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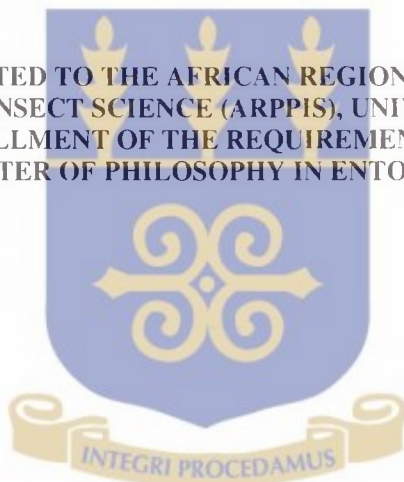


**PREVALENCE OF *TRYPANOSOMA CONGOLENSE*, *T. VIVAX* AND *T. BRUCEI*
IN CATTLE AND TSETSE-FLY, *GLOSSINA SPP* (DIPTERA; GLOSSINIDAE)
IN AN AREA OF TRYPANOCIDAL DRUG USAGE IN GHANA.**

BY

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DOCTOR OF VETERINARY MEDICINE (DVM)**

**A THESIS SUBMITTED TO THE AFRICAN REGIONAL POSTGRADUATE
PROGRAMME IN INSECT SCIENCE (ARPPIS), UNIVERSITY OF GHANA,
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD
OF MASTER OF PHILOSOPHY IN ENTOMOLOGY.**




**INSECT SCIENCE PROGRAMME
UNIVERSITY OF GHANA
LEGON.**

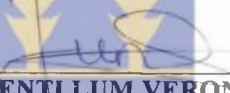
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**JOINT INTERFACULTY INTERNATIONAL PROGRAMME FOR THE
TRAINING OF ENTOMOLOGISTS IN WEST AFRICA.
COLLABORATING DEPARTMENTS: ZOOLOGY (FACULTY OF SCIENCE),
CROP SCIENCE (FACULTY OF AGRICULTURE).**


DECLARATION

I do hereby declare that except for references to other people's investigations which have been duly acknowledged, this exercise is the result of my own original research, and that this thesis, either in whole, or in part, has not been presented for another degree elsewhere.



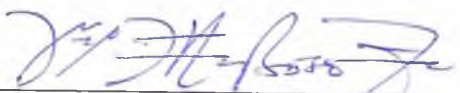


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DEDICATION

TO

LARRY, LARA AND FANWI

FOR UNCEASING PRAYERS AND LOVE



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SUMMARY

The Livestock sector in Ghana has incurred significant losses due to the African Animal Trypanosomiasis (AAT), transmitted by tsetse flies. Attempts to reduce the disease prevalence have been mainly through vector control and the use of trypanocidal drugs. An assessment of these control measures gives appreciable information on the impact of the efforts involved in such activities.

This study was therefore firstly to identify a trypanosomiasis endemic area where trypanocidal drugs are used, identify the common trypanocides used by cattle farmers and document the dosage and frequency of drug use by questionnaire. Secondly, tsetse flies were to be trapped in the endemic areas and the fly species identified by morphological features. Thirdly, it was to identify the trypanosome species infecting the vector (*Glossina spp.*) and cattle, and estimate the infection rates. Lastly, trypanosome isolates were to be obtained from infected cattle and preserved in liquid nitrogen for future use.

In all 33 cattle farmers in 17 villages in the Yilo Krobo, Manya Krobo and Asuogyaman Districts in Ghana were interviewed by questionnaire. Six trypanocides namely, Berenil[®], Diminazene[®], Ethidium[®], Novidium[®], Samorin[®] and Veriben[®] were used by the farmers, while a market survey of veterinary drugs showed that a seventh drug (Dophanil[®]) was on sale but not used by any of the farmers. In this study 38% of farmers controlled trypanosomiasis on their farms using Berenil[®] while the other diminazene-based trypanocide (Diminazene[®]) was used by 2.8% of farmers. Usage of the remaining four trypanocides (Samorin[®], Novidium[®], Ethidium[®] and Veriben[®]) was 59.2%. A high proportion of farmers (62.2%) used the trypanocides at their recommended dosage, whilst 2.7% underdosed and 35.1% overdosed. Of the six drugs in use by farmers, only Berenil[®] was found to be underdosed and used at the highest frequency (6 times/annum). Generally, the frequency of usage of trypanocides by the farmers ranged from 1-6 times per annum, but most trypanocides were administered twice yearly.

Animal trypanosomiasis was known by the farmers, who had local names for both the disease and the vector. Tsetse fly survey in the study area revealed that the flies belonged to the Palpalis group (*G. palpalis palpalis*). Dissection of the flies revealed no trypanosomes in the midgut, salivary glands or mouthparts, thereby giving a trypanosome infection rate of 0%.

A total of 397 cattle (311 females and 86 males) were sampled, however, only 6 (1.5%) were demonstrated to be infected with trypanosomes by the buffy coat technique out of which 3 were positive by the thin and thick smear methods. The trypanosome species found in cattle were *T. brucei* and *T. vivax*. Samples, which were positive for trypanosomes, were stored in liquid nitrogen using glycerol as a cryopreservative.

The results of this study revealed very low tsetse density and trypanosome infection rates in cattle, and thereby suggested that trypanosomiasis intervention measures in the study area are effective.

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The world's population is reported to be expanding by 2% each year (Gavora, 1982). As a result, there is the need to increase food production. The problem of inadequate food (especially animal protein) in developing countries is for this reason considered very important. In Ghana, average per capita of animal protein stands at 13.7g as against 59.5g in the developed countries (FAO, 1992). However the minimum daily requirement of animal protein for normal growth and healthy mental development is 34g. The main cause of this deficiency is very complex, nevertheless, animal diseases especially trypanosomiasis has been identified as a significant factor (Assoku, 1986; Urquhart, 1974; Morrison, Murray and McIntyne, 1981).

The African trypanosomiasis is an important, frequently fatal, disease of man and livestock, transmitted by tsetse flies (*Glossina spp*) with the exception of *T. evansi* and *T. equiperdum* (Mwendia, 1997). The distribution of trypanosomes is thus being influenced by the distribution of these flies.

The tsetse fly infest a variety of habitats, ranging from wooded savanna to forest, and the vegetation along banks of rivers and lakes, in 37 countries (FAO/WHO/OIE, 1982). This area of tsetse fly belt, covering about 9-10 million Km² of land or 30% of the African continent (Molyneux and Ashford, 1983), lies between latitude 15°N and 30°S (Figure 1), and occupies potentially fertile land which, if cleared, could support an additional 120 million head of cattle (Leach and Roberts, 1981; FAO/WHO/OIE, 1983; Jordan, 1986; Hornigberg, 1986). It has further been estimated that, as a result of the extensive distribution of this disease on the continent, Africa produces 70 times less protein per hectare than Europe (Allsopp, 1985).

The principal tsetse-borne trypanosomes pathogenic to livestock are *Trypanosoma congolense*, *T. vivax*, *T. brucei* and *T. simiae*. The first three-trypanosome species are

pathogenic to cattle, sheep and goats, while *T. simiae* affects pigs principally. The disease in man (sleeping sickness) is caused by *T. b. gambiense* and *T. b. rhodesiense*.

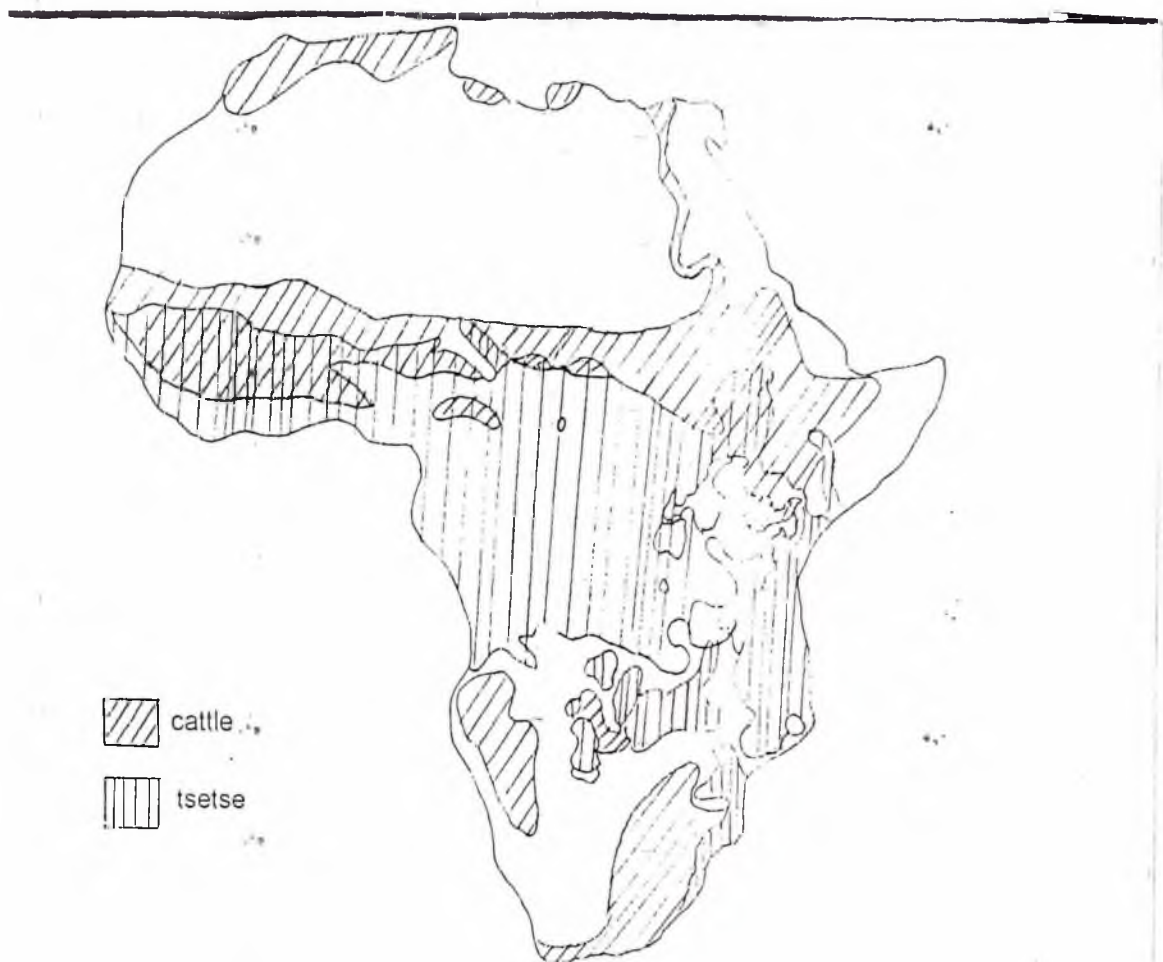


Figure 1. Map of Africa showing tsetse fly and cattle distribution.

1.1 Objectives of the study

- 1 To select trypanosomiasis endemic area where trypanocidal drugs are used and identify the common trypanocides used by cattle farmers, the dosage and frequency of its use by questionnaire and market survey.
- 2 To trap tsetse flies in trypanosome endemic areas using bi-conical traps and identify the species by morphological features.
- 3 To identify the trypanosome species in the vector (*Glossina spp.*) by dissection and estimate their infection rates.
- 4 To determine the trypanosome infection rate in cattle using the micro haematocrite centrifugation technique (MHCT).
- 5 To preserve trypanosome isolates in liquid nitrogen for future use.

1.3 Justification of the study

Tsetse-fly survey reports in Ghana have shown that trypanosome prevalence is directly related to tsetse density. Also tsetse density is independent on the amount of vegetation cover along the river system. Previous surveys by the Tsetse and Trypanosomiasis Unit and GTZ (German Society for Technical Cooperation) project have provided epidemiological information on the tsetsefly and animal trypanosomiasis in the Northern sector of the country. Focusing on activities in the Southern sector of the country, fly control was carried out by trapping and using Deltamethrine on cattle, while the farmers continued with chemotherapy as a control measure for the disease in the host (NLSP, 1999). It is important to describe the relationship between tsetse challenge and trypanosome prevalence (in both tsetse and livestock) in an area of uncontrolled trypanocidal drug usage. Such information will provide the basis for realistic decisions on the productivity of breeds of livestock under various levels of tsetse challenge after the control program. Also, this could assist livestock producers in making decisions such as those involving chemoprophylactic and therapeutic drug strategies. Generally the data obtained with this study will facilitate proper management of African animal trypanosomiasis (AAT)

CHAPTER TWO

LITERATURE REVIEW

2.1 Trypanosomiasis, Tsetsefly and Cattle

As early as 1857, David Livingstone, (cited by Hoare, 1972) suspected that trypanosomiasis of domestic animals (Nagana) was acquired from tsetse flies that fed on game animals. He noted particularly the prevalence of *Glossina morsitans* in the Zambezi basin. Nevertheless it was 40 years later before the first scientific evidence supporting the involvement of tsetse and wild game in the epidemiology of Nagana was demonstrated by the British scientist David Bruce (Bruce 1895; Hoare, 1972). The flagellate parasites were assigned to the genus *Trypanosoma*, (Bruce, 1895) and the trypanosome (*T. brucei brucei*) was later named after Bruce by Plimmer and Bradford (1899). From the onwards, tsetse flies were known to transmit trypanosomiasis. However "Mechanical" (non-cyclical) transmission of trypanosomiasis was also discovered in 1909 by Kleine, a German investigator working in German East Africa (Tanzania).

Tsetse is a Bantu word, meaning "fly destructive to cattle". Although the term "tsetse" was first applied to *G. morsitans*, it now refers to the entire *Glossina* species (Austen, 1911). The distribution of tsetse flies in the infested areas of Africa is not uniform. These are extremes ranging from abundant fly presence, presenting what may be subjectively called "heavy" trypanosomiasis challenge to livestock, to the complete absence of the fly (tsetse free localities) in which disease challenge is non-existent (Jahnke, et al., 1988). The tsetse flies act as a vital vectoral link in the transmission of trypanosomiasis between a wide range of reservoir hosts (mainly wild game) and livestock (Hoare, 1970a)

The relationship between tsetse challenge and trypanosome prevalence in livestock is complex (Whiteside, 1958; Molyneux, 1977; Jordan, 1986).

Studies on trypanosomiasis risk (challenge) are centered on evaluation of this risk

using parameters such as tsetse relative density determined from catches in biconical traps (Challier, A.C. and Laveissiere, 1973) and expressed as the mean number of tsetse caught per trap per day (TCD). Also the trypanosome infection rates in tsetse can be determined by dissecting the proboscises, salivary glands and midguts of tsetse and identifying the trypanosome types using the method of Lloyd and Johnson (1924); and the proportion of blood meals taken from the target host also determined by various techniques (Rurangirwa et al., 1986; Pant et al., 1987). The product of the above factors gives an index of tsetse challenge, which is compared with trypanosome prevalence in cattle (Clair, 1988)

2.2 The Tsetsefly

2.2.1 Morphology, Classification and Distribution

The tsetse flies are two winged insects belonging to the class Insecta, order Diptera, family Glossinidae and genus *Glossina*. The members of the genus *Glossina* are yellowish or grayish brown, dark brown to almost blackish brown flies (Glasgow, 1970). They vary in length from 7.5 millimeters (measured from the face to the end of the abdomen excluding the proboscis and wings) in the smallest species, such as *G. tachinoides* to as much as 14 millimeters in the largest species such as *G. longipennis* (Austen, 1911; Glasgow, 1970). When at rest the wings of tsetse lie closed flat over the back, one on top of the other like the blades of a pair of scissors while the ensheathed proboscis projects horizontally in front of the head. Characteristically in tsetse, the antenna has secondary branching of the hairs of the arista and the presence of a hatchet-shaped discal cell in each wing (Glasgow, 1960). The male and female are easily distinguished by the external genitalia, which in the male consist of a highly convex callus projecting from the ventral side with a visible hypopygium at the base and no projection in the female.

The genus *Glossina* comprises twenty-two recorded species and eight subspecies, which are classified under the three subgeneric groups- *morsitans*, *palpalis* and *fusca*, (Austen, 1903; Ford and Katondo, 1975,1977; Katondo, 1984; Leak and Jeannin, 1984, and Moloo, 1985). The three subgeneric groups (*morsitans*, *palpalis* and *fusca*) can be generally defined by their ecological habitats. Thus, species of the *morsitans* group are present throughout much of the savanna (grassy woodlands) of Africa. The *palpalis* group on the other hand is mainly limited to the humid areas of Africa, such as the mangrove swamps, the rain forests, and the shores and the gallery forests along rivers or lakes. The majority of the *fusca* group species are limited to the more thickly forested areas of Africa. As such, they rarely come into contact with domestic animals and hence feed very little on domestic livestock (Jordan, 1988).

It has been reported that tsetse flies are found only in Africa, including inshore islands such as Fernando Po, but excluding Madagascar (Glasgow, 1970). Nevertheless there are two fossil records on tsetse flies outside the African continent.- in Colorado in the United States of America (Buxton, 1955 ,Glasgow, 1970; Gester, 1986) as well as a documented case of the presence of *Glossina tachinoides* in Saudi Arabia, Carter (1906).

2.2.2 Feeding in the Tsetse flies

2.2.2.1 Host preference

Tsetse flies are obligate haematophagous insects with characteristic host seeking behavior (Vale and Hargrove, 1975) the disposition of the vector to feed on the vertebrate host has been considered as one of the most important factors in the epidemiology of

trypanosomiasis. Stephen, 1986 observed that the life cycle of tsetse flies permits a high level of independence in host selection. Thus, one fly species will feed on a particular type of host animal in one area, but yet on another type of animal in another location (Moloo et al, 1980; Madubunyi, 1987). Using the technique of tsetse blood meal identification introduced by Lloyd and Johnson (1924), it is now known that sheep and goats are rarely used as sources of food despite their presence in tsetse infested areas (Jordan, 1962; Leach, 1973). Although tsetse flies are known to feed on a wide range of domestic and wild animals wild pigs (suids) are a preferred source of blood for several species of *Glossina* (Jordan et al, 1961).

2.2.2.2 Feeding

A newly emerged tsetse fly is referred to as "teneral" which is, by definition, a tsetse fly that has not yet taken its first blood meal. A teneral tsetse fly is certainly not infected with trypanosomes, since the parasites are not transmitted *in utero* (Mulligan, 1970).

Temperature has been identified as the most important factor required eliciting the feeding reaction (Dethier, 1954). With temperatures substantially above ambient, flies have been successfully induced to probe quite unsuitable surfaces such as glass and paper. The flexible haustellum explores the deeper epidermal layers of the skin, an action associated with copious but intermittent outpouring of saliva through the hypopharynx (Gordon et al, 1956). This outpouring of saliva continues throughout the probing of the tissues and even as extravasated blood is sucked into the food canal by the action of the cibarial pump, and the fly starts to engorge (Stephen, 1986). It is during this issue of saliva that salivarian trypanosomes are introduced into host tissues by an infected tsetse fly (Hoare, 1972; Stephen, 1986). The salivary secretions of tsetse flies contain a highly efficient anticoagulant (Lester

and Lloyd, 1928) identified as an antithrombin (Hawkins, 1966; Parker and Mant, 1979, Klickstien, 1985). This anticoagulant and some fibrinolytic proteases facilitates the passage of blood through the fine tubes of the foregut (Bursell, 1970, and they prevent clotting of the blood during storage in the crop (Parker and Mant, 1979; Endege et al. 1989).

2.3 Trypanosomes

Morphology and Classification

Trypanosomes are species of microscopic flagellate haemoprotozoan parasites. These organisms are members of the group previously belonging to the phylum Protozoa, but recently re-assigned the kingdom Protista, subkingdom Protozoa, and phylum Sarcomastigophora (Molyneux and Ashford, 1983; Cortiss, 1984; Corliss, 1986). The trypanosomes are further classified as belonging to the class Zoomastigophorea, order Kinetoplastida, suborder Trypanosomatina, family Trypanosomatidae and the genus *Trypanosoma* (Hoare, 1972). The trypanosomes that cause the African trypanosomiasis are grouped under four subgenera, three of which are of medical and veterinary importance, namely, the *Trypanozoon*, □ brucei-type of organisms□, *Duttonella* □*T. (D.) vivax* and *T. (D.) uniforme*□, and *Nannomonas* □*T. (N.) congolense* and *T. (N.) simiae*□.

The mammalian trypanosomes that develop in tsetse are: the *Trypanozoon* namely, *T. (T.) b. gambiense*, *T. (T.) b. rhodesiense* and *T. (T.) b. brucei*); the *Pycnomonas* (*T. (P.) suis*); the *Duttonella* namely, *T. (D.) vivax* and *T. (D.) uniforme*, and the *Nannomonas* namely, *T. (N.) congolense* and *T. (N.) simiae*. The crocodile parasite, *T. grayi*, is also tsetse-borne; its developmental stages in the fly must, therefore, be recognized in order to avoid confusing it with other trypanosomes sharing the same vector.

2.4 Cyclical Development of Trypanosomes in Tsetse Flies

2.4.1 Transformation of Trypomastigotes into Procyclics in *Glossina*

An uninfected tsetse fly feeding on an infected host ingests trypanosomes contained in the blood meal. These trypanosomes undergo a series of divisions and differentiation termed "cyclical development" in the vector. These stages do not infect mammals. *Trypanosoma b. brucei* develops in the tsetse midgut, proventriculus and salivary glands, where infective metacyclic forms are produced. Parasite development in the fly takes 3 to 5 weeks. *Trypanosoma congolense* develops over 2 to 4 weeks in the tsetse midgut, proventriculus and mouthparts, where infective metacyclic forms are produced. *Trypanosoma vivax* develops entirely in tsetse mouthparts. The developmental cycle may be as short as 10 days.

Transformation of trypomastigotes to procyclic midgut forms in the fly commences immediately upon ingestion of an infective blood meal (Vickerman and Preston, 1976; Englund et al, 1982). The transformation processes involve a series of morphological and physiological changes that include: a loss of the surface coat, cessation of the synthesis of the variable surface glycoprotein, reticulation of the mitochondria, and activation of mitochondrial enzymes and the cytochrome electron-transport system, induction of procyclin synthesis, and displacement of the positions of the nucleus and kinetoplast (Barry and Vickerman, 1979; Frevert et al 1986; Roditi and Pearson, 1990). These changes enable the trypanosome to adapt to the new environment in the fly midgut.

Cyclical Development of the *T. brucei* subgroup

When a tsetse fly feeds on an animal infected with the pleomorphic brucei-type trypanosomes, it ingests trypomastigotes contained in the blood meal. The ingested organisms may consist of some long forms, intermediate forms and short stumpy forms

(Stephen, 1986). It has been determined that only the short stumpy forms, and possibly some intermediate forms with well developed mitochondria containing many cristae, are capable of establishing infections in the fly (Stephen, 1986). These bloodstream forms of the parasites undergo active transformation and division in the midgut as large procyclic trypomastigotes, which further penetrate the peritrophic membrane (PTM) of the gut to reach the ectoperitrophic space where they migrate forward to the proventriculus and cease dividing to become elongate mesocyclic trypomastigotes. In this form, the parasites traverse the PTM and migrate via the esophagus, proboscis lumen, and hypopharynx to the vector's salivary glands. Here, the parasites assume the epimastigote form and undergo multiplication during which they are anchored by their flagella to the vector's salivary gland epithelium by a process involving the production of flagellopodia over and around salivary gland microvilli (Tetley and Vickerman, 1985; Molyneux and Jefferies, 1986). The epimastigotes then differentiate into the free, non-dividing metacyclic trypomastigotes (Vickerman et al., 1988), which alone among the fly forms can infect a mammal. The entire developmental cycle takes 3-5 weeks (Vickerman et al., 1988). Infections with brucei-type trypanosomes, therefore, localize in the midgut and salivary glands of infected tsetse flies. However, brucei-type trypanosomes infect the haemolymph of *Glossina* (Mshelbwala 1972; Otieno and Darji, 1977) and the ability of these trypanosomes to penetrate the midgut wall and into the haemolymph (Evans and Ellis, 1975) have clearly indicated that cyclical development of the brucei-group of trypanosomes is not yet fully elucidated. Thus, as questioned by WHO (1979), it is possible that brucei-type trypanosomes infecting the haemolymph invade the salivary glands by other routes.

2.4.3 Cyclical Development of *T. congolense*

The *Nannomonas* subgenera have an identical cycle of development in the tsetse fly. Their cyclical development is similar to that of the *brucei* group of organisms, except that the elongate mesocyclic trypomastigotes do not proceed to the salivary glands. Instead, the epimastigotes multiply; attached to the chitinous wall of the food canal (labrum) and the premetacyclic trypomastigotes swim to the hypopharynx where they mature into metacyclics. Kaddu and Mutinga (1980) reported the penetration of the midgut cells of *G. pallidipes* by *T. congolense* organisms. A similar phenomenon in *T. brucei* infections is now known to lead to *brucei*-type trypanosome infections in the fly haemolymph. So far, however, no such occurrence has been reported in *T. congolense* infections in the tsetse fly. The development of *T. congolense* in *Glossina* from the date of the first infected blood meal to the first infective metacyclics is about 15 days (Harley and Wilson, 1968; Nantulya, Doyle and Jenni, 1978).

Cyclical Development of *T. vivax*

T. vivax, the other major tsetse-borne African trypanosome, omits the fly midgut phase altogether. Vickerman et al. (1988) suggested that the vector procyclic phase of *T. vivax* possibly occurred deep in the foregut (cibarium) and quickly transformed into epimastigotes which invade the proboscis by attaching to the inner wall of the labrum. The dividing epimastigotes then generate metacyclics in the same manner as the species of the *Nannomonas* subgenus (Vickerman, et al., 1988; Jefferies et al, 1987). In a later study, Moloo and Gray (1989) confirmed the earlier hypothesis that cyclical development of *T. vivax* was initiated at sites other than the proboscis. They observed *T. vivax* trypomastigotes, pre-epimastigotes and epimastigotes in the cibarium/esophageal region of the tsetse fly,

between 1 and 48 hrs after an infective feed, and concluded that the parasites migrated from that region to the labrum where they established an active infection that matured with infective metatrypanosomes in the hypopharynx. The time lapse between ingestion of bloodstream trypanosomes and extrusion of metacyclics in this species may be as short as 10 days.

2.5 Trypanosomiasis

2.5.1 Mechanical Transmission of the African Trypanosomiases

It has been necessary to determine the role played by tsetse flies in comparison to other biting flies in the transmission of the African trypanosomiases. Mechanical transmission of the salivarian trypanosomes is believed to involve a few genera of the haematophagous Diptera, namely, *Stomoxys*, *Haematopota*, *Chrysops* and *Lyperosa*. Among these, the *Tabanus* (family Tabanidae, genus *Haematopota*) is considered the most important since their mouthparts are suited for protecting bloodfilms, and since they inflict very painful bites which increase the frequency at which their feeding is interrupted, thereby facilitating the quick transfer of trypanosomes from one host to another as they seek to complete their meals (Mahmoud and Gray 1980; reviewed by Luckins, 1988). Jordan (1986) was of the opinion that although mechanical transmission of the African animal trypanosomiasis may be important in some localities, there is no evidence that the disease can persist in the absence of the tsetse fly. Difficulties in detecting very low-density tsetse populations were given by Wells (1972) as one of two reasons contributing to the erroneous conclusion that trypanosomiasis exists in some localities where tsetses are apparently absent. The other reason being the movement of animals from tsetse infested to tsetse free areas. Transmission of trypanosomes is mainly by *Glossina spp.*

2.5.2 Factors Influencing Transmission

2.5.2.1 The Fly

Jordan (1965) is of the opinion that trypanosome infection rates in the fly which are governed mainly by the availability of infected hosts, is the most important environmental factor influencing particularly *T. vivax* infections in the vector. However, high levels of host infection have not been observed to lead to high infection rates in wild tsetse flies, (Harley, 1966; Harley and Wilson, 1968). Some tsetse flies of the same species possess an intrinsic refractoriness to trypanosome infections (Maudlin and Dukes, 1985), and varying degrees of susceptibility between different species (Madubunyi, 1987; Maudlin and Welburn, 1987; Mooloo and Kutuza, 1988; Maudlin, 1991). This involves several factors—the intrinsic factors that relate to the innate constitution of the fly and the trypanosome (Stephen, 1986), and extrinsic factors, that are mainly environmental, such as mean ambient temperature (Mooloo et al., 1980), principal food source of tsetse species (Jordan, 1965) and variation in flexibility of different tsetse species (Roberts and Gray, 1972). Studies on selection for susceptibility in *Glossina* indicated that *T. (N.) congolense* and *T. (T.) brucei* receptivity in the fly is controlled by a stable trait which was maternally inherited (Maudlin and Dukes, 1985; Maudlin et al., 1986). This inherited character was associated with the presence of rickettsia-like-organisms (RLOs) in the cytoplasm of the fly (Maudlin and Ellis 1985; Jennings and Urquhart, 1985).

The RLOs are typical rod-shaped bacteria which occur within the cytoplasm of midgut cells of some tsetse flies, including *G. morsitans morsitans*, *G. morsitans centralis*, *G. pallidipes*, *G. fuscipes fuscipes*, *G. brevipalpis*, *G. palpalis palpalis* and *G. nigrofusca* (Pinnock and Hess, 1974; Roberts and Pell, 1976; Mooloo and Shaw, 1989; Maudlin et al., 1990). These RLOs appear to cause no obvious pathology in the fly (Shaw and Mooloo,

1991).

Glossina haemolymph has anti-Trypanosoma properties specific to salivarian pathogenic trypanosomes and not to other trypanosomes or flagellates (Croft et al 1982; East et al., 1983). It is suggested that the midgut RLOs might inhibit in some way the anti-trypanosome factors in the midgut and haemolymph, possibly lectins, leading to the establishment of *T. congolense* infections in the tsetse midgut. (Welburn and Maudlin 1989; 1990; 1991). RLOs influence trypanosome establishment in the fly midgut by producing a chitinase that generates glucosamine, which, in turn, inhibits the trypanocidal activity of the lectins (Hawking, 1977). Excess uninhibited midgut or haemolymph lectin may, however, stimulate the trypanosomes that establish in the fly midgut to mature (Matthyssens et al., 1987). Moloo and Shaw (1989) and Shaw and Moloo (1991) were unable to positively correlate RLO occurrence with susceptibility to infection of tsetse with two trypanosome species. So far, no genetic mechanism has been identified to regulate the number of midgut RLOs in tsetse flies. It is, therefore, not known whether the number is determined only by random variations in the number of RLOs transmitted from a female to her offspring; or by environmental factors such as temperature during puparial life, or availability of blood meals (Brun and Jenni, 1987).

Apart from RLOs and lectins, other tsetse midgut factors have been identified to operate against the establishment and transformation of trypanosomes in the fly. These include trypsin or a trypsin-like enzyme, which is essential for transformation of *T. b. brucei* trypomastigotes into procyclic (midgut) forms *in vitro* (Yabu and Takayanagi, 1988). Others include midgut trypanolysin and trypanoagglutinins that act against the initial establishment of the ingested parasites (Molyneux and Stiles, 1991).

Viral particles have been reported to occur in the tsetse gut and they have an influence

on trypanosome infections in the fly, and their presence seems to induce resistance to infections (Clair, 1988). Also, *Glossina* species are known to be susceptible to infection by bacteria, fungi, helminths and even other protozoa (reviewed by Nash, 1970). In the presence of certain bacteria for example there is a tendency for the fly gut flagellates to disappear (Peal and Chardome, 1954).

Also cellular and humoral immunity have been reported in insects which must cope with the immune defenses both of their vertebrate and invertebrate hosts; (reviewed by Lackie, 1988). The humoral defense system of tsetse flies differs in response due to age, sex and antigen types. Kaaya and Darji (1988)

The age of the fly at which it ingests its first infective blood meal and the principal food source of the tsetse species are also important. It is generally acknowledged that feeding tsetse on non-infected blood prior to an infective meal considerably reduces the chances of establishing mature infections in the vector (Wijers, 1958; Ward, 1968; Clarke, 1969). This is in part attributed to the physiological status of the peritrophic membrane (reviewed by Langley, 1977). Wigglesworth (1929) had long ago observed that in newly emerged *G. morsitans*, *G. tachinoides* and *G. palpalis*, the peritrophic membrane was ragged and discontinuous. It became well formed immediately after the first blood meal and contained the entire meal, suggesting that if trypanosomes were present, they could more readily traverse the endoperitrophic space to the ectoperitrophic space. Despite the diverse mechanisms that may operate against the establishment or maturation of trypanosomes in *Glossina*, it has been reported that a single trypanosome is sufficient to infect a tsetse fly (Maudlin and Welburn, 1989).

However, not all tsetse flies that ingest trypanosomes from infected animals develop mature infections, since many of such infections are aborted (Bruce et al., 1915;

Lynhurst, 1933; Vickerman et al., 1988). Nevertheless, laboratory-based tsetse infection experiments normally give very high infection rates. On the other hand, trypanosome infection rates in wild-caught tsetse flies are usually low, with some less than 10% (Jordan, 1961, 1974). Trypanosome infection rates in any species of fly is highest for *T. vivax* (5-20%), followed by *T. congolense* (2-8%) and then *T. brucei* (1%) (Jordan, 1974; Palling, 1985). This trend is believed to be due to the varying complexity of the life cycles of the different species of salivarian trypanosomes in the tsetse fly, (Hoare, 1972).

It is also established that the trypanosome infection rates in some fly species may be considerably higher than that in others. The palpalis group of tsetse flies for example, are known to be poor vectors of *T. congolense*. On the other hand, species of the fusca group can be heavily infected with trypanosomes (Jordan, 1988), but the forest ecological zone that this group inhabits makes them less important as vectors of trypanosomiasis in domestic animals. The morsitans group of tsetse flies, which occupies the savanna zone, is considered the most important vector of trypanosomiasis in livestock. It is also known that *G. fuscipes* and *G. palpalis* are hardly able to transmit trypanosomes of the subgenus *Nannomonas*, whereas *G. morsitans* and *G. pallidipes* would readily transmit these trypanosomes (Harley and Wilson, 1968). Again, *G. palpalis*, *G. tachinoides* and *G. morsitans submorsitans* are known to be efficient vectors of *T. vivax*.

2.5.2.2 The Animal

The degree of trypanosomiasis risk (challenge) to which livestock are exposed is influenced by the breed/species of animal, genetic resistance supplemented or reduced by a number of factors such as age, sex (Murray et al, 1979; Griffin and Allonby, 1979b and c)

maternally derived immunity, intensity of challenge, (Murray and Morrison, 1979), virulence of the parasite (Murray et al, 1982), previous exposure (Wilson et al,1976) and a range of stress factors such as nutrition.(Stewart,1951).

The species of livestock have a significant influence on the prevalence of trypanosome infection (d'Ieteren *et al.*, 1988). Cattle are more susceptible to *T. vivax* than sheep and goats (Ilemobade et al., 1975; ILCA, 1979; d'Ieteren *et al.*, 1988). Due to the presence of trypanotolerant breeds within the same species of animal different breeds may vary greatly in their susceptibility to trypanosomiasis (Murray, 1988). N'dama and West African Shorthorn (WAS), can survive under low to medium tsetse challenge whereas White Fulani, Zebu and other exotic species succumb to trypanosomiasis (Payne, 1973; Assoku, 1979; Vickerman and Barry, 1982). In trypanotolerant animals, as long as they do not succumb, trypanosomes may persist in their blood in sufficient numbers to allow transmission by the vector to susceptible hosts (Vickerman and Barry, 1982) causing them to be of great epidemiological importance. Trypanotolerance is a heritable trait, which is associated with the ability to control parasitaemia and to resist the development of anemia (Murray, et al 1991). No significant effects have been observed on the prevalence of trypanosomiasis in relation to the sex of the animal (Assoku 1979), while in relation to the age of animal, it is twice as high in preweaners compared to adult cows (Defly *et al.*1988). Management practices may influence the animal/fly contact.(Jordan,1986) Traditional husbandry systems (extensive system) which involve migrations (transhumance) in search of good pasture and water often exposes stock to varying degrees of trypanosomiasis (Maclennan, 1970). Movement of animals from tsetse-free areas through infested land to marketing centers exposes animals to severe trypanosomiasis challenge. The level of nutrition also serves as an important factor in determining the effects of

trypanosome infection, especially in animals, which possess some degree of tolerance to the disease (MacLennan, 1970).

2.5.2.3 Factors involving the Trypanosome

Variations in the degree of pathogenicity with different species and strains of trypanosomes have been observed (Losos and Chouinard, 1979; Leach and Roberts, 1981). Some isolates are extremely virulent producing acute disease in livestock while other strains of the same species are of low pathogenicity and produce chronic infection (Mulligan 1970; Paling, 1985). Virulence decreases from *T. vivax* to *T. congolense* (Godfrey, 1961) and *T. brucei*, which is, however, known to cause chronic infections in livestock (Stephen, 1986).

2.5.3 Tsetse surveys and estimation of trypanosomiasis challenge

Tsetse surveys are often carried out to estimate the density and distribution of tsetse flies and the severity of African animal trypanosomiasis problem. Activities carried out include: rapid survey, which gives a rough estimate from a single visit of the level of trypanosomiasis risk at sites in the survey area. This can confirm the presence of tsetse and give a provisional figure for their density from a value of tsetse/trap/day (TTD), define tsetse habitat associations and determine the main sites of cattle-tsetse contact with information on trypanosome prevalence in tsetse and parasitaemia rates in cattle.

Detailed surveys give a more reliable estimate of trypanosomiasis-tsetse challenge in terms of the disease transmission pattern, seasonal variation/distribution of the fly and trypanosome prevalence in tsetse. Stocking densities and grazing pattern of livestock are related to the health, productivity and prevalence of trypanosomiasis infections in animals.

Relating these factors to on-going population, environmental trends, farming systems and livestock management practices gives a basis for supporting decisions on realistic and practical ways of reducing challenge and improving livestock production (Snow and Ceasay, 1999). In addition, more extensive national tsetse and trypanosomiasis surveys can be carried out utilizing up to date maps and meteorological observations (rainfall, maximum and minimum temperatures, relative humidity) to compile records on tsetse density (tsetse/trap/day, TTD) or (trap/catch/day, TCD).

Techniques known as Rapid Rural Appraisal (RRA) and Participatory Rural Appraisal (PRA) have been developed (Snow and Ceasay, 1999) for rapid appraisal of tsetse/trypanosomiasis problem. These involve direct observation, questionnaire and/or interview with livestock owners/herdsmen, survey of tsetse and prevalence of trypanosome infections in livestock.

2.6 Pathology of Trypanosomiasis

2.6.1 Clinical Signs And Symptoms

In animals, African trypanosomiasis is characterized by intermittent fever, initial enlargement of superficial lymph nodes, fluctuating parasitaemia, severe loss of appetite, and progressive loss of bodily condition stunted growth, decreased fertility, and an increased rate of abortion slow/progressive development of anemia, eventually leading to heart failure (Anosa and Isoun, 1976; Murray, 1979). Typically, the disease is chronic, extending over several months, often terminating in death if untreated, although cases of spontaneous self-cure have been reported (Nantulya et al., 1984; Nantulya et al, 1986).

2.6.2 *The Chancre*

Following the successful feed of an animal by an infective tsetse fly, metacyclic trypanosomes become established in the skin initiating a local reaction in the form of a cutaneous swelling, of variable intensity called a chancre. This develops at the bite site within 5-7 days (Nyindo, 1992). Local skin reactions due to tsetse bite have long been known to occur in cattle Livingstone, 1857). The appearance of the chancre has been identified as one of the early clinical signs of trypanosome infections (Burt and Fairbairn, 1945). The development and size vary with the species and strain of trypanosomes, species of the host and status of the host. It has been suggested that this constitutes the initial site of trypanosome multiplication from where they eventually migrate into the bloodstream (Molyneux and Ashford, 1983, Akol, 1985)). An inflammatory response in reaction to the presence of trypanosomes within the chancre normally results with fever, congestion, edema and the appearance of large numbers of leukocytes and plasma cells (Nyindo, 1992). Subsequently, the lymphoid system undergoes changes characterized by generalized enlargement of lymph nodes and splenomegaly (Molyneux and Ashford, 1983). The reactions in the chancre probably represent the initial immune response to the trypanosome leading to the recognition of the first variant antigens, which are generated (Vickerman and Barry, 1982). While chancre can be introduced by all three species of tsetse transmitted trypanosomes that affect domestic livestock, the size of the reaction is also trypanosome species dependent, in that the lesions cause by *T. brucei* are more severe than those produced by *T. congolense* or by *T. vivax* (Emery et al, 1980; Akol and Murray, 1983).

2.6.3 *Anemia*

Of major clinical importance in domestic animals is anemia caused by the three major trypanosome species (Losos and Ikede 1972; Murray, 1974; Leach and Roberts, 1981;

Nyindo, 1992). Although other blood-borne parasites such as *Babesia*, *Anaplasma* and *Theileria* also cause anemia, the severity is far greater in trypanosome infections (Molyneux and Ashford, 1983). In trypanosomiasis, the anemia is a reliable indicator of the progress of the disease. Initially the anemia is hemolytic due to increased red blood cell destruction and this is associated with rising parasitaemia and lasting between 3 to 12 weeks (Holmes, 1976; Murray, 1979; Saror 1980). Treatment with trypanocidal drugs in this phase usually results with recovery (Holmes and Jennings, 1975). Later during the chronic stage, which begins between four and six months following infection, it is characterized by the apparent absence of parasites in the blood although the anemia persists but, unlike in the acute phase, response to chemotherapy is poor (Murray, 1979). Several mechanisms have been proposed to explain the anemia in animal trypanosomiasis. For example, Kimeto (1989) and Nyindo (1992) have reported that in the course of infection, released trypanosome antigens adhere to red blood cells leading to erythrophagocytosis by macrophages. Another mechanism explained by Molyneux and Ashford (1983) is that increased red blood cell fragility as a result of the fever could lead to anemia. Furthermore, pronounced splenomegaly arising from increased sequestration of blood cells (Murray, 1979) as well as haemodilution due to a rise in plasma volume (Anosa and Isoun, 1976) all contribute to the anemia.

Tissue damage is reported in the heart, skeletal muscles, central nervous system, reproductive tract, and endocrine organs (Anosa and Isoun, 1970; Ikede and Losos, 1975; Whitelaw et al 1985). The mechanism responsible for the tissue damage in trypanosomiasis are most likely to be initiated by factors such as biologically active substances released by dying trypanosomes, (Goodwin, 1970), circulating immune complexes, (Slots et al., 1977).

According to Vickerman and Barry (1982), enlargement of the spleen and lymph nodes is striking, with the spleen reaching thirty times its normal size in some infected animals.

The reproductive tract is also frequently affected leading to abortions, as well as infertility in both males and females (Nyindo, 1992). Murray (1979) reported that the anemia causes anoxia, which contributes to the widespread cellular degeneration. Also, increased vascular permeability occurs leading to perivascular edema and cellular infiltration causing severe damage and degeneration of myocardial fibers; a situation which could lead to cardiac failure (Molyneux and Ashford, 1983).

2.7 Detection of Trypanosomes in tsetse flies

2.7.1 Dissection of tsetse Flies

The standard method for identification of trypanosome infections in the tsetse fly is by dissection and microscopical examination of potentially infected tsetse organs namely proboscis, midgut and salivary glands. (Lloyd and Johnson, 1924). This is possible because tsetse-transmissible trypanosomes are found to localize in specific organs in the vector. The infections are then diagnosed at the subgeneric level according to the site of development of the trypanosomes in the vector. Thus, if trypanosomes are found in the gut and salivary glands, the infections are deemed to be due to the *Trypanozoon*. If, they are found in the gut and proboscis, then they are assumed to belong to the *Nannomonas* subgenus. Again, infections confined to only the midgut are classified as immature *Nannomonas* or *Trypanozoon* (Lloyd and Johnson, 1924), while infections confined to the proboscis alone would be ascribed to the *Duttonella* subgenus. The discovery of brucei-type infections in the haemolymph of infected tsetse flies, coupled with the very low salivary gland infection rates in the vector in areas where animals are known to be heavily infected with *T. brucei* species, led to the suggestion that the site of development of *T. brucei* infective forms in the fly is doubtful (WHO, 1979). The differential diagnosis is complicated when mixed infections

occur. Moreover, attributing tsetse midgut infections to the species of the *Trypanozoon* or *Nannomonas* subgenus could be misleading. This is because the crocodile parasite, *T. grayi*, is also known to infect the midgut of riverine tsetse (Hoare, 1929; McNamara and Snow, 1991).

Even though the method permits differentiation of trypanosome infections in the vector up to the subgeneric level only, it has been used extensively (Hoare, 1972; Stephen, 1986), and it is the method of choice for epidemiological surveys (WHO, 1979). The method, however, is not accurate enough to identify mixed or immature infections (Hoare, 1970b)

Mature trypanosome infections in *Glossina* are further differentiated, based on the morphological characteristics of infective forms in the vector through the examination of stained preparations.

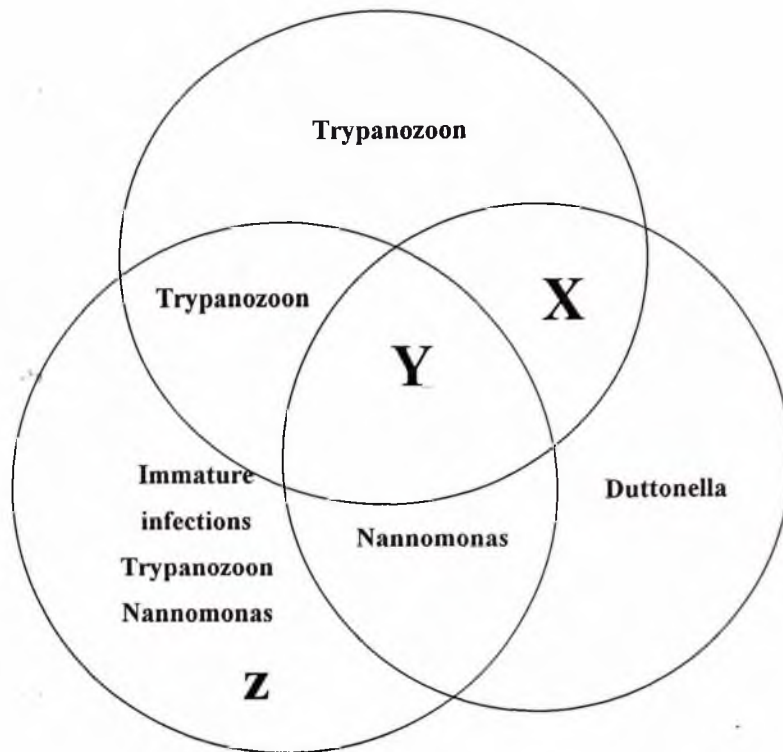


Figure 2:

Venn diagram illustrating the localization of trypanosomes of the different subgenera in the salivary glands, midgut and proboscis of infected tsetse flies:

X = mixed infections involving the *Trypanozoon* and *Duttonella*;

Y = *Trypanozoon* infections with trypanosomes located in the salivary glands and midgut, and with trypanosomes present in the proboscis, especially just after feeding;

Z = trypanosome infections that may also be due to *T. grayi*.

2.7.2 Xenodiagnosis and use of Host Restriction

Trypanosome species in the tsetse fly can also be identified by transmission to various animal hosts to determine the host range. By this method, infected tsetse flies are fed on susceptible hosts either by fly bite or by syringe inoculation of trypanosomes isolated from such flies (Moloo and Kutuza, 1974). This procedure has been used to determine the identity of quite a number of trypanosome populations. The procedure also allows the differentiation of some trypanosome species by examination of bloodstream forms in stained infected blood smears. It has been argued that, whilst *T. simiae* causes a fatal disease in pigs, *T. congolense* does not. On the other hand, *T. simiae* does not grow in rodents, whilst *T. congolense* does (Hoare, 1970a).

Blood from inoculated animals can also be sub-inoculated into other susceptible hosts. Wenyon (1926) pointed out that sub-inoculation of blood from animals with inapparent trypanosome infections into laboratory animals has been very useful in revealing infections of that nature. The good sensitivity of animal sub-inoculation in revealing mature trypanosome infections in the vector was clearly shown by Ward and Bell (1971) when they reported that feeding *T. brucei* infected tsetse flies individually on mice gave transmission rates that were five times higher than those revealed by salivary gland dissections. Moloo and Kutuza (1974) also investigated sleeping sickness in an area in Tanzania where all attempts to find the vector of the disease had failed. They triturated batches of 50 wild-caught *G. swynnertoni* by grinding them with glass powder in a mortar to which 2ml of a borate diluent was added and injected 0.5ml of the supernatant solution intraperitoneally into each of five mice, and successfully isolated nine (9) strains of brucei-subgroup trypanosomes.

Several disadvantages, however, limit the regular practice of the method. Firstly,

some trypanosome species, and sometimes-even strains, exhibit host specificity and such organisms may fail to grow in the experimental hosts used. It is recognized that whereas some *T. congolense* strains will grow in rodents, others may not (Godfrey, 1961; Mackenzie and Boyt, 1969; Young and Godfrey, 1983; Masake et al 1987). Secondly, selective pressure exerted by the *in vivo* environment in the experimental host could lead to suppression of some trypanosome sub-populations. In this way, mixed infections could easily be misidentified. Thirdly it is also known that vivax-type infections, especially the East African stocks, can easily go undetected because the experimental hosts used (laboratory rodents such as mice, guinea-pigs or rabbits) are usually refractory to that organism (Stephen, 1986). Host restriction is an expensive procedure where larger animals, such as cattle, are required and the cost of maintaining the experimental animals, further makes this method unsuitable for routine diagnosis of infections in the vector.

2.7.3 Molecular Diagnosis

2.7.3.1 Isoenzyme typing

To analyze trypanosome enzymes, soluble proteins in parasite lysates (prepared from parasites grown *in vitro* or *in vivo*) are separated by electrophoresis on thin layer starch gels. By this procedure, electrophoretic variants of the enzymes are resolved into separate bands as a result of differences in their molecular sizes and charges (Holmes and Scopes, 1974). Masters and Holmes (1975) explained that these differences were the result of genetically functional adaptations of the parasite to different environments. Analysis of the patterns (zymodemes) identified on the basis of isoenzyme profiles, has clearly demonstrated enzyme polymorphism amongst trypanosome stocks and species. The major differences were in the frequencies of the electrophoretic variants of phosphoglucomutase and peptidase. Also,

Gashumba et al., (1986) found, by isoenzyme electrophoresis, distinct differences between two stocks of *T. congolense* isolated from a sheep and a goat and 112 other stocks of the same species. By isoenzyme analysis, intra-species variations have been shown in morphologically indistinguishable trypanosomes that, however, have different behavioral characteristics (Miles et al., 1980; Gibson et al., 1980). The wide occurrence of intra-species differences makes it difficult to identify with certainty all the different species of trypanosomes by this method. Large numbers of parasites are required that must be grown *in vitro* or *in vivo* prior to analysis (WHO, 1979; Gashumba, et al., 1986). These limitations, confirms that this technique is not very suitable for direct identification of trypanosomes in tsetse flies.

2.7.3.2 Deoxyribonucleic Acid (DNA) techniques

The latest approach to identification of trypanosome species in the vector is the use of recombinant DNA probes (Majiwa and Webster, 1987; Majiwa, 1989). The technique does not require trypanosomes to be first isolated and grown before identification. To date, these probes have shown that there are five types of *T. congolense* (Kilifi type, West African riverine/forest type, Savannah type, "Godfrey" Type and Tsavo type), recognized by five different probes. It is quite likely, however, that other *T. congolense* types exist which do not react with the five probes, so that a negative result would not necessarily show that a given *Nannomonas* trypanosome population does not belong to the *T. congolense* species. The probes for *T. vivax* have also been found to hybridize with some *T. vivax* stocks (Gardiner, 1989). There is no probe yet that can hybridize with all stocks. Probes have also been derived which can distinguish between *T. simiae* and *T. congolense* in the vector (Majiwa and Webster, 1987), but those for *T. brucei* cannot distinguish between the subspecies.

Despite its high specificity, the use of recombinant DNA probes also has several disadvantages. The probes are so specific that they fail to detect some trypanosome populations within the species. Also, the technique currently employs radioactive reagents, which represent a potential biohazard, is expensive and not readily applicable in the field. It has thus been suggested that non-radioactive reagents for labeling DNA probes would diminish cost and minimize the biohazard (Leary et al., 1983; Forster, et al., 1985; Kukla et al., 1987). Recent advances in the search for non-radioactive probes have indicated that a plant-derived non-radioactive molecule, "digoxigenin", can be used in place of radioactive labels (Holtke et al., 1992).

2.7.3.3 Polymerase Chain Reaction (PCR)

This is a technique for amplification of DNA *in vitro* (Saiki et al 1985; Mullis and Faloona, 1987 and it overcomes the limits of sensitivity and specificity imposed by other techniques. Using repetitive DNA sequences, specific for each species or sub-group, as targets for amplification, PCR has been used extensively for the identification of laboratory infections in tsetse (Masiga et al, 1992) and natural infections in livestock and tsetse flies (Majiwa et al. 1994; Masiga, 1994; McNamara, Laveissiere and Masiga, 1995) The sensitivity afforded by this technique allows the easy identification of trypanosomes in the midgut proboscis and salivary glands of infected flies (Masiga et al,1995; Morlais et al,1997).

2.7.3.4 Monoclonal Antibodies (MoAb)

Monoclonal antibody (MoAb) technology was discovered by Kohler and Milstein (1975) The underlying principle involves the immortalization of a single B-lymphocyte by

fusing it with a non-secretor tumor lymphocyte *in vitro*, thereby enabling endless production of a single antibody molecule with specific reactivity to a single antigenic epitope against which it was derived, MoAbs are now being used in immunoparasitology: as probes for localization of antigen, and analysis of its organization and availability; for studies of antigenic heterogeneity (variability) in parasite populations; for detection of cloned DNA in various vectors; and for parasite typing and parasite detection (Goding, 1980; Mitchell, 1984; Sikora and Smedley, 1984). Recent studies have revealed the potential use of MoAbs for the detection of trypanosome parasites in their insect vectors (Bosompem et al., 1995a,b,c).

2.8 Diagnosis of trypanosomiasis in the Animal

2.8.1 Clinical Diagnosis

Clinical signs and symptoms of trypanosomiasis serve as a means of diagnosing the disease (Clarkson, 1968; Fiennes, 1970). Murray (1974), stated that anemia is a common feature in animals suffering from trypanosomiasis and is one of the most important to be considered during clinical diagnosis of the disease. Other clinical signs of trypanosomiasis are intermittent fever, loss of appetite and progressive loss in bodily condition (Murray, 1979). This approach has been shown to have several limitations such as many affected animals present no noticeable symptoms (Nantulya and Lindqvist, 1989) Also, presenting symptoms are not pathognomonic since a variety of diseases such as babesiosis, anaplasmosis and helminthiasis have similar manifestations (Nantulya and Lindqvist, 1989). For this reason, clinical diagnosis of trypanosomiasis should be backed by epidemiological evidence of recent contact of the affected animals with the vectors (Leach and Roberts, 1981) supported by demonstration of the parasites (Nantulya et al., 1992)

2.8.2 Parasitological Diagnosis

Direct demonstration of trypanosomes in the blood or tissue fluids of an infected animal is considered the most reliable method of diagnosing animal trypanosomiasis (Mulligan, 1970). Several techniques are used such as: microscopic examination of wet, thin and thick blood smears and rodent subinoculation (Wilson 1969) others are trypanosome concentration techniques, xenodiagnosis and culture methods. Rodent subinoculation, xenodiagnosis and the culture techniques are considered to be indirect means of parasite detection.

2.8.2.1 Direct Parasitological Diagnosis

Using this method, blood smears are made on clean glass slides and examined microscopically for the presence of trypanosomes. A wet film is prepared by placing a drop of blood onto a clean, grease-free glass slide. The blood is then covered with a glass slip and examined microscopically without staining. Wet films have been particularly useful in screening animals with relatively high levels of parasitaemia. The technique is, an inefficient method of trypanosome detection that requires immediate examination on the field. Thick smears are made by spreading a small volume of blood (4-5 μ l) over a small circular area on a microscope glass slide while thin smears are prepared by spreading a drop of blood on a slide to give a single layer of cells, followed by drying and fixing in methanol (Baker, 1970). Thick films contain much more blood per unit area; as a result, there are better chances of finding trypanosomes in such preparations. In thick and thin smears, the preparations are stained with Romanovsky-type stain (Giemsa or Leishman), which, at a pH of 7.0 - 7.2, stains the nucleus of trypanosomes red and the cytoplasm blue (Baker, 1970). The methanol fixation step results with improved preservation of the morphological characteristics of the parasite, thereby enabling species identification (Hoare, 1970a; 1970b).

2.8.2.2 Parasite Concentration Methods.

By this method it is possible to examine larger volumes (50 - 100 μ l) of blood and thereby increase the probability of detecting trypanosomes. This involves the separation of trypanosomes from host cells by processes, which normally include centrifugation such as the micro-haematocrit centrifugation technique (mHCT) (Woo, 1970) and the dark ground / phase contrast buffy coat technique (BCT) (Murray et al., (1977). Both techniques are based on the principle that trypanosomes in blood do collect at the interface of the buffy coat and the plasma (Leach and Roberts, 1981).

The mHCT involves centrifugation of heparinised blood in micro-haematocrit tubes followed by direct microscopic examination of the buffy coat / plasma interface for the presence of motile trypanosomes. One important disadvantage is that blood specimen has to be kept on ice and processed rapidly (within 4-6 hours) since the trypanosomes need to be motile to facilitate detection (Woo, 1970). These limitations of the mHCT are, however, overcome by the BCT which requires the haematocrit tube to be broken at the buffy coat / red cell interface followed by preparation of a buffy coat smear for subsequent examination under a phase contrast or dark ground microscope. This permits differentiation of some trypanosome species using morphological and behavioral characteristics (Murray *et al.*, 1977). Both the BCT and mHCT permit monitoring of the packed cell volume (PCV), which is a suitable indicator for diagnosing anemia (Paris et al., 1982). Both concentration techniques need electrically powered haematocrit centrifuges, which makes them less suitable for field use.

2.8.2.3 Indirect Parasitological Methods (*xenodiagnosis, and animal subinoculation.*)

Xenodiagnosis involves the feeding of clean *Glossina* on a suspected animal and subsequently dissecting and examining the fly for infection after the period of cyclical

development of the trypanosome. Alternatively, the flies are fed on a clean animal in which infection may later develop and be detected (Stephen, 1986). Intraperitoneal inoculation of suspected blood into rodents and subsequent examination of blood from inoculated animals during one or two months proves to be a useful diagnostic aid (Lumsden *et al.*, 1973). This method is most sensitive for detecting organisms of the *Trypanozoon* subgenus, which are usually present in low numbers (Leach and Roberts, 1981). Practical limitations of this method lie in the cost, housing and maintenance of the large number of rodents required for this study. Furthermore, *T. vivax* is usually non-infective to laboratory animals (Molyneux and Ashford, 1983; Zweygarth *et al.*, 1991) while strains of *T. congolense* with low rodent infectivity are known to exist (Godfrey *et al.*, 1965; Roberts and Gray, 1973).

In vitro culture techniques have also been used to expand trypanosome populations in the blood sample of an infected animal until detectable numbers are present. *T. vivax* is, however, known to be very difficult to culture (Zweygarth *et al.*, 1991). As a result, this method has not been adopted for general use as a diagnostic tool (Stephen, 1986).

2.8.3 Sero-Immunological Diagnostic Methods

The underlining principle in Sero-Immunological diagnosis is the specificity of antigen-antibody reactions. Several Sero-Immunological methods have been applied in the diagnosis of trypanosomiasis. These include, micro-plate ELISA dot-ELISA and agglutination assays.

2.8.3.1 Micro-Plate Enzyme-Linked Immunosorbent Assay (ELISA)

This is a solid-phase assay, which employs the property of various plastics (polyvinyl, polyacrylic or polystyrene) to adsorb monomolecular layers of proteins onto their

surface (Benjamin and Leskowitz, 1988). This binding is non-specific but saturable and is essentially irreversible (Goding and Handman, 1984).

Luckins first applied it in the diagnosis of animal trypanosomiasis in 1977. Initially the test was designed to detect circulating anti-trypanosome antibodies. The detection of anti-trypanosome antibodies, however, does not give a satisfactory diagnosis of active infection. This is because antibodies may persist for a long time following treatment (Luckins et al., 1979) or spontaneous recovery may take place, (Nantulya et al., 1986). A limitation is that the assay cannot distinguish between antibody from animals with current clinical infection and residual antibody from a previous but cured infection, and also frequently gives a false positive reaction (Nantulya and Lindqvist, 1991).

The advent of the monoclonal antibody (MoAb) technology (Köhler and Milstein, 1975) has, however, led to the development of simple MoAb-based ELISA for detecting trypanosome-circulating antigens in the host's blood (Nantulya and Lindqvist, 1989). Known as the double antibody sandwich ELISA or antigen-capture ELISA (antigen-ELISA), this technique employs trypanosome species-specific MoAbs developed against non-variable but structural and somatic antigens of the three major pathogenic trypanosome species, namely *T. brucei*, *T. congolense* and *T. vivax* (Nantulya et al., 1987). The use of antigen-ELISA has circumvented the shortcomings of the antibody ELISA. Primarily, the demonstration of specific trypanosome antigens in the serum or tissue fluids of an animal is evidence of a current infection (Komba et al., 1992). Despite these advantages, the ELISA has some important limitations. These include the need for skilled technicians, and electrically powered optical density readers. Such requirements may become a serious handicap in the use of this technique under field conditions.

2.8.3.2 Dot-Enzyme-Linked Immunosorbent Assay (Dot-ELISA)

The dot-ELISA is a highly versatile solid-phase immunoassay for antibody or antigen detection. It requires minute quantities of reagent dotted onto nitrocellulose or other suitable membranes, which avidly bind proteins (Pappas, 1988). Following successive incubation with antigen-specific antibody and an enzyme-conjugated anti-immunoglobulin, a precipitable chromogenic substrate is added. The enzyme conjugate oxidizes the substrate in the presence of hydrogen peroxide (H_2O_2) to form a clearly defined, visually readable colored dot at the reaction site. Precipitable substrates that produce blue-purple deposits are 4-chloro-1-naphthanol that is oxidized by peroxidase (Pappas et al., 1983) and 5-bromo-4-chloro-3-indol phosphate, which is catalyzed by alkaline phosphatase (Lin and Halbert, 1986). The substrate diaminobenzidine, however, gives a brown dot when oxidized by peroxidase (Hawkes et al., 1982). Color intensity is directly proportional to the amount of captured enzyme-conjugated antibody (Pappas, 1988). The dot-ELISA is rapid, easy to perform and interpret, reagent conservative, cost-effective and field portable (Pappas, 1988).

2.8.3.3 Agglutination Assay

This test involves the reaction of particulate multivalent antigens with homologous antibodies. The principle of the test is that cross-linking of the various antigen particles by antibodies leads to clumping of the antigen particles which can be visually read (Benjamin and Leskowitz, 1988), they have found useful applications in the diagnosis of a number of diseases, including trypanosomiasis.

Magnus et al., (1978) described an antibody-detection card agglutination test for trypanosomiasis (CATT) for the diagnosis of Gambian sleeping sickness caused by *T.*

gambiense. CATT has also been used successfully to detect animal trypanosomiasis (Doko et al., 1991).

Agglutination tests based on the use of latex particles sensitized with species or subgenus-specific anti-trypanosome antibodies have been developed for the detection of trypanosomal antigens (Nantulya, 1994). These tests, known as the latex agglutination antigen tests, involve the use of several kits: BRUCITEX, CONGOTEX, and VIVATEX for the diagnosis of *T. brucei*, *T. congolense* and *T. vivax* respectively; TRYPATEX as a screening assay for detecting antigens of any of these three species of trypanosomes, SURATEX for the diagnosis of *T. evansi*, and CIATT for diagnosing *T. b. gambiense* and *T. b. rhodesiense* sleeping sickness. Since agglutination reactions are rapid and easy to perform they may be regarded as a simplified and field portable form of the antigen-ELISA,

2.8.4 Deoxyribonucleic Acid (DNA) Hybridization Techniques

Recombinant DNA probes have now been developed and applied to the identification of trypanosomiasis (ole-Moi-Yoi, 1987; Majiwa, 1989). The development of DNA probes is based on the fact that certain nucleotide sequences of parasite genomic DNA are species-specific. These sequences are of two kinds, namely, those existing in a single copy and those occurring as repeat sequences (multiple copies). The latter are known as "satellite DNA" because in density centrifugation, they band separately from the bulk of the nuclear DNA owing to their high adenine-thymine content and they have no known coding function.

Probes are developed by first identifying species-specific parasite DNA fragments, isolating and reproducing them in large quantities in a plasmid or phage vector (Gibson et al., 1988). These fragments are then purified and tagged with a detectable tracer (usually a radioisotope such as ^{32}P). The probes so obtained may then be used in DNA hybridization

techniques for parasite identification. DNA hybridization reactions are extremely specific, such that most of them detect intraspecific differences (Majiwa, et al, 1993; McNamara, et al, 1991)

The repetitive nature of satellite DNA makes it possible for more labeled probe to hybridize with it, thus giving strong reactions when targeted. However, where the number of sequence repeats in the target are few, the sensitivity of the test is low and an amplification step based on DNA polymerase chain reaction may have to be incorporated to enhance sensitivity. Some disadvantages of this technique are the use of radioisotope labels which are costly and impose a biohazard. This limitation is overcome through the introduction of safer, non-radioactive labels (Majiwa, 1989).

2.9 Antigenic Variation In Trypanosomiasis Control

The antigenic properties of the trypanosome surface are located in the replaceable surface coat, which covers the entire external surface of the parasite (Vickerman, 1969) and is composed of a single glycoprotein layer, the variant surface glycoprotein or VSG, (Borst and Cross, 1982). It is this VSG molecule which is highly immunogenic and changes antigenically during the process of antigenic variation (Vickerman, 1978). In general antigenic variation is a mechanism exploited by parasitic protozoa to evade the immune response of their vertebrate hosts (Brown and Vickerman, 1986).

The VSG coat is made up of a matrix of identical glycoprotein molecules of differing amino acid sequences, Cross (1973; 1975). Due to these sequence differences, each VSG specifies a variable antigen type (VAT), which is unique in antigenic specificity. Also a single trypanosome possesses at least 1000 genes that code for VSGs (Van der Ploeg et al., 1982) and is capable of generating 10⁴ to 10⁵ VATs (Capbern et al., 1977; Van der Ploeg et al., 1982). The fact that individual trypanosomes within a population possess different VATs

meant that various MoAbs of different specificities would be needed to identify any given trypanosome population in situations where the bloodstream form trypanosome surface antigens were to be utilized.

In trypanosome infections, some VATs tend to predominate. In an immunocompetent host, such VATs are quickly eliminated as potent antibodies are produced against them. However, in a large population of dividing organisms, the gene encoding the expression of a particular VAT may be switched off in a trypanosome and a different gene controlling the expression of a different VAT may become active (Miller et al., 1984; Boothroyd, 1985). A new trypanosome population expressing a different VSG may then emerge, multiply, and produce a parasitaemic peak. As new antibodies are produced against the VSGs of the infecting organisms, those expressing the predominant VATs are rapidly eliminated leading to drastic reduction in the infecting parasite population. Once again, a few organisms that successfully acquire new surface coats as a result of gene switching, multiply as antibodies are produced against the new antigens that they express. This phenomenon of antigenic variation leads to successive parasitaemic peaks during the course of a trypanosome infection.

Apart from their unsuitability as targets for immunodiagnosis, the VSGs expressed by trypanosomes are also known to be too antigenically diverse to be useful as a basis for a vaccine against the disease (Shapiro and Pearson, 1986).

2.10 Trypanosomiasis/Tsetse Fly Control in Ghana

The control of African animal trypanosomoses (AAT) transmitted by tsetse flies is considered a priority in Ghana by virtue of the economic losses incurred by the livestock sector as a result of adverse effect of the disease on livestock production in tsetse fly infested

areas (Annual Report, 1995). Under the auspices of the National Livestock Services Project activities were carried out between 1994 and 1999 in the Coastal Savanna and the Derived Savanna agro-ecological zones of Ghana (NLSP Report, 1999). These activities sought primarily to clarify the tsetse and trypanosomoses problem, through epidemiological surveys (on trypanosomoses prevalence in cattle, tsetse distribution/abundance of the fly) to determine the effectiveness of community-base control operations. The results show that trypanosomiasis prevalence is directly related to tsetse density. It was also observed that tsetse density is dependent on the amount of vegetation cover along river systems and the availability of hosts. The tsetse and trypanosomoses component of the National Livestock Services Project has since 1994 embarked on activities aimed at promoting livestock development in tse-tsefly infested areas by: 1) determine species of tsetse flies, their spatial distribution and relative population density, 2) determine the prevalence of bovine trypanosomoses and its impact on animal health; 3) evaluate the efficacy of non-pollutant vector control methods, through the implementation of a community based pilot operation and 4) educate and mobilized rural community for sustained community participation.

In the coastal Savanna, epidemiological surveys on tsetse population and trypanosomiasis prevalence, rapid appraisals of the general perception of rural communities of the disease were conducted with the aim of initiating a sustained community participation in tsetse control activities (NLSP 1999). In the Dangme West and Eastern Region, (the Dawa, Dodowa, Afienya Sub Districts, Kpong and Somanya), it was observed that most of the potential habitats have been obliterated by human activities. Tsetse challenge and trypanosomoses prevalence in the Manya and the Yilo Krobo District could be classified as low. The Okwenya River was found to be relatively undisturbed in terms of tsetse habitat and

could be a source of infection for cattle in the Manya and Yilo Krobo areas. Tsetse fly catches and trypanosomiasis prevalence in the area before the study (NLSP Report, 1999) are shown in Table 1.

According to NLSP (1999) high proportions of Sanga (>75%) and Zebu (10%) and crossbreeds of Zebu made it necessary for intervention measures to be carried out in herds where trypanosome infection rates were more than 5%. To further reduce the tsetse population, pour on (Deltamethrin) was applied to more than 2000 cattle. Rapid appraisal six months later suggested that farmers were satisfied with the results of intervention. An overwhelming majority knew about trypanosomiasis and the associated clinical signs. Nevertheless, trypanocidal drug usage has not reduced with farmers treating cattle at least once a year, usually during the rainy season. The high patronage of trypanocides and Deltamethrine shows that the cost of intervention using chemotherapy is not a major consideration. However, it is important to educate farmers to use drugs only when it is necessary (NLSP Report, 1999)

Table 1. Tsetse challenge, trypanosomiasis prevalence and animal health assessment in various districts in southern Ghana from 1995-1998 (After NLSF, 1999)

Subdistrict	Rivers Surveyed ¹	Tsetse species and relative density ²	Number of cattle screened ³	Prevalence of tryp ⁴	Mean packed cell volume
Dodowa	Dodowa	0	136	5	34.1±2SD
Afienyi	Luledor	<i>G. palpalis</i> (ICTD)	225	8	30.5±2SD
Dawhenya	Yiribi	<i>G. palpalis</i> (ICTD)	244	6	34.1±2SD
Dawa	Yiribi	<i>G. palpalis</i> (ICTD)	205	9	35.1±2SD
Manya Krobo	Okwe	<i>G. palpalis</i> (3CTD)	30	10	33.2±2SD
Yilo Krobo	Okwe	<i>G. palpalis</i> (3CTD)	70	13	31.4±2SD

¹ Rivers selected were those with habitat for tsetse.

² Traps (Challier, A.C. and Laveissiere); CTD represents the mean catch per trap per day.

³ A systematic random sampling technique was used for selecting animals to be screened in the Krobo Districts.

⁴ The Buffy Coat Technique (BCT) was used for parasitological diagnosis.

2.11 Control of Trypanosomiasis

There are three main trypanosomiasis control methods currently in use: 1) the administration of drugs, 2) vector control and 3) the use of trypanotolerant animals. However, these have so far had a limited impact on the disease.

2.11.1 Chemotherapy

Chemotherapy of infected animals or chemoprophylaxis of animals at risk is a relatively successful method and is widely used (Okech et al., 1997). However it needs close veterinary supervision and is costly for developing trypanosomiasis endemic African countries (Dwinger, 1989). Moreover drug resistant organisms have been reported (Whiteside, 1962; Hawking, 1963).

Control of trypanosomiasis by chemotherapy in domestic livestock is currently dependent on the salts of six compounds, several of which are chemically closely related. Diminazene, homidium and isometamidium are primarily used for treatment and prophylaxis of trypanosomiasis in ruminants. The main trypanocides found on the Ghanaian market, are discussed below:

2.11.1.1 Diminazene aceturate (*Berenil*[®], *Veriben*[®], *Diminazene*[®], *Dophanil*[®])

Diminazene aceturate is an aromatic diaminadine and is marketed in combination with phenyldimethyl pyrazolone (antipyrene) a stabilizer that prolongs the activity of the compound in solution. Sensitive populations of *T. congolense* and *T. vivax* are eliminated by intramuscular administration of Diminazene aceturate at a dose of 3.5mg/kg body weight. However, higher doses may be required for clearing infection with *T. brucei* (Fussganger and Bauer, 1958). In many parts of West Africa, the maximum recommended dose of 7.0mg/kg

body weight is now generally used. Diminazene is the most commonly used therapeutic agent for trypanosomosis in livestock in Ghana. This is due to a number of reasons. Firstly, the drug is active against trypanosomes that are resistant to most other trypanocides. Secondly, low incidence of resistance has been detected as a result of using the compound, and thirdly, the drug has a higher therapeutic index than other trypanocides in most animal species.

2.11.1.2 *Homidium bromide/chloride (Novidium[®], Ethidium[®])*

Homidium belongs to the phenanthridine class of compounds and is manufactured as both the bromide (Novidium[®]) and chloride (Ethidium[®]) salts, which are equally active *in vivo*. Both salts are generally recommended for use as therapeutic agents at a dose of 1.0mg/kg body weight. However, this dose has been shown to have prophylactic activity in cattle that is effective from 2 to 19 weeks in the field. As with other prophylactic anti-trypanosomal compounds, variation in drug susceptibility in trypanosome populations and the level of trypanosome challenge are among the primary factors that determine the duration of prophylaxis (Dolan et al., 1990, 1992).

2.11.1.3 *Isometamidium chloride (Samorin[®])*

Samorin[®] is a phenanthridine aromatic amidine, formed by combining homidium with the p-aminobenzamide moiety of Diminazene, and is marketed as both the therapeutic and prophylactic agent. With the dose range recommended for prophylactic purposes (0.5-1.0mg/kg body weight), the compound has been used successfully to maintain the productivity of Zebu cattle exposed to tsetse challenge. However, considerable variation in prophylactic activity has been observed under field conditions (Peregrine, 1994).

2.11.2 Vector Control

Since the establishment of the fact that tsetse flies are vectors of human and animal trypanosomiasis, the *Glossina* species have been the target of numerous attempts to control the disease (reviewed by Molyneux, 1982). The method of vector control is complicated by the existence of 22 species of tsetse flies, each of which is adapted to different climatic and ecological conditions (Ford, 1971). Some of these measures include: elimination of the tsetse shelter and breeding sites by clearance of bushes, reduction of tsetse food sources by elimination of game animals, hand catching of tsetse flies, the use of tsetse traps aimed at reducing or possibly eliminating fly populations, the use of insecticide impregnated screens baited with odor attractants, designed to kill flies on contact, aerial and ground application of insecticides designed to kill tsetse flies in their habitats, genetic control of tsetse populations achieved by the release of sterile males into the natural fly habitat, and biological control of the flies, using organisms that are predatory or pathogenic to the *Glossina* species.

Local communities have been involved in tsetse control in Kenya, Zambia and Uganda for many years (Ssenyonga, 1994; Dietvorst, 1995) with various degrees of success. The general experience is one of partnership between public agencies or the government and communities, with a considerable financial and technical burden continuing to be met by government or donor agencies (Omolo et al., 1995). Other countries have taken up the privatization of veterinary and tsetse control services (Bastiaensen et al., 1997). The efforts aimed at controlling trypanosomiasis through the elimination of the vector have however achieved limited success. Consequently, the tsetse fly continues to play an important role in the epidemiology of both human and animal trypanosomiasis.

2.11.2.1 *Traps/Targets*

Trapping of tsetse flies was successfully developed at the beginning of the 20th century and was introduced in the late 1970s as an alternative to ground spraying. The use of traps and screens is a highly selective method of control with the advantage that it immediately reduces fly density, and is relatively harmless to the environment. Traps work by attracting passing flies, trapping them and killing them by the heat of the sun or by the use of insecticides on the traps. The efficiency of different traps [Biconical trap Chali and Lavissiere, 1973; Monoconical Vavoua trap, Pyramidal trap, Merot trap, N'Gu Trap and Epsilon trap] varies and can be greatly improved by the use of chemical attractants (Vale, 1988). Traps must not only attract flies but also encourage them to enter the trap. Maximum efficiency depends on the siting of the traps, which may be preferably placed in a visible shady clearing.

Some traps are impregnated with insecticides (Hargrove and Vale, 1979) and the persistence of the insecticide depends on climatic factors and also on the properties of the trapping material (fiber type, texture and dye) (Green, 1988; 1989). For example, deltamethrin can persist for 4-6 months when used on cotton/polyester or polyamide fabric (FAO, 1992).

Targets treated with insecticides are designed to attract flies, which on landing are killed due to the brief contact with the insecticide (Hargrove and Vale, 1979). An extension of this concept is the use of cattle treated with pour-on formulations of insecticides which form the ultimate mobile, odor-baited, insecticide treated target for catching out tsetse populations (Fox et al., 1993). Odor baited techniques may prove to be less environmentally benign and may be slow to achieve results but if managed properly they do have significant effect on tsetse populations.

2.11.2.2 *sterile insect techniques and biological control*

Sterile insect techniques (SIT) including inundative release of mass-reared, radiation sterilized tsetse males of several species have been used successfully to suppress (Politzar et al., 1980) or even to eliminate (IAEA internal report, 1997) tsetse fly populations (Olandunmade et al., 1990). The cost of rearing and sterilizing large numbers of male tsetse is high and the target population is usually reduced by some complementary method involving the use of insecticides before the release. Recently, successful SIT has been reported in trypanosomiasis control in Nigeria and Burkina Faso (Olandunmade et al., 1990; Tamboura et al., 1988). In Zanzibar (Saleh et al., 1998), the effort combined the deployment of targets, cattle dipping exercise and the release of sterile males from low flying aircraft. The use of insect growth regulators (IGRs) which acts on the principle of sterilization, rather than killing (Langley and Weidhaas, 1986), in combination with traps or targets does provide an alternative control measure. IGRs are generally arthropod specific and are usually Juvenile Hormone mimics or analogues (JHA) such as pyriproxifen, or Chitin Synthesis Inhibitor (CSI) such as triflumuron. JHA or CSI act by penetrating the adult female cuticle on contact and are transferred to the larval gut. The larva is born with either inappropriately large concentrations of JHA in its body, which prevent completion of metamorphosis or of a CSI, which prevent the larva from forming a puparium. The female continues to produce non-viable offspring in a dose-dependent manner. Contaminated males are unaffected but can transfer effective doses to females during mating (Langley et al., 1990, Langley, 1995). Special contaminating devices have been successfully used with the IGRs to suppress tsetse fly populations (Hargrove and Langley, 1990). However, the formulations are not weather proof as compared to Deltamethrine on targets.

The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* have been reported to be capable of increasing mortality in tsetse populations in the laboratory (Kaaya, 1989; Kaaya and Okech, 1990) and in the field.

Of the various techniques used in tsetse control, SIT is the most expensive, though environmentally friendly, while cattle's dipping is probably the least expensive. On the other hand, residual application of insecticides from the air is discouraged on environmental grounds.

CHAPTER THREE

GENERAL MATERIALS AND METHOD

3.1 Study Design

A study area where trypanocidal drugs are being used to control animal trypanosomiasis was selected based on information available at the Veterinary Services Department of Ghana. The use of trypanocides in the study area was confirmed by questionnaire that was also used as a tool for (Rapid Rural Appraisal) to get information on trypanocidal drug usage in the study area and the farmers' perception of the tsetse / trypanosomiasis problems as a whole. Tsetse was trapped to identify the species and determine the trypanosome infection rates by dissection. Blood samples were collected from the cattle to determine the prevalence of animal trypanosomiasis in the study area using parasitological methods (thin/thick smear and the more sensitive buffy coat technique). Trypanosome positive blood specimens were cryopreserved for future use.

3.2 Study Area/ Questionnaire Administration

Questionnaires were administered to 33 farmers in the Yilo Krobo, Manya Krobo, and Asuogyaman districts of the Eastern region of Ghana. The entire three districts are located in the coastal Savannah zone of the country. The vegetation is typically grassland with few widely scattered trees and thickets. The rainfall, 91.5 ml, humidity, 95.8% and temperatures range between 23.8 - 35.6^oc. The main source of water for both human and animal population is the Okwe River, which flows through all the three districts (Figure 3). Other sources of water were ponds and dams, and a few farmers had access to pipe-borne water. The livestock kept in the study area are mainly cattle, sheep, goats and pigs. The main activity of the population is based on crop and vegetable farming in combination with animal farming. A few game animals thrive in this with a rapidly depleting population due to farming and an

expanding human population. Tsetse-flies (*Glossina* spp.) mainly of the palpalis group have been predominant in the study area.

3.3 Tsetse-Fly Trapping

Fifteen Bi-conical traps were pitched at 300 meters distance from each other along the Okwe river for a period of 30 days in the Manya Krobo and Yilo Krobo districts, and later moved to a second side along the same river in the Asuogyaman district for sixty days. The traps were visited and emptied once daily in the mornings at 10. 00am Trapped flies were transported to the laboratory on ice and dissected within four hours of collection.

3.4 Animals

The presence and numbers of cattle, sheep, goats and pigs on study farms were Recorded between the months of January and June 1999. The sex, the age, breed and parity of the cattle were also recorded and some animals randomly selected for blood sample collection.

3.5 Blood Collection and Analysis.

Heparinized microhaematocrit capillary tubes (75mm long by 1.1mm internal diameter) were used to collect peripheral blood from the ear vein. Blood from each animal was used to fill two capillary tubes to approximately three-quarters of their lengths before the tubes were sealed at one end with cristaseal

Five milliliters (5ml) of blood was drawn from jugular vein of each animal into sterile vacutainers with Na₂EDTA as anticoagulant. All blood specimens were kept cool on ice and transported to the laboratory within six hours for analysis.

Thick and thin blood smears were prepared on clean, grease-free glass slides in the field using peripheral blood from the ear vein of each animal. Blood films were rapidly air-dried at room temperature and transported to the laboratory in a demoisturized chamber. Thin

films were fixed in methanol for three minutes whilst thick were de-haemoglobinized by immersion in distilled water for five minutes. The blood smears were then stained in 10% Giemsa (George T.Gurr Limited, England) for 30 minutes and rinsed and rinsed with water. The slides were once again air dried at room temperature and examined microscopically for the presence of trypanosomes. Stained slides were viewed under oil immersion using the x100 objective and x10 eyepiece.

3.6 The Buffy Coat Technique (BCT)

The BCT first described by Murray et al (1977) was used. Briefly, blood samples were collected into capillary tubes and processed within four hours. Blood filled capillary tubes were centrifuged at 12,000x for five minutes to obtain the buffy coat zone (leukocyte/plasma interphase), which was then examined for trypanosomes. Each capillary tube was cut with a diamond-pointed pen 1mm below the buffy coat to incorporate the uppermost layer of red blood cells. The contents of the part of the tube containing the buffy coat were then gently expressed onto a clean glass slide to include some plasma about 1 cm length above the buffy coat. The dispensed sample was then stirred to mix and cover with a glass slip. Each preparation was examined for trypanosomes by phase contrast microscopy using a 10x eyepiece and a 25x objective.

3.4 Stabilization of Trypanosomes in Blood.

A quantity of heparinised blood was collected from animals demonstrated to be infected with trypanosomes by BCT (final concentration of heparin 10unit/ml blood). Glycerol was added drop-wise to the blood whilst continually shaking the tube to give a final glycerol concentration of 10%. The sample was allowed to stand for fifteen minutes to

equilibrate and transferred into capillary tubes (half filled) approximately 70 micrometer/tube). Both ends of the tubes were sealed with cristaseal. The capillary tubes were transferred into 75x12 mm plastic test tubes together with a narrow hard paper label. Using a needle both side of the tubes were punctured to allow free flow of liquid nitrogen. The test tubes containing capillaries were inserted into a plasticine -insulated large screw cap bottle that was suspended in the vapor phase of liquid nitrogen to cool for two hours.

The samples were then transferred into a storage canister in the liquid nitrogen container.

3.8 Dissection of tsetse flies.

To dissect the tsetse salivary glands, the fly was placed on a microscope slide, ventral surface up, and 30ul of PBS added onto the head region. The thorax was then pinned down with a dissecting needle close to the base of one wing, and the head grasped with fine forceps and pulled gently, but steadily, away from the thorax in a straight line, under a dissection microscope at x 120 magnification. The salivary gland were pulled out whilst immersed in the buffer until they were out of the thorax and examined for typanosomes at x 180 magnification.

To dissect the tsetse abdomen, the fly was placed on a slide as above, and 30ul of buffer added to the abdominal area. The abdomen was then torn open at the anterior end close to thorax and elastic tissue pulled backwards toward the posterior end. The exposed gut was pulled back and a drop of PBS added into the cavity. The tissues at the anterior most part of the exposed abdominal cavity were grasped with a pair of fine forceps and pulled into the buffer on the slide. The midgut was located and examined for trypanosomes.

To examine the proboscis, the head of a tsetse fly was placed on a microscope slide, ventral surface up. The posterior base of the thecal bulb, close to the head, was pressed

gently but firmly with a dissecting needle placed almost parallel to the slide surface and pulled away from the head. The mouthpart were then teased apart and the separated labrum and hypopharynx examined for trypanosomes under a dissection microscope at x180 magnification.

3.6 Statistical Analysis

Data collected from the questionnaire and field studies discribed in the thesis were subjected to statistical analysis using the computer-based programme Epi Info version 6

CHAPTER FOUR

RESULTS

4.1 Species of Animals reared by Farmers and Farm Management

In all 33 cattle farmers in 17 villages in the Yilo Krobo, Manya Krobo and Asuogyaman Districts were interviewed by questionnaire. From the questionnaire analysis, the farmers reared either cattle alone or in combination with sheep and/or goats. Interestingly, none of the cattle farmers in the area also reared pigs. Significantly more ($P<0.05$) cattle were reared (67.9%) as compared to sheep (25.7%) and goats (6.4%).

Cattle in the study area were mainly crossbreeds of Sanga, West African Short Horn (WAS), Gudali, White Fulani, Muturu and N'dama. 79.4% of the cattle had predominantly Sanga traits. Crosses between N'dama and the other breeds made up 7.2% of the cattle population. The number of Sangas (2282) was significantly higher ($P<0.05$) than all the other breeds (594) with females dominating the population. 8.1% of the total cattle populations were calves, 81.1% cows and 10.8% bulls.

Twenty-Six (78.8%) of the farm managers made use of veterinary services. Interestingly, 21.2% of the farm managers did not engage veterinary services (Table 2), and 14.5% used assistants or neighbors. The number of farm managers who consulted Veterinary Officers was significantly more than those who did not ($P<0.05$).

Only one (3.1%) out of the 33 farm managers was female. Only 3.2% of farm managers were less than 20 years old. 48.4% were 20-50 years and 48.4% were older than 50 years. Twenty-three (69.7%) of the managers interviewed had had formal education.

4.2 Farmer's Knowledge and Importance of Trypanosomiasis

All the farmers who called it "SENYE" in the local Krobo dialect recognized trypanosomiasis as a disease. 72.7% of the farmers recognized tsetse flies, which they called "POULUE". They noted that the fly is a nuisance to their livestock. From the

Table 2. Use of Veterinary Services by Farm Managers

Age group (years)	Number of farmers	Use of Veterinary Services (%)	
		YES	NO
<20	1	1 (100)	0
20-50	16	12 (75)	4 (25)
>50	16	13 (81.2)	3 (18.8)
Total	33	26 (78.8)	7 (21.2)

questionnaire analysis, (87.9%) of the farmers were of the opinion that trypanosomiasis had a significantly high impact on the health and productivity of the animals. Twenty out of thirty (66.7%) of the farmers relied on the sale of animals as a source of income. However, 60% of them supplemented their income with earnings from crop farming. Only 10% of the farmers who sold animals relied solely on the sale of animals. Of the 10 farmers who did not sell animals, 30% relied on crop farming as a source of income while the rest were salaried workers. Generally, the farmers interviewed spent from US\$12 up to US\$200 annually in purchasing trypanocidal drugs alone. Annual mortality of cattle per farm ($n = 50$ to 500 cattle) ranged from 1-10.

4.3 Fly/Animal Contact and Tsetse Fly Control

The farmers observed that their animals encountered flies at the grazing point, drinking point and kraal. However, fly animal contact was reported to be higher (48.1%) at the grazing point compared to 34.6% at the drinking point and 7.3% at the kraal. A significantly high ($P < 0.05$) number of farmers (97%) raised their animals by free-land grazing. Of these 91.5% were involved in total grazing, 6.8% had mineral feed supplements in addition to grazing and 1.7% had concentrate feed as additive. From the questionnaire analysis, 27.4% of the farmers used ponds as the water source for the animals, 26.2% used dams and 30.9% used streams. Only 15.5% of the farmers had access to pipe-borne water for their animals.

Two out of thirty three farmers (6.1%) deliberately controlled tsetse flies. They hoped to drive away the flies at the kraal using smoke from fires made from Neem tree (*Azadirachta indica*) leaves and branches. Interestingly, 30/33 (90.9%) of the farmers used pyrethroids for spraying the animals to control ectoparasites.

4.4 Trypanocidal Drug Usage by Farmers

A market survey of veterinary drug stores in the Greater Accra Region of Ghana where the farmers in the study area purchased drugs revealed that seven (7) trypanocides namely, Berenil[®], Diminazene[®], Dophanil[®], Ethidium[®], Novidium[®], Samorin[®] and Veriben[®] were in stock for sale to farmers. Of these seven drugs, only Dophanil had not been used by any of the farmers surveyed in this study.

Figure 4 summarizes data on the number farmers who used different trypanocides and the annual frequencies of drug usage. As illustrated, most trypanocides were administered twice yearly. 38% of them controlled trypanosomiasis on their farms using Berenil[®]. The other Diminazene-based trypanocide (Diminazene[®]) was being used by 2.8% of farmers. Usage of the remaining four trypanocides (Samorin, Novidium, Ethidium and Veriben) was 59.2%. The percentage of farmers (38%) who used Berenil[®] were significantly more ($p < 0.05$) than those using any one of the other trypanocides. Even though Berenil[®] was more expensive (\$0.43/animal) compared to Diminazene[®] (\$0.3/animal) it was used by more farmers ($p < 0.05$).

As shown in Table 3, 62.2% of the farmers used the trypanocides at their recommended dosage. 2.7% underdosed whilst 35.1% overdosed. There was a significant difference between the number of farmers using the recommended dose and those who underdosed or overdosed.

Generally, the frequency of usage of trypanocides ranged from 1-6 times per annum. Table 3, summarizes the data on the dosage and frequency of use of the different trypanocides. Of the six drugs in use by farmers, only Berenil[®] was found to be underdosed and had the highest frequency of use (6 times/annum) when overdosed. All the other trypanocides were given either at normal doses or overdosed (Table 3).

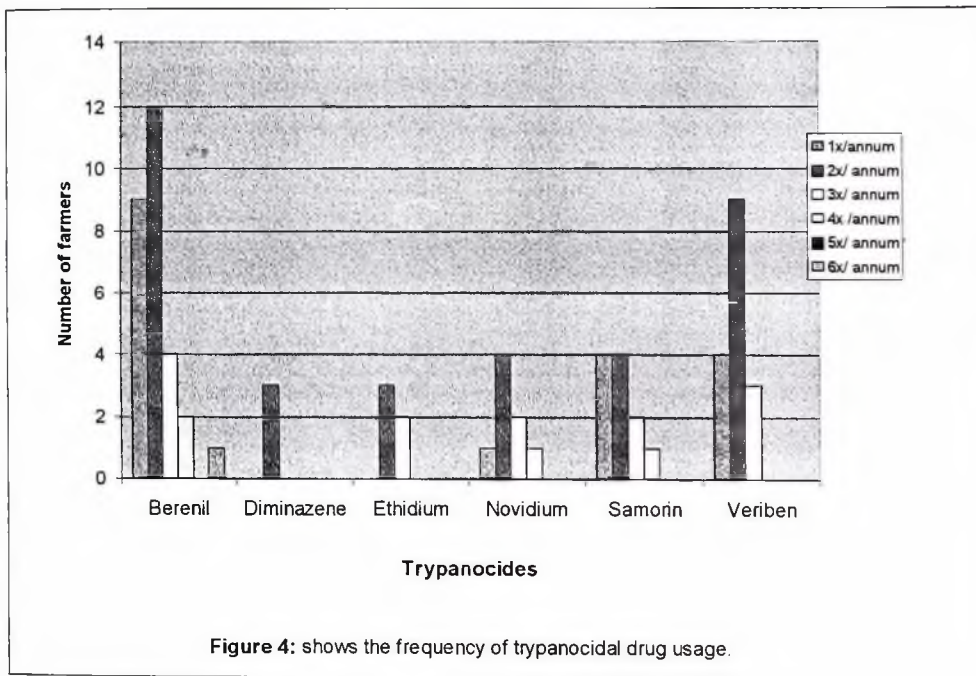


Table 3 Trypanocidal drug usage pattern

Drug	Recommended Dosage range (mg/kg body wt)	Number of farmers using different trypanocides and their frequency of use per annum		
		Under dosage	Recommended dosage	Over dosage
berenil [®]	3.5-7	1 (2)	17 (1-4)	9 (1-6)
Diminazine [®]	3.5-7		1 (2)	1 (2)
Dophanil [®]	3.5-7	0	0	0
Ethidium [®]	1.0	0	3 (2-3)	3 (2)
Novidium [®]	1.0	0	8 (1-4)	4 (2)
Samorin [®]	0.5-1.0	0	10 (1-4)	1 (1)
Veriben [®]	3.5-7	0	7 9 (1-3)	4 (2)

() Frequency of dosage of trypanocides per annum.

4.5 Trypanosome Infection Rates in Tsetse Flies as Determined by Dissection

Tsetse fly survey along the Okwe River was achieved by the use of 15 bi-conical traps. In all 15 tsetse flies (8 males and 7) females were caught. Other biting flies caught included *Tabanus*, *Stomoxys Haematopota* and *Chrysops* species. All the tsetse flies belonged to the Palpalis group (*G. palpalis palpalis*). The tsetse fly catches ranged from zero (0) per trap/catch/day (TCD) to 2/15 (0.13) TCD. The average minimum and maximum temperatures during the study period were 23.8°C and 35.6°C, respectively, with an average relative humidity of 75.8% and rainfall of 91.5mm. Figure 5, summarizes the environmental data for the study area during the period from January – June, 2000.

Two (2) of the 15 tsetse flies caught were teneral. Dissection of the 13 non-teneral flies revealed no trypanosomes in the midgut, salivary glands or mouthparts, thereby giving a trypanosome infection rate of 0%.

4.6 Trypanosomes Infection Rates in Cattle as Determined by Parasitological Techniques

A total of 397 cattle (311 females and 86 males) were sampled. Of these 34 (8.6%) were calves, 160 (40.3%) were between 1-3 yrs and 203 (51.1%) were more than 3 yrs old. Only 6 (1.5%) out of the 397 cattle screened were demonstrated to be infected with trypanosomes by the buffy coat technique and 3 were positive by the thin and thick smear methods. The trypanosome species found in cattle were *T. brucei* and *T. vivax*. The Packed Cell Volume (PCV) range for the trypanosome infected animals were 29.83 ± 1.58 compared to 30.89 ± 0.33 .

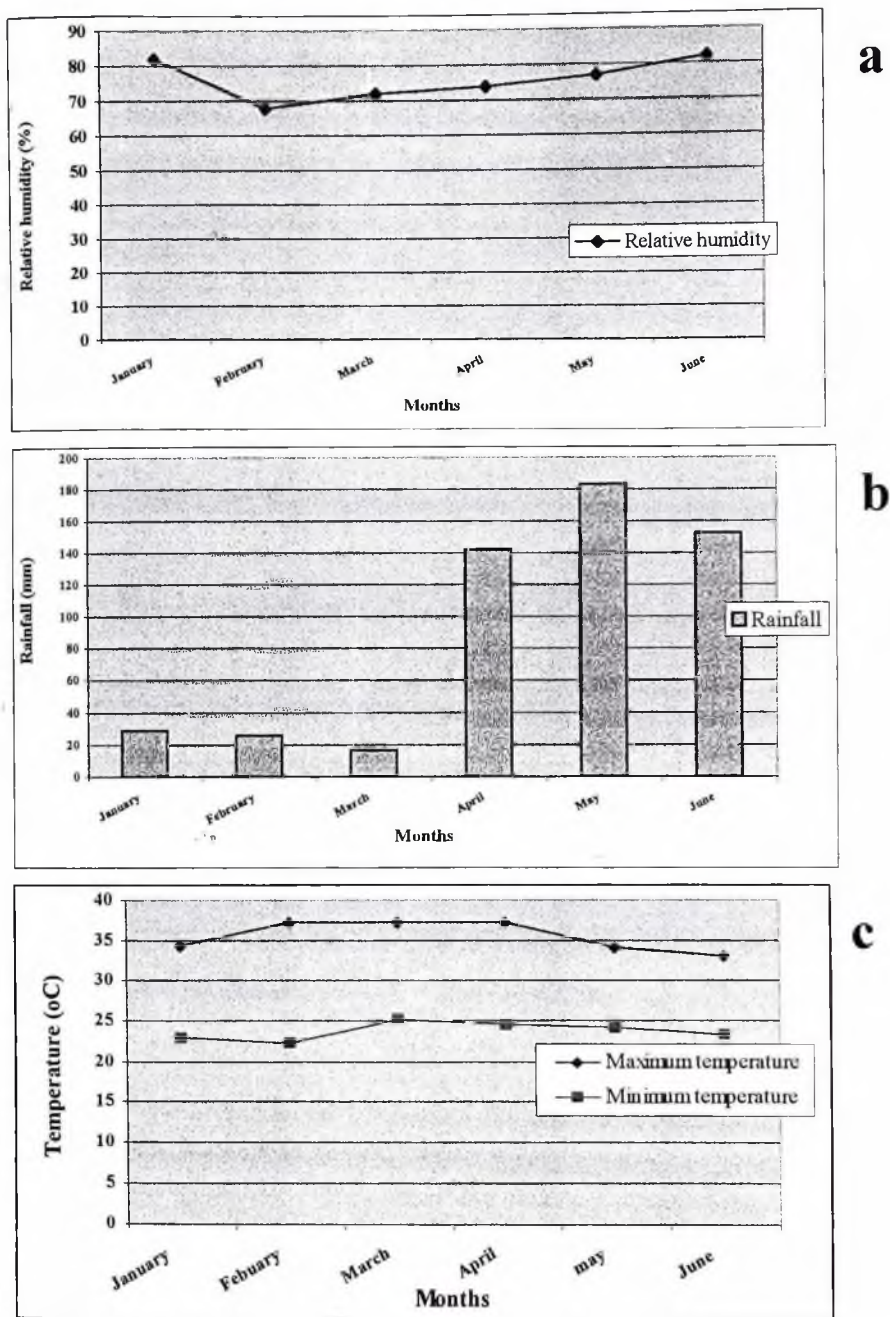


Figure 5 Summary of environmental data of the study area during the period of tsetse fly trapping (January – June, 2000): a, rainfall, b, relative humidity; c, maximum and minimum temperature.

DISCUSSION

Control of African Animal Trypanosomosis (AAT), transmitted by tsetse flies is a priority to the Livestock sector in Ghana. This is because of the significant economic losses incurred on livestock production (Doku and Seidu, 1997) in tsetse fly infested areas. Attempts have been made to reduce the disease prevalence through vector control and the use of trypanocidal drugs. The vector control effort has, however, been expensive and difficult to sustain (Agyen-Frempong, 1988). As a result, trypanocidal drug treatment is currently the main method of controlling the disease in Ghana. Again, this approach has been relatively unsuccessful and its ultimate outcome is complicated by the emergence of drug resistant strains of trypanosomes (Kupper and Wolters, 1983; Rottcher and Schillinger, 1985), and the unavailability of new drugs (Leach and Roberts, 1981). This study was therefore conducted in a trypanosomiasis endemic area in Ghana where trypanocides are used in order to obtain information on the impact of the control effort.

In this study only one out of 33 cattle farmers (3.1%) interviewed was female. A similar survey in the Gambia (Mugalla et al., 1997) showed preferential interest of women in small ruminant production (sheep and goats) compared to large ruminants (cattle). Okoth et al. (1997) reported that low level of farmer education in a community in Uganda prompted the introduction of a strategy to sensitize and mobilize the population on the importance of trypanosomiasis. In this study, a significant proportion of farmers ($P < 0.05$) had had formal education, which could enhance awareness about animal diseases including trypanosomiasis. It is therefore not surprising that most farmers knew about trypanosomiasis and tsetse flies, and appreciated the use of trypanocides and the services of trained veterinary officers. Even though all the farmers interviewed had knowledge of trypanosomiasis (which also had a local name), only 72.2% recognized tsetse flies as a nuisance or health hazard to their animals. The

ability of some farmers to recognize tsetse could be explained by the low tsetse prevalence in the study area (NLSP, 1999).

Topical application of synthetic pyrethroid insecticides to domestic livestock has been advocated as a major tool for the control of African Animal Trypanosomosis through the reduction of tsetse challenge. Striking decrease in tsetse population was achieved following application of pyrethroid insecticides to cattle in Burkina Faso (Bauer et al., 1992, 1995; Kabore et al., 1995) in Ethiopia (Leak et al., 1995) and Tanzania (Fox et al., 1993). A trial in The Gambia using spray and pour-on formulations of Deltamethrine with the objective of reducing tsetse challenge and the prevalence of trypanosome infection in village cattle proved very effective (Snow and Ceasay, 1999). A highly significant reduction was recorded in the prevalence of trypanosome infections in livestock in the intervention areas. There was an accompanying reduction in the tick burden and other insects (nuisance flies) on the animals (Snow and Ceasay, 1999). From the results of this study, a significant number of the farmers controlled ectoparasites on their farms by the use of pour-on pyrethroids. Diall et al. (1997) observed a reduction of tsetse fly populations to nearly 95% after 1 month of pyrethroid application and to near zero after 12 months. It is, therefore, likely that the low trypanosome infection rates observed in the animals screened in this study is due to the use of pyrethroids on the animals. It is important though to emphasize that only 6.1% of the farmers in the present study deliberately controlled tsetse flies. Tsetse populations may have been successfully reduced by the attempt of farmers to control other ectoparasites with pyrethroids. However, the use of smoke fires of *Azadirachta indica* by the farmers in controlling tsetse has been confirmed in studies in Kenya (Constance et al., 1996).

From a study in monitoring a pyrethroid-based trypanosomiasis control program in Kenya, a significant decline in trypanosome infection rates was observed in cattle, with a significant reduction in fly numbers (Olaho-Mukani et al., 1997; Stevenson et al., 1993). This

udy established similar trends with fly catches dropping from 3TCD before the control program (NLSP, 1999) to 0.13TCD after. Similarly, a reduction in the rate of infection in cattle occurred (11.5% to 1.3%).

Several workers including Murray (1988) Mahama (1988) and Kayang (1994) observed the dilution of the trypanotolerant gene pool in Ghana through efforts to breed larger sized cattle, and pointed out that this could eventually worsen the trypanosomiasis problem. Nevertheless, very low trypanosome infection rates were recorded in this study even though the predominant breed of cattle identified (Sanga) was trypanosusceptible. Interestingly, the trypanosomiasis control measures in place appear to be effective but the low infection rates recorded could be attributed to low sensitivity of the diagnostic tests used.

The parasitological techniques, which can detect only parasites in the circulatory system, have been reported to have low sensitivity due to the tendency of trypanosomes to sequester in tissues (Masake and Nantulya, 1991). In a comparative diagnostic study on animal trypanosomiasis in Ghana, Kayang et al. (1997), detected trypanosomal antigens in 42.7% of cattle by a latex agglutination test and 41.7% by microplate-based antigen-ELISA, but trypanosomes were demonstrated in only 7.2% using BCT. It is therefore possible that the low rate of infection observed in this study was due to the limitation of the diagnostic technique used. However recent studies by NLSP (1999) in the coastal savanna had also revealed a low level of infection. Notwithstanding, the use of genetically resistant livestock may still be important. In their study, Kayang et al. (1997) identified *T. vivax* as the most prevalent trypanosome species in cattle. Interestingly this study also recorded *T. vivax* in the few cases identified.

MacLennan (1970) observed that climatic factors affect the distribution and density of tsetse flies and, consequently, the prevalence of animal trypanosomiasis. In this study, tsetse

traps were pitched along the Okwe river where fly density is reported to be highest (NLSP, 1999). However, very few tsetse flies were caught together with other biting flies (*Tabanus*, *Stomoxys*, *Haematopota* and *Chrysops* species). In a similar study in Jos in Nigeria (Ajayi et al., 1997), an area considered tsetse and trypanosomiasis free, Kalu (1991) recorded a low tsetse catch and the few flies caught were reported to harbor no trypanosomes. Infection was therefore attributed to other biting flies (*Stomoxys* and *Tabanus* species). Nevertheless, dissection is not the most sensitive technique for detecting trypanosomes in tsetse. Methods such as PCR analysis and DNA probes can be used to confirm infection of tsetse flies (McNamara et al. 1995; Majiwa et al., 1994).

In this study, it was observed that crop farming was also carried out alongside animal farming as a source of income. With the moderate income farmers spent an average of US\$12 to US\$200 annually in purchasing trypanocidal drugs alone. Notwithstanding, annual mortality of cattle was relatively high (up to 10/500 cattle/annum). Obviously, trypanosomiasis is given a lot of importance by farmers in the study area. However, the use of trypanocides as a major control effort could create a high selection pressure for drug resistant clones of trypanosome in the animals (Geerts and Holmes, 1997). Decreasing the number of treatments has been proposed as a means of delaying the development of drug resistance (Geertz and Holmes, 1997). In the present study the trypanocides with the highest frequency of use (up to 6 times/annum) was Berenil® which may be contributing positively to the observed low trypanosome infection rates. Nevertheless, Stevenson et al. (1993) administered trypanocides as high as 12 times /annum to animals and observed that this measure might be able to control the trypanosomiasis problem temporarily but increases the levels of resistance. The underdosing of Berenil® reported in this study may further promote the emergence of resistance.

Though the control of trypanosomiasis has been hampered by unavailability of new

ugs on the market, acceptability of the new drugs by farmers is also important. A survey of Ghanaian market for trypanocides identified Dophanil[®], which was introduced in Ghana two years ago. Interestingly, none of the farmers interviewed had used this new drug. Instead, they held on to Berenil[®] which was very popular (used by 38% of the farmers) even though Dophanil[®] was cheaper and have the same active ingredient (Diminazene aceturate) as Berenil[®].

Trypanosomiasis control has potential benefits in an agro-ecological zone where there are good prospects of high crop/livestock integration and human settlement. The data collected from the Yilo Krobo, Manya Krobo and Asuogyaman districts of southern Ghana, suggest apparent success in trypanosomiasis control using trypanocides and vector control. However, the most efficient measure is to adopt an integrated disease management strategy, which has produced very impressive results (Peregrine, 1994; Fox et al., 1993). Emphasis may therefore have to be placed on community-based vector control, and rearing of trypanotolerant livestock in combination with the judicious use of trypanocides. Further studies on parasite resistance to trypanocides in the area would give a better appreciation of the problem. It is important therefore to further evaluate the trypanosome isolates preserved in this study.

In conclusion, this study provides useful information on tsetse flies, the infecting trypanosome species in the study areas, their infection rates and host-related factors that may influence the prevalence of trypanosomiasis in cattle. The data gathered could be helpful in making critical decisions on the future of trypanosomiasis control in the study areas.

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SECTION II: Animal ownership structure**2.1 Species/Number of animals**

Animal species	Number of animals				
	<10	10-50	50-100	100-500	>500
Cattle					
Sheep					
Goat					
Pigs					

2.2 Breed/Number of cattle (tick if available from records)

Age range	WASH	Sanga	Sokoto Gudali	Ndama	Muturu	White Fulani	Cross-breeds
Cow >3yrs							
Bull >3yrs							
Young cows 1- 3yrs							
Young bulls 1- 3yrs							
Calves < 1yr							

SECTION III: Herd/Flock Health

1. Have you had any disease problem before?

Yes

No

2. Do you have tsetse flies on your farm

Yes

No

3. Tsetse fly/Animal Contact

a) kraal

b) grazing

c) drinking point

3.4	Animal species			
	Cattle	Sheep	Goat	Pig
Disease				
Common diseases(list)				
Control of trypanosomiasis (Yes/No)				
Tsetsefly control (Yes/No)				
Frequency				
Use of trypanocides (Yes/No)				
Name of trypanocide				
Control of other ectoparasites				
Spraying/dipping etc (Yes/No)				
Name of acaricide				
Annual mortality (Number)				
Others and causes				

3.5 Trypanocide usage: if yes in question 3.4 indicate drug, dosage and frequency

Drug	Dosage	Frequency		
		Once yearly	2 times yearly	3 times yearly
Samorin	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dophanil	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Berenil	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Novidium	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Ethidium	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Veriben	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Diminazene	<input type="text"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

3.6 How long have you been using trypanocides?

<1yr.
 1-5yrs.
 5-10yrs.
 10yrs.

3.6 How do you get supply of drugs?.....

.....

3.7 Tsetse control: if yes in 3.4 indicate method, frequency and period

Method	Period (months)	Frequency(per year)
Trapping		
Repellent		
Others		

SECTION IV : Animal Management

1. Type of management

Management type	Animal species			
	Cattle	Sheep	Goat	Pig
Intensive				
Semi-intensive				
Free land grazing				
Backyard				
Others (specify)				

4.2 Feeding Systems

Feeding type	Animal species			
	Cattle	Sheep	Goat	Pig
Total grazing				
Grazing +Supplement				
Grazing+Fodder				
Grazing+Concentrate				
Others(specify)				

4.3 Source of water

Source	Wet Season	Dry Season
Pond		
Dam		
Stream		
Pipe-borne water		
Other(specify)		

4.4 How do you handle management problems?

Consult a Vet Extension Worker Use neighbors Personal