willadsen *et al.*, 1988). For both ticks and tick-borne diseases, vaccines have been developed. Vaccine development and production has so far focused on the economically important tick-borne diseases of cattle, such as babesiosis (*B. bovis*, *B. bigemina*), theileriosis (*T. parva*, *T. annulata*), anaplasmosis (*A. marginale*) and cowdriosis (*C. ruminantium*). The Food and Organisation has been implementing a coordinated multi-donor programme for integrated tick and tick-borne disease control in eastern, central and southern Africa. Vaccine development, production and delivery were the main focus of that programme (FAO, 1998). This programme led to the development of the three strains *T. parva* stabilate

vaccine, known as the Muguga cocktail (FAO, 1998). This vaccine has been used in combination with antibiotic treatment, known as the infection-and-treatment method (ITM). Later the mild Boleni strain was isolated for vaccine production in Zimbabwe and is currently being used without the treatment component (FAO, 1998). Existing vaccines have a high level of side effects, low coverage and expensive to produce (CRC-VT, 2001).

Lack of infrastructure for vaccine production and distribution, as well as concerns about the introduction of vaccine parasite strains into local cattle and tick populations have curtailed the use of these vaccines. More recently, research has focused on the development of subunit vaccines. Studies of immune responses to different stages of the parasites have yielded immunological probes that have been used to identify candidate vaccine antigens. Immunisation of cattle with antigens expressed in the sporozoite, schizont or merozoite stages has resulted in varying degrees of protection against challenge (Morrison and McKeever, 2006). Although the levels of protection achieved have not been sufficient to allow exploitation for vaccination, there are clearly further lines of investigation, relating to both the choice of antigens and the antigen delivery systems employed, that need to be pursued to fully explore the potential of the candidate vaccines. Improved knowledge of molecular biology and immunology of the parasites gained during the course of these studies has also opened up opportunities to refine and improve the quality of live vaccines (Morrison and McKeever, 2006).

Despite the molecular revolution in the development of vaccines against ecto-parasites, Willadsen (2001) acknowledged that there is still a long way to go before the full potential of anti-tick vaccines can be achieved and very likely need for a multiple antigen formulation. Nevertheless the pioneering work on the Bm86 based vaccines is one of the best examples of a commercial recombinant vaccine for tick and tick-borne pathogens (Willadsen 2001). The recombinant Bm86-containing vaccine against the cattle tick *B. microplus* has proved its efficacy in a number of experiments, especially when combined with acaricides in an integrated manner (García-García *et al.*, 2000).

Tellam *et al.* (2002) studied the reduced oviposition of *B. microplus* feeding on sheep vaccinated using both vitelline and the protein GP80 with which it has immunological

cross reactivity. Two vaccines against the tick *B. microplus* have been developed. They are being field tested in Brazil in collaboration with FAO, but their efficacy is not 100%, therefore there is need to have an improved and absolute vaccine that can control ticks effectively (FAO, 1998).

Jenkins (2001) has studied subunit vaccines against babesiosis and theileriosis. Because of the risk associated with using live parasites that include (the need for a cold chain, limited shelf-life, clinical reactions and reversion of attenuation), research towards subunit vaccines is rapidly advancing. However their development is complicated by several factors, such as antigenic variation and strain diversity. The generation of a protective immune response furthermore depends on other factors, for instance vaccine delivery, adjuvant used, age of the animal, etc.

The identification of protective tick antigens remains a major limitation in the development of further anti-tick vaccines as well as a significant scientific challenge. García-García *et al.* (2000) reported the isolation of the Bm95 gene from the *B. microplus* strain A, and found that Bm95 antigen from strain A was able to protect against infestations with Bm86-sensitive and Bm86-resistant tick strains. García-García *et al.* (2000) suggested that Bm95 could be a more universal antigen to protect cattle against infestations by *B. microplus* strains from different geographical areas. Almazán *et al.* (2003) conducted research on identification of protective antigens for the control of *Ixodes scapularis* infestation using cDNA expression library immunization. Saiki *et al.* (2006), have succeeded in preliminary characterisation of two *R. appendiculatus* serine protease inhibitors (RAS-1 and 2) as anti-tick vaccine cocktail candidates in cattle. Vaccination of cattle with a combination of rRAS-1 and -2 conferred significant protective immunity against ticks, resulting in 61.4% reduction in nymph engorgement rate, and in 28% and 43% increased mortality rate in adult female and male ticks, respectively.

Vaccines using live parasites have been available for over two decades, although they have not been used on a large scale. The use of live, blood derived vaccine entails some problems, including: the possible spread of silent pathogens such as bovine leukaemia virus; difficulties in standardisation of the vaccine dose; the risk of reversion to virulence

and maintenance of carrier animals which may serve as reservoirs for tick transmission and the quality control of the production, maintenance and transportation to the end user, including the necessity of cold chain (Morrison and McKeever, 2006; Varda *et al.*, 2007).

In general, attempts to develop vaccines for pathogens transmitted by arthropods have met with little or no success. Titus and co-workers (2006) observed that the saliva of arthropods that transmit diseases enhance the infectivity of the pathogens the arthropod transmits to the vertebrate hosts. Vaccinating against components of the saliva of arthropods or against antigens expressed in the gut of arthropods can protect the host from infection and decrease the viability of the arthropod (Titus *et al.*, 2006).

These results suggest that multi-subunit vaccines that target the pathogen itself as well as arthropod salivary gland components and arthropod gut antigens may be the most effective for controlling arthropod-borne pathogens as these vaccines would target several facets of the lifecycle of the pathogen. These known immunomodulators in arthropod salivary glands, have been shown to enhance infection and a limited number of examples of antiarthropod vaccines, with emphasis on three arthropods: sand flies, mosquitoes and hard ticks (Titus *et al.*, 2006).

## **2.9. Host resistance to tick infestations**

Resistance to tick infestation varies among individuals and breeds of cattle. In many subtropical and semi-arid environments in Africa, the indigenous dual purpose breeds are highly resistant to ticks, resulting in low infestation rates that cause insignificant direct losses (Paling *et al.*, 1991; Norval *et al.*, 1992; Lawrence *et al.*, 1996, Mattioli *et al.*, 1995). The phenomena of host resistance to ticks and enzootic stability to tick borne diseases are well documented (Latif and Pegram, 1992; Perry *et al.*, 1995). Generally, different indigenous breeds of cattle have certain degrees of genetic resistance to both T&TBDs. Rearing of tick resistant breeds of cattle has been encouraged as a control measure for T&TBDs such as the Ethiopian boran, Kenyan boran (de Castro 1991), Zimbabwe Nkoni (Sanga) Norval *et al.*, 1988), the Bonsmara in S. Africa (Scholtz *et al.*, 1991). The improved tick control following the use of tick resistant cattle has been demonstrated in various breeds of cattle and crossbreeds (Rick, 1962). Crossbreeds between

zebu and taurine cattle has been shown to carry fewer ticks and requires less dipping than the temperate breeds of cattle on similar pastures (Wharton *et al.*, 1969). The criteria for assessment of and quantification of resistance to ticks in cattle include; - resistant animals consistently carrying fewer ticks, the female ticks completing engorgement are fewer and smaller on the resistant animals than the susceptible ones (Willadsen, 1980; Tatchell, 1966; Latif and Pegram, 1992)

## CHAPTER THREE

### 3.0

### GENERAL MATERIALS AND METHODS

#### 3.1 The study area

This study was conducted in Pingire and Bugondo subcounties of Soroti district (Fig.3.4) located in central eastern Uganda. The district lies over 2,500 feet above sea level and covers an area of 2,662.5 sq km of which 2,256.5 sq km is land and 406 sq km is water and swamps (Ford and Katondo 1976). Soroti district borders the districts of Kamuli, Kumi, Pallisa and Lake Kyoga in the south, and the districts of Kaberamaido in the west and Katakwi in the northeast. The district has mainly savannah grassland; it receives an annual rainfall of between 1200-1500mm. There are two rainfall seasons stretching from March to May and the second short rainfall season, September to November. There are two dry seasons;- December to February and June to August. The daily minimum and maximum mean temperatures are 15°C and 27°C, respectively.

In Soroti district, an agro-pastoral farming system is practiced, which is important for livestock production that is among the main source of milk and meat consumed in Uganda. In addition to cattle, farmers keep goats and sheep under tethering, open grazing or communal grazing (Ocaido *et al.*, 2005). *Rhipicephalus appendiculatus*, *R. evertsi evertsi*, *Boophilus decoloratus* and *Amblyomma variegatum* are the major tick species of economic importance in the study area (Okello-Onen *et al.*, 1999). These tick species are capable of transmitting *Theileria parva*, *Anaplasma marginale*, *Babesia bigemina* and *Cowdria ruminantium*, respectively.

Both the Nkedi Zebu and Ankole cattle are kept together in same herds in the study areas with the presence of ticks, tick-borne diseases including ECF, babesiosis and anaplasmosis (Ocaido *et al.*, 2005). The Nkedi Zebu cattle are dual purposes breeds used for draught power, meat and milk. They can be grey, grey pied, black, black pied or light red, and they have a mean adult weight and a height of 285 kg and 104 cm respectively. A fully grown cow at a height of 101cm and 285 kg on average is substantially smaller than the mature bull (104cm and 360kg) (Felius, 1985; Magona *et al.*, 2004). The pure Ankole cow or bull has a head of medium length with characteristically long horns with a

big base, a short neck with a deep dewlap, a narrow chest with a small and cervico-thoracic hump which is barely visible on the animal, long legs (a cow stands at 130cm and bull 145 cm on average), a skinny body and a weak skeleton (Felius, 1985; Magona *et al.*, 2004). Petersen and co-workers (2004) noted that an adult cow weighs between 292 – 341 kg and has an average lactation of 220 -230 days.



**Figure 3.1** An Ankole cow and a heifer calf



**Figure 3.2** The Nkedi Zebu cattle



**Figure 3.3** A mixed herd of Nkedi Zebu and Ankole cattle

### **3.1.1 Selection of the study sites**

Selection of the study sites (sub counties and villages) and animals was done using multi-stage sampling. Kasilo county in Soroti district was purposively selected out of the four counties (Serere, Kasilo, Soroti and the municipality) because of the suitable cattle population (Table 3.1), compared to the other counties. Two subcounties with the highest cattle population were also purposively selected. Pingire with a cattle population of 13,829 and Bugondo with a cattle population of 4,900.

Three villages were then randomly selected from each subcounty and these included; Akumoi, Aswii and Osamito in Pingire sub county, and Okokoma, Kamod, and Opungure in Bugondo.

Subsequently, all herds in each selected village were identified to determine a sampling frame and herd sizes. Selection of the test cattle from a randomly selected herds was based on proportional allocation of the herd sizes with the help of the local leaders who were well known to the cattle farmers. The Nkedi Zebu and Ankole cattle were randomly selected as previously described by Feliuss, 1985 and Magona *et al.*, 2004). Randomly selected herds were assembled at designated village screening sites and were examined. For each test cattle, the breed, age, and sex were recorded.

**Table 3.1 Soroti district cattle population census, 2006**

| <b>COUNTY</b>       | <b>SUB-COUNTY</b> | <b>CATTLE POPOULATION</b> |
|---------------------|-------------------|---------------------------|
| Serere              | Atiira            | 3,980                     |
|                     | Kateta            | 11,698                    |
|                     | Kyere             | 9,370                     |
|                     | Olio              | 3,476                     |
|                     | <b>Subtotal</b>   | <b>28,524</b>             |
| Kasilo              | Pingire           | 13,829                    |
|                     | Kadungulu         | 4,890                     |
|                     | Bugondo           | 4,900                     |
|                     | <b>Subtotal</b>   | <b>23,619</b>             |
| Soroti              | Arapai            | 6,259                     |
|                     | Gweri             | 9,921                     |
|                     | Kamuda            | 3,724                     |
|                     | Tubur             | 2,679                     |
|                     | Katine            | 4,132                     |
|                     | <b>Subtotal</b>   | <b>26,715</b>             |
| Soroti Municipality | Eastern Division  | 524                       |
|                     | Northern division | 584                       |
|                     | Western division  | 325                       |
|                     | <b>Subtotal</b>   | <b>1,433</b>              |
| <b>TOTAL</b>        |                   | <b>80,291</b>             |

**Source: Soroti District Veterinary Officer's annual report, 2006**

### 3.1.2 Sample size determination

Using the Fanta sampling guide for calculating the sample sizes in observational studies or field trials involving two treatments as shown below (FANTA sampling guide, 1997).

$$n = D [(Z\alpha + Z\beta)^2 \times (P_1 (1 - P_1) + P_2 (1 - P_2)) / (P_2 - P_1)^2]$$

n = required minimum sample size per comparison group

D = design effect (assumed to be a default value of 2)

$P_1$  = the estimated level of a parameter (such as disease prevalence) measured as a proportion at the time of the study

$P_2$  = the expected level of the parameter (such as disease prevalence) for the project area such that the quantity ( $P_2 - P_1$ ) is the size of the magnitude of the difference it is desired to be able to detect

$Z\alpha$  = the Z-score corresponding to the degree of confidence with which it is desired to be able to conclude that an observed difference of size ( $P_2 - P_1$ ) would not have occurred by chance ( $\alpha$  - the level of statistical significance), and

$Z\beta$  = the z-score corresponding to the degree of confidence with which it is desired to be certain of detecting a difference of size ( $P_2 - P_1$ ) if one actually occurred ( $\beta$  - statistical power)

D = 2 (default value)

Using the assumption that there could be a difference of 25% between the Nkedi Zebu and the Ankole cattle in response to the natural tickborne infections.

Then  $P_1 = 0.5$  (50% prevalence of tickborne infection, *a priori*) and  $P_2 = 0.75$  (75%)

To be able to detect the assumed 25% difference if it actually exists then a power of 90% is chosen, along with the standard of 95% level of significance.

Accordingly,  $Z\alpha = 1.96$  and  $Z\beta = 1.28$

$n = 2[(1.96 + 1.28)^2 \times ((0.5)(0.5) + (0.75)(0.25))] / (0.75 - 0.5)^2 = 2[(8.567 \times 0.44) / 0.25^2]$   
 $= 2[(10.49 \times 0.44) / 0.0625] = 2(73.9) = 148$  head of cattle as the sample size for each breed of cattle to be studied. This was extrapolated to 151 Ankole and 380 for Nkedi Zebu cattle

A total of 531 cattle were randomly sampled and distributed as shown in Table 3.2 from the six villages, comprising of 381 Nkedi Zebu and 150 Ankole cattle .

**Table 3.2      Distribution of cattle sampled in the different villages**

| Subcounty    | Village  | Number of cattle sampled |            | Total      |
|--------------|----------|--------------------------|------------|------------|
|              |          | Ankole                   | Nkedi Zebu |            |
| Pingire      | Akumoi   | 31                       | 69         | 100        |
|              | Aswii    | 31                       | 21         | 52         |
|              | Kamod    | 14                       | 86         | 100        |
| Bugondo      | Osamito  | 39                       | 61         | 100        |
|              | Okokoma  | 18                       | 82         | 100        |
|              | Opungure | 15                       | 64         | 79         |
| <b>Total</b> |          | <b>150</b>               | <b>381</b> | <b>531</b> |

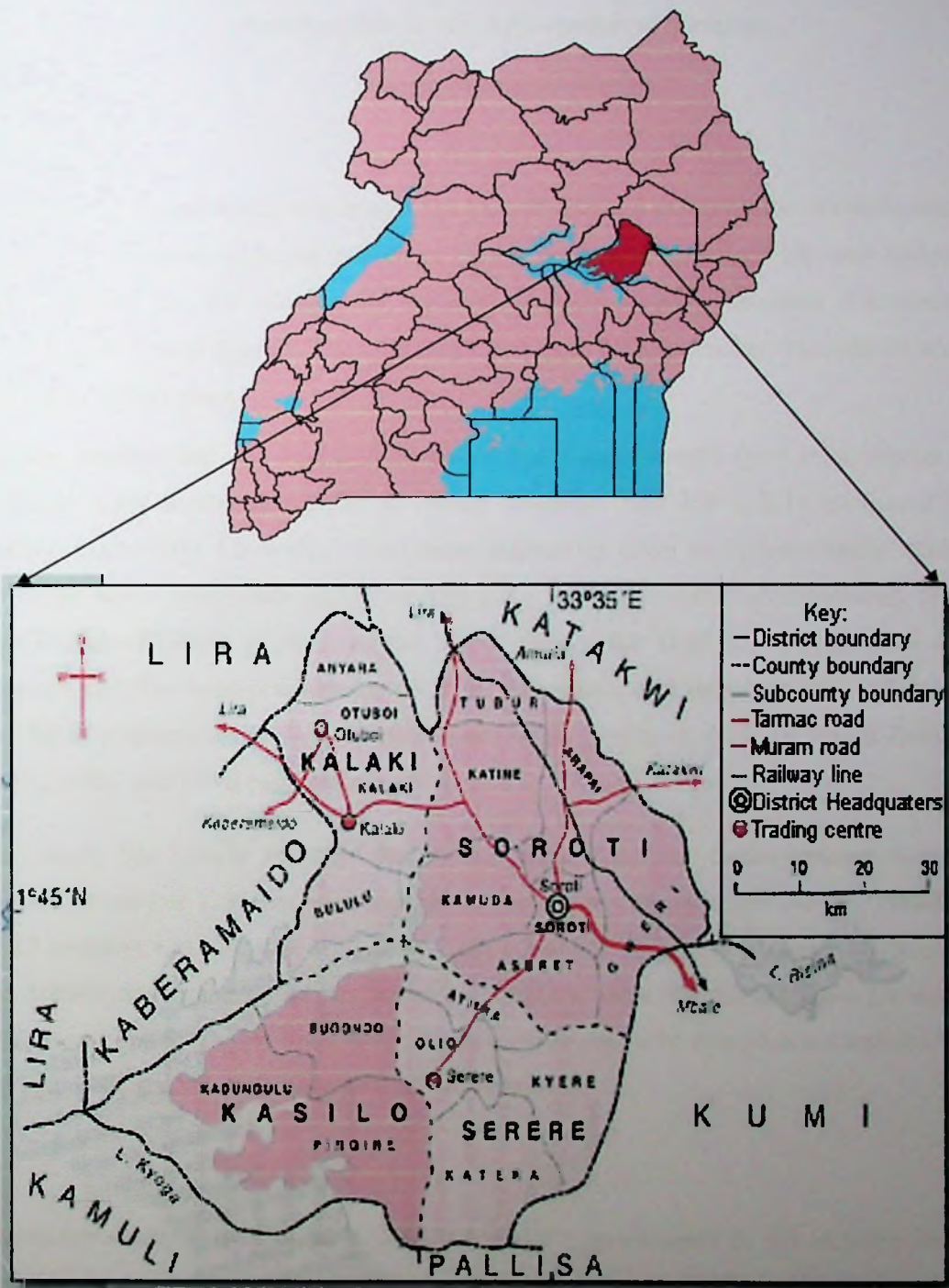
### 3.2 Collection of blood samples

About 10 ml of jugular blood was collected from the randomly selected well restrained animals and divided equally into well labelled heparinised and non heparinised vacutainer tubes (Becton-Dickinson, vacutainer system, UK). The heparinised blood was used to determine the packed cell volume (PCV) as explained in chapter 5 (5.2.1). The non heparinised blood was used to prepare serum samples. Blood samples were collected from 150 Ankole and 381 Nkedi Zebu cattle.

### 3.3 Data analysis

Data collected included antibody titres (optic density), sero-positivity status, tick counts and PCV. The data was entered in Microsoft Excel® USA, (2003) and later analysed for significant differences in the means of tick counts, PCV, and antibody titres of the respective tick borne infections using the SPSS version 12 statistical package. The means were compared using the Independent Student's t-test to obtain any significant differences at the level of  $P=0.05$  between the Nkedi Zebu and Ankole cattle, according to age group, sex and village of origin.

Figure 3.4. Map of Uganda and Soroti district, counties, sub-counties and the neighbouring districts (2006)



## CHAPTER FOUR

### 4.0 The Sero-prevalences of tickborne infections among the Nkedi Zebu and Ankole cattle in the Agro-pastoral farming System of Soroti district

#### Abstract

This cross-sectional study was conducted to establish and compare the antibody titres and sero-prevalences of tickborne infections among the Nkedi Zebu ( $n = 103$ ) and Ankole ( $n = 81$ ) cattle in the six villages of Akumoi, Aswii, Kamod, Osamito, Okokoma and Opungure in Soroti district. The antibody titres were assessed using the indirect antibody detection ELISA tests.

Results showed that, the Nkedi Zebu cattle had a significantly ( $p = 0.04$ ) higher mean antibody titres produced against *T. parva* infection ( $OD\ 1.8 \pm 0.1$ ) compared to the Ankole cattle ( $OD\ 1.2 \pm 0.1$ ). The mean antibodies titres of Ankole cattle against *B. bigemina* and *A. marginale* infections ( $OD\ 2.2 \pm 0.04$ ,  $0.05 \pm 0.05$ ), respectively were not significantly different to those of the Nkedi Zebu cattle ( $OD\ 2.1 \pm 0.05$ ,  $0.06 \pm 0.05$ ) respectively. The sero-prevalences of ECF, babesiosis and anaplasmosis (100%, 100% and 58%) respectively in Ankole cattle were quite similar to those of Nkedi Zebu cattle (99%, 100% and 57%) respectively.

This study has clearly revealed that Nkedi Zebu could be better primed to produce antibodies against *T. parva* as compared to the Ankole cattle. Likewise, the Nkedi Zebu (7-12 months) had a significantly lower tick counts of *R. appendiculatus* as compared to the Ankole cattle as reported in chapter 5, therefore, extra control strategies for tickborne infections need to be instituted if the Ankole cattle are to be reared more successfully in the agro-pastoral farming system of Soroti district.

#### 4.1 Introduction

Tickborne diseases constitutes one of the major impediments to the development and improvement of the livestock industry in eleven countries in eastern, central, and southern Africa by causing significant mortality and production losses (Mukhebi *et al.*,

1992) in susceptible exotic and cross-bred cattle. Substantial losses have also been reported among the indigenous Zebu and Sanga cattle which have been raised in *theileria* free areas, in which the disease was introduced or when they are moved to endemic areas (Henning, 1932; Stobbs, 1966, Yeoman, 1966). The national cattle herd is at constant risk of tickborne diseases and the overall loss of the calf crop due to these diseases is estimated to be 30% over the greater part of Uganda (Magona, 2004).

Zebu cattle residing for many generations in endemic areas become infected (100% morbidity) with ECF, but only a minor proportion succumb, many become carriers, and early infection with *T. parva* can affect their growth and productivity (Moll *et al.*, 1986). Of the deaths attributable to tick borne diseases, ECF is responsible for 79%, anaplasmosis 11%, and babesiosis 4.4% (Magona, 2004) in Uganda. Breed related susceptibility (resistance) to tickborne disease does exist and has been observed in several tropical areas. Studies done by Jacobsen (1983) in Zimbabwe found higher ECF induced mortality rate of 14% in crossbred calves than in the zebu calves (4.2%) and had a lower recovery rate (57%) in crossbred calves than in zebu calves (90%) in the Zanzibar. In another study in Rwanda, the mortality following experimental infection with *T. parva* was found to be higher in the crossbreds (64%), than Ankole cattle (32%) (Paling *et al.*, 1991). This difference in mortality was attributed to the genetic ability of the Ankole cattle to limit the explosive multiplication of *T. parva* microschizont (Paling *et al.*, 1991). Breed susceptibility to bovine anaplasmosis has also been observed, with exotic breeds being more susceptible than the indigenous cattle breeds (Ajayi *et al.*, 1982). Elsewhere, a sero-prevalence of bovine anaplasmosis ranging from 81% to 94% was reported across different agro ecological zones in Kenya (Deem *et al.*, 1993) and from 14.7% to 38% in Zambia (Jongejan *et al.*, 1988).

The impetus to carry out this study therefore came as a result of lack of adequate knowledge of the differences in responses to natural tickborne infections among the common indigenous breeds of Uganda and yet tickborne infections were ranked high in the disease diagnostic survey of NARO/LRSP report, (1999). The knowledge obtained from this study is expected to guide several stakeholders in the livestock industry in designing better restocking programmes and control strategies for tickborne infections.

## **4.2 Materials and Methods**

### **4.2.1 Preparation of serum samples**

The non heparinised blood was allowed to clot for about 30 minutes, the sera was obtained using a pipette and then transferred into well labeled serum tubes. The sera was kept in a cool box under ice packs and later transported to NaLIRRI, Tororo for refrigeration at less than 4°C in order to avoid spoilage by bacteria.

### **4.2.2 Serological analysis**

Randomly selected 184 (103 for Nkedi Zebu and 81 Ankole) serum samples were analysed for antibodies against *T. parva*, *A. marginale* and *B. bigemina* infections using the indirect antibody detection ELISA kits (Svanova®, Biotech AB Uppsala, Sweden). Only a total of 184 serum samples were used because only two micro-titre plates were available for each respective tick-borne micro-organism examined. The sera was screened for antibodies against the respective tick borne micro-organism using the antibody ELISA kit utilizing *T. parva* schizont antigen (polymorphic immunodominant molecule), a 19kD utilizing *A. marginale* recombinant antigen and a 200kD utilizing *B. bigemina* antigen (Katende *et al.*, 1998; Morzaria *et al.*, 1999; Tebele *et al.*, 2000).

### **4.2.3 Enzyme linked immunosorbent assay (ELISA)**

#### **4.2.3.1 Theileriosis**

The *T. parva*-Ab ELISA Kit (Svanova®, Biotech AB Uppsala, Sweden), was designed to detect specific antibodies against *T. parva* in bovine serum samples. This kit procedure is based on the Indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA). Briefly, in this procedure, serum samples were added to wells in microtitre plates coated with the recombinant antigen. If antibodies directed against *T. parva* are present in the test sample, these antibodies will bind to the antigen in the wells forming an antigen-antibody-complex.  $\alpha$ -Bovine IgG-Horseradish peroxidase (HRP) conjugate added subsequently forms a complex with the bovine anti-*T. parva* antibodies. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of a blue colour. The reaction is stopped by addition of the stop solution. The results are read by a microplate photometer reader, where the optical density (OD) is measured at 405 nm.

Materials required: Precision pipettes (range from 5 to 500  $\mu$ l), Disposable pipette tips, Distilled water, Wash bottle, Container of 1 to 2 litres for PBS-Tween, Microplate photometer of 405 nm filter, *T. parva* antigen coated microtitre plates, (sealed and stored dry), PBS-Tween Solution 20 x concentrate, Substrate Solution, Stop Solution – Contains 1% SDS, Lyophilised HRP conjugate (horseradish peroxidase mouse conjugated anti-bovine IgG, (monoclonal antibodies) A. Positive Control Serum – 0.05% merthiolate, B. Negative Control Serum – 0.05% merthiolate and 5  $\mu$ l of blood serum for each sample well.

The required reagents were prepared according the manufacturer's instructions thus: (a) PBS-Tween Buffer: PBS-Tween Solution (present in the package of the kit) 20 x concentrate 1/20 was diluted in distilled water. 500 ml was prepared per plate by adding 25 ml PBST solution to 475 ml distilled water and mixed thoroughly. Caution was taken to avoid any crystal precipitation in the bottle.

(b) Anti-Bovine IgG Conjugate: the lyophilized HRP Conjugate (present in the package of the kit) was reconstituted with 11.5 ml PBS-Tween Buffer and the buffer added carefully to the bottle. This solution was left to stand for one minute and mixed thoroughly. The remaining reconstituted conjugate was stored at -20°C and thawed and refrozen 3 times.

(c) Pre-dilution of controls and samples: For testing, the serum controls (present in the package of the kit) and serum samples were pre-diluted 1/100 in PBS-Tween Buffer (i.e. 5  $\mu$ l sample into 495  $\mu$ l of PBS-Tween buffer).

Procedure followed:

- (i) All reagents were equilibrated to room temperature 18 to 25°C (64 to 77°F) before use. Each microtitre plate was labelled with a number for correct identification.
- (ii) 100  $\mu$ l of pre-diluted Positive Control Serum (Reagent A) and Negative Control Serum (Reagent B) were added in duplicates into selected wells, respectively, as shown in Figure 4.5 and 4.6.
- (iii) 100  $\mu$ l of pre-diluted test serum sample was added to selected wells.
- (iv) The plates were sealed and incubated at 37°C (98.6°F) for 30 min.

- (v) The plates were rinsed 4 times with PBS- Tween Buffer: at each rinse cycle the wells were filled up then emptied and tap hard to remove all the remaining fluid.
- (vi) 100 µl of HRP Conjugate dilution was added to each well and the plates sealed and incubated for 37°C for 30 minutes.
- (vii) The plates were rinsed 4 times manually with PBS- Tween Buffer: at each rinse cycle the wells were filled up then emptied and tapped hard to remove all the remaining fluid.
- (viii) 100 µl Substrate Solution was added to each well incubated for 30 minutes at room temperature (18 to 25°C). Timing was started when the first well was filled.
- (ix) The reaction was stopped by adding 100 µl of Stop Solution to each well and mixed thoroughly. The stop solution was added in the same order as the Substrate Solution in step (viii).
- (x) The optical density (OD) of the controls and samples was measured at 405 nm in a microplate photometer ELISA reader (Labsystems Multiscan MS) using air as a blank. The OD values were measured within 15 minutes after the addition of Stop Solution in order to prevent fluctuation in OD values.

The OD values were expressed as percentage positivity (PP), which was calculated as follows: (OD of the test/OD of the test positive) x 100 and the cut off point for *T. parva* assay was 20PP as determined by Wright and other workers (1993). This was used to determine the sero-prevalence.

#### **4.2.3.2 Babesiosis**

*B. bigemina*-Ab ELISA Kit (Svanova® Biotech AB, Uppsala, Sweden), is designed to detect specific antibodies against *B. bigemina* in bovine serum samples. The kit procedure is based on the Indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA). The procedure was similar to that described for *T. parva*.

**Materials required:** Precision pipettes (range from 5 to 500 µl), Disposable pipette tips, Distilled water, Wash bottle, Container 1 to 2 litres for PBS-Tween, 405 nm filter microplate photometer ELISA reader, *B. bigemina* coated microtitre plates, (sealed and stored dry), PBS-Tween Solution 20 x concentrate, Substrate Solution – (ABTS), Stop Solution – Contains 1% SDS, Lyophilised HRP conjugate (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies), (a) Positive Control Serum – 0.05% merthiolate, (b) Negative Control Serum – 0.05% merthiolate, and 5 µl of blood serum.

The required reagents were prepared according the manufacturer's instructions as already described for *T. parva* on page 39.

The OD values were expressed as percentage positivity (PP), which was calculated as follows: (OD of the test/OD of the test positive) x 100 The cut off point for *B. bigemina* assays was 15PP as previously determined by Wright *et al.*, 1993. This cut off points was used determine the sero-prevalence.

#### **4.2.3.3 Anaplasmosis**

Antibody detection using enzyme linked immunosorbent assay (ELISA) SVANOVIR® *A. marginale*-Ab ELISA kit (Svanova® Biotech AB, Uppsala, Sweden), is designed to detect *A. marginale* specific antibodies (IgG1) in bovine serum samples. The test is based on an assay developed by International Livestock Research Institute (ILRI) (Morzaria *et al.*, 1999). The principle is based on the indirect enzyme linked immunosorbent assay (indirect ELISA) where the samples are added to wells in the micro-pipettes plates coated recombinant antigen. The procedure was similar to that described for *T. parva* as described on page 39.

**Materials required:** *A. marginale* coated microtitre plates, (sealed and stored dry), Lyophilized bovine IgG1-HRP conjugated mouse Ab, Sample dilution buffer Substrate solution, Stop solution, Washing buffer (20 x concentrate PBS-Tween), Serum controls and test serum samples. Procedure followed was similar to the one used for *T. parva* above except for the type of antigen used and conjugate and the OD values were expressed as percentage positivity (PP), which was calculated as follows: (OD of the

test/OD of the test positive) x 100. The cut off point for *A. marginale* assays was 15PP as previously determined by Wright and co-workers in 1993. The cut off point was used to determine the sero-prevalence.

#### 4.3 Results

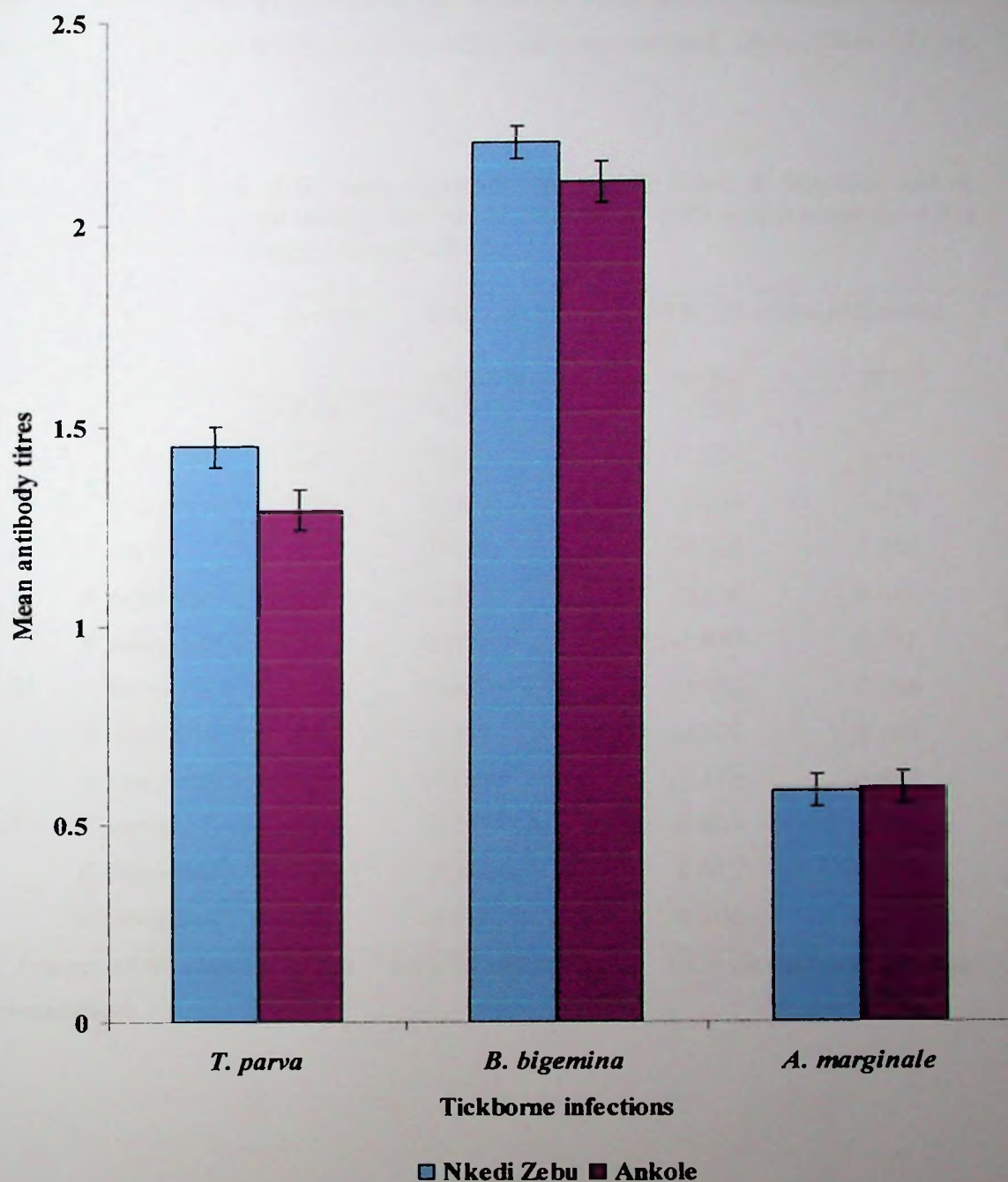
The mean antibody titres against tick borne infections (*T. parva*, *B. bigemina* and *A. marginale*) among the Nkedi Zebu (n = 103) and Ankole (n = 81) cattle were compared using the student's t test at 95% confidence interval and a significant difference was observed in the mean antibody titres against *T. parva* (p=0.04) as shown in Table 4.1.

**Table 4.1. Comparison of the mean antibody titres against *T. parva*, *B. bigemina* and *A. marginale* among the Nkedi Zebu (n=103) and Ankole (n=81) cattle, Kasilo county, Soroti district.**

| Tick-bone<br>Infection | Breed<br>of cattle | p-value | Mean | Std. error<br>of the mean | 95%CI<br>difference |
|------------------------|--------------------|---------|------|---------------------------|---------------------|
| <i>T. parva</i>        | Nkedi Zebu         | 0.04    | 1.46 | 0.05                      | 0.002 - 0.32        |
|                        | Ankole             |         | 1.29 | 0.05                      |                     |
| <i>B. bigemina</i>     | Nkedi Zebu         | 0.15    | 2.20 | 0.04                      | -0.04 - 0.23        |
|                        | Ankole             |         | 2.10 | 0.05                      |                     |
| <i>A. marginale</i>    | Nkedi Zebu         | 0.9     | 0.58 | 0.04                      | 0.14 - 0.22         |
|                        | Ankole             |         | 0.59 | 0.04                      |                     |

**p-value = level of significance, CI = confidence interval, Std. = standard**

The mean antibody titres for the Nkedi Zebu against *T. parva*, *B. bigemina* and *A. marginale* were  $1.46 \pm 0.056$ ,  $2.20 \pm 0.043$ ,  $0.58 \pm 0.048$  respectively compared to Ankole cattle that was *T. parva*, *B. bigemina* and *A. marginale*  $1.29 \pm 0.058$ ,  $2.1 \pm 0.053$ ,  $0.59 \pm 0.049$ . The Nkedi Zebu had a significantly (p=0.04) higher antibody titre to *T. parva* infection compared to the Ankole cattle (Table 4.1) and Fig 4.7. Otherwise there was no significant difference in mean antibody titres of the Nkedi Zebu against *B. bigemina* and *A. marginale* compared to that of the Ankole cattle as shown in Figure 4.9 and 4.10 respectively.



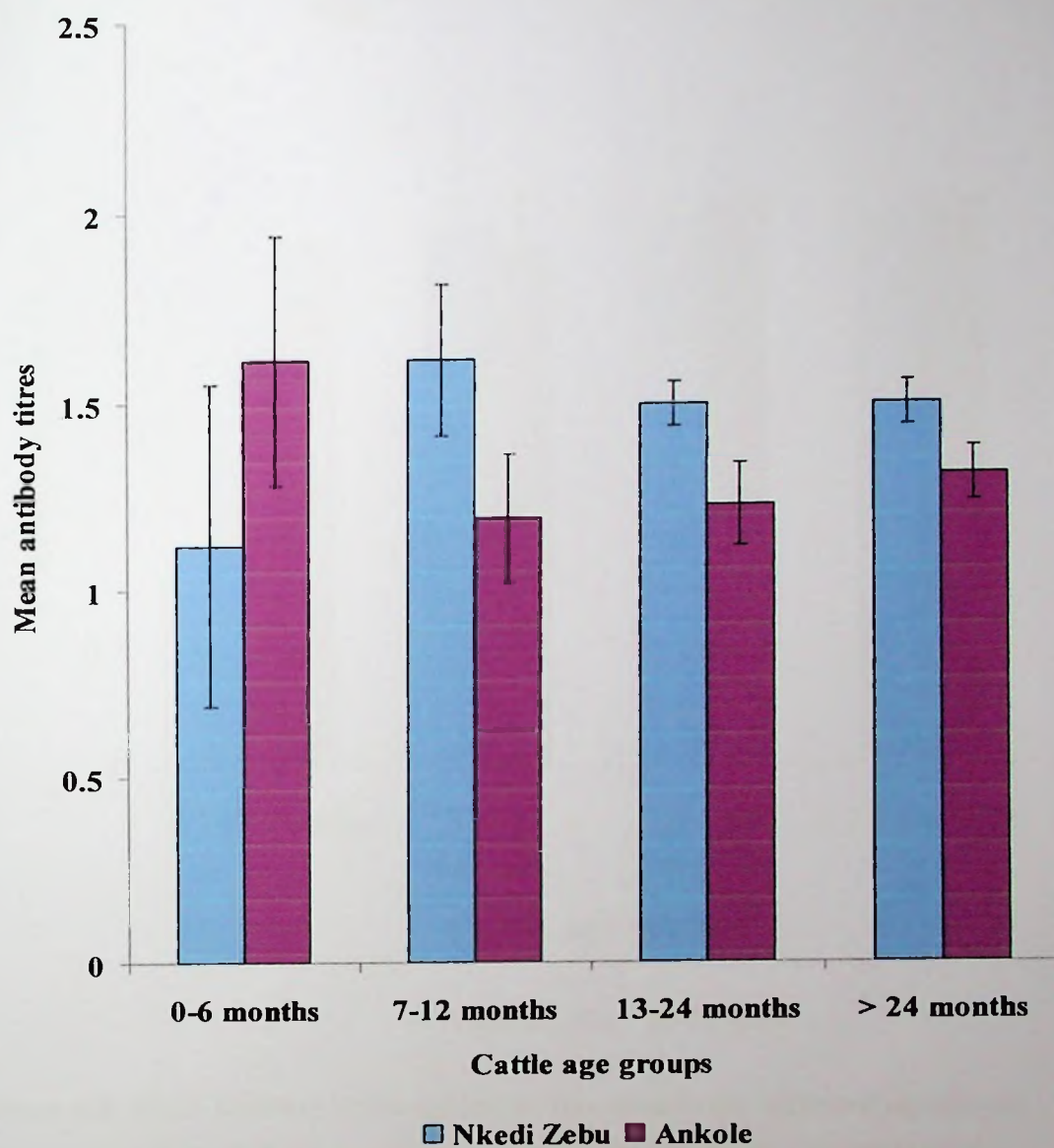
**Figure 4.1. Mean antibody titres against *T. parva*, *B. bigemina* and *A. marginale* of the Nkedi Zebu and Ankole cattle in Kasilo county, Soroti district.**

The mean antibody titres against tick borne infections (*T. parva*, *B. bigemina* and *A. marginale*) among the Nkedi Zebu (n = 103) and Ankole (n = 81) cattle were compared among the different age groups using the student's t-test at 95% confidence interval. There was no significant difference observed in the mean antibody titres (Table 4.2) and Fig. 4.8, 4.9 and 4.10).

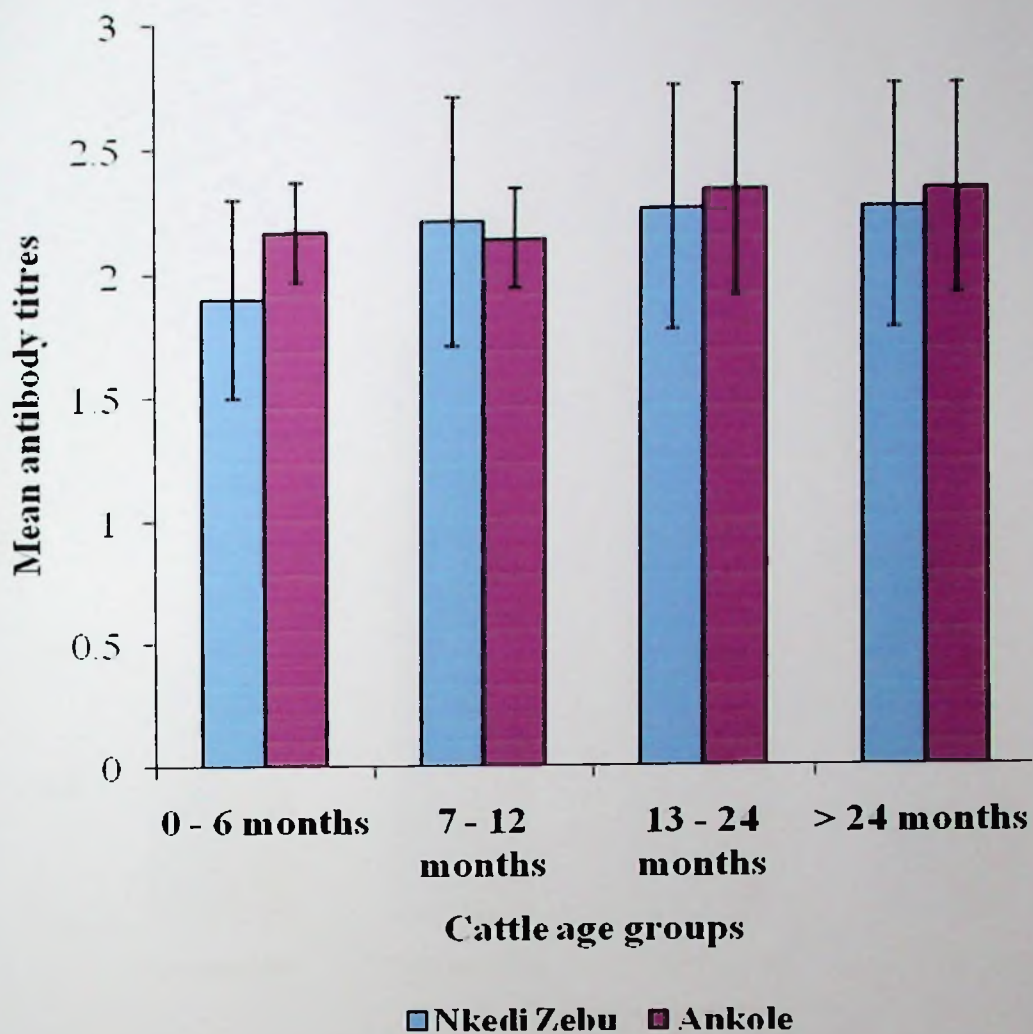
**Table 4. 2. Comparison of the mean antibody titres of *T. parva*, *B. bigemina* and *A. marginale* by age among the Nkedi Zebu (n = 103) and Ankole (n = 81) cattle, Kasilo county, Soroti district.**

| Age group | Tick-borne          | p-value | Std. error  | 95% CI of the difference |       |
|-----------|---------------------|---------|-------------|--------------------------|-------|
| (months)  | infection           |         | of the mean | lower                    | upper |
| 0 – 6     | <i>T. parva</i>     | 0.44    | 0.585       | -1.995                   | 1.014 |
|           | <i>B. bigemina</i>  | 0.37    | 0.271       | -0.960                   | 0.434 |
|           | <i>A. marginale</i> | 0.92    | 0.103       | -0.256                   | 0.276 |
| 7 – 12    | <i>T. parva</i>     | 0.17    | 0.288       | -0.221                   | 1.061 |
|           | <i>B. bigemina</i>  | 0.77    | 0.255       | -0.494                   | 0.641 |
|           | <i>A. marginale</i> | 0.67    | 0.406       | -1.079                   | 0.732 |
| 13 – 24   | <i>T. parva</i>     | 0.48    | 0.187       | -0.246                   | 0.514 |
|           | <i>B. bigemina</i>  | 0.61    | 0.128       | -0.325                   | 0.196 |
|           | <i>A. marginale</i> | 0.34    | 0.161       | -0.172                   | 0.482 |
| > 24      | <i>T. parva</i>     | 0.05    | 0.097       | -0.003                   | 0.381 |
|           | <i>B. bigemina</i>  | 0.07    | 0.085       | -0.015                   | 0.321 |
|           | <i>A. marginale</i> | 0.53    | 0.079       | -0.204                   | 0.107 |

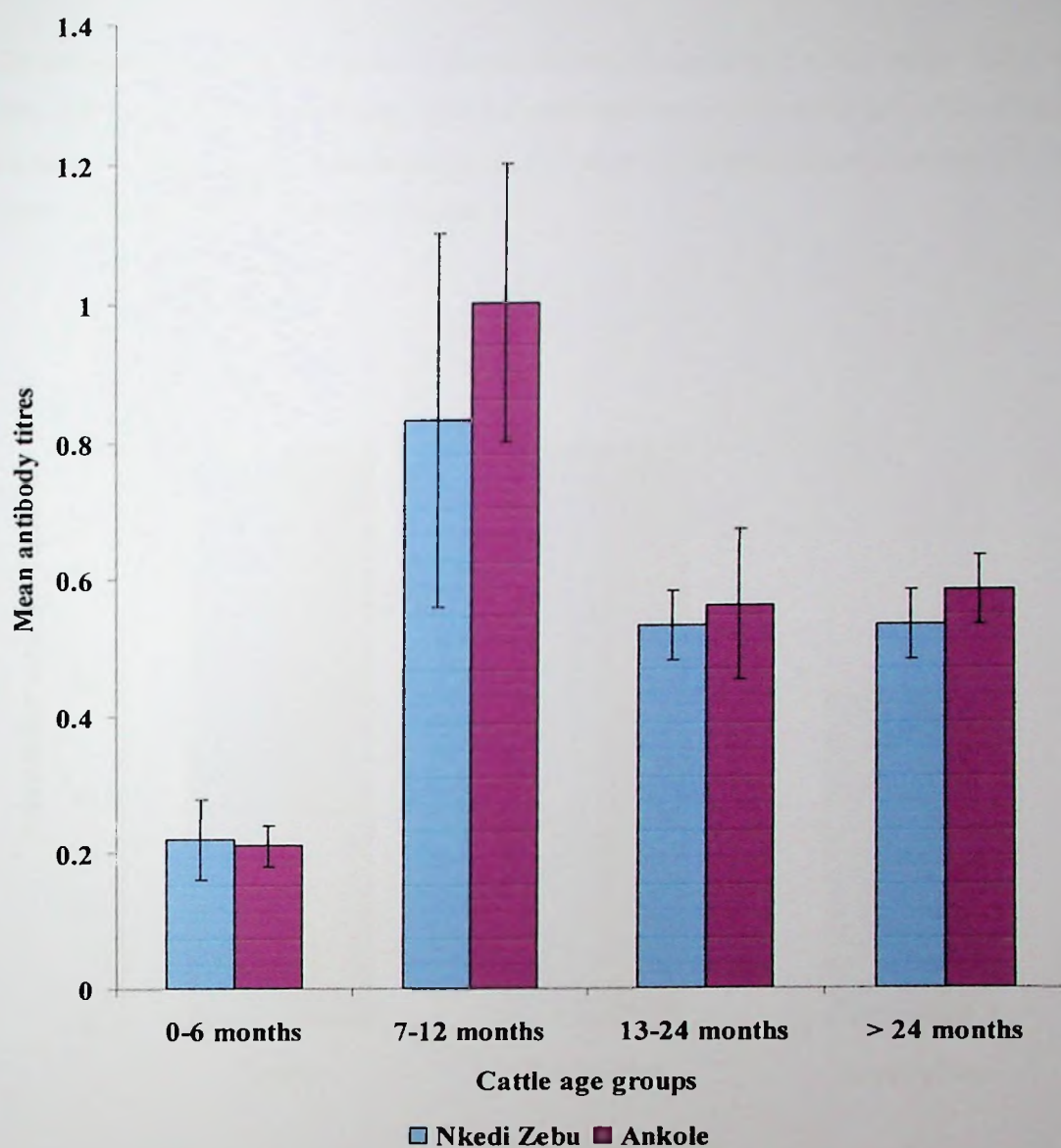
df = degrees of freedom, p-value = level of significance, CI = confidence interval, Std = standard



**Figure 4.2. Mean anti-body titres against *T. parva* in the different age groups of Nkedi Zebu and Ankole cattle in Kasilo county, Soroti district.**

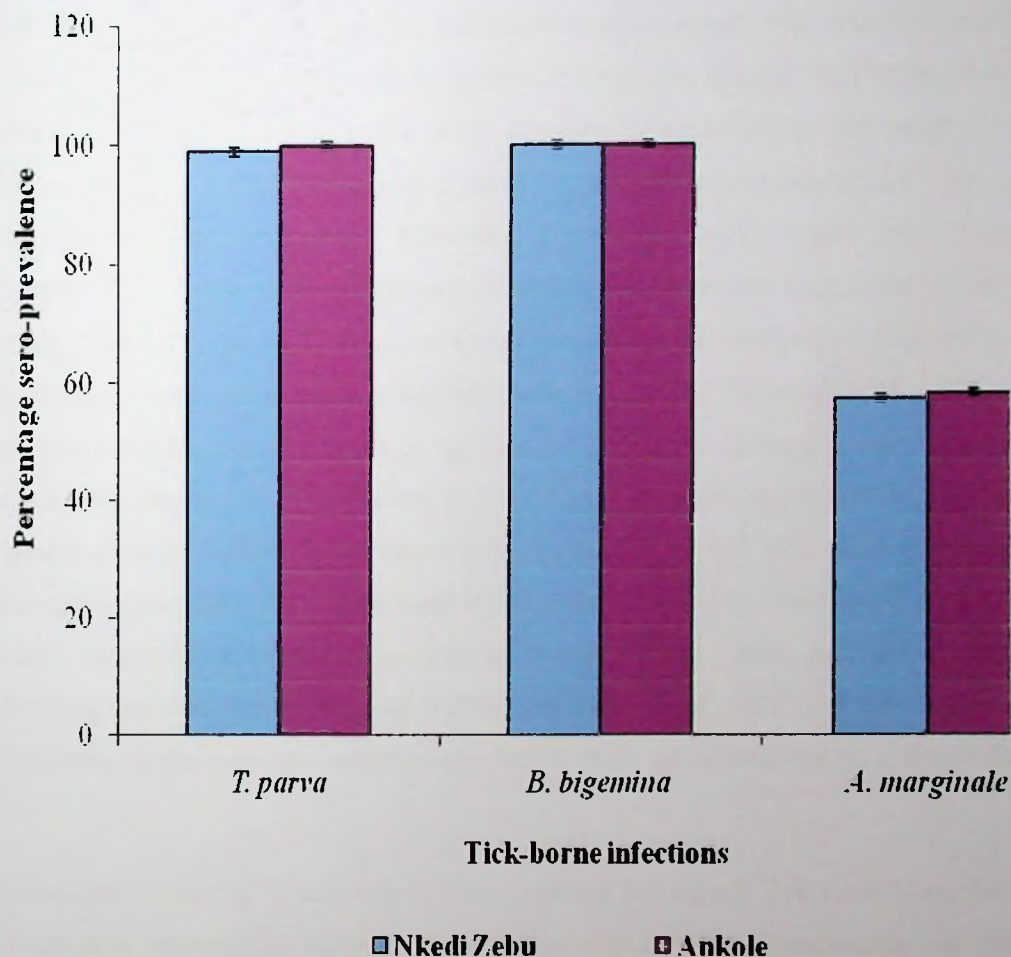


**Figure 4.3. Mean antibody titres against *B. bigemina* in the different age groups of Nkedi Zebu and Ankole cattle in Kasilo county Soroti district.**



**Figure 4. 4. Mean antibody titres against *A. marginale* in the different age groups of Nkedi Zebu and Ankole cattle in Kasilo county Soroti district.**

The sero-prevalence of *T. parva*, *B. bigemina* and *A. marginale* of the Nkedi Zebu was 99%, 100% and 57% respectively. These results were not significantly ( $p > 0.05$ ) different for those obtained for the Ankole cattle, thus *T. parva*, *B. bigemina* and *A. marginale* was 100%, 100%, 58%, respectively, (Figure 4.5).



**Figure 4.5. Sero-prevalences of tickborne infections in the Nkedi Zebu and Ankole, Kasilo county, Soroti district.**

#### 4.4 Discussion

This study revealed that there is a significant difference ( $p = 0.04$ ) in the mean antibody titres to *T. parva* infection, the Nkedi Zebu had a higher mean antibody titre than the Ankole cattle. The age (7-12 months) is the most vulnerable time because the calves are usually weaned and turned out to pastures for grazing with the older cattle, thereby exposing them to higher challenge of tick infections. Generally, no previous studies have compared antibody responses between two indigenous cattle breeds such as Ankole and Nkedi Zebu, although studies have compared exotic crosses with the indigenous cattle such as the Ankole with pure bred and exotic cross cattle. Studies by Parling *et al.* (1992) established that the Ankole cattle were capable of suppressing the multiplication of macro-schizont of *T. parva* thereby controlling the infection compared to the crossbred which had a high mortality. Likewise, previous studies which have shown that indigenous cattle have a certain degree of genetic resistance to both ticks and tick borne diseases (Norval *et al.*, 1992; Lawrence *et al.*, 1996). Resistance to tick-borne micro-organisms (TBMs) varies among different breeds of cattle. Generally, zebu (*Bos indicus*) cattle possess a higher resistance to ticks and TBMs than European (*Bos taurus*) cattle. Mattioli and others (2000) observed that the host's immune system would appear to be the single most important factor that regulates this resistance. The main effector immune mechanisms governing resistance against ticks and TBMs have been studied. The cellular immune response appears more effective and stable than humoral immunity in modulating resistance to ticks and TBMs (Mattioli *et al.*, 2000). Zebu cattle also had significantly higher serum complement levels than crossbred cattle (Matoyelo *et al.*, 2002).

The sero-prevalence of tick-borne diseases among the Nkedi Zebu and Ankole cattle in the study area implies that the natural challenge of tick-borne infections to the two breeds is similar and high in this area. This could mean that the restocking by central government and non government organisations is not being accompanied by appropriate tick and tick-borne disease control strategies (Magona *et al.*, 2004). Communal grazing commonly practiced in pastoral and agro-pastoral farming systems and the suitable climate favours the rapid multiplication of ticks, this leads to high tick burdens on cattle, and consequently the occurrence of the diseases they transmit (Rubaire-Akiiki *et al.*,

2004). The high sero-prevalence and high antibody titres for *T. parva* and *B. bigemina* revealed by this study is in agreement with previous findings which reported that more than 90% of the national cattle herd in this country is at constant risk of tick borne diseases (Magona *et al.*, 2004; Anon, 1997).

#### **4.5 Conclusions and recommendations**

This study has demonstrated that the Nkedi Zebu produced significantly ( $p < 0.05$ ) higher mean antibody titres against *T. parva* infection than the Ankole cattle in the Soroti agro-pastoral farming system. The Nkedi Zebu cattle are better primed to protect themselves against the *T. parva* infection by producing more antibodies compared to the Ankole cattle. Calves at the age of 7-12 months are turned out to graze with the older cattle which results into increased tick challenge. At this age, extra tick control measures need to be applied to the calves since they are being weaned and are first turned out to graze with the older cattle where the tick challenge increases. More studies need to be carried to establish the magnitude of protection of these antibodies to the Nkedi Zebu calves. Furthermore, there is to study which type of immunoglobulin are involved in this immunity.

## CHAPTER FIVE

### 5.0 Tick infestations among the Nkedi Zebu and Ankole cattle in the Agro-Pastoral farming system of Soroti district

#### Abstract

This cross-sectional study was conducted to determine the differences in tick counts of *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *Amblyomma variegatum*, and packed cell volumes (PCV) using the Haematocrit Centrifugation Technique (HCT). A total of 581 randomly selected cattle were investigated comprising 381 Nkedi Zebu and 150 Ankole cattle. This study was carried out in the Soroti district agro-pastoral farming villages of Akumoi, Aswii, Kamod, Osamito, Okokoma and Opungure.

The results revealed that the Ankole cattle in the age group 7-12 months (when they are first turned out to pastures with the adult cattle) had a significantly ( $p = 0.04$ ) higher mean tick count of *R. appendiculatus* ( $24.3 \pm 3.2$ ) compared to the Nkedi Zebu ( $17.8 \pm 1.3$ ). However the overall mean tick counts of *R. appendiculatus*, *B. decoloratus*, and *A. variegatum* on Ankole cattle ( $23.8 \pm 0.8$ ,  $9.6 \pm 0.6$ ,  $2.4 \pm 0.2$ ), respectively were similar ( $p > 0.05$ ) to those of Nkedi Zebu ( $24.4 \pm 0.5$ ,  $10.4 \pm 0.34$ ,  $3.1 \pm 0.1$ ), respectively.

There was generally no significant difference between the mean PCV in the two (Ankole and Nkedi Zebu) breeds of cattle and within the different age groups studied.

The Nkedi Zebu calves (7-12 months) appear to have a more developed mechanism of keeping the tick infestation lower than Ankole calves as they are first turned out to the pastures at weaning, likewise, the Nkedi Zebu had significantly higher antibody titres as compared to the Ankole cattle as reported in chapter 4, appearing to be better primed to protect themselves against *T. parva*, therefore extra tick control interventions are required for Ankole calves to be able to thrive better in this area. However in mixed herds the extra tick control should be applied to both the Ankole and Nkedi Zebu calves.

## 5.1 Introduction

The most important ticks that infest cattle and the diseases that they transmit include *Rhipicephalus appendiculatus* (ECF), *Boophilus decoloratus* (anaplasmosis and babesiosis) and *Amblyomma variegatum* (anaplasmosis). The most common tick species in order of abundance observed on adult indigenous cattle dipped once a month in Uganda include; *R. appendiculatus*, ( $61 \pm 2.3$ ), *R. evertsi*, ( $7.6 \pm 1.8$ ), *A. variegatum* ( $3.7 \pm 2.1$ ) and *B. decoloratus* ( $2.9 \pm 0.8$ ). (Okello-Onen *et al.*, 1999). Tick populations differ according to the climate and altitudes of given locations as shown by a recent study carried out in Mbale and Sironko districts revealed that the highest tick challenges were recorded in the low lands, followed by the midland and the uplands, respectively (Rubaire-Akiiki *et al.*, 2004). Similar results were obtained in the Kenyan highland (Gitau *et al.*, 1997). Climatically, the lowlands are more suitable for *R. appendiculatus* than the midlands and uplands (Matthysse and Colbo 1987). In addition, the keeping of local indigenous breeds under the free-range communal grazing system is known to increase the occurrence and spread of tick and tickborne diseases among the cattle herds (Rubaire-Akiiki *et al.*, 2004).

The location of a suitable host is a critical stage in the life history of a parasite (Belan and Bull, 1991). Ticks may locate their hosts either actively by moving towards distant stimuli or passively by waiting either on vegetation or within refuge sites until a host individual comes within reach (Waladde and Rice, 1982). The ability of livestock to control their tick burden through the acquisition of natural resistance has been known for decades (Johnston and Bancroft, 1918; Trager, 1939; Kelley, 1943; Bonsma, 1944; Macleod, 1975). Generally *B. indicus* breeds were reported to acquire natural resistance to tick infestation more effectively than those of *Bos taurus* (Riek, 1962; Francis and Little, 1964; Utech *et al.*, 1978b; Newson and Chiera, 1986; Rechav, 1987). This is due the immune response to components of the saliva of ticks, mediated by antibodies, complement and T-cell-dependant mechanisms, associated with cutaneous hypersensitivity reactions at the attachment site with increased grooming (Branagan, 1973; Willadsen, 1980; Binta and Cunningham 1984; Fivaz and Norval, 1990) that may kill or debilitate the ticks (de Catro and Newson, 1993). The immunological response is

often stronger towards species of which the host has had little or evolutionary experience (Ribeiro, 1989). Previous studies have suggested that cattle resistance to ticks is heritable (Johnson and Bancroft, 1918), although this heritability has been associated with crosses based on the zebu breed cattle (Bonsma, 1944; Heweston, 1968). Wharton and others (1970) observed that in a herd of Australian Illawarra Shorthorn (*B. taurus*) dairy cattle that acquired tick infestations naturally, heritability estimates were 39% from dam-calf correlation and 49% from full-sib correlations. Consequently, attempts have been made to breed tick-resistant cattle in Brazil (de Castro and Newson, 1993), Australia (Heweston, 1968) and South Africa (Bonsma, 1944) by balancing the desirable traits of *B. taurus* (high body weight gain and milk yield) and those of *B. indicus* (tick resistance, heat adoption, resistance to diseases and parasites). Similar observations were made by Chiera *et al.* (1985) and Latif (1984) when the weights of engorged ticks fed on resistant and susceptible host were compared. In Uganda it was recognized that some animals showed greater resistance than others to all instars and species of tick and about 50% of the total tick population infesting herds were carried by 30% of cattle (Kaiser *et al.*, 1982). The relative resistance to tick infestation of zebu cattle (*B. indicus*) in comparison to crossbred (*B. indicus* x *B. taurus*) cattle was investigated in Tanzania with all *B. indicus* breeds, belonging to Tanganyika shorthorn zebu were compared with Friesian crossbreds. Parameters to distinguish between 'tick resistant' and 'tick susceptible' cattle were tick counts on naturally exposed animals, serum complement levels and delayed skin hypersensitivity response to phytohaemagglutinin. Results showed that pure zebu cattle were less infested with ticks when compared to zebu-aurine crosses under identical field conditions. Zebu cattle also had significantly higher serum complement levels than crossbred cattle (Matoyelo *et al.*, 2002).

The desire to carry out this study, therefore, came as a result of the lack of adequate information of the differences in responses to natural tick infestations among the common indigenous breeds of Uganda and yet tick-borne infections were ranked high in the diagnostic survey of NARO/LRSP report, (1999). The knowledge obtained out of this study will be used to guide several stakeholders in designing better tick control strategies for our different indigenous breeds of cattle.

## 5.2 Materials and methods

### 5.2.1 Determination of packed cell volumes

Using the heparinised blood already obtained as explained in chapter 3 (3.2), the packed cell volumes (PCV) were determined using a Haematocrit centrifuge powered by a petrol generator in the field. Heparinised microhaematocrit capillary tubes were filled with blood (about 70 $\mu$ l), sealed at one end with plasticine and centrifuged for 5 minutes at 3000 rpm. These tubes were then wiped with clean tissue paper and the PCV (Nkedi Zebu n=380 and Ankole n=151) was determined using PCV reader (Hawksley® UK).

### 5.2.2 Tick counts

The selected cattle were not sprayed with acaricides for at least four weeks before the tick collection. Nymph and adult ticks were collected from well restrained cattle using forceps, from only one side of the body, paying particular attention to the predilection sites including the ears, head, dewlap, abdomen, anus-vulva (perineal) and the inguinal area as described by Kaiser and co-workers (1982). After collection, the ticks were placed into well labelled 100 ml universal bottles with sterile moist cotton wool and placed in a cool box with ice packs kept at a temperature of about 0°C to prevent death of the ticks and spoilage by bacteria. The ticks were later transported to the National Livestock Resources Research Institute (NaLIRRI), To roro, for identification. In the laboratory the ticks were examined using a stereo-microscope and magnifying lenses, and species identified using keys developed by Matthysse and Colbo (1987). All the tick species of *R. appendiculatus*, *B. decoloratus* and *A. variegatum* present were determined and their numbers recorded against the cattle from which they were collected.

## 5.3 Results

The mean tick counts of *R. appendiculatus*, *B. decoloratus* and *A. variegatum* among the Nkedi Zebu (n = 381) and Ankole (n = 150) cattle were compared, first without considering the different age groups and secondly in the different age groups using the student's t test at 95% confidence interval. No significant difference was observed in the first comparison as shown (Table 5.1) and yet a significant difference (p= 0.04) was observed in the mean tick counts of *R. appendiculatus* in the age groups of 7-12 months (Table 5.2 and Figures 5.2.). The mean tick counts of *B. decoloratus* and *A. variegatum*

were not significantly different among the Nkedi Zebu and the Ankole cattle (Figures 5.1, 5.3, 5.4).

**Table 5.1. Comparison of the mean tick counts among the Nkedi Zebu (n = 381) and Ankole (n = 150) cattle, Kasilo county, Soroti district.**

| Tick species             | Cattle breed | p-value | Mean  | Std. error mean | 95% CI of the difference |
|--------------------------|--------------|---------|-------|-----------------|--------------------------|
| <i>R. appendiculatus</i> | Nkedi Zebu   | 0.56    | 24.47 | 0.523           | -1.42 - 2.61             |
|                          | Ankole       |         | 23.87 | 0.880           |                          |
| <i>B. decoloratus</i>    | Nkedi Zebu   | 0.26    | 10.42 | 0.337           | -0.61 - 2.25             |
|                          | Ankole       |         | 9.60  | 0.644           |                          |
| <i>A. variegatum</i>     | Nkedi Zebu   | 0.36    | 3.14  | 0.188           | -0.99 - 0.36             |
|                          | Ankole       |         | 3.45  | 0.291           |                          |

p-value = level of significance, CI = confidence interval, Std = standard

**Table 5.2. Comparison of the mean tick counts of the Nkedi Zebu (n=381) and Ankole (n=150) cattle age groups in Kasilo county, Soroti district.**

| Age groups (months) | Tick species             | p-value | Std. error difference | 95% CI of the difference |       |
|---------------------|--------------------------|---------|-----------------------|--------------------------|-------|
| 0-6                 | <i>R. appendiculatus</i> | 0.80    | 3.42                  | -6.16                    | -7.86 |
|                     | <i>B. decoloratus</i>    | 0.26    | 2.25                  | -2.07                    | -7.17 |
|                     | <i>A. variegatum</i>     | 0.35    | 1.27                  | -3.80                    | -1.40 |
| 7-12                | <i>R. appendiculatus</i> | 0.04    | 3.03                  | -12.52                   | -0.26 |
|                     | <i>B. decoloratus</i>    | 0.64    | 2.01                  | -3.14                    | -5.01 |
|                     | <i>A. variegatum</i>     | 0.89    | 1.25                  | -2.38                    | -2.70 |
| 13-24               | <i>R. appendiculatus</i> | 0.91    | 2.11                  | -4.43                    | -3.96 |
|                     | <i>B. decoloratus</i>    | 0.62    | 1.98                  | -2.95                    | -4.92 |
|                     | <i>A. variegatum</i>     | 0.61    | 0.86                  | -2.15                    | -1.27 |
| > 24                | <i>R. appendiculatus</i> | 0.21    | 349.0                 | 0.21                     | -4.01 |
|                     | <i>B. decoloratus</i>    | 0.45    | 0.83                  | -1.02                    | -2.27 |
|                     | <i>A. variegatum</i>     | 0.56    | 0.42                  | -1.07                    | -0.58 |

p-value = level of significance, CI = confidence interval, Significant difference (p<0.05)

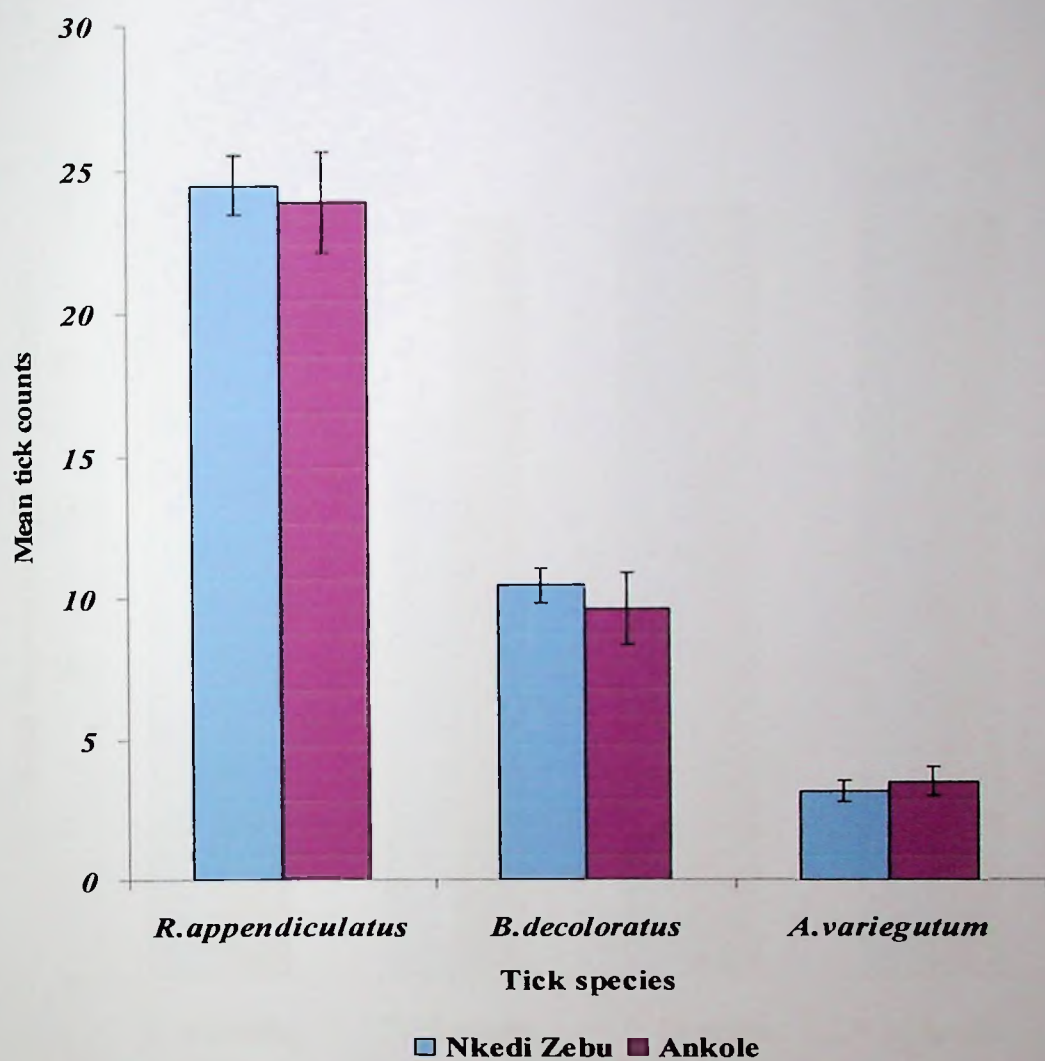


Figure 5.1. Mean tick counts of *R. appendiculatus*, *B. decoloratus* and *A. variegatum* on the Nkedi Zebu and Ankole cattle, Kasilo county, Soroti district.

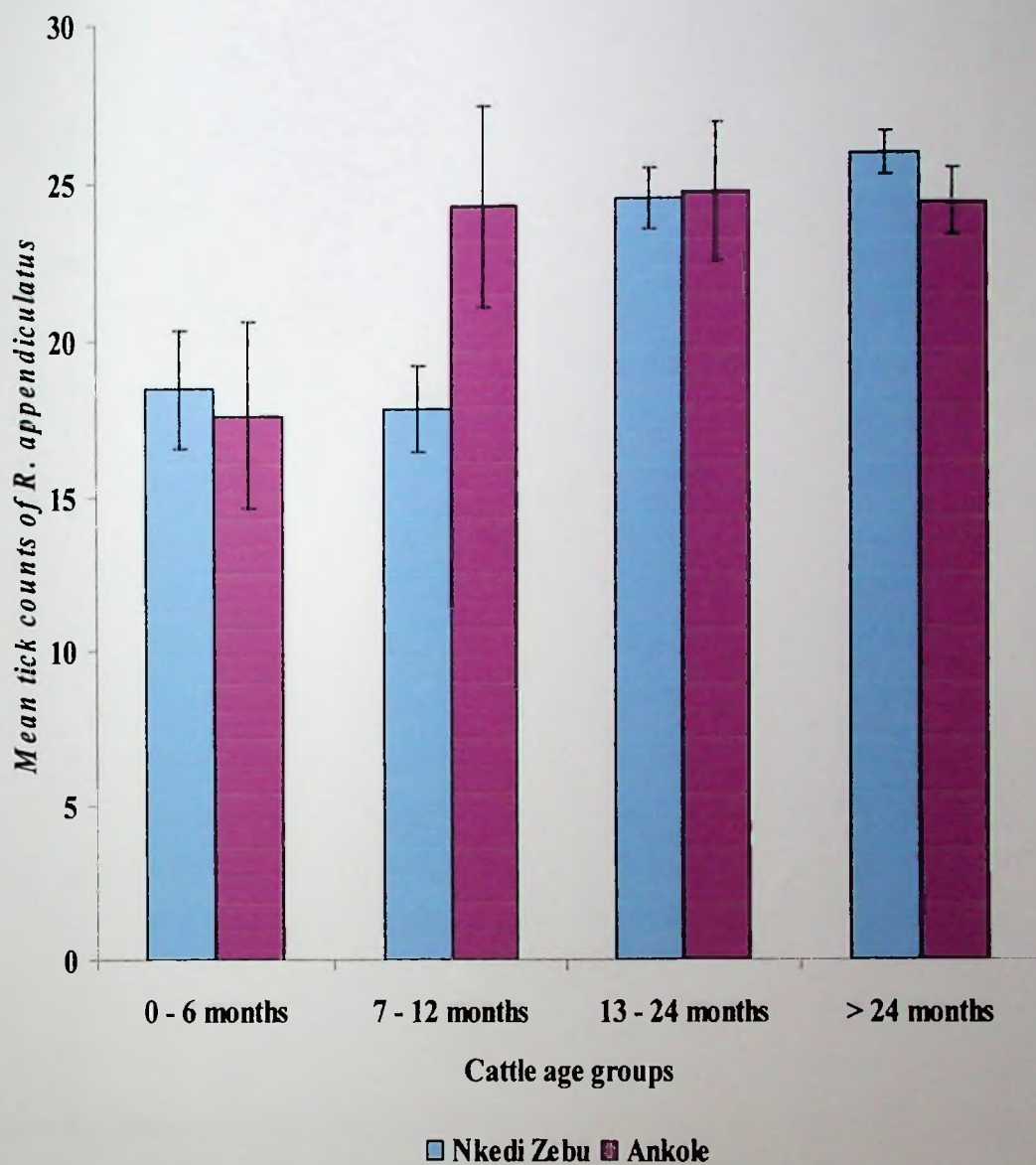


Figure 5.2. Mean tick counts of *R. appendiculatus* on the different age groups of Nkedi Zebu and Ankole, Kasilo county, Soroti district.

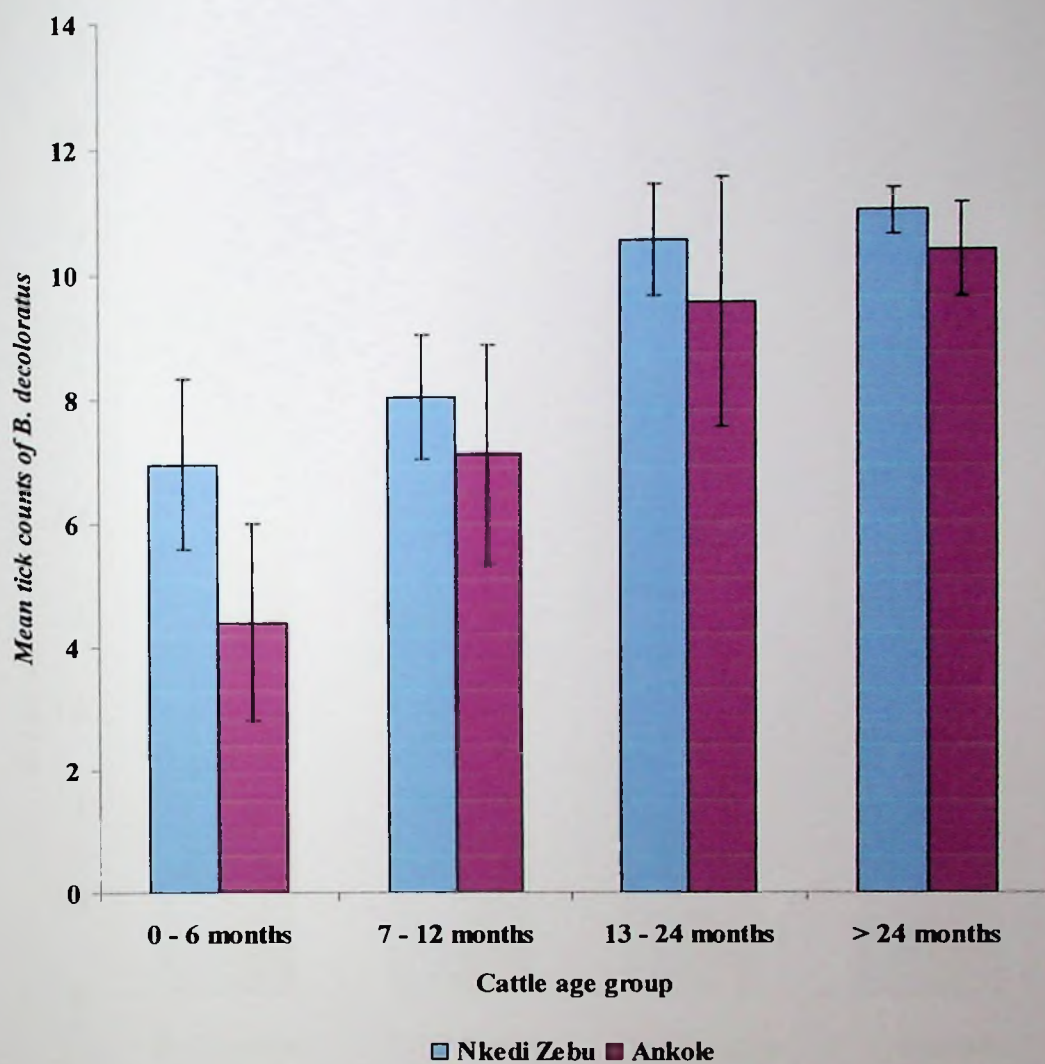


Figure 5.3. Mean tick counts of *B. decoloratus* on the different age groups of Nkedi Zebu and Ankole cattle, Kasilo county, Soroti district.

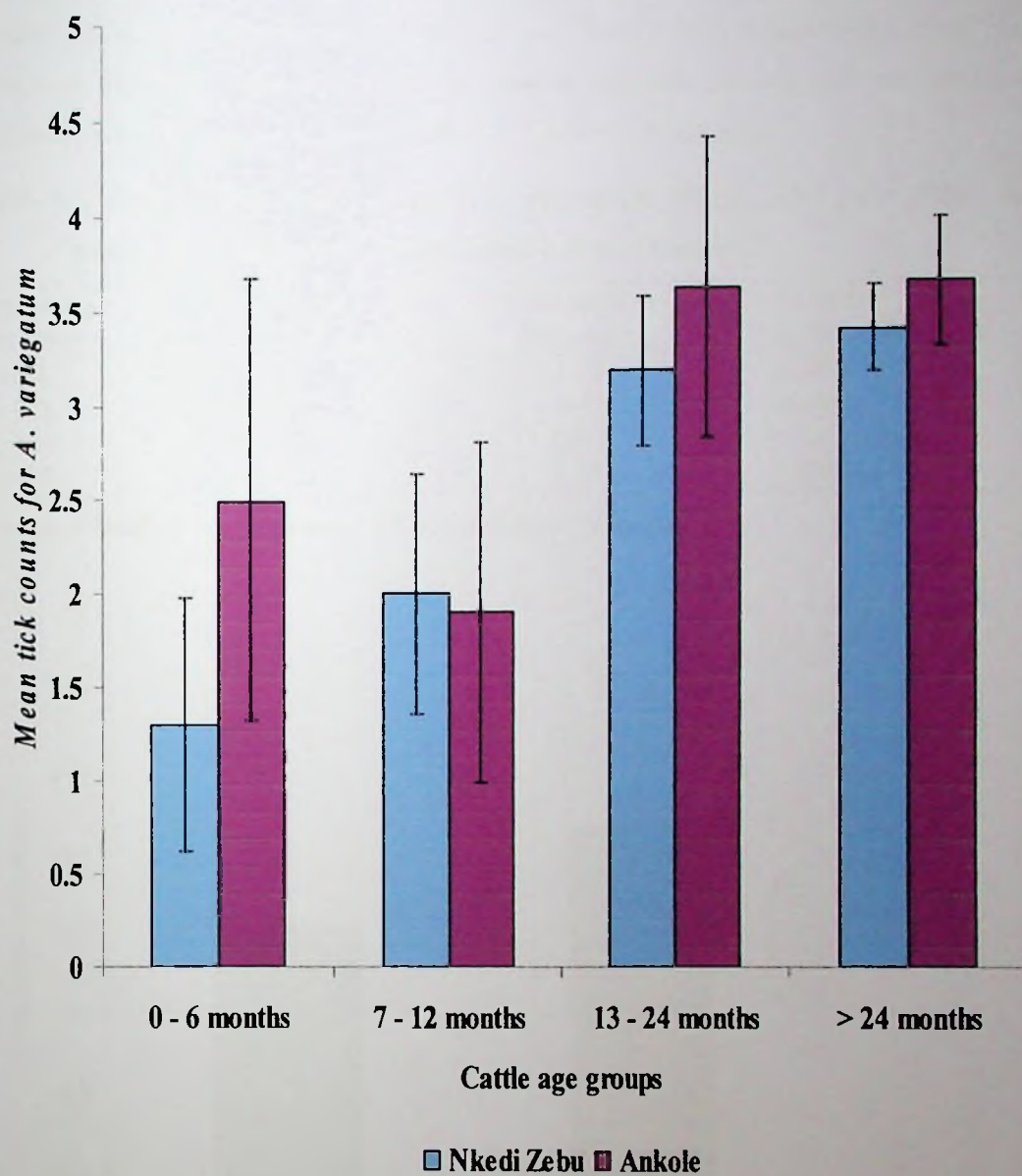


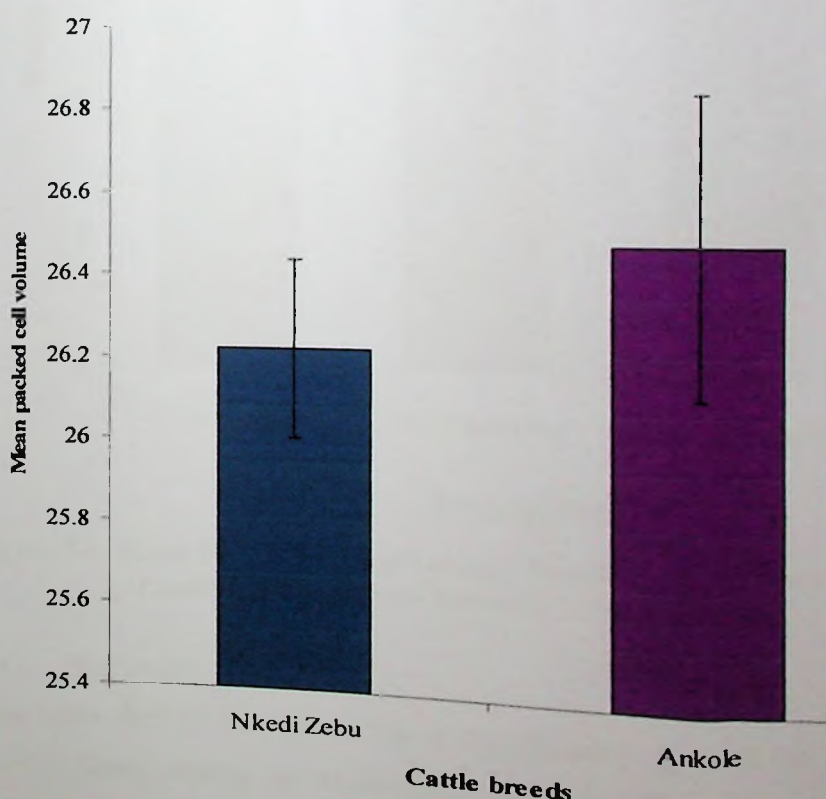
Figure 5.4. Mean tick counts of *A. variegatum* in the different age groups of Nkedi Zebu and Ankole, Kasilo county, Soroti district

The mean packed cell volumes (PCV) were obtained from 38 Ankole cattle and these were  $26.2 \pm 0.21$  and  $26.4 \pm 0.36$  compared using the student's t test at 95% confidence interval difference was observed as shown (Table 5.1, Figures 5.1 and 5.2)

**Table 5.3. Comparison of the mean PCV among the Nkedi Ankole (n = 150) cattle, Kasilo county, Soroti district.**

| Age group (months) | p-value | Standard error difference |
|--------------------|---------|---------------------------|
| 0 – 6              | 0.63    | 1.76                      |
| 7 – 12             | 0.17    | 1.92                      |
| 13 – 24            | 0.07    | 0.93                      |
| > 24               | 0.50    | 0.48                      |

p-value = level of significance, CI = confidence interval



**Figure 5.5. Mean PCV of the Nkedi Zebu and Ankole cattle, K district.**

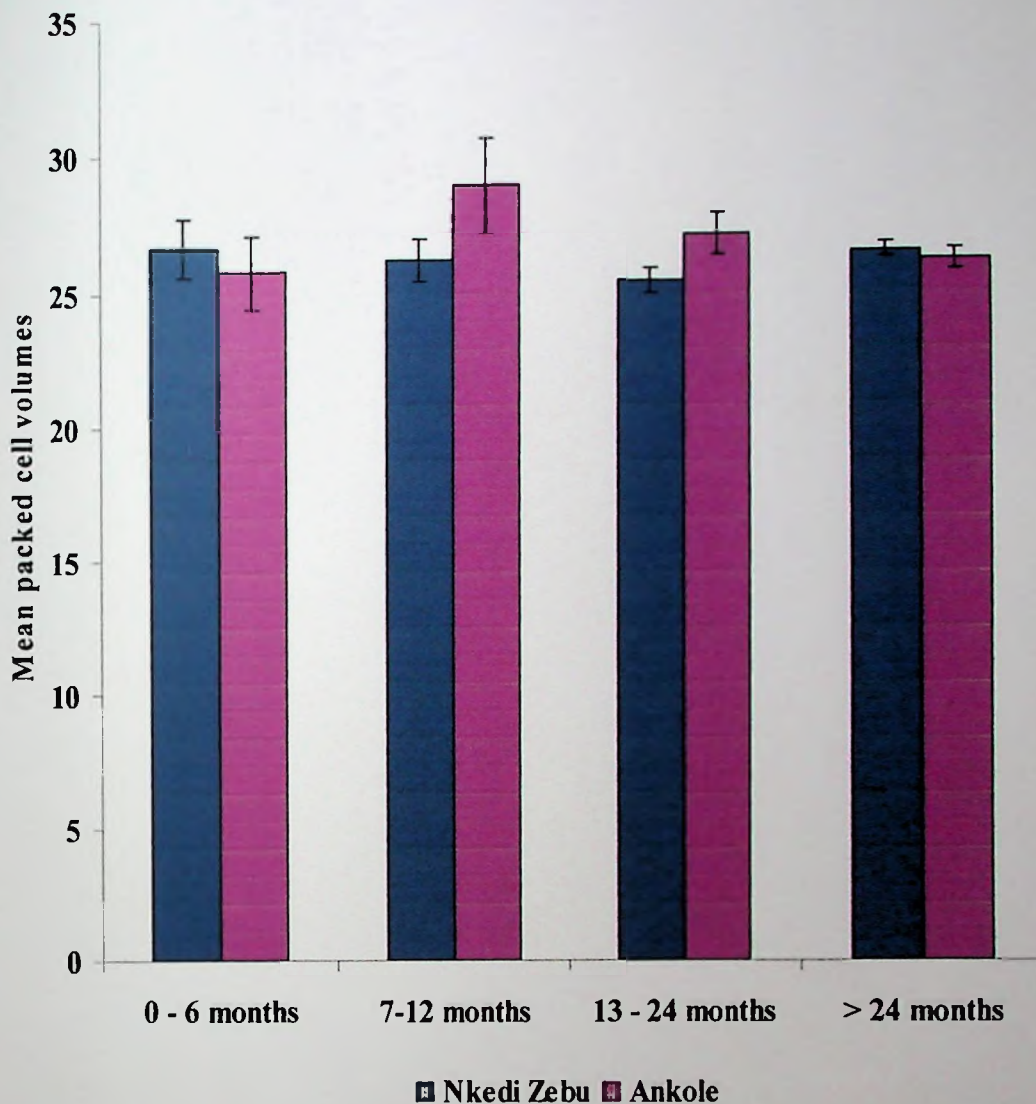


Figure 5.6. Mean PCV of the Ankole and Nkedi Zebu in the different age groups in Kasilo county, Soroti district.

#### 5.4 Discussion

This study showed that there was a significantly ( $p = 0.04$ ) higher infestation of *R. appendiculatus* among the Ankole than the Nkedi Zebu cattle in the age group of 7-12 months. Usually, at this age that the calves are weaned turned out to pasture to graze with the older cattle (Magona, 2004). This finding is in agreement with previous studies which reported differences in tick counts between different indigenous breeds of cattle

(Matoyelo *et al.*, 2002; Okello Onen *et al.*, 1999; Mattioli *et al.*, 1995). Studies have showed that highly resistant cattle especially Zebu cattle in Africa keep tick populations low (de Castro, 1991). Several indigenous breeds have been found to have high levels of resistance, including Africander, Bonsmara, Nkoni and Brahman (S. Africa), Nkoni (Zimbabwe), Boran and East African Zebu (Kenya), Butana and Kenana (Sudan) and Boran and Horro (Ethiopia) (Scholtz *et al.*, 1991; de Castro 1991; Latif *et al.*, 1991).

The lower mean tick counts among the Nkedi Zebu cattle could be attributed to the innate ability to both immunological response to components of the saliva of feeding ticks, mediated by antibodies, complement and T-cell dependant mechanisms, cutaneous hypersensitivity reactions, and mechanical activities such as increased grooming activity as reported by earlier workers (Branagan, 1973; Willadsen, 1980; Binta and Cunningham, 1984; Fivaz and Norval, 1990) that may kill or debilitate the ticks (de Castro and Newson, 1993). This is closely related to findings in chapter 4 in which the Nkedi Zebu were observed to have had a significantly higher mean antibody titres to *T. parva* as compared to the Ankole cattle.

There was no significant difference between the mean PCV of the Nkedi Zebu and Ankole cattle. The PCV values are indicative of a mild degree of anaemia, which could be attributed to the high mean tick counts among other causes, as related to previous studies which indicated a high tick burden among the agro-pastoral farmers (Ocaido *et al.*, 2005).

## **5.5 Conclusions and recommendations**

The Ankole cattle in the age group of 7-12 months had a higher mean tick count of *R. appendiculatus* ticks as compared to the Nkedi Zebu cattle in the same age group, extra tick control measures need to be applied on the calves of the age group of 7-12 months as they are first turned out at weaning to graze with the older cattle. This is the most vulnerable age for *T. parva* infection as exhibited by the higher antibody titres (chapter 4). This should apply to both the Nkedi Zebu and Ankole as long as they are in the same herds.

## CHAPTER SIX

### 6.0

### GENERAL DISCUSSION

Most (>95%) of the cattle in Uganda are of the Zebu or Sanga breeds (Magona and Mayende, 2002). In the east of the country, where the study was done, most of the cattle are of the Nkedi Zebu breed although the Ankole breed (Sanga) were brought in as result of a restocking programme (Fèvre *et al.*, 2001). The Ankole cattle have the good attributes of faster growth, and higher birth weight, weight gain, higher milk production and carcase weight compared to the Nkedi Zebu (Petersen *et al.*, 2004) This study was carried out in Soroti district, Pingire and Bugondo sub-counties with the main aim of comparing the responses of the Nkedi Zebu and Ankole cattle to natural ticks and tickborne infections under the same environmental conditions based on tick counts, the antibody titres and the packed cell volumes.

The sero-prevalence of theileriosis and babesiosis among the Nkedi Zebu and Ankole cattle (sero-prevalence of theileriosis and babesiosis of the Nkedi Zebu was 99% and 100%, respectively, and in the Ankole cattle, the sero-prevalence of theileriosis, and babesiosis was 100%, and 100% respectively), implies that the tick challenge in this area is high and probably because restocking by central government and non government organisations is not being accompanied by appropriate tick and tick-borne disease control strategies (Magona *et al.*, 2004). Rubaire-Akiiki and others (2004) reported that communal grazing under the pastoral farming system is characterised by high tick burdens on cattle and with the suitable climate which favours the rapid multiplication of ticks and consequently, the occurrence of the diseases they transmit. These high sero-prevalence and high antibody titres for tick borne infections revealed by this study is in agreement with previous findings which reported that more than 90% of the national cattle herd in this country is at constant risk of tick borne diseases (Anon, 1997; Magona *et al.*, 2004).

This study has also shown that there was a significant difference ( $p = 0.04$ ) in the mean antibody titre to *T. parva* infection whereby the Nkedi Zebu had a higher mean antibody titre than the Ankole cattle.

This study has also shown that there was a significantly ( $p = 0.04$ ) higher infestation of *R. appendiculatus* among the Ankole than the Nkedi Zebu cattle in the age group of 7-12 months. This is the age at which the calves are weaned and turned out to pasture to graze with the older cattle (Magona, 2004). This observation is in agreement with previous studies which have reported differences in tick counts between different indigenous local breed cattle (Mattioli *et al.*, 1995; Okello Onen *et al.*, 1999; Matoyelo *et al.*, 2002). There is now adequate evidence that cellular immune responses are essential in modulating resistance to ticks and tick-borne infections (Mattioli *et al.*, 2000). Similarities between the immune mechanisms employed by trypanotolerant *N'Dama* cattle, when infected with trypanosomes, and those elicited by tick bites and tick-borne infections occur, particularly at the skin level in the early phases of parasitic invasion. Moreover, there is evidence that in the *N'Dama* breed, resistance against ticks *per se* has a genetic basis (Mattioli *et al.*, 2000). Infection is maintained in a population by continuous exposure to ticks; cattle become infected as calves, when they acquire a degree of age related resistance or colostral immunity, which minimises the effects of diseases, and there after become immune (Norval *et al.*, 1992; Lawrence *et al.*, 1996). The immunological response is often stronger towards species of which the host has had little or evolutionary experience (Ribeiro, 1989).

Generally there was an increase in the mean tick counts of all the tick species in older cattle than the young cattle. This could probably be due to movement of older cattle for longer distances in search for pastures resulting in an increase in the tick exposure and, therefore, a higher infestation. The most abundant tick species shown by this study, in a decreasing order was *R. appendiculatus*, *B. decoloratus* and *A. variegatum*. This is in agreement with previous studies (Okello-Onen *et al.*, 1996; Rubaire Akiiki *et al.*, 2004; Ocaido *et al.*, 2005). *R. appendiculatus* ticks were the most abundant tick species among the Nkedi Zebu and Ankole cattle in this study. This maybe because cattle are widely believed to be the primary hosts for this tick species and cattle resistance to this tick has been reported to be lower under field condition (Lightfoot and Norval 1982; Kaiser *et al.*, 1982). Ocaido *et al.* (2005) reported similar mean tick counts in a socio-economic and livestock disease survey of agro-pastoral communities in Serere county, Soroti district. This study has also shown that the mean tick counts were quite higher than those

obtained in similar studies carried in Uganda. For example Otim *et al.*, 2004, in a study to establish the disease and vector constraints affecting cattle production in pastoral communities of Ssembabule district, *R. appendiculatus* had a mean tick count of  $3.7 \pm 0.28$  and *A. variegatum* had a mean tick count of  $0.24 \pm 0.012$ . In another study, Ocaido *et al.*, 2005, indicated that among other reasons this could be due to the break down of tick control infrastructure due to the previous civil unrest (1986-1996).

There was no significant difference between the mean PCV of the two (Ankole and Nkedi Zebu) breeds of cattle and within the different age groups. PCV values obtained in this study were indicative of a mild degree of anaemia which could be attributed to the high mean tick counts. Other causes could be helminthosis and trypanosomosis, although these were not studied. This is in agreement with previous study by Ocaido and others (2005) carried out in Serere county, Soroti district, which reported anaemia as one of the major clinical signs of tick infestations and tick borne diseases. In acute and chronic forms of babesiosis, haemoglobinuria and anaemia are evident (Urquhart *et al.*, 1996; Losos, 1986); in acute and chronic anaplasmosis anaemia is a common syndrome (Ristic and Carson, 1977) and in ECF anaemia and haemorrhages are characteristic (Moloo *et al.*, 2001; Urquhart *et al.*, 1992; Shannon, 1977).

This study has revealed that there are significant differences in the response to natural tick challenges and tick-borne infections in theileriosis (ECF), but not babesiosis and anaplasmosis among the Nkedi Zebu and Ankole breeds of cattle in the agro-pastoral farming system of Soroti district.

## CHAPTER SEVEN

### 7.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

#### 7.1 General conclusions

In the Soroti district and generally in the eastern part of Uganda, where this study was conducted, most of the cattle are of the Nkedi Zebu breed (Zebu breed) but the Ankole breed (Sanga) was a recent introduction restocking restocking programmes (Magona, 2004; Fèvre *et al.*, 2001).

In this study, it has been demonstrated that there was a significant difference in the mean tick counts of *R. appendiculatus* in calves of the age group of 7-12 months among the Nkedi Zebu and the Ankole cattle in Soroti district. The Ankole cattle had a higher mean tick count of *R. appendiculatus* as compared to the Nkedi Zebu cattle. This is probably due to the innate ability of the Nkedi Zebu to both immunological response to tick saliva, associated with cutaneous hypersensitivity reactions at the attachment site and increased grooming activity (Branagan, 1973; Willdsen, 1980; Binta and Cunningham 1984; Fivaz and Norval, 1990) that may kill or debilitate the ticks (de Castro and Newson, 1993).

Secondly, the present study has demonstrated that there was a significant difference ( $p=0.04$ ) in the mean antibody titres produced against *T. parva* infection among the Nkedi Zebu and Ankole cattle. The Nkedi Zebu cattle had a higher mean antibody titre produced against *T. parva* as compared to the Ankole cattle. This, therefore, implies that the Nkedi Zebu are better primed to protect themselves against the *T. parva* infection on tick challenge compared to the Ankole cattle in the Soroti agro-pastoral farming system.

There were no significant differences in the mean PCV, mean tick counts of *B. decoloratus*, and *A. variegatum* and the sero-prevalences of theileriosis, babesiosis and anaplasmosis infections among the Nkedi Zebu and the Ankole cattle in all the age groups in Soroti district agro-pastoral farming system.

## 7.2 General recommendations

Since the Ankole cattle in the age group of 7-12 months had a higher mean tick count of *R. appendiculatus* tick as compared to the Nkedi Zebu cattle in the same age group and also since the Nkedi Zebu cattle had a higher mean antibody production against *T. parva* infection as compared to the Ankole cattle, extra tick control measures need to be applied on the calves of the age group of 7-12 months as they are weaned and turned out to graze with the older cattle. This should apply to both breeds in the herds, since it is during this time when they are most vulnerable.

Due to the high tick burdens in Soroti district agro-pastoral farming system, there is need to develop and disseminate sustainable tick control strategies through community participation since studies have indicated that growth of livestock can be increased by 20-30% when tick control strategies are applied in the pastoral and agro-pastoral areas (Ocaido *et al.*, 2003). Community participation in the control of ticks and tick borne diseases has been demonstrated to be successful in pastoral and agro-pastoral areas (Ocaido *et al.*, 2003; Oloo *et al.*, (1999).

If livestock farmers in the industry are to be encouraged to adopt keeping of Ankole cattle because of their superior qualities in terms higher birth weight, growth rate, milk yield, and carcase weight (Petersen *et al.*, 2004) as a way forward to increased livestock production and productivity in the eastern pastoral and agro-pastoral areas, more attention on tick control on calves of the age group of 7-12 months be observed.

Using molecular techniques, studies should be done to establish the nature and magnitude of protection conferred by the antibodies produced against the tick-borne infections and the exact protective mechanisms the Nkedi Zebu apply to keep the tick infestations lower than the Ankole cattle. Further studies need to be carried out to establish the magnitude in differences to TBDs between the indigenous, crosses and pure bred cattle under controlled conditions using molecular techniques in longitudinal observations. There is need to establish the age at which the calves start to sero-convert to tick-borne infections.

## REFERENCES

- Abdel-Shafy S, Zayed AA. (2002) *In vitro* acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on eggs, immature and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). *Vet Parasitol.*; **106**:89–96.
- Ajayi, S. A., Fabiyi, J.P., Umo, I. (1982). Clinical bovine Anaplasmosis and Babesiosis in Friesians, in an outbreak and its control. *World Animal Review* **42**: 41- 42.
- Ajayi, S A., Oyctunde, I L., Ogbonna, G.A., Dipeolu, O. O. (1987). Bovine Anaplasmosis: clinical, haematological and blood biochemical changes in experimentally infected Nigerian cattle. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux* **40**, 41-47.
- Allsopp, B.A., Carrington, M., Baylis, H., Sohal, S., Dolan, T.T. and Iams, K. (1989). Improved characterisation of *T. parva* isolates using the polymerase chain reaction and oligonucleotide probes. *Molecular and Biochemical Parasitology*, **35**: 137-148.
- Almazán C, Kocan KM, Bergman DK, García-García JC, Blouin EF, del la Fuente J. (2003). Identification of protective antigens for the control of *Ixodes scapularis* infestation using cDNA expression library immunization. *Vaccine*. **21**(13-14):1492–1501
- Angus BM. (1996) The history of the cattle tick *Boophilus microplus* in Australia and achievements in its control. *Australian Society of Parasitology*; **26**:1341–1355.
- Anon (1996) Livestock and Food Security. In: World Food Summit Rome 13<sup>th</sup> -17<sup>th</sup> November, 1996. FAO, Rome, 23.
- Anon. (1997). *National Tick and Tick borne Disease Control Strategy*, Uganda.
- Babes V., (1888) Sur l'haemoglobinuria bacterine de bœufs. *Compt Rend Acad Sci* (Paris) **107**:692-700.
- Barnett, S.F (1961) The Control of Ticks on Livestock. Rome, Italy: *Food and Agriculture Organization of the United Nations*; p. 115. Agriculture Studies No. 54.
- Barnett, S.F (1968). Theileriosis. In: Infectious blood diseases of man and Animals. Academic Press , New York, Ed. M. Ristic and D. Weinman, **2**: 269-328.
- Belan, I and Bull, C.M. (1991). Host detection by four Australian tick species. *Journal of Parasitology*, **77**: 337-340
- Billiouw, M., Mataa, L., Marcotty, T., Chaka, G., Brandt, J., Berkvens, D. (1999). The current epidemiological status of bovine theileriosis in eastern Zambia. *Tropical Medicine and International Health* **4**: A28b-A33.

- Binta, M.G. and Cunningham, M.P. (1984). Cutaneous responses of cattle to extracts from *R. appendiculatus* larvae. *Veterinary Parasitology*, **15**:67-73.
- Bonsma, J.C. (1944). Hereditary heartwater-resistant characteristics in cattle. *Farming in South Africa* **19**:71-96.
- Bram R A. (1983) Tick-borne livestock diseases and their vectors: The global problem. In: Ticks and tick-borne diseases, *FAO Animal Production and Health Paper No. 36. Food and Agriculture Organization, Rome*, pp. 7-11.
- Bram RA, George JE, Reichard RE, Tabachnick WJ. (2002). The threat of foreign arthropod-borne pathogens to livestock in the United States. *Journal of Medical Entomology*, **39**:405-416.
- Branagan D. (1973), Observations on the development and survival of the ixodid ticks *Rhipicephalus appendiculatus* under quasi natural conditions in Kenya. *Tropical Animal Health Production*. **5**(3):153-165.
- Bruce, D.D., Hamerton, A E., BATERMAN, H. R., Mackie, F.P. (1910). Amakebe: a disease of calves in Uganda. *Proceedings of the Royal Veterinary Society B*, **82**:256-272.
- Callow, L. L. (1979). Some aspects of the epidemiology and control of bovine Babesiosis in Australia. *Journal of South African Veterinary Association*, **50**: 353-356.
- Chiera JW, Cunningham MP, Dolan TT, Newson RM, Radley DE, Young AS. (1985b) Survival of *Rhipicephalus appendiculatus* and persistence of *Theileria parva* in the field. *International Journal of Parasitology*. **14**:48-49.
- Conrad, P.A., ole-MoiYoi, O.K., Baldwin, C.L., Dolan, T.T., O'Callaghan, C., Njamunggeh, R.E.G., Grootenhuys, J.G., Stagg, D.A., Leitch, B.L. and Young, A.S. (1989). Characterization of buffalo-derived theilerial parasites with monoclonal antibodies and DNA probes. *Parasitology* **98**:179-188.
- CRC-VT. (2001) Australian Government Cooperative Research Centres Program. Research Program: *Vaccine Applications, Tick Fever*.
- De Castro J J. (1991). Resistance to ixodid ticks in cattle with an assessment of its role in tick control in Africa. In: Oxford R.F.E and Owen J. B (eds), *Breeding for disease resistance in farm animals*. CABI, Wallingford, UK
- De Castro, J.J, (1997), sustainable tick and tick borne disease control in livestock improvement in developing countries. *Veterinary Parasitology* **71**: 77-79.
- De Castro, J.J., Newson, R.M., (1993). Host resistance in cattle tick control. *Parasitology today* **9** :13-17.
- De Vos, A. J. (1979). Epidemiology and control of bovine Babesiosis in South Africa. *Journal of South African Veterinary Association*, **50**: 357-362.

- Sam, S. L., Perry, B.D., Katende, J. M., McDermott, J. J., Mahan, S. M., Maloo, S. H., Morzaria, S. P., Musoke, A. J., Rowland, G. J. (1993). Variations in prevalence rates of tick-borne diseases in zebu cattle by agroecological zone: implications of east coast fever immunizations. *Preventive Veterinary Medicine*, 16: 171-187.
- Kamans, G. (1950). Transmission of Anaplasmosis. *American Journal of Veterinary Research*, 11: 5-16.
- rummond RO. (1983) Tick-borne livestock diseases and their vectors. Chemical control of ticks. *Wld Anim Rev*, (FAO).;36:28-33.
- uncan IM. (1991) Tick control on cattle with flumethrin pour-on through a Duncan applicator. *J S Afr Vet Assoc*.;63(3):125-127.
- gbe-Nwiyi, T. N., Salako, M.A. Otukonyong, E. E., (1997). Observations on naturally occurring bovine Anaplasmosis in arid zone of north-Eastern Nigeria: prevalence, haematological and pathological changes. *Studies and Researches in Veterinary Medicines*, 5: 95-99.
- FANTA sampling guide (Food and Nutrition Technical Assistance Project, 1997) Academy for Educational Development 1825 Connecticut Ave., NW Washington, DC 20009-5721 [www.fantaproject.org](http://www.fantaproject.org). Retrieved on Sept.02th , 2007.
- FAO. (1998) ( <http://www.fao.org/ag/AGA/AGAH/PD/pages/tick01.htm>).
- Felius, M. (1985). Genus *Bos*: Cattle Breeds of the World. *Rahway, NJ*: MSD Agrovvet.
- Fernandes F F. (2001) Toxicological effects and resistance to pyrethroids in *Boophilus microplus* from Goias. *Brazil Arq Bras Med Vet Zoot*; 53: 538-543.
- Fèvre E.M., Coleman P.G., Odiit M.D., Magona J., Welburn S.C., Woolhouse M.E.J., (2001). The origins of a new sleeping sickness outbreak (caused by *Trypanosoma brucei* infection) in eastern Uganda. *The Lancet* 358:625-628.
- Fivaz, B.H., and Norval, R.A.H. (1990) Immunity of the ox to the brown ear tick, (*Rhipicephalus appendiculatus*). *Experimental and applied Acrology*, 8: 51-63.
- Francis, J and Little, D. A. (1964). Resistance of droughtmaster cattle to tick infestation and babesiosis. *Australian Veterinary Journal*, 40; 247-253.
- Ford, J and Katondo, J (1976). The description of climatic conditions in Uganda. *Atlas of Uganda*, 6<sup>th</sup> edition, department of Lands and surveys, Uganda, pp16-21.
- García-García JC, Montero C, Redondo M, Vargas M, Canales M, Boue O, Rodríguez M, Joglar M, Machado H, González IL, (2000) Control of ticks resistant to immunization with Bm86 in cattle vaccinated with the recombinant antigen Bm95 isolated from the cattle tick, *Boophilus microplus*. *Vaccine*. 18 (21):2275-2287.

- George J E.** (2000) Present and future technologies for tick control. *Ann N Y Acad Sci.*;916:583–588.
- George J E, Pound J E, Davey R B** (2004). Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology*; 129:S353-66
- Gitau G K, McDermott J J, Waltner-Toews D, Lissemore K D, Osumo J M, Muriuki D.**(1994). Factors influencing calf morbidity and mortality in smallholder dairy farms in Kiambu district of Kenya. *Preventive Veterinary Medicine*, 21:167–177.
- Gitau GK, Perry BD, Katende JM, McDermott JJ, Morzaria SP, Young AS.**( 1997). The prevalence of serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a District, Kenya; a cross-sectional study. *Preventive Veterinary Medicine*, 30:95–107.
- Gitau, G. K., McDermott, J. J., Katende, J. M., O'Callgham, C. J., Brown, R.N., Perry, B.D.,** (2000). The differences in the epidemiology of theileriosis on smallholder dairy farms in contrasting agro-ecological and grazing strata of highland Kenya. *Epidemiology and Infection*, 124: 325-335.
- Graf J.F, Gogolewski R, Leach-Bing N, Sabatini G.A, Molento M.B, Bordin E.L, Arantes GJ.** (2004) Tick control: an industry point of view. *Parasitology*. 129:S427–S442.
- Graham O H, and Hourrigan J L.** (1977) Eradication programs for the arthropod parasites of livestock. *Journal of Medical Entomology*. 13: 629–658.
- Gray, J. S.,** (1985). Ticks: their economic importance and methods of control. *Outlook on Agriculture*, 14(3): 136-142.
- Henning, M. W.** (1932). Animal Diseases in South Africa. 1st Ed, *Central News Agency Ltd., Cape Town*, 298-329.
- Heweston, R.W.,** (1968). Resistance of cattle to cattle tick, *Boophilus microplus* 11. The inheritance of of cattle tick to experimental infestations. *Australian journal of Agricultural Research*, 19: 497-505.
- Hidebrandt, D.** (1981). The organ and vascular pathology of Babesiosis. In: Ristic, M. and Kreier, J. P. (Eds), *Babesiosis. Academic Press Inc.*, 459-473.
- Irvin, A. D., Cunningham, M.P.** (1981). Theileria infections in *Rhipicephalus appendiculatus* ticks collected in the field. Advances in the control of Theileriosis. Proceedings of the International Conference held in Nairobi, on 9th to 13th Feb. 1981. Martinus, Nijhoff publication, The Hague, Netherlands. Pp63-65.
- Jacobsen, P** (1983). East Coast Fever as a cause of calf mortality in Zanzibar. *Tropical Animal Health and Production*, 51: 43-46.

- Jenkins M C (2001). Advances and prospects for subunit vaccines against protozoa of veterinary importance. *Vet Parasitol.* **101**:291–310.
- Johnston, T.H. and Bancroft, M.J. (1918). A tick resistant condition in cattle. *Proceeding of the Royal Society of Queensland*, **30**: 219-317.
- Jongejan, F., Perry, B.D., Moorhouse, P.D.S., Musisi, F. L., Pegram, R. G., Snacken, M., (1988). Epidemiology of bovine Babesiosis and Anaplasmosis in Zambia. *Tropical Animals Health and Production*, **20**: 234-242.
- Jongejan, F and Uilenberg, G. (2004) The global importance of ticks. *Parasitology* **129**: S3-S14.
- Kaiser, N M., Sutherst, R. W., Bourne, A S., (1982). Relationship between ticks and zebu cattle in southern Uganda. *Tropical Animal Health and Production*, **14**: 63-74.
- Kambarage, D. M., (1995). East Coast Fever as a continued constraint to livestock improvement in Tanzania: A case study. *Tropical Animal Health and Production*, **27**: 145-149.
- Katende, J., Morzaria, S., Toye, P., Skilton, R., Nene , V., Nkonge, C., Musoke , A. (1998). An enzyme-linked immunosorbent assay for detection of *Theileria parva* antibodies in cattle using a recombinant polymorphic immunodominant molecule. *Parasitology Research*, **84**: 408-416.
- Kaufmann, J., Pfister, K. (1990). The seasonal epidemiology of gastrointestinal nematodes in N' Dama cattle in the Gambia. *Veterinary Parasitology*, **37**: 45-54.
- Kaufmann, J., Dwinger, R.H., Hallebeck, A., Van Dijk, B, Pfister. K (1992). The interaction of *T. congolence* and *Haemonchus* infection in trypanotolerant N'dama cattle. *Veterinary Parasitology*. **43**.157-170.
- Kelley R B. (1943). Zebu cross cattle in Northern Australia. An ecological experiment. *CSIR Australia Bulletin*.
- Kiptoon, J.C., Masha, J. B., Shatry, A. M ., Wolff, W. A. (1983). The clinical signs of East Coast Fever (bovine *Theileria parva* infection) examination of 96 suspected field cases of the disease. *Kenyan Veterinarian*, **7**: 9-11.
- Laffont CM, Alvinerie M, Bousquet-Melou A, Toutain PL. (2001) Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle. *International Journal Parasitology*. **31**(14):1687–1692.
- Latif A A. (1984). Resistance to natural tick infestations in different breeds of cattle in the Sudan. *Insect Science and Application* **5**:95-97.
- Latif AA, Pegram RG. (1992) Naturally acquired host-resistance in tick control in Africa. *Insect Sci & Appl.*; **13**:505–513.

- Latif A A, Punyua D K, Nokoe S and Capstick P B. (1991). Tick infestations on zebu cattle in Western Kenya: Individual host variation. *Journal of Medical Entomology*, **28**:114-121
- Latif, A. A., Rowlands, G. J., Punyua, D. K. Hassan, S M. Capstick, P B. (1995). An epidemiological study of tick-borne diseases and their effects on productivity of Zebu cattle under traditional management on Rusinga Island, Western Kenya. *Preventive Veterinary Medicine*, **22**: 169-181.
- Lawrence, J A, Musisi, F. L., Mfitilodze, M. W., Tjornehoj, K., Whiteland, A. P., Kafuwa, P. T., Chamambala, K. E. (1996). Integrated tick and tick-borne diseases control trials in crossbred dairy cattle in Malawi. *Tropical Animal Health and Production*, **28**: 280-288.
- Lightfoot, C J., and Norval R. A. I (1982) Tick problems in the wild life in Zimbabwe. Factors influencing the occurrence and abundance of *R. appendiculatus*. *Zimbabwe Veterinary Journal* **13**: 11-20
- Losos, G. J. (1986). Infectious Tropical Diseases of Domestic Animals. 1st edition, Churchill Livingstone Inc., New York.
- MacLeod, J. (1975). Apparent host selection by some African tick species. *Oecologia*, **3**:359-370.
- Maritim, A. C., Kariuki, D.P., Young, A.S. and Mutugi, J.J., (1989). The importance of a carrier state of *T. parva* on the epidemiology of theileriosis and its control by immunization. In: *Theileriosis in eastern, central and southern Africa, Preceedings of A workshop on ECF Immunization in Lilongwe, Malawi*. ILRAD, Nairobi . Ed, T.T.Dolan, 121-128
- Magona, J. W., (2004a). Point of care diagnosis of bovine trypanosomosis, tick-borne diseases and helminthosis with emphasis on portable anaemia detection devices and decision support system. *PhD thesis submitted to Faculty of Veterinary Medicine, University of Glasgow*. Pp 189
- Magona, J. W., Mayende, J.S.P., (2002). Occurrence of concurrent trypanosomosis, theileriosis, anaplasmosis and helminthosis in Friesian, Zebu and Sahiwal cattle in Uganda. *Ondestepoort Journal of Veterinary Research*, **69**,133-140.
- Magona, J. W., Walubengo, J., Odimim, T. J., (2004b). Differences in susceptibility to trypanosome infection between Nkedi Zebu and Ankole cattle, under field conditions in Uganda. *Annals of Tropical Veterinary Medicine and Parasitology*, **98**: 785-792.
- Matthysse, J G., Colbo, M. H., (1987). The ixodid tick of Uganda. Entomolical society of America, college park, MD
- Martin S W, Meek H A and Willeberg P (1987) Veterinary epidemiology: *Principles and Methods*. Iowa State University Press, Ames, Iowa. P. 45.

- Matoyelo, J. A., Gwakisa, P.S., Gwamaka, M., Chilongola, J., Silayo, R. S., Mtenga, K., Maselle, M. and Kambarage, D.M. (2002). Induction of acquired immunity in Pastoral Zebu cattle against ECF after natural infection by early diagnosis and early treatment (EDET). *Journal of Applied Research in Veterinary Medicine*, 1(2): 136-142.
- Mattioli, R. C., Bah, M., Kora S., Cassama, M., Clifford, D. J. (1995). Susceptibility to different tick genera in Gambian N'Dama and Gobra Zebu cattle exposed to naturally occurring tick infestations. *Tropical Animal Health and Production*, 27: 95-105.
- Mattioli, R.C., Pandey, V.S., Murray, M., Fitzpatrick, J.L.(2000). Immunogenetic influences on tick resistances in African cattle with particular reference to trypanotolerant N'Dama (*Bos taurus*) and trypanosusceptible Gobra Zebu (*Bos indicus*) cattle. *Acta Tropica*. 75(3): 263 - 272.
- Matthysse, J.G., Colbo, M.H. (1987). The ixodid ticks of Uganda. *Entomological society of America*, College Park, Maryland, 426pp.
- Matthewson MD, Baker JAF.( 1975) Arsenic resistance in species of multihost ticks in the Republic of South Africa and Swaziland. *Journal of South African Veterinary Association*. 46, 341-344.
- Millar JA, Davey RB, Oehler DD, Pound JM, George JE.( 2001) The Ivermectin was delivered orally in the case of *Boophilus annulatus* on cattle in South Texas. *Journal of Economic Entomology*. 94:1622-1627.
- Mohler, JR. (1906) The Cattle Tick in Its Relation to Southern Agriculture. Washington D.C: *United States Department, Agriculture Farmer's Bulletin*, P. 258.
- Moll, G., Lohding, A., Young, A.S. and Leitch, B.L. (1986). Epidemiology of theileriosis in calves in an endemic area of Kenya. *Veterinary Parasitology* 19:255-273.
- Moloo, S. H., Rowland, G. J., Thorpe, W., Gettinby, G., Perry, B.D. (2001) A longitudinal study of disease incidence and case fatality risks on smallholder dairy farms in coastal Kenya. *Preventive Veterinary Medicine*, 52: 17-29.
- Moran, M., (1996). Epidemiology of ticks and Tick-borne diseases in Uganda: Future Research needs and priorities. In: "Epidemiology of ticks and Tick-borne diseases in eastern, central, and southern Africa". *Proceeding of a workshop held in Harere*, 12-13<sup>th</sup> March 1996.
- Morrison W. I., McKeever D. J., (2006) Current status of vaccine development against theileria parasites. *Parasitology* 133 suppl. S168-87.
- Morzaria, S P., Katende, J., Musoke, A., Nene, R., Skilton, R., Bishops, R. (1999). Development of sero-diagnostic and molecular tools for the control of important tick-borne pathogens of cattle in Africa. *Parasitologia* 41, 73-80

- Mulci, C. M., Rege, J. E. O., Kruska, R. (1989). An examination of the incidences of East Coast Fever (ECF), Anaplasmosis and Babesiosis in the bovine in Kabete area of Kiambu districts of Kenya. *Bulletin of Animal Health and Production in Africa*, **37**, 213-216.
- Mulenga A, Sugimoto C, Onuma (2000). Issues in tick vaccine development: identification and characterization of potential candidate vaccine antigens. *Microbes Infect.*(2) **11**,1353-61.
- Mukhebi, A.W., Perry, B.D. and Kruska, R. (1992). Estimated economics of theileriosis control in Africa. *Preventive Veterinary Medicine*, **12**, 73-85.
- Mwase, E T.;Pegram, R G.; Mathers, T N. (1990) New Strategies for Controlling Ticks. In: Curtis CF., editor. *Appropriate Technology in Vector Control*. FL, USA: Boca Raton;. pp. 93-102.
- NARO/Livestock System Research Programme (LSRP) Report (1999). Ministry of Agriculture Animal Industry and Fisheries (MAAIF), Entebbe, Uganda.
- Ndi, C., Bayemi, P. H., Ekue, F. N., Tarounga, B. (1991). Preliminary observations on tick and tick-borne diseases in the North West Province of Cameroun. In: Babesiosis and Anaplasmosis. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux* **44**, 263-265.
- Neitz, W.O. (1957). A consolidation of our knowledge of the transmission of tick-borne diseases. *Onderstepoort Journal of Veterinary Research* **27**:115-163.
- Newson, R.M and Chiera, J.W. (1986). Some effects of acquired resistance in cattle on population of *Rhipicephalus appendiculatus* in the field. In: *Ticks and tick borne diseases: Proceedings of An International Workshop on the Ecology of Ticks and Epidemiology of tick borne diseases, Nyanga, Zimbabwe*, ACIAR Proceeding No. 17 Australian centre for international Agricultural Research, Canberra, Ed, R.W., Sutherst, 72-75.
- Nolan J. (1990) Acaricide resistance in single and multi-host ticks and strategies for control. *Parasitology*; **32**:145-153.
- Norval, R.A.I., Fivaz, BH., Lawrence, JA., Dailleccourt, T. (1983). Epidemiology of tick borne diseases of cattle in Zimbabwe. I Babesiosis. *Tropical animal health and production*, **15**: 87-94.
- Norval, R.A.I., Fivaz, BH., Lawrence, JA., Brown, AF (1984). Epidemiology of tick borne diseases of cattle in Zimbabwe. III *T. parva* gp. *Tropical animal health and production*, **17**: 19-28.
- Norval, R.A.I, Lawrence JA, Young AS, Perry BD, Dolan TT, Scott J.( 1991) *Theileria parva*: influence of vector, parasite and host relationships on the nature and distribution of theileriosis in southern Africa. *Parasitology*.; **102**:247-356.

- Norval, R. A. I, Perry, B. D. Young, A S. (1992). The Epidemiology of Theileriosis in Africa. Academic press, London.
- Norval, R.A.I., Sutherst, R.W., Kurki, J., Gibson, J.D. and Kerr, J.D. (1988). The effect of the brown ear tick *Rhipicephalus appendiculatus* on the growth of Sanga and European breed cattle. *Veterinary Parasitology* 30:149-164.
- Ocaido, M., Siefert, L. and Baranga, J., (1996) Disease surveillance in mixed livestock and game areas around Lake Mburo National Park in Uganda. *South African Journal of Wildlife Research*, 26: 133-135.
- Ocaido, M., Otim, C P., Okuna, N.M., Erume, J., Ssekitto, C.M.B., Wafula, R .Z .O., Kakaire,D.W., Walubengo, J and Monrad, J. (2005). Socio-economic and livestock disease survey of agro-pastoral communities in Serere County, Soroti District, Uganda. *Livestock Research for Rural Development. Volume17, Art. 93*. Retrieved on September 6, 2007.  
<http://www.cipav.org.co/lrrd/lrrd17/8/ocai17093.htm>
- Ocaido, M., Otim, C P., Okuna, N.M., Erume, J., Ssekitto, C.M.B., Wafula, R .Z .O., Kakaire, D.W., Okello-Obwangmoi, Musisi G, Okure S., Monrad, J. Ebiaru W (2003). Control of trypanosomosis and tick borne diseases in agro pastoral areas in Uganda through community participation: A report of two phases. *Proceeding of the LSRP Annual Scientific Workshop*. Kampala Uganda
- Okello-Onen, J (1995), Cattle dynamics and the impact of tick control on productivity of indigenous cattle under ranch conditions in Uganda. *PhD thesis, Makerere University, Kampala*, pp184
- Okello-Onen J, Tukahirwa EM, Ssenyonga GSZ, Perry BD, Katende JM, Musisi G, and Mwayi MT. (1994). Epidemiology of *Theileria parva* under ranch conditions in Uganda. In: Rowlands GJ, Kyule MN and Perry BD editors. *Proceedings of the 7th International Symposium on Veterinary Epidemiology and Economics*, Nairobi, 15–19 August. *The Kenyan Veterinarian*. (Special Issue). 18(2):362–365.
- Okello-Onen, J., Heuer, C., Perry, B. D., Tukahirwa, E. M., Ssenyonga, G. S. Z., Heinonen, R., Bode, E. (1996). Evidence of endemic stability of cattle to East Coast Fever on a Commercial ranch in Uganda. In: *Proceedings of the second conference on tick-borne Pathogens at the host vector interface: A global perspective* held at Kruger, South Africa, pp. 146-152.
- Okello-Onen, J., Rutagwenda, T., Unger, F., Bohle, W., Musinguzi, C., Mwayi., Erima, S., Musisi G. (1998). The status of ticks and tick-borne diseases under pastoral farming system in Uganda. In: *Proceedings of the ninth International Congress of Parasitology*, Makuhari Messe, Chiba, Japan, August 24th -28th pp. 537-541.
- Okello-Onen, J., Tukahirwa, E. M., Perry, B. D., Rowland, G. J., Nagda, S. N., Musisi, G., Bode, E., Heinonen, R., Mwayi, W., Opuda-Asibo, J. (1999).

Population dynamics of ticks on indigenous cattle in a pastoral dry to semi-arid rangeland zone of Uganda. *Experimental and Applied Acarology*, **23**: 79-88

**Okello-Onen, J., Tukahirwa, E. M., Perry, B. D., Rowland, G. J., Nagda, S. N., Musisi, G., Bode, E., Heinonen, R., Mwayi, W., Opuda-Asibo, J.** (2003). The impact of tick control on the productivity of indigenous cattle under ranch conditions in Uganda. *Tropical Animal Health and Production*, **35**:237-247.

**Okiria R., Okuna, NM., Magona, JW., Mayende, JSP** (2002). Sustainability of tsetse control by subsequent treatment of 10% of a previously treated Ugandan cattle population with 1% w/v of deltamethrin. *Tropical Animal Health and Production*, **34**: 105 – 114.

**Okoth J.O. Kimura, E K., Kapaata R.,** (1993), A new approach to community participation in tsetse control in Busoga sleepingsickness focus, Uganda. In *OAU/STRC*, 209.

**Okoth J.O. Omare Okurut A., Ebonyu F** (1999). The use of theatre to mobilise and sensitize rural communities to participate in tsetse control in Bugiri district, Uganda: A case study In *OAU/STRC*, 480-483.

**Oloo, G O., Olet, P A Olaho-Mukhani W** (1999). Tsetse and trypanosomosis control methods in Kenya. In *IAEA*, 19-22.

**Omuse, J. K.,** (1978). A comparative haematological picture of field cases of East coast fever, Anaplasmosis and Babesiosis in bovines around Kabete. In; Wilde, J. K. H. (Ed), *Proceedings of an international conference on tick-borne diseases and their vectors. Centre for tropical Veterinary Medicine*, 1976, Edinburgh, UK. pp181-187.

**Otim, C. P.,** (1997). Aspects of tick immunobiology, *A PhD thesis submitted to the Department of Biological Sciences, University of Salford, Salford, UK*. Pp167

**Otim, C. P.,** (2000) Advances in disease control: Ticks and tick-borne diseases. *Uganda Journal of Agricultural Sciences*, **5**: 79-85.

**Otim, C.P., Kakaire, D.W., Ssekitto C.M.B., Barasa, R., and Wejuli, P.** (2005). Epidemiological implications of Tick-borne diseases in smallholder dairy production in southeastern Uganda following tsetse and Trypanosomosis control. *Proceedings of the 28th international conference on research and control of Trypanosomosis*. Addis Ababa. Ethiopia

**Otim, C P., M Ocaido, N M Okuna, J Erume, C Ssekitto, R Z O Wafula, D Kakaire, J Walubengo, A Okello, A Mugisha and J Monrad** (2004). Disease and vector constraints affecting cattle production in pastoral communities of Ssembabule district, Uganda. *Livestock Research for Rural Development* **4**: 10-16.

- Paling, R. W., Mpangala, C., Luttinhuizen, B Sibomana, G. (1991). Exposure of ankole and crossbred cattle to theileriosis in Rwanda. *Tropical Animal Health and Production*, **23**, 203-214.
- Perry B D.(1994) The role of epidemiology and economics in the control of tick-borne diseases of livestock in Africa. *The Kenyan Veterinarian*. **18**:7-10.
- Perry, B. D., Young, A. S. (1995). The past and future roles of epidemiology and economics in the control of tick-borne diseases of livestock in Africa: the case of theileriosis. *Preventive Veterinary Medicine*, **25**: 107-120.
- Petersen P.H. D.B. Ndumu, G.H. Kiwuwa, M.L. Kyomo, D.K.N. Semambo, G.J.Rowlands, S.N. Nagda & H. Nakimbugwe (2004) Characteristics of Ankole Longhorn cattle and their production environments in South Western Uganda: milk offtake and body measurements, *Animal genetic resources information 34 Food and Agriculture Organization of The United Nations Organisation*
- Potgieter, F. T., Stoltz, H. H. (1994). Bovine Anaplasmosis. In: Coetzer, J. A., Thompson, G. R and Tustin, R C. (Eds), *Infectious diseases of livestock with special reference to Southern Africa*. Volume I, Oxford University Press, Cape Town. pp 408-430.
- Rechav Y. (1987). Resistance of Brahman and Hereford cattle to African ticks with reference to serum gammaglobulin levels and blood composition. *Experimental Applied Acarology* **3**:219- 232.
- Rechav Y, Clarke, F.C., Els D A., Dauth, J. (1991). Development of resistance in laboratory animal to adult tick *R evertsi evertsi*. *Medical and Veterinary Entomolgy*, **5**: 29-34
- Rehacek, J (1965). Development of animal viruses and rickettsiae in ticks and mites. *Ann. Rev. Entomology*, **10**:1-24.
- Riberio, J.M.C., (1989). Role of saliva in tick/host interactions. *Experimental Applied Acarology* **7**:15-20.
- Rick R F. (1962). Studies on the reactions of animals to infestation with ticks. VI Resistance of cattle to infestation with the tick *Boophilus microplus*. *Australian Journal of Agricultural Research* **13**:532-551.
- Ristic, M. (1968). Anaplasmosis. In: Weinman, D., and Ristic, M., (Eds), *Infectious blood diseases of man and animals*, **II**. Academic Press Inc., New York, London
- Ristic, M. (1980). Anaplasmosis. In: Bovine medicine and surgery, Amstutz AE, (Ed). Santa Barbra, CA: *American Veterinary Publication*, pp 324-348.

- Shanahan GJ, and Hart RJ.** (1966) Change in response of *Lucilia cuprina* (Weid.) to organophosphorus insecticides in Australia. *Nature*. **212**(5069):1466–1467.
- Shannon D.** (1977). Field cases of East Coast Fever in grade cattle in Uganda. *Tropical Animal Health and Production*, **9**: 29-35.
- Solomon K R.** (1983). Acaricide resistance in ticks. *Advances in Veterinary Sciences and Comparative Medicine*.; **27**:273–296.
- Spickett A M** (1998). Acaricides and resistance. *Veterinary Ectoparasitology and Protozoology*. **1**:1–13.
- Spickett A M, Fivaz B H.** (1992) A survey of cattle tick control practice in the Eastern Cape Province of South Africa. *Onderstepoort Journal of Veterinary Research*. **59**:203–210.
- Stobbs T. H.,** (1966). The introduction of Boran cattle into an ECF endemic area. *East African Agricultural and Forestry Journal*, **31**:298-304.
- Sutherest R.W.** (1987). The role of models in tick control. In: *Proceedings of International Conferences on Veterinary Preventive Medicine and Animal Production Australian*, Australian Veterinary Association, Ed. by K.L. Hughes, 32-37.
- Ssenyonga, G. S. Z., Kakoma, I., Montenegro-James, S., Nyeko, P. J., Buga, J.** (1991). Anaplasmosis in Uganda. II, Prevalence of bovine Anaplasmosis in Uganda. *Annals of Tropical Medicine and Parasitology*, **85**: 305-308.
- Sseruga J.N, Jonsson N.N, Bock R.E, More S.J.** (2003) Serological evidence of exposure to tick fever organisms in young cattle on Queensland dairy farms. *Australian Veterinary Journal*. **81**:147–152.
- Tatchell R J.** (1986) Interactions between ticks and their hosts. In: Howell J M (ed), *Proceedings of the 6<sup>th</sup> International Congress on Parasitology, Australia, Brisbane*, 1986. Australian National University, Canberra Australia, 597-606
- Tatchell R J.**(1992) Ecology in relation to integrated tick management. *Insect Science and Application*. **13**:511–561.
- Tebele, N., Skilton, R.A., Katende, J., Wells, C. W., Nene V., McElwain, T., Morzaria, S. P., Musoke, A J.** (2000). Cloning characterisation, and expression of a 200 Kilodalton diagnostic antigen of *Babesia bigemina*. *Journal of Clinical Microbiology*, **38**, 2240-2247.
- Tellam RL, Kemp D, Riding G, Briscoe S, Smith D, Sharp D, Irving D, Willadsen P.** (2002) Reduced oviposition of *Boophilus microplus* feeding on sheep vaccinated with vitellin. *Vet Parasitol*. **103**:141–156.

- Theiler, A.** (1910). *Anaplasma marginale*. The marginal points in the blood of cattle suffering from specific disease. *Government Veterinary Bacteriology*, Transvaal. South Africa, 6-64.
- Tickcost** (1999), Economic impact of ticks and associated diseases on cattle: in Africa, Asia and Australia ILRI/ACIAR.
- Titus R G, Bishop J V, Mejia J S.** (2006). The immunomodulatory factors of arthropod saliva and the potential for these factors to serve as vaccine target to prevent pathogens transmission. *Parasite Immunology* 28:131-41.
- Trager, W.** (1939). Acquired immunity in ticks. *Journal of Parasitology*, 25: 57-81.
- Uilenberg, G.** (1981). Theileria species of domestic animals. In: *Advances in the control of theileriosis: Proceedings of an International conference at ILRAD, Nairobi*. Martinus Nijhoff Publishers, The Hague, Ed. A.D. Irvin, M.P. Cunningham and A.S. Young, 4-37.
- Utech K B, Wharton R H and Kerr J D.** (1978). Resistance to *Boophilus microplus* in different breeds of cattle. *Australian Journal of Agricultural Research* 29:885-895.
- Urquhart, G. M., Armour, J. L., Duncan, J. L., Jennings, F. W.** (1996). *Veterinary Parasitology*. 2nd edition, Blackwell Sciences Ltd, Oxford.
- Varda Shkap, Albertus J. de Vos, Erich Zwerygarth and Frans Jongejan.** (2007). Attenuated vaccine for tropical theileriosis, babesiosis and heartwater: the continuing necessity. *TRENDS in Parasitology* 23:391-458.
- Wagland, B.M** (1978). Host resistance to cattle ticks (*Boophilus microplus*) in Brahman (*Bos indicus*) cattle. II. The dynamics of resistance in previously unexposed and exposed cattle. *Australian Journal of Agricultural Research*, 29 395-400
- Waladde, S.M. and Rice, M.J.** (1982). The sensory basis of tick feeding behaviour. *Physiology of Ticks*. Pergamon Press, Eds. F.D. Obenchain and R. Galun, 71-118
- Wamukoya, J. P. O.** (1992). The status of tick infestation of livestock and tick control methods in Kenya. *Insect Science and its Applications*, 13, 665-670.
- Wharton R H.** (1967) Acaricide resistance and cattle tick control. *Australian Veterinary Journal*; 43(9):394-398.
- Wharton R H. Hary K R S, Wilkinison P R, Utech K B and Kelley B M** (1969). A comparison of cattle tick control by pasture spelling, planned dipping, and tick resistant cattle. *Australian Journal of Agricultural research* 20: 783-793.
- Wharton R H.** (1983). Tick-borne livestock diseases and their vectors. Acaricide resistance and alternative methods of tick control. *World Animal Review*, (FAO);36:34-41.

- Wharton R H and Roulston W J. (1970) Resistance of ticks to chemicals. *Annual Review of Entomology*. **15**(1):381-405.
- Willadsen P. (1980). Immunity to ticks. *Advances in Parasitology*, **18**:293-313.
- Willadsen P. (2001). The molecular revolution in the development of vaccines against ectoparasites. *Veterinary Parasitology*. **101**:353-367.
- Willadsen P, Kemp D H. (1988) Vaccination with concealed antigens for tick control. *Parasitology Today*. **4**:196-198.
- Wright, P.F., Nelson, E., Van Rooij, E.M. A., Lelenta M., Jeggo, M.H., (1993). The standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibodies in infectious disease diagnosis. *Revue scientifique et Technique Office Internationale de Epizootique* **27**: 435-450
- Yeoman, G. H., (1966). Field vector studies of epizootic East Coast Fever. 1. A quantitative relationship *Rhipicephalus appendiculatus* and the epizooticity of East Coast Fever. *Bulletin of Epizootic Diseases of Africa*, **41**:5-27.
- Young, A S., Branagan, D., Brown, C.G.D., Burrridge, M.J., Cunningham, M.P., Purnell, R.E. (1973). Preliminary observations on the theilerial species pathogenic to cattle isolated from buffalo (*Syncerus caffer*) in Tanzania. *British Veterinary Journal*, **129**: 382-389.
- Young, A S., Grocock, C M., Karuiki, D P. (1988). Integrated tick control of ticks and tick-borne diseases of cattle in Africa. *Parasitology* **96**: 403-432.