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DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL

SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES UNIVERSITY OF GHANA



**FACTORS ASSOCIATED WITH AFRICAN ANIMAL TRYPANOSOME
INFECTION IN CATTLE IN KIANG WEST DISTRICT, LOWER RIVER REGION,**

THE GAMBIA

By

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN

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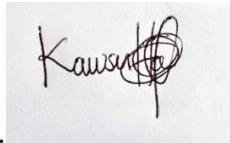
MPHIL APPLIED EPIDEMIOLOGY AND DISEASE CONTROL DEGREE

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DECLARATION

I, Kawsu Sanyang, do declare that this thesis under the guidance of my primary and secondary supervisors, Dr Samuel Sackey and Prof.Col E. A. Afari (Rtd), School of Public Health, University of Ghana, Legon, is my work and that it has not been submitted for any other degree or professional qualification, except as specified



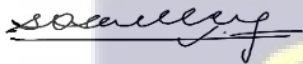
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Date 15/12/2021

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DEDICATION

This thesis is wholeheartedly dedicated to my lovely parents, who have been my source of inspiration and strength. Also, to my brothers and sisters home and abroad, relatives, mentors, friends, and all GFELTP cohort 13 members who have shared their words of advice and encouragement to finish this thesis work. Finally, I thank God for the guidance, power of the mind and gift of life.



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ABSTRACT

Background:

African Animal Trypanosome (AAT) infection is caused by hemoparasites (Trypanosomes) that are transmitted by the Tsetse fly. In the Gambia, cattle production produces the highest livestock output that contributes 54% to the total agricultural GDP which accounts for 34 % of National Gross Domestic Productivity (GDP). However, diseases including AAT infection cause huge losses to cattle production with 53.4% deaths annually. This study determined the prevalence of risk factors associated with AAT infection in cattle in Kiang West District, The Gambia.

Methods

A cross-sectional study involving a sample size of 384 cattle from four randomly selected villages of Kiang West District was conducted from February 2020 to June 2021. Blood samples collected were screened for AAT infection using buffy coat and Giemsa staining methods. Data on animal demographic and husbandry factors were collected using a structured questionnaire and statistically analysed using univariate and multivariate logistic regression techniques.

Results:

The overall prevalence of AAT infection was 11.7% (45/384), of which 57.78% (26/45) were *Trypanosome vivax*, 24.40% (11/45) were *Trypanosome congolense* and 4.44% (2 /45) were *Trypanosome brucei*. Mix-infection (T. congolense and T. vivax) was 33.33% (6/45). Among the study villages, Jifarong had 33.3% (15/45) AAT infections. A total of 29.95% (115/384) of cattle had anaemia. AAT infection was significantly associated with crossbreed [**aOR=11.9 ;95% CI: 1.87-74.85**] and anaemia [**aOR= 2.0; 95% CI: 1.07 – 3.89**]. The age and sex of cattle were not associated with AAT infection.

Conclusion:

There is a high prevalence of AAT infection in Kiang West District Lower River Region, The Gambia and *Trypanosome vivax* was found to be the most dominant species among causing infection in cattle. Crossbreed cattle and anaemia condition were found the associated risk factors to AAT in cattle. To minimize the burden of AAT on cattle production in the Gambia, we recommend farmers adopt the rearing of trypanotolerant breeds (Ndama) of cattle and implement husbandry practices that lessen anaemia in cattle.



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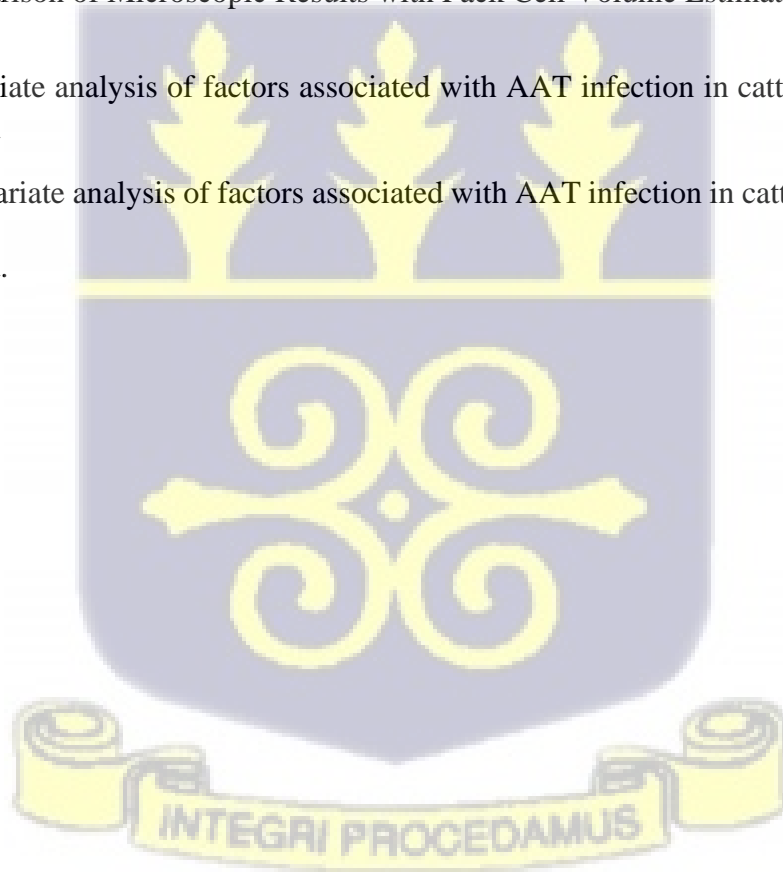
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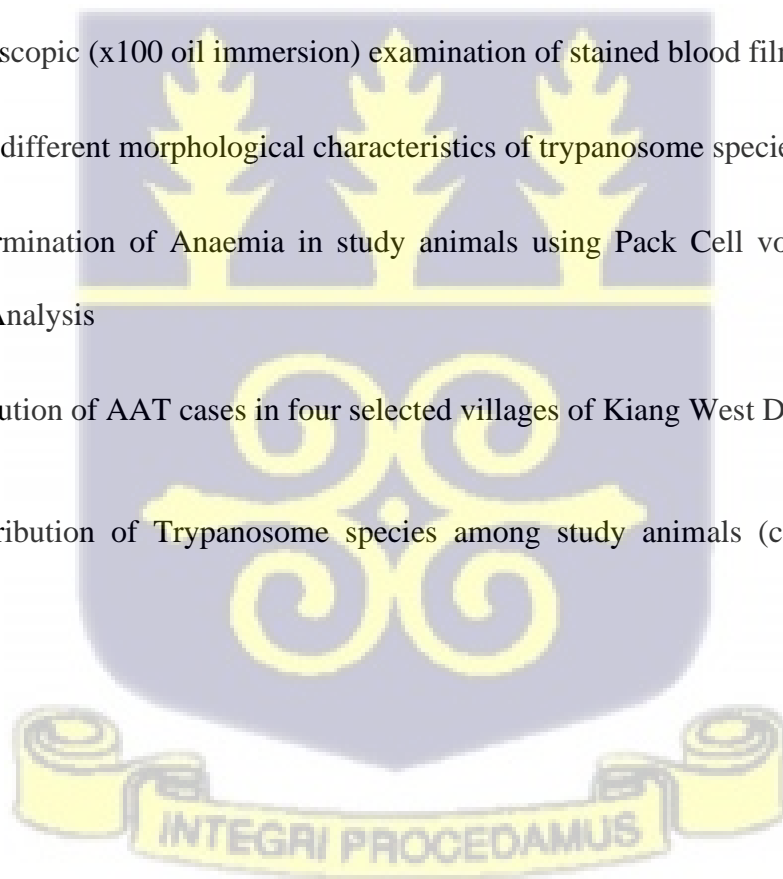
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LIST OF ABBREVIATION

AAT - African Animal Trypanosomiasis

GFELTP -Ghana Field Epidemiology and Laboratory Training Program

SSA - Sub-Saharan Africa

WAHO - West African Health Organisation

WALIC – West African Livestock Innovation Centre

DLS - Department of Livestock Services

CVL - Central Veterinary Laboratory

GBOS - Gambia Bureau of Statistic

CFSPH – Centre for Food Security and Public Health

VSG - Variant Specific Glycoprotein

PARPs - Procyclic Acidic Repetitive Proteins

CNS - central nervous system

HAT - Human African Trypanosomosis

PCV - Pack Cell volume

OIE - World Organisation for Animal Health ()

DNA - Deoxyribonucleic Acid

SIT - Sterile Insect Technique

EDTA - Ethylenediaminetetraacetic aci



CHAPTER ONE

INTRODUCTION

1.1 Background

African Animal Trypanosomosis (AAT) infection is a vector-borne disease caused by unicellular protozoan parasites of the genus *Trypanosoma* (order Kinetoplastida) (Diarra et al., 2019). The parasites are primarily transmitted by a tsetse fly vector to livestock species, wildlife and human. (Desquesnes, 2017). Amongst livestock species, the disease is highly fatal to the cattle population and causes huge economic losses in terms of value and productivity (Desquesnes, 2017).

Furthermore, the disease has also constrained agricultural production in more than 10 million km² in sub-Saharan Africa including ecological zones that hold the continent's greatest potential for expanded agricultural production (Diarra et al., 2019). It has been estimated that a total AAT infection causes over 3 million cattle mortalities in sub-Saharan Africa annually. (Assefa & Shibeshi, 2018).

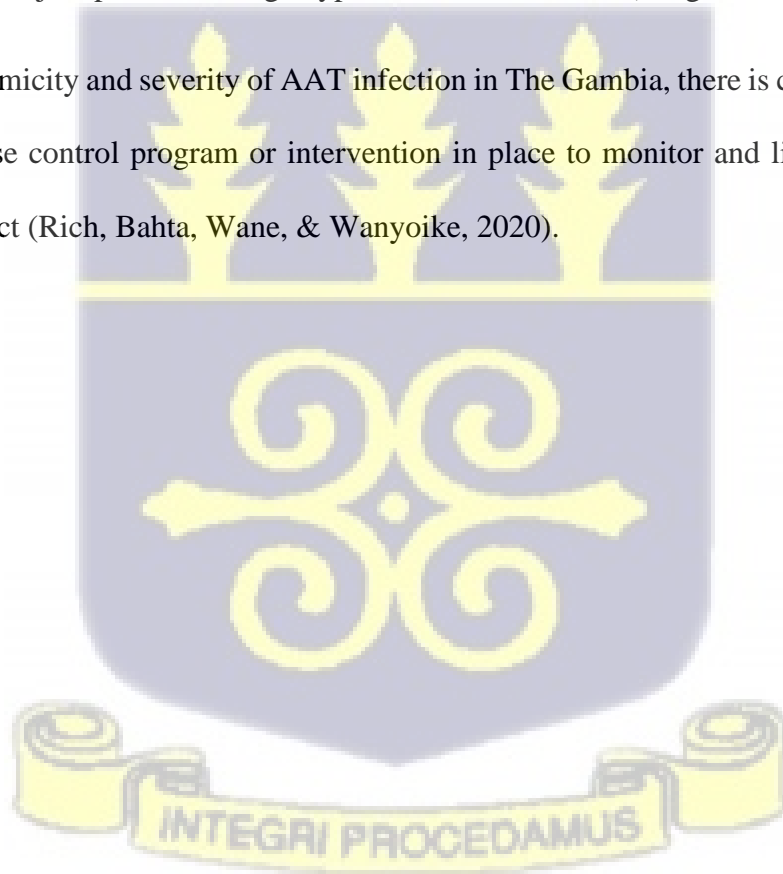
Even though the disease has been designated as a notifiable disease by the World Organisation for Animal Health (OIE) (i.e., a disease that should be reported to OIE as soon as it is suspected or detected) and that significant efforts have been made to study and control AAT, the disease continues to be a major obstacle to the development of more sustainable and cost-effective crop-livestock agricultural systems in sub-Saharan Africa (Diall et al., 2017)

Unfortunately, The Gambia remains one of Sub-Saharan Africa's most vulnerable countries to trypanosome infection (Touray, Ceesay, & Njai, 2010). Although efforts have been made some years ago by the Government of the Gambia with the establishment of the *International Trypanotolerant Center* (ITC) which is a non-autonomous, non-profit oriented regional

livestock agricultural research institute to conduct research and promotion of trypanotolerant breed of cattle to mitigate the impacts of AAT infection on livestock species. However, due to acute funding challenges, ITC could no longer carry out this mandate in the Gambia thus leaving AAT infection prevention and control in a handicap situation.

Recently, annual reports from the Department of Livestock Services recorded a total of 1065 cases of AAT infections, with over 80 % detected in cattle species (Department of Livestock Services, Ministry of Agriculture, 2020). Furthermore, studies conducted in Kiang West District on small ruminant species of livestock reveal an overall prevalence of 12.5% Tsetse-transmitted trypanosome infection with that *T. congolense* and *T. vivax* and to a lesser degree *T. brucei* identified as the major species causing Trypanosome infection in (Kargbo & Kuye, 2020).

Despite the endemicity and severity of AAT infection in The Gambia, there is currently no well-structured disease control program or intervention in place to monitor and limit the disease's spread and impact (Rich, Bahta, Wane, & Wanyoike, 2020).



1.2 Problem statement

In Sub-Saharan Africa, more than 55 million herd of cattle are at risk of AAT infection and over 60 million people are at risk of acquiring sleeping sickness (SSA) (Muhanguzi et al., 2017). The disease has also inflicted havoc on different livestock species and caused an economic loss to poor rural farmers in almost all countries within the Tsetse fly belt (Wilson G., 1963).

In the Gambia, AAT infection is endemic in most rural agricultural communities and has caused a significant reduction in the production capacity of cattle in terms of daily milk and beef supply (Rich et al., 2020). The infection has also reduced the draught power of cattle and the market value of its essential by-products such as hides and skins (Touray et al., 2010). In addition, AAT infection has been identified as one of the major livestock priority diseases that causes the highest number of losses to cattle populations in terms of mortalities in the Gambia (DLS, 2016).

Some of the studies conducted on equine species (horses, donkeys) and small ruminants (sheep, goats) indicated a high prevalence and incidences of AAT infection in The Gambia. In equine, an overall AAT infection prevalence of 91% was identified (Pinchbeck et al., 2008), while an overall prevalence of 12.5% was identified in small ruminants in The Gambia (Kargbo & Kuye, 2020).

AAT has been endemic in the Gambia due to several factors. Some of these factors include the dynamic nature of the distribution and abundance of Tsetse fly (AAT vector), trypanosomes, ecological disturbances, ineffective disease surveillance systems, poor diagnostic capacities, and inadequate control measures (Pinchbeck et al., 2008). These factors have a significant economic impact on cattle production, either directly or indirectly, by decreasing the animals'

production capacity or by increasing the rate of cattle losses attributable to mortalities (Rich et al., 2020). Without enough information and scientific data to clarify the relationship between some of these factors and AAT in cattle, the Gambia's cattle population will continue to decline due to death, poor rural farmers' economic growth will be slowed, and the livestock sector's output and contribution to national GDP will be significantly lowered.

Therefore, this study was set up to determine the factors associated with AAT infection in the cattle population to determine its current prevalence, the type of species in circulation in cattle and the association between husbandry factors and the infection.



1.3 Conceptual framework

The conceptual framework in **Figure 1** presents some of the risk factors associated with AAT infection in cattle. These factors include animal demographic factors, environmental factors, husbandry factors, and policy systems

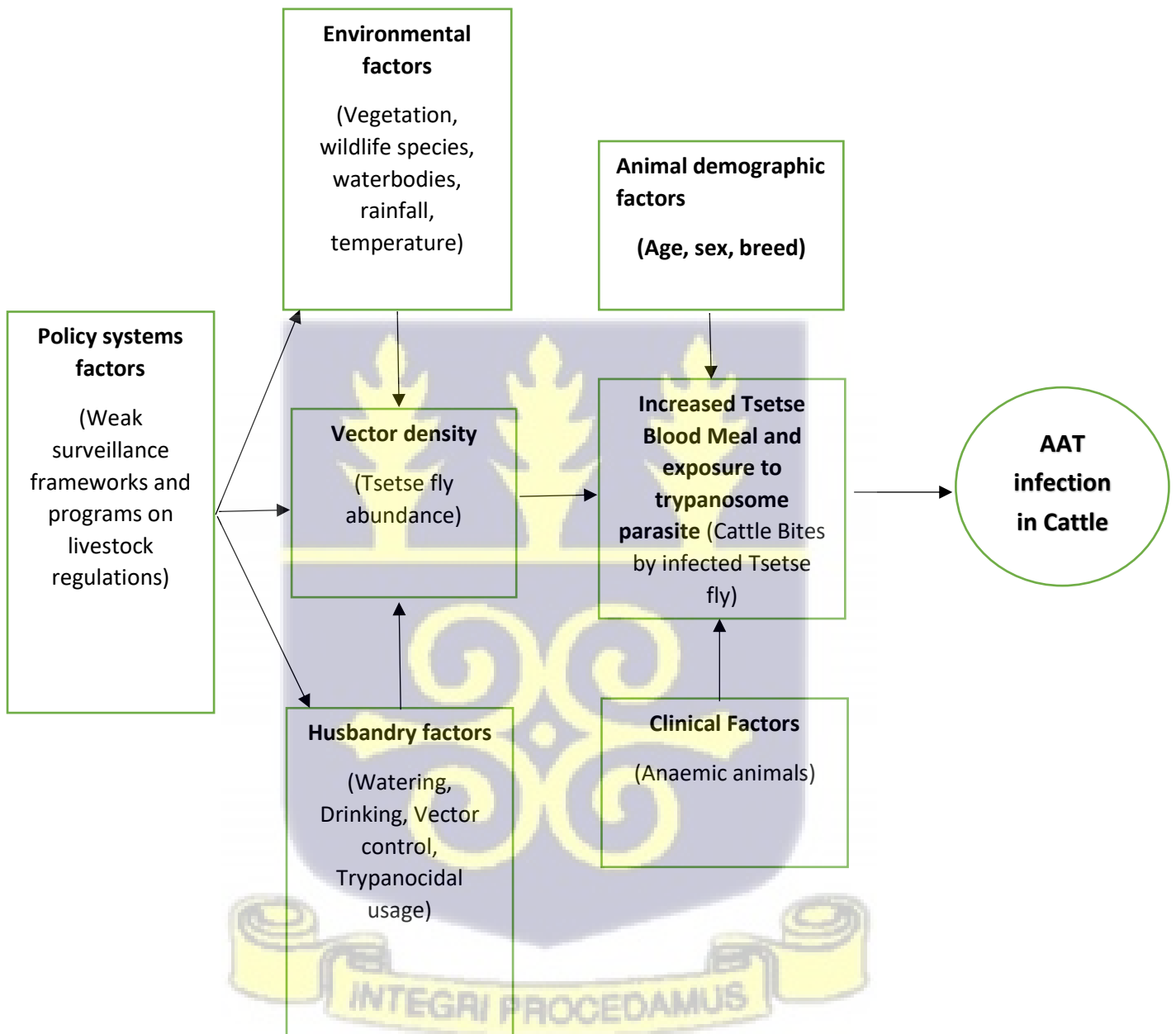


Figure 1: Conceptual Framework on factors associated with African Animal Trypanosome Infection in cattle

1.3.1 Animal Demographic Factors

Cattle's vulnerability to AAT infection can be greatly influenced by their age, gender, and breed. Such demographic factors can play a major role in the grazing activities, techniques used by farmers and herdsman during husbandry practices and disease control and prevention. For instance, farmers that practice traditional husbandry systems (Extensive grazing) usually focus more female animals on the farm with one or two or very few males for mating purposes. Such choices made by farmers automatically creates a more focus on bulls (male cattle) than female cattle leading, thus protecting the male cattle from exposure to Tsetse fly-infested suspected areas. Some farmers in certain instances prioritizes male cattle over female cattle when having to administer Trypanocidal drugs this is due to a very similar notion of limited male cattle as compared to female cattle therefore deserving more attention and care. This phenomenon has put female cattle in a situation where they are more susceptible to Tsetse bites and, as a result, AAT infection.

When it comes to cattle's age, the situation is similar. Adult cattle are more likely to be exposed to Tsetse fly bites than younger cattle because younger cattle are kept close to homes or backyards within designated locations where they are rarely exposed to Tsetse fly bites, whereas adult cattle are usually allowed to graze freely within woodland environments where they are more likely to be exposed to Tsetse fly bites and eventually develop AAT.

1.3.2 Clinical Factors

The animal's anaemia status is also an important clinical factor caused by other priority livestock disease and poor husbandry practices that usually reinforces cattle susceptibility to AAT (Mamoudou, Njanloga, Hayatou, Suh, & Achukwi, 2016). Anaemia, regardless of the source, causes cattle to become lethargic, weak, and occasionally anorexic, making them less active to protect themselves from Tsetse bites while feeding and grazing in Tsetse fly density areas.

1.3.2 Husbandry Practices

1.3.2.1 Grazing and Watering Practices

Most cattle in the Gambia are raised under traditional management systems whereby animals are tethered individually at night on grounds close to the homestead and herded during the daytime to approximately graze 6-8 h in natural unimproved pasture and vegetation (Agyemang, Dwinger, Touray, Jeannin, & Fofana, 1990).

During such grazing activities, animals get exposed to infected tsetse fly bites, which eventually leads to AAT infection. Similarly, for drinking, most animals are taken to ponds and riversides for drinking and during this period they mostly mingle and associate with wildlife species that are highly susceptible to Tsetse bites and AAT infection, and as a result, there is always a possibility of transmission of the pathogen by the vector from susceptible wildlife to cattle during a blood meal(Edmond et al., 2021)

1.3.2.2 Vector control Practices

The use of several vector control methods such as the application of insecticides in the form of odour-baited traps, aerosols by air or ground spraying, and in some instances dipping or pour-on solution all contribute to a significant reduction of Tsetse effects on animals. However, due to the cost of most of these products, many poor rural farmers can't afford to purchase these thus leaving their animals to Tsetse exposure within communities(WHO-TDR, 2004)



1.3.3 Environmental factors

1.3.3.1 Temperature

The effects of climate change are predicted to be worse for the developing world where challenging socioeconomic and political environments are exacerbated by a lack of epidemiological studies on zoonotic diseases (Naicker, 2011). Arthropod vectors such as tsetse flies (*Glossina* spp.) are the most sensitive to climatic temperature variability (Naicker, 2011). The entire live cycle of the fly is dependent on ambient temperatures. They survive well at an optimal temperature of 25°C- 26°C but has the potential to survive at a maximum temperature of up to about 38 degrees Celsius (FAO, 1982). Usually, the adult vector gets damaged at temperatures above 38 °C and hardly survives a normal life at 17°C (FAO, 1982). With an abundant vector presence due to higher survival rates, there is a likelihood of transmission of the AAT amongst the livestock population.

1.3. 3.2 Rainfall

The soil moisture during the rainy season provides a suitable condition for the breeding and development of the Tsetse fly. Following the hatching of the eggs into larvae within the fly, they are well deposited and burrowed into moist soil where they continue their development into pupae and eventually emerging as adult flies.

1.3.4 Policy factors

Poorly implemented policies and surveillance systems play a big role in the emergence and re-emergence of livestock diseases like AAT infection in cattle populations. Unfortunately, Gambia continues to be low on both trained veterinary professionals and the equipment required to track the spread of crucial diseases like AAT infection. Surveillance officers, veterinarians,

and equipment such as modern-technology-equipped laboratories (PCR) are all in insufficient supply, making disease diagnosis, prevention, and control a challenge (Rich et al., 2020).

1.5 Justification

In The Gambia, AAT has posed a severe food insecurity threats as well as zoonotic potentials on farmers and general population (Münstermann, 2004). Unfortunately, there is no available scientific data on morbidities and mortalities cause by AAT infection to cattle population and its zoonotic impacts on human population in the Gambia. However, there is the urgent need to maintain and improve the trend of cattle production contribution to national GDP, through the control and eradication of AAT infection in the Gambia.

However, there is no available data on the current prevalence of AAT infection in cattle and its associated risk factors. This situation has greatly limited the implementation of appropriate AAT infection prevention and control strategies in cattle population in The Gambia

Findings from these studies will provide significant appropriate data on factors associated with AAT in cattle population in The Gambia that can be used by all stakeholders to implement appropriate disease prevention and control strategies that are needed to reduce poverty and hunger on poor rural communities and enhance economic growth of Agricultural GDP in the Gambia

Furthermore, this study will provide information on the best husbandry practices in terms of grazing and animal drinking patterns and the use of appropriate vector control measures that are necessary to promote the growth and development of cattle production

There is also a need to identify individual trypanosome species that are in circulation within the cattle population. This will guide farmers and livestock personnel in making appropriate treatment strategies that will limit the current misuse of Trypanocidal drugs and reduce drug resistance.

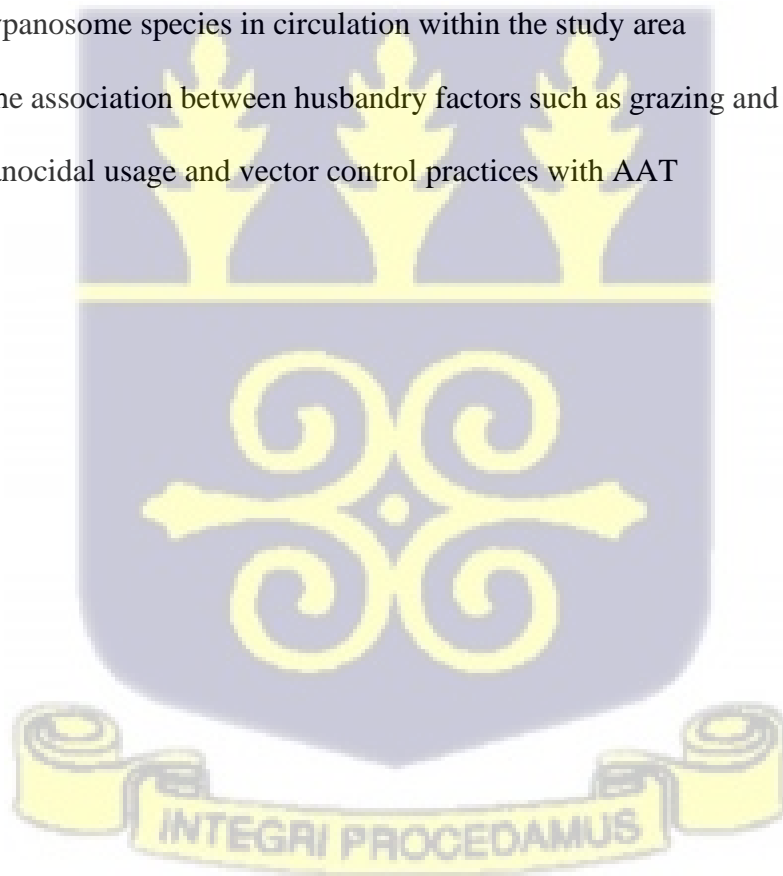
1.4 Objectives

1.4.1 General objective

To determine risk factors associated with African Animal Trypanosomiasis in cattle in Kiang West District, Lower River Region in the Gambia

1.4.2 Specific objectives

1. To determine the prevalence of AAT among the cattle population
2. To assess the association between the age, sex, breed, and anaemia condition of cattle with AAT
3. To examine trypanosome species in circulation within the study area
4. To determine the association between husbandry factors such as grazing and drinking patterns of animals, Trypanocidal usage and vector control practices with AAT



CHAPTER TWO

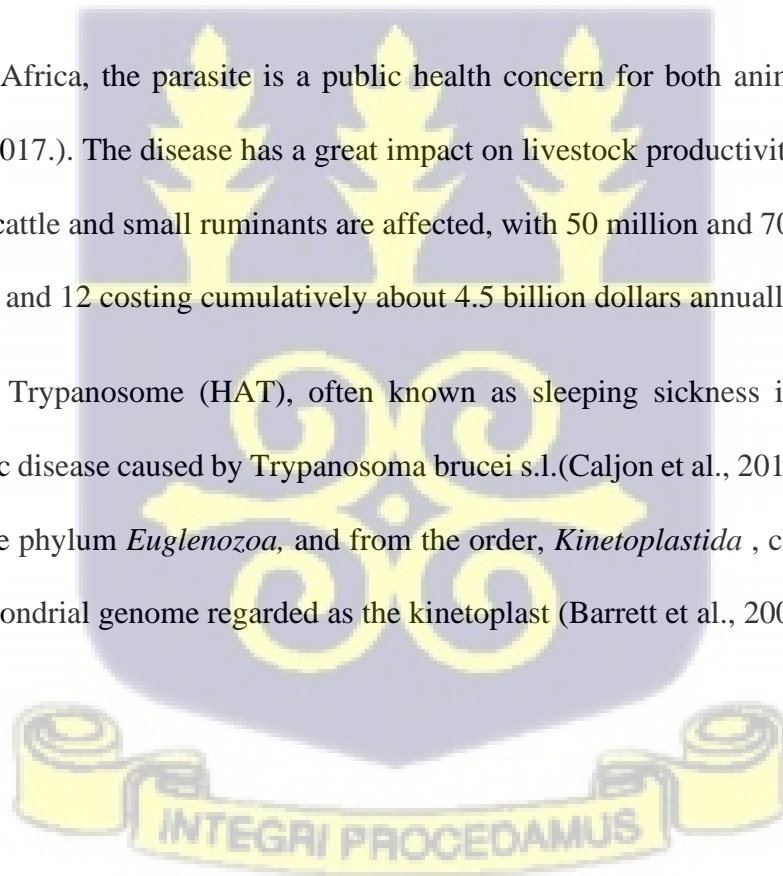
LITERATURE REVIEW

2.1 Etiology of African Animal Trypanosomosis (AAT)

African Animal trypanosome is a disease complex that is caused by protozoan parasites of the genus *Trypanosoma*, family Trypanosomastidae and order Kinetoplastida (Cox et al., 2010). Some of the key species that cause infection in cattle and other livestock species are *Trypanosoma brucei*, *T. congolense* or *T. vivax*. However, the parasites are obligate protozoans and they can infect all vertebrates including wildlife species (Cayla, Rojas, Silvester, Venter, & Matthews, 2019).

In Sub-Saharan Africa, the parasite is a public health concern for both animals and humans (Philippe et al., 2017.). The disease has a great impact on livestock productivity in sub-Saharan Africa. Mostly, cattle and small ruminants are affected, with 50 million and 70 million being at risk respectively and 12 costing cumulatively about 4.5 billion dollars annually (FAO., 2014)

Human African Trypanosome (HAT), often known as sleeping sickness in humans, is an endemic parasitic disease caused by *Trypanosoma brucei* s.l. (Caljon et al., 2016). The parasites are found in the phylum *Euglenozoa*, and from the order, *Kinetoplastida*, characterized by a modified mitochondrial genome regarded as the kinetoplast (Barrett et al., 2003)



2.1.1 Morphology

African Animal Trypanosome is a single cell organism that differs in sizes ranging from 8µm to 50 µm in length (Satoskar, Simon, Hotez, & Tsuji, 2009). All the activities of the organism are carried out within this single cell.

The protoplasm of the parasites is unique and comprises three parts. The outer protective layer is known as the pellicle, cell envelope and cell membrane. Its inside is made up of the cytoplasm which forms most of the cell. The organism has a nucleus like other living cells, and it's located within the cytoplasm. The nucleus serves as the command centre of the cell containing the DNA (deoxyribonucleic acid). The nucleus also serves as the and reproduction organelle of the organism.

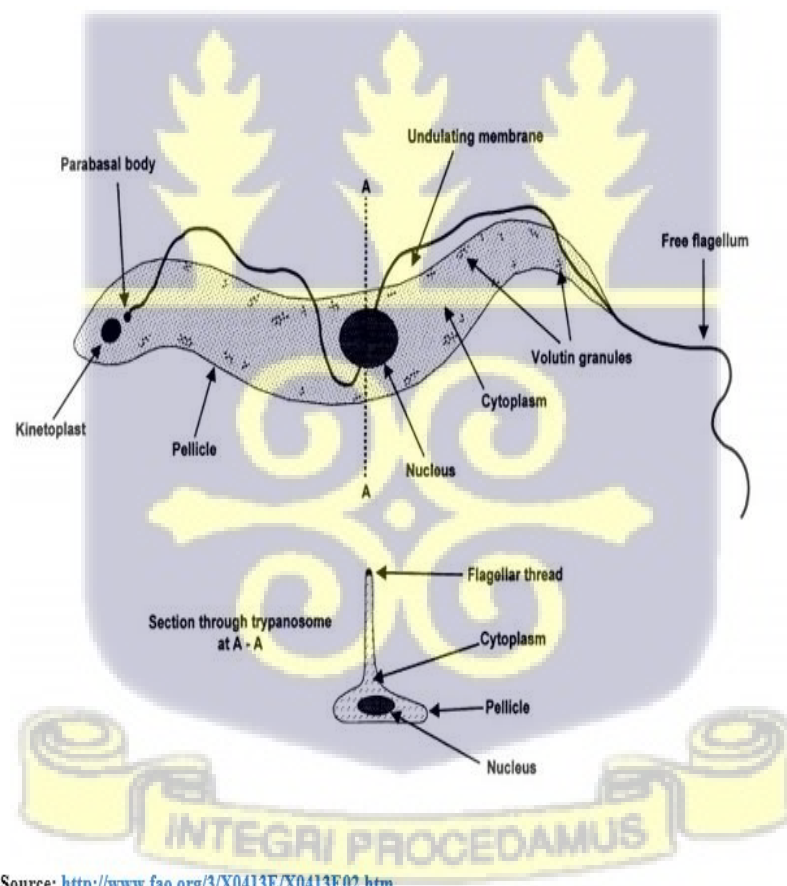


Figure 2: General structures of African Animal Trypanosome

2.1.2 Life cycle AAT infection

The life cycle of the African Animal trypanosome occurs between the Vector (Tsetse fly) and the mammalian host (animals or human) (Baral, 2010). The pathogen exists in different forms at different stages of their life cycle. When the vectors (Tsetse fly) bites and inject the infective stage of the parasite in the metacyclic form into the mammalian host, it quickly transforms into a stage known as the blood-stage Trypomastigotes (Baral, 2010). From the Blood -stage Trypomastigotes, the organism undergoes a binary fission division in the interstitial spaces at the site of vector bite followed by a rapid cell cycle re-entry and exchange of the restricted repertoire for antigenic variation(Baral, 2010). This stage is followed by another transformation into a heterogeneous form comprising the proliferative slender form and non-proliferative stumpy form (Baral, 2010).

During a blood meal, the Vector (Tsetse fly) ingests along the bloodstream trypomastigote from the mammalian host and inside the midgut of the vector, the organism changes by losing a surface molecule known as the Variant Specific Glycoprotein (VSG) and expresses its surface protein called the Procyclic Acidic Repetitive Protein (PARPs or procyclin) and this leads to the formation of a procyclic trypomastigote form (Baral, 2010).

By the end of proliferation in the midgut of the vector, the procyclic trypomastigote migrates to the salivary glands and attaches itself to the glands and rebuild a VSG coat before being released into the salivary gland lumen in the epimastigote form, which afterwards multiplies and transforms into metacyclic trypomastigote (Baral, 2010). After this stage of transformation within the vector, the parasite gets ready and waits for possible inoculation into any mammalian host to continue its life cycle. **Figure 3** below shows the complete life cycle of AAT infection.

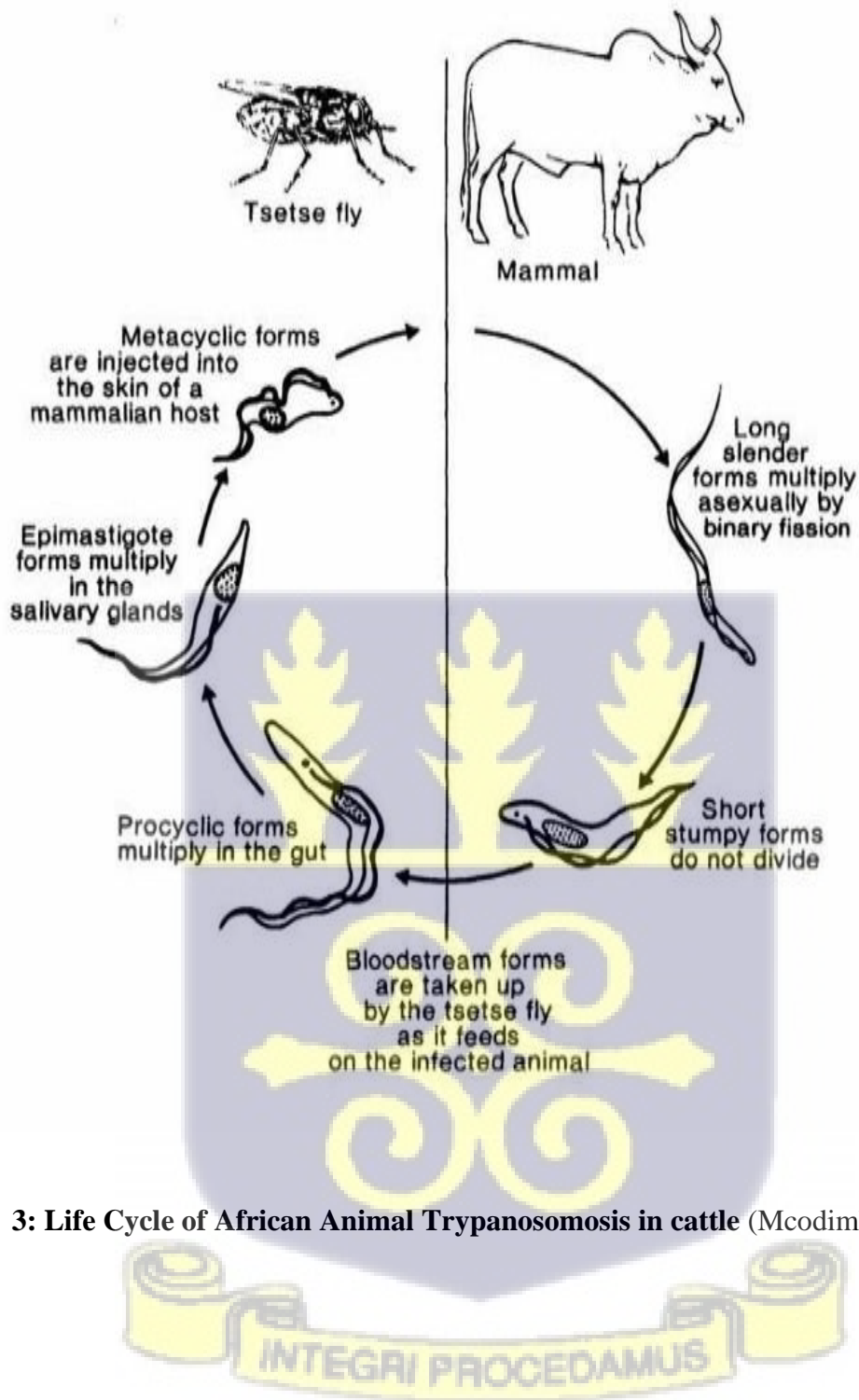


Figure 3: Life Cycle of African Animal Trypanosomosis in cattle (Mcodimba, 2006).

2.2 Pathogenesis and clinical manifestation of AAT infection

After the vector (tsetse fly) injects the trypanosome parasite (metacyclic trypomastigote form) into the epidermis of a mammalian host (cattle), the parasite develops for a few days before entering the lymph nodes and bloodstream, where it divides quickly by binary fission (Caljon et al., 2016). The pathogen eventually attaches to endothelial cells and sets in capillaries and small blood vessels, causing symptoms such as intermittent fever, anaemia, oedema, lacrimation, enlarged lymph nodes, abortion, decreased fertility, loss of appetite, and weight loss (Muhanguzi, Mugenyi, Bigirwa, Kamusiime, & Kitibwa, 2017).

2.3 Zoonotic potential

AAT infection has been classified as a critical zoonotic disease that requires prompt intervention. It causes a severe impediment to cattle productivity and a major public health risk for humans. Some of the species responsible for Trypanosome infection in humans are *Trypanosoma brucei*, which may resist the naturally occurring trypanolytic factor (APOL I) and cause infections in people that can be fatal.

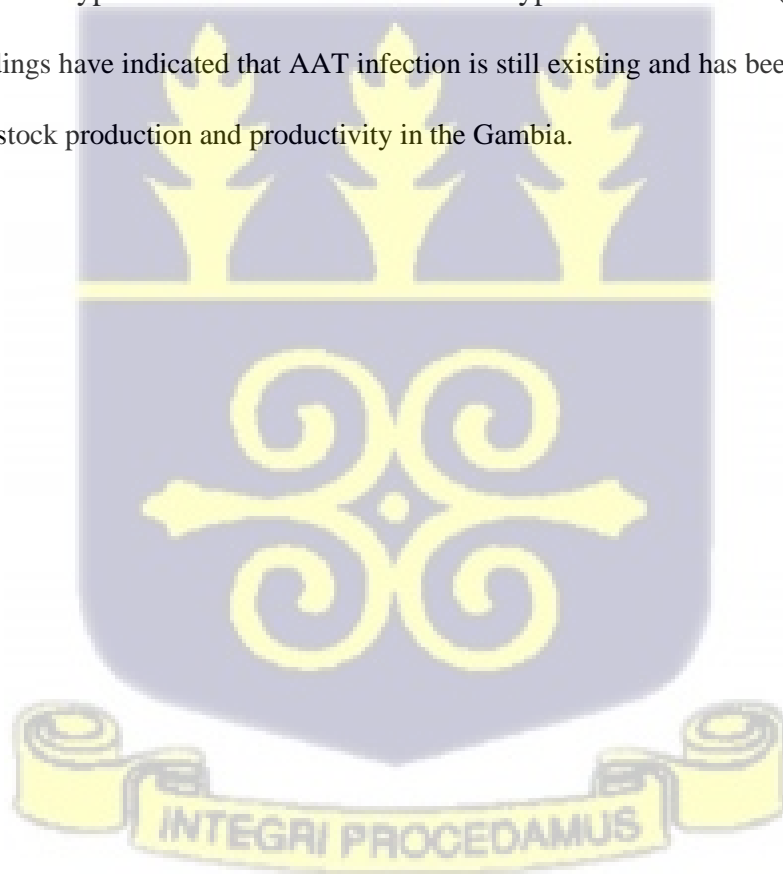
In humans, the disease is manifested in two stages (Matovu et al., 2020). The first stage is regarded as the hemolymphatic stage which is associated with nonspecific symptoms such as fever, headache, or fatigue (Berthier et al., 2016). The second phase is characterized as the meningoencephalitic stage, which occurs when the disease invades the central nervous system (CNS) and causes neurological problems and eventually leading to coma and death (Berthier et al., 2016).



2.4 African Animal Trypanosomosis in the Gambia

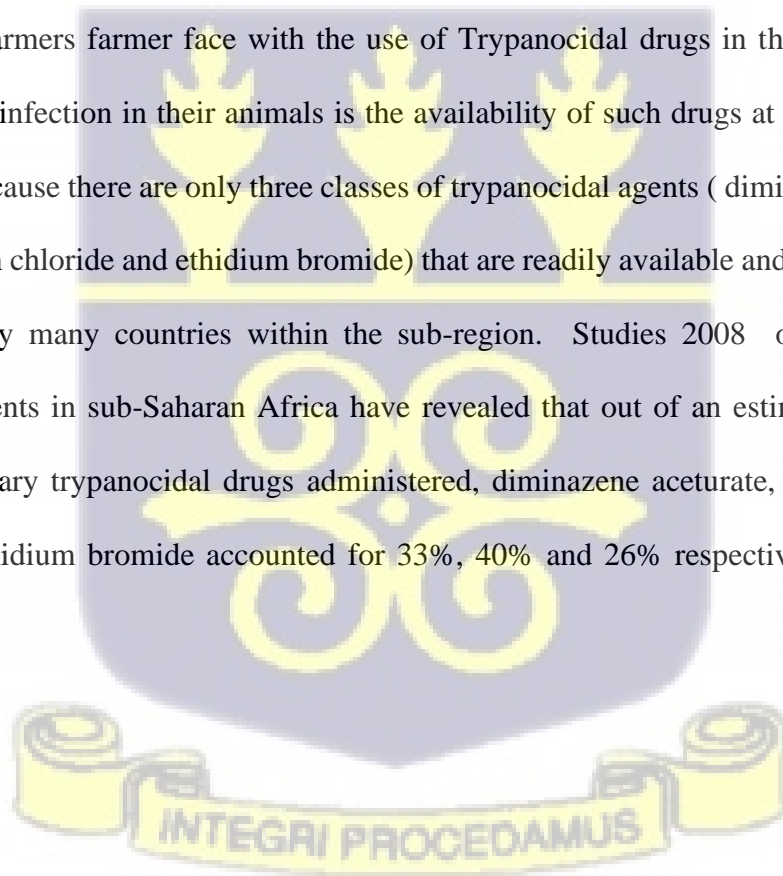
In the Gambia, livestock farmers rely heavily on livestock production for their social and economic growth. However, the existence of infectious Transboundary Animal Diseases (TADs) such as AAT infection, have significant effect production and productivity of diverse livestock species such as cattle, sheep, goats, and pigs in different regions of the country. Although the prevalence of AAT infection in small ruminates (goats and sheep) was as low as 1% to 0% (Kargbo & Kuye, 2020). However, in other livestock, the prevalence of AAT infection is significantly high.

Studies conducted in the Central River Region of the country on working horses and donkeys reveals a trypanosome prevalence of 91%; with an infection rate of 31% for *Trypanosoma congolense* Savannah, 87% for *Trypanosoma vivax* and 18% for *Trypanosoma brucei* (Pinchbeck et al., 2008). These findings have indicated that AAT infection is still existing and has been one of the major constraints to livestock production and productivity in the Gambia.



2.5 AAT infection Prevention and Control

Throughout the twentieth century, several attempts were undertaken to control AAT by altering the transmission vector (Tsetse fly) through the use of sterile insect release technique, the elimination of fly habitat, the use of Tsetse traps, the use of insecticide-treated cattle, and coordinated mass insecticide spraying (Yaro, Munyard, Stear, & Groth, 2016). However, the use of Trypanocidal drugs that target the parasite in host species has been the most effective and frequently used methodology to control AAT infection in livestock species (Vitouley et al., 2012). Unfortunately, despite the massive success of Trypanocidal drugs, there is a significant public health issue about their resistance, which is attributable to drug misuse by untrained livestock officers and farmers with insufficient knowledge about drugs (Holmes et al., 2004). Another issue farmers farmer face with the use of Trypanocidal drugs in the prevention and control of AAT infection in their animals is the availability of such drugs at a very expensive price. This is because there are only three classes of trypanocidal agents (diminazene aceturate, isomethamidium chloride and ethidium bromide) that are readily available and proven effective and approved by many countries within the sub-region. Studies 2008 on the usages of trypanocidal agents in sub-Saharan Africa have revealed that out of an estimated 35 million doses of veterinary trypanocidal drugs administered, diminazene aceturate, isomethamidium chloride and ethidium bromide accounted for 33%, 40% and 26% respectively (Yaro et al., 2016).



CHAPTER THREE

METHODS

3.1 Study design

A cross-sectional study was conducted from February 2020 to June 2021 to determine the factors associated with AAT in cattle in Kiang West District Lower River Region The Gambia. Purposive sampling was used to select the study district (Kiang West District) based on the endemicity and abundance of Tsetse fly vector in the area (Kargbo & Kuye, 2020). Simple random sampling method was used in selection of study villages and herds. The age study animals was estimated based on dentition technique given by Torell and Dr. Ben Bruce (Torell, Bruce, & Kvasnicka, 2003)

Data on clinical factors and AAT in cattle from laboratory analysis was collected into Microsoft excel. Data on animal demographic factors, such as grazing and watering pattern, Trypanocidal usage and vectors control measures were collected using structured questionnaires designed on KoboCollect software application. Data collected was analysed using STATA version 16.0 and Microsoft office 2010 software. Descriptive statistic was performed using mean and standard deviation, and univariate and multivariate logistic regression was performed to determine the for the association between AAT and independent variables for these studies

3.2 Study Area

The study was conducted in the Kiang West District. It is one of the six districts of the Lower River Region of The Gambia. The region is one of the five administrative regions of the country located south bank of River Gambia. It is the largest district within the region with a total of thirty -four (34) villages. (GBOS 2013 Census.).

Geographically, the district is on latitude 13 degrees north and -16 degrees west. It has a total area of 709 km² with an estimated human population of 14,990 inhabitants (GBOS 2013

Census.). The district has 6793 heads of cattle that are reared extensively, with a system of communal herding(Touray et al., 2010)

The district is bordered with Kiang Central District to the East, Bintang Bolong to the West, south with northern Senegal and north with the River Gambia. The livelihood of the people is primarily dependent on mixed subsistence livestock and crop farming. The district is unique in having one of the country's largest and most important national parks namely Kiang West National Park with a perimeter size of 125kmsq. **Figure 4** below shows the district of Kiang West, The Gambia and **Figure 5** shows the location of the four selected villages within the district.

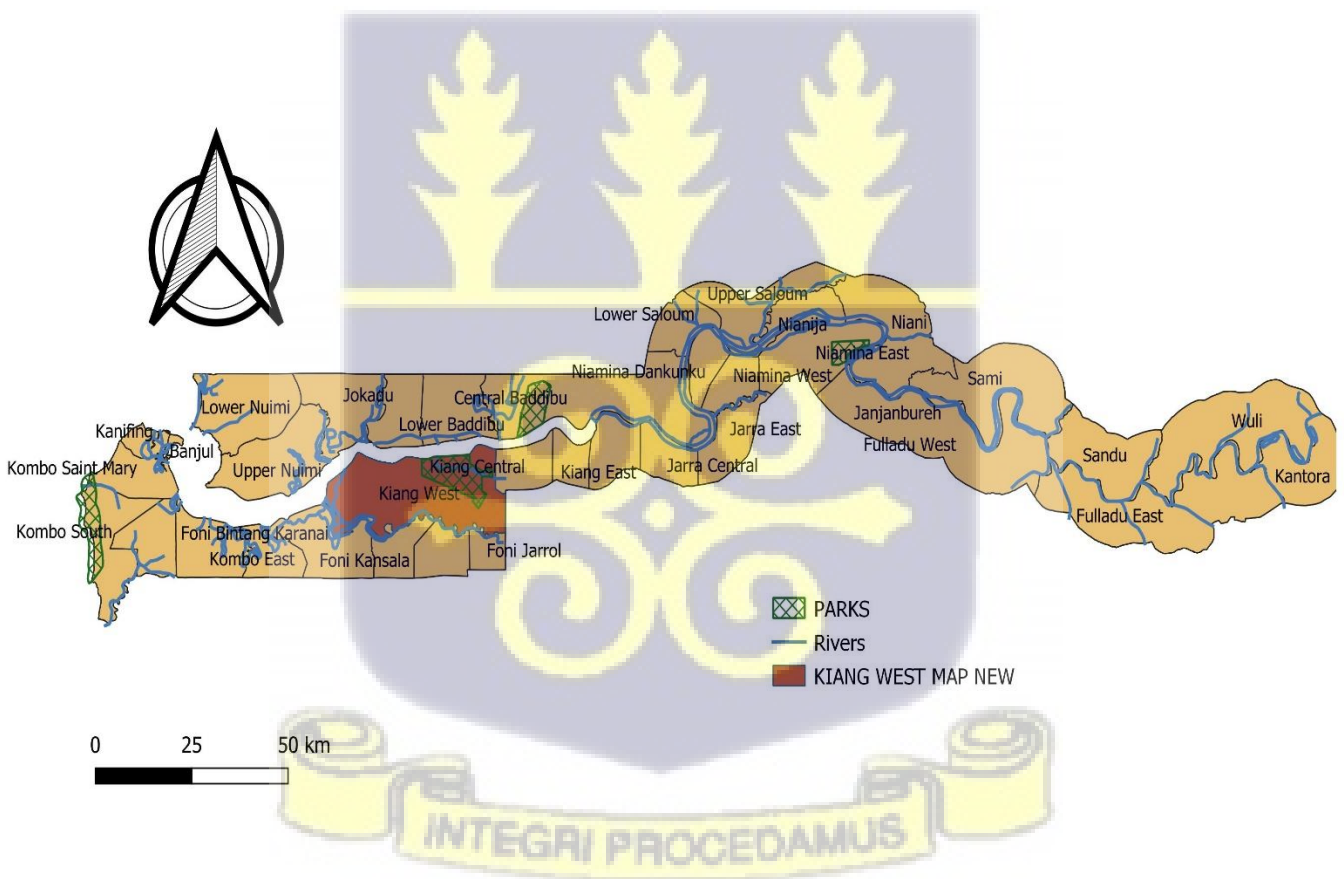


Figure 4: Map of Lower River Region with the Administrative Districts including Kiang West District (source: https://en.wikipedia.org/wiki/Kiang_West)

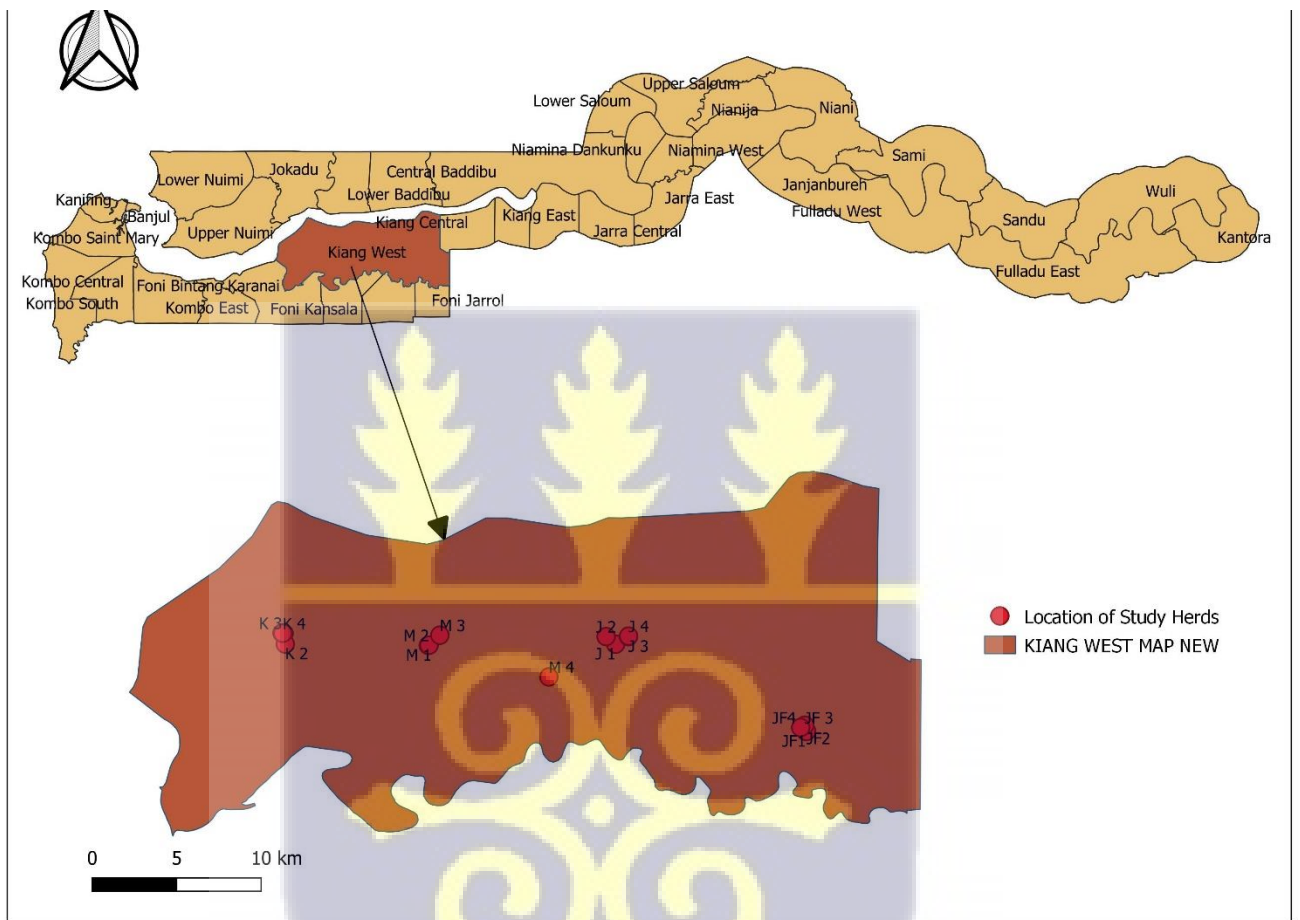


Figure 5: Map of showing Selected Villages within Kiang West District (source : (Brawen J et al . 2017))

3.3 Study Population

The study population was made up of cattle of all age groups, sex, and breed that are managed under the traditional husbandry system.

3.3.1 Inclusion criteria

Cattle of all ages, sex and breed that are found within the Kiang West District.

3.3.2 Exclusion criteria

Cattle that had had Trypanocidal drugs within 20-21 days before sample collection

3.4 Study Variables

The dependent variable for this study was African Animal Trypanosome Infection while the independent variables were animal age, sex, breed, presence of anaemia, grazing pattern, watering pattern, Trypanocidal Usage and Vector Control measures. The study variables and their operational definition is given in **Table 1a-b** below.



Table 1a: Operational Definition and scale of Measurement for study variables

Variables	Operational Definition	Scale of Measurement	Source of Data	
Dependent Variable	AAT infection	Whether the sample examine contains AAT infection of not	Binary	Results from whole Blood Sample analysis
			- AAT positive - AAT negative	
Independent variables	Age	The total years lived by the animal (cattle) from time of birth until the time of data collection for this study	Nominal	Observation of animal dentition
			- 0-3 years (Young) 4-9 years (Adult)	
	Sex	Being either a male animal or a female animal	Nominal	Animal Observation
			- Male - Female	
	Breed	Being either a Zebu, Ndama or Cross-breed animal	Nominal	Animal Observation
			- Zebu - N'dama -Crossbreed	
	PCV	Packed Cell volume estimates whole blood	Nominal	Whole blood sample PCV estimation using Hematocrit reading method
			- Anaemic -Non-anaemic	

Table 1b: Operational Definition and scale of Measurement for study variables

Variables		Operational Definition	Scale of Measurement	Source of Data
Independent variables	Grazing pattern	Nature feeding cattle are subjected	Binary - Extensive Intensive	Interview of farmers/herdsmen
	Watering pattern	source of water for drinking of cattle	Binary - Home Away	Interview of farmers/herdsmen
	Trypanocidal Usage	Type of Trypanocidal agent given to cattle before this study	Binary - Administered - Not Administered	Interview of farmers/herdsmen
Vector control measure	Implementation of any form of vector (tsetse fly) control method	Binary - Employed - Not employed	Interview of farmers/herdsmen	

3.5 Sample Size determination

The sample size was determined using the formula given by Michael Thustfield (Veterinary Epidemiology, 2018 .), based on the expected prevalence of 50%, with a confidence interval level of 95%, and 5% desired absolute precision.

$$n = \frac{1.95^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision



3.6 Sampling method

The study district was selected purposively due to finding from previous studies identifying the district as a Tsetse fly (disease vector) endemic zone (Kargbo & Kuye, 2020). We used a multistage sampling process to obtain individual study animals. Study villages were selected by a simple random sampling technique using Microsoft Excel® 2010 (Micro-soft Corp., Redmond, WA, USA) after obtaining a sampling frame that comprised of a complete list of all villages (34) and their geo-reference positions from the Department of Livestock Services, The Gambia.

From each of the villages, four (4) herds were selected using a simple random technique without replacement (**Table 2**). We selected a total of 16 herds that were involved in this study. The average size of a herd was 70 head of cattle. We finally selected individual study units by using individual herds per village as strata and randomly selected animals proportional to each herd size (stratified random sampling technique).

Each herd was visited a month before sample collection to seek consent from herdowners in participating in the study

Table 2: List of Villages and Herds in Kiang West District, Lower River Region, The Gambia

S/N	VILLAGES	NO. OF HERD	NO. SELECTED HERD
1	Jali	7	4
2	Jifarong	8	4
3	Manduar	5	4
4	Karantaba	10	4
Total		30	16

3.6.1 Blood Sample collection and Examination

A total of 384 blood samples were collected from the jugular veins of each animal (cattle) using sterile sharp 14-gauge needles. Collected blood was quickly transferred into clean and dry vacutainer tubes containing Ethylenediaminetetraacetic acid (EDTA) anticoagulant. Samples were packed in a cooler with ice packs and transported to the West African Livestock Innovation Centre (WALIC) field veterinary Laboratory for processing and analysis (**Figure 6**).



Figure 6: Blood sample collection from individual study animals from a herd

3.6.2 Parasitological Examination

Phase-contrast Technique (Murray Method) was used as a parasitological technique to analyse the collected blood samples. A total volume of 70 µl of blood was transferred into capillary tubes (25X1.5mm), using a Cristal seal one end of the tube was sealed before placing into a microhematocrit centrifuge for centrifugation. The seal capillary tubes were centrifuged at 9000 revolutions per minute (rpm) for 5 minutes. After spinning, the capillary tubes were cut with a diamond-tipped pencil 1mm below the buffy coat layer (to include the RBC) and the uppermost content was extruded onto a clean microscope slide and covered with a coverslip (22X22mm). The slide was then examined using a phase-contrast microscope at a magnification of x40. Total examination of the slide was conducted by thoroughly scanning over 200 fields of preparation to find the presence of motile trypanosomes.

Morphological characteristic of Trypanosome

T. vivax: Under a wet smear the parasite looks large, extremely active, transverses the whole field very quickly, passing occasionally.

T. brucei: Under wet preparation, rapid movement in confined areas, undulating membrane traps the light into pockets moving along the body.

T. congolense: Under wet smear, parasite looks small, sluggish, and adheres to Red Blood Cells (RBCs)

3.6.3 Giemsa Stained -Thick Blood films technique

This technique was used to identify the AAT species. It was carried out by placing a drop of blood (5-10 microliters) on a clean microscope slide and spreading it over an area of 20cm followed by air drying at room temperature before staining for 30 minutes using 4% diluted Giemsa Stain in phosphate buffer saline (PBS) pH 7.5. The stained smears were then washed

with buffered water and examined at x100 oil immersion objective for identification of different morphological characteristics of trypanosome species (**Figure 7**)



Figure 7: Microscopic (x100 oil immersion) examination of stained blood films for identification of different morphological characteristics of trypanosome species.

3.6.4 Haematological Examination

Pack Cell Volume (PCV) estimation technique was conducted on all collected blood samples. This was achieved by filling a capillary tube with Whole blood up to 2/3rd to 3/4th (70µl volume) and sealing at one end using Cristal seal. The sealed tubes were placed into a microhematocrit centrifuge and spun at 9000 rpm for 5 minutes. Using a Haematocrit Reader, the tubes were read and PCV values were obtained to estimate the anaemia condition of each animal. The criteria used for this estimation were standards on references based on the calculation of PCV % ranges for Cattle. Such criteria include **Normal PCV (%)** 24 – 46, **Anaemic indicator (%)** Reading: < 24, and **Invalid PCV Results (%)** Reading: >46



Figure 8: Determination of Anaemia in study animals using Pack Cell volume (PCV %)

Hematological Analysis

3.7 Data processing and Analysis

Data collected during this study period was entered into a Microsoft Excel sheet and coded. Data was summarised and statically analysed by using Stata version 16.0 software. Descriptive analysis was used to summarise data on husbandry practices. The proportion of AAT was calculated as the number of AAT divided by the total number of samples examined for the study multiplied by 100. Univariate Logistic regression was used to assess the association between the dependent variable (AT infection) and the independent variables such as age, sex and breed and anaemia condition of the animals. For the multivariate analysis, a stepwise forward regression was used to select variables. Variables with a p-value less than 0.05 were retained in the final model. The corresponding variables with P-value ($p < 0.05$) at 95% confidence interval were then considered statistically significant.

3.7 Ethical consideration

3.7.1 Ethic Office for Livestock Research

Ethical approval was obtained from National Agricultural Research Institute (NARI) Ethics board with reference number Ref: NARI/DLS/01/(02). NARI is an institution set by an act of parliament of the Republic of Gambia mandated for approving any scientific research that involves plants and Livestock species in the country.

Before sample collection, the purpose, method, benefit, and risk that might arise from these studies were clearly explained to each herd owner and their consent was sought. Approval was granted voluntarily by the herd owner.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of study animals

Out of 384 animals (cattle) sampled, 69.27% (266/384) were female and 75% (288/384) were adults. The mean age of the cattle in months was 45 (SD± 21.65). N'dama Breed comprised 98.70% (376/384) of total animals that were sampled while crossbreed comprised 1.30% (5/384) of total animal sampled. A total of 29.96% (116/384) cattle were found to be anaemic in this study. The mean PCV% observed was 25.92708 (SD± 4.5). (**Table 3**).

4.2 Husbandry Characteristics of study animals

All the animals sampled for these studies were reared under an extensive (free-range) grazing system and 31.25 % (120 /384) were under husbandry systems that employed vector control measures. Among animals that had prior exposure to Trypanocidal agents, 75 % (288/384) were exposed to Diaminazine aceturate drugs (**Table 4**)

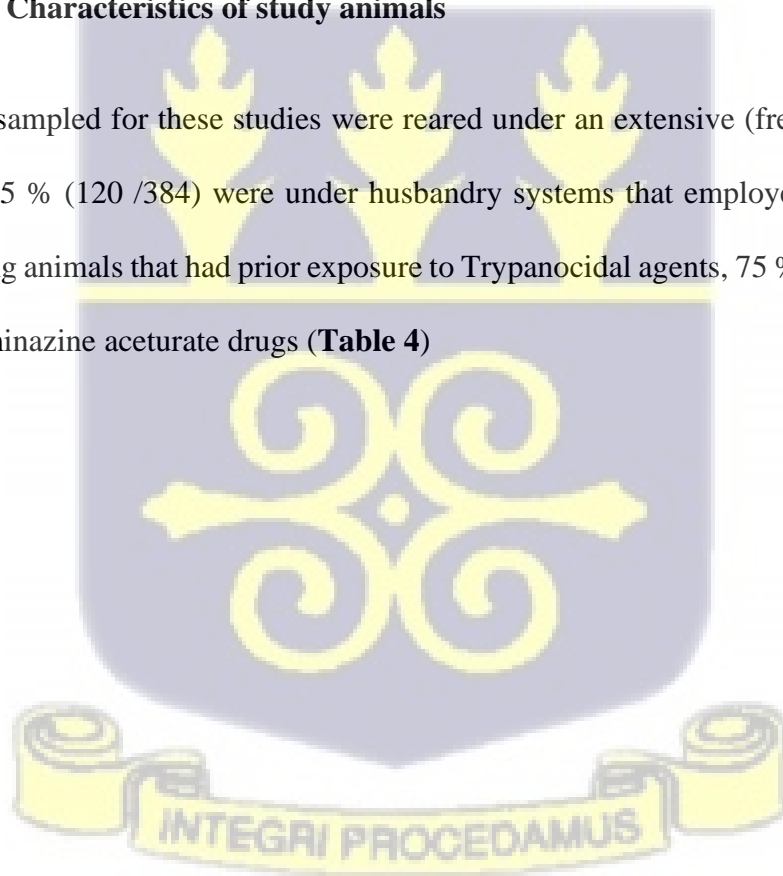


Table 3: Frequency distribution of animal demographic and clinical characteristic in Kiang West District, Lower River Region, The Gambia, 2020-2021

Variables	Frequency (n)	Percentage (%)
Age group (years)		
0-3	96	25.00
4-8	288	75.00
Sex		
Male	118	30.73
Female	266	69.27
Breed		
Cross	5	1.30
Ndama	379	98.70
PCV		
Anaemic	115	29.95
Non-Anaemic	269	70.05

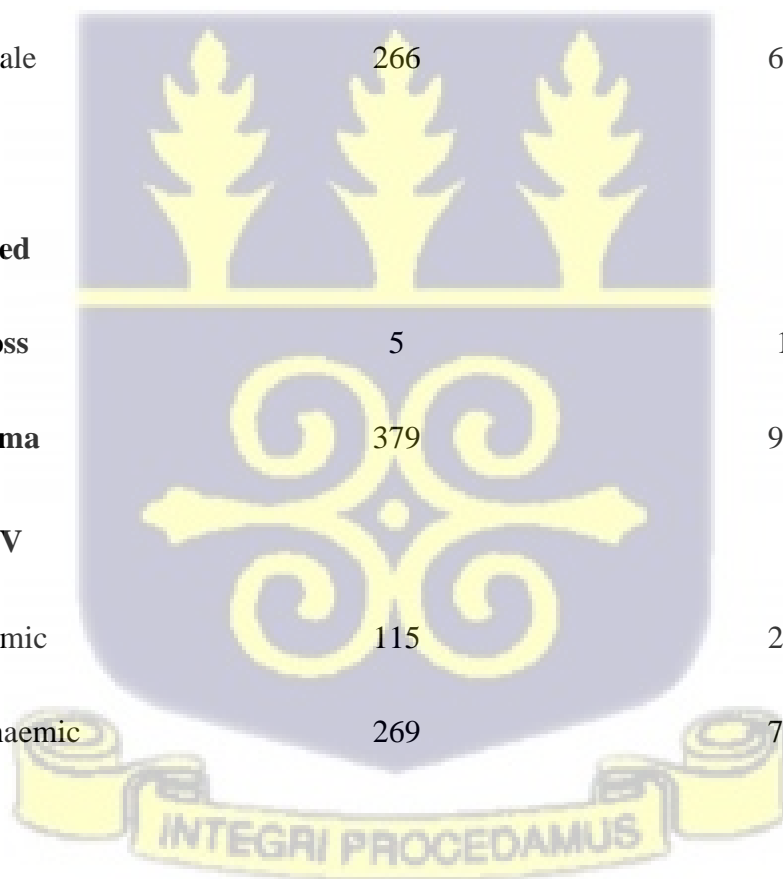


Table 4: Distribution of animal husbandry characteristics in Kiang West District, Lower River Region, The Gambia, 2020-2021

Variables	Frequency (n)	Percentage (%)
Vector Control		
Employed	120	31.25
Not - employed	264	38.75
Trypanocidal Type		
Diminazen Aceturate	288	75.00
Isometamidium	96	25.00
Watering		
Home	96	25.00
Away	288	75.00
Grazing		
Extensive	384	100.00
Intensive	0	0.00

4.3 Prevalence of AAT Infection

A total number of 45 cattle sample in Kiang West District were infected with AAT infection and these cases includes both Ndama and crossbreed species. Of this, an overall prevalence of AAT infection was 11.72 % (95% CI: 0.08-0.15) was observed. Amongst the study villages, Jifarong had 33.33% (15/45) and Jali with 15.56% (7/45) of the AAT cases (**Figure 9**). Cattle that were of "Ndama" breeds constituted 11.1% (42/379) total AAT infection while cattle that were crossbreed had 60.0% (3/5). Results from the Pack Cell volume analysis (PCV) showed that cattle that were found anaemic constitute 44.44 (20/45) of the AAT infections. Among the different species of trypanosome diagnosed, *T. vivax* and *T. brucei* comprised 57.78 (26/45) and 4.44 (2 /45) of total AAT infections respectively. Mix-Infection among the cases involves *T. congolense* and *T. vivax* species of trypanosomes 33.33 (6/45) (**Table 5**).

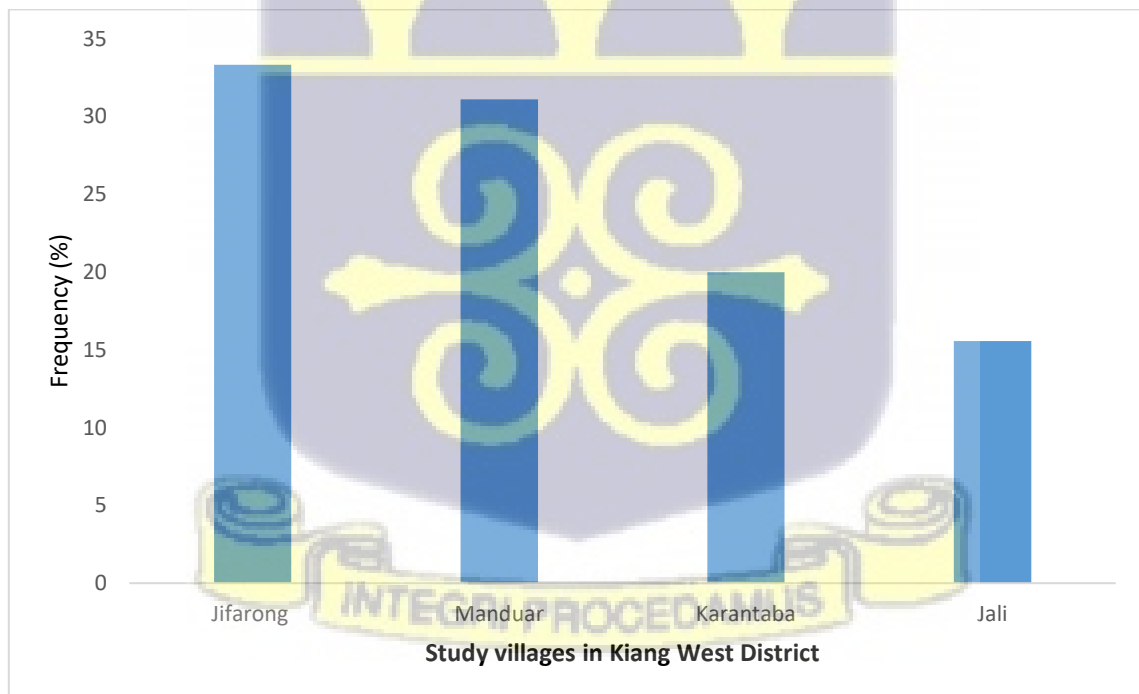


Figure 9: Distribution of AAT cases in four selected villages of Kiang West District, Gambia

Table 5: Comparison of Microscopic Results with Pack Cell Volume Estimation

AAT species	PCV <24 , (%)	PCV 24-46 , (%)	Microscopy, (%)
	Anaemic	Non -Anaemic	
T. vivax	38.46 (10/20)	61.54(16/25)	57.78 (26/45)
T. congolense	54.55 (6/20)	45.45(5/25)	24.40 (11/45)
T. brucei	50 (1 /20)	50 (1/25)	4.44 (2 /45)
Mix-Infection (T.congolense +T.vivax)	50 (3/20)	50 (3/25)	33.33 (6/45)
Total	44.44 (20/40)	55.56 (25/25)	100 (45/45)

4.3.1 Prevalence AAT infection by Trypanosome species

Out of 374 total animals (cattle) that were sampled from the selected villages with the study district (Kiang West District) , 45 animals (cattle) were infected with various species AAT (*T. brucie*, *T.congolense* or *T. vivax*). Of this, the overall prevalence of AAT observed was 11.72% (45/384), and with the three key species identified, *Trypanosome vivax* constituted 57.7% (26/45) , *Trypanosome congolense* constituted 24.44% (11/45) and *Trypanosome brucei* constituted 4.44 % (2/45). The mixed infection observed was 13.33 % (6/45) and involves *T.vivax* and *T. congolense* species (Figure 10).

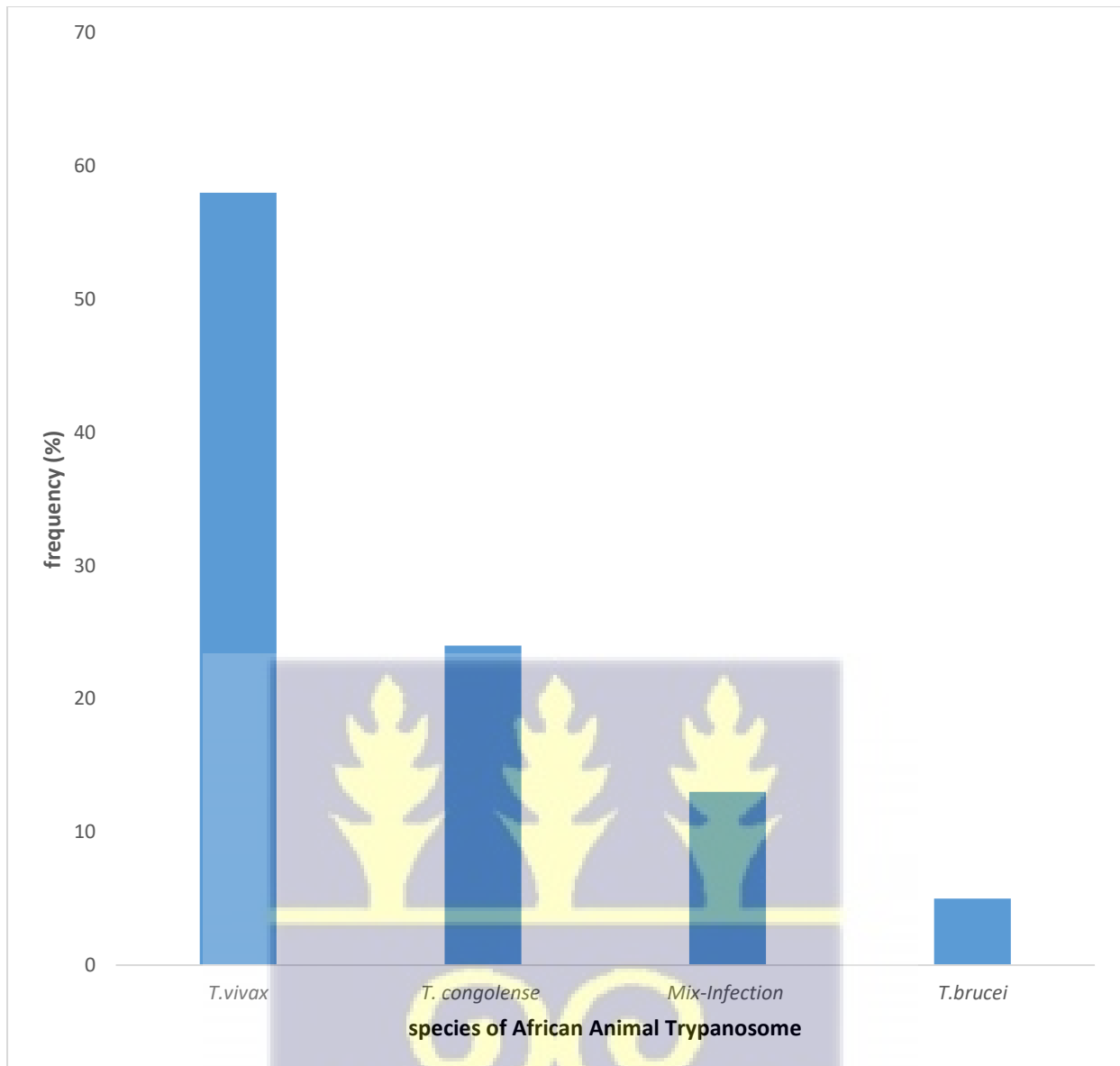


Figure 10: Distribution of Trypanosome species among study animals (cattle) with AAT infection



4.4 Univariate Analysis of association between risk factors and AAT infection

From the univariate logistic regression analysis, there was statistical significance observed in cross breeds cattle (**cOR = 12.0, 95% CI: 1.95 – 74.11**), and anaemia condition in animals (**cOR = 2.1, 95% CI: 1.09-3.87**), however no statistical significance was observed in adult cattle (**cOR: 1.2, CI: 0.56 – 2.50**), male cattle (**cOR = 0.1, 95% CI: 0.39 – 1.60**) (Table 6).

4.5 Multivariate analysis of factors associated with AAT infection

Variables that were tested with a p-value <0.05 in the univariate logistic regression analysis were entered into the multivariate logistic regression analysis. Cattle breeds and pack cell volume were the only 2 significant variables at the univariate level of analysis that was statistically significant. All 2 variables remained significant at the multivariate level of analysis.

Adjusting for pack cell volume there were 11.85 folds increased odds of AAT among crossbreed compared to Ndama (aOR= 11.85, 95% CI: 1.87 – 74.85). Controlling for cattle breed, there were 2.04 times increased odds of AAT among anaemic cattle compared to non-anaemic cattle (aOR= 2.04, 95% CI: 1.07 – 3.89) (Table 7).

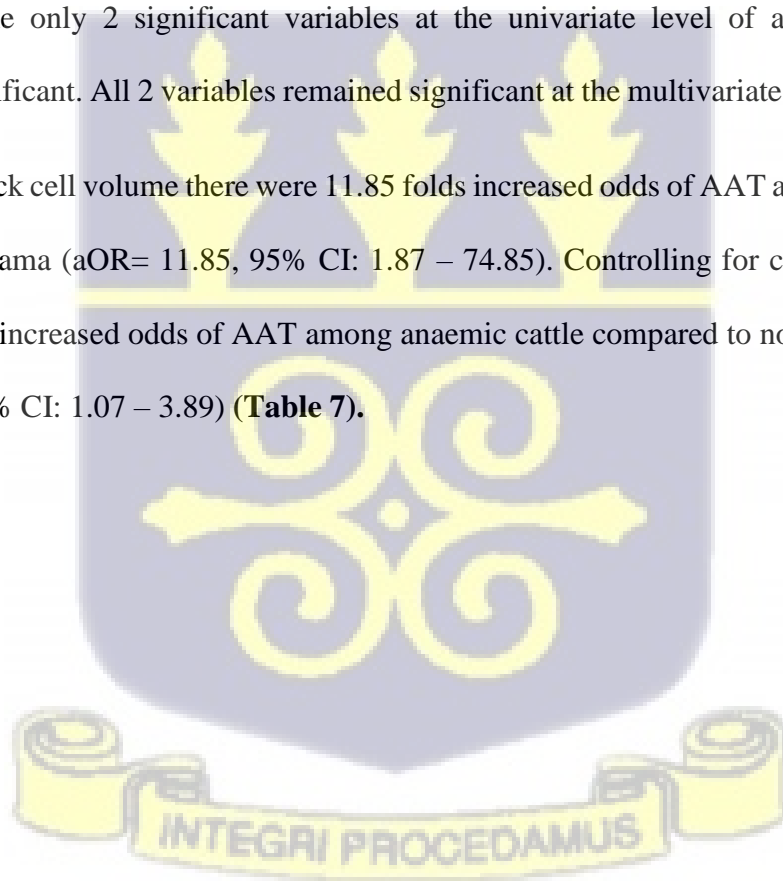


Table 6: Univariate analysis of factors associated with AAT infection in cattle in Kiang West District, Gambia

Variables	AAT Infection		Crude Odd Ratio	95% CI	P-Value
	Positive, n (%)	Negative, n (%)			
Age					
Adult (4-9)	35 (12.15)	253 (87.85)	1.2	0.56 – 2.50	0.647
Young (0-3)	10(10.42)	86 (89.58)	Reference		
Sex					
Male	12 (10.17)	106(89.83)	0.1	0.39 – 1.60	0.53
Female	33 (12.41)	233 (87.59)	Reference		
Breed					
Crossbreed	3 (60.00)	2 (40.00)	12	1.95 – 74.11	0.007*
N'dama	42 (11.08)	337 (88.92)	Reference		
PCV)					
Anaemic	20 (17.39)	95 (82.61)	2.1	1.09 – 3.87	0.026*
Non-Anemic	25 (9.29)	244 (90.71)	Reference		
Watering					
Away	45(11.72)	339(88.28)	NA		
Home	0 (0.00)	0 (0.00)			
Grazing					
Extensive	45(11.72)	339 (88.28)	NA		
Intensive	0 (0.00)	0 (0.00)			
Trypanocidal Type Used					
Isometamidium	12(12.50)	84(87.50)	1.1	0.54 – 2.23	0.784
Diaminazine aceturate	33(11.46)	255(88.54)	Reference		
Vector Control					
Employed	35(13.26)	229(87.74)	1.68	0.80 – 3.52	0.168
Not Employed	10(8.33)	110(91.69)	Reference		

Table 7: Multivariate analysis of factors associated with AAT infection in cattle in Kiang West District, Gambia.

Variables	AAT Infection		Adjusted Odds Ratio (aOR)	95% CI	P-value
	Positive, n (%)	Negative, n (%)			
Breed					
Crossbreed	3 (60.00)	2 (40.00)	11.85	1.87 - 74.85	0.009*
N'dama	42 (11.08)	337 (88.92)	Reference		
Pack cell volume (PCV)					
Anaemic	20 (17.39)	95 (82.61)	2.04	1.07 - 3.89	0.03*
Non-anaemic	25 (9.29)	244 (90.71)	Reference		

***Statistically significant**



CHAPTER FIVE

5.1 DISCUSSION

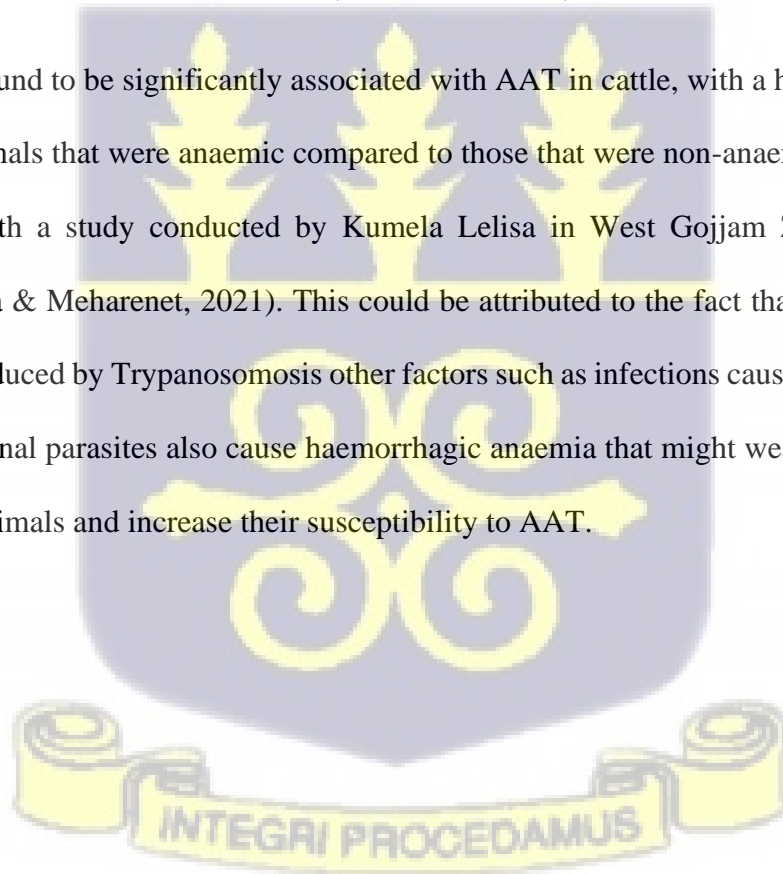
The overall prevalence of AAT in the study area was 11.72 % (95 % CI: 0.08- 0.15 %). This finding is lower than the prevalence of 12% in a study conducted by Alpha Kagbo in the central region, The Gambia (Kargbo & Kuye, 2020). A similar study conducted by Dennis Muhanguzi in Tororo District, south-eastern Uganda, had a higher overall AAT prevalence of 15.3 % (95 % CI: 12.2-19.1 %) (Muhanguzi et al., 2014). This difference in prevalence could be attributed to the fact that both studies conducted by Alpha Kagbo and Dennis Muhanguzi used a molecular diagnostic technique such as the internal transcribed spacer (ITS1) - Polymerase Chain Reaction (ITS1- PCR), which has higher sensitivity compared to a microscopic parasitological diagnostic technique that is used in this study. Another reason that could be attributed to the differences in vegetation types and seasonal variation between Gambia and Uganda. Such factors are known to influence tsetse populations and, as a result, the prevalence of trypanosome infections. Nonetheless, the prevalence rate of 11.72% recorded during this study could therefore constitute a huge economic loss to the poor rural farmers and significantly affect the Livestock sector output particularly in cattle production and productivity of in the country.

Among species observed in this study, *Trypanosome vivax* was found to be the most dominant species compared to *Trypanosome congolense* and *Trypanosome brucei*. Studies conducted by Abdoumoumini Mamoudou in the North region of Cameroon also revealed similar findings (Mamoudou et al., 2016). Another study conducted by Jessica Nakayima in Adidome and Koforidua in Ghana also supported this study finding where *T. vivax* was the dominating trypanosome species in infected cattle hosts (Nakayima et al., 2012).

These findings on higher *T. vivax* cases can be explained in two folds. First, it could be due to its ability to undergo a non-cyclical or mechanical transmission via other blood-sucking flies such as tabanid or stable flies (*Stomoxys* spp) (Jones & Alberto, 2001). Secondly, it could be attributed to its high pathogenicity in cattle compared to other Trypanosome species. (Dwinger & Hall, 2000).

Type of breed of cattle and anaemia were found to be associated risk factors of AAT in cattle in the Kiang west district. Cattle that were crossbreed had a higher association to AAT compared to cattle that were of the Ndama breed. This finding was similar to a study conducted by Daniel Kizza in Murchison Falls National Park, Uganda, in which the breed of the animal was significantly associated with the infection (Kizza et al., 2021).

Anaemia was found to be significantly associated with AAT in cattle, with a higher prevalence observed in animals that were anaemic compared to those that were non-anaemic. This finding is consistent with a study conducted by Kumela Lelisa in West Gojjam Zone, Northwest Ethiopia (Lelisa & Meharennet, 2021). This could be attributed to the fact that in combination with anaemia induced by Trypanosomosis other factors such as infections caused by fasciolosis, and gastrointestinal parasites also cause haemorrhagic anaemia that might weaken the immune system of the animals and increase their susceptibility to AAT.



5.2 CONCLUSION

In conclusion, AAT is found to be highly prevalent in cattle in Kiang west district, Lower River Region of The Gambia. This signals a significant threat to cattle production and productivity within the study area. Furthermore, this study also showed that AAT is significantly associated with anaemia condition and breed of cattle within Kiang west district. In addition, *T. vivax* species were the predominant species in the area compared to other pathogenic species causing AAT.



5.3 RECOMMENDATION

Government of The Gambia – Department of Livestock Services should implement a Trypanosome control and eradication strategy through a sound multisectoral surveillance system that will target to minimize the current trend of parasites in livestock and as wildlife species.

In addition the Department of Livestock Services, should strengthen cordial working with Livestock farmers and encourage them to make rational decisions in choosing breeds of cattle that are less susceptible to AAT such as the Ndama cattle breeds

The Department of Livestock Services together with the National Agricultural Research Institute – The Gambia (NARI) should conduct further research to investigate the status of Ndama cattle breeds to find out if such breeds are not potential carriers of pathogenic AAT species. This is crucial since findings from this study has indicated a significant number of AAT in Ndama since significant infection but as findings from this research indicated a significant number of AAT infection if Ndama cattle breeds

The Ministry of Health , Department of Livestock Services, and the Department of Parks and Wildlife should implement One health strategy geared prevention and control of zoonotic disease including AAT control strategy that minimize its on agricultural production and productivity as well as its impacts on public health. .



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APPENDICES

Appendix I



National Agricultural Research Institute (NARI)
PMB 526 Serrekunda, The Gambia, West Africa.
Tel: (220) 4484 926/ 4484 925/ 4484925 Fax/dh: 4484921

Ref:NARI/DLS/01/(02)

May 07, 2021

Mr. Kawsu Sanyang
M.Phil. in Applied Epidemiology and Disease Control
School of Public Health,
University of Ghana
Legon.

ETHICS APPROVAL LETTER

Dear Kawsu,

**Subject: Factors associated with trypanosomiasis in cattle in Kiang West District,
Lower River Region, The Gambia**

I am writing in response to the request for the approval for the study on “Factors associated with trypanosomiasis in cattle in Kiang West District, Lower River Region, The Gambia” as part of your thesis.

After a close review of your proposal, I write to confirm the NARI Board’s approval of the study and I do not require further approval.

This is conditional on the project, designed to examined the risk factors associated with African Animal Trypanosome infection in cattle in Kiang West District, Lower River Region, The Gambia, being conducted in accordance with the study protocol which was provided to the Board of Directors on the 26th April 2021 and in accordance with all conditions stated in applicable NARI policies and procedures.

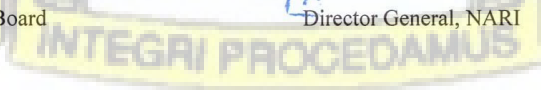
Any major change to the status of the project (including changes to the research team) or project amendments must be notified to the Board of Directors.

If you need any further information, please do not hesitate to contact us.

Yours sincerely,

Chairman, NARI Board

DIRECTOR GENERAL
Director General, NARI



Appendix II



Cattle Drinking from a pond in Kiang West District, Lower River Region, The Gambia, 2021



Cattle herd in Kiang West District, Lower River Region, The Gambia, 2021



Data collection from Livestock Farmer in Kiang West District Lower River Region, The Gambia 2021

Appendix III:

Questionnaires for assessing husbandry factors such as grazing, watering, vector control measures and Trypanocidal usage

Name of Livestock Farmer/ Herdsmen:

Contact Number:.....

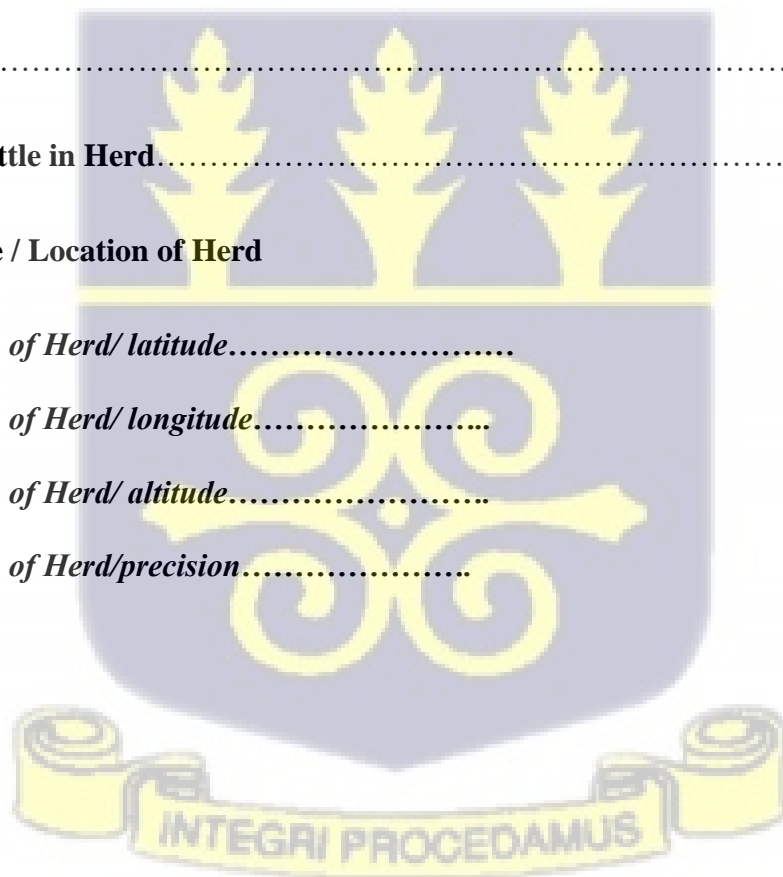
Name Village/settlement

Herd Number

Total No. of Cattle in Herd.....

GPS coordinate / Location of Herd

- *Location of Herd/ latitude*.....
- *Location of Herd/ longitude*.....
- *Location of Herd/ altitude*.....
- *Location of Herd/precision*.....



1. What type of grazing system do you practice?

- a. Zero Grazing
- b. Extensive Grazing

2. Is vegetation available and accessible to cattle throughout the year?

- a. Yes
- b. No

3. Is vegetation use by other animals within or outside the settlement?

- a. Yes
- b. No

4. Where do your cattle get water to drink?

- a. Home
- b. Away from home (River, Pond , stream or lakes)

5. If away from home, is the drinking place for your cattle used by other livestock?

- a. Yes
- b. No

6. How many times do your cattle drink from the water source?

- a. Once a day
- b. Two or many Times a day

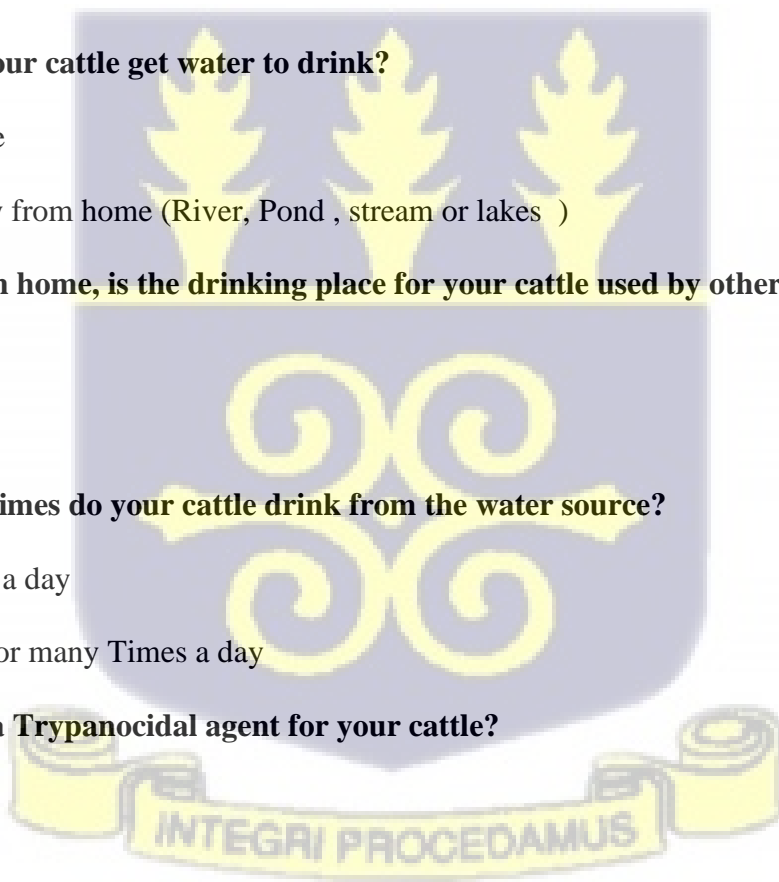
7. Do you use a Trypanocidal agent for your cattle?

- a. Yes
- b. No

8. If yes, What type of Trypanocidal agent?

9. How often do you use the Trypanocidal agent in a year?

10. Who administers the Trypanocidal agent?



11. Who administers the Trypanocidal agent?

- a. Self
- b. Livestock personnel

12. When did you last use the Trypanocidal agent on your animals?

13. Can you Identify AAT infection in cattle?

- a. Yes
- b. No

14. Do you employ vector control measures?

Questionnaire's submission date:

validation status.....

The questionnaire administered by

