SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES,
UNIVERSITY OF GHANA

HUMAN AND PORCINE TRYPANOSOMIASIS IN THE NEW JUABEN MUNICIPALITY IN EASTERN REGION OF GHANA

By

KOFI AFAKYE

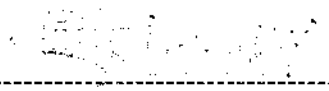
(10325589)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY IN PUBLIC HEALTH (MPHIL APPLIED EPIDEMIOLOGY AND DISEASE CONTROL)

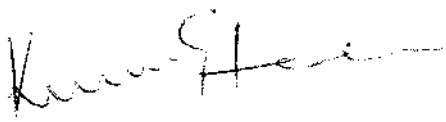
JULY, 2011

DECLARATION

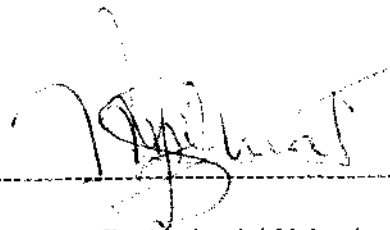
I, Kofi Afakye, do hereby declare that except for references to works done by other investigators which have been duly acknowledged, this thesis is the result of my own original research carried out under supervision, and has not been presented, either in whole or in part, for another degree elsewhere.

CANDIDATE: 

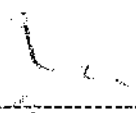
Kofi Afakye

SUPERVISOR: 

Professor George Kwame Aning

SUPERVISOR: 

Dr. Nathaniel Yebuah

SUPERVISOR: 

Dr. George Bonsu

DEDICATION

This piece of work is dedicated to The Lord Almighty, who made everything possible for me. To my mother Mary Adwoa Adanse of blessed memory and my father Louis Kofi Afakye, for their prayers and support. It is also dedicated to my children Maame, Paapa, Nana, Tutu and Tutuwaa for their living prayers, patience and understanding whilst I was away. Again it goes to Florence Iddrisa Nzilanye for her wonderful support in diverse ways.

ACKNOWLEDGEMENT

I appreciate the enormous support and dedication of my supervisors Professor George Kwame Aning, Dr. Nathaniel Yebuah and Dr. George Bonsu. Prof Aning, you are really a father, your patience and encouragement are very much appreciated.

My utmost thanks go to Dr. Mark Hansen, Ag. Director of Veterinary Services, Dr Charles I. Mahama, Head of PATTEC (Ghana), Dr. Martin Abavana, (PATTEC), Dr. Benita Anderson (PATTEC), Dr. Joseph Osafo Duah, Regional Veterinary Officer (Eastern Region), Dr. Andy Sonn, (VSD), Mr. Joseph Otchere, (NMIMR), Mr. Thomas Azurago (GHS), Mr. Gabriel Adukpo, Municipal Director of Agriculture (New Juaben municipality).

I am greatly indebted to Dr. Christine Clark, the Head of Department of Epidemiology, School of Public Health, University of Ghana, Prof. Edwin Afari, Coordinator of the Ghana Field Epidemiology and Laboratory Training Programme (GFELTP), Dr. Fred Wurapa, Director, African Field Epidemiology Network (AFENET), Dr. Chima Ohuabunwo, Resident Director of the (GFELTP), Dr. Samuel Sackey, Senior Lecturer at the Department of Epidemiology, Mr. David Mensah and Mrs. Cynthia Afetsi and all my lecturers at the School of Public Health for their inputs and encouragement in this work.

My special gratitude to my Cohort 3 colleagues of the GFELTP; Dr Sherry Johnson, Dr. William Tasiame, Mr. Brain Worlanyo Pida, Mr. Dodzi Amelor, Dr. Otu Nyarko and Dr. Carl Osei for the friendship and encouragement to get through the programme. Above all, I thank the God Almighty for bringing me this far.

ABSTRACT

Introduction: Trypanosomiasis is a zoonotic, vector-borne parasitic disease caused by species of the genus *Trypanosoma (T.)* and mainly transmitted cyclically by the genus *Glossina (G.)* (Tsetse fly). About 45,000 human cases are reported annually in Africa, and livestock production is decreased by 20-40%. In Ghana, 0.2% sero-prevalence has been reported in humans, and the prevalence in cattle ranges between 5-50%. In 2006, Trypanosome species were detected in pigs in the New Juaben municipality and there was a likelihood of their transmission into humans. We conducted epidemiological studies in Human African Trypanosomiasis and Animal African Trypanosomiasis in the New Juaben municipality to generate information for developing intervention strategies.

Methods: Cross-sectional study was conducted from October 2010 to June 2011 in New Juaben municipality, using simple random sampling to select study participants. Tsetse flies were trapped and blood samples taken from humans and pigs for serology and parasitology respectively. We measured the prevalence of human and porcine Trypanosomiasis, identified the Trypanosome and Tsetse fly species and determined fly apparent densities. Semi-structured questionnaires were used for demographic and behavioural data, and to assess the general knowledge of participants. We performed descriptive statistics, univariate and bivariate analyses using EpiData and SPSS.

Results: Of 352 human-participants 199(56.2%) were females. The dominant age-group was 11-20 years and majority (43.8%) was farmers. Participants' knowledge of Trypanosomiasis was good [269(76.4%)]. Most [128(37.5%)] of the 341 pigs were

weaners, and all 2,141 Tsetse-flies caught were identified as *Glossina palpalis palpalis* with fly apparent-density of 14.46 *Glossina*/trap/day.

Sero-prevalence in humans was 0.7%. In pigs, overall prevalence was 56.0% with *T. vivax, T. congolense* and *T. brucei* groups being the species identified. The biotope and sub-municipality in which pigs are reared were significant risk factors (95% CI:1.239-10.925; OR=3.679) and (95% CI:1.351-10.149; OR=3.703) respectively. However, keeping piglets was protective (95% CI: 0.078-0.514; OR=0.2)

Conclusion: More than half of the pigs are infected with Trypanosomiasis. The presence of *T. brucei* group and domestic pigs as potential reservoir poses great threat to public health in the municipality. We recommend intensification of health and veterinary education, and integrated control of Tsetse and Trypanosomiasis in Eastern Region.

Table of Content

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAT – Africa Animal Trypanosomiasis

CATT – Card Agglutination Test for Trypanosomiasis

CSF – Cerebro-spinal Fluid

DALY – Disability Adjusted Life Years

DNA – Deoxyribonucleic Acid

ELISA – Enzyme-linked Immunosorbent Assay

FAD- Fly Apparent Density

FAO – Food and Agricultural Organisation

FTA – Flinders Technical Associates

GHS – Ghana Health Service

GPS – Global Positioning System

HAT – Human African Trypanosomiasis

HCT – Haematocrit Centrifugation Technique

IFAT – Indirect Flourescent Antibody Test

OAU – Organisation of African Unity

OIE – International Organisation of Epizootics

PATTEC – Pan-African Tsetse and Trypanosomiasis Eradication Campaign

TTCU – Tsetse and Trypanosomiasis Control Unit

VSG – Variable Surface Glycoprotein

VSD – Veterinary Services Directorate

WHO – World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Trypanosomiasis is a vector-borne parasitic disease complex caused by several species of protozoan parasites of the genus *Trypanosoma (T.)* in many parts of the tropics and sub-tropics. In Africa, Trypanosomes are mainly transmitted cyclically by the tsetse fly genus *Glossina (G.),* but also transmitted mechanically by other biting flies (tabanids, stomoxys and others) (OIE, 2008).

Tsetse-transmitted Trypanosomiasis affects both man and animals, causing Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT), respectively in these hosts. WHO (1998), has commented on the complex nature of the epidemiology of the human disease arising from two species of the infecting Trypanosome in various parts of Africa. In East and Southern Africa, HAT, described as Rhodesian sleeping sickness is caused by *Trypanosoma brucei (T. b.) rhodesiense,* which runs an acute disease course leading to death of infected persons within few weeks or months (WHO, 1998). In West and Central Africa, HAT is caused by *T. b. gambiense* and is transmitted principally by Tsetse flies, *Glossina palpalis* and *G. tachinoides.*

Animal African Trypanosomiasis is mainly caused by *T. congolense, T. vivax* and, to a lesser extent, *T. brucei brucei* with *G. morsitans, G. palpalis* and *G. fusca* groups as primary vectors. It is found in the Tsetse fly-infested area of Africa which extends from the southern edge of the Sahara desert (lat. 15° N.) to Angola, Zimbabwe, and

Mozambique (lat. 20° S.) (Roder *et al.*,1984). Livestock development in this area is impeded by the effect of Trypanosomiasis. Control of AAT is through prophylactic and curative chemotherapy, control of the vector and use of trypanotolerant livestock (Mahama *et al.*, 2003).

The three AAT Trypanosomes are considered to be nonpathogenic for humans. *Trypanosoma brucei brucei*, although not causing human disease, is closely related to *T. b. gambiense* and *T. b. rhodesiense* which are the causes of human sleeping sickness. In East and Southern Africa, *T. b. rhodesiense* does not only cause human sleeping sickness but, also infects cattle, bushbuck (*Tragelaphus scriptus*), and probably many other wild animals that may serve as reservoirs of the parasite. Similarly, in West and Central Africa, humans are major hosts of *T. b. gambiense* but it also infects pigs (WHO, 1998). 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 Ghana, more attention is given to cattle as reservoir of Trypanosomiasis, as has been observed in other African countries (Fevre *et al.*, 2005), and therefore intervention efforts are directed more towards bovine Trypanosomiasis. 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; Simarro *et al.*, 2001; Nkinin *et al.*, 2002). Workers like Waiswa *et al.* (2003) speculated that so much effort is 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. In West Africa, domestic pig has been incriminated as animal reservoir hosts for *T. b. gambiense* (Mehlitz *et al.*, 1982). 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.

1.2 PROBLEM STATEMENT

It has been established that *Trypanosoma* species are circulating in the pig populations in some districts in Eastern Region and the Tsetse fly vector have been identified in those areas. According to Simarro *et al.*, (2001), pigs could play epidemiological role of Trypanosomiasis in humans and animals. However, people in these tsetse-infested areas continue to work on farms, close to water bodies and close to pigs, thereby being exposed to tsetse fly bites. Thus a likelihood of transmission of Trypanosomes from the animal host to the human host exists. This can cause epidemics of Trypanosomiasis in the human populations. It is therefore imperative to establish which particular types of Tsetse fly have infested the area and which species of Trypanosome are circulating in the pigs in the affected communities. It is also important to establish the prevalence of the infection in the human and pig populations in these districts.

1.3 JUSTIFICATION

The study will generate data on the potential transmission dynamics which could occur between the vector, the human and the animal hosts. It will also provide information on the vector and parasite burden in their hosts, as well as the spatial distribution of Tsetse fly in the specific affected areas. Such data could be used to formulate policies and strategies to mitigate the public health concerns that have arisen, and also serve as a basis for effective local interventional planning for the control of Tsetse fly and Trypanosomiasis.

1.4 OBJECTIVES

1.4.1 Main objective

The main objective of the study is to generate epidemiological information on human and porcine Trypanosomiasis in the New Juaben municipality of the Eastern region of Ghana for developing intervention strategies through entomological and parasitological surveys.

1.4.2 Specific objectives

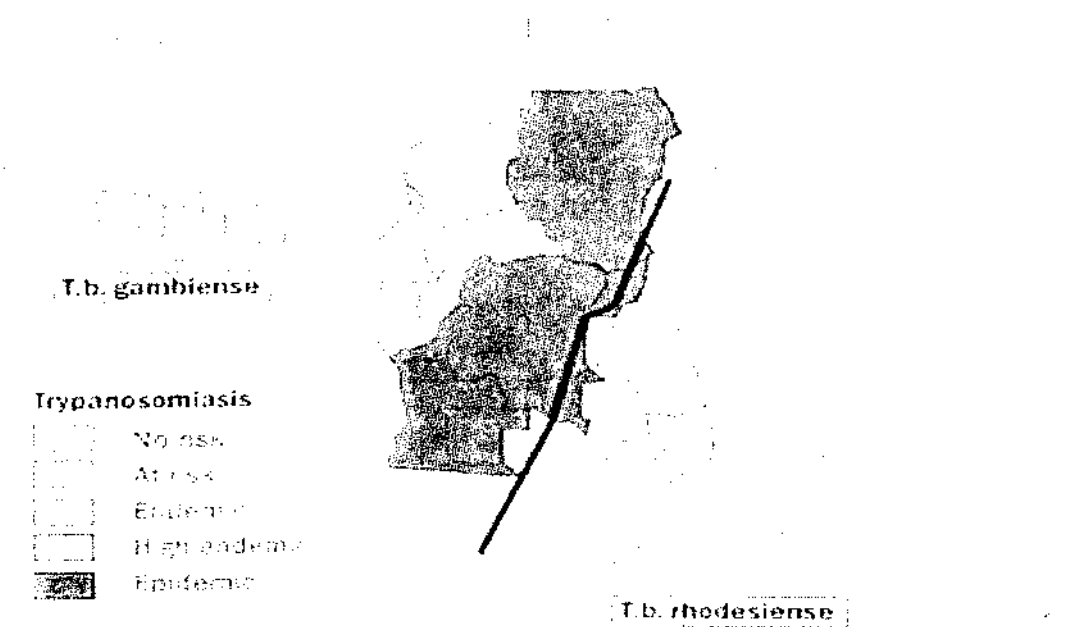
- To identify the species of Tsetse fly and their apparent density
- To identify the species of *Trypanosoma* in the pigs and human hosts
- To determine the Trypanosomiasis prevalence in pigs and humans
- To assess the community knowledge regarding the effects of Trypanosomiasis and control methods.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DISTRIBUTION OF TSETSE-TRANSMITTED TRYPANOSOMIASIS

Human African Trypanosomiasis (HAT) is the only vector-borne parasitic human disease whose geographical distribution is limited to Africa (WHO, 2005), especially in regions harbouring tsetse flies that can transmit the disease. More than 90% of Gambian sleeping sickness cases have been reported in West and Central African countries (WHO, 2010) (Figure 2.1).



(Source: WHO CPE)

Figure 2.1. Geographic distribution of major endemic foci of *T. b. gambiense* and *T. b. rhodesiense* Human African Trypanosomiasis in Africa (1995).

The two forms of the disease do not overlap except in Uganda where both forms of HAT occur (Reto and Oliver, 2006). However, there are indications that this may change in the near future because the areas of their occurrence are expanding (Picozzi *et al.*, 2005). The Gambian Trypanosomiasis has a wider geographical spread (77.8% of HAT endemic countries) than Rhodesian disease (WHO, 1998).

2.2 PUBLIC HEALTH AND ECONOMIC IMPORTANCE

According to WHO, (1998) about 300,000 to 500,000 people are affected by HAT, an average of 45,000 cases are reported annually while up to 60 million people in 36 countries are at risk of contracting the disease. However, less than 10% of the populations at risk are currently under surveillance with regular examination, have access to a health centre that can provide diagnostic facilities, and are protected by vector control interventions. The disability adjusted life years (DALYs) lost due to HAT is 2.05 million (WHO, 2000).

The disease is associated with secondary preventability because screening of people facilitates early detection and treatment which yields excellent results. However, in the absence of effective screening, most people with sleeping sickness - an estimated 80% - die before they can ever be diagnosed. There is no vaccine for HAT and chemotherapeutic agents for treatment are expensive, difficult to administer in remote areas and exhibit poor safety profiles.

Apart from the burden of human suffering, livestock can be severely affected by the disease, causing in them abortion, infertility in male animals, stunted growth and death. It

can reduce cattle production by 20-40% which results in an estimated annual economic loss of US$ 4.5 billion in Africa (Swallow, 1998). Within this region, some 46–62 million head of cattle and other livestock species are at risk of the disease. The impact of this tsetse-transmitted disease extends in sub-Saharan Africa over some 10 million km^2, out of which 70% represents natural pasture for grazing. The optimal use of these grazing fields for livestock production is hampered by Trypanosomiasis, thereby affecting animal traction for cart and plough. Also affected is the availability of livestock products like milk, meat, manure and hide. The absence of all these over a greater part of the African continent means a major constraint to the progress of development in this region (WHO, 2005).

2.3 TRYPANOSOMIASIS IN GHANA

Human African Trypanosomiasis (HAT) epidemics occurred in Ghana in 1940 where surveys showed 3% prevalence in Lawra and about 5% in Kintampo, Tumu, Wa, Gambaga and Yendi without mortality (Morris and Morris, 1949). Between 1986 and 2009, a total of 35 cases were reported from all the regions in the country (Ghana Health Service, 2009), and between 1994 and 1998, four cases with one fatality were 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 in cattle, pigs and horses. More than 60% of the land surface area of Ghana had been infested with tsetse flies (genus *Glossina*) 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 indicated 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 Northern Region recorded 5% prevalence as against 50% prevalence in high risk areas like West Mamprusi and Damongo districts (TTCU, 1997). Comparing two adjacent districts Dangme East and Dangme West in the coastal savanna zone, the prevalence was higher in the former, where riverine vegetation was prevalent, than in the latter where riverine vegetation was almost absent. A study carried out in the rain forest zone of the Eastern Region in 1993 showed 1.6% prevalence in cattle (Dankwa, 1993).

2.4 THE VECTOR – GLOSSINA (TSETSE FLY)

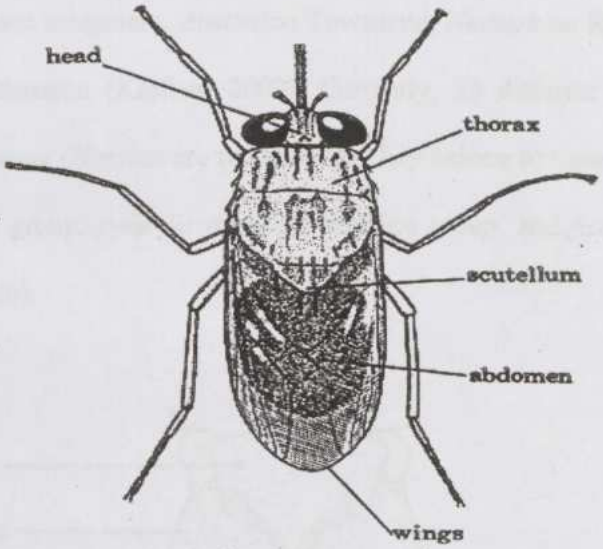
Tsetse flies (*Glossina*) belong to the Phylum *Arthropoda*, Order *Diptera* and Family *Glossinadae*. *Glossina* is the only genus of the family *Glossinadae* Weidemann 1830 (FAO, 1982a). Tsetse flies infest semi-arid, sub-humid and humid lowlands of about 37 countries in sub-Saharan Africa covering an area of about 8.7 million km^2 (Figure 2.2) but only certain species transmit the disease.

Source: FAO Corporate Document Repository

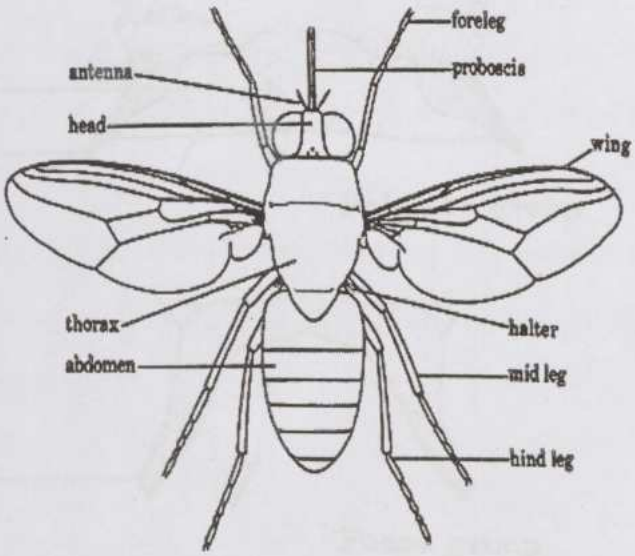
Figure 2.2 Global distribution of Tsetse fly (indicated by red colour)

When a Tsetse fly hatches from its pupal case it is free from Trypanosomes. Until its first blood-meal, it is called a teneral fly after which it turns to adult fly. The common anatomical distinguishing feature of Tsetse fly is that the wings fold completely when resting so that one wing lies directly on top of the other over the abdomen in a scissor format (Figure 2.3a). Tsetse flies also have a long proboscis, which extends directly forward and is attached by a distinct bulb to the bottom of their head.

a)



b)

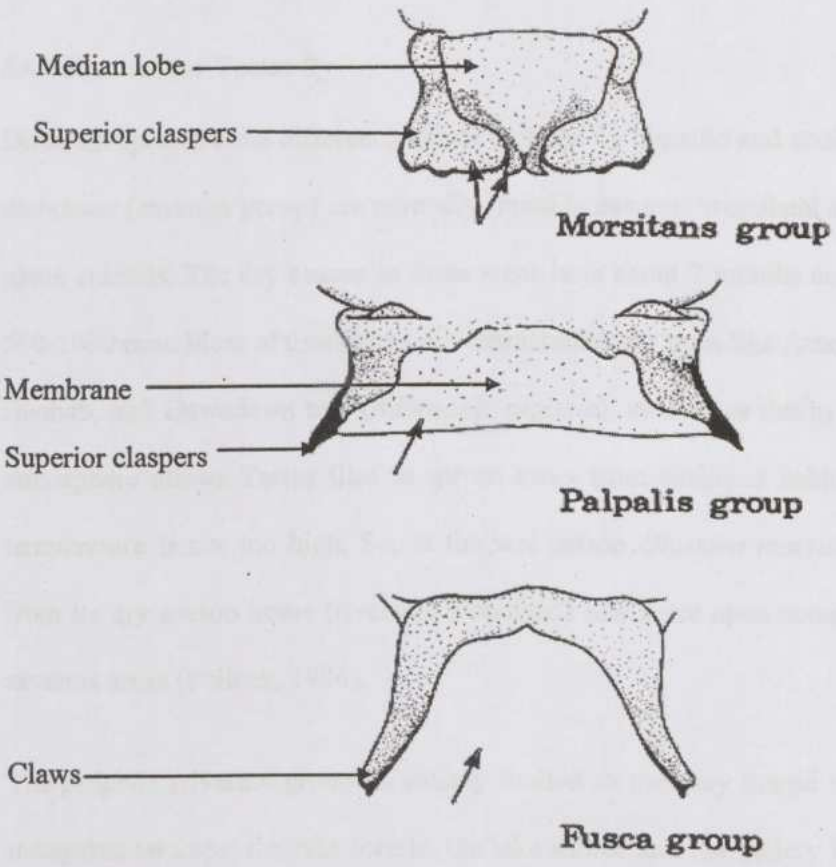


Source: Food and Agricultural Organisation

Figure 2.3 *Glossina* dorsal views. a) With wings folded b) With wings spread out

2.4.1 The Species of *Glossina*

There are three extant subgenera, *Austenina* Townsend, *Nemorhina* Robineau-Desvoidy, and *Glossina* Wiedemann (Krafsur, 2009). Currently, 23 different species and eight subspecies of the genus *Glossina* are recognized. They belong to three groups: *morsitans* group or savannah group, *palpalis* group or riverine group, and *fusca* group or forest group (Pollock, 1986).



Source: FAO Corporate Document Repository

Figure 2.4. The types of superior claspers in *Glossina*, as shown in *morsitans* group, *palpalis* group and *fusca* group.

According to Pollock (1986), these species groups differ in the morphology of their male genitalia (Figure 2.4 above). In the *morsitans* group the ends of the superior claspers are wide plates and the gap between the superior claspers is filled by the median lobes. In the *palpalis* group, the ends of the superior claspers are narrow claws with a membrane filling the gap between them. With the *fusca* group, the ends are claws and nothing fills the gap between them.

2.4.2 Habitat for Tsetse fly

Different species have different habitats dictated by climatic and ecological factors. The *morsitans* (savanna group) are normally found in savanna woodland and where there are game animals. The dry season in these areas lasts about 7 months and annual rainfall is 500-1000 mm. Most of these areas are characterized by trees like Acacia (*Cassia siamia)*, Baobab, and Dawadawa tree (*Adansonia digitata*), as well as thorny thickets. A humid atmosphere allows Tsetse flies to spread away from sheltered habitats, so long as the temperature is not too high. So, in the wet season *Glossina morsitans* disperses away from its dry season home (riverside woodland) into more open country, in the northern savanna areas (Pollock, 1986).

The *palpalis* (riverine group) is mainly limited to the very humid areas of Africa, the mangrove swamps, the rain forests, the lake shores and the gallery forests along rivers. Generally when members of this group penetrate into drier areas, they do not move far away from free water (rivers and lakes), but in more humid areas they may not have to live so close to free water.

The *fusca* (forest group) is predominantly located in the thick forests. They are found in waterside evergreen thickets and forest islands in savannas (Clausen *et al.*, 1998) and can have areas of overlap with the *palpalis* group.

Studies in Ghana have shown that the *morsitan* 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. As human activities in the forests increase and the game animals retreat, the *morsitans* group also moves along with them for protection. In contrast, resilience of the *palpalis* group in the presence of man is based on their adaptability to change from feeding on the large species of wild animals to the less obvious components of the wild fauna, such as reptiles, as well as to man and his domestic animals (Plucknett and Smith, 1987).

2.4.3 Host Preference of Tsetse fly

Studying the host preference of Tsetse fly in East Africa, Clausen *et al.* (1998), confirmed what earlier authors had said about the opportunistic feeding habit of the *palpalis* group. They reported that the group members (*G. palpalis, G. tachinoides* and *G. fuscippes*) were identified to have fed on wide range of hosts including domestic pigs, primates, monitor lizards, reptiles, ruminants and birds. In the Western Region of Ghana, Tsetse flies were seen feeding on domestic fowls and toads (Abavana, personal

communication). The identification of avian species as a source of food for *G. f. fuscipes* has also been reported in Cameroun (Okoth and Kapaata, 1986). The major hosts for the *morsitans* group are warthog, wild ruminants, hippopotamus, man and domestic ruminants, while the *fusca* group feed mainly on bushbuck (Weitz, 1963), rhinoceros and buffalo (Pollock, 1986). Certain environmental factors such as an overlap of habitat and activity of fly and host may contribute to the strong preference of *Glossina* for feeding on specific host (Mihok *et al.*, 1996).

2.4.4 Vector Competence

The ability to transmit disease varies among *Glossina* species and depends on both the species and Trypanosomes they transmit. *Glossina morsitans* is a good vector of *T. congolense* whereas *G. palpalis* is a poor vector (Moloo and Kutuza, 1988). Conversely, *G. palpalis* is the main vector of *Trypanosoma brucei gambiense* (Hoare, 1972) the causative agent of Human African Trypanosomiasis, whereas *Glossina morsitans* is not (Maudlin *et al.*, 1986). Because of *Glossina palpalis'* close association with man and domestic animals in the peri-domestic habitats, risks of human sleeping sickness are obviously increased when domestic pigs are healthy carriers of *T. gambiense* (Pollock, 1986).

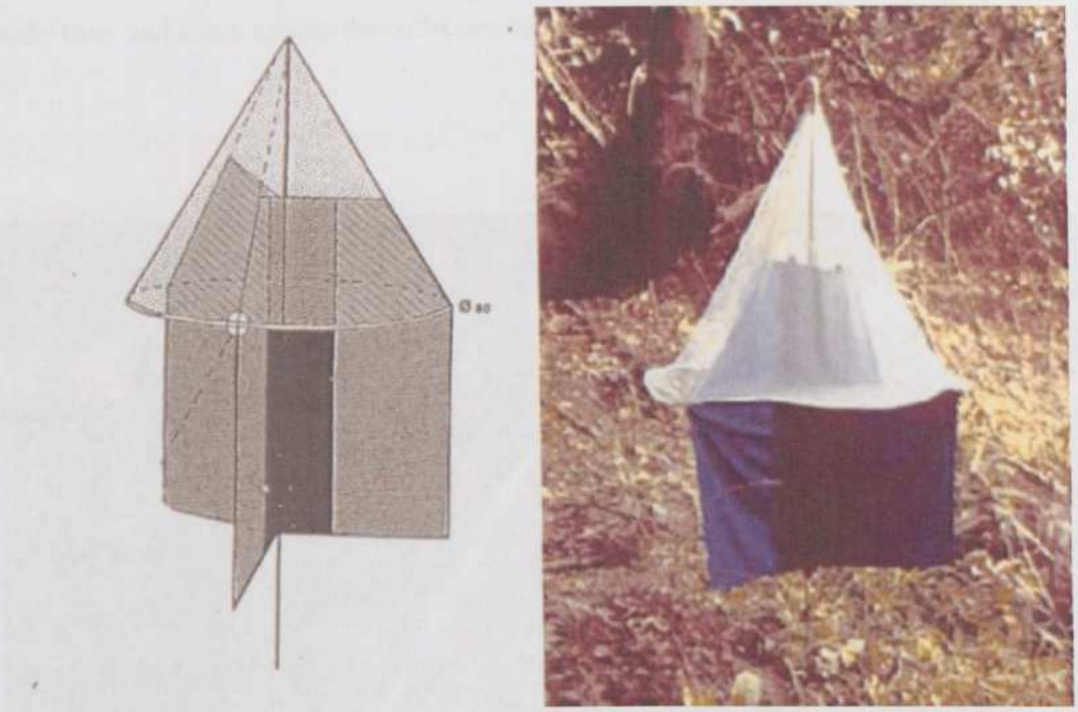
2.4.5 Trapping Tsetse flies

Tsetse flies of both sexes bite mostly during the day. Traps are used to capture flies. The attractiveness of traps for Tsetse flies depends on their shape, size, colour and colour pattern, and this differs from species to species. They are attracted to dark colours, generally blue or black, and attack preferentially moving hosts. Certain substances have an attractive odour for flies and therefore can increase the ability of traps to attract and also the number of flies caught (Vale, 1974). For example, acetone, phenolic molecules, carbon dioxide, bovine or buffalo urine are known to attract Tsetse flies (Spath, 1995).

The type of trap and time interval for harvesting depends on the objectives of the exercise. The number of flies caught by traps varies greatly from day to day, from season to season and from place to place. Traps use blue and black cloth in a shape that attracts flies and then funnels them upwards into a trap cage which may be monoconical or biconical in shape.

2.4.5.1 The mono-conical (Vavoua) traps

The mono-conical (Vavoua) trap (Fig. 2.5) 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.



Source: Vestergaard Frandsen

Figure 2.5. Monoconical (Vavoua) Trap

2.4.5.2 The bi-conical (Challier - Laveissiere) traps

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. 2.6). 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.

Figure 2.6 Biconical (Challier-Laveissiere) Trap

2.4.6 Fly Apparent Density

Fly apparent density (FAD) is a measure of the population of the Tsetse flies in an area. It does not give an accurate population size but an estimate. It is calculated as number of flies per trap per day (FTP). Fly densities are determined by the availability of hosts and suitable habitats (WHO, 1998), and is influenced by the behaviour of the Tsetse fly species involved and prevailing microclimatic conditions both in time and space. The most important climatic factors are temperature and humidity. Temperature range of 17-35^{0}C and 60-70% humidity are tolerated by adult flies (Pollock, 1986). One of the determinants of persistence or otherwise of Trypanosomiasis in an area has conceived of as pattern of host-fly contact. This in turn depends on factors such as fly apparent density, their spatial distribution, absence of alternative source of blood meal for flies and certain behavioural activities of the host (Khonde *et al.,* 1997). There is no dispute about correlation between Tsetse fly challenge, measured as the product of apparent density and percentage of Tsetse fly infected, and the prevalence of Trypanosomiasis infection (Leak *et al.,* 1990; Rawlings *et al.,* 1991).

2.5 THE CAUSATIVE AGENT – *TRYPANOSOMA*

Trypanosomes are haemoflagellate protozoans that inhabit the blood plasma, the lymph and various tissues of their hosts. The genus *Trypanosoma* belongs to the protozoan branch, order *Kinetoplastida*, and family *Trypanosomatidae* (OIE, 2008). Tsetse-transmitted Trypanosomes belong to the salivarian section of three subgenera. These are

Nannomonas – a group of small Trypanosomes including *T. congolense* with medium-sized marginal kinetoplasts, no free flagella, and poorly developed undulating membranes; *Duttonella* – a group of Trypanosomes including *T. vivax* with large terminal kinetoplasts, distinct free flagella, and inconspicuous undulating membranes and *Trypanozoon* (example *T. brucei*), a group of polymorphic Typanosomes occurring as short, stumpy organisms without flagella, long slender organisms with distinct flagella, and intermediate forms that are usually flagellated (Pollock, 1986).

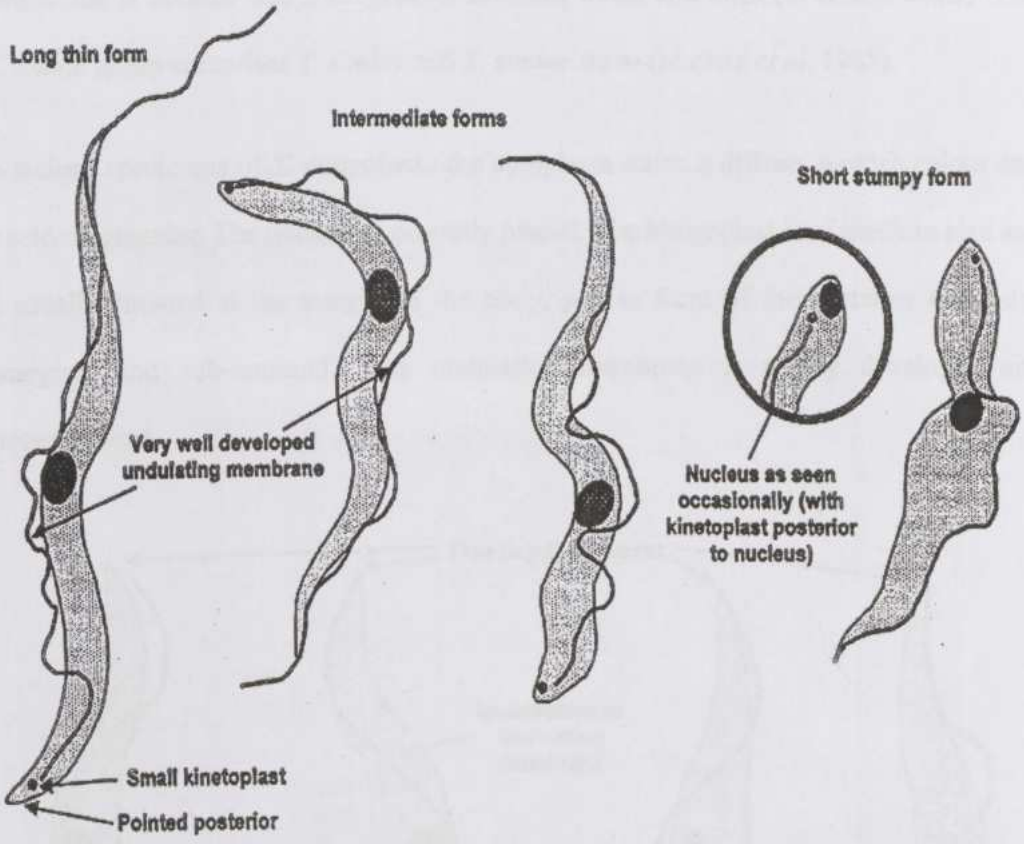
2.5.1 Species of *Trypanosoma*

2.5.1.1 Trypanosoma brucei group

The subgenus *Trypanozoon* consists of 3 species: *Trypanosoma brucei*, *T. evansi* and *T. equiperdum.* Of the three species, only *T. brucei* group exhibits pleomorphism and has the ability to develop inside the vector Tsetse fly (Wendy, 2003). It is therefore the only species among the three that is cyclically transmitted by Tsetse fly. It is further subdivided into 3 subspecies (Hoare, 1972) – *T. b. gambiense, T. b. rhodesiense* and *T. b. brucei. Trypanosoma brucei* is a spindle-shaped cell with a single flagellum (Fig. 2.7). This flagellum runs along the cell membrane and extends beyond the anterior part of the cell. The base of the flagellum is associated with the kinetoplast. The long and thin trypomastigote is the only form to be observed in the mammalian host, whereas the short and stumpy epimastigote form occurs during the development phase in the Tsetse fly. During the entire life cycle, *T. brucei* cells multiply by binary fission and are considered to be exclusively extracellular. (Chappuis *et al.*, 2005).

Morphologically, these subspecies are identical (Losos, 1986), and therefore are defined by extrinsic factors (pathogenicity, distribution and host range): *Trypanosoma brucei gambiense* is responsible for the chronic form of HAT in Western and Central Africa, *T. b. rhodesiense* is the agent of the acute form of HAT in East Africa, and *T. b. brucei* does not infect humans but causes animal Trypanosomiasis (nagana) in cattle (Mathurin *et al.*, 2009). The normal human serum has trypanocidal effect on *T. b. brucei* whereas *rhodesiense* and *gambiense* Trypanosomes have resistance against normal human serum.

Animals can serve as reservoir for the *T. b. rhodesiense* and *T. b. gambiense* without showing signs of the disease. *T. b. gambiense* can maintain a wide range of reservoir hosts because it has the capacity to proliferate and persist in alternative hosts in the absence of symptoms with prolonged maintenance of infectivity to the vectors (Mehlitz, 1986). This Trypanosome can conserve its infectivity to human even after 10 cyclical transmissions in pigs (Van Hoof., 1947). In addition, the transmissibility index of *T. b. gambiense* group 1 is increased when this parasite is transmitted to different hosts (Van Hoof,, 1947). Therefore, the infectivity of *T. b. gambiense* group 1 for human is enhanced during human-fly-pig transmission cycle. The host may however, differ from one endemic area to another.



Source: FAO Corporate Document Repository

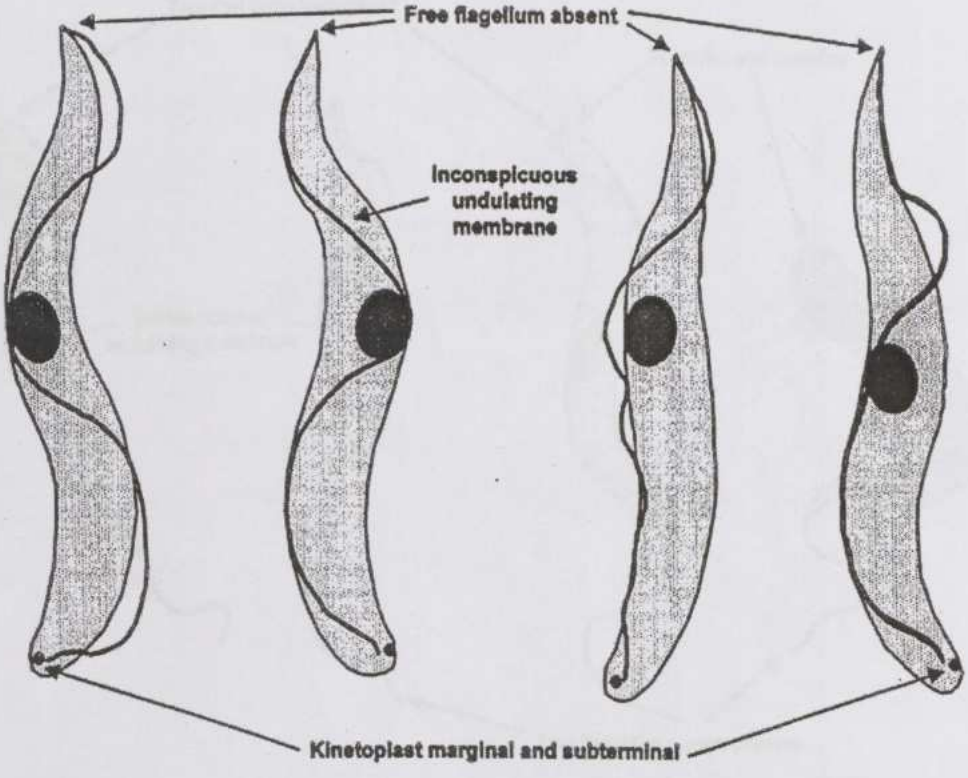
Figure 2.7 *Trypanosoma brucei* blood stream form

2.5.1.2 Trypanosoma congolense group

Trypanosomes of subgenus *Nannomonas* are defined by their developmental cycle in the Tsetse fly, which involves the midgut and proboscis. They are the smallest bloodstream forms and are split into 3 species: *Trypanosoma congolense* (Figure 2.8), which has a wide range of ungulate hosts, *T. simiae*, for which pigs are regarded as the most important host (Hoare, 1972) and *T. godfreyi*. Molecular characterisation reveals that *T.*

congolense is divided into 3 subgroups: savanna, forest and kilifi (or Kenya coast). The *T. simiae* group comprises *T. simiae* and *T. simiae tsavo* (Majiwa *et al*, 1985).

In stained specimens of *T. congolense* the cytoplasm stains a diffuse, pinkish colour and is seldom granular. The nucleus is centrally placed. The kinetoplast is of medium size and is usually situated at the margin of the body, just in front of the posterior extremity (marginal and sub-terminal). The undulating membrane is poorly developed and inconspicuous.

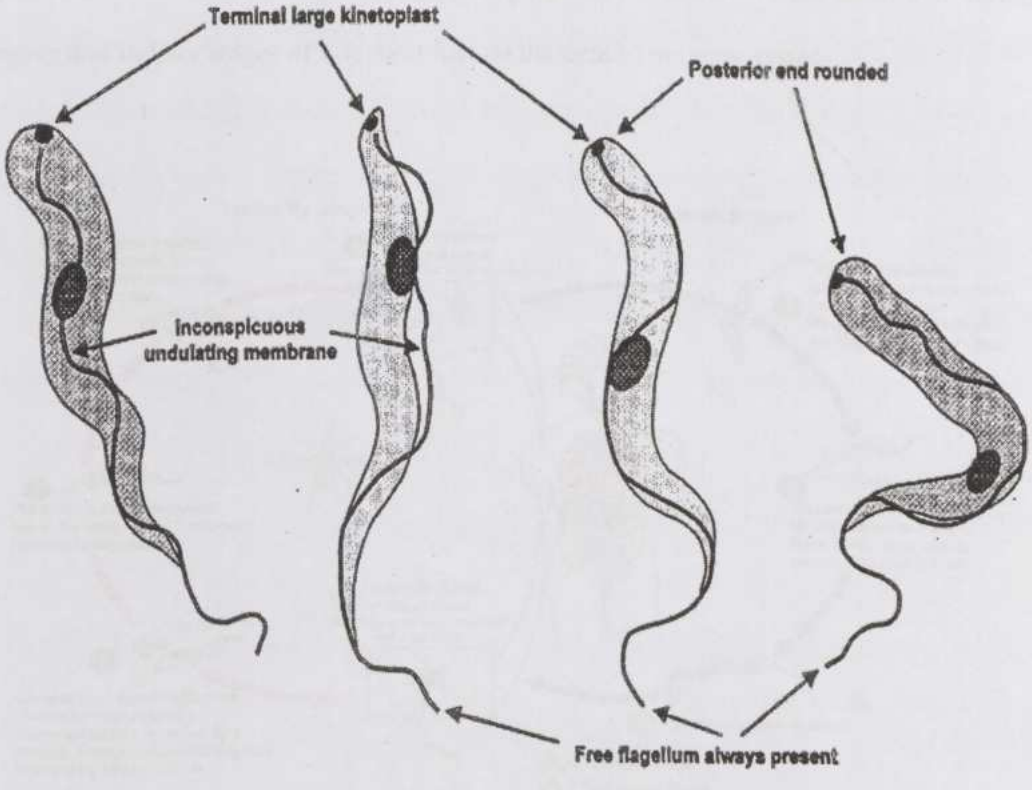


Source: FAO Corporate Document Repository

Figure 2.8. *Trypanosoma congolense* as seen in a stained blood smear

2.5.1.3 Trypanosoma vivax group

The *T.vivax* as seen in the blood of mammals is essentially monomorphic, with a free flagellum. Its length, including the free flagellum, varies from 18 to at least 26 μm. A typical specimen shows a large and terminal kinetoplast (Fig. 2.9). It is much larger than any of the other pathogenic species, and this is a distinguishing feature. The nucleus is centrally placed, but the bulk of the cytoplasm is found in the posterior part of the body. The posterior extremity is swollen and blunt.

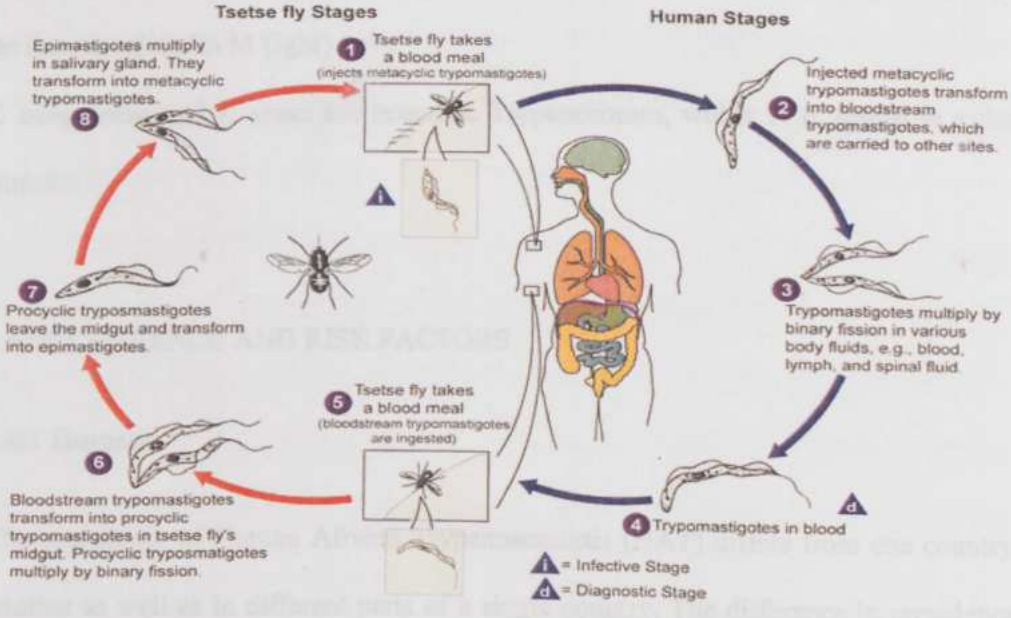


Source: FAO Corporate Document Repository

Figure 2.9 *Trypanosoma vivax* blood stream forms

2.5.2 The Life Cycle of *Trypanosoma*

Figure 2.10 illustrates the typical developmental cycle of a cyclically transmitted Trypanosome in man (or other mammalian host) and the Tsetse vector. Infection of the mammalian host starts with the bite of an infected Tsetse fly (*Glossina* species.), 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.



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

Figure 2.10. Diagrammatic representation of the life cycle of *T. b. gambiense* and *T. b. rhodesiense* in humans and the Tsetse fly.

Trypomastigotes are taken up by the male and female Tsetse fly when it sucks blood. The parasites develop in the mid-gut of the fly into procyclic trypomastigotes and multiply. From about 2-3 weeks, the trypomastigotes move to the salivary glands transforming through epimastigotes into metacyclic trypomastigotes. The Tsetse fly remains infective for life - about 3 months.

In the mammalian host, Trypanosomes can escape the host's immune response by sequential expression of antigenically distinct variable surface glycoproteins (VSGs). Through this mechanism, a small but sufficient fraction of the parasite population is able to evade the mammalian host's humoral immune response and proliferate until the new surface antigen coat is recognized by a new generation of specific antibodies, mainly of the immunoglobulin M (IgM) type.

T. congolense and *T. vivax* are haematic Trypanosomes, whilst *T. b. brucei* is a tissue parasite.

2.6 OCCURRENCE AND RISK FACTORS

2.6.1 Humans

The prevalence of Human African Trypanosomiasis (HAT) differs from one country to another as well as in different parts of a single country. The difference in prevalence is influenced by factors emanating from the host, vector, parasite and environment. Their

influence is not only due to their individual effect but also as a result of the complex interactions that exist between them (Moore *et al.*, 1999).

The people most exposed to the Tsetse fly and therefore the disease, are in rural populations that depend on agriculture, fishing, animal husbandry or hunting for their living (WHO, 2010). Analysis of the localization and frequency of human–fly contact shows that infection with human Trypanosomiasis is related to behavioural risk factors, and the behaviour associated with forest activities is in turn strongly related to age, gender and culture (Laveissie`re *et al.*, 1986b). The importance of age as risk factor for the disease seems also to depend on the epidemiological situation, and although sleeping sickness appears more often in adults than children (Moore *et al.*, 1999), this difference varies according to prevalence levels (Jannin *et al.*, 1992). Age and sex as risk factors could be linked with the type of activities the person is engaged in traditionally. Khonde *et al.* (1997) showed that the risk of HAT in children was correlated to a history of HAT in the mother but not in the father because during the first 2 years of life, children spend a lot of time with their mother and could be exposed to Tsetse bite when collecting water from riverside. In Urambo district, Tanzania, it was demonstrated that adult males were most likely to suffer from HAT because most of them were engaged in activities such as honey gathering, timbering, fishing, hunting and herding and therefore were more exposed to Tsetse fly bites (Sindato *et al.*, 2008).

Differences in disease prevalence according to ethnic group are a consequence of each group's distinctive pattern of agricultural activity and way of life, which modifies the

level of human–fly contact (Sane *et al.,* 1999). In the Vavoua focus (Western-central part of Ivory Coast), the prevalence among the Mossi (originating from Burkina Faso) was 6.1% whereas it was only 0.6% among the Baoule (Laveissie`re *et al.,* 1994b). This difference was presented as a consequence of agricultural activities and way of life between the two groups, the Mossi being much more in contact with Tsetse flies. Daily activities have been frequently described as important risk factors for HAT in forest areas responsible not only for higher prevalence rates (Moore *et al.,* 1999) but also for both spatial and familial cases clustering (Khonde *et al.,* 1997).

Prevalence of the disease seems not to be correlated with the apparent density of Tsetse flies (Sane´ *et al.*, 1999), but rather with the Tsetse challenge of an area. Human-vector contact occurs mostly in forested rivers and shores but is also peri-domestic when huts are built in or near plantations (Burri and Brun, 2003), especially when members of *G. palpalis* group are the dominant vectors. It was reported that after an African Swine Fever epidemic had killed numerous pigs in a village in Congo, Tsetse flies left their usual resting sites and moved closer to the houses, thus increasing man-fly contact. (Gouteux *et al.,* 1987). Asonganyi *et al.* (1991) also reported that the reactivation of an old sleeping sickness focus in Mamfe´ (Cameroon) after 1982 seemed to be related to an earlier African Swine Fever epidemic that killed all the pigs from this focus.

2.6.2 Pigs

The prevalence of Trypanosomiasis in pigs will largely depend on the species of the Tsetse fly and the Trypanosome available. The probability of a host contracting Trypanosomiasis depends on the rate at which it is fed upon by infected Tsetse flies [Rogers, 1988]. In a comparative study in which cattle, pigs and goats were involved and *G. morsitans morsitans* the only vector, the prevalence of Trypanosomiasis in pigs was reported as 6.5% using Polymerase Chain Reaction (PCR) (Simukokoa *et al.*, 2007). Pigs were initially considered resistant to *T. vivax* (Losos, 1986; Simukokoa *et al.*, 2007; Stephen, 1986). However, recent studies by Musa *et al.* (2005) in Kenya recorded 19.2% prevalence of Trypanosomiasis in pigs and out of ten pigs carrying Trypanosomes, five were infected with *T. vivax* and three with *T. brucei*. Similarly in Uganda, three *T. vivax* infections were detected from twenty-two pigs by Balyeidhusa *et al.* (2006). Three years later Biryomumaisho *et al.* (2009) also reported *T. vivax* infection of nine pigs. When dealing with areas where *G. palpalis* are the dominant vectors, the availability or otherwise of domestic pigs becomes a great concern since they can serve as 'living screens' for humans.

Apart from the cyclical transmission, *T. vivax* can be mechanically transmitted. This mechanical (non-cyclical) transmission occurs when feeding process of a biting fly is interrupted. Aning, (1993) mentioned that high occurrence of *T. vivax* in an animal population could be an indication of less Tsetse fly and more mechanical transmitters involved in the Trypanosome transmission.

2.7 CLINICAL PRESENTATION

2.7.1 Humans

The signs and symptoms of Human African Trypansomiasis depend on the host and the sub-species of Trypanosome. Classically, the progression of HAT can be divided into three stages: the bite reaction (chancre), parasitemia (blood and lymphoid tissues) and central nervous system (CNS) stages. First, a non-pustular, painful, itchy chancre forms 1-3 weeks after the bite and lasts for 1-2 weeks (Fig 2.11). The early stage is usually characterized by malaria-like symptoms, including fatigue, headache, recurrent fever, and anaemia after 2-3 weeks of bite. These are followed by generalized pain, weakness, cramps and swelling of neck lymph nodes. In advanced stages, the disease affects the central nervous system, causing severe neurological and mental disorders and making the individual dependent on other people. These include apathy, mental dullness, tremors, convulsions, sleepiness, and coma. There is rapid weight loss and death a few months later from malnutrition, heart failure, or pneumonia. In the case of *T. brucei rhodesiense* infections, there is no coma or nervous system symptoms as patient probably dies before these can develop (WHO, 1998)

2.7.2 Pigs

The cardinal clinical sign is anaemia. With the haematic Trypanosomes (*T. congolense* group) within a week of infection there is decrease in packed cell volume (PCV), intermittent fever, oedema, lacrimation, enlarged lymph nodes, loss of appetite and

weight. These may lead to early death in acute cases or to digestive and/or nervous signs with emaciation and eventually death in chronic forms (OIE, 2008). Abortion may be seen, and infertility of males and females may be a sequel.

The severity of the clinical response is dependent on the breed of affected pig and the dose and virulence of the infecting Trypanosome. Stress, in the form of poor nutrition or concurrent disease, plays a prominent role in the disease process.

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. b. brucei* are often asymptomatic (Ilemobade and Balogun, 1981).

2.8 DIAGNOSIS OF TRYPANOSOMIASIS

2.8.1. In Humans

The diagnosis of HAT follows a three-step pathway: screening, diagnostic confirmation, and staging (Chappuis *et al.*, 2005).

2.8.1.1 Screening

Screening involves active case detection using different tools depending on the disease type. Field screening of Rhodesian sleeping sickness relies on clinical symptoms and signs. In the case of Gambian sleeping sickness, antibody detection tests are currently used in mass-screening for the identification of sero-positive individuals on whom to focus parasitological examinations (André *et al.,* 2000). The most commonly used test in the field is the Card Agglutination Test for Trypanosomiasis (CATT), developed for *T. b. gambiense* specific antibody detection (Magnus *et al.,* 1978). CATT is a fast and simple agglutination assay for detection of *T. b. gambiense*-specific antibodies in the blood, plasma, serum, or Cerebro-spinal fluid (CSF) of HAT patients. It relies on the presence of anti-trypanosomal antibody to agglutinate intact, stained and preserved Trypanosomes. The reported sensitivity of the CATT on undiluted whole blood (CATT-wb) varies from 87 to 98%, and the negative predictive value is excellent during mass population screening (Penchenier *et al.*, 2003).

André *et al.* (2000) reported that non-*T. b. gambiense* Trypanosomes being harboured by human patients could play a role in transient, fluctuating or even long-lasting CATT seropositivity in the human population.

2.8.1.2 Diagnostic Confirmation

Parasitological diagnosis is made by microscopic examination of lymph node aspirate, blood, or CSF. It provides direct evidence for Trypanosome infection and therefore allows a definite diagnosis. There are usually many more parasites in *T. b. rhodesiense*

than in *T. b. gambiense* infections. Parasite numbers in *T. b. gambiense* infection can vary between more than 10,000 Trypanosomes/ml, which is easily detectable, and less than 100 Trypanosomes/ml, being below the detection limit of the most sensitive methods in use. Serial examination of blood on consecutive days can increase the test sensitivity. Tests may be carried on lymph node aspirate, wet and thick blood films using the microhaematocrit centrifugation technique, and buffy coat (Chappuis *et al.*, 2005).

2.8.1.3 Staging

The stage of progression of Trypanosomiasis is determined so as to suggest which chemotherapy would be appropriate for the patient. In the absence of sufficiently specific clinical signs and blood tests indicating the evolution from first- to second-stage HAT, staging of patients still relies on examination of CSF obtained by lumbar puncture. It is a vital step in the diagnosis process. According to WHO recommendations, second-stage HAT is defined by the presence in the CSF of one or more of the following: (i) raised white blood cell count (5 cells/l), (ii) Trypanosomes, and (iii) increased protein content (370 mg/liter, as measured by the dye-binding protein assay) (WHO, 1998).

2.8.1.4 Use of Molecular Method

Other different methods are used in diagnosing Trypanosomiasis. The Polymerase Chain Reaction (PCR) test is a molecular diagnostic method involving Deoxyribonucleic acid (DNA) amplification that is widely used to confirm sero-positives after CATT. It is also used in analyzing CSF during staging. In principle, PCR can be applied to any patient sample that may contain Trypanosome DNA, such as whole blood or buffy coat, lymph

node fluid or CSF. Samples should be stabilized in special buffers or on Flinders Technical Associates (FTA) filter paper (FP). The FTA-FP (Whatman, Australia) is particularly convenient since it is easy to handle and also protects DNA from degradation, unlike ordinary filter paper. However, on account of costs PCR is restricted to research purposes (Chappuis *et al.*, 2005).

2.8.2. Animals

A variety of diagnostic tests are available (Toure, 1976) but they vary in their sensitivity and specificity, cost and the ease with which they can be applied (Paris *et al.*, 1982). The choice of a particular test will be guided by economic principles and the availability of expertise, but especially by the diagnostic requirement (OIE, 2008).

2.8.2.1 Identification of the Agent

Direct Examination Techniques (DETs): The simplest 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 other two. 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.

Parasite Concentration Techniques (PCTs): By increasing the volume of blood to be examined and by concentrating the Trypanosomes sensitivity of the PCTs is improved. The PCTs usually used are Microhaematocrit centrifugation technique (Woo method) (Woo, 1970) and dark-ground/phase-contrast buffy coat technique (Murray method) (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.

PCR Techniques: 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 DNAs (OIE, 2008). False-negative results may occur when the parasitaemia is very low (< 1 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).

2.8.2.2 Serological tests

Several antibody detection techniques have been developed to detect trypanosomal antibodies for the diagnosis of animal Trypanosomiasis, with variable sensitivity and specificity. The methods of choice are the Indirect Fluorescent Antibody Test (IFAT) (Katende *et al.*, 1987), and the trypanosomal antibody-detection Enzyme-linked Immunosorbent Assay (ELISA). Both the IFAT and antibody-detection ELISA have been adapted for the analysis of blood samples collected on filter paper. Both antibody-detection 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.

Most of the tests are based on detection of anti-trypanosomal antibodies in patients' sera and hence cannot distinguish between an active infection and a cured. Also, they are not sufficiently specific to reveal conclusively the identity of the infecting Trypanosome species.

To overcome these challenges, 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 blood-stream form Trypanosomes. When these MoAbs are used in an antigen-trapping ELISA, diagnosis is sensitive and specific enabling many latent infections to be detected.

2.9 PREVENTION AND CONTROL

Due to various ecological, behavioural, political and economic factors the focus of Trypanosomiasis control is different for different regions in Africa: in Western and Central Africa the target of control is the disease, whereas in Eastern and Southern Africa the target of control is the vector (Allsopp, 2001). However, an integrated approach gives better results (WHO, 2005). The latter consists of continuous surveillance of the human population at risk, passive and active case detection and treatment, reduction of animal reservoirs through selective or mass treatment of livestock and intense Tsetse fly control in highly endemic and epidemic areas.

The first ever and largest vector control campaign to control Tsetse and Trypanosomiases in Ghana dates back to 1930, when vegetation clearing and game population control methods were used (Mahama *et al.*, 2003). Over the years Tsetse control methods have included ground spraying with insecticides, use of trypanotolerant animals, insecticide impregnated traps and screens/targets, insecticide-treated animals (moving targets) as well as chemotherapy. Most of these campaigns have been led by Tsetse and Trypanosomiasis Control Unit (TTCU) of the Veterinary Services Department who carry out active surveillance of the disease. The initial objective was to control the disease to an optimum level of containment and management (Veterinary Services Department, 1990).

A new programme of Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was initiated by African Heads of State and Government, who met in July 2000 at the Organisation of African Unity (OAU) Summit in Lomé, Togo (PATTEC, 2001). The objective is to eradicate Trypanosomiasis from the African continent. Its strategies, among others are: conduct of surveys on Tsetse fly and Trypanosomiasis; identification of zones of infestation which are isolated physically; systematic elimination of the confined populations of Tsetse fly by integrated use of conventional methods; detection and treatment of trypanosomiases in other areas to promote economic and developmental activities (PATTEC, 2001).

The zoning, sequential aerial spraying and subsequent use of sterile insect technique (SIT) are methods to be employed and will be implemented in phases. However, the

detection of the disease and use of chemotherapy and insecticide impregnated targets for management and control will be carried out at where and when needed.

The recent introduction of insecticides-impregnated black nets in Ghana (Bauer *et al.*, 2006) are proving promising as means of reducing the Tsetse fly populations.

2.10 COMMUNITIES KNOWLEDGE OF TRYPANOSOMIASIS

2.10.1 Levels of Community Knowledge

Studies have shown that most of the people in areas where Trypanosomiasis occurs are knowledgeable about the disease both in humans and in animals (Affognon, 2007; Kinung'hi *et al.*, 2006; Sindato *et al.*, 2008). Communities' understanding of Trypanosomiasis and its vector could be influenced by several factors such as the presence or otherwise of the condition in the area, the kind of host that is mostly affected (human or animal) and an encounter with the disease or vector by the people in practical terms. The knowledge level of Trypanosomiasis therefore varies from one geographical area to the other (Affognon, 2007), and among different occupational groups in the same area. In a study in Mali and Burkina Faso, Affognon (2007) noted that cattle farmers in these countries had higher level of knowledge of Trypanosomiasis as compared with cattle farmers in most sub-Saharan African countries. Kinung'hi *et al.* (2006) attributed high level of knowledge among questionnaire respondents to their experience from earlier outbreak of HAT and the education they received from healthcare personnel. Communities may show different knowledge levels of Trypanosomiasis in different

aspects of the disease. In a study in Tanzania, Sindato *et al.* (2008) indicated that most of the community members were very well informed about transmission of Trypanosomiasis however, they lacked knowledge in the role played by domestic and wild animals in the epidemiology of the disease.

2.10.2 Usefulness of Community Knowledge

Community knowledge in Trypanosomiasis could be used in diverse ways to benefit the local community and the state or country as a whole. Community participation and ownership in any local programme implementation is crucial for its sustainability. One of the reasons for minimal community's involvement in planning and execution of disease control programmes is lack of knowledge of the disease (Mboera *et al.*, 2007). In their study to assess the factors influencing individual and community participation in the control of Tsetse flies and HAT, Sindato *et al.* (2008), found out that the willingness of people to give their labour and money towards control activities was overwhelming if their knowledge level were high. Another area where community knowledge is becoming more important and necessary is participatory research. When community members are knowledgeable, they are willing and able to contribute meaningfully to influence the choice of research options by researchers to the benefit of their communities (Catley and Irungu, 2000). Furthermore, community knowledge in Trypanosmiasis empowers members to become self-supporting in managing the disease at all levels and are able to adhere to preventive practices by engaging themselves in less risk activities.

CHAPTER THREE

3.0 METHODS

3.1 STUDY DESIGN

A cross-sectional study was adopted and conducted between October 2010 and June 2011 in the New Juaben Municipality. Tsetse fly populations and apparent densities were determined. The prevalence of Trypanosomiasis in the human and pig populations were measured at a point in time and associations between dependent and independent variables ascertained. Using semi-structured questionnaires, the general knowledge of the selected communities of Trypanosomiasis was assessed.

3.2 STUDY AREA

3.2.1 Climate, Vegetation and Drainage

The study was carried out in the New Juaben municipality (Fig. 3.1), one of the twenty-one (21) districts in the Eastern Region of Ghana. The municipality has a surface land area of 110 sq km. The vegetation is semi-deciduous rain forest and enjoys bimodal rainfall pattern ranging between 1200 and 1700mm (Ghana Meteorological Agency, 2010). The major rainy season extends from May to July and the minor season is from September to November. The dry season spans from December to March. The daily temperature ranges from 20^0C to 32^0C with an average relative humidity of 80%.

The area is hilly with numerous low-lying swamps and streams across the entire municipality. These streams meander under the canopies of cocoa and oil palm plantations as well as secondary forests and traverse through crop farms, giving a wet

nature to the land. These streams serve as tributaries to River Densu which has been dammed to provide domestic and industrial water for the urban communities. Some of the rural communities fetch water from the streams for domestic purposes. The entire municipality is drained by River Densu and its tributaries.

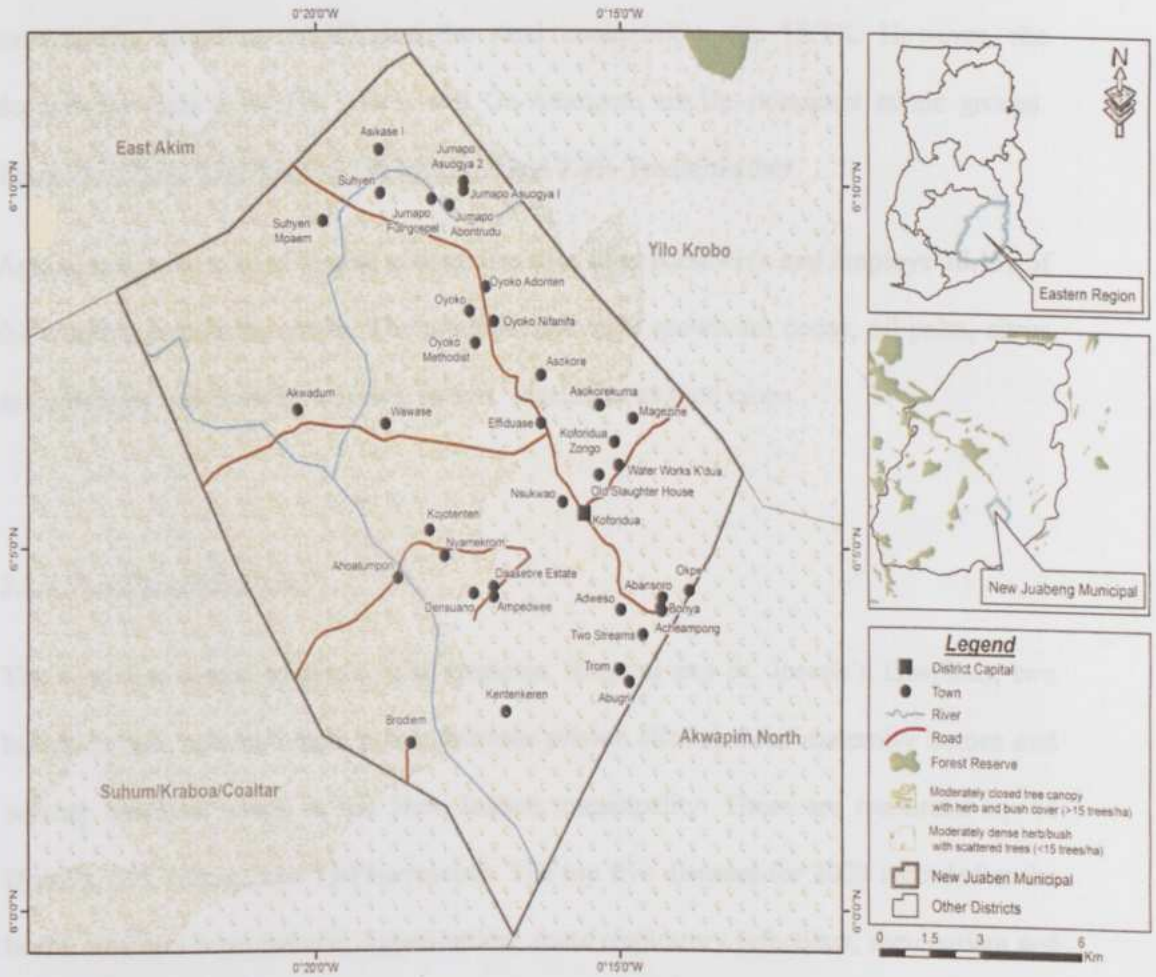


Figure 3.1 Map of New Juaben Municipality (left) within Eastern Region of Ghana (right)

3.2.2 Human Socio-demographic Characteristics

Some demographic, occupational and economic production data in the New Juaben municipality have been provided by the Ghana Statistical Service (2000): The total human population is 136,768 with 51.5% being females. The urban communities in the municipality constitute 84.3% and the rural communities are 15.7%. However, the dependency ratio is 64.7%. Akans and Ga-Adangme are the dominant ethnic groups. There are 82.6% Christians, 6.1% Muslims and 2.4% Traditionalists.

Agriculture is the second largest economic sector after commerce and employs 26.1% of the working population group. The predominant crops grown are cocoa, oil palm, citrus as cash crops and plantain, cassava, maize, vegetables as food crops.

3.2.3 Healthcare Delivery

There are two major hospitals (The Regional Hospital and St. Joseph's Hospital), two health centres, sixteen health posts, fourteen private clinics, three maternity homes and seventy chemical shops in the New Juaben municipality. These are resourced by 44 Doctors, 387 Nurses, and 13 Pharmacists. The top five diseases for 2009 recorded at the health facilities were malaria, hypertension, acute respiratory infections, rheumatism and joint pains, and skin diseases and ulcers (New Juaben Municipal Health Directorate, 2010)

2.3.4 Animal Production and Health

Livestock production in the municipality includes large ruminants (cattle), small ruminants (sheep and goats) and monogastrics (pigs, rabbits and poultry). Pig production in the municipality is by intensive system and pigs are always kept in pig-sties. These pig-sties are mostly located within the compounds of owners, either inside the community or the hamlets in Cocoa and Oil palm plantations. The 2009 livestock census indicated 3,566 pigs and 298 pig-sties with holdings ranging between one and 166 animals. Other livestock populations are 5,694 sheep, 8,688 goats and 457 cattle (Veterinary Services Directorate, 2009).

Some known diseases of veterinary importance in the municipality include Mange, Trypanosomiasis, Helminthiasis, Rabies, Peste des petits ruminants (PPR) and New Castle Disease. Animal health services for the entire municipality are provided by one Veterinary Officer with seven Veterinary Technical Officers, a Regional Veterinary Laboratory and three private veterinary input outlets (Veterinary Services Directorate, 2009).

3.3 VARIABLES

3.3.1 Dependent Variables

The dependent variables were the apparent density of the Tsetse flies, positive test for Trypanosomes in humans and also in pigs, as well as the packed cell volume (PCV) of the pigs. Others were the species of the Trypanosomes circulating in the pig populations.

3.3.2 Independent Variables

In humans, the independent variables included socio-demograhic factors of the study participants: sex, age, place of residence, and educational status. The behavioural factors studied included clothing pattern, other occupation, location of work and time allocated to work (daily duration).

Independent variables in pig data were sex, age group, breed, type of housing, feeding and watering, farm sanitation, and number of abortions/still births. Independent variables in the Tsetse fly data included, species of flies, sex, and developmental status. With regards to environmental factors the study focused on biotopes (vegetation type, land condition and drainage were considered).

3.4 SAMPLING

3.4.1 Study Population

The study covered people living in the municipality as well as the pig and Tsetse fly populations in the area. These included 136,768 people and 3,566 pigs in the municipality.

3.4.1.1 Exclusion Criteria

People who are already on Trypanosomiasis chemotherapy and people who are physically and mentally unable to give consent and are not accompanied by parent or guardian were excluded from the study.

3.4.2 Sample Size Determination

The following formula was used to obtain the sample size of pig and human participants.

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

- where n= sample size,
- z= risk of type 1 error (=1.96 at 95% confidence level)
- p= expected prevalence of Trypanosomiasis in pigs and humans
- 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),

- $n = 1.96^2 \times 0.737 \times (1-0.737) / 0.05^2$
- n = 298 pigs

A total of 341 pigs were selected for the study to make room for non-response and other circumstances.

In humans, using 50% (0.5) as presumed prevalence of Trypanosomiasis, a sample size of 384 was planned to be taken. In order to cater for drop-outs and non-response, 16 more participants were added to get a total of 400 participants. Blood samples were taken from 558 participants but only 352 of them consented to respond to the questionnaire.

3.4.3 Sampling Method

The region and the municipality were purposefully selected for the study. This is because more outbreaks of Trypanosomiasis in pigs were reported in the Eastern Region in 2006 where majority of pig farmers in the New Juaben Municipality lost more than half of their standing stock. The effect of the outbreak was more severe in this municipality. Only the communities (enumeration areas) where pigs were reared were selected for the study

3.4.3.1 Sampling Humans

For purposes of this study the municipality was divided into five sub-municipalities as shown on Figure 3.2. The communities (enumeration areas) in the sub-municipalities were selected using probability proportional to size (PPS). The number of human participants to be drawn from each selected community was also determined by this method. The method was used so as to minimize the effect of differences in population sizes of the communities and therefore offer equal opportunities for all the eligible participants to be selected. A bottle was spun in the centre of each of the selected enumeration areas and the immediate compound in the direction of the bottle-head was counted as the first of those that will be selected successively in that direction. In each compound, all the households were listed and one was randomly picked. In the selected household, all the members who qualified and gave consent to be part of the study were listed and one randomly picked. Where in a compound no household gave consent to be part of the study, we moved to the next compound in the row. In situations where the compounds in a row were not up to the number of participants required from that

enumeration area, the bottle was spun once again and the new direction was followed accordingly.

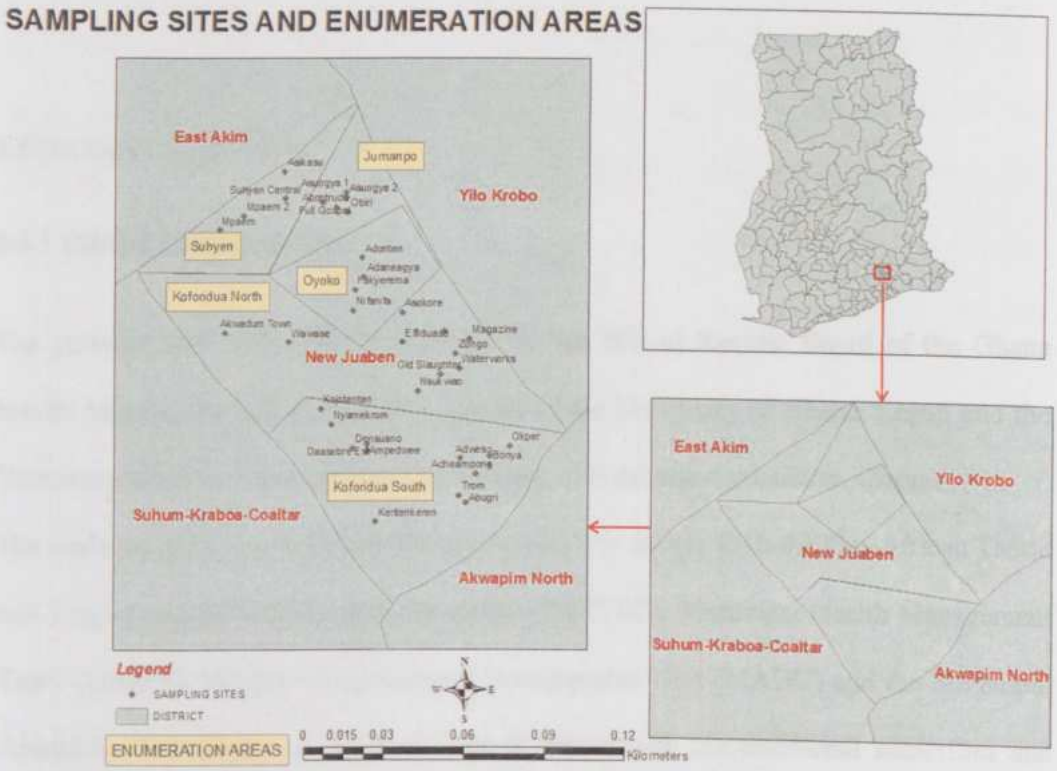


Figure 3.2 Distribution of sampling sites and enumeration areas, New Juaben municipality, 2011

3.4.3.2 Sampling Pigs

Random sampling was used to select the pigs. All the 298 pig houses in the municipality were included in the study. Sample frame was generated from the cumulative number of pigs in the pig houses. The random number table was then used to select the sample size of 341 pigs from the pig houses. The selected random numbers, each of which was not to

be greater than the sample frame, were then used to select the pigs from the pig houses to which they belonged. In the pig house all the pigs were counted serially and the one that corresponded to the selected random number was picked for the study.

3.5 DATA COLLECTION

3.5.1 Ethical Considerations

The protocol was reviewed for approval by the Ethical Review Board of the Ghana Health Service, the School of Public Health of the University of Ghana, Legon and the Veterinary Services Directorate of the Ministry of Food and Agriculture, Ghana.

The study protocol was followed and permission was sought from the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC), Municipal Health Management Team (MHMT), Municipal Agricultural Development Unit (MADU) and the Municipal Assembly. The subject of the study was discussed with the traditional authorities and community leaders in order to elicite their views and also to ensure their consent and cooperation in the study.

Verbal and written informed consent was duly sought from the study participants. All collected data were coded with numbers and securely stored to ensure privacy and confidentiality. Further use and storage of left-over samples were agreed upon with participants at the feedback section after the Card Agglutination Test for Trypanosomiasis (CATT) diagnostic test had been completed. Their participation was

voluntary, and they were free to opt out or give no response to any question. Contact address and telephone numbers were given to the participants on their result cards for further clarification. Participants were asked questions about their daily activities, pig production practices (for pig farmers) and knowledge about Tsetse fly and Trypanosomiasis. The quantity of blood to be taken from each of the participants and their pigs (where applicable) was made known to them before the exercise. The risks involved in sample collection by pricking were also discussed with the selected participants. The duration of engaging participants, as well as their benefit and rights were earlier made known to them and then fulfilled after the exercise. Individuals (humans and pigs) with a positive parasitaemia were treated free of charge. Each consenting individual or a witness was asked to append his/her signature or left thumbprint before the questionnaires were administered.

3.5.2 Questionnaire Survey

Structured questionnaires were administered to eligible and consenting participants for socio-demographic and behavioural data and to assess the knowledge of community members of the occurrence of Tsetse flies and Trypanosomiasis, and their control. The owners of the selected pigs were also interviewed on livestock production constraints, herd composition, husbandry practices, farm sanitation and control methods of Trypanosomiasis. All the selected sites for the entomological and parasitological data collection in both human and pig surveys were geo-referenced using Global Positioning System (GPS) device (GARMIN eTrex Legend® H).

3.5.3 Entomological Survey

3.5.3.1 Biotopes

Based on the vegetation type, land-use patterns, and other ecological features, four different biotopes were identified. A feature common to all these biotopes was presence of pig sties with pigs. These biotopes were:

1. Cocoa and oil palm plantations with vegetation canopies A (Fig 3.2). Cocoa and oil palm are the two major cash crops grown in the municipality. They normally form dense canopies at heights ranging from 4 to 7 metres and are sometimes interspersed with streams. High humidity is maintained in such areas.
2. Low-lying shrubs B. These areas are characterized by shrubs and grasses with isolated trees. They are usually used for food crop farming and therefore trees are not allowed to grow to form canopies.
3. Edge of communities C. These are the areas around the boundaries of towns and villages. The land is used for animal rearing rather than crop farming. Bushes at these areas sometimes overgrow but they are intermittently weeded off. Pig concentration is higher here than all other biotopes.
4. Residential areas inside communities D. People continue to keep pigs at their compounds even within communities. The only shrubs are the live fences, and human activities are more pronounced.

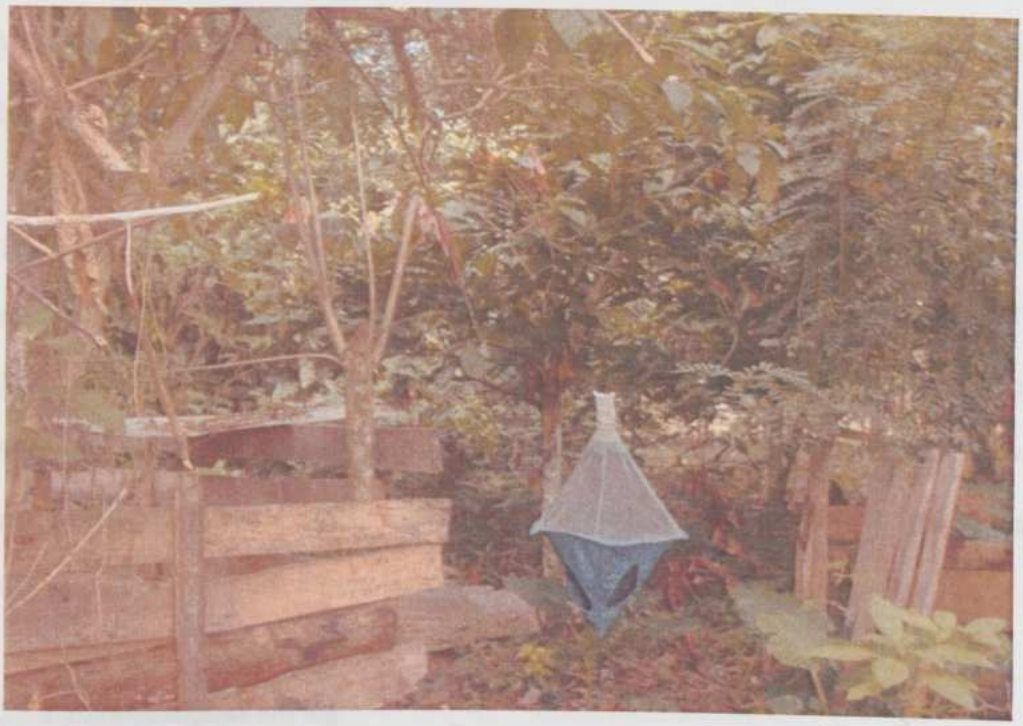


Figure 3.3 Biotope A - Cocoa plantation with vegetation canopies

3.5.3.2 Tsetse fly Trapping

A total of 148 un-baited bi-conical traps (Brightwell *et al.*, 1987) with blue-black material were deployed near pig sties at intervals more than 200m for 24 hours. The entomological survey was conducted during the minor rainy season, precisely in October, 2010. Traps were put under shades cleared of weeds. The bases of the poles were smeared with grease to prevent ants from climbing into the trapping chamber to attack the captured flies. 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 immediately sent to the Eastern Regional

Veterinary laboratory. The species, sex, and teneral status of Tsetse flies were identified based on morphological characteristics described by Leak (1999).

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, 1987). 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/TxD. 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.

3.5.4 Parasitological Survey

3.5.4.1 Humans Participants

Collection of Blood Samples

Each participant was given a HAT Survey Card with his/her serial number and sent to the blood sampling table where their capillary blood was taken after cleaning the ring finger with alcohol-soaked cotton swab and pricking with a sterile single use blood lancet. The first drop of blood was wiped off and a heparinised capillary tube was filled about three quarter of its length, avoiding bubbles. The tube was inclined several times in order to mix the blood with the heparin. The capillary tube was placed horizontally on a capillary tube holder which could take 10 samples. After filling the holder with 10 blood samples they were passed on to the Technician who performed the tests. Samples were taken serially, making sure that no body jumped the queue.

Reconstitution of the Card Agglutination Test for Trypanosomiasis (CATT) Antigen

CATT/*T.b.gambiense* (Prince Leopold Institute of Tropical Medicine) field test kit was used. Using a syringe 2.5ml of CATT buffer (Phosphate Buffered Saline, pH 7.2) was added to a vial of freeze dried CATT antigen; 0.5ml of CATT buffer to a vial of the positive and negative controls. The vial was immediately shaken for a few seconds so as to obtain a homogenous suspension. After reconstitution of each vial of CATT antigen one drop of the positive and negative controls were tested to check the quality of the antigen.

Screening of Whole Blood

A ten-piece test card was used. One drop of blood from the capillary tube was placed on a test area of the card. This was repeated in the remaining nine test areas for each of the samples. One drop of the CATT antigen was placed on each test sample. Using a stirring rod, each mixture was mixed and spread out to about 1mm from the edge of the test area. The stirring rod was wiped clean after each use. The test card was placed on a flat bed orbital rotator and rotated for 5 minutes at 60 rpm. After the reaction the results were read before removing the card from the rotator. Whole blood CATT positive cases were further investigated by taking venous blood for plasma dilution.

3.5.4.2 Parasitological Survey in Pigs

Collection of Blood Samples

Each selected pig was restrained, the ear vein identified and the site cleaned with alcohol-soaked cotton swab. Using a sterile single-use blood lancet, the vein was pricked and two

heparinised haemocrit capillary tubes were filled with blood to about three quarter full (70µl). The tubes were then placed on jelly-seal holder with labels. The samples were put on ice and sent immediately to the Eastern Regional Veterinary laboratory for analysis using Buffy coat technique (Woo, 1970; Murray *et al.*, 1977).

Examination of Blood Samples by Buffy Coat Technique

The sealed heparinised capillary tubes with blood samples were placed in a microhaematocrit centrifuge with the sealed ends pointing towards outside. Centrifugation was at 9000 g for 5 minutes. After centrifugation, the capillary tubes were cut with a diamond tipped pencil, 1 mm below the Buffy coat, to include the top layer of Red Blood Cells (RBCs). The Buffy coat and the uppermost layer RBCs were then extruded on to a clean microscope slide and checked to be sure that the Buffy coats were not sticking to the capillary tubes. Cover-slips (22 × 22 mm) were placed on them and approximately 200 fields of the preparation were examined for the presence of motile Trypanosomes with a dark-ground microscope with a ×40 objective lens. Thin and thick blood smears (Woo, 1970) were prepared from the Buffy coat.

Determination of Packed Cell Volume (PCV)

The microhaematocrit capillary tubes were placed in a haematocrit reader to determine the packed cell volume (PCV). The length of the packed red blood cells column was expressed as a percentage of the total volume of blood.

Preparation of Thick Blood Smear

These 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, using the corner of another slide. 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 4% diluted Giemsa stain in phosphate buffered saline (PBS), pH 7.2. the manufacturer's directions were followed but with some variation to obtain optimal results. The stained smear was then washed with buffered water and examined at ×700 total magnification (OIE, 2008).

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 (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 4% 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 an x100 oil-immersion objective lens. After a trypanosome had been detected, approximately 20 extra fields were investigated to determine if more than one species was present (OIE, 2008).

3.5.5 Pre-testing of Questionnaire

The questionnaire was validated by pre-testing in a community outside, but similar to, the selected areas. Repeated questions were checked, ambiguities removed and complex questions simplified. This exercise was also used to strengthen the training for the field team.

3.5.6 Data Quality

Design of questionnaire was done with systematic checks to avoid errors. Collected data were entered into the computer within two days and checked for completeness and any internal inconsistencies were corrected. The raw data were edited and coded with numbers and were entered twice on spread sheet to check for mismatch and out-of-range errors.

Specimens were transported in a cold box on ice after collection and immediately examined on arrival. The rest of blood samples were stored at -70°C until required.

3.6 DATA PROCESSING AND ANALYSIS

We carried out initial exploratory analysis to obtain descriptive statistics in the form of frequencies and percentages using appropriate tables and charts. Maps were generated from the GPS coordinates to indicate the spatial distribution of flies, pigs and the communities participating in the study. Categorical or qualitative variables (e.g. biotope and disease status) were analysed using Chi-square test for possible associations between the dependent and the independent factors whilst Student t-test was used to compare

quantitative variables like the means of PCV of the parasitological positive and negative pigs. The fly catches and the mean apparent densities were not normally distributed and therefore non-parametric Kruskal Wallis test was applied to compare them statistically (Chan, 2003). The relationship between binary dependent and independent variables was expressed by binary logistic regression. Logistic regression was run for the analysis of infection status (outcome) and its predictor (explanatory) variables. We set statistical significance level at a p-value less than 0.05 or at 95% confidence interval (CI). Data was entered using EpiData version 3.1 and exported to Epi info version 3.5.1 and Statistical Package for Social Scientists (SPSS) version 16 for analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 SURVEY IN HUMANS

4.1.1 Age and Sex Distribution

Questionnaires were administered to 352 participants of whom 199 (56.5%) were females (Table 4.1). Their ages ranged from 3 months to 87 years with a mean of 37.0 years (SD=±19.8). Age group 11-20 years was the most frequent, 69 (19.6%) and the least were persons above 70 years 18 (5.1%) There was no age difference between the female and male participants (p=0.24).

Table 4.1: Age distribution of respondents, New Juaben municipality, 2011

Age Groups	Frequency		Total (Percent)	Mean (yrs)	Standard deviation	P - value
	Male	Female				
<10years	11	15	26 (7.4)			
11-20"	29	40	69 (19.6)			
21-30"	19	33	52 (14.8)			
31-40"	21	28	49 (13.9)			
41-50"	30	26	56 (15.9)			
51-60"	20	37	57 (16.2)			
61-70"	12	13	25 (7.1)			
>70"	12	6	18 (5.1)			
Total	**154**	**198**	**352 (100)**	**37.0**	**19.8**	**0.24**

4.1.2 Educational level of Participants

Table 4.2 shows that most of the participants 247 (70.1%) were either at basic school level or ended their education at that level and only 15 (4.3%) attained tertiary education. Females represented 130 (53.9%) of those at basic school level. However, the difference in educational level between the males and females was significant (χ^2=11.755; p<0.05).

Table 4.2 Highest educational level attained by sex of participants, New Juaben municipality, 2011

Educational level	Frequency		Total	Percentage	P-value
	Male	Female			
Basic	117 (46.1)	130 (53.9)	**247 (100)**	70.1	
Secondary	16 (51.6)	15 (48.4)	**31 (100)**	8.8	
Tertiary	7 (45.5)	8 (54.5)	**15 (100)**	4.3	
None and Don't know	14 (35.9)	45 (64.1)	**59 (100)**	16.8	
Total	**154 (43.8)**	**198 (56.2)**	**352 (100)**	**100**	0.008

4.1.3 Ethnicity

There was diversity of ethnic groupings which reflected diverse pre-occupations of the people Figure 4.3). The Akans represented the biggest group of 163 (46.3%) and the least 5 (1.4%) were the Grushi ethnic group.

Table 4.3 Distribution of Ethnic groups among the participants, New Juaben municipality, 2011

Ethnic Group	Frequency	Percentage
Akans	163	46.3
Guans	40	11.4
Ga/Adangme	82	23.3
Eves	51	14.5
Grushi	5	1.4
Others	11	3.1
Total	**352**	**100.0**

4.1.4 Occupation of Participants

Participants were engaged in more than one activity and each one was recorded (Figure 4.1). The most common occupation was crop farming which represented 154 (43.8%), whereas 60 (17.0%) were engaged in livestock farming. Salary workers formed the least group [17 (4.8)]

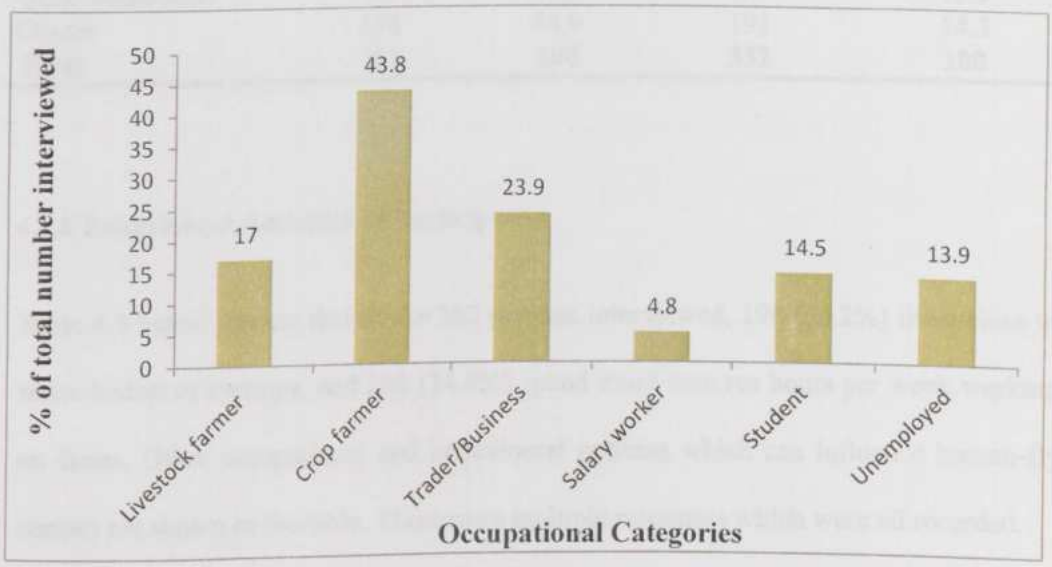


Figure 4.1 Distribution of occupational categories among participants, New Juaben municipality, 2011.

4.1.5 Sources of Income

Sources of income were recorded separately as main and supplementary sources since participants were engaged in multiple occupations (See Table 4.4 below). Respondents that used cash crops (cocoa, oil palm, citrus) as main source of income were about three times the number of those that derived their supplementary income from them. However, more people depended on livestock as supplementary source of income than as main source.

Table 4.4 Main and Supplementary sources of income of participants, New Juaben municipality, 2011

Sources of income	As main source	% main source	As supplementary	% supplementary
Cash crop	35	9.9	11	3.1
Food crop	58	16.5	57	16.2
Livestock/poultry	6	1.7	27	7.7
Agro-processing	9	2.6	11	3.1
Non-agric enterprise	45	12.8	17	4.8
Salary/wages	15	4.3	1	0.3
Gifts/remittances	26	7.4	37	10.5
Others	158	44.9	191	54.3
Total	**352**	**100**	**352**	**100**

4.1.6 Behavioural Activities of Participants

Table 4.5 below depicts that of the 352 persons interviewed, 198 (56.2%) lived close to water bodies or swamps, and 262 (74.4%) spend more than ten hours per week working on farms. Other occupational and behavioural patterns which can influence human-fly contact are shown in the table. There were multiple responses which were all recorded.

Table 4.5: Proportion of respondents engaged in behavioural activities/lifestyles that can influence human-fly contact, New Juaben municipality, 2011

Activity/Life style	% of Respondents
Living close to: pig-rearing sites	45.7
Water bodies or swamps	56.2
Plantations with canopies	83.8
Working on farm	74.4
Fetching water from riverside	35.8
Working close to water bodies	41.5
Working/resting without top dress	61.1
Fishing	4.0
Hunting	4.3

4.1.7 Community Knowledge of Trypanosomiasis

According to Table 4.6 below, out of the 352 persons interviewed, 269 (76.4%) had heard of Trypanosomiasis (sleeping sickness) and among them 232 (86.0%, n=269) mentioned humans and animals as hosts that are affected by the disease (Table 4.6). Awareness of Trypanosomiasis was similar among males and females ($p=0.056$). A majority (342 or 97.2%) of the respondents had heard of Tsetse fly and 328 (93.2%) had seen the fly. Even though 261 (74.4%) of the persons interviewed worked on farm, there was no significant association ($p>0.05$) between working on farm and encountering Tsetse fly. Of those who knew Tsetse fly, 216 (65.8%, n=328) linked it with wet and bushy environment. Those who knew that transmission is through Tsetse fly bite were 298 (84.6%).

The frequently mentioned symptoms of Trypanosomiasis were change in sleeping pattern 259 (73.6%), emaciation in humans 261 (74.1%), emaciation in pigs 261 (75.5%), abortion in humans 179 (50.9%), and abortion in pigs 169 (48.0%). Most of the respondents 254 (72.2%) said Trypanosomiasis can be treated. However, only about half of them [178 (50.6%)], knew the method used for its control.

Table 4.6 Community knowledge level of Trypanosomiasis, New Juaben municipality, 2011

Knowledge Area	Yes (%)§	No (%)§	Don't know (%)§
Heard of Tsetse fly	342 (97.2)	9 (2.6)	1 (0.3)
Ever seen Tsetse fly	328 (93.2)	23 (6.5)	1 (0.3)
Transmitted by Tsetse fly	298 (84.6)	5 (1.3)	49 (14.1)
Affects both humans and animals	280 (79.5)	35 (10.0)	37 (10.5)
Heard of Trypanosomiasis	269 (76.4)	82 (23.3)	1 (0.3)
Wet and bushy habitat	220 (62.5)	103 (29.3)	29 (8.2)
Signs and symptoms:			
• Emaciation in animals	264 (75.0)	17 (4.8)	71 (20.2)
• Emaciation in humans	261 (74.1)	23 (6.5)	68 (19.3)
• Change in sleeping pattern	259 (73.6)	16 (4.5)	77 (21.9)
• Abortion in humans	179 (50.9)	44 (12.5)	129 (36.6)
• Abortion in animals	169 (48.0)	46 (13.1)	137 (38.9)
Trypanosomiasis can be treated	254 (72.2)	51 (14.4)	47 (13.4)
Controlled by destroying Tsetse fly	178 (50.6)	147 (41.7)	27 (7.7)

§ = Figures in parenthesis are row percentages

4.1.8 Laboratory Analysis of Samples taken from Humans

4.1.9.1 Card Agglutination Trypanosomosis Test (CATT).

A total of 558 blood samples were taken using finger prick method. Four (0.72%) of these samples were weakly positive for the CATT test. Confirmatory test on 3 of them using two-fold dilution of venous blood from these subjects showed negative results (Table 4.7). Two (50%) of the CATT positives were females aged between 22 and 43 years. The former was a Seamstress and the later, a Trader. One of the male positive cases (a 3-month old baby) was being breastfed and the other was 8 year old pupil. Both female CATT-positive cases resided at Jumapo and the males in Kojotenten community.

Table 4.7: Results of Card Agglutination Trypanosomosis Test (CATT) for Human African Trypanosomosis (HAT), New Juaben municipality, 2011

Sub-municipality	Number tested	Sex		CATT		Dilution test		
		F	M	Neg.	Pos.	<1/4	=1/4	>1/4
Koforidua North	87	48	39	87	0	0	0	0
Koforidua South	116	56	60	114	2	0	0	0
Oyoko	35	22	13	35	0	0	0	0
Jumapo	69	38	31	67	2	0	0	0
Suhyen	45	34	11	45	0	0	0	0
Total	352	198	154	348	4	0	0	0
% Total	**100**	**56.2**	**43.8**	**98.9**	**1.1**	**0**	**0**	**0**

4.2 ENTOMOLOGICAL SURVEY

4.2.1 Fly Species and Apparent Density

A total of 2,141 Tsetse flies were caught in 37 trapping sites during the survey. The only Tsetse fly species was *Glossina palpalis palpalis*. The sites were grouped into four different biotopes (A, B, C, D) based on vegetation and other environmental characteristics. The trapping done over a four-day period resulted in daily mean fly catches shown in Table 4.8. The highest catch of 1496 (69.9%) was recorded at Biotope A (areas at cocoa or oil palm plantations with dense vegetation canopies) (Table 4.8). Day 2 to Day 3 recorded a reduction in fly catches.

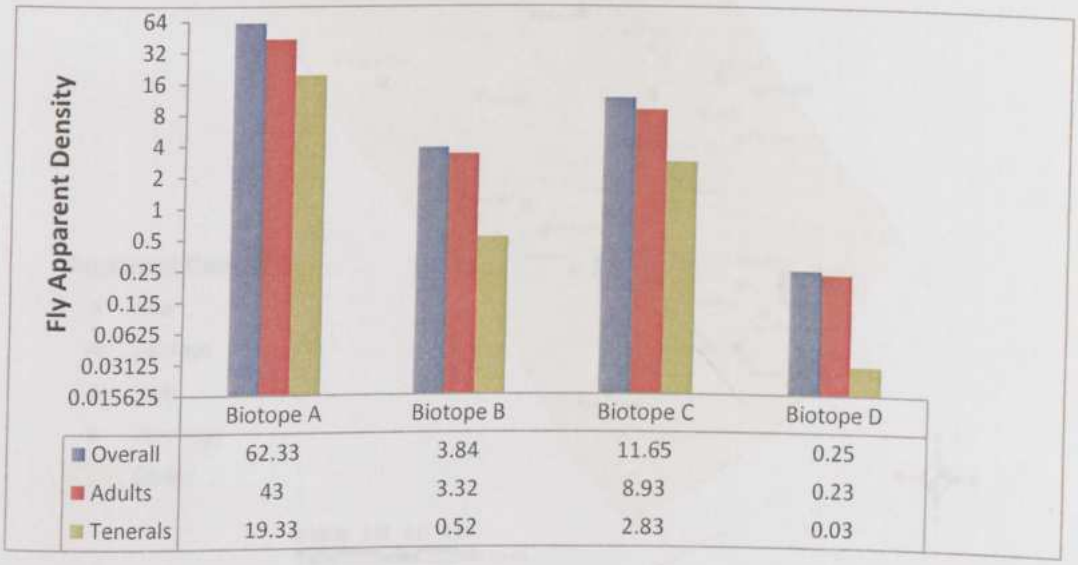
Table 4.8: Daily Tsetse catch in four different biotopes using bi-conical trap, New Juaben Municipality, 2010

Biotope*	Day 1	Day 2	Day 3	Day 4	Total	% Total	P value
A	289	390	340	477	**1496**	**69.9**	H Test
B	24	40	37	68	**169**	**7.9**	Statistics
C	80	119	100	167	**466**	**21.7**	= 16.006
D	0	4	1	5	**10**	**0.5**	
Total	**393**	**553**	**478**	**717**	**2141**	**100**	
% Total	**18.4**	**25.9**	**22.3**	**33.4**	**100**		

**Biotopes: A. Cocoa/Oil palm Plantation with dense canopy; B. Low-lying shrubs without canopy; C. Edge of communities; D. Residential areas within communities*

The fly mean apparent density (MAD) was calculated as average number of Tsetse flies caught per trap per day. It differed significantly (Kruskal Wallis test, $p<0.05$) among the biotopes. Figure 4.2 below illustrates the fly density of the adults and tenerals flies on a logarithmic scale. It clearly shows a very high concentration of teneral flies in the areas with the cocoa and oil palm plantations. The apparent densities were grouped into five

levels: <1 very low; 1-5 low; 6-10 average; 11-20 high; >20 very high. Apparent densities more than 100.0 were recorded in about 8% of the selected trapping sites, all of which were from communities in and around Jumapo. No fly was captured in 46% of the trapping site



	Biotope A	Biotope B	Biotope C	Biotope D
Overall	62.33	3.84	11.65	0.25
Adults	43	3.32	8.93	0.23
Tenerals	19.33	0.52	2.83	0.03

**Biotopes: A. Cocoa/Oil palm Plantation with dense canopy; B. Low-lying shrubs without canopy; C. Edge of communities; D. Residential areas within communities*

Figure 4.2: Fly Apparent Densities by Developmental status and Biotopes*, New Juaben municipality, 2010

Figure 4.3 below depicts the distribution of fly apparent density among the trapping sites and the sub-municipalities. Very high values were seen in the Jumapo sub-municipality.

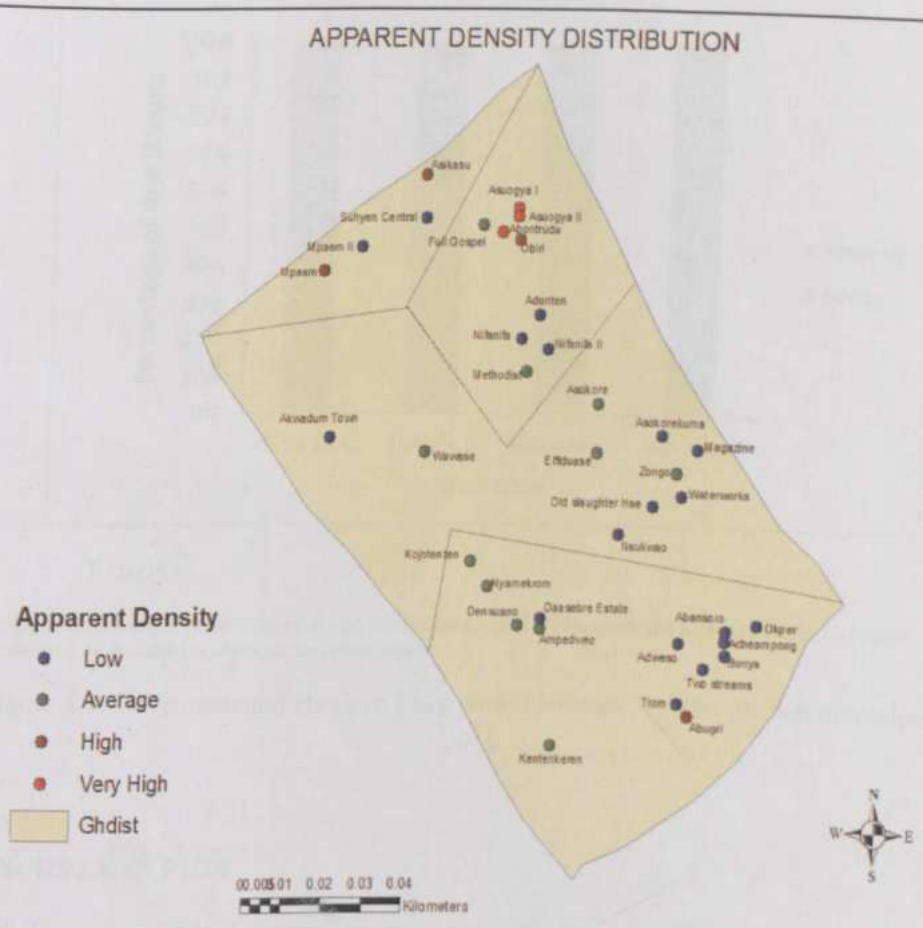
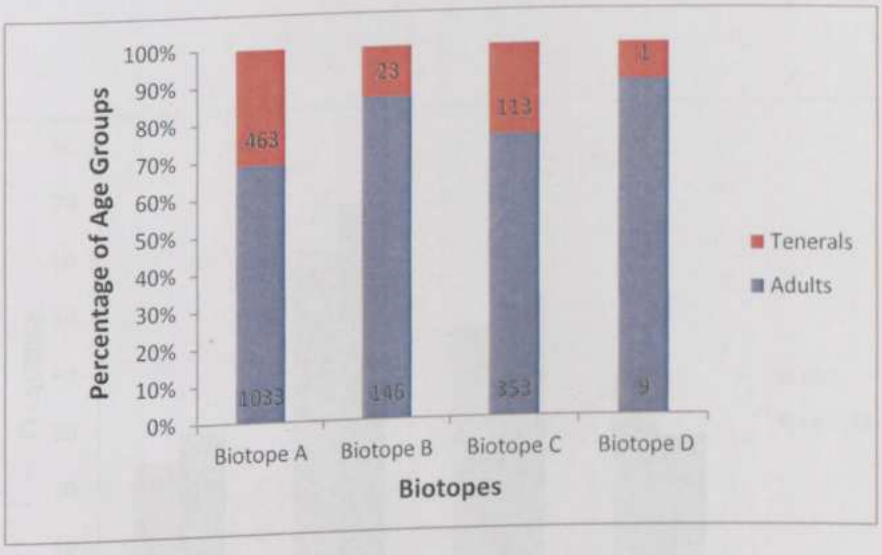


Figure 4.3 Distribution of Fly Apparent Density by trapping sites, New Juaben municipality, 2010

4.2.2 Fly Developmental status and Sex structure

The developmental status (teneral or adult) of the flies were significantly different (Kruskal Wallis test, $p<0.05$) in all biotopes. Biotope A (Cocoa/oil palm plantation with dense canopies) showed the highest infestation of teneral flies. In these areas, the tenerals constituted 31% of the fly population (Figure 4.4).



P<0.05

**Biotopes: A. Cocoa/Oil palm Plantation with dense canopy; B. Low-lying shrubs without canopy; C. Edge of communities; D. Residential areas within communities*

Figure 4.4: Developmental status of Flies within Biotopes*, New Juaben municipality, 2010

4.3 SURVEY IN PIGS

4.3.1 Descriptive Characteristics of Pigs

4.3.1.1 Breeds, Sex and Age

The findings revealed that of the 341 pigs that were sampled, 328(96.2%) were kept for commercial purposes. Most of the pigs 315 (92.4%) were crossbreeds between the Large White and Landrace (exotic) breeds. Female pigs accounted for 173 (50.7%) of the total pigs sampled. Four age groups identified were piglets (<2 months), weaners (2-5 months), growers (6-11 months) and finishers (12 months and above) (Figure 4.5).

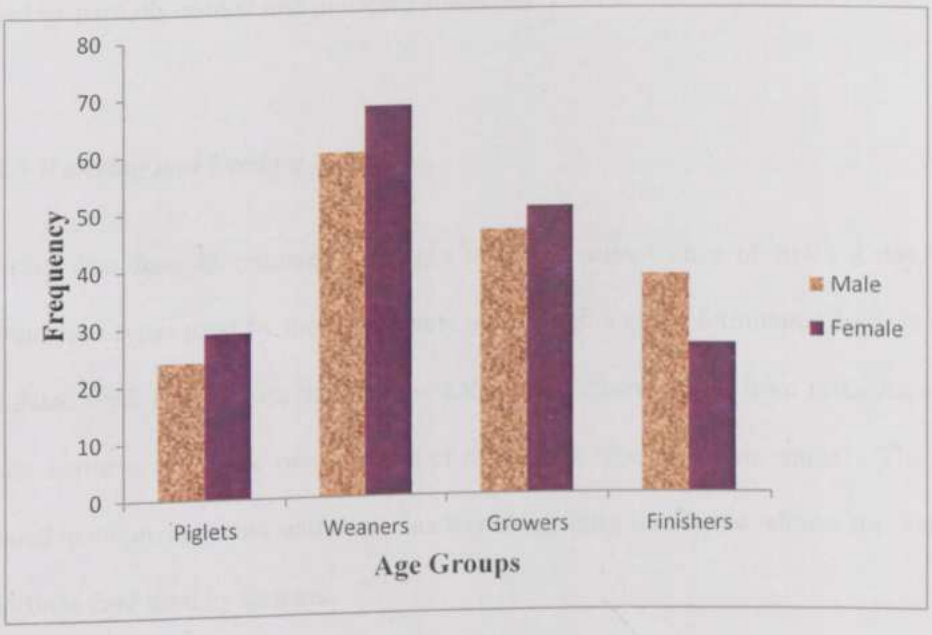


Figure 4.5: Age and Sex Structure of Pigs, New Juaben municipality, 2010

4.3.1.2 Housing

Two main husbandry systems were being practised in the municipality – intensive and semi-intensive. Pigs that were raised under intensive system of production were 302 (88.5%). Under these systems, 288 (85.4%) of the pigs were housed by structures made entirely from wooden boards, and 53 (14.6%) were housed by structures made from cemented walls of varying heights. The wooden structures had spaces in between the boards that could allow in flies. The cemented walls were however, solid. Three types of roofing of the pig houses were identified – fully-roofed, partially-roofed and unroofed.

The fully-roofed structures housed 131 (38.4%) of the pigs, and 105 (30.8%) were housed by partially-roofed and unroofed structures.

4.3.1.3 Watering and Feeding

Watering was done in removable troughs and was served once or twice a day. The different feed types used by the pig farmers were commercially formulated feed, farmer-formulated feed, peels (cassava, plantain, and yam), left-over food from restaurants and foliage. Farmers could use two or more of these feed types for their animals. The feed appeared poor in nutrients and were inadequate. Figure 4.6 below shows the various proportions feed used by farmers.

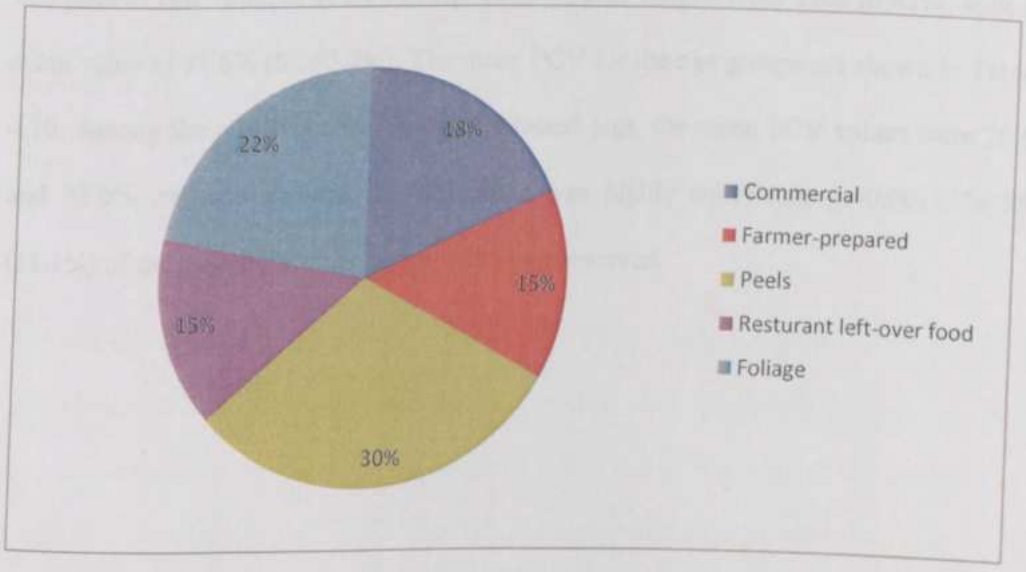


Figure 4.6: Type of Feed used for Pigs, New Juaben municipality, 2010

4.3.1.4 Farm Hygiene and Pig Health

Most of the pig farmers cleaned their pigsties once a day and wastes were disposed close to the sties which attracted more flies to them. Out of the 341 pigs sampled, 15 (4.3%) were protected from flies with mosquito nets put around the pigsties. A total of 26 (7.7%) pigs had received prophylactic treatment for Trypanosomiasis within the past twelve months and none had received curative treatment within the same period. Sixty-five (19.2%) of the sampled pigs were in various pigsties where abortions had ever occurred.

4.3.2. Laboratory Analysis on Samples taken from Pigs

4.3.2.1 Haematological Analysis

The packed cell volumes (PCV) of the sampled pigs ranged from 12% to 47%, with a mean value of 31.6% (SD=5.7%). The mean PCV for the age groups are shown in Table 4.10. Among the positively and negatively tested pigs, the mean PCV values were 28.5 and 35.0%, respectively and the difference was highly significant (p=0.001). In 38 (11.1%) of the pigs, PCV value below 25% was observed.

4.3.2.2 Parasitological Analysis

The overall prevalence of porcine Trypanosomiasis and the relative prevalence of different Trypanosome species for the age groups are shown in Table 4.9. The laboratory analyses identified three different species of Trypanosomes among the pig populations in the New Juaben municipality. *Trypanosoma vivax* was the most prevalent species, followed by *T. congolense* and *T. brucei*. Mixed infections of *T. vivax* and *T. congolense* were also recorded. The difference in relative prevalences was highly significant ($p<0.001$).

Table 4.9: Distribution of Trypanosome species by pig age groups, New Juaben municipality, 2011

Age Group (months)	Age Groups Frequency	Mean PCV	Positive by Buffy Coat	Trypanosome Species*			
				T.b	T.v	T.c	Mixed
Piglet (<3)	53 (15.5)	32.72	18 (34.0)	1	8	9	0
Weaner (3-5)	128 (37.5)	30.79	73 (57.0)	4	34	32	3
Grower (6-10)	96 (28.2)	31.84	61 (63.5)	7	29	20	5
Finishers (>10)	64 (18.8)	30.66	39 (60.9)	2	21	16	0
Total	**341 (100)**	**31.36**	**191 (56.0)**	**14(4.1)**	**92(27.0)**	**77(22.6)**	**8(2.3)**

**T.b. – Trypanosoma brucei; T.v – Trypanosoma vivax; T.c – Trypanosoma congolense*

The age-specific prevalence of Trypanosome infections varied significantly ($\chi^2=13.35$; $p=0.004$). The highest age-specific prevalence was seen in growers (Table 4.9). The relative prevalence of the various Trypanosome species among the age groups also showed significant difference ($p=0.03$). The most prevalent Trypanosome species in the piglets was *T. congolense*, and in the weaners, growers and finishers it was *T. vivax*. However, infection rates among male and female pigs were similar ($p>0.05$).

4.3.2.3 Porcine Trypanosomiasis in the Sub-municipalities

Figure 4.7 shows distribution of porcine Trypanosomiasis among the sub-municipalities where significant difference (χ^2=62.1; p<0.001) was observed in the prevalence. The highest prevalence [28/35 (80.0%)] was in Oyoko municipality.

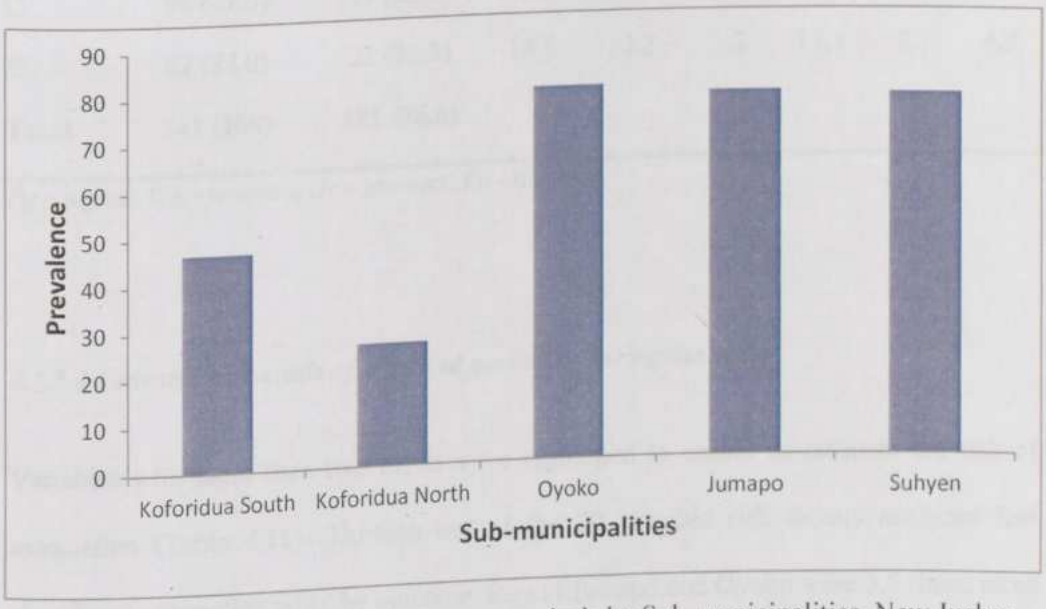


Figure 4.7 Prevalence of Porcine Trypanosomiasis by Sub-municipalities, New Juaben municipality, 2011

Table 4.10 shows the data on the distribution of Trypanosomiasis among the biotopes. With the exception of Biotope D, all the others recorded more than fifty percent prevalence and the difference was highly significant (χ^2=45.22; p<0.001).

Table 4.10: Distribution of Positively Tested Pigs by Biotopes in the New Juaben municipality, 2010

Biotopes	Pigs Frequency	Positive by Buffy Coat Technique	Pig Sex[α] (%)		Pig Age Groups[§] (%)			
			F	M	Pg	Wn	Gr	Fn
A	71 (20.8)	56 (78.9)	45.1	33.8	2.8	15.5	49.3	11.3
B	94 (27.6)	54 (57.4)	24.5	33.0	4.3	24.5	9.6	19.1
C	94 (27.6)	59 (62.8)	30.9	31.9	11.7	26.6	14.9	9.6
D	82 (24.0)	22 (26.8)	14.6	12.2	1.2	17.1	3.7	4.9
Total	**341 (100)**	**191 (56.0)**						

Pg – piglets, Wn – weaners, Gr – growers, Fn - finishers

4.3.2.4 Univariate analysis of observed variables during the study

Variables with more than two levels were regrouped to enable us estimate the risk of association (Table 4.11). Thirteen out of the 20 possible risk factors analysed had significant association with the outcome. Pigs at Jumapo and Oyoko were 3.5 times more likely than those elsewhere to be infected with Trypanosome (95%, CI:1.9–6.5) and (95%, CI:1.5-8.3). The findings of the study also showed that keeping pigs at areas with 'very low' fly apparent density is protective as compared to areas with higher fly apparent densities (95%, CI: 0.24-0.59). Similarly, piglets as compared to other age groups are significantly protected from Trypanosome infection.

Table 4.11 Univariate analysis of porcine Trypanosomiasis risk factors, New Juaben municipality, 2011

Independent Variables	Trypanosomes Frequency	Odd Ratio	Confidence Interval	P value
Sub-municipalities: (Pearson x^2= 62.105; p = 0.000)				
Suhyen	35 (77.8)	3.141	5.100 – 6.576	0.002
Jumapo	54 (78.3)	3.547	1.909 – 6.591	0.000
Oyoko	28 (80.0)	3.509	1.488 – 8.277	0.003
Koforidua North	20 (26.3)	0.196	0.111 – 0.347	0.000
Koforidua South	54 (46.6)	0.559	0.356 – 0.880	0.012
Biotopes: (Pearson x^2= 45.223; p = 0.000)				
Biotope D	22 (26.8)	0.195	0.113 – 0.339	0.000
Biotope C	59 (62.8)	1.146	0.902 – 2.391	0.121
Biotope B	54 (57.4)	1.084	0.671 – 1.751	0.742
Biotope A	56 (78.9)	3.733	2.013 – 6.924	0.000
Sex of pig	94 (56.0)	0.995	0.649 – 1.526	0.983
Age of Pig: (Pearson x^2= 13.352; p = 0.004)				
Adult	39 (60.9)	1.283	0.736 – 2.235	0.378
Grower	61 (63.5)	1.542	0.949 – 2.505	0.080
Weaner	73 (57.0)	1.069	0.687 – 1.663	0.769
Piglets	18 (34.0)	0.342	0.185 – 0.633	0.000
Fly Apparent Density: (Pearson x^2= 35.635; p = 0.000)				
FAD 'very high'	51 (79.7)	3.839	1.998 – 7.375	0.000
FAD 'high'	29 (80.6)	3.657	1.555 – 8.603	0.002
FAD 'average'	16 (57.1)	1.051	0.481 – 2.296	0.900
FAD 'low'	36 (49.3)	0.709	0.422 – 1.192	0.194
FAD 'very low'	59 (42.1)	0.381	0.244 – 0.594	0.000
Sanitation: (Pearson x^2= 19.766; p = 0.000)				
Cleaning of pigsty	65 (61.9)	0.346	0.215 – 0.557	0.000

4.3.2.5 Multivariate Analysis with Logistic Regression

To assess the effect of potential risk factors of Trypanosomiasis in pigs, logistic regression was used. The final regression model showed that sub-municipality, biotope and age of pig were factors that determined the occurrence of Trypanosome infections in the pigs (Table 4.12). At 95% confidence interval (CI), pigs in Suhyen as compared to those in Koforidua South were four times more likely to be infected with Trypanosome at p=0.001 significant level (OR=4.3). Similarly, pigs kept under cocoa and oil palm plantations with canopies compared to those kept at residential areas inside communities were three times more likely to be parasitological positive (95%, CI: 1.35-10.15; OR=3.7). However, piglets in the municipality as compared to finishers were protective (95%, CI: 0.07-0.51; OR=0.20).

Table 4.12 Logistic regression model for multivariate analysis

Factor	Coefficient	P Value	Odd Ratio	95.0% C.I. for OR	
				Lower	Upper
SubMunicipality		*.000*			
Koforidua North	-.704	.067	.494	.233	1.051
Oyoko	1.303	.019	3.679	1.239	10.925
Jumapo	1.398	.002	4.045	1.651	9.914
Suhyen	1.462	.001	4.316	1.784	10.438
Biotope		*.003*			
Biotope A	1.309	.011	3.703	1.351	10.149
Biotope B	1.239	.001	3.451	1.670	7.131
Biotope C	1.222	.004	3.395	1.476	7.811
PigAge		*.003*			
Piglets	-1.609	.001	.200	.078	.514
Weaners	-.196	.603	.822	.393	1.721
Growers	-.193	.638	.824	.369	1.843
Constant	-.745	.107	.475		

CHAPTER FIVE

5.0 DISCUSSIONS

Tsetse-transmitted Trypanosomiasis is a neglected tropical disease of zoonotic and public health importance in Africa. Recent outbreaks of Trypanosomiasis in pigs in the Eastern Region of Ghana have raised public health concerns. However, there is inadequate data and information for effective planning for control. In view of this, we assessed the situation of human and porcine Trypanosomiasis and the Tsetse fly vector in the New Juaben municipality of Eastern Region.

The present study detected only *Glossina palpalis palpalis*, and estimated Tsetse fly apparent density of 14.46 *Glossina*/trap/day and Trypanosome infection rate of 56% in pigs. However, sero-prevalence of only 0.7% was observed in the human populations. No Trypanosome parasite was detected in the blood samples obtained from humans. The community knowledge level of Trypanosomiasis was good.

In New Juaben municipality, *Glossina palpalis palpalis* was found to be the only Tsetse fly species. This result is similar to what was observed in Cameroon by 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 zone 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). Thus, the present findings of *G. palpalis* dominance in the municipality are more likely to be as a result of the continuous and vigorous land use that the area has been subjected to over the years.

The global fly apparent density of 14.46 recorded in the municipality was much higher than reported by Grebaut *et al.* (2004). In assessing the high-risk areas of Human African Trypanosomiasis in the Bipimbi focus in the forest zone of Southern Cameroun, these authors recorded fly apparent density of 2.3 *Glossina*/trap/day. This finding in Cameroun was made during the rainy season in a cocoa growing area similar to the area of the present study. The difference in fly apparent densities could be explained by the fact that Bipimbi is a long-standing HAT focus and a number of Tsetse fly control activities might have been implemented to keep the Tsetse fly populations low. Mahama *et al.* (2004) reported 1.8 fly apparent density in Savelugu District in the Northern Region of Ghana. They observed that this low figure could be attributed to vigorous human activities which have caused fragmentations and disappearance of habitats for Tsetse fly in that area.

The highest fly apparent density was recorded in Biotope A (Cocoa and oil palm plantations with dense vegetation canopies), followed by Biotope C (Areas at edge of communities). This is in agreement with findings from forest zone of Southern Cameroun by Grebaut *et al.* (2004), where the highest densities were recorded in the wettest

biotopes. This could be explained by the fact that cocoa and oil palm plantations, with their dense canopies are capable of providing the right conditions of humidity, temperature and feeding hosts for the maintenance of Tsetse fly populations.

The two biotopes (A and C) had something in common: the high concentration of pigs. This could play a role in attracting Tsetse flies considering the opportunistic feeding nature of the *G. palpalis* group (Clausen *et al.,* 1998). The high prevalence of teneral flies in Biotopes A and C indicates that these are reproduction sites of Tsetse flies. The areas are characterized by well-sheltered places with loose sandy to clayey alluvial soils which are very favourable for the development of the pupae of the Tsetse fly (Pollock, 1986).

Very low serological prevalence among the human population in the municipality was detected by CATT. When these weak positive cases were subjected to further blood dilution test for confirmation, they were proven negative. These findings are consistent with observations in the Upper West and Western regions of Ghana (Ghana Health Service, 2009). In these regions, using CATT/*T. b. gambiense* diagnostic method, 0.3 and 0.1% sero-prevalence of HAT respectively were recorded. However, they were all proven negative by further confirmatory test. It is possible to find false-positive results in patients with malaria and other parasitic diseases such as transient infection by non-human Trypanosomes (Miezan *et al.*, 1991). This study did not however, investigate the health history of the four persons found with positive serology. Confirmatory test using molecular methods with relatively higher sensitivity would possibly have generated different outcomes (Ghana Health Service, 2009).

The very low number of sero-positivity in humans did not allow a rated quantitative appreciation of the situation. However, most of the dwellers in Oyoko, Jumapo and Suhyen sub-municipalities were engaged in behavioural activities that could enhance human-fly contact. They lived close to pigs, cocoa and oil palm plantations and to water bodies and swamps. They also worked for long hours on farms and without top dresses. Analyzing the localization and frequency of human-fly contact, Laveissiere *et al.* (1986b), demonstrated that these human behavioural risk factors have correlation with human Trypanosomiasis. Even though the high fly apparent densities in the municipality might not have direct correlation with HAT infection (Sane *et al.*, 1999), it has been shown that the interaction which exists between behavioural risk factors and presence of the vector can influence HAT infection rates (Moore *et al.*, 1999).

The communities demonstrated a high level of knowledge of human and porcine Trypananosomiasis as well as of the vector. Similar results were observed by Sindato *et al.* (2008), when they investigated factors influencing individual and community participation in the control of Tsetse fly and HAT in Urambo district in Tanzania. This surprisingly high level of knowledge in the New Juaben municipality could be a manifestation of an impact of recent increased activities of Veterinary Services Department (VSD) in that part of the region. Since the detection of porcine Trypanosomiasis outbreaks and increased Tsetse fly bites on humans at Oyoko in the year 2006, VSD has intensified health and veterinary education on Trypanosomiasis in the municipality. This package of knowledge is seen as great asset for the community with respect to the control of Tsetse fly and Trypanosomiasis in the municipality. In such

programmes, community participation and ownership are essential elements for sustainability. In their study in Tanzania, Sindato *et al.* (2008) demonstrated that knowledge in HAT, the vector and its control measures could increase a community's willingness to contribute labour and money to control programmes. Even in the absence of special control programmes, such knowledge could also become useful when applied to personal preventive strategies against Trypanosomiasis.

Trypanosomosis in pigs has not been given the necessary attention as has been given to the disease in domestic ruminants. Few studies that have been conducted indicated different infection rates of porcine Trypanosomiasis. The findings of this study in the New Juaben municipality corroborate with the observations by Mehlitz (1979). This author revealed by the haematocrit centrifugation technique (HCT) a prevalence of 56.4% in Liberia. However, the result is lower than 73.7% reported in Cameroun by Simo *et al.* (2006), but much higher than the 4% and 3.5% prevalence observed by Boniface *et al.* (2011), and Biryomumaisho *et al.* (2009), respectively. The main reason for these differences may be that in their study, Simo *et al.* (2006) used molecular methods in the parasite detection which increased the sensitivity of the test.

The high Trypanosomiasis prevalence in the present study could be attributed to the production system under which these pigs were kept. The farmers practice intensive system where pigs are confined in sties with provision of water and feed. It was observed that the housing units were made of wooden boards with spaces that allowed flies to have access to the pigs. The feeding and watering were poor in terms of quality and quantity

and this can affect the immuno-competency of the pigs. The dung was heaped close to the sties thereby attracting more flies. All these factors are known to increase the tsetse challenge on the pigs. The results of the present study show that Trypanosomiasis in Ghana is important not only in cattle but also in the pigs where even higher prevalence could be recorded.

There was an association between age of the pigs and the Trypanosome infection rate, however, its significance was largely contributed by piglet age group (χ^2=13.35, p=0.004). The study shows that keeping piglets was significantly protective as compared to the other age groups. This result was in agreement with the observations by Shimelis *et al.* (2005) in Ethiopia. In their study, they reported 17.2% Trypanosome infection rate in adults as against 11.0% in younger age group. This comparatively low prevalence of Trypanosomiasis, observed in young pigs has previously been explained by either an inherent resistance to Trypanosome infections in young animals or Tsetse feeding preferences for adult pigs due to size and olfactory cues (Vale, 1974). However as animals remain infected unless treated, or could be re-infected after auto-recovery, the higher prevalence of Trypanosomiasis observed in adult pigs in the present study may simply be a result of older pigs having been exposed to tsetse for a longer time-span and thus having a higher cumulative risk of infection.

The findings of this study show that *T. vivax* was the most prevalent in the pigs. The result was in agreement with a study by Ng'ayo *et al.* (2005) in Western Kenya in which they intended to determine the animal reservoirs of human sleeping sickness. In their

study, of the ten pigs infected with Trypanosomes, three carried *T. brucei* and five had *T. vivax*. The reason for high *T. vivax* infection rate could be that in addition to the cyclical transmission by tsetse flies, there can be mechanical transmission of *T. vivax* by other biting flies in the municipality (Aning, 1993). However, the predominance of *T. vivax* in our findings is contrary to other studies where *T. brucei* infections other than *T. vivax* were predominant in the pig populations (Beatrix *et al.*, 2011) elsewhere in West Africa. Prevalence rates of 38.0% and 40.0% porcine Trypanosomiasis were due to *T. brucei* in Liberia (Mehlitz, 1979) and Cameroun (Simo *et al.*, 2006) respectively.

The presence of the *T. brucei* group in pigs in the municipality poses a serious public health risk in view of the fact that pigs are potential reservoirs for *T. brucei* sub-species infective for humans (Waiswa *et al.*, 2003b; Simo *et al.*, 2006). It has been demonstrated that this Trypanosome conserves its infectivity to human even after 10 cyclical transmissions in pigs (Van Hoof *et al.*, 1947). In addition, these authors reported that the transmissibility index of *T. b. gambiense* group 1 is increased when this parasite is transmitted to different hosts (Van Hoof *et al.*, 1947). Therefore, the infectivity of *T. b. gambiense* group 1 for human is enhanced during human-fly-pig transmission cycle. The *G. palpalis* group, which has proven to be a very good transmitter of *T. brucei* (Hoare, 1972), was the sole Tsetse fly species identified in the municipality with very high catches. This could thus increase the risk for humans. Nevertheless, this study did not go to the sub-species level which could be done by using molecular methods with high specificity.

Twenty-seven percent of the pigs were infected with *T. congolense* group. This result is in accord with those found in Liberia by Mehlitz (1979). The pathogenic *T. congolense* group of parasites consists mainly of *T. congolense, T. simiae* and *T. godfreyi,* but the method employed in the study could not differentiate between these species. We could probably rule out the presence of *T. simiae* in these pigs because, despite the high parasitaemia observed in the animals, no severe symptoms of Trypanosomiasis and deaths were seen, phenonmeno which is characteristic of *T. simiae* infections. Pigs are known to be very susceptible to *T. simiae,* and previous findings have shown that, except in cases of *T. simiae* infections, pigs infected by *T. congolense* or/and *T. brucei* are often asymptomatic (Ilemobade and Balogun, 1981).

The highest prevalence of Trypanosome infections (78.9%) was seen in Biotope A (areas at cocoa and oil palm plantations with vegetation canopy) and the difference in prevalence among the biotopes was significant (χ^2, $p<0.001$). The difference in infection rate could be as a result of difference in fly apparent density which was five to ten-fold higher than the other biotopes. Similar observations were made by Shimelis *et al.* (2005) in Ethiopia. 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 packed cell volumes (PCV) of the positively and negatively tested pigs were statistically different (χ^2, $p<0.001$). This result is 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 parasitemic animals is reported by several authors (Leak, 1987). Similarly, Van den Bossche (2000) reported that the regression analysis of herd average PCV of parasitologically positive herds showed a decrease with the increasing prevalence of Trypanosome infection. Animal African Trypanosomiasis (AAT) control aims at reducing the prevalence of infection with a concomitant increase in the herd average PCV (Bauer, 2001). Therefore, the knowledge of the relationship between the prevalence of Trypanosome infection and herd average PCV could be a useful tool to assess the impact of control interventions. However, the herd average PCV is affected by factors other than Trypanosomiasis. These confounding factors are not always identifiable but they are likely to affect both Trypanosomiasis positive and negative animals.

Other factors considered to affect PCV values in animals in the New Juaben municipality were helminthiasis, tick-borne diseases and nutritional imbalances. There will be a need for further studies to address these factors.

Our findings indicated that, the major factors that contributed to porcine Trypanosomiasis in the New Juaben municipality are the sub-municipality in which the pigs were raised, the biotope and the age of the pigs.

5.1 LIMITATIONS

The study was conducted during the rainy season and there were intermittent rains during some of the Tsetse fly trapping days. This could cause low catches of the flies.

We were unable to follow-up the serological CATT/*T. gambiense* test with a molecular test on the human blood samples. The parasitological test on human blood sample was therefore, not done.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The results showed that *Glossina palpalis palpalis* is the only Tsetse species with fly apparent density of 14.46 *Glossina*/trap/day. The preferred habitat of the Tsetse flies was the cocoa and oil palm plantations with dense canopies. However, they were also attracted to peri-domestic environment.

The prevalence of Trypanosmiasis in the pigs wass found to be 56.0%, and the parasites pecies identified were *T. vivax, T. congolense* and *T. brucei* groups. Porcine Trypanosomiasis was more prevalent in Suhyen, Jumapo and Oyoko sub-municipalities and the predominant trypanosome species was *T. vivax* group. Feeding, housing and farm sanitation practices were generally poor among the pig farmers.

Trypanosomiasis sero-prevalence of 0.7% was detected among the people in the New Juaben municipality using CATT/*T. gambiense* test. The behavioural activities of the community members enhanced human-fly contact. However, the knowledge level of the people as far as Trypanosomiasis and its vector were concerned was quite good. This knowledge could be tapped for community-based control measures.

With the presence of the *T. brucei* group 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 in the municipality.

6.2 RECOMMENDATIONS

Based on the findings of this study in the New Juaben municipality, the following are recommended:

- Municipal Assembly should take leadership role and with stakeholders, initiate an integrated control of tsetse and Trypanosomiasis in the municipality
- Ghana Health Service (GHS) and Veterinary Services Directorate (VSD) should intensify health education and awareness creation on Human African Trypanosomiasis (HAT) and Animal African Trypanosomiasis (AAT) in the New Juaben municipality and the Eastern Region as a whole.
- A robust integrated surveillance system for HAT and AAT, where the zoonotic nature of the disease will be emphasized, should be established by GHS/VSD and made operational not only in the municipality but in the entire Eastern Region.
- Veterinary Services Directorate should intensify surveillance on other opportunistic ecto-parasites and endo-parasites in the area.
- Human resource capacity building on Trypanosomiasis both in the Health and Veterinary sectors should be considered priority by GHS and VSD
- Laboratories should be well equipped to take up the challenge of human and porcine Trypanosomiasis in the municipality
- The study should be replicated in the other districts for baseline data, and further research done to include speciation and risk factors in animals.

REFERENCES

Affognon, H. D. Economic analysis of trypanocide use in villages under risk of drug resistance in West Africa. PhD Thesis, University of Hannover, 2007.

Allsopp, R. Options for vector control against trypanosomiasis in Africa. *TRENDS in Parasitology.* (2001) **17**-1.

André, G., Vincent, J., Eddy, M., Claude, L.,Veerle, L., N'Guessan, P., N'Dri, L., Nestor, Van M. and Philippe, B. Follow-up of Card Agglutination Trypanosomiasis Test (CATT) positive but apparently aparasitaemic individuals in Côte d'Ivoire: Evidence for a complex and heterogeneous population. *Tropical Medicine and International Health,* (2000) **5**: 11; 786–793.

Aning, K. G. The overgrazing pressures on land can be shared by the forest zone – trypanosomosis is not the impediment. A paper presented at the *19th Annual Ghana Veterinary Medical Association Congress*, Atlantic Hotel, Takoradi 6-8 October, 1993.

Asonganyi, T., Hengy, C., Louis, J.P., and Ghogomu, N.A. Reactivation of an old sleeping sickness focus in Mamfe (Cameroon): epidemiological, immunological and parasitological finding. *Revue Epidemiologie et Sante Publique.* (1991) **39**; wq55–62.

Balyeidhusa, A.S.P., Enyaru, J.C.K., Matovu, E., Nerima, B., Akol, M. and Sebikali, C. Molecular differential diagnosis of African trypanosomosis in domestic animals in North-West Uganda, in *Proceedings of the 11th International Congress of Parasitology*, Glasgow, Medimond, (2006) 7–12.

Bauer, B., Gitau, D., Oloo, F.P., and Karanja, S.M. Evaluation of a preliminary title to protect zero-grazed dairy cattle with insecticide-treated mosquito netting in Western Kenya *Tropical Animal Health and Production* (2006) **38**: 29–34

Beatrix, von W., Noreen, M., Kim, P., Fèvre, E. M., Barend, M. de C. B., Ian, G. H. and Susan, C. W. Factors Associated with Acquisition of Human Infective and Animal Infective Trypanosome Infections in Domestic Livestock in Western Kenya. *PLoS Neglected Tropical Disease.* 2011 January; **5(1)**: 941

Biryomumaisho, S., Melville, S.E., Katunguka-Rwakishaya, E. and Lubega, G.W. Detection of natural *Trypanosoma vivax* infections in pigs with microhaematocrit centrifugation and amplification of ITS1 rDNA. *Onderstepoort Journal of Veterinary Research,* (2009) **76**:285–289

Boniface, M, A., Augustine, O. I., Ikechukwu, A. I. and Paschal, U. U. Prevalence and haemato-biochemical parameters of trypanosome-infected pigs at Nsukka, Nigeria. *Comparative Clinical Pathology* (2011) **20**:15–18

Brightwell, R., Dransfield, R.D., Korku, C.A., Golder, T.K., Tarimo, S.A. and Mugnai, D. A new trap for *Glossina pallidipes*. *Tropical Pest Management*, (1987) **33**: 151-159.

Burri, C., and Brun, R. Human African trypanosomiasis, *In* G. C. Cook and Z. Alimuddin (ed.), *Manson's Tropical Diseases*, 21st ed. Elsevier Science, Edinburgh, United Kingdom, (2003) 1303–1323.

Chan, Y. H. Basic Statistics for Doctors. *Singapore Medical Journal*, (2003) **44(8):** 391-396

Chappuis, F., Loutan, L., Simarro, P., Lejon, V. and Buscher, P. Options for Field Diagnosis of Human African Trypanosomiasis. *Clinical Microbiology Reviews*, (2005) **18:** 133–146.

Clausen, P. H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F. and Taak, C . S. Host preferences of tsetse (Diptera: *Glossinidae*) based on bloodmeal identifications *Medical and Veterinary Entomology* (1998) **12**: 169–180.

Dankwa, D. A preliminary report on the prevalence of trypanosomes in cattle in the Eastern region of Ghana. *Paper presented at the 6th Annual Ghana Society of Animal Production*, University of Cape Coast 22-25th August, 1993.

FAO, Basic Biology and Anatomy of the Tsetsefly, Volume 1 of the *FAO training manual for tsetse control personnel.* FAO, 1982a.

Fe`vre, E.M. A burgeoning epidemic of sleeping sickness in Uganda. *Lancet* (2005) **366:** 745–747

Ghana Health Service, Presentation on Human African Trypanosomiasis (HAT) 18 – 21 Nov. 2009

Ghana Statistical Survey Department, 2000.

Gouteux, J.P., Eouzan, J.P. and Noireau, F. Modification du comportement de Glossina palpalis palpalis lie´ a` l'e´pizootie de peste porcine de 1982 dans un village congolais. *Acta Tropica.* (1987) **44**: 333–337)

Grebaut, P., Mbida, J.A., Antonio, K.C., Njiokou, F., Penchenier, L., and Laveissiére, C. Spatial and Temporal Patterns of Human African Trypanosomosis (HAT) Transmission Risk in the Bipindi Focus, in the Forest Zone of Southern Cameroon *Vector-Borne and Zoonotic Diseases* (2004) **4**: 3

Hoare, C. A. The trypanosomes of mammals, a zoological monograph. Blackwell Scientific Publications, Oxford, England. 1972.

Ilemobade, A. A. and Balogun, T. F. Pig trypanosomiasis: Effects of infection on feed intake, live weight gain and carcass triats. *Tropical Animal Health and Production.* (1981) **13**: 128–136.

Jannin, J., Penchenier, L. and Eozenou, P. Recrudescence actuelle de la trypanosomiase humaine dans le foyer de la Shanga (Cuvette) au Congo. *Bulletin de la Socie´te´ de Pathologie Exotique* (1992) **85**: 31–38

Katende, J.M., Musoke, A.J., Nantulya, V.M. and Goddeeris, B.M.. A new method for fixation and preservation of trypanosomal antigens for use in the indirect immunofluorescence antibody test for diagnosis of bovine trypanosomiasis. *Tropical Medicine and Parasitology*, (1987) 38: 41–44.

Khonde, N., Pe´pin, J., Niyonsenga, T. and De Wals, P. Familial aggregation of *Trypanosoma brucei gambiense* trypanosomiasis in a very high incidence community in Zaire. *Transactions of the Royal Society of Tropical Medicine and Hygiene* (1997) **91**: 521–524.

Kinung'hi, S.M., Malele, I.I., Kibona, S.N., Matemba, L.E., Sahani., J.K., Kishamawe, C. and Mlengeya, T.D.K. Knowledge, attitude and practices on tsetse and sleeping sickness among communities living in and around Serengeti National Park. *Tanzania Health Research Bulletin* (2006) **8:** 168-176.

Krafsur, E.S. Tsetse flies: Genetics, evolution, and role as vectors. Review. *Infection, Genetics and Evolution* (2009) **9**: 124–141

Laveissie`re, C., Hervouet, J. P. and Couret, D. Localisation et fre´quence du contact homme/glossine en secrteur forestier de Coˆ te d'Ivoire. (2) Le facteur humain et la transmission de la trypanosomiase. Cahier ORSTOM, *Se´rie Entomologie Me´dicale et Parasitologie* (1986b) **24:** 45–57

Laveissière, C. and Grebaut, P. The trapping of tsetse flies (Diptera: *Glossinidae*). Improvement of a model: the Vavoua trap. *Tropical Medicine and Parasitology*, (1990) **41**:185-192.

Laveissie`re, C., Gre´baut, P. and Lemasson, J. J. Les communaute´s rurales et la lutte contre la maladie du sommeil en fore^t de Co^te d'Ivoire. *WHO, Geneva. WHO/TRYP/* (1994b) **94:** 166.

Leak, S.K.A., Woume, K.A., Colardelle, C., Duffera, W., Feron, A., Mulingo, M., Tikubet, G., Toure, M. and Yangari G., Determination of tsetse challenge and its relationship with trypanosomosis prevalence. In: *Livestock production in tsetse infested areas of Africa*. Nairobi, Kenya, *ATLN,* (1987) 43-52.

Leak, S.G.A., Collardelle, C., Coulibaly, L., Dumont, P., Feron, A., Hecker, P., d'leteren, G.D., Jeannin, P., Minengu, M., Minja, S., Mulatu, W., Nankodaba, G., Ordner, G., Rowlands, G.J., Sauveroche, B., Tikubet, G. and Trail, J.C.M., Relationships between tsetse challenge and trypanosome prevalence in trypanotolerant and susceptible cattle. *Insect Science Applied*, (1990) **1**: 293-299.

Leak, S.G.A. Tsetse biology and ecology: Their role in the epidemiology and control of trypanosomosis. Wallingford, UK, CABI Publishing and ILRI, (1990) 152-210.

Losos, G.J. Infectious tropical diseases of domestic animals. In *Protozoal diseases.* Avon, UK: The Bath Press. 1986.

Magnus, E., Vervoort, T. and Van Meirvenne, N. A card agglutination test with stained trypanosomes for serological diagnosis of *Trypanosoma brucei gambiense* trypanosomiasis. *Annales de la Société Belge de Médecine Tropicale* (1978) **58**: 169–176.

Magona, J. W., Kakaire, D. W. and Mayende, J. S. P. Prevalence and distribution of animal trypanosomosis on Buvuma Islands in Lake Victoria, Uganda. (1999).

Mahama, C.I., Mohammed, H. A., Abavana, M. Sidibé, I., Koné, A. and Geerts, S. Tsetse and Trypanosomoses in Ghana in the Twentieth Century: a Review. *Revue d'Elevage Medicine Veterinaire des Pays Tropicaux,,* (2003) **56 (1-2)** : 27-32

Mahama, C. I., Desquesnes, M., Dia, M. L., Losson, B., De Deken, R. and Geerts S., A cross-sectional epidemiological survey of bovine trypanosomosis and its vectors in the Savelugu and West Mamprusi districts of northern Ghana. *Veterinary Parasitology* (2004) **122:** 1-13

Majiwa, P.A.O., Masake, R.A., Nantulya, V.N., Hamers, R. and Matthyssons, G. *Trypanosoma congolense*: identification of two karyotypic groups. *EMBO Journal* (1985) **4**:3307-3313

Mathurin, K., Thierry, De M., Bruno, B., Philippe, S., Mamadou, C., Dramane, K., Ge´rard, C., Francisco, J. A. and Vincent, J. Population genetics of *Trypanosoma brucei gambiense*, the agent of sleeping sickness in Western Africa. *Proceedings of the National Academy of Sciences.* January 6, (2009) **106: 1**; 209–214

Maudlin, I., Dukes, P., Luckins, A. G. and Hudson, K. M. Extrachromosomal inheritance of susceptibility to trypanosome infection in tsetse flies. II. Susceptibility of selected lines of *Glossina morsitans morsitans* to different stocks and species of trypanosome. *Annals of Tropical Medicine and Parasitology* (1986) **80:**97-105

Mboera, L.E.G., Rumisha, S.F., Senkoro, K.P., Mayala, B.K., Shayo, E.H. and Kisinza, W.N. Knowledge and health information communication among communities living in and around Serengeti National Park in Tanzania. *Tanzania Health Research Bulletin (*2007) **8**: 168-176.

Mehlitz, D. Trypanosome infections in domestic animals in Liberia. *Tropenmedizin und Parasitologie.* (1979) **30:** 212–219

Mehlitz, D., Zillmann, U., Scott, C.M. and Godfrey, D.G. Epidemiological studies on the animal reservoir of gambiense sleeping sickness. 3: Characterization of Trypanozoon stocks by isoenzymes and sensitivity to human serum. *Tropenmedizin und Parasitologie* (1982) **33**: 113–118.

Mehilitz, D. Le reservoir animal de lad maladie du sommeil a *T.b. gambiense. Etudes et syntheses de I'IEMVT,* (1986) **18**: 156X

Miezan, T., Doua, F., Cattand, P. and de Raadt, P. Evaluation of Testryp CATT applied to blood samples on filter paper and on diluted blood in a focus of trypanosomiasis due to *Trypanosoma brucei gambiense* in the Ivory Coast. *Bulletin of World Health Organization.* (1991) **69**:603–606.

Ghana Meteorological Agency, 2009.

Mihok, S., Moloo, S.K., Oden'y, J.O., Brett, R.A., Rakwar, J.G., Munyoki, E., Kiilu, J. and Kyorku, C.A. Attractiveness of black rhinoceros (*Diceros bicornis*) to tsetse flies (*Glossina* spp.) (Diptera: *Glossinidae*) and other biting flies. *Bulletin of Entomological Research*, (1996) **86**: 33–41.

Moloo, S. K., and Kutuza, S. B. Comparative study on the infection rates of different laboratory strains of *Glossina* species by *Trypanosoma congolense. Medical and Veterinary Entomology* (1988) **2**:253-257

Moore, A., Richer, M., Enrile, M., Losio, E., Roberts, J. and Levy, D. Resurgence of sleeping sickness in Tambura county, Sudan. *American Journal of Tropical Medicine and Hygiene* (1999) **61**: 315–318.

Morris, K. R. S. The control of trypanosomiasis by entomological means. *Bulletin of Entomological Research*, (1947) **37**: 201-247.

Morris, K. R. S. and Morris, M. G. The use of traps against tsetse flies in West Africa. *Bulletin of Entomological Research*, (1949) **39**: 501-531

Murray, M., Murray, P.K. and Mcintyre, W.I.M. An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* (1977) 71: 325–326.

Nantulya, V. M., Musoke, A. J., Rurangirwa, F. R., Saigar, N. and Minja, S. H. Monoclonal antibodies that distinguish *Trypanosoma congolense, T. vivax* and *T. brucei. Parasite Immunolgy*, (1987) **9:4**; 421-431

New Juaben Municipal Health Directorate, 2010. Verbal communication

Ng'ayo, O. M., Zablon, K. N., Eucharia, U. K., Geoffrey, M. M., Ellie, O. O. and Daniel, K. M. Detection of trypanosomes in small ruminants and pigs in western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology and Disease* (2005) **4**:5

Nkinin, S. W., Njiokou, F., Penchenier, L., Grebaut, P., Simo, G. and Herder, S. Characterization of *Trypanosoma brucei* s.l. subspecies by isoenzymes in domestic pigs from the Fontem Sleeping Sickness focus of Cameroon. *Acta Tropica* (2002) **81(3):** 225 – 232

Offori, E. D., Tsetse flies in Ghana. *Ghana Journal of Science*. (1964) **4**: 1-8.

Okoth, J.O. and Kapaata, R. Trypanosome infection rates in *Glossina fuscipes fuscipes* Newst. in the Busoga sleeping sickness focus, Uganda. *Annals of Tropical Medicine and Parasitology* (1986) **80:** 459–461

Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) Action Plan, June 2001

Paris, J., Murray, M. and Mcoimba, F. A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomosis in cattle. *Acta Tropica* (1982) **39:** 307-316

Penchenier, L., Grebaut, P., Njokou, F., Eboo, V. E. and Bu¨scher, P. Evaluation of LATEX/T.b. gambiense for mass screening of *Trypanosoma brucei gambiense* sleeping sickness in Central Africa. *Acta Tropica* (2003) **85**:31–37.

Picozzi, K., Fevre, E. M. and Odiit, M. Sleeping sickness in Uganda: a thin line between two fatal diseases. *British Medical Journal,* (2005) **331**:1238–1241.1

Plucknett, D.L. and Smith, M.J.H. Livestock Prod in Tsetse affected areas of Africa. Proceedings of meeting, by ILCA/ILRAD in Nairobi, Kenya (1987) 23-27

Pollock, J.N. Training Manual for Tsetse Control Personnel, Vol. 1*: Tsetse biology, systematics and distribution; techniques.* FAO Corporate Document Repository, 1986

Sindato, C., Kimbita, E.N. and Kibona S.N. Factors influencing individual and community participation in the control of tsetse flies and human African trypanosomiasis in Urambo District, Tanzania *Tanzania Journal of Health Research* (2008) **10**: 1.

Späth, J. Olfactory attractants for West African tsetse flies, Glossina spp. (Diptera: *Glossinidae*). *Tropical Medicine and Parasitology*. (1995) **46**:253-257.

Statistical Services Department, Ghana, 2000 Population Census.

Stephen, L.E. *Trypanosoma vivax* Trypanosomiasis: a veterinary perspective, 1st ed. Oxford: Pergamon Press. 1986.

Swallow, B.M. Impacts of African Animal Trypanosomosis on Migration, Livestock and Crop Production. ILRI (International Livestock Research Institute), (1998) 19.

Toure, S.M. Diagnostic des trypanosomiases animales. *Revue d'Elevage Medicine Veterinaire des Pays Tropicaux*, (1976) 30: 1–10.

TTCU, Country Report: Ghana. In: *Proc. International Scientific Council for the Research and Control of Trypanosomiasis,* Maputo, Mozambique, (1997): 82-87.

TTCU, Progress report on tsetse and trypanosomiasis research in Ghana. Pong Tamale, Ghana, Veterinary Services Department, (2001) 20.

Vale, G. A. Proceedings: Attractants for controlling and surveying tsetse populations. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* (1974) **68**:11.

Van den Bossche, P. The development of a new strategy for the sustainable control of bovine trypanosomiasis in Southern Africa. Thesis, University of Pretoria, Pretoria, South Africa, 2000.

Van Hoof, L., Observations on trypanosomiasis in the Belgian Congo. 2. Variation in virulence and pathogenicity for man and animals. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* (1947) **40**: 743–751

Veterinary Services Department, Annual Report. VSD – Ghana, 1990

Veterinary Services Directorate, Annual Report. VSD, Ghana, 2009

Waiswa, C., Olaho-Mukani, W., and Katunguka-Rwakishaya E. Domestic animals as reservoirs for sleeping sickness in three endemic foci in south–eastern Uganda. *Annals of Tropical Medicine and Parasitology*, (2003) 97: 2, 149–155

Weitz, B. The feeding habits of *Glossina*. *Bulletin of the World Health Organization*, (1963) **28**, 711–729.

Wendy, G. Species concepts for trypanosomes: from morphological to molecular definitions? *Kinetoplastid Biology and Disease* (2003) **2**: 2-10.

WHO, Control and surveillance of African trypanosomiasis. Report of WHO expert committee. *WHO technical report series, Geneva,* World Health Organization, 1998

WHO, The world health report, Health systems: Improving performance, Geneva, World Health Organization, 2000

WHO, Control of Human African Trypanosomiasis: A Strategy for the African Region. *Report of Who Regional Committee for Africa, Fifty-fifth session*, Mozambique, World Health Organization, 2005

WHO, African trypanosomiasis (sleeping sickness) Fact sheet N°259 October 2010

Woo, P.T.K. The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica*, (1970) **27**: 384–386.

OIE, Terrestrial Manual (2008) Chapter 2.4.18

APPENDICES

Respondents' Informed Consent Form and Questionnaire

Ghana Field Epidemiology and Laboratory Training Program (FELTP), School of Public Health, College of Health Sciences, University of Ghana, Legon.

Project Title: ***Human and Porcine Trypanosomosis in the New Juaben Municipality, Eastern Region – Ghana.***

Investigator: ***Kofi Afakye***

Respondent ID No ☐☐☐

INDIVIDUAL QUESTIONNAIRE

Questionnaire Number:

Respondent ID Number:

District/Municipality:

Community:

Enumeration Area Number:

House Number:

Household Number:

Consent Form

Dear Participant,

Introduction: My name is .....from..... I am here to collect information for research on Human and Porcine Trypanosomosis, diseases which can cause large economic and social loss. This information will be used to assess the potential impact of this condition in the people of New Juaben Municipality and will trigger up public health interventions for control and prevention for the benefit of the municipality.

Random selection: You/your child have been randomly selected to be part of this study and this is why we would like to interview you.

Confidentiality: The information you provide is totally confidential and will not be disclosed to anyone. Samples and questionnaires will be coded, and the use of left-over samples will be agreed upon with you and stored at -70°C. No response that you give will be specifically connected to you but rather be combined with the views of the entire population. You may be contacted by the Research Team again only if it becomes necessary to clarify information on the study.

Voluntary: Your participation is voluntary and you can withdraw from the study after having agreed to participate without being asked to explain. You are free to refuse to answer any question that is asked in the questionnaire. If you have any questions about this study you may ask me or contact the Lead Investigator, Dr. Kofi Afakye, a resident of Ghana Field Epidemiology and Laboratory Training Programme (Ghana FELTP), at the School of Public Health, University of Ghana, on 024-4476361 or 026-8703781

What's involved: You will be asked some questions about your daily activities, pig production practices (for pig farmers) and knowledge about tsetse fly and trypanosomosis/sleeping sickness. You will have three to five drops (75-125 µl) of blood taken after a finger prick to test for Human African Trypanosomiasis (Sleeping sickness) and/or taken from your pigs to test for Porcine Trypanosomosis. Excess after use will be immediately autoclaved and safely discarded.

Possible risks: This research poses no risk to you. The finger prick may give you a little pain and also some of your time will be taken up by the interview.

Possible benefits: By participating in this study, you would know whether you have sleeping sickness or otherwise. If you are tested positive, you will receive free treatment. The pigs would also receive free deworming and the positively tested ones will be treated against trypanosomosis free of charge. The results will be available to you, on request, after about two months.

Your rights: You have every right to ask any question or to opt out. You can also seek for clarification at any time during the study.

Consent to participate: Signing this consent form indicates that you have read/been read to you and understand what will be expected of you and are willing to participate in this survey.

Read by Participant		Read by Interviewer	
Agreed		Refused	

Signatures: I hereby provide INFORMED CONSENT to take part in this Human and Porcine Trypanosomosis study.

Name (first name only): _____ Sign or left thumb-print: _____

Witness (first name): _____ Sign _____

Date: _____

Respondent ID Number

MODULE 1: DEMOGRAPHICS

Qu.No.	Questions	Response	Code
1.1	Location (house number)		D1
1.2	Description of location (to facilitate call-back)		D2
1.3	Name of Household Head		D3
1.4	Respondent's First name		D4
1.5	Respondent's Family name		D5
1.6	Contact phone number		D6
1.7	Sex	Male .....1 Female 2	D7
1.8	What is your date of birth?	⌊⌊ ⌋⌋ ⌊⌊ ⌋⌋ ⌊⌊⌊⌊ ⌋⌋⌋⌋ dd mm yy *(If Don't know, code 77-77-7777 and go to Q1.9. If known, go to Q1.11)*	D8
1.9	How old were you at your last birthday?	Age (in complete years).....	D9
1.10	If you don't know your age, please estimate how old you are? *Interviewer should estimate maximum age and narrow down until respondent agrees appropriate age range*	<1.....1 1-5.....2 6-10.....3 11-15.....4 16-20.....5 21-30.....6 31-40.....7 41-50.....8 >50.....9	D10
1.11	What is the highest level of school you attended?	Primary.....1 Middle / JSS.....2 Secondary / SSS.....3 Voc./Comm./Tech./.....4 Post Secondary.....5 Tertiary.....6 None .....7 Other (specify).....96 Don't know.....77	D11
1.12	What is your religion?	Catholic.....1 Protestant.....2 Pentecostal/Charismatic.....3 SDA/Jehovah Witness etc.....4	D12

		Moslem. .....5 Traditiomnal religion.....6 Spiritual church.....7 No. Religion.....8 Other (specify)_____96	D12other
1.13	To which ethnic group do you belong?	Akan.....1 Guan.....2 Ga/Dangme.....3 Ewe.....4 Krobo.....5 Mole Dagbani.....6 Other (specify)_____96	D13 D13 other
1.14	Occupation	Unemployed.....1 Student.....2 Unskilled labour.....3 Trade/Business.....4 Livestock Farming.....5 Crop Farming.....6 Hunting .....7 Salary worker.....8 Palm fruit processor.....9 Dry season farming.....10 Other (specify)_____96	D14 D14 other
1.15	What is your main source of income and livelihood?	Salary/wages .....1 Cash crop .....2 Food crop .....3 Livestock/poultry .....4 Agro-processing.....5 Non-agric enterprise .....6 Gifts/remittances.....7 Others (Specify) 96	D15
1.16	What are your supplementary sources of income?	Salary/wages .....1 Cash crop .....2 Food crop .....3 Livestock/poultry .....4 Agro-processing.....5 Non-agric enterprise .....6 Gifts/remittances.....7 Others (Specify) 96	D16 D16other

MODULE 2: HUMAN FACTORS

2.1	Do you live close to wet area/water body?	Yes …1 No …2	H1
2.2	Do you live close to pig rearing site?	Yes …1 No …2	H2
2.3	Do you live close to plantation/vegetational growth?	Yes …1 No …2	H3
2.4	For how long have you lived at your present residence?	_____Years	H4
2.5	Have you ever received blood transfusion?	Yes …1 No …2	H5
	Mention the duration of the following activities in a day for Q2.6 – 2.14		
2.6	Working on farm	___hrs…1 N/A…2	H6
2.7	Fetching water from riverside	___hrs…1 N/A…2	H7
2.8	Working close to river/swamp	___hrs…1 N/A…2	H8
2.9	Washing by the riverside	___hrs…1 N/A…2	H9
2.10	Working with livestock	___hrs…1 N/A…2	H10
2.11	Working or resting in the compound without top dress	___hrs…1 N/A…2	H11
2.12	Fishing	___hrs…1 N/A…2	H12
2.13	Hunting	___hrs…1 N/A…2	H13
2.14	Working close to pig sty where pigs are kept	___hrs…1 N/A…2	H14
2.15	Have you ever experienced nodule?	Yes …1 No …2	H15
2.16	If yes to Q2.15, did you treat?	Yes …1 No …2	H16
2.17	Have you ever experienced intermittent fever?	Yes …1 No …2	H17
2.18	If yes to Q2.17, what was diagnosed?		H18

MODULE 3: PIG DATA

Q.No.	Question	Response	Code
3.1	How many years have you been keeping pigs?	_____Years……1	P1
3.2	What are your reasons for keeping pigs?	Commercial ……1 Tradition ……2 Food ……3 Social ……4	P2
3.3	What is the pig population (including piglets)? *Help respondent to count if necessary*		P3
3.4	What is the total number of males?		P4
3.5	What is the total number of females?		P5
3.6	How many are piglets?		P6
3.7	How many are growers?		P7
3.8	How many are adults?		P8
3.9	What breed of pigs do you have?	Exotic……1	

		Indigeneous.....2 Crossbreed.....3	P9
3.10	What is the system of pig production?	Free range.....1 Semi-intensive.....2 Intensive.....3	P10
3.11	If pigs are housed, which housing materials did you use?	Wood.....1 Cement walls.....2 Metal railings.....3 Mud.....4	P11
3.12	Roofing:	Open-top.....1 Roofed.....2 Partially roofed.....3	P12
3.13	Is location of your pig sty close to river/stream/swamp?	Yes .....1 No 2	P13
3.14	Is location of your pig sty close to plantation/vegetational canopy?	Yes .....1 No 2	P14
3.15	What do you feed your pigs on? *(Tick as many as respondent would mention)*	Commercial feed.....1 Self-prepared.....2 Peels.....3 Slurry.....4 Foliage 5	P15
3.16	How often do you clean the pig sty?	Once a day.....1 Twice a day.....2 Not daily.....3	P16
3.17	Where do you dispose of waste materials?	Close to sty.....1 Far from sty.....2 Bury deep in a pit.....3	P17
3.18	Do you experience abortions/stillbirths in your pigs?	Yes .....1 No .....2 Not observed 3	P18
3.19	Have you ever done curative treatment against trypanosomosis for your pigs?	Yes ...1 No ...2	P19
3.20	Have you ever done prophylactic treatment of trypanosomosis?	Yes ...1 No ...2	P20
3.21	How many births did you have from one sow last year?	None .....1 One .....2 Two .....3 Not applicable.....4	P21
3.22	How many pigs died last year *(total number of deaths in all ages)*? *(Help respondent find the quantity)*	(Give number) .....1 Don't know 2	P22
3.23	Are your pigs protected from flies by any method?	Yes ...1 No ...2	P23

MODULE 4: KNOWLEDGE IN TRYPANOSOMIASIS

4.1	Have you ever heard of trypanosomiasis/sleeping sickness? *(Probe to be certain respondent has never heard of it)*	Yes .....1 No 2	K1
4.2	Have you heard of tsetse fly? *(Probe to be certain respondent has never heard of it)*	Yes .....1 No 2	K2
4.3	Have you ever seen tsetse fly? *(Probe to be certain respondent has never seen it)*	Yes .....1 No 2	K3
4.4	Which of the following are affected by the disease?	Only humans.....1 Only animals.....2 Both humans and animals.....3 Don.t know 77	K4
4.5	Can a person bitten by tsetse fly get trypanosomiasis?	Yes .....1 No .....2 Don.t know 77	K5
4.6	Can an animal bitten by tsetse fly get trypanosomiasis?	Yes .....1 No .....2 Don.t know 77	K6
4.7	If a person is bitten by an infected animal, can he/she get trypanosomiasis?	Yes .....1 No .....2 Don.t know 77	K7
4.8	Can someone who consumes meat of an infected pig trypanosomiasis?	Yes .....1 No .....2 Don.t know 77	K8
4.9	Can an infected pig directly transmit the trypanosome to another pig without a bite from tsetsefly?	Yes .....1 No .....2 Don.t know 77	K9
4.10	Can an infected pig directly transmit trypanosomes to humans without a bite from tsetsefly?	Yes .....1 No .....2 Don.t know 77	K10
4.11	Can an infected person directly transmit trypanosomes to another person without a bite from tsetsefly?	Yes .....1 No .....2 Don.t know.....77	K11
4.12	Which area do tsetse flies like most?	Dry and weeded area .....1 Wet and bushy area .....2 Don.t know.....77	K12
	Can trypanosomosis cause the following (Q4.13-Q4.17):		
4.13	Change in sleeping pattern of affected persons? *(Explain: feeling sleepy during the day and not feeling sleepy at night)*	Yes .....1 No .....2 Don.t know.....77	K13

4.14	Abortion in humans?	Yes.....1 No .....2 Don.t know.....77	K14
4.15	Abortion in animals?	Yes .....1 No .....2 Don.t know 77	K15
4.16	Emaciation in humans?	Yes .....1 No .....2 Don.t know 77	K16
4.17	Emaciation in animals?	Yes .....1 No .....2 Don.t know 77	K17
4.18	Trypanosomosis can be treated	Only in humans.....1 Only in animals.....2 In both humans and animals....3 None of them .....4 Don't know.....77	K18
4.19	What is the best method for preventing trypanosomosis/sleeping sickness?	Destroy pigs.....1 Destroy tsetse fly .....2 Destroy pigs and tsetse flies...3 None of them .....4 Don't know 77	K19
THANK YOU VERY MUCH FOR YOUR TIME AND PATIENCE			

Questionnaire checked by (Supervisor).....

Signed .....