

**PREVALENCE AND CONTROL OF ANAPLASMOSIS IN CATTLE:
A CASE OF GULU DISTRICT**

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DECLARATION

I Otimong Joseph do here by declare that this work is purely mine and has not been presented for a degree award in any university.

Signature  Date 13.12.2024

APPROVAL

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DEDICATION

I hereby dedicate my thankful feelings to the closer people who are always on my side from day one of being a student at Gulu University; brother Odeke Nathan, for the financial support during the entire course. My supervisors for their academic support; Assoc. Professor Elly Kurobuza Ndyomugyenye and Dr. Aliro Tony, may the almighty continue protecting and guiding you abundantly. Special thanks go to the acting DVO; Mr. Opio Alfred, of Gulu district, research assistants and cattle farmers for their merciful support.

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TABLE OF CONTENTS

DECLARATION.....	i
APPROVAL	ii
DEDICATION.....	iii
ACKNOWLEDGEMENT.....	iv
TABLE OF CONTENTS	v
LIST OF FIGURES	ix
LIST OF TABLES	x
ACRONYMS	xi
ABSTRACT.....	xiii
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 Background.....	1
1.2 Statement of the problem.....	3
1.3. General Objective	4
1.4 Specific objectives	4
1.5 Research questions.....	4
1.6 Conceptual framework of the case study	4
1.7 Significance of the study.....	5
1.8 Scope of the study.....	6
1.8.1 Geographical scope.....	6
1.8.2 Content scope.....	6
1.8.3 Time scope	6
CHAPTER TWO	7
LITERATURE REVIEW	7

2.1 Prevalence and distribution of Anaplasmosis in cattle	7
2.2 Life cycle of Anaplasma in cattle	8
2.3 Transmission of Anaplasmosis in cattle	9
2.3 Reservoir hosts of Anaplasmosis	11
2.4. History of Anaplasmosis in cattle	11
2.5 Clinical signs of Anaplasmosis in cattle	12
2.6 Diagnosis of Anaplasmosis in cattle	12
2.7 Risk factors associated with Anaplasmosis in cattle.....	14
2.8 Control measures of Anaplasmosis in cattle	14
2.8.1 Grazing management of cattle	14
2.8.2 Chemotherapeutic or prophylactic treatment of cattle.....	15
2.8.3 Use of acaricide against ticks in cattle.....	15
2.8.4 Use of vaccines against Anaplasmosis in cattle.....	16
2.8.5 Use of medicinal plants for treatment of Anaplasmosis in cattle	16
CHAPTER THREE	18
MATERIALS AND METHODS	18
3.1 Study area.....	18
3.2 Study animals.....	20
3.3 Study design.....	20
3.3.1 Sample size determination	21
3.3.2 Selection of sub-counties and respondents	21
3.4 Data collection	22
3.4. 1 Blood sample (determination of the prevalence of Anaplasmosis in cattle)	22
3.4.1.1 Collection of blood samples.....	22
3.4.1.2 Laboratory analysis of blood	22

3.4.2 Determination of risk factors associated with Anaplasmosis in cattle	23
3.4.3 Assessment of control measures for Anaplasmosis in cattle	23
3.5 Statistical data analysis	23
3.6 Ethical considerations	24
CHAPTER FOUR.....	25
RESULTS	25
4.1 Determination of the prevalence of Anaplasmosis in cattle	25
4.1.2 Socio-demographic characteristics of cattle farmers	25
4.2 Risk factors associated with Anaplasmosis in cattle in Gulu district	26
4.3 Control measures of Anaplasmosis infection in cattle in Gulu district.	28
4.3.1 Spraying	28
4.3.1.1 Frequency of spraying.....	28
4.3.1.3 Types of acaricides used for spraying cattle	28
4.3.1.4 Alternation of acaricides	29
4.3.2 Chemotherapeutic control of Anaplasmosis in cattle	29
4.3.2.1 Who treats the cattle?.....	29
4.3.2.2 Drugs used in the treatment of Anaplasmosis infection	29
4.3.2.3: Rationale of the dosage of drug use.....	30
4.3.2.4 Use of ethno medicinal drugs by cattle farmers.....	30
CHAPTER FIVE	31
DISCUSSION OF RESULTS	31
5.1 Prevalence of Anaplasmosis in cattle in Gulu district	31
5.2 Associated risk factors of Anaplasmosis in cattle in Gulu district	31
5.3 Control measures of Anaplasmosis infection among cattle in Gulu district.....	33
CHAPTER SIX	35

SUMMARY, CONCLUSION AND RECOMMENDATION.....	35
6.1 Summary	35
6.2 Conclusions.....	35
6.3 Recommendation	36
REFERENCES.....	37
APPENDICES	47
Appendix 1: Summary of laboratory information on cattle in Gulu district.....	47
Appendix 2: Questionnaire	49
Appendix 3: Ethical clearance	52
Appendix 4: COVID-19 risk management plan for the study	54
Appendix V: Plagiarism Check	57

LIST OF FIGURES

Figure 1. 1: Conceptual framework of the case study	5
Figure 1. 2: Diagrammatic representation of the life cycle of Anaplasma in cattle (Kocan, 1999)	9
Figure 1. 3: Anaplasma on blood smear -Giemsa stain (Dhruba, 2020).....	13
Figure 3. 1: Map of Uganda showing the location of Gulu district (UBOS, 2014).....	19
Figure 3. 2: Map of Gulu district showing location of sub-counties where the study was conducted (UBOS, 2021).....	20

LIST OF TABLES

Table 4. 1 Prevalenceof Anaplasmosis in cattle in Gulu district	25
Table 4.2 Risk factors associated with Anaplasmosis in cattle	27
Table 4.4 Spraying of cattle	28
Table 4.5 Frequency of spraying animals against Anaplasmosis infection	28
Table 4.6 Types of acaricides used for spraying cattle	28
Table 4.7 Alternation of acaricides	29
Table 4.8 who treats the cattle?.....	29
Table 4.9 Drugs used in treating Anaplasmosis infection	30
Table 4.10 Rationale of the dosage of drugs used	30
Table 4.11 Use of ethno medicinal drugs by farmers	30

ACRONYMS

AHT	Animal Health Technicians
A. centrale	Anaplasmosis centrale
A. marginale	Anaplasmosis marginale
CAO	Chief Administrative Officer
DVO	District Veterinary Officer
ECF	East Coast Fever
EDTA	Ethylenedianetetracetic Acid
E.g	For example
cELISA	Competitive enzyme-linked immunosorbent assay
EVM	Ethno Veterinary Medicine
FAO	Food and Agriculture Organization
g	grams
GDP	Gross Domestic Product
GUREC	Gulu University Research Ethics Committee
Hrs	Hours
ILRI	International Livestock Research Organization
LC1	Local Council One
Mls	Milliliters
NCHHS	National Census and House Hold Survey
MSP1	Major Surface Protein 1
MSP5	Major Surface Protein 5
MOH	Ministry Of Health
NGOs	Non-Government Organizations

OIE	World Health Organization for Livestock
OR	Odds Ratio
PCV	Packed Cell Volume
RBCs	Red Blood Cells
R	Rhiciphellus
SPSS	Statistical Package for Social Sciences
SOPs	Standard Operational Procedures
UBOS	Uganda Bureau of Statistics
USA	United States of America
%	Percentage
PCR	Polymerase Chain Reactions
qPCR	duplex Polymerase Chain Reactions
RFLP	Restriction fragment length polymorphisms
RLBH	Reverse line-Blot Hybridization
LAMP	loop-mediated isothermal amplification

ABSTRACT

Tick-borne diseases present a major economic burden to communities across East Africa, especially Anaplasmosis affecting livestock production in Uganda. This study aims at determining the prevalence, risk factors and common control measures of Anaplasmosis in cattle, Gulu district. Samples of blood were collected from 384 cattle in a period of three months and examined microscopically using Giemsa staining procedures. The overall prevalence of cattle with Anaplasmosis was 17.97%. Locality, cattle not injected during treatment and cattle breeds had no significant difference ($P>0.05$) with prevalence of Anaplasmosis. However, higher prevalence of Anaplasmosis was recorded among cross breed (26.47%) and least in Small East African zebu breeds (16.13%) of cattle. Body condition, tick infestation, cattle of age group less than one year (28.95%) and previously injected with needles observed variations that were statistically significant ($P<0.05$), with highest prevalence's of 59.65%, 20.54% and 28.95% respectively. There was statistically significant ($P<0.05$) difference in risk of infection, indicating that different factors influenced the prevalence of Anaplasmosis in the study area. The major control measures were mainly; spraying with Amitix (62%), treatment of cattle using oxytetracycline (77%), with inappropriate acaricide and other drug usage, inadequate veterinary staff and infrequent spraying of cattle. In conclusion, study showed that endemic stability of Anaplasmosis in Gulu district was due to inappropriate control measures employed by cattle farmers. The study recommends that cattle farmers should be sensitized on rationale and effective use of acaricides during spraying and drugs during treatment of infected cattle.

Key words: *Anaplasmosis, Cattle, Control measures, Prevalence, Risk factors,*

CHAPTER ONE

INTRODUCTION

1.1 Background

Anaplasmosis is among the important tick borne diseases affecting livestock globally, and of economic burden due to its higher mortality rate and morbidity (Mubashir *et al.* 2022). In Sub-Saharan Africa, Anaplasmosis is of endemic stability due to its pathogen complexity with the environment, host and herd status that are modified by community activities (Tawana *et al.* 2022; Kasozi *et al.* 2014). Its prevalence is generally high among exotic dairy breeds of cattle (Costa *et al.* 2016b).

Anaplasmosis infection is mostly rampant in countries of East Africa; Burundi (Nyabongo *et al.* 2021), Uganda (Kasozi *et al.* 2021), causing economic losses in respect to mortality, reduced carcass weight, abortion, reduced milk production and veterinary costs (Aubry & Geale, 2010; Kocan. 2009). In Tanzania, majority (80%) of cattle are susceptible to tick-borne diseases, contributing an economic loss of US\$ 248 million and 0.92 million mortality rate annually (Kivaria, 2006).

In Uganda cattle population is 14.6 million with constant rate of 10% of exotic cattle breeds contributing 4.2% to the National GDP (UBOS 2019). Studies have shown that in Uganda, Anaplasmosis prevalence rates second to East Coast Fever among the tickborne diseases (Byaruhanga *et al.* 2018; Mugisha *et al.* 2005; Ocaido *et al.* 2009a; Muhanguzi *et al.* 2014b; Rubaire *et al.* 2004), with high mortality rate of adult cattle within the country (Ocaido *et al.* 2005). Studies, indicated overall seroprevalence of Anaplasmosis in different regions in Uganda; (58-62)% Central, South-Western and North-Eastern (Weny *et al.* 2017a) and 83% North-Western (Byaruhanga *et al.* 2018), thus showing high potential of endemic

stability. Anaplasmosis leads to high financial losses, linked to abnormal treatment costs and high prices of acaricides leading to high mortality rate of cattle, because most farmers may not be able to purchase the acaricides and buy drugs (Okello *et al.* 1998; Ocaido *et al.* 2009b). Anaplasmosis affects not only cattle but also other ruminant animals like: small ruminants, buffaloes (Tumwebaze *et al.* 2020). Its common clinical signs are; fever, abortion, lacrimation, dysponia, loss of weight, icterus, nervousness and hypoxia (Akure, 2019). Transmission can be by ticks and formites, instruments like needles, surgical knife, burdizo especially when conducting activities such as; tattooing, tagging, castration and dehorning (Mugisha *et al.* 2005; (Akure, 2019) and placental transmission through dam to calf (Costa *et al.* 2016a).

Anaplasmosis can be prevented by control of ticks and fleas that transmits the pathogen by use of acaricide (Byaruhanga, 2017; Kasaija *et al.* 2021a), chemotherapeutic treatment of cattle showing signs of the infection using antibiotics such as Imidocarb dipropionate, Oxytetracycline; vaccination of cattle using antigens inform of vaccines (Otim, 2000; Kocan 2003; Fuente *et al.* 2003); use of Ethno medicinal plants (Angwech *et al.*, 2011). However, all control measures have failed due to financial crisis of cattle keepers on weekly acaricides application (Irene *et al.* 2021). In addition, control measures have failed because most tick borne control programs are developed mainly for project European breeds without taking in to considerations the knowledge and perceptions of indigenous cattle keepers and the current control measures being used (Kasaija *et al.* 2021a).

Recent studies showed that farmers in Northern Uganda use ethno medicinal plants and chemotherapeutic drugs for treatment of tick-borne diseases and acaricide for ticks, information on prevalence, risk factors and control measures imposed by farmers against Anaplasmosis (Chenyambuga *et al.* 2010). Studies shows that tick-borne diseases varies with

intensity of management in ruminants (Angwech *et al.* 2011). Generally, there is insufficient data regarding prevalence, associated risk factors and control measures of Anaplasmosis in Gulu district. The data obtained will aid government programs during implementation of new technologies against infections as far as cattle production is concerned. *A. centrale*, is spread by the *African Rhipicephalus simus* (Kocan 2003b; Fuente, *et al.* 2003b), which is being used as live vaccine during its sub-acute stage of Anaplasmosis, for treatment of *A. marginale* in certain countries (Sakyi *et al.* 2015). Besides cattle, Anaplasmosis affects a wide range of hosts including small ruminants (Christina & Batiha, 2020), buffalos and other wild ruminants (Tumwebaze *et al.*, 2020). Studies reveal that Anaplasmosis in cattle depends on seasonal changes, during wet seasons than dry season due to high reproductivity rate of hematophagous insects like; *Tabanus*, *Stomoxys calcitrans*, *Culicoides* and mosquitoes, which are mechanical transmitters of pathogens (Kasaija *et al.* 2021).

1.2 Statement of the problem

In Uganda, research studies still prove Anaplasmosis as a constraint to cattle productivity, causing economic losses to farmers, such as; morbidity, high mortality rate and high treatment costs (Ssekitto & Mwayi, 2003; Kasaija *et al.* 2021). Studies, conducted in different regions of Uganda have shown high potential of endemic stability of Anaplasmosis; South-western (58%), North-eastern (62%) (Weny *et al.* 2017a) and North-western (83%) (Byaruhanga *et al.* 2018), which could be attributed to differences in ecological zones, seasonal changes, cross breeding of animals with no routine veterinary care and production systems (Rubaire *et al.* 2006). However, the body condition score of cattle in Gulu district is poor suggesting high prevalence of tick borne diseases, particularly Anaplasmosis (Kasozi *et al.* 2014). Despite its economic importance, the body condition of cattle still remains poor, suggesting high prevalence of tick borne diseases, especially Anaplasmosis which could be

attributed to insufficient published research on the prevalence and control of Anaplasmosis since the control measures applied are not comparable to the magnitude of the disease problem (Chenyambuga *et al.* 2010). This information gap hinders the development of effective control strategies, exacerbating the problem and perpetuating poverty among cattle keepers. Therefore, this study focuses on addressing information gaps pertaining the prevalence, risk factors and control measures of Anaplasmosis that would be of use for gaining insights on the burden of the infection in the district, such that informed solutions and policies can be designed.

1.3. General Objective

To assess the prevalence and control measures of Anaplasmosis among cattle in Gulu district

1.4 Specific objectives

- 1) To determine the prevalence of Anaplasmosis in cattle
- 2) To determine the risk factors associated with Anaplasmosis in cattle in Gulu district
- 3) To assess the control measures of Anaplasmosis infection in cattle in Gulu district

1.5 Research questions

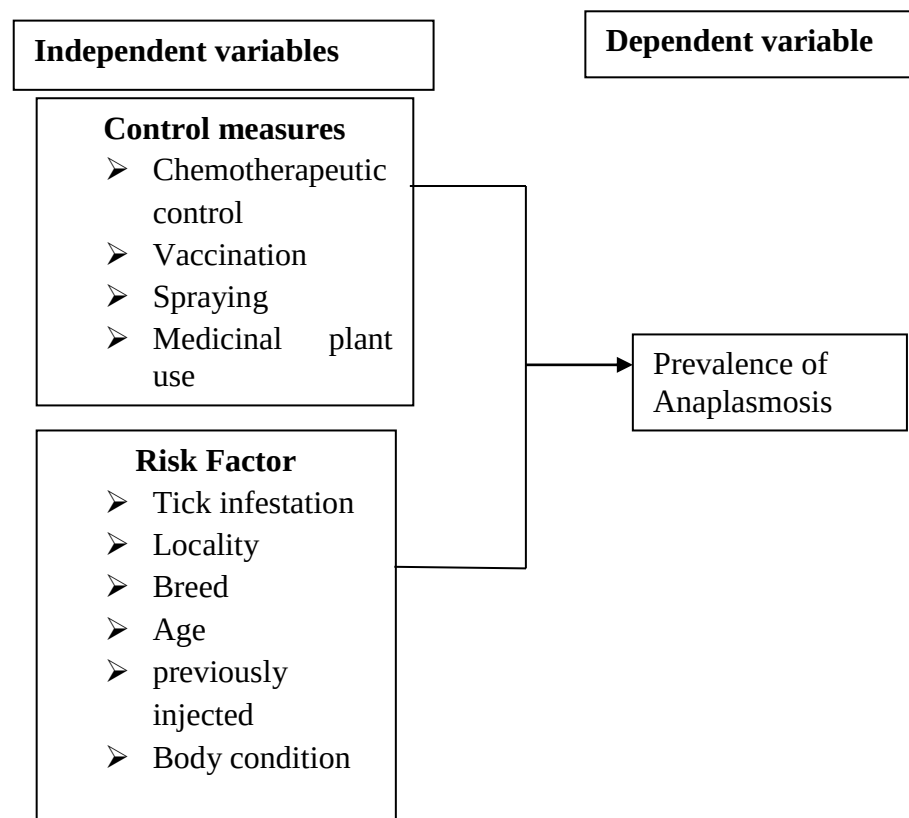
- 1) What is the prevalence of Anaplasmosis in cattle in Gulu district?
- 2) What risk factors are associated with prevalence of Anaplasmosis in cattle in Gulu district?
- 3) What are the control measures of Anaplasmosis infection in cattle in Gulu district?

1.6 Conceptual framework of the case study

In this research study, the independent variables are; control measures (chemotherapeutic drug use, vaccination, acaricides use and use of medicinal plants) and associated risk factors

such as breed, locality, age and tick infestation directly affect the prevalence of Anaplasmosis among cattle in Gulu district as shown in the figure 1.1 below;

Figure 1. 1: Conceptual framework of the case study



1.7 Significance of the study

The results of this study will provide an updated data on prevalence of Anaplasmosis, risk factors and control measures used by cattle farmers against the infection in Gulu district. This information will aid district officials, government, NGOs and researchers in designing strategic control measures of the infection that will contribute to poverty reduction through improvement of cattle production, increased income to farmers to purchase food items hence Zero hunger. Also it will promote government projects especially Parish Development Model (PDM), since there will total management of the infection, hence improving the livelihood of cattle farmers in the study area. Finally, improves farmer's decisions on cattle management, tick control, and disease prevention leading to more effective and efficient farming practices.

1.8 Scope of the study

1.8.1 Geographical scope

The study was conducted in Gulu district, specifically in four selected sub counties of Awach, Palaro, Paicho and Unyama.

1.8.2 Content scope

The study was specifically conducted on cattle farmers of herd size 5-10 animals, where blood samples were picked from selected cattle with pretested questionnaires inclusive. Therefore, there was involvement of laboratory work and survey in order to determine the prevalence of Anaplasmosis, risk factors and control measures used by cattle farmers against the infection.

1.8.3 Time scope

The study was carried out within a period of two months from September to October of 2023 during wet season.

CHAPTER TWO

LITERATURE REVIEW

2.1 Prevalence and distribution of Anaplasmosis in cattle

Anaplasmosis is a zoonotic tickborne disease affecting livestock production, leading to economic losses because of its ability to cause high morbidity and mortality rate (Mubashir *et al.* 2022), hence global concern. Anaplasmosis is highly rampant in Sub-Saharan Africa, due to its pathogen complexity with the environment, host and herd status that are modified by community activities (Tawana *et al.* 2022; Kasozi *et al.* 2014). Its prevalence is generally high among exotic dairy breeds of cattle (Costa *et al.* 2016b). In East Africa, the common causal agents of Anaplasmosis are; *Anaplasma marginale* and *Anaplasma centrale* which is spread by ticks called *Boophilus decoloratus* (Wesonga *et al.* 2010). Anaplasmosis is rampant most in East African countries such as; Burundi (Nyabongo *et al.* 2021), Uganda (Kasozi *et al.* 2021& Angwech *et al.* 2011),and Tanzania (Kivaria, 2006). In Tanzania, majority (80%) of cattle are susceptible to tick-borne diseases, contributing an economic loss of US\$ 248 million and 0.92 million mortality rate annually (Kivaria, 2006). causing economic losses in respect to mortality, reduced carcass(beef) weight, decreased milk production and abortion (Aubry & Geale, (2010; Kocan, 2009). In Uganda the cattle population is at 14.6 million (UBOS 2019), with constant rate of 10% of exotic cattle breeds contributing 4.2% to the National GDP (UBOS 2019). Studies have shown that in Uganda, Anaplasmosis ranks second to ECF, as one of the tick borne diseases hindering livestock production(Byaruhanga *et al.* 2018; Mugisha *et al.* 2005; Ocaido *et al.* 2009a; Muhanguzi *et al.* 2014b; Rubaire *et al.* 2004), with high mortalities within the country (Ocaido *et al.* 2005).

Studies, have found out overall seroprevalence in different regions of Uganda where central, South-western and North-eastern was (58-62%) (Weny *et al.* 2017a) and North-western was

(83%) (Byaruhanga *et al.* 2018), thus showing high potential of endemic stability. The disease is prevalent in the tropics and sub tropics where it stagnates cattle productivity by causing economic losses like; mortality, weight loss and abortion (Aubry & Geale, 2010 ; Kocan,2009). It's also prevalent during wet seasons than dry season due to high reproductivity rate of hematophagous insects, such as; ticks and fleas that are biological transmitters of pathogen (Byaruhanga *et al.* 2018).

Other than cattle, Anaplasmosis infection, affects a wide range of animal species; small ruminants, buffalos and other wild foregut fermenters (Christina & Batiha, 2020). Its transmitted mechanically by; tattooing, tagging, castration and dehorning(Aubry & Geale, 2010; Kocan, 2011), and biologically by tick vector called *Boophilus decoloratus*, (Costa *et al.* 2016).

2.2 Life cycle of Anaplasma in cattle

Life cycle of Anaplasma start by biting tick to infected animals and get the rickettsial pathogens by feeding on blood of the carrier's host as shown in Figure 1.2. Cattle infected with Anaplasmosis pathogen takes approximately 7-60 days to emerge depending on dosage infectiveness(Kocan, 2009). *A. marginale* is a strictly intra-erythrocytic microbe in which the infected erythrocyte contains a membrane-bound inclusion, called initial body, with (4-8) *rickettsia* (Kocan,2003; Fuente, *et al.* 2003b). During acute phase of pathogen, *A. marginale* undergoes replication through binary fission to form dense structures and others persist until it invades as much as 10⁹erythrocytes per ml of blood at 70% infection rate (Palmfjord, 2015). The infected erythrocytes cause hemolytic anemia and icterus with no haemoglobinaemia or hemoglobinuria (Palmfjord, 2015). It was revealed that 15% of infected erythrocytes can make an animal's clinical signs to be shown (Jaswal *et al.* 2015).

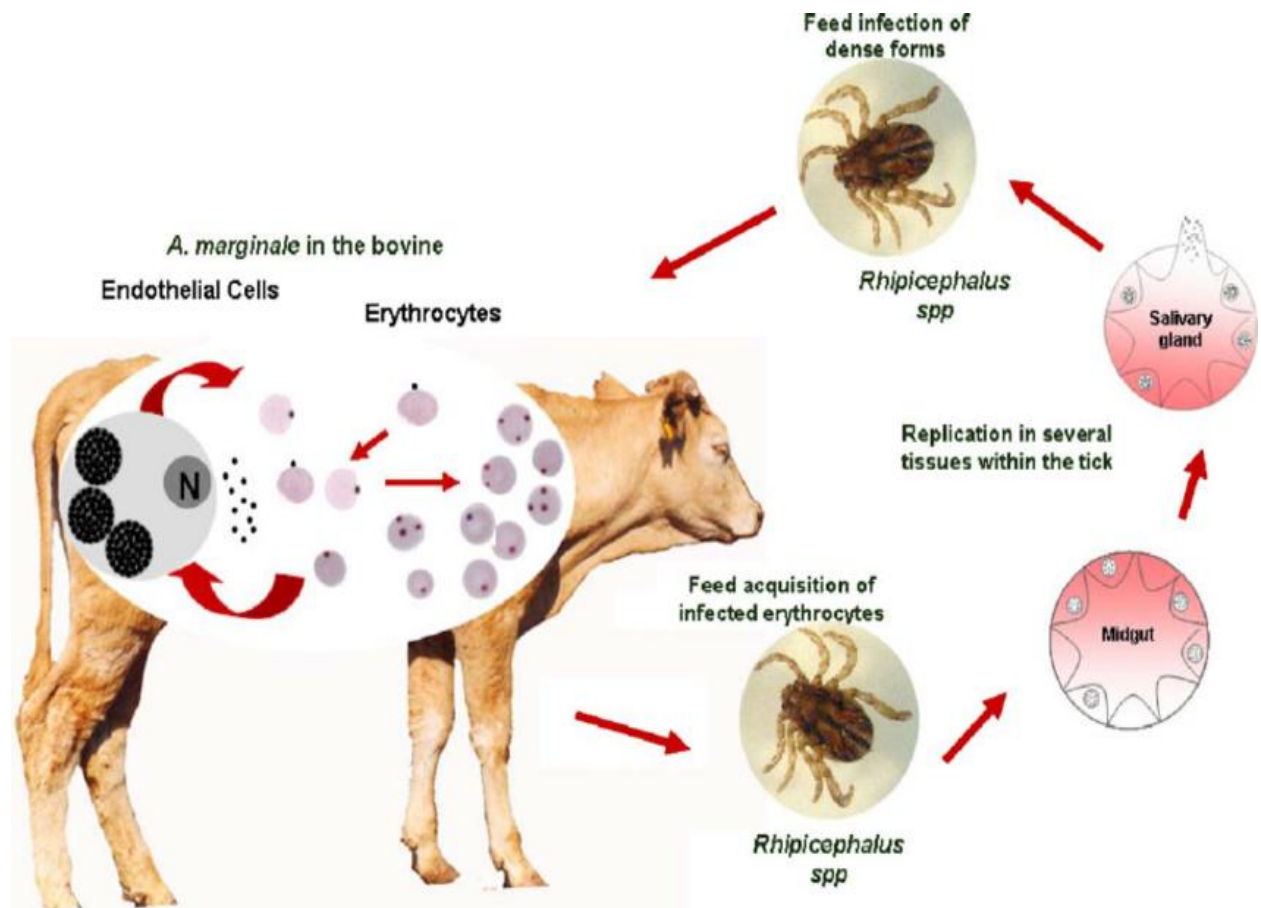


Figure 1. 2: Diagrammatic representation of the life cycle of *Anaplasma* in cattle (Kocan, 1999)

2.3 Transmission of Anaplasmosis in cattle

Anaplasmosis can be transmitted mechanically means by either; blood-contaminated formites or by blood sucking flies which relies on *rickettsemia* levels during their feeding (Scoles *et al.* 2005). Earlier on, studies indicated that ticks are more effective in transmitting Anaplasmosis biologically than stomoxys scalcitrans , and stable fly that are mechanical transmitters of the infection (Kocan *et al.* 2010a). However, some strains of *Anaplasma marginale* are not transmissible by ticks (Aubry & Geale, 2010). In general, *A. marginale* is spread by *Rhipicephalus* spp., *Dermacentor* spp. and *Ixodes ricinus*, but has not been proven to be transmitted by *Amblyomma* spp. (Kocan, 2009).

Anaplasmosis is transmitted mechanically by biting dipterans, use of contaminated needles, dehorning saws, nose tongs, tattooing instruments, ear tagging devices and surgical instruments (Byaruhanga *et al.* 2018).

Recently, Byaruhanga, (2017) provided evidence that the Florida strain of *A. marginale*, which is not transmissible by ticks, was highly retained in fly mouthparts than was the St. Maries strain, which is tick transmissible. This form of mechanical transmission is likely the major route of dissemination for *A. marginale* in certain areas of the world where tick vectors are absent (Scoles & Miller, 2008), especially in areas of the U.S. where geographic isolates of *A. marginale* are not infective for ticks and or tick vector populations are limited (Kocan, 2009a).

Biological transmission of Anaplasmosis is by ticks, with over 20 species incriminated as vectors worldwide (Scoles *et al.* 2005). Studies showed unknown species of ticks that could be vectors of Anaplasmosis infection amongst cattle and wildlife species in Europe (Kocan *et al.* 2010b), within a stage (Intrastadial), while transovarial transmission from one tick generation to the next has not been shown to occur (Mari & De, 2014). Interstadial transmission of *A. marginale* has been demonstrated by the 3-host ticks, *D. andersoni* *D. variabilis* and one-host tick *R. annulatus* in the United States of America but the vector competence for *A. marginale* may vary among or within tick species (Kocan, 2009). However, there was significant variation in infection of tick midguts of *D. andersoni* originating from different geographic regions (Kocan, 2009).

Intrastadial transmission of *A. marginale* has been shown to be effected by male *Dermacentor* reticulatus ticks (Eriks *et al.* 1993). Furthermore , studies have stated that male *Dermacentor* ticks may play an important role in the biological transmission of *A. marginale* because they become persistently infected with the pathogen and can transmit *A. marginale* repeatedly

when they transfer among cattle (Kocan, 2009), Ticks and cattle continuously infected generally serve as reservoir hosts for Anaplasmosis (Kocan *et al.* 2010a).

2.3 Reservoir hosts of Anaplasmosis

The most common reservoirs of Anaplasmosis are; cattle, goats, sheep, water buffalo, American bison ,white-tailed deer and Rocky Mountain elk (Kuttler, 2023; Kocan, 2009). However, white-tailed deer in South-eastern United States of America, were found experimentally susceptible to infection with Anaplasmosis, but did not appear to be exposed naturally, even at enzootic sites (Kocan *et al.* 2010a; Aubry & Geale (2010).Persistently infected wildlife species can be a reservoir of Anaplasmosis and becomes a factor in the spread of Anaplasmosis, thus serving as a source of infective blood both mechanical and biological transmission (Palmfjord, 2015).

2.4. History of Anaplasmosis in cattle

Anaplasmosis was first discovered in Europe in 1996 in Slovenia and reported in Poland in 2001(Mari & De, 2014). Besides, it was called yellow bag describing gall sickness in 1910 (Byaruhanga, 2017). Later, *Anaplasma* bacteria were first discovered in the blood of South African cattle as; *A. marginale* and *A. centrale* (Kocan, 2003; Fuente *et al.* 2003). Other important species were discovered; *Anaplasma ovis*, *Anaplasma platys* (Kocan *et al.* 2015). However, organisms were classified basing on genetic analyses of 16S rRNA genes, groESL and surface protein genes, and organisms have two families: *Anaplasmataceae* and *Rickettsiaceae* (Kocan *et al.* 2015). According to Mubashir *et al.* (2022), genetic study showed, Anaplasmosis as a rickettsial bacterial pathogens belonging to *Anaplasmataceae*. However, both organisms of the family *Rickettsiaceae* and *Anaplasmataceae* are all obligate intracellular bacteria that grew freely within the cytoplasm of eukaryotic cells and are found

exclusively within membrane-bound vacuoles in the host cell cytoplasm (Mubashir *et al.* 2022).

2.5 Clinical signs of Anaplasmosis in cattle

In cattle, Anaplasmosis is commonly recognized as gall sickness, and its severity is related to the age and breed of the animal (Jaswal *et al.* 2015; Kocan, 2003; Fuente *et al.* 2003a). Cattle between age bracket of 1-2 years show moderate symptoms and severe in older cattle (Weny *et al.* 2017b). The disease is characterized by; fever, loss of weight, icterus, hypoxia and nervousness, dysponia, lacrimation and abortion (Kocan *et al.* 2010a; Akure, 2019). The occurrence of clinical signs varies in periods; prepatent period ranging from 15–36 days, (Kocan, 2003; Fuente *et al.* 2003a), chronic period (Aubry & Geale, 2011), with doubling of *Rickettsemia* after every 24 hours during the exponential growth phase (Alicja, 2022). Pregnant cows may abort, surviving cattle convalesce over several weeks, during which hematologic parameters gradually return to normal but cattle that survive the acute phase turn to be carriers (Palmfjord, 2015).

2.6 Diagnosis of Anaplasmosis in cattle

Diagnosis of Anaplasmosis is based on clinical examination, anamnesis, physical examination, laboratory examination, in the form of hematology and blood smears, presence of vectors, cattle management practices, time of the year, and other aspects considered in the clinical history (Kocan *et al.* 2010a). Early diagnosis may improve the chances of a good outcome and reduce the economic losses in an infected herd (Hendrix *et al.* 2019). The infection is characterized by; anemia, fever, lethargy, low production, constraining reproductive activity as a result of low energy and oxygen transport (Hendrix *et al.* 2019; Aubry & Geale, 2010; Kocan. 2009). It is best done clinically by laboratory test, after presumptive diagnosis (Aubry & Geale, 2010). For example; microscopic examination of

blood sample stained with Giemsa, but its dismay is that it requires expertise, and errors (Kocan, 2009). Serological tests, its applicable in monitoring the development of antibodies at first infection or immunization, epidemiological purposes and during trading exercise in order get disease free cattle (Kocan, 2009).

The molecular diagnosis facilitates the identification of pathogen, when the infection is not quantifiable at microscope and allows the procurement of Deoxyribonucleic acid (DNA) for genomic identification (Salinas *et al.* 2023). Here several genes are used as target for molecular amplification by Polymerase chain reaction (PCR), including msp5 gene as the elected gene for standard detection, followed by msp1a, msp4 (Salinas *et al.* 2023). Other molecular test have been developed for *A. marginale* detection, such as multiplex Polymerase chain reaction (PCR), qPCR (duplex qPCR, PCR followed by restriction fragment length polymorphisms (PCR-RFLP), reverse line-blot hybridization (RLBH) (Kocan, 2009), loop-mediated isothermal amplification (LAMP), and Enzyme-linked immunosorbent assays (ELISA) to permit the uncovering of parasites in blood, tissues or ticks with optimal sensitivity, specificity and consistency(Hassan, 2021). *Anaplasma species* can be seen in smear and cell as shown in the Figure 3 below;

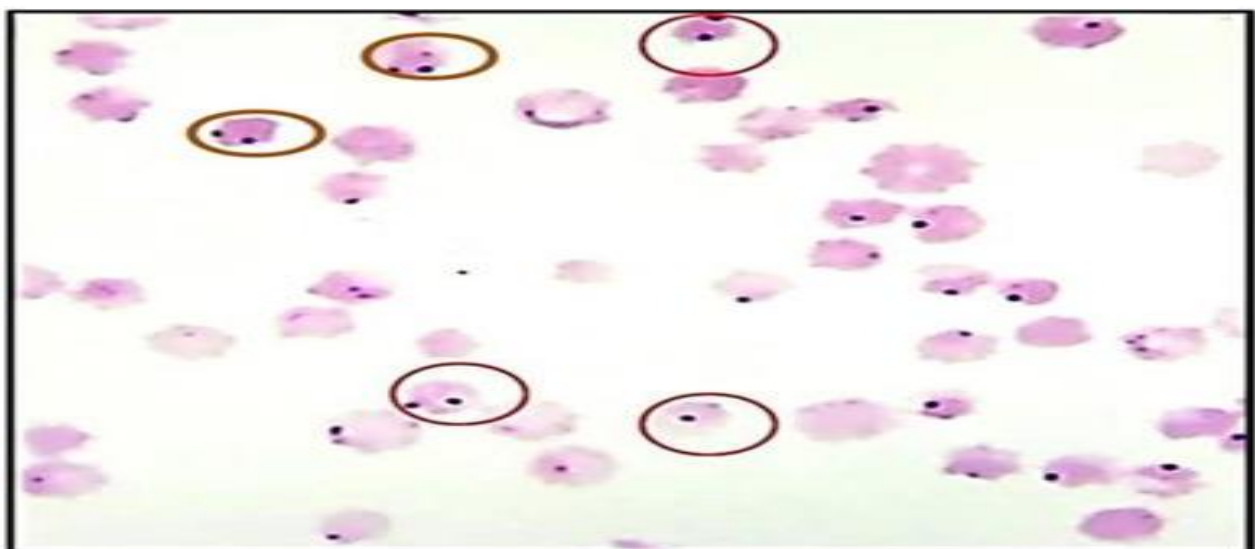


Figure 1. 3: Anaplasma on blood smear -Giemsa stain (Dhruba, 2020)

2.7 Risk factors associated with Anaplasmosis in cattle

Anaplasmosis is generally associated with various factors such as: tick infestation possessing ticks as vectors of *Anaplasma* species (Okafor *et al.*, 2018); Age of the animal in which young cattle of less than 2 years old being more susceptible to Anaplasmosis (Okafor *et al.* 2018); Breed of the animal where exotic breeds are more prone to the infection (Okafor *et al.* 2018); season in which Anaplasmosis showed high prevalence during wet season when tick populations are higher (Kumar *et al.* 2015); Geography, showed that cattle grazing on areas with high tick populations such as near wooded or bushy areas were at risk of Anaplasmosis (Kocan *et al.* 2010); Poor animal husbandry practices like inadequate tick control and poor nutrition (Kocan, De La Fuente, *et al.* 2003); Co-infection with other tick-borne diseases such as babesiosis (Okafor *et al.* 2018).

2.8 Control measures of Anaplasmosis in cattle

Control measures for Anaplasmosis in cattle include actions towards control of tick vector, rotational grazing, chemoprophylaxis treatments, vaccination, immunization and acaricide use (Kocan, 2003; Fuente *et al.* 2003b). However, immunization has not been practically intense in terms of dipping using acaricide which has resulted in resistance and failure in affectivity (Kocan *et al.* 2000).

2.8.1 Grazing management of cattle

According to some studies, proper grazing management practices such as rotational grazing is a controversial practice as it works best if pastures are left to fallow for at least 60 days (Byaruhanga *et al.* 2020). Horizontal transmission due to blood transfer from one animal to another by flies can be reduced by a number of management practices of Anaplasmosis, like; use of repellents, manure management, bio pesticides, insecticides, and traps (Salinas *et al.*

2023). Iatrogenic transmission is one of the most unattended forms of dispersion of diseases (Salinas *et al.* 2023). Changing needles between animals, disinfection of ear-taggers and other tools that are contacts get in contact with blood are the most simple and economical practices to curtail this form of horizontal transmission (Reinbold *et al.* 2010).

2.8.2 Chemotherapeutic or prophylactic treatment of cattle

Treatment of cattle infected with Anaplasmosis can be done with two basic antibiotics; Oxytetracycline and Imidocarb dipropionate (Otim, 2000; Kocan, 2003; Fuente, *et al.* 2003). Oxytetracycline and Imidocarb dipropionate(Imizol), blocks erythrocytes from taking up Inositol leading to starvation of hemoparasite, thus preventing replication in the cytoplasm of host cells to take place (Otim, 2000).In Uganda, Oxytetracycline is widely used drug because it is cheaper and easily accessible (Byaruhanga, 2017). Studies in Uganda, reveal that many cattle owners facing challenges such as; inadequate knowledge of Anaplasmosis, high costs of drugs, lack of veterinary support (Okello *et al.* 1998; Kasozi *et al.* 2021; Kasaija *et al.* 2021b). Therefore, under dosage of cattle is a common practice that can bring development of carriers in cattle herds (Kasaija *et al.* 2021b). Also studies showed use of tick repellent chemicals such as pyrethroids to cattle (Stafford *et al.* 2017)

2.8.3 Use of acaricide against ticks in cattle

Control of Anaplasmosis is mainly by regular use of chemical acaricide such as Amitraz, cypermethrin and deltamethrin (Byaruhanga, 2017; Irene *et al.*, 2021; Chenyambuga *et al.* 2010), can help in reducing tick populations and subsequent transmission of Anaplasmosis. Therefore alternate application of acaricide reduces the infection because it avoids issues of acaricide tick resistance development, which are attributed to mutations in genes related to drug susceptibility (Byaruhanga *et al.* 2020).

2.8.4 Use of vaccines against Anaplasmosis in cattle

Much as Anaplasmosis poses high economic constraints to cattle farmers, there is not yet readily available vaccine against the infection (Spare *et al.* 2020; Aubry & Geale, 2011; Byaruhanga, 2017). Vaccines for control of Anaplasmosis are under way. Attempts to boost animals immunity towards the infection within ruminants are underway using live vaccines and killed vaccines due to same variation of major surface protein (MSP) 2, and conserved CD4⁺ T-cell epitopes among two species of *Anaplasma* (Aubry & Geale, 2011; Kasaija *et al.* 2020). Cattle that have been vaccinated can be have continuous infections, which induces protective immunity within cattle (Kocan, 2009b). However, Studies conducted in Uganda , shows that vaccine efficacy and effectiveness against Anaplasmosis had positive results to be adopted by state regulatory authorities (Kabi *et al.* 2022).

2.8.5 Use of medicinal plants for treatment of Anaplasmosis in cattle

Studies carried out in Karamoja, indicate that over 209 plant species and 18 non-plant species are distributed over 116 genera and 54 families and are of medicinal use for the treatment of Anaplasmosis infection (Chenyambuga *et al.* 2010). Cattle farmers generally use Ethno-veterinary Medicines (EVM) for the treatment of Anaplasmosis using different plants such as parts such as; leaves and roots especially when there is insufficiency of veterinary healthcare workers (McCorkle & Mathias, 2016). However, the common plants used are: Argemone Mexicana (stem is pounded and mixed with soda ash and little fed to cow 0.25kg/1xday in 3days); Cissus grandifolia, fed to animal handful of pounded plant, 1 time(x)/day, for 2-3 days (Kioko *et al.* 2015). Other control measures include; culling of infected animals in order to remove those animals that can be source of infection in the herd (Okafor *et al.* 2018). Also, integrated pest management in order to combine tick control,

vaccination and other management practices can help reduce the incidence of Anaplasmosis (Stafford *et al.* 2017).

In general, Anaplasmosis is a global disease of concern of ruminants, causing significant losses economically within the livestock industries mainly due to its high morbidity and mortality (Kumar *et al.* 2015). However there is little information on prevalence, associated risk factors and control measures of Anaplasmosis in cattle in Gulu district. Therefore, this study aims to investigate the prevalence its associated risk factors and control measures of Anaplasmosis in cattle in Gulu district.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Gulu district, which is bordered by Pader district to the East, Amuru district to the West, Lamwo to the North and Omoro district to the South. Gulu district is located in the Northern part of Uganda (Figure 3.1). Gulu district has total area of 3,452.1 km² (1,332.9 square miles), with a total population of 307,700 (UBOS 2018) and density of 114.9/km² (298/square miles) (UBOS, 2018). The coordinates of the district are, 02°49'50.0"N, 32°19'13.0"E (Latitude: 2.830556; Longitude: 32.320278). The district headquarters in the city of Gulu are 333 kilometers (207 mi), by road, north of Uganda's capital city, Kampala. The economic activity of 90 per cent of the population in the district is subsistence agriculture. However, the annual estimated livestock population is 288,885, with total number of cattle being 27,899 and, 304,851 litres of milk (Gulu District Natural Resources profiling, 2021-2022).



Figure 3. 1: Map of Uganda showing the location of Gulu district (UBOS, 2014)

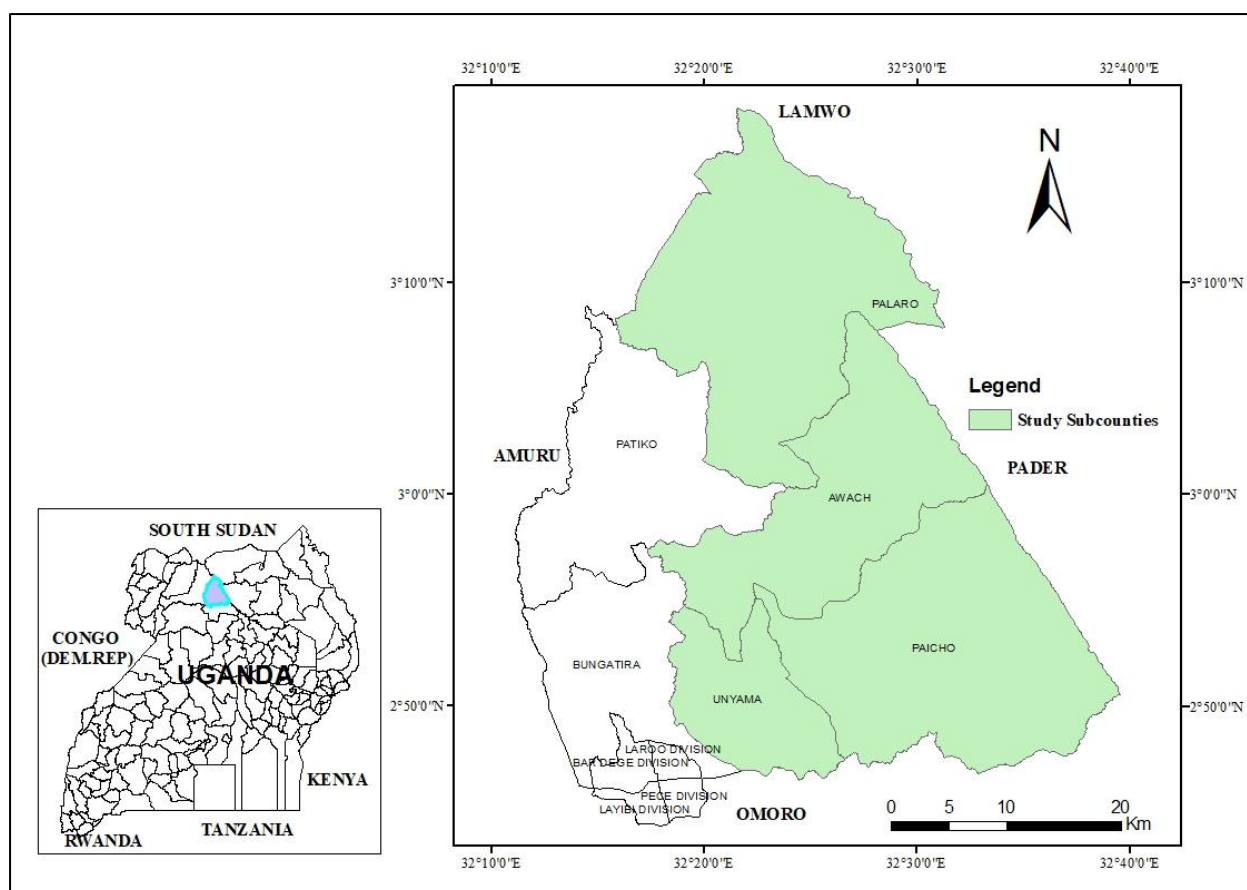


Figure 3. 2: Map of Gulu district showing location of sub-counties where the study was conducted (UBOS, 2021).

3.2 Study animals

The study animals comprised of 384 heads of cattle which were randomly selected from the four sub counties in Gulu district. The cattle breeds were categorized as; cross breeds (34) and Zebu short horn (310) and Ankole long horn (40), from which blood samples collected.

3.3 Study design

A cross sectional study was conducted for two months was adapted to this study. The study only targeted 100 farmers keeping at least 5-10 animals in the district. Four sub-counties were purposively selected from the district. The sub-counties were; Awach, Unyama, Palaro and Paicho. The sub-counties were selected for the study because most farmers had good representation of cattle, easily accessible, beneficiaries from Non-government organizations

and livestock restocking government projects that distributed improved cattle breeds for livelihood improvement of households with respect to other sub counties within the district.

3.3.1 Sample size determination

The sample size was calculated by using the formular given by Thrusfield (2007). Since the study was the first of its kind, the expected prevalence of Anaplasmosis in cattle in the study area using 50% precision value. The parameters used were of 95% confidence interval and 5% desired level of precision.

$$N = 1.96^2 \times \frac{p_{ex}(1 - p_{ex})}{d^2}$$

Where; N = required sample size, p_{ex} = expected prevalence, d²=desired absolute precision

By substituting these values in the formular, the sample size of the study population was 384 cattle as shown below;

$$N=1.96^2 \times \frac{0.5 (1-0.5)}{0.05^2} = 384$$

Thus the minimum sample size required for this study was 384 cattle.

3.3.2 Selection of sub-counties and respondents

Four (04) sub counties of; Unyama, Paicho, Awach and Palaro that were purposively selected from the eight (08) sub counties, based on high cattle population and high death rates due to tick borne cases in the district (Angwech et al., 2011). In each of sub counties, veterinary health professionals availed a list of all cattle farmers with at least 5-10 animals, which simplified the accessibility of 100 cattle farmers that were selected using systematic sampling.

3.4 Data collection

3.4. 1 Blood sample (determination of the prevalence of Anaplasmosis in cattle)

3.4.1.1 Collection of blood samples

A total of 384 blood samples were collected from cattle within four purposively selected sub-counties (Awach, Palaro, Unyama, and Palaro). Each animal was properly restrained. 3mls of blood sample were withdrawn from jugular vein directly in to Ethylenediaminetetracetic Acid vacutainer tube from each animal. During sampling, each sample in tube was properly labeled indicating animals history (location, age, sex, breed, body condition, tick infestation and previous injection). Blood samples were placed in ice box containing ice packs in it, and transported to Gulu University biological sciences laboratory on the same day for laboratory analysis.

3.4.1.2 Laboratory analysis of blood

Thick and thin dried blood smear were made on slide for each of the animal following procedures described by Emberth (1986). In each of 3mls of blood sample collected, two (2) drops of blood sample were put on slide. Blood smears were stained in Giemsa and placed in staining racks for 30 minutes, later dipped in methyl alcohol for two minutes, washed with water and air dried. Oil drop was placed on the smear and slide examined under oil immersion lens using a light microscope (Olympus CX23) of 100x for the presence of *Anaplasma* species (*Anaplasma marginale* and *Anaplasma centrale*). However, *A. marginale* inclusion were identified as small, irregular shaped organisms (0.3-0.5µm) located along the margin of RBCs, *A. centrale* were identified as slightly larger (0.4-0.6 µm) and more elongated located at the center of the RBCs (Emberth, 1986). This method is effective and

less costly as compared to other methods in determining the prevalence of infections in blood smears of animals.

3.4.2 Determination of risk factors associated with Anaplasmosis in cattle

Determination of risk factors associated with Anaplasmosis in cattle was done as with objective 1 of the research study, as described by Emberth (1986). A semi structured questionnaire was administered detailing animals and animal's bio data; sex, age, breed, date, location, presence of ticks, injected or not previously injected as risk factors associated with Anaplasmosis in cattle. The prevalence of Anaplasmosis (Objective 1) was compared in relationship to animals' bio-data in order to find out which risk factors were significantly different.

3.4.3 Assessment of control measures for Anaplasmosis in cattle

Data were collected using a pretested semi structured questionnaire in which 10 cattle farmers of Paicho sub-county were sampled randomly in order to check for its accuracy and minimize errors. Multi stage sampling was used for selection of four sub counties in the district. Both purposive sampling were used to select villages and famers in order to get data and to avoid biasness. The data included: level of education, herd size, chemotherapeutic use (type of medicines, dosage, drugs used), spraying (acaricide used, alternate use, efficiency of use). Household heads were selected as respondents to get required information on each of the selected cattle herd farmers. For easy administration of the questionnaire, it was translated in to the Luo language of the study area.

3.5 Statistical data analysis

All the data were entered to Microsoft office excel and analyzed using Statistical package for the social sciences (version 22). Data on prevalence of Anaplasmosis in cattle were analysed

using descriptive statistics and Chi square test at the, confidence level of 95 % and at a significance level of $P \leq 0.05$. Chi square tests were used in order to determine the significant effect of associated risk factors. That's to say breed, age, sex, locality, body conditions and tick infestation on the prevalence of Anaplasmosis infection. Significant differences were ($P \leq 0.05$). Data obtained on control measures was analysed using descriptive statistics and Chi square test ($P \leq 0.05$). The prevalence of Anaplasmosis in cattle was calculated as the number of positive animals that were examined microscopically by thin smear Giemsa stain divided by the total number of animals examined multiplied by 100% as shown by formula below;

$$P = \frac{\text{No. of cattle infected with Anaplasmosis}}{\text{Total number of cattle examined}} \times 100\%$$

Where, P is the prevalence of Anaplasmosis in cattle.

3.6 Ethical considerations

The study obtained approval from Gulu University Research Ethics Committee (GUREC), under application number GUREC-2023-534. The following ethical considerations were observed: Respondents consent to participate in the study confirmed by either signature or thumb print on the question; during data collection, the aim of the study was clearly explained to the respondents; research assistants considered research obligations by emphasizing that study be conducted with integrity, honesty, clarity, minimal risks and cultural values of the participant; and adherence to the principle of research ethics by considering respondents rights to take part in the study or kindly withdraw confidentiality by providing each of the respondents with a copy of consent form detailing chairperson for Gulu University Research Ethic Committees for more clarification.

CHAPTER FOUR

RESULTS

4.1 Determination of the prevalence of Anaplasmosis in cattle

Overall, 384 cattle were examined for Anaplasmosis, 69 animals tested positive for the disease indicating a prevalence of 17.97%, with *A. marginale* recording highest prevalence of 15.89%, *A.centrale*(1.820%), and mixed-infection (0.260%) as indicated in Table 4.1.

Table 4.1: The prevalence of Anaplasmosis in cattle based on Anaplasma species (N=384)

Total Number of cattle examined	Anaplasma species	Positive	Prevalence of Anaplasmosis (%)
384	<i>A.marginale</i>	61	15.89%
	<i>A.centrale</i>	7	1.82%
	Mixed-infection	1	0.26%
	Total positive	69	17.97%

4.1.2 Socio-demographic characteristics of cattle farmers

Most cattle farmers in the study were males (75%), and females were few (25%). Regarding level of education, majority of respondent (65%) attained primary level, 16% secondary level, 8% tertiary and none were 11%. Most farmers have kept cattle (50%) in period of greater than ten (10) years and others (25%) between <5 years to (>5-10) years.

Table 4.2: Socio-demographic characteristics of cattle farmers

Variable	Category	% respondent
Gender	Male	75
	Female	25
Education level	Primary	65
	Secondary	16
	Tertiary	8
	None	11
Experience of keeping cattle	<5	25
	>5-10	25
	>10	50

4.2 Risk factors associated with Anaplasmosis in cattle in Gulu district

Regarding sex and age of cattle, the highest prevalence was recorded for male (23.61%) and cattle of less than one year (28.95%), respectively (Table 4.2). The lowest prevalence was observed in males (14.58%) and cattle of 1-2 years (9.52%), with statistically significant difference ($p < 0.05$) for both factors. There were no significant association ($p > 0.05$) of prevalence of Anaplasmosis in cattle breeds. Results of body condition were statistically significant ($p < 0.005$), where cattle with poor body condition had the highest prevalence (59.65%) while those with good condition posted the lowest prevalence (2.990%). The observed variation based on previous injection of the animals examined was statistically significant ($p < 0.05$). Animals that had been previously injected for any treatment had the highest prevalence (47.73%), while those that had not been injected registered the lowest prevalence (14.12%). Based on locality, results were not significant variation ($p > 0.05$) regarding the prevalence of Anaplasmosis across the selected sub-counties.

Table 4.3: Risk factors associated with Anaplasmosis in cattle

Associated risk factors	Number of cattle examined (N=384)	Positive cases obtained	Prevalence of infection (%)	Chi square(X²)	P-value
Sex				4.9763	0.026
Female	144	34	23.61		
Male	240	35	14.58		
Age				10.3261	0.006
Less than one year	76	22	28.95		
1-) years	84	8	9.52		
More than 2 years	224	39	17.41		
Breed				3.7207	0.156
Local	310	50	16.13		
Long horn	40	10	25.00		
Ankole					
Cross breed	34	9	26.47		
Body condition				138.9214	≤0.001
Poor	57	34	59.65		
Medium	59	27	45.76		
Good	268	8	2.99		
Previous Injection				29.8559	≤0.001
Injected	44	21	47.73		
Not injected	340	48	14.12		
Locality				4.5690	0.206
Awach	100	15	15		
Unyama	100	16	16		
Paicho	100	25	25		
Palaro	84	13	15.48		
Tick Infestation				12.0163	0.001
Yes	336	69	20.54		
No	48	0	0.00		

4.3 Control measures of Anaplasmosis infection in cattle in Gulu district.

4.3.1 Spraying

The majority (97%) of cattle farmers were spraying their cattle (Table 4.4).

Table 4.4: Spraying of cattle

Spraying	No. cattle farmers (N=100)	%farmers spraying	Cumulative
Yes	97	97	97
No	3	3	100

4.3.1.1 Frequency of spraying

Farmers (31%) sprayed their animals more than a month and only 3% never spray at all (Table 4.5).

Table 4.5: Frequency of spraying animals against Anaplasmosis infection

Frequency of spraying	No. of spraying interval (N=100)	% spraying (N=100)	Cumulative
Weekly	27	27.00	27
Monthly	15	15.00	42
Twice a month	24	24.00	66
Never	3	3.00	69
More than a month	31	31.00	100

4.3.1.3 Types of acaricides used for spraying cattle

Table 4.6 shows that majority of cattle farmers (62%) use only *amitix* to spray against Anaplasmosis infection while only 2% of the farmers use all the acaricides (i.e. *amitix*, *milbitraz* and *alfapor*).

Table 4.6: Types of acaricides used for spraying cattle

Type of acaricides used	No. Acaricides used (N=100)	% acaricides used (N=100)	Cumulative
<i>Amitix</i>	62	62	62
<i>Milbitraz</i>	22	22.00	84
<i>Alfapor</i>	3	3.00	87
Does not know	11	11.00	98
All	2	2.00	100

4.3.1.4 Alternation of acaricides

Majority (71%) of cattle farmers do not alternate the acaricides when spraying their cattle (Table 4.6)

Table 4.7: Alternation of acaricides

Acaricides alternated or not	No. Acaricides alternation (N=100)	%Acaricides alternation (N=100)	Cumulative
No	71	71.00	71
Yes	29	29.00	100

4.3.2 Chemotherapeutic control of Anaplasmosis in cattle

4.3.2.1 Who treats the cattle?

Findings showed that a relatively high number of cattle farmers (47%) rely on paraprofessionals on treatment of their cattle (Table4.8), while least number of farmers (10%) seek help from government veterinary officers.

Table 4.8: Who treats the cattle?

Who treats the cattle	No. service providers	%No. service providers	Cumulative
Government vets	10	10.00	10
Myself	25	25.00	35
Assistance from paraprofessionals	47	47.00	82
Quacks	18	18.00	100

4.3.2.2 Drugs used in the treatment of Anaplasmosis infection

Table 4.9 shows most (77%) of cattle farmers use oxytetracycline for Anaplasmosis infection, while a few (2%) use botanical medicinal herbs.

Table 4.9: Drugs used in treatment of Anaplasmosis infection

Drugs used in cattle treatment	No. Drug use (N=100)	%Drug use (N=100)	Cumulative
<i>Oxytetracycline/ Berenil</i>	77	77.00	77
<i>Imidocarb dipropionate</i>	17	17.00	94
<i>Botanical medicinal herbs</i>	2	2.00	96
<i>Oxytetracycline and Imidocarb-dipropionate</i>	4	4.00	100

4.3.2.3: Rationale of the dosage of drug use

Study results showed that most (65%) cattle farmers offer drugs to animals only once a day and a few (6%) give their animals each drug for three days (Table 4.10), instead of recommended regiment of one offer for three days in case of tetracycline, single or twice per day for imidocarb dipropionate, and varied amounts depending on the level of infection when using ethno-medicinal drugs.

Table 4.10: Rationale of the dosage of drug use

Rationale of drug use(day)	No. Drug rationale (N=100)	%Drug rationale (N=100)	Cumulative
Once	65	65.00	65
Two days each	29	29.00	94
Three days each	6	6.00	100

4.3.2.4 Use of ethno medicinal drugs by cattle farmers

The present results indicated that most (66%) farmers were not using ethno-medicinal drugs and only 32% were using ethno-medicinal drugs as control for Anaplasmosis in cattle (Table 4.11).

Table 4.11: Use of Ethno medicinal drugs by farmers

Whether a farmer uses ethno-medicinal drugs	No. Ethno-medicinal use(N=100)	%Ethno-medicinal use(N=100)	Cumulative
Yes	32	32.00	32
No	66	66.00	98
Non	2	2.00	100

CHAPTER FIVE

DISCUSSION OF RESULTS

5.1 Prevalence of Anaplasmosis in cattle in Gulu district

The study obtained an overall prevalence of 17.97% (Table 4.1) for Anaplasmosis infection in cattle was recorded, agreeing with the findings of Kispotta *et al.*(2016);Byaruhanga *et al.* (2015a); Badshah *et al.*(2023), who reported the prevalence of 18.5%, 17-28% and 19.79%, respectively. However, current results were lower than the 30.8% prevalence reported by Palmfjord.(2015),due to endemic instability of Anaplasmosis in Kiruhura district Palmfjord, (2015). Similarly, the previous study was higher than 14.4% and 10.4% reported by Kasozi *et al.* (2014), and Angwech *et al.* (2011), respectively. This difference was probably due to abundance of ticks, misuse of drugs and acaricides, inadequate veterinary services, intensity of management and variations in sampling periods.

5.2 Associated risk factors of Anaplasmosis in cattle in Gulu district

Present findings indicated a significantly higher prevalence of 23.61% in female and 14.58% in male of Anaplasmosis in cattle(Table 4.3), agreeing with findings of Markos & Abdurahaman, (2018), Kispotta *et al.*(2016) and Atif *et al.* (2013),who reported higher proportion in female than male (11.9% and 10.9%), (13.48% and 2.52%), (10.8% and7%), respectively. Higher prevalence in female cattle were attributed to hormonal imbalances due to milk production, pregnancy, depressing the immune system of the animal (Markos & Abdurahaman, 2018; Faruque *et al.* 2022).

Regarding age, the present findings indicated significantly higher prevalence of Anaplasmosis of 28.95% in younger animals of less than one year and 17.41% in cattle greater than 2years of age (Table 4.3), and this concurs with findings of Badshah *et al.*(2023),

who reported prevalence of 26.78% and respectively 12.35%. This disagrees with the findings of Markos & Abdurahaman, (2018), who reported higher prevalence in cattle greater than two years (12.8%) than young ones (8.7%). These disparities could be due to relatively weaker immune systems ($P>0.05$) in both young and older cattle.

The study reveals that there were not significant difference among the breeds of cattle and, this concurs with findings of Kispotta *et al.* (2016) and Badshah *et al.* (2023), who reported the difference in the susceptibility of cattle breeds to Anaplasmosis due to inherent resistance against the causative agent, and breed itself.

Current findings recorded significantly higher prevalence of 59.65%, in cattle with poor body condition, and lowest (2.990%) in cattle with good body condition (Table 4.3) and, this concurs with findings of Markos & Abdurahaman, (2018), who reported 15.7%, and 4.080% for poor, and good body condition but with no statistically significant association ($P>0.05$) observed. This could be associated with the disease, other chronic infections and malnutrition of the animals which interferes with immune system due to inadequate nutrition or poor management systems, rendering them highly susceptible to infection.

The current study indicated significantly higher prevalence of 47.78% in previously injected animals, concurring with findings of Markos & Abdurahaman, (2018), who reported 16.3%. This could be associated with reuse of contaminated needles and instruments during treatment of cattle. However, according to study by one report, a used needle on an infected animal leads to a 60% chance of the other animal getting the infected in case of use of the same needle (Markos & Abdurahaman, 2018). In case of our country, during vaccination most animals are injected with one non-disposable needle without disinfecting. Furthermore, study findings indicated higher significant prevalence (20.52%) in tick-infested cattle compared to tick-free animals ($\leq 0.00\%$). These results concur with the findings of Kocan,

(2009). This might be because ticks are vectors of pathogens that cause Anaplasmosis, hence the high disease risk in tick-infested cattle.

5.3 Control measures of Anaplasmosis infection among cattle in Gulu district

Current study findings indicated that most farmers use spraying as a control measure for Anaplasmosis infection and this results agree with findings of Irene *et al.* (2021); Byaruhanga *et al.* (2015b); Kasaija *et al.* (2021a), who found out that spraying was mostly used method of acaricide application against vectors of Anaplasmosis infection among cattle in Uganda. Unfortunately, most of them sprayed their cattle ones more than a month (Table 4.5), signifying high increase in the parasites load and disease spread.

However, research by Irene *et al.* (2021), in southwestern Uganda revealed that herdsmen spraying of cattle is done once a week and dependent on seasons due to perceived increase in tick infestation by farmers in their research area.

Regarding acaricide use, findings indicated high use of Amitix (62%) and lowest in Alfapor (3%), this concur with findings of Byaruhanga *et al.* (2015b); Ocaido *et al.* (2009a); Kocan *et al.* (2000); Kasaija *et al.* (2021a). This could be due to availability of Amitix, cost effective and efficacious, respectively. Contrasting with findings of Tesfaye & Abate, (2023), reported high preference in Ivermectin by farmers. Further, the present study indicated that most farmers did not periodically alternate the acaricide (71%), concurring with the findings of Tesfaye & Abate, (2023) and Kabi *et al.* (2014), who reported that farmers did not periodically alternate the type of acaricides they use and tend to switch acaricide use based on season. However, this may lead to acaricide tick resistance, which may reduce their effectiveness requiring higher doses and frequent applications, placing a cost burden on the farmer.

The current study recorded relatively high number of paraprofessionals (47%) and only 10% of farmers were using government veterinary officers to treat their animals (Table 4.8). Farmers argued that government veterinary officers are few and in most cases, unreachable especially when there are emergencies at their herds.

Furthermore, findings indicated high use of tetracycline (77%) in control of Anaplasmosis and 2% use botanical medicinal herbs (Table 4.8). This agrees with findings of Byaruhanga *et al.* (2015b), who reported 100%, use of oxytetracycline. This could be attributed to availability and low prices of oxytetracycline drugs to farmers. However, farmers were inappropriately using drugs during treatment of infected cattle (Table 4.10), implying, under dosing which often contributes to the development of resistance as pathogens are not exposed to sufficient drug concentrations that warrant complete and effective elimination, hence the continued presence and potential spread of the disease. However, according to Payne, (2017), findings indicated dosage rate of 10 mg/kg daily for 3 days when using tetracycline intravenously along with vitamin C, which yielded positive results.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The major aim of the study was to determine the prevalence and control measures of Anaplasmosis in Gulu district. The study showed that; majority of the respondents were males (75%) with formal education (65%) in primary level. The study recorded high prevalence(17.97%), of Anaplasmosis in cattle (Table 4.1), with *Anaplasma marginale* (15.89%) being dominant over *Anaplasma centrale*(1.82%) in female cattle(23.61%), of age less than one year (28.95%) were most infected. Poor body condition (59.65%), cattle injected with needle (47.7%), and tick infestation (20.52%), recorded high significant prevalence of Anaplasmosis in Gulu district. Majority of the respondents were spraying (97%) their cattle using Amitix (62%), more than a month (31%), without periodically alternating the acaricide (71%) (Table4.6). 47% of the respondents got assistance from paraprofessionals and by self (25%), using mostly oxytetracycline (77%) with mostly one injection administered (65%) to infected cattle.

6.2 Conclusions

The current findings have revealed high significant prevalence of Anaplasmosis in Gulu district where female cattle were mostly affected. Age group of less than one year, poor body condition, previously injected and infested by ticks were prone to infection. Majority of the respondents sprayed their cattle with acaricide (Amitix) inappropriately. The respondents received assistance mainly from paraprofessionals and by themselves during treatment of sick animals. This is evidenced by few veterinary personnel, in Gulu district. Therefore, implies

that significantly different factors influence the prevalence of Anaplasmosis in Gulu district, justifying its continued presence and spread.

6.3 Recommendation

- 1) Due to inappropriate control measures of Anaplasmosis in Gulu district, policies need to be put in place, in order to curb the disease through implementation of laws governing drug use and handling.
- 2) Research on seasonal dynamics of the pathogen needs to be conducted especially for dry season.
- 3) Herders should be encouraged to properly use acaricides by following manufacturers' recommendations to avoid acaricide tick resistance.
- 4) Research on Anaplasmosis infection using more sensitive tests such as; ELISA and PCR should be done in order to facilitate identification of pathogens when the infection is not quantifiable by microscopy.
- 5) Communities should be sensitized on appropriate control measures of the Anaplasmosis infection in respect to acaricide use, drug use and handling, and simple diagnosis procedure of sick animals.

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APPENDICES

Appendix 1: Summary of laboratory information on cattle in Gulu district

Variable	Category	Frequency	%
Sex	Female	143	37.2
	Male	241	62.8
Breed	Short horn zebu	310	80.7
	Longhorn Ankole	40	10.4
	Crossbreed	34	8.9
Body condition	Poor	57	14.8
	Medium	59	15.4
	Good	268	69.8
Age	<1 year	76	76
	(1-2)years	84	84
	>2years	224	224
Previous injection	Injected	44	11.5
	Not injected	340	88.5
Tick infestation	Yes	336	87.5
	No	48	12.5
Spraying	Weekly	27	27
	Monthly	15	15
	Twice month	34	34
	Never	3	3
	> Month	31	31
Type of acaricide used	Amitix	62	62
	Milbitraz	22	22

	Alfapor	3	3
	Doesn't know	11	11
	All	2	2
Acaricide alteration	No	71	71
	Yes	29	29
Use of ethno-medicinal plants	No	66	66
	Yes	32	32
	None	2	2
Chemotherapeutic drugs used	Tetracycline	77	77
	Imidocarb-dipropionate	17	17
	Botanical med. Drugs	2	2
	Tetracycline &		
	Imidocarb dipropionate	4	4
Who treats cattle?	Government vets	10	10
	Myself	25	25
	Paraprofessionals	47	47
	Quacks	18	18

Appendix 2: Questionnaire

APPENDICES

APPENDIX I: QUESTIONNAIRE

Instructions

Circle appropriate options and fill in gaps

Section A

1. Socio-demographic factors of respondent

Name.....

Sex a) Male b) Female

Level of education

Primary c) tertiary

Secondary d) None

Experience of keeping animals

<5yrs b) >5-10 yrs c) >10yrs

Section B (Tick any one or two)

2. Associated risk factors of anaplasmosis in cattle

Locality.....

Breed: a) Crossbreed ☐ b) Local breed ☐

Sex: a) Male ☐ b) Female ☐

Age of bovine

1<year ☐

1-2 years ☐ c) > 2 years ☐

Body condition

Good ☐

Medium ☐ c) Poor ☐

Tick infestation

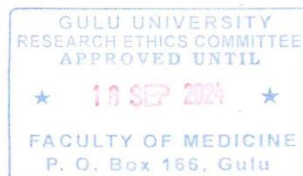
Yes ☐ b) No ☐

Ticks found on the cattle.

a) Brown ear tick b) Blue ticks c) Bont ticks

3.0 Control measures of anaplasmosis in cattle

3.1 Vector control by spraying of ticks



3.1.1 How often are the animals being sprayed?

- a) Weekly c) Two weeks e) Monthly and above
b) Twice a week d) Three weeks f) Never

3.1.2 Do you always use different acaricide for spraying your cattle?

- a) No b) Yes

3.1.3 What are the common acaricide often used?

- a)
b)
c)

3.1.4 Common acaricide used/pour on used

.....
.....
.....

3.2 How do they treat their cattle against anaplasmosis?

- a) Assistance from government vets c) Buy drugs and treat by own
b) Private vet e) Animal Health assistant

3.2.1 Chemotherapeutic controls

- a) Use of conventional medicine c) Both
b) Never d) Use of botanical medicines

3.6 Botanical medicines used by farmers for treatment of anaplasmosis

Name (local)	Scientific name	Plant parts used
.....		
.....		
.....		

3.2.2 Rationale of use

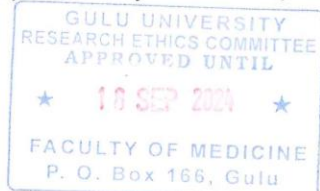
- c) Cupful and water c) others
d) Spoonful and water

3.3 What is the common prophylaxis drugs being used

- a) Oxytetracycline c) Albendazole d) Penicillin e) Both a & b
b) Imidocarb dipropionate d) others

3.3.1 What the rationale of the dosage of drugs was used?

- a) Ones a day b) Three days c) Two days



30

3.3.2 What is the dosage of treatment of sick cattle against anaplasmosis?

- a) Over dose b) Under dose c) Right dose

4.0 What are the constraints of anaplasmosis to farmers?

- a) Loss of weight b) mortality c) Abortion
d) High cost of treatment c) low milk production



Appendix 3: Ethical clearance

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RESEARCH ETHICS COMMITTEE

18/09/2023

To: Otimong Joseph

0788198452

Type: Initial Review

Re: GUREC-2023-534: Prevalence and control measures of anaplasmosis in cattle. A case study in Gulu district

I am pleased to inform you that at the **97th** convened meeting on **23/03/2023**, the Gulu University REC meeting voted to approve the above referenced application.

Approval of the research is for the period of **18/09/2023** to **18/09/2024**.

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes.
3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC.
4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
5. Continuing review application must be submitted to the REC **eight weeks** prior to the expiration date of **18/09/2024** in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study.
6. The REC application number assigned to the research should be cited in any correspondence with the REC of record.
7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

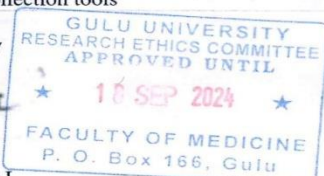
The following is the list of all documents approved in this application by Gulu University REC:



No.	Document Title	Language	Version Number	Version Date
1	Protocol	English	4.0	2023-09-18
2	COVID-19 risk management plan for the study	English	3.0	2023-09-02
3	Informed Consent forms	Luo	3.0	2023-09-02
4	Informed Consent forms	English	3.0	2023-02
5	Data collection tools	English	2.0	2023-07-06

Yours Sincerely

[Handwritten Signature]



Dr. Gerald OBAI

For: Gulu University REC

Appendix 4: COVID-19 risk management plan for the study

Version 3.0

Measures for the Prevention and control of risk of spread of COVID-19 during the implementation of research

Study Title: Prevalence and control measures of Anaplasmosis in cattle: A case study in Gulu District.

Principal Investigator (s): Otimong Joseph

Introduction:

The novel Corona virus is transmitted from human to human through droplets and direct or close personal contact with an infected individual.

Novel Corona virus signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death.

Implementation of the prevention and control measures

In the implementation of the research activities, the investigators are committed to ensuring the safety of its research team, research participants and communities where the study will be conducted. The study team undertakes to comply with the Standard Operating Procedures issued by the Ministry of Health, and presidential directives to mitigate against the risk of infection of COVID-19, rapidly detect and effectively respond to any COVID-19 case that may occur in the process of carrying out the study, screening, face covering, physical distancing, and good hand hygiene.

Procedures to be followed during the implementation of the research

Prior to data collection and Training

Health guidance: There will be training sessions dedicated for COVID-19 sensitization and awareness, to equip the research team with knowledge of signs and symptoms of the COVID- 19, and preventive measures such as hand hygiene before placing and removing the mask, as well as storage of the mask, social distancing. The research team will circulate notices, posters, charts on common signs and symptoms of COVID-19 as provided by the Ministry of Health, and develop and/or use existing plan for the appropriate referral pathway for identified and/or suspected cases.

1. **Personal Screening:** All researchers, research assistants, research participants and any other individuals engaged in research activities will screen temperature daily for fever.

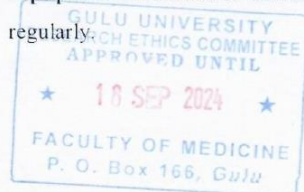


Screening for temperature will be carried out during planning meetings, trainings, community outreach. Any individual found with COVID 19 symptoms will be withdrawn and referred to the COVID-19 task force for further assessment and management. The withdrawn individual will be allowed to return only if they present a valid certification of their COVID-19 status showing negative results.

2. **Wearing face coverings:** All researchers and research assistants and any other individuals engaged in research activities that require interaction with fellow researchers or research participants or the community member will have to wear a face mask that fully covers the mouth and nose at all times. The study will provide face masks for individuals who do not have.
3. **Physical Distancing:** There will be social distancing during the training, meetings, community outreach of at minimum of 2 metres. During breaks, the team will not be allowed to congregate in common areas.
4. **Good hygiene:** All team members will be required to wash their hands or use hand sanitizers before and after entering the training room, and other common areas. Hand washing equipment shall be supplied and made available at all times. All surfaces and equipment shall be sanitized frequently.

During data collection

1. **Personal Protective Equipment (PPE):** All research assistants involved in tracing of respondents and in in-person interviews and research participants will use PPE including a properly fitted face mask. Enumerators/Researchers and research participants will wash hands with soap and water or use hand sanitizer prior to the interview. Handshakes and hugging are prohibited. Physical distancing of at least 2metres in all research related activities shall be observed.
2. **Consent Process:** The Researcher or Research assistants will observe social distancing when taking consent (while 2 metres apart) upon arrival to the participant, and will have to wash hands thoroughly with soap and clean water or use hand sanitizer prior to the interview. Finally, Participant will then be sensitized on covid-19 and presented with preventive measures.
3. **Equipment sanitization:** shared devices used to record or capture data shall be sanitized regularly.



During Dissemination/Community engagement

1. **Community Engagement:** The research team shall carry educational materials on prevention of COVID-19 in a language understood by the participants. These materials shall also have visual images to support understanding. Enumerators/Researchers and research participants will wash hands with soap and water or use hand sanitizer prior to the interview. Screening for temperature will be carried out prior to conducting community engagements.
2. **Equipment sanitization:** The shared devices used during community engagements shall be sanitized regularly.

The preventive and control measures will continuously be reviewed based on new information and guidelines communicated by the Ministry of Health



Appendix V: Plagiarism Check

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PLAGIARISM CHECK

Declaration for Originality Form

This form must be completed and signed for all scholarly work produced.

Name: OTIMONG JOSEPH (MR.)

Personal File No.(staff) Reg. (student) 21/U/3399/MSA/PS

Faculty/Institute: AGRICULTURE AND ENVIRONMENT, GULU UNIVERSITY

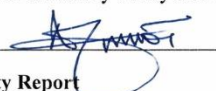
Department: ANIMAL PRODUCTION AND RANGE MANAGEMENT

Title and bibliographic description of the work

PREVALENCE AND CONTROL OF ANAPLASMOSIS IN CATTLE: A CASE OF GULU DISTRICT

Declaration.

1. I understand what plagiarism is and I am aware of the University policy on this regard,
2. I declare that this ..., **Dissertation** ... (thesis, Proposal, essay, assignment, paper, report etc.) is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my work has been used, this has been properly acknowledged and referenced in accordance with Gulu University requirements,
3. I have not sought or used the services of any professional agency to produce this work,
4. I have not allowed, and shall not allow anyone to copy my work with the intention of passing it off as his/her own,
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Signature 

Date 12th 09 2024

Originality Report

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- ☒ Minor
☐ Moderate
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