

**TRYPANOSOME PREVALENCE IN PIGS AND  
TSETSE FLIES FROM JOMORO DISTRICT IN THE  
WESTERN REGION OF GHANA**

**BY**

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**DECLARATION**

This thesis is wholly an outcome of research work undertaken by me, Apaatah Francis in the Department of Animal Biology and Conservation Sciences, University of Ghana, under the supervision of Dr. Langbong Bimi, Dr. Alexander Egyir-Yawson and Dr. Fred Aboagye-Antwi.

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## DEDICATION

To Almighty God Jehovah Rapha is all the Glory. This work is dedicated to my parents and siblings for the encouragement and support they rendered during my studies, and to my guardians, Dr. and Mrs. Mensa for supporting my early childhood education that made me reach the level of tertiary education and finally to Miss Naomi Naa Narkey Donkor for her immeasurable, inspiration, love and prayers support.



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**LIST OF ABBREVIATIONS AND ACRONYMS**

<b>AAT</b>	African Animal trypanosomiasis
<b>BCT</b>	Buffy Coat Technique
<b>BNARI</b>	Biotechnology and Nuclear Agriculture Research Institute
<b>CAT</b>	Card Agglutination Test
<b>CPE</b>	Control, Prevention and Eradication
<b>CATT</b>	Card Agglutination Test for Trypanosomiasis
<b>CTAB</b>	Cetyl Trimethylammonium Bromide
<b>DABCS</b>	Department of Animal Biology and Conservation Sciences
<b>DALYs</b>	Disability Adjusted Life Years
<b>DDT</b>	Dichloro- Diphenyl-Trichloroethane
<b>DNA</b>	Deoxyribonucleic Acid
<b>DNTPs</b>	Deoxynucleotide-triphosphates
<b>EDTA</b>	Ethylendiaminetetraacetic acid
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay
<b>F/T/D</b>	(fly/trap/day).
<b>FAO</b>	Food and Agriculture Organisation of the United Nations
<b>GAEC</b>	Ghana Atomic Energy Commission
<b>GHS</b>	Ghana Health Service

<b>HAT</b>	Human African Trypanosomiasis
<b>HCT</b>	Haematocrit Centrifugation Technique
<b>IAEA</b>	International Atomic Energy Agency
<b>IFAT</b>	Indirect Fluorescent Antibody Test
<b>ILARD</b>	International Laboratory for Research in Animal diseases
<b>JDA</b>	Jomoro District Assembly
<b>MHCT</b>	Micro Haematocrit Centrifugation Technique
<b>MoAbs</b>	Monoclonal antibodies
<b>NMIMR</b>	Noguchi Memorial Institute For Medical Research.
<b>OIE</b>	Office International des Epizootics,
<b>PAAT</b>	Programme Against African Trypanosomiasis
<b>PATTEC</b>	Pan African Tsetse and Trypanosomiasis Eradication Campaign
<b>PBS</b>	Phosphate Buffered Saline
<b>PCR</b>	Polymerase chain reaction
<b>PCV</b>	Packed Cell Volume
<b>RPM</b>	Revolutions per minute
<b>SAT</b>	Sequential aerosol technique
<b>SIT</b>	Sterile Insect Technique
<b>SPSS</b>	Statistical Package for Social Sciences

<b>SSA</b>	Sub-Saharan Africa
<b>SS</b>	Sleeping Sickness
<b>TBE</b>	Tris-Borate-EDTA
<b>TE</b>	Tris-EDTA
<b>TTCU</b>	Tsetse and Trypanosomiasis Control Unit
<b>UV</b>	Ultra Violet
<b>V</b>	Volts
<b>WHO</b>	World Health Organization

## ABSTRACT

The study was carried out from October to November 2013 in Jomoro district of the Western Region of Ghana to assess the prevalence of trypanosomes in pigs and tsetse flies. Besides, the study identifies tsetse fly species and their apparent density in the study area. The methods employed during the study were molecular technique and microscopy of stained blood smears for parasitological study. Entomological surveys were also conducted using a total of 16 un-baited biconical traps for the collection of tsetse flies. A total of 300 pigs were randomly selected from the study population and examined for the presence of trypanosomes. The PCV value of each animal was also measured using haematocrit reader. From the entomological survey, DNA was isolated from 300 flies and subjected to PCR for trypanosome detection and identification. An overall prevalence of trypanosomes in the study area was 4.33% and 0.77% for pigs and tsetse flies respectively. None of the blood samples was positive for the presence of trypanosome species by microscopy of stained blood smears. Out of positive cases, mixed infection between *Trypanosoma congolense* Forest and *Trypanosoma vivax* showed the highest prevalence (46.15%) of trypanosome species followed by *Trypanosoma vivax* (15.38%), while of *Trypanosoma congolense* and a mixed infection of *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei gambiense* were the least with (7.70%) each. The study also demonstrated no variations in prevalence among different age groups and between both sexes which were statistically insignificant ( $p > 0.05$ ). The prevalence rate in good and poor body conditioned animals were 1.9 % and 20 %, respectively with a statistical significant difference ( $P < 0.05$ ) among them. Mean packed cell volume (PCV) of parasitaemic pigs (29.33%) was significantly ( $P < 0.05$ ) lower than that of aparasitaemic pigs (37.75%). The entomological survey showed that *Glossina palpalis palpalis* was the only tsetse species

with fly apparent density of 18.36 fly/trap/day found in the study area. Diagnosis of trypanosomiasis in tsetse or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing control operations. Therefore, the results of this study should be used to define the strategy of disease control in places where tsetse and trypanosomiasis challenge were reported. Although the present study revealed low prevalence, implementing control of trypanosomiasis with an integrated approaches have paramount importance in the study sites.

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background to the Study

African trypanosomiasis is an infectious disease of humans and domestic animals that causes a significant negative impact on food production and economic growth in many parts of the world, particularly in sub-Saharan Africa (Taylor, 1998). The tsetse and trypanosomiasis problem to man and livestock lies at the heart of Africa's poverty. Estimates of gross national per capita income show that 20 of the world's 25 poorest countries are affected by tsetse-transmitted trypanosomiasis (Cattand *et al.*, 2010). Trypanosomiasis is estimated to cost sub-Saharan Africa approximately US \$4 billion each year, calculated as direct losses in meat production, milk yield, loss in traction, transport and programmes that control the disease (Budd, 1999). By depriving agricultural communities the use of animals for traction, only small areas of land can be tilled by hand, leaving the affected communities vulnerable to food shortages, starvation and famine (FAO, 2003).

The causative agents of the disease trypanosomiasis are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina sp.*). Trypanosomes are unicellular, microscopic and elongated protozoa that move by the help of a single flagellum at the base of which is found a characteristic structure known as kinetoplast. They are obligatory parasites usually having two hosts, and multiply in the body fluids, especially blood, of vertebrate host and live in the digestive tract of invertebrate host which is generally a biting insect (ILARD, 1989).

### 1.1.1 Human African Trypanosomiasis (HAT)

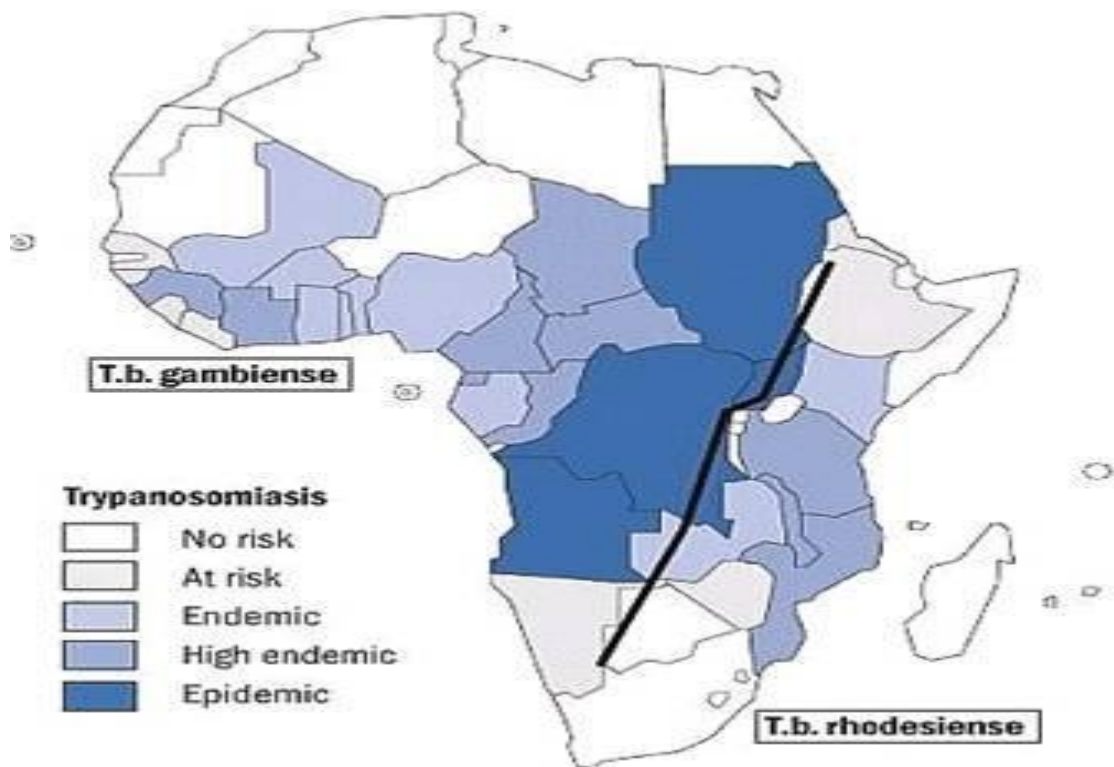
Human African Trypanosomiasis (HAT) also known as sleeping sickness is a zoonotic disease affecting poor, rural communities living in the tsetse fly-infested regions of Africa. Sleeping sickness spreads more easily in poor settings, generally occurring in remote rural areas where health systems are weak or non-existent. The rural populations that depend on agriculture, fishing, animal husbandry or hunting are the most exposed to tsetse bites and therefore to the disease. Displacement of populations, war and poverty are other important factors that lead to increase in transmission (WHO, 1997).

Human African trypanosomiasis or sleeping sickness is caused by two subspecies of *Trypanosoma brucei* (*T. b*), *T. brucei gambiense* (*T. b gambiense*) and *T. brucei rhodesiense* (*T. b rhodesiense*), while the third subspecies, *T. brucei brucei* (*T.b brucei*), is only infectious to animals. *T. b. gambiense* is responsible for the chronic form of sleeping sickness and it occurs in 24 countries in West and Central Africa. This form currently accounts for over 90% of reported cases of sleeping sickness, whereas *T. b. rhodesiense* gives rise to the acute form of the disease and is found in 13 countries in Eastern and Southern Africa. Nowadays, *T. b. rhodesiense* infection represents fewer than 10% of reported cases of the sleeping sickness infection (WHO, 2006). There are two distinct stages during the course of the disease. The first or early stage of the disease, also known as the haemolympathic phase, is defined by the restriction of the trypanosomes to the blood and lymph system (WHO, 1998). The symptoms of this stage are fever, headaches, joint pains and itching. The second or late stage of the disease, also known as the neurological phase, is characterised by the presence of the parasites in the cerebrospinal fluid. In general, this is when the typical signs of the disease occur: confusion, disturbed sleep patterns, sensory disturbances, extreme lethargy, poor condition and coma. If left untreated, sleeping sickness patients die within months when

infected with *T. b. rhodesiense* or within some few years when infected with *T. b. gambiense*. Wild and domestic animals play a major role as parasite reservoirs for human infections with trypanosomes (Simo *et al.*, 2006).

According to the World Health Organization (WHO)'s global burden of disease estimates (Fig.1.1), Human African Trypanosomiasis caused 1.5 million disability adjusted life years (DALYs) in 2002 within the area of tsetse fly distribution, which ranks it much lower than most infectious diseases in Africa but high among parasitic diseases (WHO, 2004).

At the beginning of the 20th Century, huge sleeping sickness epidemics devastated vast areas of the African continent. In the 1960s, prevalence of the disease had been successfully reduced to less than 0.1% in all endemic countries through the historic campaigns executed by the former colonial powers. Soon after independence, however, national governments failed to sustain such programmes due to lack of or diversion of resources to other more pressing health problems. The breakdown of specialized mobile teams and health facilities in several countries, as a consequence of war and civic strife or change in health policy, resulted in dramatic resurgence of the disease, which distribution corresponds closely with that of major conflicts in sub-Saharan Africa (PATTEC, 2001).



**Figure 1.1. Geographic distribution of major endemic foci of *T. b. gambiense* and *T. b. rhodesiense* human African trypanosomiasis in Africa (1995).**

(Source: WHO/CPE)

### 1.1.2 African Animal Trypanosomiasis (AAT)

African Animal Trypanosomiasis (AAT) or “Nagana” is a very important disease of domestic livestock in sub-Saharan Africa. According to the Food and Agriculture Organization (FAO) of the United Nations, it is probably the only disease which has profoundly affected the settlement and economic development of a major part of a continent. Trypanosomiasis in livestock has a significant impact on agricultural productivity and is caused mainly by *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma simiae*, *Trypanosoma evansi* and *Trypanosoma brucei brucei*. *Trypanosoma evansi*, which is most closely related to *Trypanosoma brucei brucei*, is not transmitted by tsetse flies but mechanically transmitted by biting flies (Masiga *et al.*, 1992).

All domestic animals can be affected by nagana and the symptoms are fever, listlessness, emaciation, hair loss, oedema, anaemia, paralysis and discharge from the eyes. As the illness progresses the animals weaken more and more and eventually become unfit for work, hence the name of the disease Nagana which is a Zulu word that means "powerless/useless". Because of nagana, livestock farming is very difficult within the tsetse belt (Winkle, 2005).

African Animal Trypanosomiasis occurs in 37 sub-Saharan Africa (SSA) covering about 10 million km<sup>2</sup>, an area which corresponds approximately to one-third of Africa's total land area out of which 70% represents natural pasture for grazing (Mattioli, 2000). An estimated 45 to 60 million cattle and tens of millions of small ruminants are at risk from trypanosomiasis (Gilbert *et al.*, 2001). FAO estimates that about three million cattle die each year due to AAT (FAO, 2000).

Over the past decade, domestic pigs have become an increasingly popular livestock strategy in Africa. Free range pig production is particularly attractive to subsistence farmers as it requires low input and space. Pig keeping is considered profitable since there is a perception that they demand few or even zero inputs. Both domestic and wild pigs can become infected with various species of tsetse transmitted trypanosomes, but do not usually show symptoms of disease pathology unless infected with *Trypanosoma simiae*, which is highly pathogenic for domestic pigs (Ilemobade and Balogun, 1981). Pigs are an essential source of income and for household consumption in rural areas of the Western Region of Ghana but the persisting threat of African trypanosomiasis may at any time jeopardize a productive pig husbandry as was experienced in a recent outbreak

in Eastern Region of Ghana (Bauer *et al.* 2011). In West and Central Africa, *T. b. gambiense* does not only cause human sleeping sickness but also infects pigs and probably many other wild animals that may serve as reservoirs of the parasite. Even though AAT can affect many species of mammals especially livestock, from a public health point of view, the disease is particularly important in domestic pigs in West and Central Africa (WHO, 1998). In a HAT survey carried out in the Upper West Region of Ghana in 2007, the only trypanosome isolated from one person was identified as *T. b. brucei* (Ghana Health Service, 2009) which is the sub-species that affects animals, especially domestic pigs.

### **1.1.3 Trypanosome Detection Method**

A major constraint to studies on the epidemiology of trypanosomiasis is the lack of suitable diagnostic tools. The diagnosis of trypanosomiasis is usually based on the demonstration of the parasites in the blood, supplemented by haematological, biochemical, serological and molecular tests. Different diagnostic tests demonstrating the presence of trypanosomes (wet-film test, microhaematocrit centrifugation /dark-ground/phase-contrast and buffy coat technique) are available which vary in their sensitivity and specificity. Some of these techniques cannot always detect ongoing infections as the level of parasitaemia is often low and fluctuating, particularly during the chronic stage of the disease (Nantulya, 1990). Moreover, the actual sensitivity and specificity of these techniques is directly dependent on the volume of the blood examined and the skill and experience of the examiner (OIE, 2000). However, these parasitological tests can only detect 20-30% positive cases and sometimes identification of trypanosome species is difficult. To overcome these problems in the diagnosis of trypanosomiasis, different serological tests (Indirect fluorescent antibody test, antibody/antigen detection enzyme-linked immunosorbent assay and card agglutination

test) have been developed that involve the detection of either trypanosomal antibodies or antigens. Despite all these tests, detection of either trypanosomal antigen or antibody has shown poor results (Nantulya, 1989).

In recent years, Deoxyribonucleic Acid (DNA) based technologies including Polymerase Chain Reaction (PCR) have been used for the diagnosis of trypanosomiasis (OIE, 2000; Uilenberg, 1998). PCR has evolved as one of the most specific and sensitive methods for the diagnosis of infectious diseases, and many applications of PCR for detecting pathogenic microorganisms have been reported (Bromidge *et al.*, 1993). This technique has been also used for large scale analysis of trypanosomes samples (Hide and Tait, 1991).

## **1.2 Statement of the Problem**

Various tsetse control programmes such as the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) has been put in place to control the vector and the disease. Nevertheless, livestock losses due to tsetse and tsetse-transmitted trypanosomes are still being experienced in Ghana (PATTEC, 2001).

In Ghana, more attention is given to cattle as reservoir of trypanosomes, as has been observed in other African countries (Fevre *et al.*, 2005), and therefore intervention efforts are directed more towards bovine trypanosomiasis. Few studies that were done between 1995 and 2004 in Ghana focused on cattle (TTCU, 1997; Dankwa, 1993; Mahama *et al.*, 2004). However, studies elsewhere have shown that domestic pigs could play an important role in the epidemiology of trypanosomiasis both in humans and animals (Mehlitz *et al.*, 1982; Magona *et al.*, 1999; Nkinin *et al.*, 2002). Workers like

Waiswa *et al.* (2003a) speculated that efforts directed towards bovine trypanosomiasis and the limited attention given to the other domestic animals like pigs might be the reason for persistence of sleeping sickness in south-eastern Uganda. Their study in Uganda showed that 13.9% of the pigs, 5.0% of the cattle, and 0.4% of the small ruminants investigated were found to be infected with parasites of the *T. brucei* subgroup, which is an important species in human trypanosomiasis (Waiswa *et al.*, 2003b). Therefore domestic pigs in tsetse infested areas of Western Region may be important reservoirs for the transmission of human trypanosomiasis.

### **1.3 Justification**

Agriculture plays an important role in the socio-economic development of Ghana. It is one of the sectors that employ about 60 % of the Ghanaian working force. It is the bedrock of the economy since it contributes to ensuring food security, provides raw material for local industries and generates the needed foreign exchange and incomes. Of crucial importance is the greater use of animal traction to enable increased cultivation and to provide transport in rural areas for crops, fertilizer and other goods. A major constraint to achieving this goal is tsetse-transmitted trypanosomiasis.

The renewed interest and efforts to eliminate trypanosomiasis has created new demands for information on the epidemiology of African trypanosomiasis and has necessitated more frequent updating of available data on the distribution and burden of the disease, and the target population for diagnostics and chemotherapy, in order to develop a global access strategy for these tools (PATTEC, 2001). African trypanosomiasis has been accorded little attention and priority by politicians and donors, essentially because it is a rural problem, which occurs only in Africa, and mainly affects the poor. However, its

negative impact on the history and socio-economic development of individuals, households and communities in endemic countries has the potential of negating the achievements of one of the Millennium Development Goals of halving poverty and food insecurity by 2015 (PATTEC, 2001).

Scanty information exists about the status of trypanosomiasis in Ghana, especially regarding prevalence, diversity and molecular epidemiology. If the Programme Against African Trypanosomiasis (PAAT) initiative is to succeed in Western Region, some empirical information on the dynamics of the disease is needed. This information can be inferred from information on the state of reservoirs and the species of trypanosomes they are infected with. Since no specific study has been done in this direction, the present study is timely.

It is necessary to furnish decision makers and other stakeholders with vital information regarding the prevalence of trypanosomes and diversity of tsetse flies in the district for possible policy interventions.

## **1.4 General Objective**

The primary objective of this study is to determine the incidence and identify the different species of trypanosomes affecting pigs and tsetse flies in some areas of the Western Region.

### **1.4.1 Specific Objectives**

1. To determine the prevalence of trypanosome infection based on location, age, sex and body condition of the pigs.
2. To compare the sensitivity of microscopy and conventional PCR in the detection of trypanosomes in pig blood samples from the various study sites.
3. To identify the species of tsetse fly and their apparent density at the study area.
4. To compare the mean Packed Cell Volume of infected and non-infected animals.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1. The Vector, Tsetse Fly

##### 2.1.1. Classification and Distinguishing Characteristics

The vector, tsetse fly, can be classified in the order *Diptera* (the two-winged flies), family *Glossinidae*, and within the genus: *Glossina*. There are about 23 species and 8 sub-species of *Glossina* identified so far (Moloo, 1993; Leak, 1999). Only a few species are vectors of human sleeping sickness but all are potential vectors of animal trypanosomiasis. From a morphological point of view, tsetse flies are elongated and robust, of various shades of brown ranging from yellowish to greyish to dark or blackish brown in colour and about 6 to 16mm long excluding the proboscis. Useful features for identification include: wings being held closed over the abdomen fully overlapping one another, a piercing proboscis which sticks out horizontally from the front of head, widely separated compound eyes and the hairs on the arista of antenna have further hairs branching off them (Robertson, 2004).

The historical classification of tsetse flies, based on morphological criteria, divides the species into three groups (Pollock, 1986). The Fusca group flies (subgenus *Austenina*) tend to occur in the lowland rainforests of West and Central Africa. The genitalia of the females have an external armature with five plates. Most species in this subgenus have a well-developed signum (Leak, 1999). The Palpalis group (subgenus *Nemorhina*) is found in the riverine galleries of West and Central Africa but can extend into savannah regions between river systems; *G. palpalis* and *G. tachinoides* are important AAT vectors in this group. The genitalia of the females have an external armature with six

plates. Males are identified by superior claspers which have narrow claws with a membrane filling the gap between them. (Phelps and Lovemore, 1994; Leak, 1999). The Morsitans group (subgenus *Glossina*) occur in a variety of savannah habitats lying between the forest edges and desert and includes several important vectors of AAT including *Glossina morsitans* spp., *G. pallidipes* and *G. austeni*. The genitalia of the females have an external armature with three plates. Males in this subgenus are identified by the morphology of the superior claspers. The ends of the superior claspers have wide plates and the gap between the superior claspers is filled by the median lobes.

### **2.1.2 Distribution of Tsetse Flies in Africa**

Tsetse flies (genus *Glossina*) occur exclusively in sub-Saharan Africa over an area extending on both sides of the equator from 15<sup>0</sup>N to 29<sup>0</sup>S latitude (Ford, 1971) Fig. 2.1. According to Molloo (1985), most of the *fuscus* and *palpalis* group of tsetse flies are concentrated in west and central Africa, while most of the morsitans group of tsetse species are found in eastern and southern Africa. Tsetse flies are absent from islands close to the African continent, except the island of Zanzibar, where *G. austeni* was present but has been eliminated (Vreysen *et al.*, 2000) and the loose islands of Guinea and might be infesting other small islands around Africa but not yet reported.

The general distribution of tsetse flies, determined principally by climate and influenced by altitude, vegetation and the presence of suitable host animals, has been known for a long time (Leak, 1999). Each of these factors may directly affect the birth, death or migration rates of the vector and thus the population size (Hay *et al.*, 1996). The limit of distribution is closely correlated with the tropical savannah (summer rain) climate, which follows the 508mm annual isohyet. Climate, though dependent on latitude, is modified by altitude and has a great effect on the vegetation, which is vital for providing

shade and maintaining a suitable microclimate for tsetse as well as a habitat for their vertebrate hosts. Altitude influences tsetse distribution through its effect on climate, particularly temperature (Leak, 1999).

The most favourable temperature for *Glossina* is between 21<sup>0</sup>C and 24<sup>0</sup>C for the adult stage while too high (>35<sup>0</sup>c) or too low temperature (<14<sup>0</sup>c) hinder puparia from completing their development. The influence of humidity and rainfall can be partly seen in the relatively high mortality rates at the end of the dry season and the low mortality rates during the rainy season (Pollock, 1986). However, during the onset of the dry season changes in host distribution, amount of available shade, number and kinds of predators, changes in temperature (<sup>0</sup>C) and changes in hours of sunshine must also be taken into consideration (Leak,1999).



**Figure 2.1 Global distribution of Tsetse fly (indicated by red colour)**

*Source: FAO Corporate Document Repository*

### **2.1.3 History of Trypanosomiasis and Tsetse Fly Distribution in Ghana**

Sleeping sickness infections appeared to have hit Ghana, the then Gold Coast in 1884 with an outbreak in the Lawra area, in what was the north-western fringe of the Asante Empire. Colonial officials did not investigate the disease, however, until after 1903, when the Ugandan epidemic prompted the Colonial Office to sponsor investigations across the African colonies. Thus prompted, Gold Coast officials who first found *G. palpalis* around Kumasi in 1901 (Morris 1947). In 1906, the Ghana Medical Department officials found trypanosomes in a patient's blood for the first time in the Northern Territories (Gambaga). By 1907/8 the disease appeared to be on the rise; field officers reported severe outbreaks around out-stations in Brong-Ahafo (Morris, 1947). They did note, however, that the disease seemed to be more significant in Ashanti than the Colony or the Northern Territories, and that it “almost assumed an epidemic form” in Wenchi and Sunyani.

The variation in the prevalence of trypanosomiasis is still a major feature of the epidemiology of the disease in Ghana and is consistent with the correlation between tsetse apparent density and the risk of trypanosomiasis described by Rogers in 1985. Between 1986 and 2009, a total of 35 cases of Human African Trypanosomiasis were reported from all the regions in the country (Ghana Health Service, 2009), and between 1994 and 1998, four cases with one fatality was reported in the Western Region. An active case search conducted among 21,656 individuals in Upper West and Western Region during 2008 and 2009 showed 44 (0.2%) positive cases by Card Agglutination Test for Trypanosomiasis (CATT/*T. b. gambiense*). However, only one of these was

confirmed by molecular test at the WHO Reference Laboratory in Geneva (Ghana Health Service, 2009).

African Animal Trypanosomiasis (AAT) in Ghana was first identified in 1909 following high mortalities and morbidity in cattle, pigs, sheep and horses. More than 60% of the land surface area of Ghana had been infested with tsetse flies of various species which have direct correlation with the occurrence and distribution of animal trypanosomiasis in the country. From 1995 through 2001, trypanosomiasis surveys conducted across the country pointed out differences in prevalence of bovine trypanosomiasis in areas under different risk levels. Low risk areas like Dangme West district in the Greater-Accra Region and Talensi-Nabdam district in the Upper East Region recorded 5% prevalence as against 50% prevalence in high risk areas like West Mamprusi and Damongo districts in the Northern Region (TTCU, 1997). Outbreaks of bovine trypanosomiasis, where entire immigrant herds were decimated in a matter of months (Abavana, pers. commun. 2002) have been reported; the herds had been brought from the tsetse-free areas of Accra plains to the high trypanosome challenge areas of the Brong Ahafo Region (Sene District). In the Western Region of Ghana, tsetse flies were seen feeding on domestic fowls and toads (Abavana, personal communication).

The first attempt at field research on the distribution of tsetse flies (Simpson, 1914) showed that ten out of the thirty-one species and subspecies of tsetse fly which have been identified in sub-Saharan Africa (SSA) are found in Ghana (Fig. 2.2). A more comprehensive description of the occurrence of these species according to vegetation types and climate was given by Nash in 1948. During the period between 1978 and 1983, tsetse surveys were carried out to determine the distribution of tsetse flies in the northern half of the country between latitude 8°N and 11°N (Draeger, 1983 ). Studies in

Ghana have shown that the *morsitans* group is more distributed in the Sene and Damongo districts in the Brong Ahafo and Northern Regions respectively where game is abundant, while *Glossina tachinoides* (*Palpalis* group) are dominant species in the Upper East, Northern and part of Upper West Regions (Offori, 1964; TTCU, 2001). The *Glossina palpalis gambiense* (*Palpalis* group) coexists in small proportions with the *G. tachinoides* but are completely absent in areas where there are no vegetation canopies. Between 1983 and 2000 other tsetse surveys were conducted in the coastal and derived savannah zones by the Tsetse and Trypanosomiasis Control Unit (TTCU) of the Veterinary Services of Ghana (TTCU, 1996). It was observed that *Glossina longipalpis* known to transmit bovine trypanosomiasis occurs mainly in secondary forest in Ashanti and the western region and also in the semi-savannah regions bordering on the Accra plains. According to Morris (1947) who studied the species in the Takoradi area, this fly tends to avoid wet evergreen forest of the extreme west of Ghana in preference for the transitional type of vegetation in the adjacent area.

*Glossina palpalis* is found in almost every type of vegetation in Ghana. However it is normally closely associated with water and will not normally travel long distances away from rivers and streams. Water-side clearing therefore reduces its numbers considerably and this method has been used to advantage in the drier parts of Northern Ghana. In the South, Brong-Ahafo, Ashanti, Western Region and parts of the Volta Region where the vegetation is tropical or near-tropical forest, clearing is not so feasible (Offori, 1964).

*G. caliginea* is one of the species which has a limited distribution in Ghana and is not readily encountered. It has so far been recorded only from the forests of Western Region and Brong-Ahafo. According to several investigators *G. caliginea* shows a tendency to associate with rivers and streams. It is also a ferocious biter, preferring to bite the lower

parts of the legs (Offori, 1964). *G. fusca* is a forest type. However, in Nigeria, Page (1959) recorded it from forest islands within savannah country. In Ghana it appears that its main home is the thick forests of Western Ashanti and Brong-Ahafo. Offori (1964) caught one specimen at Pokuase by means of a trap in an area which cannot be considered true forest. Two other Specimens have also been obtained from an area close to the Akosombo dam site.

*Glossina nigrofusca* is a forest species found in Ghana, but it tends to prefer the outskirts where the true forest gives way to savannah country. The importance of this species is yet to be fully assessed, but, according to Austen and Hegh (1922), the fly attacks man and seems to prefer biting the legs. There are also records of this species attacking elephants in large swarms (Buxton 1955). Compared with other species, *G. nigrofusca* is of little or no known economic importance in Ghana.

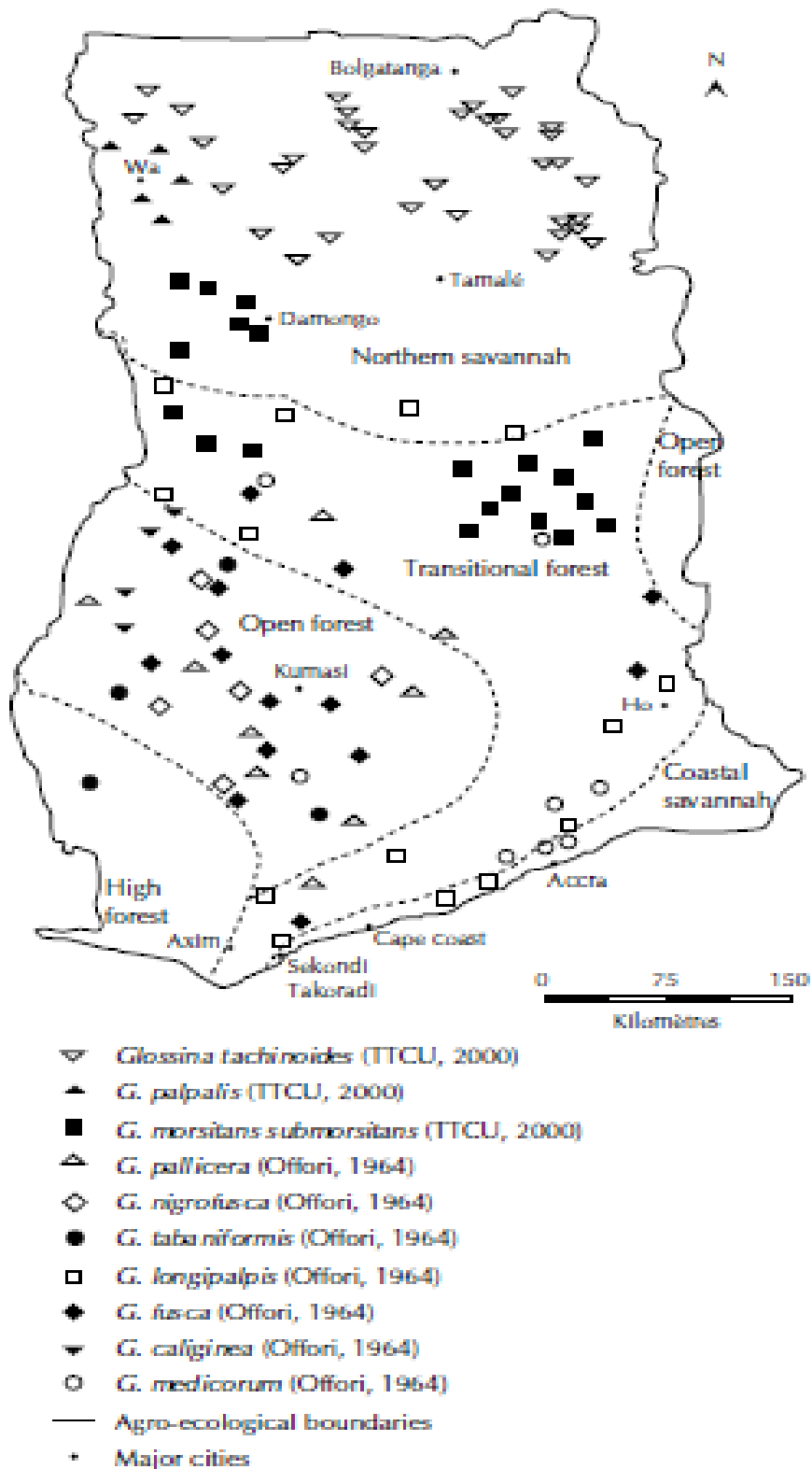


Figure 2.2: Tsetse distribution in Ghana.

Source: (Mahama *et al.*, 2003)

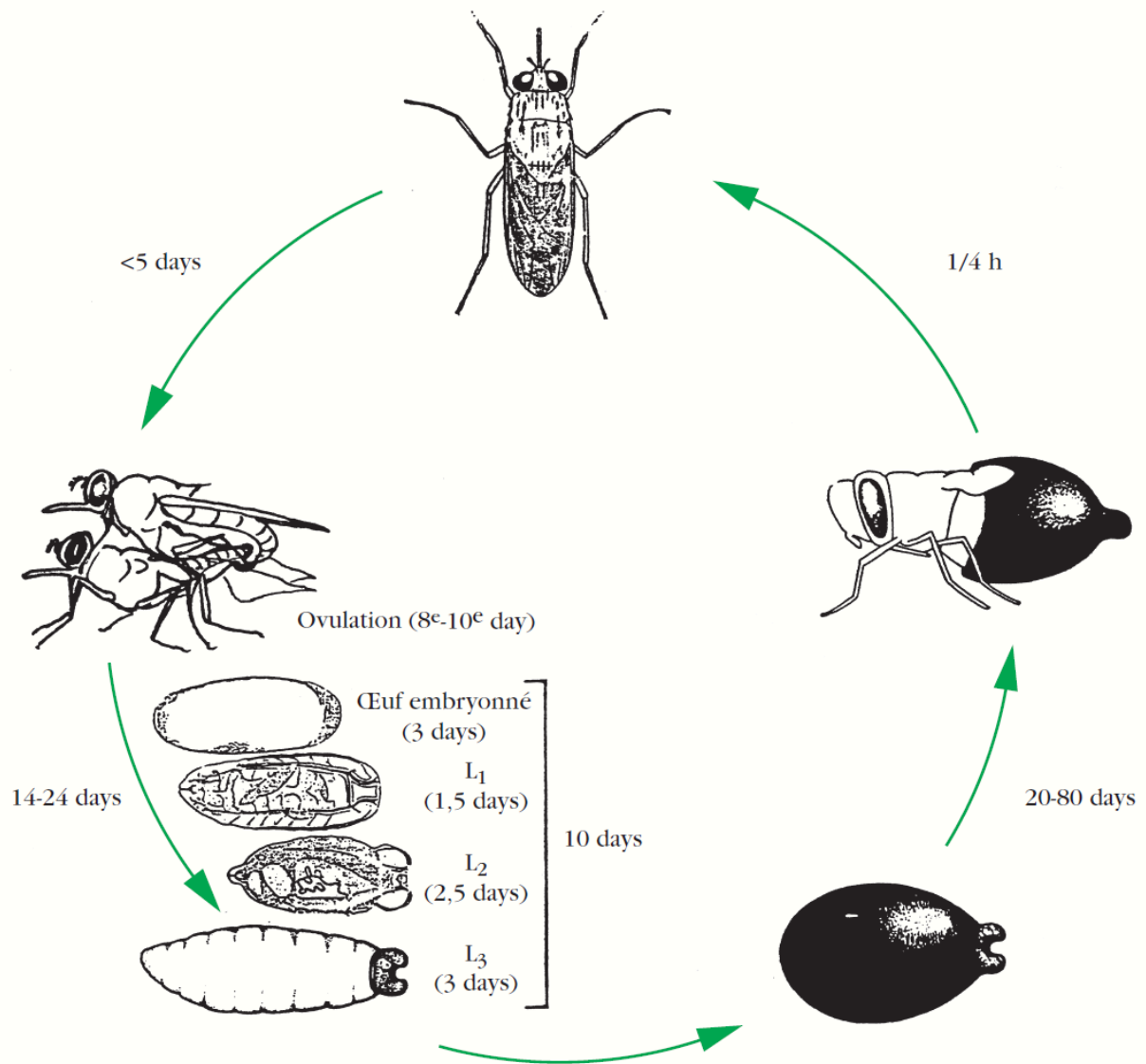
#### **2.1.4 Biology of Tsetse Flies**

Tsetse flies have a form of reproduction called adenotrophic viviparity in which the egg hatches within the female and the larva develops in the female by feeding on food from modified accessory glands (Robertson, 2004; Leak, 1999). Females have longer life-span than males in the field. During her life-span a female can theoretically give birth to only a maximum of 8-10 offspring so tsetse flies are rather like human beings in that they make a large investment per offspring so that juvenile mortality is low. However, this means that they can't produce many offspring (Robertson, 2004). Consequently, tsetse flies have a very low rate of reproduction and therefore, termed as k-strategists and they differ in terms of growth and reproductive strategies from most insect species which produce large numbers of eggs and termed as r-strategists (Leak, 1999).

Mating takes between 1-2 hours during which time a spermatophore is formed within the female's uterus using secretions from the male. Just before copulation ends, the male ejaculates sperm into the spermatophore. Within the subsequent few hours, the sperm moves from the spermatophore up the paired spermathecal ducts into the paired spermatheca. These sperms serve the female throughout her life so she doesn't have to mate again. Males are able to mate a number of times with different females (Robertson, 2004).

In the uterus the egg is fertilized by a sperm from the spermatheca (gained during earlier mating with a male). After on average, about 3.5 days of development in the egg, the 1<sup>st</sup> instar larva breaks out of the egg case (Robertson, 2004). Then the larva develops in the female's uterus by feeding on food from modified accessory glands. It passes through two moults to reach the 3rd instar larva and it is then 'larviposited' by the female. The female finds a suitable place to lay the larva. Once the larva is expelled from the uterus,

it moves actively in the soil for pupation (Buxton, 1955). Generally, the pupae are placed 2 to 8 cm deep into the soil where its skin hardens and blackens into a puparium and within the puparium, pupation and metamorphosis take place. The puparial period can range from 20 days at 30°C to 47 days at 20°C. Development in the puparium is generally unsuccessful below about 17°C and above about 32°C. The entire life cycle from egg to adult usually takes about 30 days (Leak, 1999; Robertson, 2004). The exterior membrane of the pupa, the puparium, carries the two black polypneustic lobes at its posterior end, enabling differentiation of tsetse pupae from other Diptera Muscoidae. The entire process of metamorphosis occurs within the puparium, leading to the adult form of the insect (Fig. 2.3). When the tsetse fly emerges, it is sexually matured and mating can occur when they are only a few days old (48 to 72 hours post eclosion from the pupae).



**Fig. 2.3: Reproductive Cycle of a Tsetse Fly (adapted from Cuisance, 1989)**

## 2.2 The Parasite and Disease

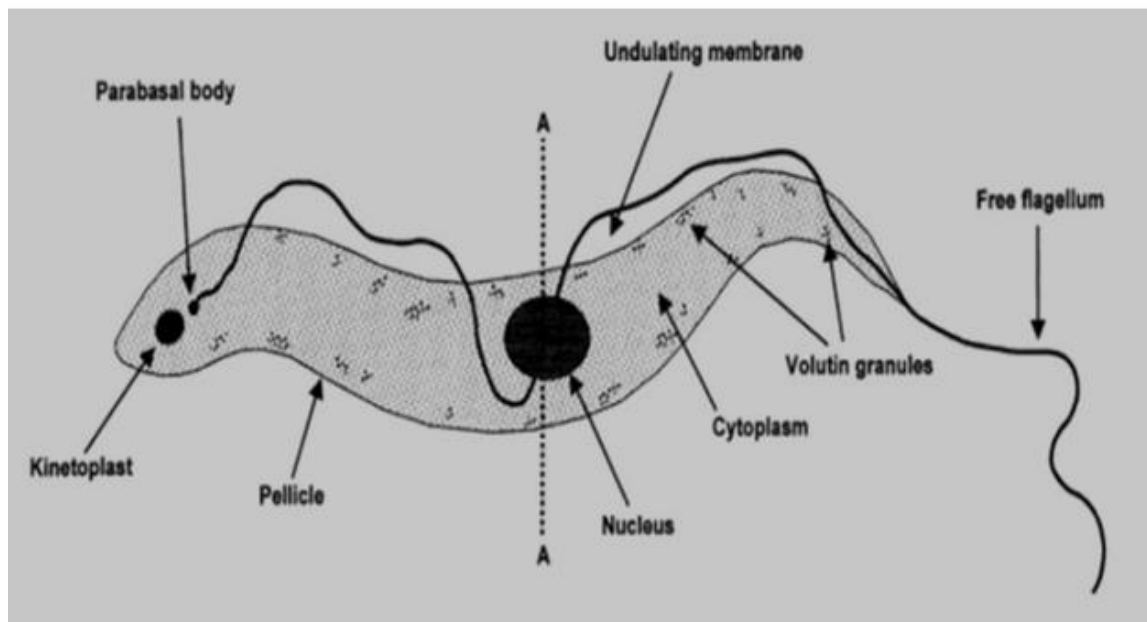
### 2.2.1 Trypanosomes

Trypanosomes are flagellated protozoa which belong to the family *Trypanosomatidae*. The family consists of several genera and many species; all are parasitic in habit. The species which parasitize vertebrates require a vector for transmission (Adam *et al.*, 1979). Trypanosomes live and multiply in the bloodstream, lymphatic vessels, and

tissues, including the cardiac muscle and the central nervous system. The most common form of *Trypanosomatidae* is the bloodstream form of the mammalian parasites (Itard, 1989).

### **2.2.2. Classification and Morphology**

Trypanosomes are whip-like unicellular organisms, of 8-50µm in size, depending on the species. Figure 2.4 is a schematic representation sketch of a trypanosome (trypomastigote) (Uilenberg, 1998). The length and position of the trypanosome's flagellum is variable. A number of cellular organelles can be clearly seen under an electron microscope. In the trypomastigote, the kinetoplast is situated near the posterior end, the flagellum arises from the parabasal body, the undulating membrane is seen along the length of the body and the nucleus is placed in the centre. The kinetoplast is distinct and well-defined, and its size and position differs among species. It plays an important role in reproduction, metabolism and in the cyclic transmission of trypanosomes. The flagellum is used for movement through the blood plasma and tissue fluid (Adam *et al.*, 1979 Uilenberg, 1998).



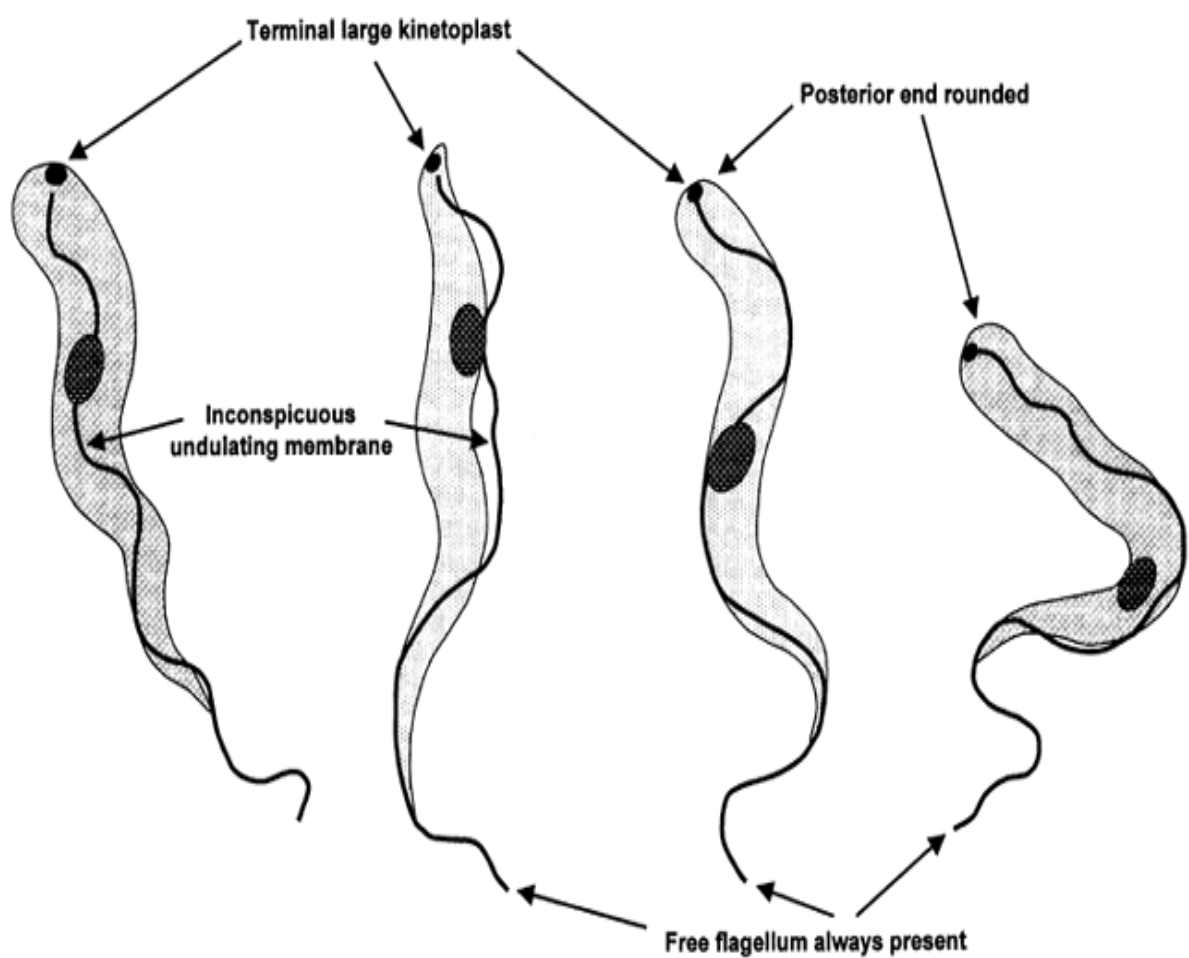
**Fig. 2.4: A Simplified Drawing of a Trypanosome (Taken from Uilenberg, 1998)**

Pathogenic trypanosomes in the genus *Trypanosoma* are divided according to their development in the vector and transmission by either the saliva (Salivarian group) or transmission by faecal contamination (Stercoraria) (Adam *et al.*, 1979; Losos, 1986; Mulligan, 1970). The Salivarian type consists of species of veterinary and medical importance and can be separated into four groups or sub genera (Duttonella, Nannomonas, Trypanozoon and Pycnomonas) according to their morphology in the blood and their pattern of development in the tsetse fly (Adam *et al.*, 1979; Mulligan 1970).

#### **2.2.2.1 Subgenus Duttonella**

*Trypanosoma vivax* which has a mean length of 20-26 $\mu$ m, a long free flagellum and a large terminally placed kinetoplast, distinguishing it from the other pathogenic salivarian trypanosomes, are found in this group. *T. vivax* is a very mobile and “lively” parasite. It crosses the field of a microscope rapidly, which makes it difficult to follow its movement under microscope (Stephen, 1986). It is much larger than any of the other

pathogenic species, and this is a distinguishing feature. The nucleus is centrally placed. The posterior extremity is swollen and blunt. The undulating membrane is inconspicuous (Fig. 2.5). *T. uniformes* is another example of trypanosomes in this group which is much smaller in size (from 12 to 20)  $\mu\text{m}$  but similar to that of *T. vivax* (Molyneux and Ashford, 1983).



**Figure 2.5** *Trypanosoma vivax* blood stream forms

*Source: FAO Corporate Document Repository*

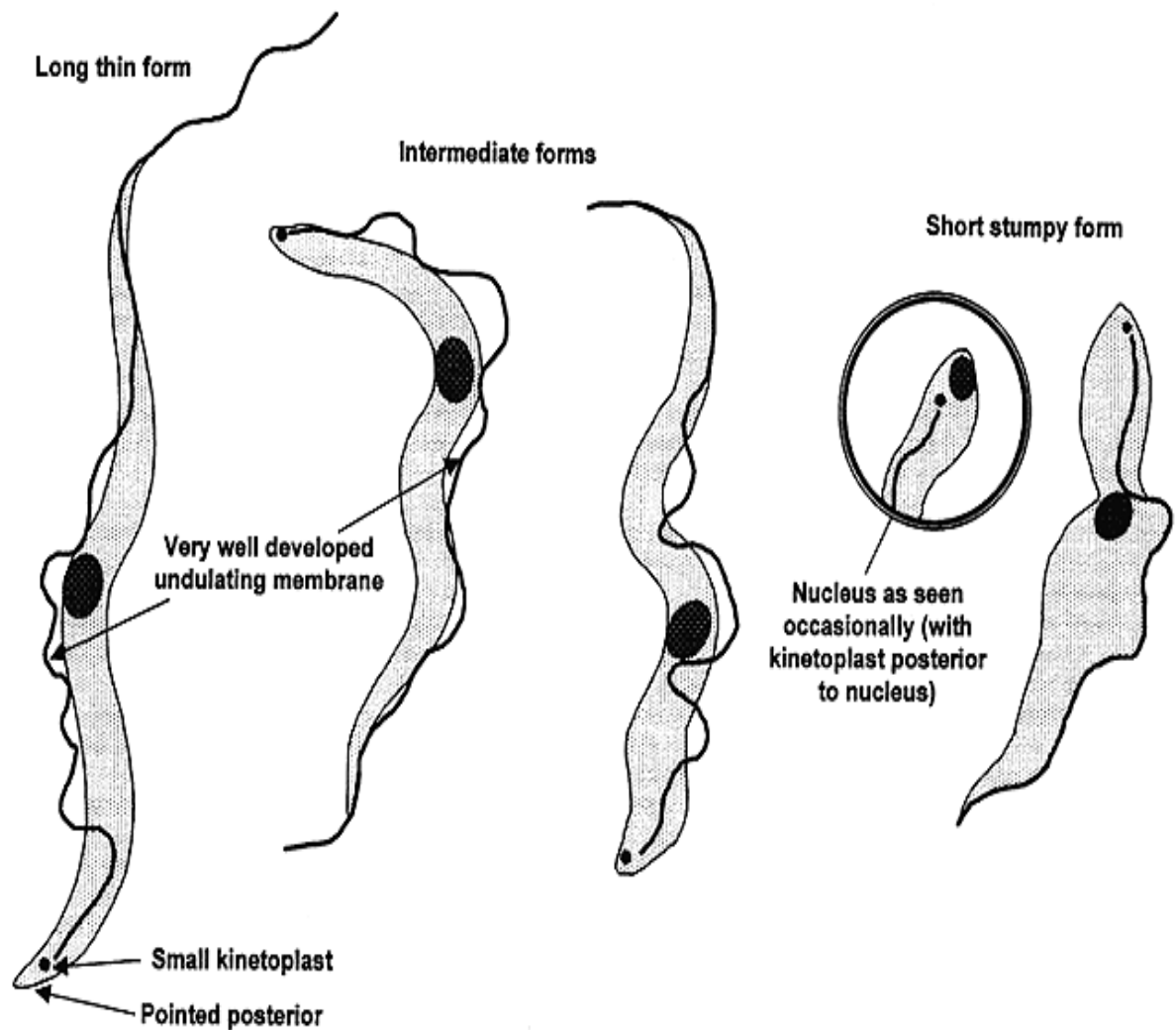
### 2.2.2.2 Subgenus *Pycnomonas*

The subgenus *Pycnomonas* is represented by a single species, *Trypanosoma suis*, which is characterized by stout monomorphic forms with a free flagellum and a small sub terminal kinetoplast. It develops in *Glossina* species like the tsetse-borne species of Trypanozoon, in the mid gut and salivary glands. *T. suis* is the least known pathogenic trypanosome. Pigs are the only domestic mammalian hosts (Mulligan, 1970).

### 2.2.2.3 Subgenus *Trypanozoon*

The subgenus *Trypanozoon* is the most homogeneous group of salivarian trypanosomes, represented conventionally by species which are morphologically indistinguishable but differ in biological features. In Africa, there are about five species, three of which (*Trypanosoma brucei brucei*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*) (Fig. 2.6) are tsetse-borne and develop in the mid gut and salivary glands of the vector while the remaining two are transmitted directly; one (*T. evansi*) by mechanical inoculators (Tabanid flies) and the other (*T. equiperdum*) by contact between the mammalian hosts. The blood forms of *Trypanozoon* measure from 11 $\mu$ m to 42 $\mu$ m (Mulligan, 1970) in total length. They are typically polymorphic, being represented by three forms: (a) slender trypanosomes (mean length 23-30 $\mu$ m) (Mulligan, 1970) possessing a free flagellum and a well-developed undulating membrane, elongated nucleus, sub terminal kinetoplast and narrow posterior end drawn out to a blunt point or sometimes truncated ;(b) stumpy trypanosomes (mean lengths 17-22 $\mu$ m) which are stout and usually without a free flagellum, well developed undulating membrane, rounded nucleus (displaced to the posterior end in posteronuclear forms), near broadly rounded or obtusely pointed posterior ended kinetoplast; and (c) intermediate forms varying in length between the two previously mentioned types. A free flagellum, of varying length,

is always present. The nucleus is centrally placed. The posterior end is somewhat variable in shape, but usually bluntly pointed. The kinetoplast is close to the posterior extremity (Hoare, 1972).



**Figure 2.6** *Trypanosoma brucei* blood stream form

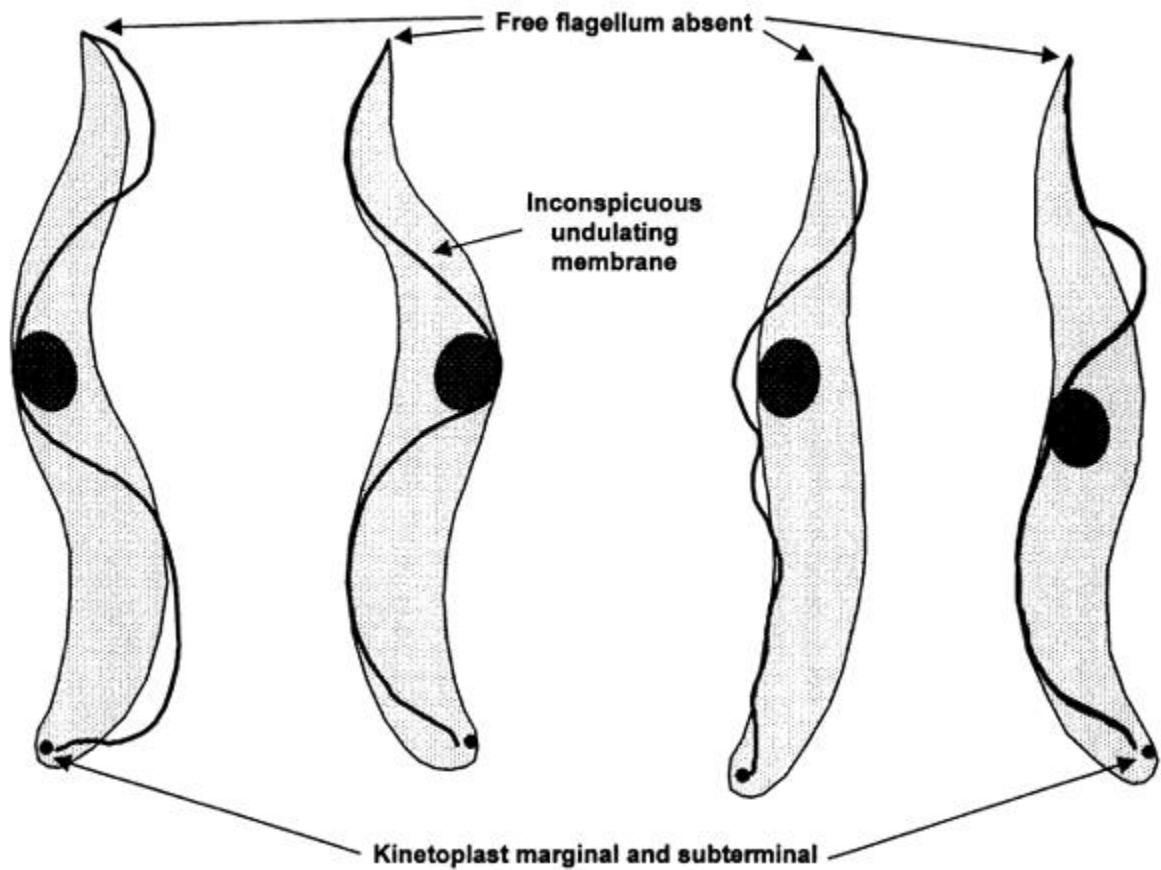
*Source: FAO Corporate Document Repository*

#### 2.2.2.4 The subgenus *Nannomonas*

This is the smallest of the pathogenic trypanosomes, with a length of 8 $\mu$ m -24 $\mu$ m. The blood forms are monomorphic, in that they lack a free flagellum. Generally two variants

are seen, a shorter form 9  $\mu\text{m}$  -18 $\mu\text{m}$ , the typical *congolense* type, and a longer form (up to 25)  $\mu\text{m}$ , with individuals intermediate in length between the shorter and the longer forms (Hoare, 1972). The posterior end of the body is either rounded (in the smaller forms) or obtusely pointed (in the longer ones). The nucleus is situated near the middle of the body (Mulligan, 1970). The subgenus *Nannomonas* contains two recognized species; *T. congolense* and *T. simiae* (Fig. 2.7) and a proposed new one, *T. godfreyi* (McNamara *et al.*, 1994). All the three species are morphologically identical but differ with respect to host specificity and disease symptoms in the host. *T. simiae* and *T. godfreyi* are generally associated with infection in pigs. *Nannomonas* trypanosomes are very active in fresh blood films but do not tend to move far across the microscope field. They also demonstrate agglutinating properties by tending to adhere to each other as well as to host tissues *in vivo* (Stephen, 1986).

Recent studies have resulted in a subdivision of the *T. congolense* species into several ‘types’, distinguished by isoenzymatic differences and molecular techniques. These are *T. congolense* savannah type, *T. congolense* Tsavo type, *T. congolense* forest type and *T. congolense* Kilifi type (Majiwa *et al.*, 1985; Majiwa *et al.*, 1993).



**Figure 2.7** *Trypanosoma congolense* as seen in a stained blood smear

*Source: FAO Corporate Document Repository*

### 2.2.3 Epidemiology

African Animal Trypanosomiasis (AAT) is widely distributed in Africa. The fact that the disease is transmitted mechanically as well as cyclically has certainly expanded the disease distribution out of the tsetse belt area. The epidemiology of AAT in tsetse infected areas of Africa is determined by four biological factors, namely: trypanosomes, tsetse flies, reservoir hosts and livestock. However, cattle are the domestic species in which the disease is most frequently diagnosed and treated. When dealing with tsetse-transmitted trypanosomiasis, much depends on the distribution and the vectorial capacity of *Glossina* species responsible for transmission. Of the three groups of *Glossina*, the

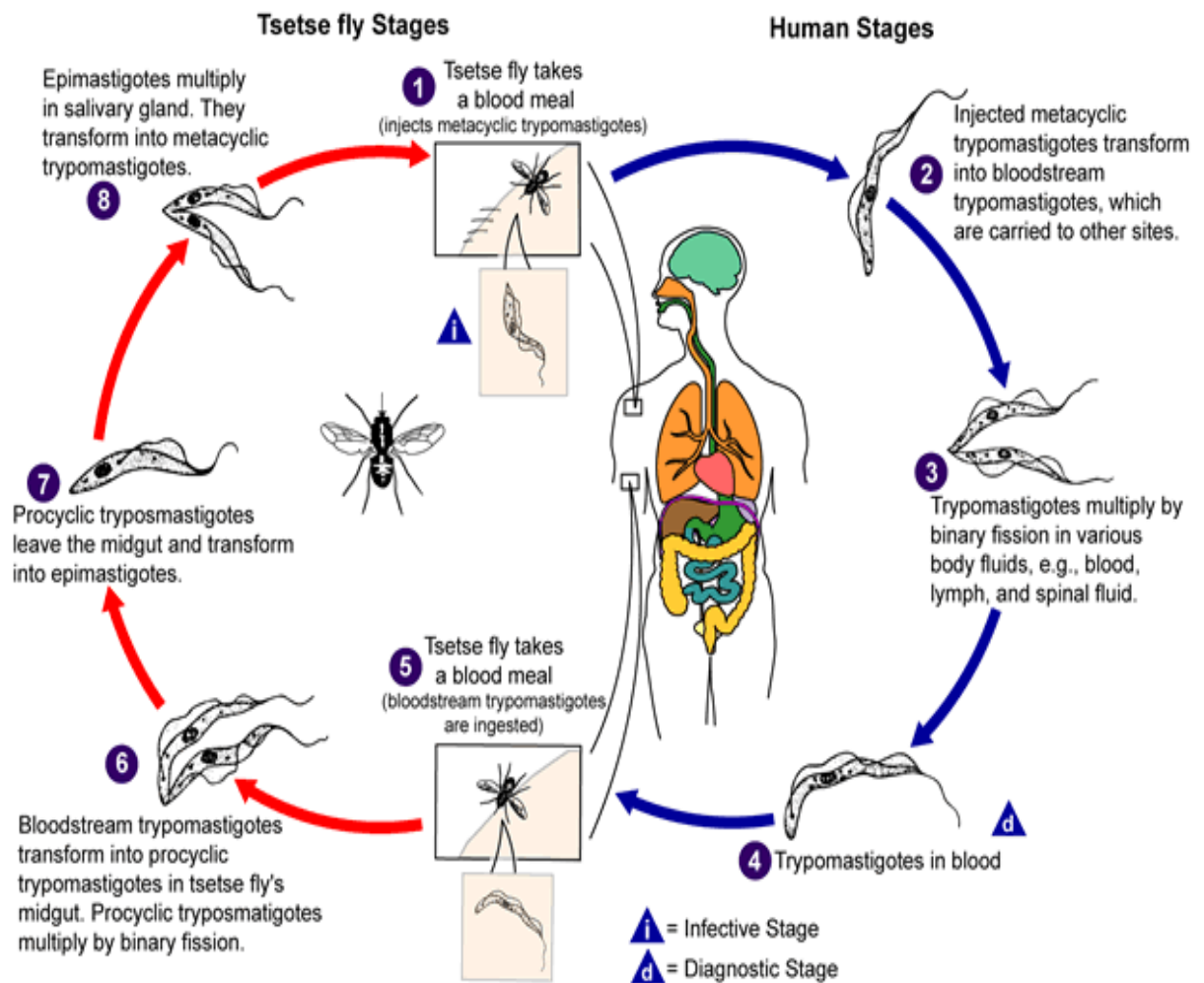
savannah and riverine species are the most important since they inhabit areas suitable for grazing and watering. Biting flies such as *Stomoxys* and *Tabanus* may act as mechanical vectors, but their significance in Africa is still undefined. However, in Central and South America, *T. vivax* is thought to be transmitted readily by such mechanical vectors (Urquhart *et al.*, 1987). The fact that the parasite affects not only cattle but also wild animals which constitute the reservoirs of the disease, makes the epidemiology of animal trypanosomiasis extremely complicated (Hoare, 1972; Uilenberg, 1998).

The occurrence of trypanosomiasis within the overall tsetse infested area is irregular because of differences in animal husbandry practices which affect the nature of the contact between tsetse flies and livestock, and because of variation in the distribution and density of particular groups and species of tsetse flies which differ in their capacities to transmit pathogenic trypanosomes. The pattern of the disease is also affected by differences in the distribution of pathogenic trypanosomes; *T. congolense*, *T. vivax* and *T. brucei* are always found within tsetse infested areas. *T. simiae*, *T. uniforme*, and *T. suis* occur less frequently (Robertson, 1976).

#### **2.2.4. Life Cycle of Trypanosomes**

Trypanosomes reproduce asexually by binary fission, that is, the division of one parent cell into two daughter cells. They can also reproduce sexually by which genetic exchange takes place between two of them. This process is known to take place in the tsetse vectors but the frequency of its occurrence is unknown (Jenni *et al.*, 1986; Uilenberg, 1998) and the process is not obligatory. Trypanosomes require two hosts to complete their lifecycle which is made up of different developmental forms. That is the vertebrate or the mammalian host and the tsetse vector.

Infection of the mammalian host starts with the bite of an infected tsetse fly (*Glossina* spp.), which injects the metacyclic (infective) trypomastigote form of the parasite in its saliva before taking its blood meal. The parasites develop into long slender trypomastigotes which multiply at the site of inoculation and later in the blood, lymphatic system and tissue fluid by binary fission. The bloodstream trypomastigotes are carried to the heart and various organs and in later stages of infection invade the central nervous system. Trypomastigotes are taken up by the male or female tsetse fly when it takes a blood. The parasites develop in the mid-gut of the fly into procyclic trypomastigotes and multiply. From about 2-3 weeks, procyclic the trypomastigotes move to the salivary glands transforming through epimastigotes into metacyclic trypomastigotes (Fig. 2.8). The tsetse fly remains infective for life - about 3 months.



**Figure 2.8** Diagrammatic representation of the life cycle of *T. b. gambiense* and *T. b. rhodesiense* in humans and the tsetse fly.

(Source: Alexander J. da Silva and Melanie Moser, Centres for Disease Control, Public Health Image Library. Atlanta).

### 2.2.5. Pathogenesis and clinical signs

Initial replication of trypanosomes happens at the site of inoculation in the skin; this causes a swelling and a sore (chancre). The clinical sign observed in AAT is anaemia. Within a week of infection with the haematic trypanosomes (*T. congolense* and *T. vivax*) there is usually an intermittent fever, oedema, lacrimation, enlarged lymph nodes, loss of appetite and weight with pronounced decrease in packed cell volume (PCV),

haemoglobin and red blood cells, and within 2 – 3 months the PCVs may drop to below 30 percent of their preinfection values. Acute infections associated with high parasitaemia may lead to the death of an animal still in good body condition. However, chronic trypanosomiasis is associated with progressive emaciation and eventually, cachexia (reduction in vitality and strength of body and mind). This is usually accompanied by low levels of parasitaemia and death in untreated cases (Coetzer *et al.*, 1994).

In the domestic pig, *T. simiae* produces a hyperacute, disease. After a short incubation period, death occurs very rapidly and at post-mortem examination there is complete capillary breakdown with haemorrhages and congestion in various organs throughout the carcass (OIE, 2008). Infections with *T. congolense* and *T. brucei* are often asymptomatic (Ilemobade and Balogun, 1981). Recently, however, there have been several reports of severe acute *T. brucei* infections in pigs. One case report (Agu and Bajeh, 1986) mentioned anorexia, emaciation, pale mucous membranes and death within 1-3 months due to *T. brucei*. The same authors also conducted an experimental infection with the same strain and observed an undulating parasitaemia for several weeks and intermittent pyrexia (a rise in the temperature of the body) (Agu and Bajeh, 1987). The pigs became progressively emaciated, some showed nervous signs and were recumbent in the terminal stages.

### **2.2.6 Diagnosis of trypanosomiasis in pigs**

Routine diagnosis of trypanosomiasis in the field is undertaken via clinical signs and knowledge of the endemicity of the disease in the area (Blood *et al.*, 1989; Coetzer *et al.*,

1994). However, other more accurate methods of diagnosis include: parasitology, serology and DNA-based methods.

#### **2.2.6.1 Parasitological Test**

The simplest parasitological techniques are examination of wet, thick or thin films of fresh blood. Amongst them, stained thin blood films are generally regarded as more specific but less sensitive than the wet and thick blood films. The actual specificity and sensitivity of these techniques is directly dependent on the volume of blood actually examined and the skill and experience of the microscopist. The major disadvantage of these tests is the low sensitivity as only parasite levels above 100,000 per ml can be detected (Paris *et al.*, 1982).

In the Micro Haematocrit Centrifugation Technique (MHCT), trypanosomes are concentrated at the buffy coat of a capillary tube after centrifugation and capillaries can directly be examined under the microscope (Woo, 1970). A modification of the MHCT is the Buffy Coat Technique (BCT): where the buffy coat is expelled onto a glass slide and subsequently examined under background (Murray *et al.*, 1977). Compared with the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record (OIE, 2008). Both tests are particularly useful in that the packed cell volume (PCV) can be assessed at the same time.

#### **2.2.6.2 Serological tests**

A large number of serological tests have been used to indicate infections with trypanosomes indirectly. However, few of them have found practical application. The

most commonly used techniques are: Indirect Fluorescent Antibody Test (IFAT), Enzyme-Linked Immunosorbent Assay (ELISA) and Card Agglutination Test (CAT). With the use of the above serological techniques, the responsible *Trypanosoma* species may be identified within certain limitations (Seifert, 1996). Both the IFAT and ELISA have been adapted for the analysis of blood samples collected on filter paper. In addition, both tests have high sensitivity and genus specificity but their species specificity is generally low. They detect immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection.

To overcome these problems in the diagnosis of trypanosomiasis, Nantulya *et al.*, (1987) developed monoclonal antibodies (MoAbs) that can distinguish *T.brucei*, *T.congolense* and *T.vivax*. These monoclonal antibodies recognize antigens that are specific to the procyclic stages as well as those expressed by bloodstream-form of trypanosomes. When these MoAbs are used in an antigen-trapping ELISA, diagnosis is sensitive and specific enabling many latent infections to be detected.

### **2.2.6.3 Molecular Techniques**

With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed (Wuyts *et al.*, 1995; Clausen *et al.*, 1998, Donelson *et al.*, 1998). PCR is reported to be more sensitive than conventional parasitological techniques in a number of hosts and has the advantage that it can identify parasites at the species level (Wuyts *et al.*, 1995; Desquesnes and Davila, (2002). With PCR methods, specific repetitive nuclear DNA sequences can be amplified for trypanosomes and specific primer sets are available. The procedure is extremely sensitive, but false-positive results may occur as a result of

contamination of samples with other DNA (OIE, 2008). False-negative results may occur when the parasitaemia is very low ( $< 10$  trypanosome/ml of blood), which occurs frequently in chronic infections; they may also occur when the specificity of the primers is too high, so that not all isolates of a particular trypanosome species are recognized (OIE, 2008). The method also requires that the genome of the parasite be known in order for primers to be designed to flank conserved regions of genes.

### **2.3. Control of Tsetse and Trypanosomiasis**

Prevention and control of tsetse-transmitted trypanosomiasis depends on methods directed to the vectors, the host and the parasites. Each of these approaches is useful but has important limitations, such as expense, environmental pollution and drug resistance. Vector control is the most reliable means of disease control since it removes the threat of trypanosomiasis on a permanent basis. Many vector control methods including woody vegetation clearance to remove tsetse shelter, and large scale application of insecticides by air (non-persistent and persistent formulations) and ground spraying (only persistent insecticides) and large wild life elimination (to deny tsetse its source of food i.e. blood), could be applied (SIT, 1996).

#### **2.3.1 Vaccination**

Despite extraordinary research efforts directed at the development of vaccines against trypanosomes no vaccine has so far been developed. Due to the fact that the trypanosome has genes that can code for many different surface – coat glycoprotein, and changes its surface coat to evade the antibodies, it has been very difficult to produce vaccine for immunization from trypanosome (Uilenberg, 1998). The one area where

there has been some progress has been in the development of an anti-disease vaccine. Here efforts had been directed towards preventing the pathogenic effects of the parasite rather than infection itself (Authie *et al.*, 2001).

### 2.3.2 Chemotherapy

The application of antitrypanosomal drugs have been the most widely practised means of controlling trypanosomiasis in domestic livestock since the early 1950s, either as curative or prophylactic drugs. A programme to eradicate tsetse flies from some 9 million km<sup>2</sup> of Africa is highly ambitious. It will be complex, take many years and possibly cost some US\$ 20 billion (Budd, 1999).

Drugs currently recommended for chemotherapy of animal trypanosomiasis come from only three closely related groups. These are the phenanthridines, isometamidium, and homidium, and the aromatic diamidine, diminazene. Only isometamidium and homidium are recommended for prophylaxis (Peregrine, 1994). A very widely used chemotherapeutic drug is Diminazine Aceturate (Berenil), which is effective against all three African animal trypanosomes. The Isometamidium drugs are also excellent chemotherapeutic agents as are the quaternary ammonium trypanocides Antrycide, Ethidium and Prothidium.

For Human trypanosomiasis the WHO has recommended a two phased treatment according to the disease stage, first phase treatments: Suramine: discovered in 1921, is used in treatment of the initial phase of *T.b. rhodesiense*. There are certain undesirable effects, especially on the digestive tract. Pentamidine: discovered in 1941, is used in treatment of the initial phase of *T.b. gambiense* sleeping sickness. In spite of a few

undesirable effects, it is well tolerated by patients. Future production is guaranteed by an agreement between WHO and Aventis. Then when the disease enters the neurological phase comes the second phase treatments: Melarsoprol: discovered in 1949, is at present the only drug available on the market to treat the advanced stage of sleeping sickness, no matter which parasite is the cause. It is the last arsenical derivative in existence. Eflornithine was registered in 1990, and is the alternative to melarsoprol treatment. It is effective only against *T.b. gambiense*. The regimen is strict and hard to apply. Production ceased in 1999. Aventis company gave the licence to WHO, which has undertaken several initiatives to seek a new source of production, (Burchmore *et al.*, 2004).

### **2.3.3 Bush clearing**

There are certain types of vegetation that enable micro-climate establishment of both temperature and humidity that makes habitats suitable for certain types of tsetse flies. Clearing of such vegetation therefore causes the tsetse species concerned to disappear (Finelle, 1974). This method of trypanosomiasis control is labour intensive, can lead to soil erosion, and needs slashing of regenerated vegetation.

### **2.3.4 Insecticide use in tsetse control**

The most successful tsetse control programmes carried in Africa were based on the insecticides either by ground or aerial spraying. The insecticides that have most frequently been used were the organochlorines such as Dichloro- Diphenyl- Trichloroethane (DDT) and dieldrin, pyrethroids (deltamethrin, permethrin, and alphasmethrin), and avermectins (ivermectin). Endosulphan was also used extensively (Ormerod, 1986). Many efforts have been deployed to improve the spraying techniques.

### 2.3.5 Sequential aerosol technique (SAT)

Sequential aerosol technique is more environmental friendly than ground spraying or conventional aerial spraying. It needs planes that fly at an approximate speed of 250-300 km/h and the flight should be 10 to 15m above the canopy. Tsetse flies are killed by direct contact with the insecticide micro-droplets; there is no residual effect and treatments must be repeated until all newly emerging adults are affected. The SAT was used successfully in Botswana to eliminate tsetse from the Okavango Delta (Kgori *et al.*, 2006) and in Zimbabwe, where 48,000 km<sup>2</sup> of savannah area were cleared in the 1980's (Shereni, 1990). The SAT application in tsetse control campaigns was very successful, but it needs institutional support and national commitment aiming at eradication because of the high cost of this technique.

### 2.3.6 Traps and Targets

Tsetse control by use of traps offers some of the best realistic approaches for control of many *Glossina* species. Traps can be made from local materials, for example cone traps made from old vehicle tyres or biconical tsetse traps made from black cloth with the upper cone made from palm reed netting. A number of different traps have been developed for capturing tsetse-flies in large numbers.

The mono-conical (Vavoua) trap (Fig. 2.9) was first designed in Vavoua, Ivory Coast (Laveissière and Grebaut, 1990). It consists of a mosquito netting cone attached to a circular piece of galvanized metal wire and placed above three screens joined together at angles of 120°. Each screen is two-thirds blue and one-third black, the black joining together in the middle. The flies land on the screens, fly upwards towards the light, pass through the upper cone and get trapped in the cage where they are collected.

The bi-conical (Challier-Laveissiere) trap consists of two fabric cones, approximately equal in size, joined base to base and supported by a vertical pole passing through the middle (Fig. 2.10). It is an efficient trap for most riverine tsetse. The bottom cone of blue cotton fabric is divided into four segments by dark cloth partitions, visible through four slits in the blue cloth. The upper cone is made of strong mosquito netting. At the top of the vertical pole is mounted a small cone made of a wire frame and covered with mosquito netting. This small cone is open at the base and has a small exit hole at the top. It acts as a non-return device, and leads to a collecting cage at the top of the trap. Flies attracted to the trap enter through the dark slits at the base, and move up into the collecting cage at the top (Okoth, 1986).

The pyramidal trap consists of a pyramid of transparent white mosquito netting surmounting two black and two blue screens arranged in the form of a cross. It was developed in the Congo (Gouteux and Lancien, 1986) and is currently being extensively used in Uganda (Fig. 2.11). If provided with a catching device at the top this trap can be used without an insecticide and is then suitable for areas with high rainfall. In large-scale programmes it offers the advantage that it is very compact for storage. It can be given its final shape in the field by extending the screens with two sticks.



**Figure 2.9: Monoconical (Vavoua) Trap**

*Source: Vestergaard Frandsen*



**Figure 2.10: Biconical (Challier-Laveissiere) Trap**



**Fig. 2.11: Pyramidal Trap (Gouteux and Lancien 1986)**

### **2.3.7 Sterile Insect Technique (SIT)**

One of the latest methods of control is the Sterile Insect Technique (SIT) involving continuous release of sterile insects among the indigenous insect population at rates sufficient to result in a reduction in biotic potential of the target population. The mating

of released sterile male insects with indigenous fertile female insects causes infertility in the target population (SIT, 1996). It is a cumbersome venture, involving the use of skilled manpower (Jordan, 1995 and Alsop, 1994). With this method of tsetse fly control there is a need to have regional effort. However, in an event of eradicating tsetse flies, SIT can be used for mopping up low density foci of tsetse flies remaining (Jordan, 1995 and Alsop, 1994).

The technique requires large numbers of tsetse of the target species to be reared in laboratory colonies and sterilized with a source of radiation while in puparia and the sterile males emerging from the puparia are released in large numbers, over a long period, into the area from which tsetse are to be eradicated (Leak, 1999). The sterile insect technique (SIT) has been used successfully against *G. austeni* in Zanzibar. During this campaign, 60,000 irradiated male flies were being released per week. From 1995-1996, 5.5 million sterile males were released in total (Robertson, 2004). In practice, number of wild tsetse have to be reduced by other means, such as insecticide-impregnated traps or targets, before the release of sterile males so that the numbers of sterile males required are within acceptable limits (Leak, 1999).

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Study Design

A cross-sectional study was conducted between October and November 2013 in the Jomoro district of the western region of Ghana to determine the prevalence of trypanosomes in pigs and tsetse flies and transmission rate on pig populations which were measured at a point in time and their interactions demonstrated.

#### 3.2 The Study Area

##### 3.2.1 Climate, Vegetation and Drainage

The Jomoro District is one of the twenty-two (22) districts in the Western Region of Ghana. Sampling was carried out in four villages within the District and the selected villages with their corresponding global position systems include: Ezinlibo (N 05.01449 °, W 002.72064 °), Mpataba (N 05.09570 °, W 002.59226 °), Nawulley (N 05.03798 °, W 002.72401 °), and Tikobo No.1. (N 05.05396 °, W 002.70091 °). These villages were selected on the basis of having reported cases of tsetse fly bites by pig farmers in those villages within a period of about two years prior to the study. Figure 3.1 indicates the map of the study area in Jomoro District.

The District is located in the South-western corner of the Western Region of Ghana. It is bounded on the South by Latitude 4.80° N and the Atlantic Ocean (Gulf of Guinea) and in the North it is bounded by Latitude 5.21° N and the Nini River. It also lies between Longitude 2.35° W to the East and 3.07° W to the West. The district covers an area of 1344 km<sup>2</sup> (JDA, 2009), representing about 5.6% of the total area of the Western

Region. It shares boundaries with Wassa-Amenfi and Aowin-Suaman to the North, Nzema East District to the East and Ivory Coast to the West and the Gulf of Guinea at the South (JDA, 2009). The south-central part of the district, including the Ankasa Forest Reserve, is an area of rolling granite topography consisting of frequent steep-sided small round hills rising from 200 to 600 feet or no flat uplands and no broad valleys. Around the coastal area, the relief is lower consisting of flattish upland areas and steep valleys. A minor relief feature is the one formed by a ridge of highland running northwest to southeast from the Tano to Bonyere that terminates on its northern side in the Nawulley scarp (JDA, 2009).

The district has extensive tropical rainforest vegetation with the coastal vegetation being largely mangrove swamp. The vegetation is not uniform throughout but can be divided into a number of belts which differ in their floristic composition and general character. The distribution of these-belts is connected with rainfall and soil acidity. The Ankasa Forest Reserve is the major Forest Reserve in the District, and is one of the largest designated areas for conservation in Ghana. The park is recognized for its high plant diversity, indigenous forest birds, monkeys especially chimpanzee and forest elephants. Lumbering activities remain a big challenge in the area and these can be found around Mpataba, Nuba, Ankasa, Tikobo No.1, Ellenda and Anwiafutu area. The activities of timber extractors in the district have had negative effect in depleting economic trees as a result of the neglect of afforestation and reforestation programmes and the destruction of young trees all leading to micro climate change and ecological imbalance (JDA, 2009). Although gaps in climatic records on the district make them unreliable for planning purposes, the district is believed to be the wettest part of the country with a mean annual precipitation of 1732 mm. Temperature in the district is generally high with a mean monthly temperature of 26 °C (JDA, 2009). Relative humidity throughout the district is

about 90% during the night and falls to about 75% in the afternoons. The district is characterized by two rainy seasons from April to July and September to November. There is a short dry spell in August and longer dry period in December to January. Although February and March are hot, and dry. The climate is classified as Equatorial Monsoon. Some of the major rivers and their tributaries that drain through the district include: Tano, Ankasa, Suhwen, Elloin and Amanzulle River. The other water body of importance is the Dwenye Lagoon (JDA, 2009). The gallery forests along the banks of the streams and rivers that run through the study sites provide a suitable home for riverine species of tsetse fly such as *G. palpalis palpalis* and *G. tachinoides* which prefer more humid areas.

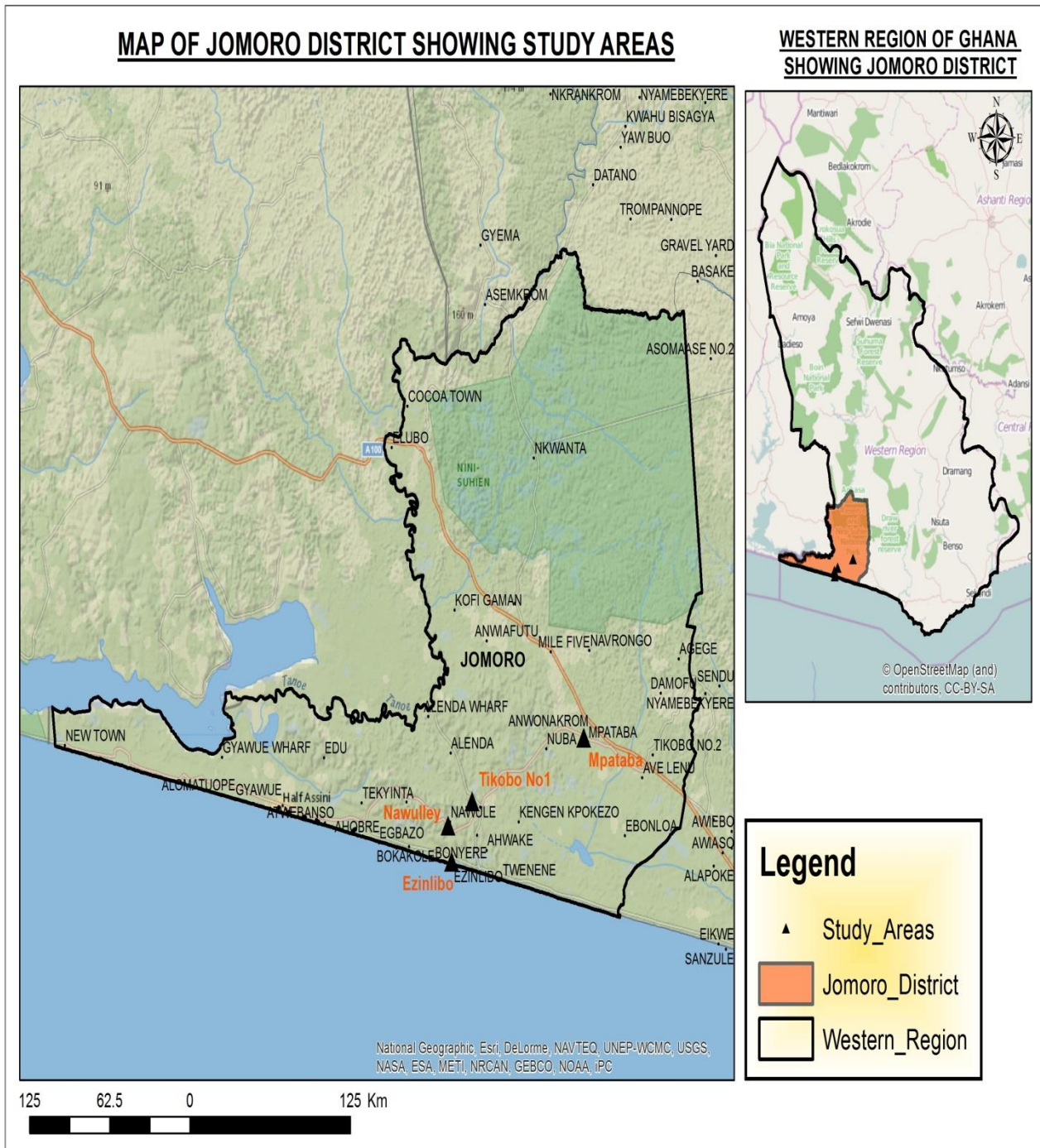


Figure 3.1 Map of Jomoro District Within Western Region of Ghana Showing The Study Areas.

### **3.2.2 Animal Production and Health**

Small ruminants (sheep and goats) abound in the district. However there are only few cattle in the area because according to sources, (Ishak, 2013 Personal Communication), the nature of the vegetation at the study areas are not convenient for cattle grazing. Pigs are reared more intensively by the youth who see it as a means of raising money for other ventures. Coconut chaff, a by-product from the oil extraction, forms the main feed ingredient. Pig production in the district is by small holder farmers who keep between ten to thirty pigs. Pig-sties are mostly located within the compounds of the owners, either inside the community or the hamlets in cocoa and oil palm plantations. Most of the pigs are crossbreeds between the local breed, large white, and landrace (exotic) breeds.

Some known diseases of veterinary importance which turn to affect pig production in the district include: Trypanosomiasis, Helminthiasis and tick borne diseases (Babesiosis). Animal health services for the entire district are provided by one Veterinary Officer with five Veterinary Technical Officers and a district veterinary laboratory with inadequate facilities which renders veterinary services for the entire District (Veterinary Services Directorate, 2009).

## **3.3 Data Collection**

### **3.3.1 Sample Size Determination**

The following formula was used to obtain the sample size of pigs to be sampled from the study areas (Thrusfield, 2005).

$$n = z^2 p (1-p) / d^2$$

- Where  $n$  = sample size,
- $z$  = risk of type 1 error (=1.96 at 95% confidence level)
- $p$  = expected prevalence of trypanosomiasis in pigs
- $d$  = absolute precision = 5% = 0.05

Using 73.7% (0.737) as prevalence of trypanosomiasis among pigs in a West African country (Simo *et al.*, 2006),

- $n = 1.962 \times 0.737 \times (1 - 0.737) / 0.05^2$

- $n = 298$  pigs

A total of 300 pigs were selected for the study to make room for non-response and other circumstances.

### 3.3.2 Sampling Method

A proportional simple random sampling technique was used to determine pigs to be sampled to represent all the study population at various sites. The study was conducted on 300 pigs traditionally managed under small holder system. The study animals were of different age groups and both sexes. The age of the sampled animals was determined based on the owners information and were grouped into two as follows : < 1 year and  $\geq 1$  year old. The body condition of animals were recorded by classifying the animals into two groups as good and poor based on the appearance of ribs and dorsal spines using the method described by Nicholson and Butterworth (1986).

The study protocol was followed and permission was sought from the District Veterinary Services Directorate of the Ministry of Food and Agriculture and the Jomoro District Assembly. Thereafter, verbal informed consent was obtained from each owner of the pigs prior to the extraction of the blood samples by the field team. The quantity of blood to be taken from their pigs (where applicable) was made known to them before the exercise. Only pig owners who agreed to participate in the study had their animals sampled.

### **3.3.3 Entomological survey**

Entomological study was carried out to assess the apparent density and species of tsetse in the study area. During the study, a total of 16 un-baited bi-conical traps (Brightwell *et al.*, 1987) with blue-black material were deployed near pig sties which seem suitable habitat for tsetse flies at intervals more than 200m for 24 hours. The entomological survey was conducted during the minor rainy season, between October and November, 2013. Traps were put under shades cleared of weeds to avoid undue fly mortality. The base of each trap pole was smeared with grease in order to prevent the ants from climbing up the pole. Flies were collected over a period of four days at all sites to minimize effect of day-to-day variations (WHO, 1998). Harvesting was done exactly 24 hours from the time each trap was set and the flies were then sorted out by sex and species and preserved in 70% ethanol until DNA was extracted. The species of tsetse was identified based on the characteristic morphology (FAO, 2000). Other biting flies were also separated according to their morphological characteristics such as size, colour, proboscis and wing venation structures at the genus level (Langridge, 1976). Sexing was done for tsetse fly by observing the posterior end of the ventral aspect of abdomen by hand lens. Male flies were identified by an enlarged hypophageum.

The apparent density which is relative to the type of sampling tool (trap) used is expressed as the average number of flies caught per trap per day (flies/trap/day) or FTD (Leak, 1999). The apparent density was calculated by traps used to catch them (T) and the number of days for which traps were operational (D). So,  $FTD = F/T \times D$ . If a trap was not operational for some reason, e.g. it was vandalized, blown down or destroyed by pigs, that trap-day was excluded from the sum of trap days. Season, altitude, trap numbers and vegetation types were recorded.

### **3.3.4 Parasitological Survey**

#### **Collection of Blood Samples**

Each sampled pig was restrained, the ear vein was identified and the site cleaned with cotton soaked with alcohol. The skin surface was completely dried before stabbing a visible vein on the outer ear with a sterilized needle. Approximately 5ml of whole blood was collected in a vacutainer tube which contained Ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The place of stabbing was plugged with cotton soaked in 70% ethanol to stop haemorrhage. After sampling, the vacutainer tubes were placed in a cool box containing ice packs and transported to the vector genetics laboratory at the Ghana Atomic Energy Commission (GAEC) for analysis. From each vacutainer tube, blood was transferred into two capillary tubes to about three quarter full (70 $\mu$ l) which were sealed at one end with “Cristaseal” (Hawxley) and was used for packed cell volume (PCV) determination.

### **3.4 Laboratory Analysis of Samples**

#### **3.4.1 Parasitological Analysis**

##### **3.4.1.1 Measuring of packed cell volume (PCV)**

The capillary tubes were placed in microhaematocrit centrifuge. The tubes were loaded symmetrically to ensure good balance and centrifuged at 12,000 rpm for 5 minutes. Tubes were then placed in microhaematocrit reader and the readings were expressed as a percentage of packed red cells to the total volume of whole blood following the standard procedure described by Murray and Dexter (1977). Animals with PCV < 32% were considered to be anaemic.

##### **3.4.1.2 Preparation of Thick Blood Smear**

Thick blood smears were prepared by placing a drop of blood (5–10 µl) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter. The film was dried thoroughly by rapidly waving in the air and, without fixation, was dehaemoglobinised by immersion in distilled water for a few seconds and dried before staining. It was stained for 30 minutes with 10% diluted Giemsa stain in phosphate buffered saline (PBS), pH 7.2. The manufacturer's directions were followed but with some variation, example varying the timing and the concentration of the Giemsa solution simultaneously to obtain optimal results. The stained smear was then washed with buffered water and examined under X100 oil immersion objective lens (1000X magnification). At least 20 fields were observed before declaring a slide as negative (OIE, 2008).

### **3.4.1.3 Preparation of Thin Blood Smear**

About 5 µl of blood from the microhaematocrit capillary tube was placed on a clean microscope slide approximately 20 mm from one end (allowing for space to apply the thick smear). It was spread with the edge of another slide which was placed at an angle of (approximately 30°) to the first slide and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader, which was then pushed to the other end of the slide in a fairly rapid but smooth motion. The slide was dried quickly by waving in the air and protected from dust and flies. It was then fixed for 3 minutes in methanol, and stained for 30 minutes with 10% diluted Giemsa stain in PBS, pH 7.2. After staining, the slide was washed gently under tap water and allowed to dry. Between 50 and 100 fields of the stained thin smear were examined, with x100 oil-immersion objective lens to detect the presence of trypanosomes (OIE, 2008).

### **3.4.2 Molecular Analysis**

#### **3.4.2.1 DNA Extraction from Whole Blood**

DNA was extracted from pig blood using the CHELEX Method of extraction as described by Penchenier *et al.* (1996) with few modifications. 10 µl of whole blood was dispensed into a 1.5 ml eppendorf tube. 1ml of sterile water (dd H<sub>2</sub>O) was added and then vortexed briefly. This was incubated at room temperature for 30 minutes and then after centrifuged at a speed of 10,000 rpm for 3 minutes. Without disturbing the substrate, all the supernatant was removed and discarded, leaving the substrate in the tube. 200 µl of 5% Chelex suspension was added to the sample and vortexed for 30seconds. The mixture was incubated at 56<sup>0</sup>C for 30 minutes. The sample was vortexed at high speed for 10 seconds and incubated again at a temperature of 100<sup>0</sup>C for 8 minutes. The mixture was vortexed again at a high speed for another 10 seconds and

centrifuged at 10,000 rpm for 3 minutes. The supernatant was transferred into new 1.5ml micro centrifuge tubes and stored at  $-20^{\circ}\text{C}$  until PCR amplification.

### **Preparation of Cetyl Trimethylammonium bromide (CTAB)**

A 40ml of distilled water was added to 0.5g of CTAB in sterile bottle. The mixture was swirled under water bath until CTAB was dissolved. 1M Tris, 5M NaCl, 0.5M EDTA were added to CTAB solution. 100 $\mu\text{l}$  of  $\beta$ -mercaptoethanol was then added in a hood.

#### **3.4.2.2 DNA Extraction from Tsetse Fly**

Alcohol preserved samples of tsetse flies were placed in 1.5ml eppendorf tubes and washed three times in double distilled water. Liquid nitrogen was added to the samples and crushed using micro pestle. 550 $\mu\text{l}$  of CTAB mixture was added to each sample in the tubes and capped. The samples were heated in a water bath at  $65^{\circ}\text{C}$  for 60 minutes and then centrifuged for 7 minutes at 13,000 revolutions per minutes (rpm). The upper phase was transferred into new tubes and 550 $\mu\text{l}$  of chloroform was added to each sample and finger vortexed. The samples were then centrifuged for 15 minutes at 13,000rpm. The upper phase was transferred into new tubes containing 750 $\mu\text{l}$  of cold isopropanol and left in the freezer overnight. The samples were then centrifuged for 30 minutes at 13,000rpm. The supernatant was decanted into a beaker and 200 $\mu\text{l}$  of cold 70% ethanol was added to wash the DNA pellet. Samples were then centrifuged for 5min at 7,000 rpm. The supernatant was decanted and the inverted tubes were kept on kimwipes for 30min until the DNA dried. The DNA pellet were resuspended in 50 $\mu\text{l}$  TE buffer and stored at  $-20^{\circ}\text{C}$  until PCR amplification.

### 3.4.2.3 PCR Amplification of DNA

#### Identification of Trypanosomes Using Generic Primers

Amplification of trypanosome DNA was carried out in a total volume of 20µl PCR mixture containing 2.0µl of 10X PCR buffer (Promega, USA); 1.0µl of 3mM MgCl<sub>2</sub> (Sigma-Genosys, UK); 1.6µl of 2.0mM of mixed dNTPs (Bioline, UK); 0.25µl each of the primers with a concentration of 20 µM, 0.1µl of 5U/ µl Taq DNA polymerase (Promega, USA). The tubes containing the mixture were subjected to 40 cycles of amplification in a Techne TC 412 thermal cycler (Fig. 3.2). During each cycle the sample of DNA was denatured at 94 °C for 1minute, annealed at 50 °C for 2 minutes, and extended at 72 °C for 2 minutes. Prior to the cycling and at the end of the cycling the mixture was subjected to incubation at 94 °C for 3 min and final extension at 72 °C for 10 min, respectively. PCR reactions were carried out in 0.2ml eppendorf tubes using Techne, TC 412 and Bio-rad C1000 thermal cycler (Fig. 3.3). This cycler condition lasted for 4 hours 30 seconds.



**Figure 3.2: PCR Techne, TC 412 Thermal Cycler Used For Amplification**



**Figure 3.3: PCR Bio-rad Thermal Cycler Used For Amplification**

#### **3.4.2.4 Agarose Gel Preparation and Electrophoresis**

2.0 g of agarose was added to 100 mls of 1x Tris Borate EDTA (TBE) buffer (36 Tris, 30 mM  $\text{NaH}_2\text{PO}_4$ , 1 mM EDTA, pH 8.0) in an Erlenmeyer flask. The mixture was heated in a microwave oven on high setting for 2 minutes or until mixture began to boil. The molten gel solution was removed carefully from microwave using a folded paper towel to hold the neck of the Erlenmeyer flask. The combs were placed in gel tray about 1 inch from one end of the tray and were positioned vertically in such a way that the teeth were about 1-2 mm above the surface of the tray. Before pouring, a drop of ethidium bromide was added to the gel at this point to facilitate visualization of DNA

after electrophoresis. The gel solution was poured into the tray to a depth of about 5 mm and was allowed to solidify in about 20-30 minutes at room temperature.

The DNA samples were prepared during the process of gel solidification. A small piece of Para film was cut and placed on the bench near the gel, 5  $\mu$ l of 6 X loading dye/tracking dye (Final conc. 1X in 50  $\mu$ l sample), was spotted onto the Para film for each sample to be loaded onto the gel in order to increase the sample density and to provide a visible marker to monitor the progress of electrophoresis. 5 $\mu$ l of PCR mixture was drawn into a pipette tip, and was pipetted up and down onto a spot of loading dye to mix. On data sheet was recorded where to load the dye samples (1-13) wells. A 100 bp DNA ladder was loaded into the last well and the numbered dye samples were loaded into the first 12 wells. The gel was placed in the electrophoresis chamber filled with sufficient 1X TBE buffer such that the level of the liquid just covered the gel. The electrophoresis was carried out at a constant voltage of 100V for 40 minutes. Following electrophoresis the gel was visualized and photographed on Hoefer, Macro Vue UV-25 ultra violet transilluminator (Figure 3.4).



**Figure 3.4: High Performance Ultra Violet Transilluminator**

**Table 3.1 The Generic Primers Used To Identify Positive Samples**

Name of primer	Sequence 5_ to 3_
Tryp 1	AAGCCAAGTCATCCATCG
Tryp 2	TAGAGGAAGCAAAAG
Tryp 3	TGCAATTATTGGTCGCGC
Tryp 4	CTTTGCTGCGTTCTT

### Identification of Trypanosome Using Species-Specific Primers

Species specific identification of trypanosomes was carried out by using the method described by Simon *et al.*, (1994) with few modifications. The PCR reaction was carried out in a total volume of 20µl PCR mix containing 2.0µl of the genomic DNA of an individual pig blood and tsetse fly sample; 2.0µl of 10 X PCR buffer (Promega, USA); 1.0µl of 3mM MgCl<sub>2</sub> (Sigma-Genosys, UK); 1.6µl of 2.0mM of mixed dNTPs (Bioline, UK); 0.25µl each of the individual species specific primers with a concentration of 20 µM, 0.1µl of 5U/µl *Taq* DNA polymerase (Promega, USA).

The cycling conditions were as follows: a 5minutes denaturation step at a temperature of 94<sup>0</sup>C followed by 40 cycles of 30 seconds at 94<sup>0</sup>C, 30 seconds at 53<sup>0</sup>C for *T. vivax*, 30 seconds at 60<sup>0</sup> C for *T. congolense savannah*, *T.congolense forest* and *T. simiae*, 30 seconds at 55<sup>0</sup> C for *T.brucei* and 1min at 72<sup>0</sup>C and a final extension step of 10min at 72<sup>0</sup>C. PCR reactions were carried out in 0.2ml eppendorf tubes using Techne, TC 412 and Bio-rad C1000 thermal cycler. Following the PCR cycles, 5µl of PCR product from each reaction was then analyzed by 2 % of agarose gel electrophoresis stained with ethidium bromide. The electrophoresis was carried out at a constant voltage of 100V for 40 minutes. PCR products were visualized under UV illumination and documented by photography.

**Table 3.2 Species Specific Primers for the Identification of Specific Trypanosomes.**

<i>Trypanosome species</i>	Primer	Sequence 5_ to _3	Length of product (bp)	References
<i>T. congolense savannah</i>	TCS1	CGAGAACGGGCACTTTGCGA	369	Masiga <i>et al.</i> (1992)
	TCS2	GGACAAACAAATCCCGCACA		
<i>T. congolense forest</i>	TCF1	GTGCCCAAATTTGAAGTGAT	350	Masiga <i>et al.</i> (1992)
	TCF2	ACTCAAATCGTGACCTCG		
<i>T. simiae</i>	TSM1	CCGGTCAAAAACGCATT	437	Masiga <i>et al.</i> (1992)
	TSM2	AGTCGCCCCGGAGTCGAT		
<i>T. brucei gambiense</i>	TBR1	CAATATTAACAATGCGCAG	450	Moser <i>et al.</i> (1989)
	TBR2	CCATTTATTAGCTTTGTTGC		
<i>T. vivax W/A</i>	TVWA	GTGCTCCATGTGCCACGTTG	175	Masiga <i>et al.</i> (1994)
	TVWB	CATATGGTCTGGGAGCGGGT		

NB: *T. congolense forest* = TCF, *T. congolense savannah* = TCS, *T. simiae* = TSM, *T. brucei gambiense* = TBR, *T. vivax West African type* = TVW, 1 and A =Forward primer, 2 and B = Reverse primer.

### **3.5 Data Processing and Analysis**

An initial exploratory analysis was carried out to obtain descriptive statistics in the form of frequencies and percentages using appropriate tables and charts. Data on individual animals, entomological and parasitological examination results was uploaded into Ms-excel spread sheet to create a data base and analysed using SPSS software version 17.0. Chi-square analysis was done to determine prevalence on sex, age and body condition basis. Two sample t-tests were utilized to compare the mean PCV values of the parasitaemic and aparasitaemic animals. A 95% confidence interval and 5% absolute precision was used to determine whether there was significant difference between measured parameters ( $P < 0.05$ ).

## CHAPTER FOUR

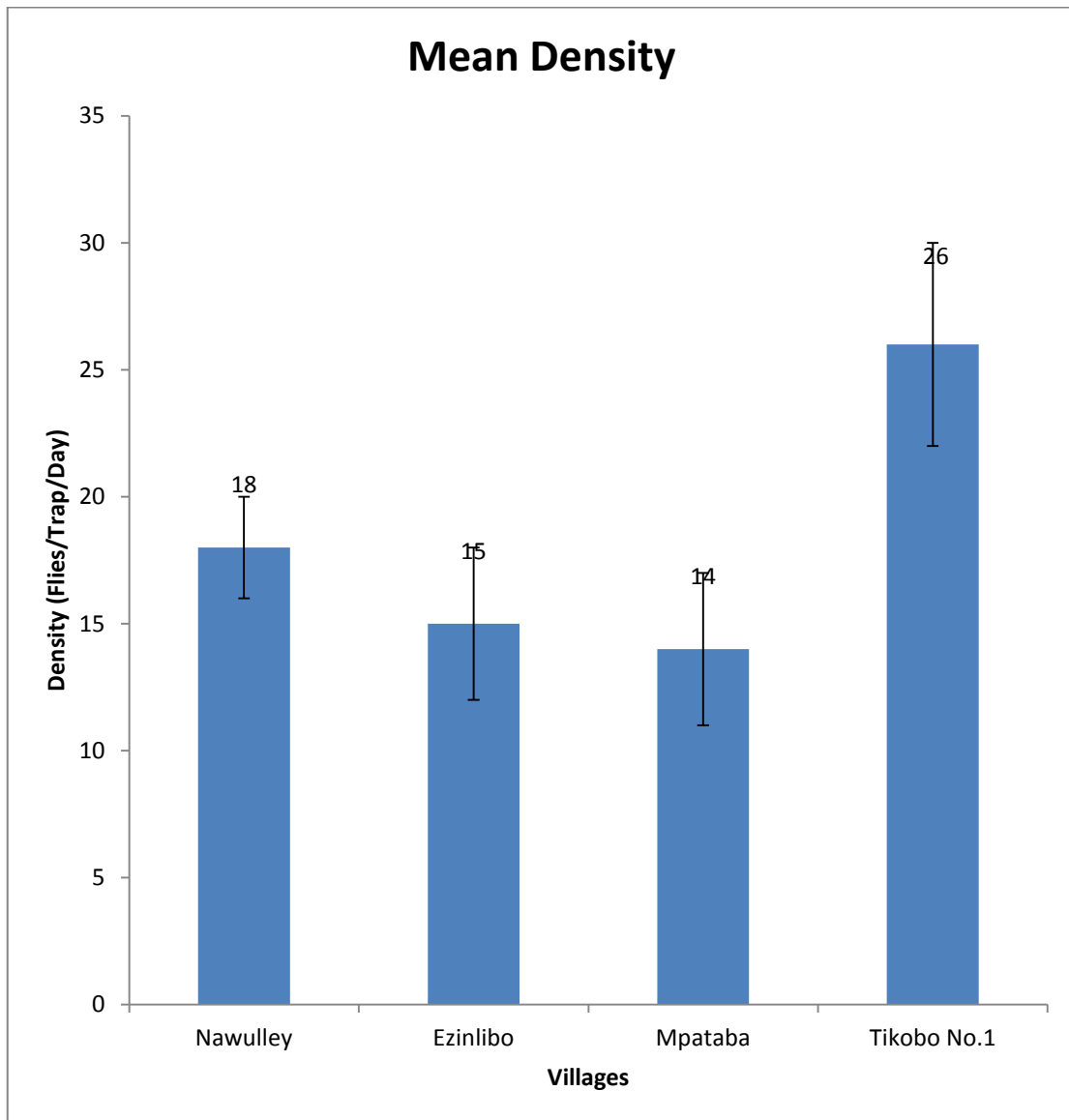
### 4.0 RESULTS

#### 4.1 Entomological Findings

A total of 1,210 Tsetse and other biting flies were caught during the study period from different study sites. Out of the total, 1,175 (97%) belonged to tsetse fly of the genus *Glossina*, and the remaining 35 (3%) belonging to the genus *Tabanus*. Of the *Glossina* genus collected, only *Glossina palpalis palpalis* was identified in the survey site with the overall apparent density of 18.36 F/T/D (fly/trap/day). The highest fly density was observed in Tikobo No.1 [418 flies (26.13 F/T/D)] and the lowest recorded in Mpataba [228 flies (14.25F/T/D)] (Table 4.1). Despite the highest fly density recorded in Tikobo No.1 and the lowest in Mpataba, no significant differences in the mean fly density were observed among the selected villages ( $P > 0.05$ ) (Figure 4.1). From the total tsetse fly trapped, females represented the larger proportion. Out of a total of 1175 tsetse flies captured, 466 (39.65%) flies were males and the rest 709 (60.35%) flies were females. The difference observed between both sexes was statistically significant ( $P < 0.05$ ) (Table 4.1).

**Table 4.1: Total Flies (*Glossina palpalis palpalis*) Catch and Their Apparent Densities in Various Study Sites in the Jomoro District.**

<b>Village</b>	<b>Total No of Tsetse flies</b>	<b>Females</b>	<b>Males</b>	<b>Tsetse Apparent Density</b>	<b>Other Biting Flies (Tabanus)</b>
Nawulley	291	168	123	18.18	12
Ezinlibo	238	138	101	14.87	0
Mpataba	228	132	96	14.25	7
Tikobo					
No 1	418	272	146	26.13	16
<b>Total</b>	<b>1175</b>	<b>709</b>	<b>466</b>	<b>18.36</b>	<b>35</b>



**Figure 4.1: Mean Density of Tsetse Flies in Different Areas of the Survey Sites in the Jomoro District.**

## 4.2 Laboratory Analysis of Blood Samples Taken From Pigs

### 4.2.1 Haematological Findings

The analysis of Packed Cell Volume (PCV) values in the animals examined for trypanosome infection ranged from 20% to 48%. The results showed that the mean PCV value for the parasitaemic pigs was 29.33% whilst the mean PCV value for the aparasitaemic pigs was 37.75% and pigs having PCV < 32% (anaemic) was 65 (21.7%) whilst in the pigs having PCV  $\geq$  32% (non-anaemic) was 235 (78.3%) and the differences were highly significant  $P < 0.05$  as indicated in Table 4.2.

**Table 4.2: Comparison of Mean PCV between Infected and Non-Infected Pigs**

Condition	No. Examined	Mean PCV	S E	T - Test	P Value
Infected	13	29.33	1.71		
				4.85	0.003
Non-Infected	287	37.75	0.25		
Total	300	34.2	0.27		

#### 4.2.2 Parasitological Findings

A total of 300 pig blood samples were collected during the study period from different study sites. Out of the total, 127 (42.33%) were males whilst the remaining 173 (57.67%) were females as indicated in Table 4.3. The results obtained by microscopic examination of the Giemsa-stained Thin and Thick blood smears from the Vector Genetics Laboratory at the Ghana Atomic Energy commission (GAEC) with the optimization of the protocol was the same as a confirmatory test obtained from the Department of Animal Biology and Conservation Sciences (DABCS) and the Noguchi Memorial Institute For Medical Research (NMIMR). No positive results came out of the microscopic examination from all the total blood samples using both Thin and Thick blood films (Table 4.4).

**Table 4.3: Total Number of Pigs Sampled in Different Areas of survey sites in the Jomoro District.**

Village	No of Pigs		
	Sampled	Male	Female
Nawulley	96	40	56
Ezinlibo	77	38	39
Mpataba	60	26	34
Tikobo No.1	67	23	44
Total	300	127	173

**Table 4.4: Comparison of Results Between Microscopic Examination and Polymerase Chain Reaction (PCR) For the Detection of Trypanosome Species in Pigs.**

<b>Test Performed</b>	<b>No. Examined</b>	<b>No. of Positives</b>	<b>Prevalence (%)</b>	<b>Percentage Efficiency</b>
Microscopic Examination	300	0	0	0
PCR Examination	300	13	4.33	100

#### **4.2.3 Molecular Findings from the Pig Blood Samples.**

In all, three hundred (300) blood samples were analysed using PCR technique to detect the presence of trypanosomes, out of which 13 tested positive giving an overall prevalence rate of 4.33% when generic primers (Table 3.2) were used (Table 4.4). Only three out of the five species specific primers (Table 3.3) were able to identify specific species of trypanosomes of the DNA positives from the pig blood samples as *T. congolense* Forest, *T. vivax* and *T. brucei gambiense*.

The predominant trypanosomes species were the mixed infection of *T. congolense* Forest and *T. vivax* six (46.15%) followed by *T. vivax* two (15.38%). A single infection of *T. congolense* Forest and a mixed infection among *T. congolense* Forest, *T. vivax* and *T. brucei* were least recorded, one (7.70%). Three (3) out of the thirteen (13) DNA samples which were identified as trypanosome positives by generic primers could not be identified by the species specific primers (Table 4.5).

**Table 4.5: Trypanosome Species Identified From Pig Blood Samples from Different Survey Sites in Jomoro District Using PCR.**

Trypanosome species	No of positive	Prevalence (%)
<i>T. CF</i>	1	7.70
<i>T. BR</i>	0	0.00
<i>T. V</i>	2	15.38
<i>T. CF + TV</i>	6	46.15
<i>T. CF + TV + T. BR</i>	1	7.70
UN Identified	3	23.07

*T C F* = *Trypanosoma congolense* Forest, *T BR* = *Trypanosoma brucei*, *T V* = *Trypanosoma vivax*.

The prevalence of trypanosomes within the four villages during study period was 6.5%, 5.0%, 4.5% and 2.1% at Ezinlibo, Mpataba, Tikobo No.1 and Nawulley, respectively. Despite the occurrence of highest prevalence in Ezinlibo (6.5%) and the lowest in Nawulley (2.1%), significant difference ( $P = 0.5852$ ) was not observed (Figure 4.2).

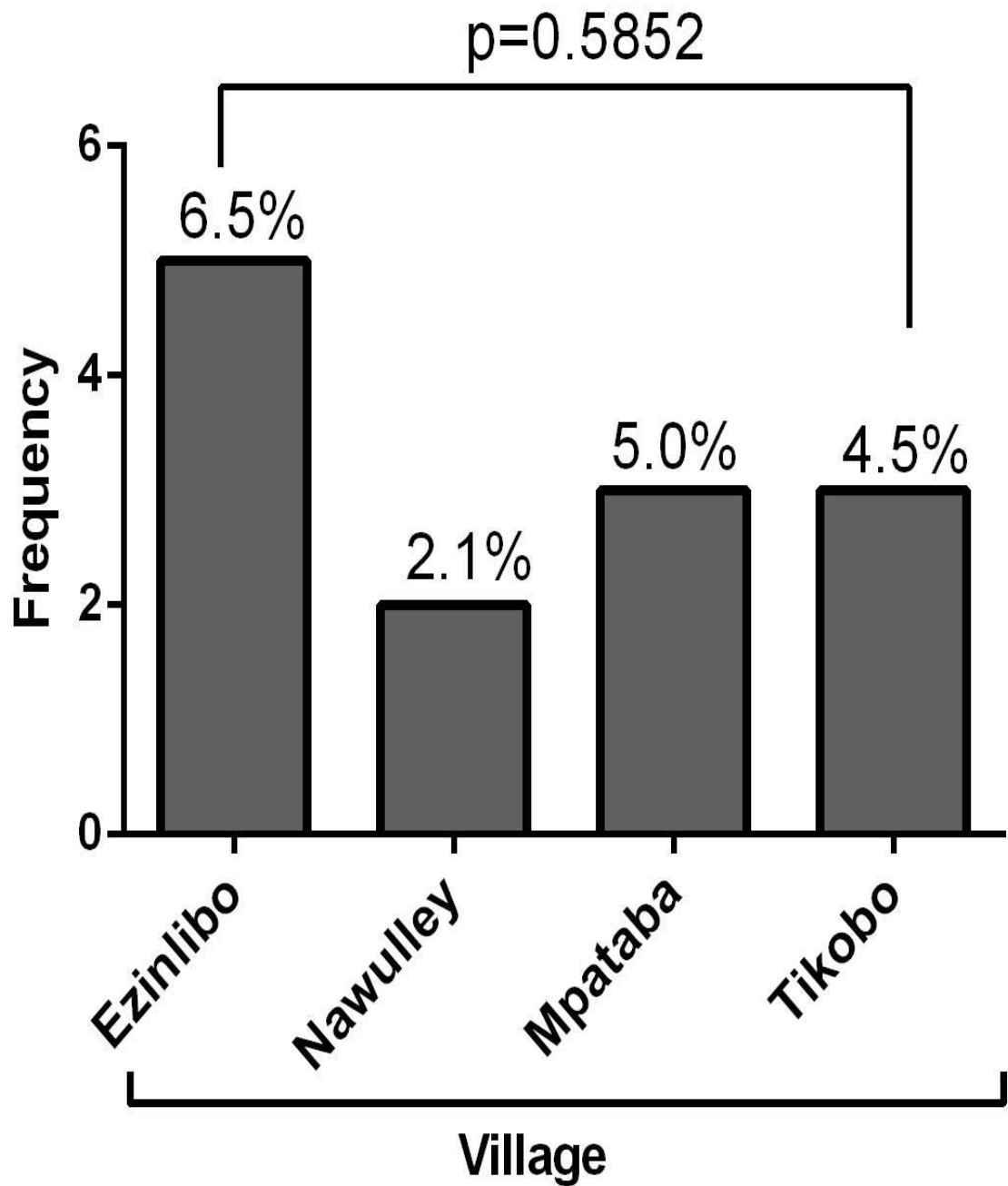


Figure 4.2: Trypanosome Prevalence from Different Study Sites.

The overall prevalence rates of trypanosome infection in the different sexes, ages and body condition score are shown in Table 4.6. In the present study, the rate of trypanosome infection in pigs was higher in the males (6.3%) than their female (2.9%) counterparts but the association was not statistically significant ( $P > 0.05$ ). The differences may be due to chance. Similarly, there was no significant association in the prevalence of trypanosome infection between young (4.9%) and adult (3.2%) animals. With respect to body condition score, the prevalence was 1.9% and 20%, in good and poor body condition respectively with a significant variation ( $P < 0.05$ ) between them.

**Table 4.6: Trypanosome Prevalence in Relation to Age, Sex and Body Conditions of the Pigs.**

Parameter	Total examined	Positive cases	P values
	(n=300)	(n=13)	
<b>Age</b>			
young (<1year)	205(68.3)	10(4.9)	0.7611
adult (>1 year)	95(31.7)	3(3.2)	
<b>Sex</b>			
male	127(42.3)	8(6.3)	0.2521
female	173(57.7)	5(2.9)	
<b>Body condition</b>			
good	260(86.7)	5(1.9)	0.001
poor	40(13.3)	8(20)	

Data is presented as frequencies and percentages. Proportions were compared using chi square and Fishers exact test where necessary.

### 4.2.3 Molecular Findings from the Tsetse Fly Samples.

Molecular analysis of trypanosomes from tsetse flies using generic primers gave a total of 2 out 300 flies representing 0.77% for trypanosomes (Table 4.7). Five of the species specific primers (Table 3.3) were used for the identification of specific trypanosome species; two identified only one of the two tsetse fly DNA positives as mixed infection between *Trypanosoma congolense* Forest and *Trypanosoma brucei gambiense*. The remaining one (1) DNA positive sample could not be identified by any of the species specific primers that were used (Table 4.8). The sexes of the flies that tested positive for the presence of trypanosomes were one (1) female and one (1) male from Nawulley and Tikobo No.1 respectively.

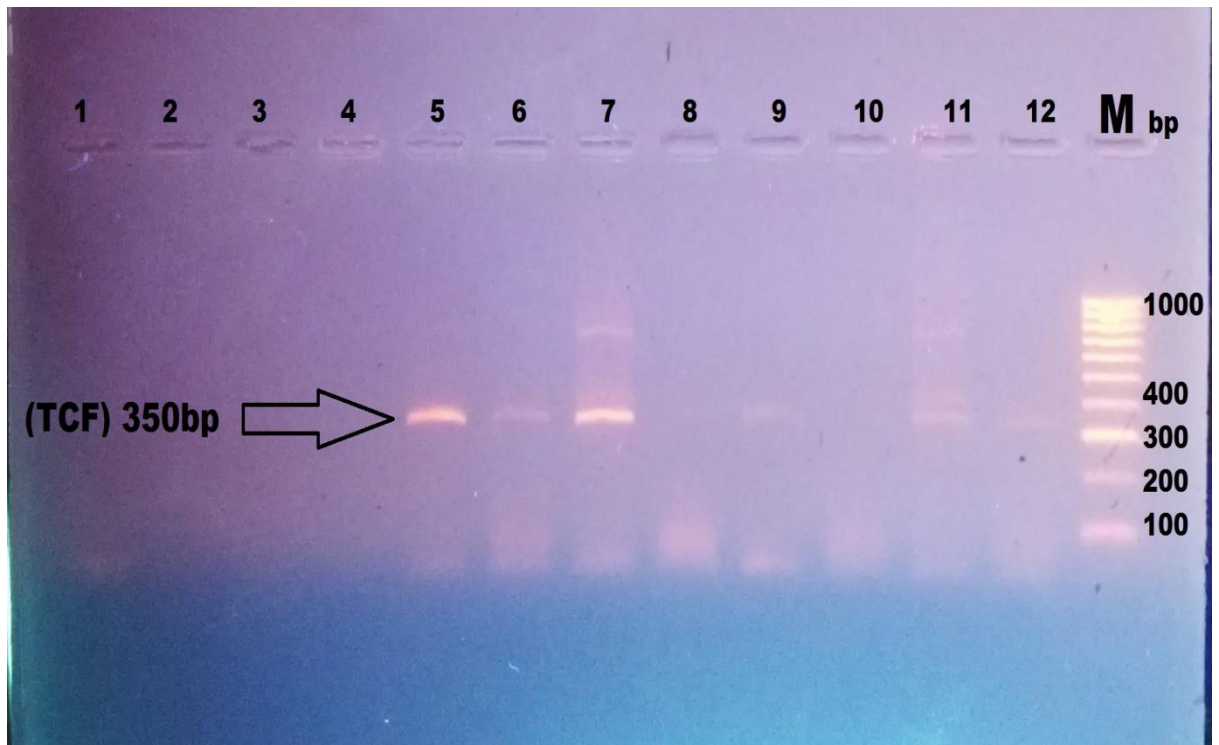
**Table 4.7: Trypanosome Prevalence in Pigs and Wild Tsetse Flies Using Molecular Techniques.**

Host	No. Examined	No. of Positives	Prevalence (%)	$\chi^2$	P Value
Pig	300	13	4.33		
				8.067	0.005
Tsetse					
Fly	300	2	0.70		

**Table 4.8: Trypanosome Species Identified From Tsetse Fly Samples from Different Survey Sites in Jomoro District.**

<b>Trypanosome Species</b>	<b>No. of Positive</b>	<b>%of Positive</b>
<i>T C F</i>	0	0
<i>T B R</i>	0	0
<i>T V</i>	0	0
<i>T C F + T V</i>	0	0
<i>T C F + T V + T B R</i>	0	0
<i>T C F + T B R</i>	1	50
<i>Un- Identified</i>	1	50

*T C F* = *Trypanosoma congolense* Forest, *T B R* = *Trypanosoma brucei*, *T V* = *Trypanosoma vivax*.



**Figure 4.3: *T. congolense* Forest Identified Using Species Specific Primer from Pig DNA on an Ethidium Bromide Stained Gel Electrophoresis.**

## CHAPTER FIVE

### 5.0 DISCUSSION

The present study assessed the prevalence and species of trypanosomes in pigs and tsetse fly species in the Jomoro District of the Western Region of Ghana. The ecological parameters such as availability of food sources, relative humidity, and vegetation type and vector control interventions greatly influenced the prevalence and incidence of the vector and its parasite. Fly apparent density alone does not constitute trypanosome challenge to be used to measure infection rates. This will involve estimation of the fly infection rates and blood meal analysis. However, the study could not estimate these factors due to logistical and time constraints.

The level of infection rate is determined by the density of tsetse fly population in the area and the level of their contact with the host. This is further influenced by the vectorial capacity of the fly and availability of its preferred host, which is not necessarily domestic livestock (Radostits *et al.*, 2007). The average tsetse fly density in the area was 18.36 F/T/D. In Ghana, Afakye (2012) reports 14.46 F/T/D in the New Juaben municipality of the Eastern Region of Ghana as against 18.36 F/T/D in this present in the Jomoro District of the Western Region of Ghana. Such increment could possibly be attributed to favourable environmental conditions such as enough moisture, vegetation growth, and suitable habitat during the study period. Moreover, in the present study, there were no significant variations in the tsetse fly apparent density among the selected villages. This could be due to the fact that the selected villages were close to each other in such a way that both climatic and environmental factors that have influence on tsetse fly population density from one study location to another may not be very different.

Only *Glossina palpalis palpalis* was observed and the only mechanical transmitters of trypanosomiasis found in the study area was *Tabanus*. The result of the tsetse fly species identified agrees with earlier findings by Combey (2014) who observed only *G. palpalis palpalis* in the survey area. According to Mahama, *et al.*, (2003), the Palpalis group of tsetse flies has for many years exhibited greater tenacity to their environment and still survive and transmit animal trypanosomes in areas where the other groups of tsetse flies have disappeared.

Similarly, *Glossina palpalis palpalis* was found to be the only tsetse fly species in the New Juaben municipality of the Eastern Region of Ghana by Afakye (2012). He assessed the situation of human and porcine trypanosomiasis and the tsetse fly vector in the New Juaben municipality of Eastern Region. This result is also in agreement with the findings of Grebaut *et al.* (2004) in the forest zone of Southern Cameroun. In their study, 99% of the 4,559 tsetse flies captured were *G. palpalis palpalis* and 1% comprised *G. nigrofusca nigrofusca* and *G. fusca fusca*. These tsetse fly species were also formerly identified in the forest zones of Ghana as reported by earlier authors (Offori, 1964) but *G. nigrofusca nigrofusca* and *G. fusca fusca* were not identified in this present study. This suggests that human modification of the environment is more favourable to opportunistic species such as *G. palpalis* group than to other *Glossina* species, such as *G. fusca*, whose ecological requirements and feeding patterns in particular are more narrowly defined (Pollock, 1986).

Sex ratio of the flies was assessed in this study and higher numbers of female flies were recorded. The presence of high number females might result in high population density

which is an indicative for future high infection rate. This finding confirms earlier findings by Combey (2014), who assessed the distribution of trypanosomes and characterized available species of tsetse flies in three ecological zones in Ghana. Similar results have been reported by Msangi (1999), Mohammed-Ahemed and Dairri (1987) and Leak (1999) which showed that in an unbiased sampling, female would comprise between 70 to 80% of the mean population. The high proportion of female is most probably attributed to the fact that they live longer (mean female fly life span being eight weeks, but only four weeks in males); hence more females could be caught as reported by Leak *et al* (1987) and Terzu (2004).

In order to understand the epidemiology of trypanosomiasis, the various species of trypanosomes that infect tsetse fly have to be identified accurately using methods that are highly specific and sensitive. The classical technique for identification of trypanosomes relies on dissection and microscopical examination of the potentially infected tsetse organs such as proboscis, midgut and salivary glands where the parasites will be located (Lloyd and Johnson, 1924). The method is labour intensive and the detection requires large amount of trypanosomes. Moreover, visual examination does not allow the identification of parasites below the subgenus level and cannot detect immature and mixed infections. Molecular biology methods have to a larger extent overcome the limitations of the old methods (Masiga *et al.*, 1992).

The present study revealed an overall infection rate of 0.77% when generic and species specific primers were used to identify trypanosome from extracted DNA from entire flies. The results are in agreement with the previous finding by Combey, 2014 which was 0.9%. This work characterized trypanosome species from wild tsetse flies using molecular techniques from three ecological zones in Ghana including the present study

area. Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 – 20% of the flies, each is infected for life, and their presence in any number makes the rearing of pigs and other livestock extremely difficult (Urquhart et al., 1987). The similarity of the prevalence between these studies might be ascribed to the fact that the flies are there in their numbers but most of them do not carry the trypanosome parasites. According to Jordan, (1974), infection rates of trypanosomes are usually low in tsetse fly populations with infestation rates being influenced by factors such as the parasite species, vector species, host and the environment. Moreover, there are a number of barriers to both establishment and maturation process of trypanosomes and thus only a small proportion of these infections reach maturity (Maudlin and Welburn, 1989). It is also possible that these trypanosomes may be held back by the fly's immune response (Askoy, 2003). These could possibly be the factors that accounted for the low infection rate of trypanosomes from the wild tsetse flies recorded in this work.

The present study also revealed the infection rate to be equal between male and female flies when the same number of the sexes were compared. This could probably mean that both male and female flies have equal chances of picking up the parasites from the vertebrate hosts since both are blood feeders. Samdi *et al.*, (2011) in Nigeria, reported higher infection rates in males than females. Mihok *et al.*, (1992) suggests that female flies have higher infection rates than males as they live longer and thus have higher chances of getting infection. However, this relationship has not been established. In contrast, Marlene and Mckean, (1996) explains that more males may be infected than females as they are involved in sex activities and competition than females. Whether more males or females are infected in field tsetse populations, this study found no statistical difference with variation in sex.

The current study also revealed that 50% (n=1) of the total tsetse DNA samples that were positive for various trypanosomes using generic primers were identified by species specific primers. A single mixed infection between *T. congolense* Forest and *T. brucei gambiense* was identified while the remaining 50% (n=1) could not be identified. This finding is in line with previous reports by other authors (Combey, 2014; Solano *et al.*, 1995; Morlaise *et al.*, 1998). Much lower rates (13%) of unidentified species were also recorded by Lehane *et al.*, (2000). However higher identification level of 80% was recorded by Lefrancois *et al.*, (1998) from wild tsetse flies from Burkina Faso. Malele *et al.*, (2003) observed that high number of unidentified species of trypanosomes may be due to the fact that a high proportion of the samples may have contained unknown trypanosome species that could not be identified by the current range of species-specific primers that were used for the PCR analysis. Degradation of target DNA may also account for the failure of some samples to be amplified efficiently.

Mixed infections found in the tsetse could have occurred through several possible sources. Firstly, the tsetse may have fed on a host infected with only Forest-type *T. congolense* and subsequently on another host infected with only *T. brucei gambiense*. This would have provided a midgut infection, which may later have developed into a mature infection consisting of a mixture of the 2 genotypic groups. Alternatively, the tsetse may have fed on a host with a pre-established mixed infection consisting of both Forest- type *T. congolense* and *T. brucei gambiense* (Nakayima *et al.*, 2012).

From the results, it is evident that PCR is more sensitive than the microscopic examination of blood for diagnosis of trypanosomiasis in pigs as a higher percentage of positive cases (4.33%) were detected as compared to microscopic examination, which

revealed a positive percentage of (0%). These findings are in line with (Basagoudanavar *et al.*, 1998; Nahla *et al.*, 2011).

There are several factors that may contribute to this outcome of low sensitivity of the microscopic examination. One major factor is that the time interval between the blood collection and examination was too long that is some few days were elapsed before the blood samples were examined. Trypanosomes are rapidly killed by direct sunlight but can survive for a few hours (4-6 hours) after collection when the sample is kept in a cool and dark place. It is therefore essential to keep the time between sampling and examination as short as possible to avoid immobilization and subsequent lysis of trypanosomes in the blood samples (Thumbi *et al.*, 2008).

Another important factor that might have influenced this outcome is the stage of parasite infection. Trypanosomiasis that can be encountered in the field can be in acute or chronic form. In the acute phase, the usual clinical signs in natural hosts are intermittent fever accompanied by accumulation of parasites in the blood (parasitaemia) (Luckins, 1998). However, as the disease progresses to the chronic phase the number of parasites in the blood disappears. The low levels of parasitaemia prevent parasitological diagnosis of trypanosomes (Soulsby, 1982). Experimentally, groups of mice were infected with *T. evansi*, isolated from a naturally infected local camel and blood samples were collected every day for 30 days post-infection. Direct microscopy and PCR were applied to detect the level of parasitaemia by Ijaz *et al.* (1998). Results showed that during the acute phase of infection, parasites were detected by PCR three days earlier than by microscopy. Furthermore, the infected mice were consistently positive by PCR during the chronic phase while the parasites could not be demonstrated during this period using microscopic examination. These findings suggested that PCR may be applied to pig

trypanosomiasis during both acute and chronic phase of the infection. Nantulya (1990) and Desquesnes (2004) stated that detection of trypanosomes is unsuccessful when the numbers of parasites are too low, as is the case with chronic infections. Furthermore, PCR amplification allowed identification of immature infections and accurate diagnosis of four mixed infections, which could not be detected by parasitological examination. These might be the probable reasons why no trypanosomes were detected in the blood smear of 300 pigs in the present study.

The overall prevalence of pig trypanosomiasis detected by PCR in the study area was 4.33%, which is lower than previous reports: 28.6% in the Koforidua and Adidome areas in the southern part of Ghana (Nakayima *et al.* 2012) and 56% in the New Juaben municipality of the Eastern Region of Ghana (Afakye, 2012). The prevalence of trypanosome infection according to Solano *et al.*, (1995), Mahama *et al.*, (2003) and others is generally variable and can be reduced to less than 10% of the original level following rigorous application of control methods, which includes selective bush clearing and chemotherapy. This could possibly be the factors that accounted for the low infection rate of trypanosomes from the pigs and wild tsetse flies recorded in this work.

The overall prevalence recorded in the present study is in line with the findings of Teka *et al* (2012) who also recorded an overall infection rate of 4.43% in a study conducted at Fura and Eligo villages of Gamo Gofa Zone in the Southern rift valley of Ethiopia. The similarity of the prevalence between these studies might be comparably attributed to the similarities in the environmental and the climatic conditions such as temperature, rainfall, relative humidity and the vegetation type of the two study sites.

Among the different spp. of trypanosomes recorded during the study, *T. Congolense* causing mixed infection with *T.vivax* six (46.15%) was the most predominant trypanosome species followed by *T. vivax* two (15.38%) and one (7.70%) for *T. congolense*. Mixed infection of *T. Congolense*, *T.vivax* and *T.b gambiense* also accounted for one (7.70%) of the trypanosome species identified. The mixed infections with two or three different *Trypanosoma* species were commonly observed among the studied animals. This molecular epidemiological work confirmed the abundance of mixed infections in the field, which could not have been detected by the classical parasitological methods. The presence of mixed infection was also consistent with the findings of Mahama *et al.* (2004) and Nakayima *et al.* (2012) who carried out molecular epidemiological studies on animal trypanosomiasis in Ghana. Mixed infections could be a result of high chances of trypanosome infections by tsetse flies and/or a case of chronic infection in susceptible hosts. This study showed higher infection of *T. congolense* and *T. vivax*. Such observation could possibly be attributed to the presence of major cyclical vectors such as *Glossina* species (*palpalis*) and other biting flies (*Tabanus*) in the area. Since the transmission of *T.congolense* is cyclical, it requires the presence of tsetse flies, whereas the transmission of *T.vivax* is mechanically by biting flies according to Abebe (2005).

The results also indicate that three (23.07%) out of the total number of positives detected by generic primers could not be identified by species specific primers. This finding is in agreement with Lefrancois *et al.*, (1998) who recorded 20% unidentified trypanosome species from wild tsetse flies from Burkina Faso. However higher identification level of

87% was recorded by Lehane *et al.*, (2000). In this study, two out of the five species-specific primers used could not identify any trypanosome species from the total blood samples examined. This could possibly mean that those species are not found in the present study area.

For PCR success, an essential amplification condition has to be fulfilled thus the use of suitable primers. So far, specific primers are not available for all trypanosome species that infect wild tsetse and pigs, just those for trypanosomes of medical or veterinary importance exist and it is possible that other trypanosome species can exist in the study area. There could be another factor such as new mutations or variants in the system. (Adams *et al.*, 2010).

In the present study, there was no significant difference ( $p > 0.05$ ) in the prevalence of trypanosome infection in all the four study sites. These may be because the climatic and agro ecological conditions which influence tsetse fly distributions in the selected villages may be homogeneous. The occurrence of trypanosomiasis frequently corresponds with the fly density (occurrence of the vectors) which is in turn dependent on those climatic factors such as temperature, humidity and vegetation coverage of the area (Leak, 1999; Ababayehu, 2011).

In the present finding, most of the trypanosome infected pigs were animals less than 1 year old ( $n=205$ ) young animals compared with that of adult animals ( $n=95$ )  $\geq 1$  year old. Significant difference was not observed in the prevalence rate of infection between age groups. This variation could be due to the fact that young animals show greater susceptibility to infection than sexually mature ones that have development of better immune system to respond to various infections. Onset of increased resistance often

coincides with development of maturity due to changing hormonal balance and the immune system (Taylor, 1998). A similar proportional trend was reported by Teka *et al.* (2012) who conducted a cross-sectional study aimed at determining the prevalence of bovine trypanosomiasis and apparent tsetse density in two selected villages of Arba Minch Woreda in Ethiopia. However, the pattern of trypanosome prevalence in different age groups, observed in this study is not consistent with the findings of Desquesnes *et al.* (1999) who argued that older animals had more chances to be exposed to tsetse fly bites than younger ones.

Higher infection rates were observed in male animals than females in the present study but the difference was not significant ( $p > 0.05$ ). Similar findings were reported by Daya and Abebe (2008) and Tadesse and Tsegaye (2010) where they observed no significant difference in trypanosome infection between males and females. This could be due to the similarity in the agro ecological zones of the study locations that supported proliferation of both the tsetse and other biting flies that feed on both sexes of the pigs indiscriminately.

Infection rate in poor body condition animals was significantly ( $P < 0.05$ ) higher than in good body condition animals. This is also in agreement with the reports of (Ali and Bitew, 2011) and (Mekuria and Gadissa, 2011). Abebe and Wolde (2010) also reported significantly higher prevalence of trypanosomiasis in donkeys with poor body condition than with good body condition animals. This is due to the chronic nature of the disease that result in anaemia and depletion of body condition and/or the predisposition of trypanosome infected animals to other concurrent infections that further aggravate the emaciation.

The mean Packed Cell Volume (PCV) value of the studied animals was significantly different ( $P < 0.05$ ), varying between parasitaemic (29.33%) and aparasitaemic (37.75%) animals. The differences observed among the studied animals were not as a result of chance but other factors such as low levels of haemoglobin and red blood cell. Anaemia is one of the most common indicators of trypanosomiasis in livestock (Stephen, 1986). The level of anaemia or PCV usually gives a reliable indication of the disease states and productive performance of infected animals. This implies that those animals with lower PCV than the reference limit ( $< 32\%$ ) in the present study are at risk of contracting other opportunistic infections if left untreated.

This result was consistent with the previous result reported by Afakye (2012) in the New Juaben Municipality of the Eastern Ghana. This is also in agreement with other studies elsewhere (Rowlands *et al.* 1995). These authors in Ghibe observed that with a decrease in the PCV value, the proportion of infected animals increased and hence the mean PCV was a good indicator for the health status of herds in trypanosomiasis endemic areas. The lower mean PCV value of infected animals is reported by several authors (Leak *et al.*, 1987). Similarly, Van den Bossche (2006) reported that the regression analysis of herd average PCV of parasitologically positive herds showed a decrease with the increasing prevalence of trypanosome infection. In addition, cattle and other domestic animals with high prevalence of trypanosomes have low PCV / haematocrit levels (Mahama *et al.*, 2004).

The presence of trypanosome negative animals with mean PCV values of  $< 32\%$  which is below the lowest reference limits for porcine species may be due to inadequate detection method used (Murray *et al.*, 1977) or delayed recovery from anaemic situation after recent treatment with trypanocidal drugs or factors other than trypanosomiasis,

such as nutritional imbalances, helminthiasis, fasciolosis and tick borne diseases. In fact, the difference in mean PCV between parasitaemic and aparasitaemic animals indicated that trypanosomiasis may be involved in adversely lowering the PCV values of infected animals. Therefore, knowing the vector - parasite interaction and having a full understanding of the complex relationships between tsetse flies (*Glossina* spp.), and the trypanosomes that they transmit is crucial in future designing and implementation of control strategies.

## CHAPTER SIX

### 6.0 CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 CONCLUSION

Trypanosome infection in livestock is of considerable economic importance. The existing data regarding the prevalence and epidemiology of trypanosomiasis in pigs as well as the absence of reliable and sensitive diagnostic methods is a setback in the effective control of the disease. This study aimed at providing information on the trypanosome species and their prevalence in pigs and tsetse flies in the Jomoro District of the Western Region of Ghana. Besides, it identifies tsetse species and their apparent densities in the study sites.

The results showed that the overall prevalence of trypanosomes in pigs and tsetse flies were 4.33% and 0.77% respectively by PCR method of diagnosis. However, all the blood samples analyzed using microscopy of stained blood smears were found to be negative for trypanosomes. This could be due to the time interval used to analyse the blood samples which was too long besides the factors mentioned earlier. The different species of trypanosomes identified in the blood samples of the pigs were *T. brucei gambiense*, *T. vivax* and *T. congolense* Forest while in tsetse flies a single mixed infection of *T. brucei gambiense*, and *T. congolense* Forest was recorded. The prevalence of trypanosomes within the four villages during study period was 6.5%, 5.0%, 4.5% and 2.1% at Ezinlibo, Mpataba, Tikobo No.1 and Nawulley, respectively. The difference in results demonstrated that PCR is a more sensitive and reliable method for the diagnosis of trypanosome infection than the parasitological method of examination.

The results showed that *Glossina palpalis* is the only tsetse fly species and *Tabanus* with fly apparent densities of 18.36 F/T/D (fly/trap/day) and 0.55 F/T/D (fly/trap/day) respectively were identified from the study areas. The high number of fly apparent density recorded in this work implies that the District is likely going to experience endemic status of the trypanosome infection in the near future if proper control measures are not put in place to eradicate these flies in the affected communities.

With the presence of the *T. brucei* subspecies where human-infective species are found, *G. palpalis palpalis* which is a good transmitter of human trypanosomiasis and the domestic pig which is potential reservoir for human trypanosomiasis, there is a threat to public health and piggery industry in the district.

In summary, pig trypanosomiasis is an important zoonotic disease and a potential threat affecting the health and productivity of the piggery industry in the district. It has a tremendous negative impact on PCV and body condition of the animals and hence priority should be given to the control of trypanosomiasis by targeting the vector and the parasite through integrated disease management strategy and awareness creation. The results of the present study shows that trypanosomiasis in Ghana is important not only in cattle but also in the pigs where even higher prevalence could be recorded.

## 6.2 RECOMMENDATIONS

Based on the findings of this study in the Jomoro District, the following are recommended:

This study recommends that the PCR technique should be adopted as a routine method for diagnosis of trypanosome infection in livestock in all research centres in Ghana. This implies that all the research centres should be supplied with all the facilities to conduct PCR analysis.

Entomological surveys should be conducted at different seasons to understand seasonal dynamics of the vectors and the associated trypanosomiasis risk. Data on seasonal variations of potential vectors can be integrated into epidemiological models to facilitate better understanding of the relative importance of cyclical and mechanical vectors in the Jomoro District.

The study should be replicated in the remaining 21 districts of the Western Region of Ghana for baseline data, and further research done to include other livestock such as cattle, sheep and goats that are reared in the area.

Ghana Health Service (GHS) and Veterinary Services Directorate (VSD) should intensify health education and awareness creation on Human African Trypanosomiasis and African Animal Trypanosomiasis in the district and the region as a whole.

Laboratories should be well equipped to take up the challenge of human and animal trypanosomiasis in the district.

Continuous community-based tsetse monitoring and more trypanosomiasis surveillance programmes should be instituted in tsetse infested regions such as the Western Region of Ghana.

Attempt should be made to expand government and private veterinary services to serve the district properly.

Since the only *Glossina* species identified in this study was *Glossina palpalis*, therefore, designing and implementing tsetse control should be targeted on the vectors of the Palpalis group of tsetse flies (*G. palpalis palpalis*) rather than controlling the whole species, hence the cost of tsetse control and the time of operation will be reduced.

There should be a further research into the knowledge of the relationship between the prevalence of trypanosome infection and herd average Packed Cell Volume (PCV).

Approaches that simultaneously control several zoonotic diseases should be embraced to avert the impacts on which trypanosome infection exerts on animal health, pig production and public health in the study area.

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## APPENDICES

**Appendix I:** Data on Entomological Survey from Different Study Sites in Jomoro District.

<b>Village</b>	<b>Total No. of flies</b>	<b>Species</b>	<b>GPS</b>	<b>Elevation</b>
Nawulley	291	G p p	N 05.03798 W 002.72401	16 metres
Ezinlibo	238	G p p	N 05.01449 W 002.72064	20 metres
Mpataba	228	G p p	N 05.09570 W 002.59226	20 metres
Tikobo				
No 1	418	G p p	N 05.05396 W 002.70091	19 metres

**Appendix II: Daily Fly Catch from Different Survey Sites in Jomoro District.**

<b>Sampling Site</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Total</b>
Nawulley	96	77	58	60	291
Ezinlibo	90	49	54	45	238
Mpataba	79	28	64	57	228
Tikobo No 1	150	102	84	82	418

**Appendix III: Ages, Sex And Body Conditions Of Pigs Sampled from Different Sites.**

SITE	AGE		SEX		BODY CONDITION	
	YOUNG	ADULT	MALE	FEMALE	GOOD	POOR
NAWULLEY	72	24	39	57	90	6
EZINLIBO	47	30	38	39	67	13
MPATABA	40	20	26	34	50	12
TIKOBONO 1	46	21	24	43	58	9
TOTAL	205	95	127	173	260	40

**Appendix IV: Populations Characteristics of the Jomoro District**

Sex					Type of locality	
Age Group	Both Sexes	Male	Female	Sex Ratio	Urban	Rural
All Ages	150,107	73,561	76,546	96.1	53084	97,023
0 – 14	60,046	30,711	29,335	104.7	20,176	39,870
15 – 64	83,467	40,250	43,217	93.1	30,803	52664
65+	6,594	2,600	3,994	65.1	2,105	4,489
Age-dependency ratio	79.84	82.76	77.12		72.33	84.23

**Source: Ghana Statistical Service, 2010 Population and Housing Census.**

**Appendix V: Preparation of Reagents and Solutions****Preparation of 70% Ethanol**

A 100ml of 70% Ethanol was prepared by adding 30mls distilled water to 70mls of absolute Ethanol.

**Preparation of Cetyl Trimethylammonium bromide (CTAB)**

A 40ml of distilled water was added to 0.5g of CTAB in sterile bottle. The mixture was swirled under hot tap water until CTAB was dissolved. 1M Tris, 5M NaCl, 0.5M EDTA were added to CTAB solution. 100 $\mu$ l of  $\beta$ -mercaptoethanol was then added in a hood.

**Preparation of 0.5M EDTA pH 8.0**

A stock solution of 0.5M EDTA was prepared by dissolving 93.05g of EDTA in 400ml of ddH<sub>2</sub>O. The pH of the solution was adjusted with 5M NaOH to pH 8.0, and the volume was adjusted to 500ml.

**Preparation of Tris Borate EDTA (TBE)**

The 1x stock solution of TBE buffer was prepared by weighing and dissolving 53.99g Trizma-Base, 27.50g Boric acid and 2.92g of EDTA in 900ml of dH<sub>2</sub>O and the solution was adjusted to 1L.

**Preparation of 1L Tris EDTA (TE)**

To prepare 1L of TE-1 buffer; 10ml of 1M Tris and 200ml of 0.5M EDTA, pH 8 were diluted in 790ml of dH<sub>2</sub>O.

**Appendix VI: Parasitological Survey From Mpataba in the Jomoro District.**



**Appendix VII: Entomological Survey From Tikobo No. 1 in the Jomoro District.**

