CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

Over the past century an enormous amount of information related to tsetse and tsetse-transmitted trypanosomosis has been published. To present a comprehensive overview of all this is impossible and, in the context of this work, not necessary. Hence this literature review is restricted to published information related to the epidemiology, prevalence, impact and control of bovine trypanosomosis. Whereas some of the literature deals with general aspects of tsetse and bovine trypanosomosis an attempt has been made to focus, as much as possible, on the tsetse species and bovine trypanosomosis in southern Africa.

1.2 The role of tsetse in the epidemiology of tsetse-transmitted trypanosomosis

The epidemiology of tsetse-transmitted trypanosomosis is complex and, because of the focal nature of the disease, varies spatially (Buxton, 1955; Mullighan, 1970). A number of analytical and simulation models have been proposed to describe the disease complex (Habtemariam *et al.*, 1983a; 1983b; Rogers, 1988; Milligan and Baker, 1988). The analytical models describe the number of new cases of trypanosomosis that could arise from a single case at the present time. This "basic reproductive rate" is determined by tsetse-related variables such as (i) the *host-tsetse contact*, (ii) the *prevalence of trypanosomal infections in tsetse*, (iii) the *density of the vector*, (iv) the *coefficient of transmission of a trypanosomal infection* or the proportion of infected bites that give rise to infection (Rogers, 1988). Each of these variables is discussed below in detail.

1.2.1 The host-tsetse contact

Both male and female tsetse flies are obligate blood feeders. They feed only on the blood of vertebrates, mainly mammals, and need a blood meal at regular intervals. The energy gained from a blood meal can either be converted entirely into fat, which represents an energy store, or it can contribute to the growth of flight musculature in young flies or of the larva within the mature female (Rogers and Randolph, 1978). The fat reserve is converted to proline to fuel flight activity essential for locating hosts, larviposition sites and mates (Bursell *et al.*, 1974).

During feeding, tsetse flies with mature trypanosomal infections in their mouthparts or salivary glands, are capable of transmitting the disease. The frequency with which a particular animal species becomes infected will depend on (i) the host preference of the fly (host preference), (ii) the ease with which the tsetse can feed on that particular species (probing response) and (iii) the frequency of feeding (feeding interval).

1.2.1.1 Host preference

Since tsetse flies have only sufficient energy reserves for limited periods of daily activity (Bursell and Taylor, 1980), potential hosts should have a similar

behavioural pattern and must be present in the same habitats as the flies. The animals that are rarely fed upon are usually found in open grass country whereas a large proportion of the preferred hosts are browsers. Many surveys to determine the host preference of tsetse flies have been conducted (Weitz and Glasgow, 1956; Weitz, 1963; Okiwelu, 1977a; Boyt, 1978; Snow and Boreham, 1979; Tarimo *et al.*, 1981; Okiwelu and Maiga, 1981; Robertson, 1983; Dagnogo *et al.*, 1985; Baldry *et al.*, 1987; Okoth and Kapaata, 1988; Küppe*et al.* 1990; Moloo, 1993; Gouteux *et al.*, 1994; Sasaki *et al.*, 1995; Makumi *et al.*, 1996; Clausen *et al.*, 1998). Results indicate that the host preference undergoes substantial spatial and, often, temporal variations. Nevertheless, it is possible to make certain generalizations about the feeding habits of the different species of *Glossina*. Weitz (1963) grouped tsetse species according to those that fed mainly on (i) suids, (ii) bovids, (iii) suids and bovids, (iv) mammals other than suids and bovids, and lastly, (v) on most available hosts including man. Although this grouping has been criticized (Moloo, 1993) it can be used to make assumptions as to the probable feeding habits in an area where potential host animals are known.

The feeding habits of *G. m. morsitans* and *G. pallidipes* in Zimbabwe have been subject of extensive studies (Robertson, 1983). In most game areas, warthog (*Phacochoerus aethiopicus*) and kudu (*Tragelaphus strepsiceros*) were identified as the most important hosts for both tsetse species. Other animals frequently fed upon were: bushbuck (*Tragelaphus scriptus*), bushpig (*Potamochoerus porcus*), buffalo (*Syncerus caffer*) and elephant (*Loxodonta africana*). A survey conducted in the Central Province of Zambia, showed that *G. m. morsitans* takes approximately 62% of its feeds from suids (mainly warthog) (Okiwelu, 1977a). A similar survey conducted in the Luangwa Valley of the Eastern Province indicated that the proportion of feeds taken on suids was far less (*ca.* 30%) (Rottcher, 1975).

Tsetse are capable of quickly adapting to new hosts that were either previously not present or not considered as favoured hosts. This phenomenon is well-known in West Africa where tsetse, of the *palpalis* group and also the *morsitans* group, have adapted to feeding on peri-domestic animals such as pigs and dogs (Baldry, 1980). This

phenomenon has, however, also been observed in southern Africa. For example, within five months of selective elimination of warthogs in the Sengwa Wildlife Research Area of Zimbabwe, *G. m. morsitans* switched its diet from 80% warthog to a diet of mainly kudu and elephants (Vale and Cumming, 1976). Even in the presence of game, tsetse can take a large proportion of their feeds on domestic animals. Cattle and donkeys can be particularly good hosts. In South Africa (KwaZulu-Natal Province), for example, the increased contact between *G. brevipalpis* and cattle has resulted in an increased proportion of feeds taken on cattle by this tsetse species (Kappmeier *et al.*, 1998). The high proportion of feeds on cattle in some areas suggests that cattle alone can maintain a tsetse population (Pilson and Harley, 1959; Robertson, 1983). Goats and sheep, on the other hand, are less frequently fed upon by *G. m. morsitans* and *G. pallidipes* (Boyt *et al.*, 1972; Boyt *et al.*, 1978; Pilson *et al.*, 1978).

1.2.1.2 Probing response

Notwithstanding the tsetse's efficiency in finding the source of an odour, the proportion of flies that engorges on a potential host animal can vary substantially and has a major bearing on the epidemiology of tsetse-transmitted trypanosomosis (Ford, 1960).

A crucial factor is the host's tolerance of tsetse attack. Host irritability, resulting in defensive behaviours such as kicking, stamping, head movements and skin rippling can affect significantly the feeding success of the fly. This explains the differences in the proportion of tsetse that engorge on various host species (Ford, 1960; Hargrove, 1976; Vale, 1977; Pilson *et al.*, 1978; Boyt *et al.*, 1978; Snow, 1980; Torr, 1994). For example, the proportion of flies that engorges on certain antelopes, such as impala (*Aepyceros melampus*), or goats is very low (Table 1.2.1). These animals are nervous and ripple their skin and flick their tail when attacked by tsetse. Hence the low proportion of flies that engorge on these hosts. Cattle, on the other hand, are much more tolerant of tsetse bites. Therefore, the feeding success on oxen can be as high as 50% (Table 1.2.1).

Table 1.2.1: Proportion of tsetse feeding on various host species.

Host species	Proportion of tsetse feeding	Source
Ox	0.47	Leggate and Pilson, 1961
	0.22-0.24	Dean *et al.*, 1969
	0.38	Hargrove, 1976
	0.37	Vale, 1977
	0.17-0.27	Baylis *et al.*, 1994
Donkey	0.47-0.56	Vale, 1977
Goat	0.00-0.02	Vale, 1977
Impala	0.00	Vale, 1977
Warthog	0.14-0.28	Vale, 1977
	0.26	Torr, 1994

Irrespective of the host species, the condition of an individual animal can also affect the proportion of tsetse that engorge. For example, Baylis and Nambiro (1993a) observed that the feeding success of *G. pallidipes* is higher on trypanosome-infected than uninfected cattle. This could be explained by a reduction in defensive reactions of sick animals.

Finally, several studies have reported that the tsetse's feeding success may be dependent on the population density of tsetse. A greater density of tsetse causes higher levels of host activity (Vale, 1977; Baylis *et al.*, 1994; Torr, 1994; Baylis, 1996) and, hence, a decrease in tsetse feeding success (Vale, 1977; Baylis *et al.*, 1994; Baylis and Mwabi, 1995; Baylis, 1996).

1.2.1.3 Feeding frequency

Estimates of the feeding interval of tsetse vary widely. Earlier studies, based on mark-recapture exercises, estimated the feeding intervals between 3 and 8 days (Jackson, 1933; Glasgow, 1961; Jackson, 1954; Rogers, 1977). Recently, a more

analytical approach using fat and haematin levels has been adopted. After ingestion of a blood meal, the haematin content (a measure of the blood meal residue) of the tsetse fly is high. The decline in the haematin content over time (expressed logarithmically) after blood meal uptake is linear (Randolph and Rogers, 1978). Therefore, the frequency distribution of haematin content of field-caught tsetse should give an estimate of the mean time since feeding. Using this method, a feeding interval of approximately 3-4 days has been estimated for several tsetse species (Randolph and Rogers, 1978; Randolph *et al.*, 1991a, b). However, the above method assumes that tsetse flies take blood meals only when the previous meal has been digested completely. Challenging this assumption, Langley and Wall (1990) estimated that male *G. m. morsitans* may feed as frequently as every 38 hours. Obviously, the best approach to estimating feeding intervals is to examine the fat and haematin contents of tsetse at the time of feeding. Using this method, Baylis and Nambiro (1993b) found that the mean feeding interval for male *G. pallidipes* varied between 42-60 hours. Hargrove and Packer (1993) used a differential equation model for blood meal metabolism which described accurately the changes in fat levels in laboratory *G. m. morsitans* and the relationship between fat and haematin in the field. They predicted a fairly similar mean feeding interval of 54-65 hours for *G. pallidipes*.

1.2.2 The prevalence of trypanosomal infections in tsetse

Although African animal trypanosomosis is mainly associated with tsetse, it can be transmitted mechanically, at least in the laboratory. There is circumstantial evidence that it occurs in the field also (Wells, 1972).

1.2.2.1 Mechanical transmission of trypanosomes

In mechanical transmission, a haematophagous insect becomes contaminated with an infectious agent during normal feeding behaviour, and the agent may persist on the mouthparts until the next feed without undergoing any biological development. Effective mechanical vectors usually are interrupted frequently during feeding, are highly mobile and have large mouthparts to transfer agents (Foil, 1989). Many different species of haematophagous diptera, including *Glossina* species, have been

implicated in the possible mechanical transmission of trypanosomes (Wells, 1972; Roberts *et al.*, 1989). In South and Central America, *T. vivax* is transmitted efficiently by Stomoxydinae, Tabanidae and Hippoboscidae (Foil, 1989; Raymond, 1990; Otte and Abuabara, 1991). Mechanical transmission of *T. vivax* has also been suggested on the African continent (Roeder *et al.*, 1984; D'Amico *et al.*, 1996). *Trypanosoma congolense* has been detected in the mouthparts of several tabanid species in Burkina Faso suggesting the possible mechanical transmission of the parasite (Solano and Amsler-Delafosse, 1995). Finally, *T. brucei* is transmitted efficiently by various *Stomoxys* species in the laboratory (Mihok *et al.*, 1995). Despite the potential of mechanical transmission in African animal trypanosomosis, many reports of mechanical transmission have subsequently been discounted by the discovery of low density tsetse populations. Moreover, several field transmission experiments, including a trial conducted in south east Zimbabwe (Boyt *et al.*, 1970), have failed to demonstrate unequivocally mechanical transmission of *T. congolense* (Wells, 1972). The role of mechanical transmission in the epidemiology of bovine trypanosomosis is, therefore, not clear and requires further investigation. However, in most areas of southern Africa where tsetse have been eradicated although biting flies are abundant, trypanosomosis is absent. This suggests a minor role of biting flies in the transmission of the disease.

1.2.2.2 Cyclical development of trypanosomes in the tsetse fly

In cyclical transmission, trypanosomes undergo substantial morphological and metabolic changes within the vector. *Trypanosoma vivax* has the simplest life cycle in tsetse (Gardiner, 1989). Its development is normally restricted to the mouthparts and the development cycle is completed in about 5-14 days (Davies, 1977, Woolhouse *et al.*, 1993, 1994; Woolhouse and Hargrove, 1998). *Trypanosoma congolense* develops in the midgut and the proboscis and takes a longer, but variable, time to complete its development. Estimates of the developmental period range from 7-40 days (Nantulya *et al.*, 1978; Woolhouse *et al.*, 1993, 1994; Dale *et al.*, 1995; Kazadi *et* al., 1998; Woolhouse and Hargrove, 1998). Species of the subgenus *Trypanozoon* have the most complicated cycle of development. It takes place in the midgut and the salivary glands of the tsetse fly and takes between 17 and 45 days (Hoare, 1970).

During the development in the tsetse fly, trypanosomes of the subgenera *Nannomonas* and *Trypanozoon* undergo substantial morphological and metabolic changes. They adapt themselves first to the physico-chemical environment in the insect vector and finally to life in the host (Vickerman, 1985; Vickerman *et al.*, 1988). The transformation of bloodstream trypanosomes into procyclic or midgut forms is a crucial first step in the establishment of a trypanosomal infection. This transformation proceeds rapidly in the posterior part of the midgut, the first procyclic forms appearing about 11 hours after ingestion (Turner *et al.*, 1988; Van den Abbeele *et al*, 1996). Factors known to influence this process include trypanolysins and trypsin or trypsin-like molecules in the fly's midgut (Imbuga *et al.*, 1992a, b), the type of host blood at the time of the infective feed (Moloo, 1981; Rickman & Kolala, 1982; Mulla & Rickman, 1988; Mihok *et al.*, 1993) and blood composition (Maudlin *et al.*, 1984; Gingrich *et al.*, 1985; Nguu *et al.*, 1996). The tsetse fly's immune system also plays an important role. A humoral defense mechanism, involving lectins, is implicated in the establishment of midgut infections (Maudlin and Welburn, 1987, 1988). In invertebrates, lectins bind to specific carbohydrate groups on cell surfaces of various organisms including trypanosomes and may cause agglutination, lysis and death (Jackson *et al.*, 1978; Croft *et al.*, 1982; Jackson and Diggs, 1983; Ibrahim *et al.*, 1984; Mutharia and Pearson, 1987). The procyclic form has a coat of procyclin and uses proline as source of energy (Roditi and Pearson, 1990). A procyclic infection does not always progress to maturation, the mechanism of which is complex. The midgut procyclics are free swimming; they move to the ectoperitrophic space to form an actively dividing population. They lose their glycoprotein coat and move forward to the proventriculus where they stop dividing. The proventricular 'mesocyclic' trypanosomes are longer than their procyclic precursors; they reinvade the endoperitrophic space and, in the case of members of the subgenus *Nannomonas*, move via the oesophagus to the hypopharynx where they attach and complete their development to become coated metacyclics. Members of the subgenus *Trypanozoon* move from the hypopharynx to the salivary glands where they complete development.

Although no classical sexual processes in the life cycle of trypanosomes have been described, it has been shown that gene exchange does occur within the tsetse fly

(Jenni *et al.*, 1986, Sternberg and Tait, 1990). The frequency of occurrence and epidemiological importance of this sexual cycle is still under investigation.

1.2.2.3 Methods to detect trypanosomal infections in tsetse

The most commonly used technique, employed in epidemiological surveys, to detect and characterize trypanosomal infections in tsetse, involves the dissection and microscopic examination of the vectors organs' in which the different subgenera of trypanosomes are known to reside (Lloyd and Johnson, 1924; Willet, 1955). Trypanosome species are identified according to their location in the fly. Infections due to *T. vivax* are found in the hypopharynx and labrum. *Trypanosoma congolense* infections are found in the hypopharynx, labrum and midgut, while *T. brucei* infections are confined to the hypopharynx, labrum, midgut and salivary gland. The method is easy and inexpensive but may underestimate the prevalence of infected flies (Otieno, 1983; Jefferies *et al.*, 1987). Moreover, the method cannot distinguish a mature *T. congolense* infection from a combination of immature midgut infections and mature *T. vivax* infections, neither can it identify mixed mature infections. The species specificity and sensitivity of the "dissection method" can be improved by inoculation of the infected organs into laboratory rodents (Tarimo *et al.*, 1987) or by feeding infected tsetse on susceptible animals (Nitcheman and Jacquiet, 1990). Since all mature trypanosomes are extruded when feeding, tsetse flies with mature trypanosomal infections can also be identified by inducing probing on a glass slide (Burtt, 1946c). The collected saliva can then be examined microscopically (Otieno and Darji, 1979; Gidudu *et al.*, 1995; Kazadi *et al.*, 1995). This method does not, however, enable species identification.

The development of specific DNA probes to identify trypanosomes improved the accuracy of the identification (Ole-MoiYoi, 1987; Mcnamara and Snow, 1990; Majiwa and Otieno, 1990; Masiga *et al.*, 1992; 1996; Woolhouse *et al.*, 1993; 1994). However, the method requires large numbers of trypanosomes, which are not always available in the mouthparts or salivary glands. The Polymerase Chain Reaction (PCR) has overcome this shortcoming and has been used successfully in various epidemiological studies (Solano *et al.*, 1995; Reifenberg *et al.*, 1997; Lefrançois *et al.*,

1998; Morlais *et al.*, 1998). A dot-ELISA was also developed to identify of trypanosome species in infected tsetse flies but this has not been used extensively (Bosompen *et al.*, 1996).

1.2.2.4 Factors affecting the prevalence of trypanosomal infections in tsetse

Various endogenous, ecological and parasite and host-related factors have been identified to influence the potential of trypanosomes to develop in tsetse flies (Jordan, 1974; Molyneux, 1976, ~~1980~~; Lambrecht, 1980).

There is contradictory evidence on the role of the tsetse's *sex* on its vectorial capacity. In field situations, female tsetse usually have a higher infection rate than males. This is attributed to the longer survival of females and, hence, the higher probability of picking up and maturing an infection. However, in laboratory experiments, some researchers have observed a significantly higher infection rate in females than in males (Makumyaviri *et al.*, 1984; Mihok *et al*, 1992) whereas others did not (Burtt, 1946b; Kazadi *et al.*, 1991).

Temperature exerts marked influence on the infection rates of salivarian trypanosomes in tsetse under laboratory conditions (Burtt, 1946a; Ndegwa *et al.*, 1992). The epidemiological importance of this phenomenon is not very clear. However, Ford and Leggate (19~~55~~61) found a positive correlation, associated with increasing mean annual temperature, between the infection rates of tsetse and the distance from the median of the tsetse belt in Africa.

Infectability of tsetse is known to be associated with the fly's *age*, the highest infection rates with *Trypanozoon* and *Nannomonas* infections normally being found in tsetse flies that have had their first blood meal on an infected host within 32 hours after eclosion (Wijers, 1958; Harley, 1971b; Jordan, 1976; Makumyaviri *et al.*, 1984; Distelmans *et al.*, 1982; Mwangelwa *et al.*, 1987). On the other hand, *Glossina* spp. can be infected at any age with trypanosomes from the *Duttonella* subgenus (Jordan, 1976). Welburn and Maudlin (1992) attributed the greater susceptibility of teneral flies (or flies that have never taken a blood meal) to midgut infections with *T. congolense* to the role of

rickettsia-like organisms potentiating the teneral's susceptibility to infection. Notwithstanding the teneral's higher susceptibility to midgut infections, field data show that the prevalence of mature infections increases with age for both the *Nannomonas* and *Duttonella* subgenera of trypanosomes (Harley, 1966; Woolhouse *et al.*, 1993; 1994; Leak and Rowlands, 1997; Woolhouse and Hargrove, 1998; Msangi *et al.*, 1998). This age-specific increase in the prevalence of trypanosomal infections in tsetse has important epidemiological consequences. The age structure of the tsetse population is thus an important factor in the determination of challenge.

1.2.2.5 The prevalence of trypanosomal infections in southern African tsetse species

Various studies have been conducted to determine the prevalence of trypanosomal infections in *G. m. morsitans* and *G. pallidipes* (Table 1.2.2). Despite the difference in location of fly capture and the season of capture, infection rates are variable but generally low.

Table 1.2.2: Prevalence of trypanosomal infections in the mouthparts of *G. m. morsitans* and *G. pallidipes* in southern Africa.

Tsetse species	Country	Average prevalence (%)	Source
G. m. morsitans	Zimbabwe	11.0	Chorley, 1929
	Zimbabwe	14.4	Leggate, 1963
	Zambia	7.9	Clarke, 1969
	Zambia	2.8	Okiwelu, 1977a
	Zambia	24.7	Willemse *et al.*, 1983
G. pallidipes	Zimbabwe	6.7	Leggate, 1963
	Zambia	12.7	Willemse *et al.*, 1983
	Zimbabwe	5.5	Woolhouse *et al.*, 1993
	Zambia	9.3	Woolhouse *et al.*, 1994

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1.2.3 The density of the tsetse population

An important variable in the epidemiology of trypanosomosis and probably the most important component of challenge is the density of the tsetse population, a factor that is usually unknown. It can be estimated with a variety of sampling methods. Unfortunately, these sampling methods are often biased with respect to certain tsetse species, age, sex or nutritional state (Vale, 1974; Vale and Phelps, 1978; Langley and Wall, 1990; Hargrove, 1991; Hargrove and Packer, 1993; Van den Bossche and Hargrove, 1999). Hence, the sample does not represent a true picture of the composition of the tsetse population and can only be used as an index of tsetse abundance. Despite the questionable value of samples collected with various sampling devices, within site comparisons of samples usually give a good indication of temporal variations in population density. The product of the index of abundance and the proportion of infected flies or the "index of challenge" generally correlates well with the incidence of trypanosomal infections in susceptible hosts (Claxton *et al.*, 1993; Leak *et al.*, 1988; Nankodaba *et al.*, 1988; Leak *et al.*, 1993; Rawlings *et al.*, 1994).

However, there are complex temporal and spatial aspects to the cattle/fly contact that should be taken into consideration and may explain discrepancies in the relationship between overall fly abundance and incidence of infection in a particular herd. For example, cattle/fly contact is influenced by cattle management practices, such as tethering or herding away from fly-infested areas. Such practices are common for nomadic cattle livestock owners (Leak, 1998). In some situations, the use of stock routes has enhanced the risk of trypanosomal infections in cattle (Jordan, 1965; Jordan, 1986). The spatial heterogeneity in contact between cattle and tsetse was investigated in The Gambia by calculating the tsetse density in relation to host density (index of exposure) in various sites for a particular time period (Wacher *et al.*, 1993; 1994). Results from this study indicated that individual herds based at the same village may experience a 5- to 10-fold variation in the degree of challenge (Wacher *et al.*, 1994).

Tsetse flies have strong diurnal activity patterns with respect to most sampling techniques (Leggate and Pilson, 1961; Power, 1964; Harley, 1965; Pilson and Pilson, 1967; Cuisance and Itard, 1973; Crump and Brady, 1979; Rowcliffe and Finlayson, 1982; Mwangelwa *et al.*, 1990; Owaga *et al.*, 1993; Kyorku and Brady, 1994). Similar patterns were observed when feeding activity of *G. pallidipes* on a tethered ox was monitored (Leggate and Pilson, 1961). This suggests that grazing cattle during periods of low feeding activity would decrease the chance of infection. Studies in The Gambia, however, have suggested that management of grazing times is unlikely to eliminate the risk of trypanosomosis transmission (Rawlings *et al.*, 1994).

1.2.4 The coefficient of transmission of a trypanosomal infection

Estimates of the efficiency of natural transmission of trypanosomal infections from tsetse to susceptible hosts vary widely but the efficiency is probably very low (Table 1.2.3).

Table 1.2.3: Coefficient of transmission (C.T.) of trypanosomes from infected tsetse to susceptible hosts.

Trypanosome species	C.T.	Source
T. congolense	0.22[+]	Harley and Wilson, 1968
	0.67[+]	Wilson *et al.*, 1972
	0.18[°]	Otieno and Darji, 1979
	0.46[+]	Rogers, 1988
	0.20[°]	Milligan and Baker, 1988
	0.008[•]	Baylis, 1997
T. vivax	0.23[+]	Wilson *et al.*, 1972
	0.46[+]	Rogers, 1988
	0.20[°]	Milligan and Baker, 1988
	0.024[•]	Baylis, 1997

[+] Estimates derived from injecting infected probosces into laboratory animals
[°] Estimates based on the number of trypanosomes extruded
[•] Estimate based on natural transmission

The probability of any infected blood meal eventually giving rise to a mature infection in a fly is also low (0.17 and 0.025 for *T. vivax* and *T. congolense*, respectively) (Rogers, 1988). Studies in the Luangwa Valley of Zambia and the Zambezi Valley of Zimbabwe have indicated that one in every 80 blood meals taken by *G. pallidipes* results in a successful infection in the fly (Woolhouse *et al.*, 1993; 1994). Similar estimates were obtained for *G. pallidipes* in Kenya (Tarimo Nesbitt *et al.*, 1991).

1.2.5 The interaction between tsetse and cattle in southern Africa

Detailed information is available on the behaviour of most Southern African tsetse species of economic importance (Phelps and Lovemore, 1994). Nevertheless, knowledge of the fly-related variables determining the interaction between *G. m. morsitans*, the major vector of bovine trypanosomosis in southern Africa, and cattle is poor. Data available on, for example, the seasonal distribution and abundance of the tsetse species and factors affecting those variables were collected in wildlife areas that cannot be compared with areas where the cattle/tsetse interface occurs (Pilson and Pilson, 1967). Although the epidemiology of trypanosomal infections in *G. pallidipes* has been investigated thoroughly (Woolhouse *et al.*, 1993, 1994) no information is available on the prevalence of trypanosomal infections in *G. m. morsitans*. However, a sound understanding of the dynamics of the tsetse population and the epidemiology of the disease is required for the successful management and localised control of bovine trypanosomosis.

1.3 The prevalence of tsetse-transmitted trypanosomosis in the host

1.3.1 Trypanosome development in the host

As the infective tsetse fly feeds, metacyclic trypanosomes and saliva pass through the hypopharynx and are inoculated intradermally. It is here that the infection and the induction of immunity is established (Akol and Murray, 1982; Dwinger *et al.*, 1988b). Trypanosomes multiply in the skin, and local skin reactions or chancres are often observed at the sites of the tsetse bite in goats but not in cattle. They are the first clinical indication of a trypanosomal infection but are not a prerequisite for establishment of an infection (Roberts *et al.*, 1969). The chancre is a raised, indurated, hot, painful swelling that may attain a diameter of 100 mm in 10-12 days; it regresses 10-15 days later (Akol and Murray, 1982). The composition of the cells within the chancre suggests that the reaction consists of an initial inflammatory reaction followed by an immune response. A major route of dissemination of trypanosomes from the skin to the general circulation is via the afferent lymphatics causing enlarged lymph nodes by about 7 days after infection (Luckins and Gray, 1979; Luckins *et al.*, 1994). The trypanosomes reach the blood via the draining lymphatics within a few days (Luckins and Gray, 1979; Luckins *et al.*, 1994). However, they cannot be detected in the peripheral blood until approximately 10-16 days post infection (Gray and Luckins, 1980; Akol *et al.*, 1986).

The early stages of infections with African animal trypanosomes in hosts are characterized by periodic fluctuations in the numbers of trypanosomes in the peripheral blood (Edwards *et al.*, 1956; Wijers, 1959; Godfrey, 1961). These are caused by the immunological response of the infected animals to the trypanosomal infection. The trypanosome stage in the blood possesses an electron-dense surface coat that covers the membrane and is present in all mammalian stages of the parasite. It consists of tightly packed antigenic molecules known as the Variable Surface Glycoproteins (VSG) or Variable Antigen Type (VAT) (Turner, 1985). It is absent during cyclical development in the tsetse fly until the infective metacyclic stage is reached in the tsetse's mouthparts. After an infected fly has bitten a suitable host, the

first antigens to appear in the blood is the metacyclic type (metacyclic VAT or M-VAT) (Luckins *et al.*, 1994).

When an animal is infected with a given trypanosome population, it mounts a protective antibody response against the specific bloodstream VAT resulting in a decline in the parasitaemia. However, some trypanosomes will produce a different VSG that cannot be destroyed by the antibodies of the initial immunological response. Consequently, this new VAT will give rise to an increasing parasitaemic wave. When the host mounts an immunological response to this new VAT, the parasitaemia again goes into remission. The consecutive replacing of the VSG coat is called antigenic variation which provides an outstanding mechanism for evasion of immune responses (Turner, 1985).

New VATs appear in the blood every few days (Uilenberg and Giret, 1972). The total number of VATs that can arise from a single trypanosome during the course of an infection has been estimated to be in excess of a hundred and the number of antigen genes is probably higher than 1000 (Nantulya, 1986; Pays, 1989). The sequence of expression of VATs tends to be quite stable in clonally-derived trypanosomes and characterizes a trypanosome strain or serodeme (Nantulya *et al.*, 1979; Nantulya, 1986). It is determined by gene activation (Pays, 1989).

Both IgG and IgM classes of antibody are involved in the immunological response of the infected host (Authié *et al.*, 1993). However, studies of trypanosome resistance in wildlife have indicated that serum factors other than antibodies may also affect the viability, multiplication and differentiation of trypanosomes (Mulla and Rickman, 1988). IgM are much more effective than IgG antibodies in the initial response to the infection. Nevertheless, in the later stages of infection IgG becomes as effective as IgM in neutralising trypanosomes. The levels of IgG in trypanosusceptible cattle breeds are usually low and transient (Authié *et al.*, 1993). A distinct population of IgM antibodies is of lower specificity and reacts with both trypanosome and non-trypanosome antigens (Williams *et al.*, 1996). These antibodies are likely to mediate

pathology rather than protection and only occur in trypanosusceptible breeds (Taylor, 1998).

1.3.2 Methods to detect trypanosomal infections in the host

The specific diagnosis of bovine trypanosomosis is notoriously difficult. Not only are there no specific clinical signs, but the intermittent and frequently low parasitaemias make detection of the parasites difficult. Economic principles, the availability of expertise and the diagnostic requirements will guide the choice of a particular diagnostic test. The diagnostic method will differ between situations depending, for example, upon whether species-specific diagnosis is required or whether surveys are conducted simply to determine the presence or absence of the disease. Often a combination of diagnostic tests is needed to obtain the required results.

1.3.2.1 Parasitological diagnosis

The parasite detection methods for trypanosomosis are highly specific but their diagnostic sensitivity (the proportion of infections that the methods detect) varies between tests (Paris *et al.*, 1982) and, for a particular test, is determined by the level of parasitaemia (Desquesnes and Tresse, 1996). The body fluid most commonly examined is blood, either capillary blood from the tip of the tail or venous blood from an ear vein or from the jugular vein. Lymph, aspirated from a punctured superficial lymph node (usually the superficial cervical), provides useful supplementary diagnostic material. The diagnostic sensitivity of wet blood smears (Boyt, 1986) is low but can be improved significantly by lysing the red blood cells before examination using a powerful haemolytic agent such as sodium dodecyl sulphate (SDS) (Ndao *et al.*, 1995).

More commonly, for routine diagnosis in veterinary practice, thick and thin smears of blood or lymph are prepared (Boyt, 1986). The unfixed de-haemoglobinized thick smear allows approximately 120 times more blood to be scanned than a thin smear (Killick-Kendrick, 1968) and, thus, has higher diagnostic sensitivity. The thin smear permits accurate speciation of the parasites. Despite the thin smear's species

specificity the method is relatively insensitive in detecting infection and results are delayed.

The probability of detecting trypanosomal infections in a sample of infected animals can be improved by increasing the volume of blood to be examined and concentrating the trypanosomes. The microhaematocrit centrifugation technique or Woo-method (Woo, 1970) is more sensitive but identification of trypanosome species is difficult. Alternatively, the buffy coat and the uppermost layer of red blood cells can be extruded onto a clean microscope slide and covered with a cover slip (buffy coat technique or Murray method (Murray *et al.*, 1977)). The two concentration methods are the most sensitive for detecting *T. congolense* and *T. vivax* infections (Paris *et al.*, 1982). The sensitivity of the concentration methods can be further improved by using the buffy coat double centrifugation technique (Kratzer and Ondiek, 1989). A modification of the microhaematocrit centrifugation technique is the Quantitative Buffy Coat method (QBC) (Bailey and Smith, 1992). The method has been used to diagnose *T. b. gambiense* infections but is too expensive for routine use in the diagnosis of animal trypanosomosis. The microhaematocrit centrifugation and buffy coat techniques are particularly useful in that the haematocrit or packed cell volume (PCV) can be assessed after centrifugation. The PCV of individual animals and the average PCV of herds can be determined (Hall *et al.*, 1983). At the herd level, the haematocrit profile or the herd average PCV is a useful indicator of infection and herd health. However, other factors such as nutrition and fasciolosis may also cause anaemia on a herd basis. Therefore, it is important to establish the haematocrit profile of negative herds before relying on it as an indicator of trypanosomosis in a herd.

The subinoculation of blood into rodents, usually mice or rats, allows a greater proportion of, especially, *T. brucei* infections to be detected than by direct examination of the buffy coat (Boyt, 1986). For practical reasons, subinoculation of blood into laboratory or other animals is not used as a routine diagnostic procedure. The method is expensive and diagnosis is not immediate. Furthermore, since rodents are refractory to *T. vivax* and not all *T. congolense* and *T. brucei* infections become established in the new host, even this method has serious limitations (Leeflang *et al.*,

1976). Mixed trypanosomal infections may also remain undetected. A procedure for the *in vitro* cultivation of *T. brucei* from the blood of infected animals has been described but success has been variable. Moreover, the method needs sophisticated equipment, yields results only after a considerable delay and is certainly not suitable for widespread use. A recently described kit for *in vitro* isolation (KIVI) of trypanosomes has proved promising for isolating and amplifying *T. b. gambiense* in humans, domestic and game animals (Truc *et al.*, 1992). The test's value in isolating *T. congolense* and *T. vivax* is still unknown. Since it is based on the cultivation of procyclic forms of trypanosomes, species differentiation is not possible (Komoin-Oka *et al.*, 1994).

A miniature anion-exchange technique has been described for field use in the diagnosis of human trypanosomosis (Lumsden *et al.*, 1979; Lanham & Godfrey, 1970), but is too cumbersome for routine use in veterinary practice. The use of a minicentrifuge with buffy coat technique has been advocated (Kelley and Schillinger, 1983), but it is not satisfactory in bovine practice where large numbers of samples often have to be examined.

1.3.2.2 Anti-trypanosomal antibody detection tests

The development of anti-trypanosomal antibody detection techniques has been a major improvement in the serodiagnosis of trypanosomosis. The indirect immunofluorescent antibody test (IFAT) (Wilson, 1969) has been and still is used widely to diagnose trypanosomosis (Ooijen, 1986). The test has undergone several modifications so that it can differentiate, to a limited extent, between trypanosome species in ruminants (Katende *et al.*, 1987). The serodiagnosis of trypanosomosis has greatly benefited from the introduction of enzyme immunoassays. The enzyme-linked immunosorbent assay (ELISA) was first used to detect antibodies against *T. b. rhodesiense* in humans (Voller *et al.*, 1975). It was further developed for use in animal trypanosomosis (Luckins, 1977) and was recently modified for large-scale use in trypanosomosis surveys (Hopkins *et al.*, 1998). Antigens can be prepared using bloodstream forms or procyclic trypanosomes (Greiner *et al.*, 1997b). The ELISA compares favourably with the IFAT (Luckins and Mehlitz, 1978) and has been found

to give results that correlate with the local history of trypanocide usage (Connor and Halliwell, 1987). However, even if a trypanosomal infection has been cured, anti-trypanosomal antibodies persist for several months (Bocquentin *et al.*, 1990) and antibody detection tests do not distinguish between current and past infections. They can only provide a presumptive diagnosis. Although the prevalence of anti-trypanosomal antibodies often increases with increasing prevalence of trypanosomal infections in a herd, antibody detection methods are not suitable for monitoring disease challenge in trypanosomosis endemic areas. Nevertheless, antibody detection tests, especially the antibody-detection ELISA, are very useful tools for determining the distribution of trypanosomosis. This is especially the case in areas where disease prevalence is low and where trypanocidal drugs are used frequently. Unfortunately, the test has hardly been used for this purpose.

1.3.2.3 Trypanosome antigen or DNA detection tests

Another alternative to the parasitological diagnosis of nagana is the use of assays to detect trypanosome-specific antigen or species-specific, or sub-species-specific DNA. An antigen detection enzyme-linked immunosorbent assay (antigen ELISA) test for trypanosomosis has been described (Nantulya *et al.*, 1987) but field evaluations of the test have given inconsistent results. Additional work is needed to develop the test for routine diagnosis of trypanosomosis. A polymerase chain reaction (PCR) has been developed for the diagnosis of infections with African trypanosomes in humans and animals (Gibson, 1994). Specific repetitive nuclear DNA sequences can be amplified for *T. vivax* and each of the three *T. congolense* subgroups (Moser *et al.*, 1989; Desquesnes, 1997). A common primer set is available for detection of the three *T. brucei* subspecies. The test requires specialized equipment and highly trained personnel; consequently, it is not suitable for use in most laboratories. Sample collection has been simplified by adapting the test using blood spotted on filter papers (Katakura *et al.*, 1997) so that a large number of samples can be processed at one time. This makes the test potentially suitable for large-scale surveys. However, the cost of PCR analyses prohibits its routine use in veterinary investigation. The PCR technique's ability to detect latent or mixed trypanosomal infections in different hosts

renders it suitable for research into the complex relationships between trypanosomes and their vectors and hosts (Reifenberg *et al.*, 1997).

1.3.3 The distribution of bovine trypanosomosis in southern Africa

Accurate and up to date information on the distribution of tsetse and/or bovine trypanosomosis in southern Africa, and elsewhere, is not available. Moreover, the little information that is available has been obtained through erratic tsetse and/or trypanosomosis surveys of low sensitivity. This leaves many areas where trypanosomosis is present but unidentified. In areas where the disease has been detected, the magnitude of the problem is likely to be quantified poorly and underestimated. Hence, available information on the distribution is unreliable for the development of a strategy for control. The recent development of a more sensitive and practical indirect method (anti-trypanosomal antibody detection Enzyme-Linked Immunosorbent Assay (antibody ELISA)) to detect the presence of bovine trypanosomosis (Hopkins *et al.*, 1998), offers the possibility of determining more accurately the distribution of the disease even in areas where trypanocidal drugs are used systematically. In areas where cattle are present, this may be a useful adjunct to tsetse surveys. Moreover, this indirect diagnostic method may be a practical monitoring tool for the effectiveness of vector control interventions. Unfortunately, the usefulness of this indirect method has not yet been fully assessed and interpretation of results needs to be improved.

1.4 Impact of tsetse-transmitted bovine trypanosomosis

1.4.1 Pathogenesis of bovine trypanosomosis

The pathogenesis of bovine trypanosomosis depends on three main factors (i) anaemia, (ii) tissue lesions notably myocarditis and myositis, (iii) immunosuppression (Urquhart, 1980).

1.4.1.1 Anaemia

In susceptible cattle breeds, the development of anaemia is a cardinal sign of trypanosomosis and the aetiology is similar in all species (Murray and Dexter, 1988). In cattle infected with *T. congolense*, increased red blood cell breakdown commences with the development of parasitaemia. The level and the duration of the parasitaemia often determine the severity of the anaemia (Murray *et al.*, 1979b, c). The course of the anaemia in cattle differs depending on the phase (Dargie *et al.*, 1979). During the first or acute phase, a rapidly developing haemolytic, often macrocytic and normochromic, anaemia develops over a period of, on average, 6 weeks after infection (Naylor, 1971). The anaemia occurs largely as a result of the removal from the circulation of damaged erythrocytes by cells of the, often hyperplastic, mononuclear phagocytic system (Murray and Morrison, 1979). Several mechanisms have been identified as being responsible for the erythrocyte destruction. They include, haemolysins and enzymes produced by trypanosomes, fever, complement and trypanosomal antigen (Murray *et al.*, 1979b). By the end of the acute phase, the PCV may be reduced to 15-20%. Severely affected animals will succumb. There are several possible sequelae to the acute phase of infection. Cattle that survive may gradually recover from the anaemia over several months, whereas some remain chronically infected and progress into the second or chronic phase. During the chronic phase, the rate of erythrocyte destruction continues to be high and the packed cell volume remains low (20-25%) with often low levels of parasitaemia. The anaemia is usually normocytic and normochromic (Naylor, 1971). While in the acute phase of the disease erythropoiesis is increased, as the disease progresses red cell synthesis is less than expected for the degree of anaemia resulting in dyshaemopoiesis (Dargie *et al.*, 1979). The trapping of iron in the phagocytes is believed to contribute to the failure of

erythropoiesis (Dargie, 1978). This chronic anaemia is by far the most common form of anaemia in trypanosomosis endemic areas. Animals lose weight and condition and extensive haemosiderosis occurs as a result of erythrophagocytosis.

Besides the development of anaemia, *T. congolense* and *T. vivax* infections also induce other haematological changes in cattle. Leukopenia associated with neutropenia is often observed in infected animals (Williams *et al.*, 1991). A lymphocytopenia manifests itself in most cattle and thrombocytopenia commonly develops rapidly during the first wave of parasitaemia (Wellde *et al.*, 1978; Davies, 1982).

1.4.1.2 Myocarditis and myositis

Many tissues and organs are damaged during the course of a trypanosomal infection. The pathogenesis of tissue damage depends on the species of trypanosome involved and its tissue invasiveness. *Trypanosoma congolense* and *T. vivax* are mainly intravascular parasites. They induce changes in the endothelium of capillaries, and so indirectly damage adjacent tissues. *Trypanosoma brucei*, on the other hand, is distributed both in the circulation and in the tissue. Its presence in the extravascular compartment is associated with marked lesions in parasitized tissues (Losos and Ikede, 1972; Murray and Morrison, 1979). One vital organ that is consistently damaged by all three trypanosome species is the heart. Cattle deaths from trypanosomosis are frequently the result of congestive heart failure brought about by a combination of anaemia, myocarditis and circulatory disturbances. Myositis of the skeletal muscle is partly cause of the emaciation characteristic of the disease (Urquhart, 1980).

1.4.1.3 Immunosuppression

The antibody responses of *T. congolense* or *T. vivax*-infected cattle to non-trypanosomal antigen are often depressed (Holmes *et al.*, 1974). The exact mechanism involved in this trypanosome-induced immunosuppression is not clear. It has been suggested that it is due to the specific effect of trypanosomes on the B-lymphocyte population making the B-cells unable to respond to other antigens (Holmes *et al.*,

1974; Murray, 1974). This state of immunosuppression renders trypanosome-infected animals more susceptible of other infections. Moreover, immune response of trypanosome-infected cattle to contagious bovine pleuropneumonia (Rurangirwa *et al.*, 1978; Ilemobade *et al.*, 1982), foot and mouth disease (Scott *et al.*, 1977; Sharpe *et al.*, 1982), clostridial (Scott *et al.*, 1977), rinderpest (Rurangirwa *et al.*, 1980) and louping-ill (Whitelaw *et al.*, 1979) vaccine is suppressed. However, immunosuppression does not necessarily impede the effectiveness of vaccinations (Scott *et al.*,1977; Rurangirwa *et al.*, 1980). Moreover, the competence of the immune system is largely restored by chemotherapy on the day of vaccination (Whitelaw *et al*, 1979; Rurangirwa *et al.*, 1979).

Trypanosomosis also causes widespread endocrine malfunction in cattle (Gombe, 1989). Abnormalities of the thyroids, ovaries, testes, adrenals and pituitary have been observed in trypanosome-infected cattle.

Trypanosomosis rapidly impairs thyroid gland function in susceptible cattle breeds. Reduced thyroxin levels are observed in the early stages of the infection (Mutayoba *et al.*, 1988a). In the more chronic stages of *T. congolense* or *T. brucei* infections, histopathological degenerative changes such as leucocyte infiltration, fibrosis and atrophy often occur (Fiennes, 1970; Losos and Ikede, 1972).

Trypanosome-induced ovarian anomalies such as cysts, fibrosis, reduced numbers of follicles and a persistence of the corpus luteum have been reported (Mutayoba *et al.*, 1988b). As a result, trypanosome-infected cows often have an irregular oestrus cycle and may be infertile or sterile. Trypanosomal infections during pregnancy may lead to endometritis, foetal death, abortion, still birth and neonatal death. Degenerative changes have also been observed in the male reproductive organs. This is especially the case in the testes and the epididymides of *T. congolense* and *T. vivax* infected bulls. This may lead to atrophy and aspermia (Sekoni *et al.*, 1990). However, the effect of trypanosomal infections on female reproductive organs is usually reversible. For example, cows that have received a curative diminazene aceturate treatment may resume cyclical ovarian activity within 4 months after treatment (Llewelyn *et al.*,

1988). Where there is severe degeneration of the testes and epididymes chemotherapy may be ineffective and infertility problems may persist (Sekoni, 1990).

Fiennes (1970) reported subcapsular cell infiltration in the adrenal gland of trypanosome-infected cattle. In *T. congolense*-infected goats, marked hypertrophy of the cortical zones, in the initial phase of the infection, followed by adrenocortical atrophy has been observed (Mutayoba *et al.*, 1988b). Focal necrosis, mononuclear infiltration and fibrosis have been described in the pituitary glands of trypanosome-infected domestic animals (Gombe, 1989).

The mechanisms of the trypanosome-induced ovarian, pituitary, thyroid and adrenal dysfunction are poorly understood. No parasites have been detected in the parenchymatous areas of those organs. Therefore, the cause of the lesions is believed to be the effect of anaemia, prolonged fever, thrombosis, the general wasting of body organs and imbalances in the endocrine systems (Ikede *et al.*, 1988).

1.4.2 Immunity to bovine trypanosomosis

1.4.2.1 Trypanotolerance

Certain breeds of cattle, sheep and goats, as well as many species of wild animals, can survive and produce better than other breeds in endemic tsetse-infested areas without the aid of chemotherapy. The majority of the "trypanotolerant" cattle breeds are confined to West and Central Africa and belong to the, humpless, *Bos taurus* type. Trypanotolerance is defined as the relative capacity of an animal to control the intensity, prevalence and duration of the parasitaemia and to limit the pathological effect of the parasites, the most prominent of which is anaemia (Murray *et al.*, 1981; 1982; Authié, 1994). These characteristics become very obvious 30 to 50 days after infection (Paling *et al.*, 1991). Few mechanisms have been identified to explain how trypanotolerant breeds account for their superior control of parasitaemia and anaemia. The sustained antibody response to trypanosome antigens in trypanotolerant breeds is probably the most prominent immunological feature. The superior humoral response of trypanotolerant cattle breeds may also result in the neutralisation of parasite products that are responsible for pathology (d'Ieteren *et al*, 1998). The absence of the non-specific IgM, which is likely to mediate pathology rather than protection, in trypanotolerant breeds may explain

the less severe pathology caused by trypanosomosis in trypanotolerant breeds (Williams *et al.*, 1996). Finally, trypanotolerant cattle maintain higher complement levels during trypanosome infection than susceptible breeds (Authié and Pober, 1990). While it is generally accepted that trypanotolerance, as an innate characteristic, is under genetic control, the stability of the characteristic can be affected by external factors such as overwork, malnutrition, intercurrent disease, pregnancy, parturition and lactation (d'Ieteren *et al*, 1998).

1.4.2.2 Acquired tolerance to trypanosomal infections

Although trypanotolerance is mostly associated with *Bos taurus*, there is evidence that, in some areas, Zebu cattle or *Bos indicus* have developed a degree of immunity to trypanosomosis (Dolan, 1998). There are no direct methods for measuring the development of such immunity. It can be achieved, to some extent, by assessing indirectly the performance of animals under a defined trypanosome challenge on the basis of variables such ability to maintain normal blood values, trypanocidal drug requirements and productivity.

It has been observed, on several occasions, that trypanosusceptible cattle breeds which survive trypanosomosis with or without chemotherapy, are subsequently more resistant to rechallenge (often referred to as nonsterile immunity or tolerance). Bevan (1928) was the first to suggest that such "nonsterile immunity" could be induced in cattle by administering trypanocides after infection with strains of *T. congolense*. Experiments conducted in Uganda in the 1970s, renewed the interest in this concept of nonsterile immunity (Wilson *et al.*, 1976). The principle was exploited successfully in Kenya, Ethiopia, Mozambique and Zimbabwe (Boyt, 1967; Bourn and Scott, 1978; Akol and Murray, 1985; Wellde *et al.*, 1989).

The mechanisms of nonsterile immunity still require further elucidation but it is attributed partly to the development of specific immunity against most or all metacyclic trypanosomes (M-VATs) of the various serodemes in a particular location (Masake *et al.*, 1987; Frame *et al.*, 1990). Another

contributing factor may be antigenic cross-reactivity of VATs from different serodemes (iso-VATs) (Murray *et al.*, 1982).

Age appears to play a significant role in tolerance of trypanosomosis. Various researchers have confirmed that young calves are more resistant to infection (Fiennes, 1970). Colostral antibodies have been demonstrated in goats and calves (Whitelaw and Jordt, 1985; Dwinger *et al.*, 1992). In goats, the antibodies protected newborn kids against homologous challenge, but provided no protection against heterologous challenge (Mehlitz *et al.*, 1983).

A range of stress factors increases susceptibility to trypanosomosis. These include pregnancy, parturition, lactation, nutrition, overwork and intercurrent disease. The physiological states of late pregnancy and lactation predispose cows to trypanosome infections and affect their ability to maintain PCV levels and body weights (Murray *et al.*, 1981; Ogwu and Njoku, 1987; Agyemang *et al.*, 1992). Research in sheep showed that adequate energy uptake enhances the ability of the infected animals to withstand the adverse effects of infection by promoting body weight gains and moderating the severity of the pathophysiological changes associated with trypanosomosis (Katunguka-Rwakishaya *et al.*, 1995). In arid conditions when fodder is in short supply, animals may have to trek many miles in order to obtain sufficient food. Under these circumstances, it is likely that infected animals suffering from anaemia and myocardial lesions are less able to cope and that their poor nutritional status will exacerbate the disease. Overwork also constitutes a stress which may exacerbate the severity of disease (Connor, 1994a). Finally, intercurrent disease is stressful; trypanosome-infected animals with helminthosis or another disease are more severely affected than those with either disease alone (Griffin *et al.*, 1981; Agyemang *et al.*, 1997).

1.4.3 Effect of bovine trypanosomosis on productivity

Tsetse-transmitted trypanosomosis is recognized as an important constraint to agricultural development in large parts of Africa. The effects of the disease can be either direct or indirect and have serious socio-economic implications. The direct impacts are those on (i) livestock productivity, (ii) livestock management and impacts

on (iii) migration and (iv) settlement (Swallow, 1998). Indirect effects can be aggregated into four groups. They are the effects on crop production, land use, ecosystem structure and function and human welfare (Swallow, 1998). The direct and indirect socio-economic impacts of nagana are often difficult to quantify. Nevertheless, the socio-economic impact of the disease and expected socio-economic impact of control interventions are essential components of planning for cost-effective control. Sustainable control can only be achieved when the benefits accruing from the control intervention are larger than its cost (Salmon and Barrett, 1994; Swallow and Woudyalew, 1994). The impacts that are the easiest to quantify are the direct effects of the disease on livestock productivity. Nevertheless, few studies have been conducted to assess the direct effects of trypanosomosis on livestock productivity. Most of the available information on the impact of trypanosomosis on cattle productivity is data collected before and after tsetse or trypanosomosis control interventions. The results of these studies suggest that the most consistent and quantifiable impact of bovine trypanosomosis in susceptible cattle breeds is on birth and mortality rate (Table 1.4.1).

Table 1.4.1: Effect of bovine trypanosomosis on various production variables.

Production parameter	Source
Mortality rate calf	Trail *et al.*, 1985
	Camus, 1991
	Fox *et al.*, 1993
Mortality rate adult	Fox *et al.*, 1993
	Jemal and Hugh-Jones, 1995
Calf growth rate	Trail *et al.*, 1985
Calving rate	Camus, 1991
	Fox *et al.*, 1993
	Jemal and Hugh-Jones, 1995
Calving interval	Trail *et al.*, 1990
	Rowlands *et al.*, 1994
	Agyemang *et al.*, 1997

1.4.4 The impact of bovine trypanosomosis in southern Africa

Most Governments in southern Africa recognize bovine trypanosomosis as a serious constraint to development and a serious threat to the agricultural sector. In Zambia, for example, bovine trypanosomosis is listed as a disease of National importance. In Mozambique, trypanosomosis is considered as a serious threat to the cattle-restocking programme. In Zimbabwe, the financial implications for the communal and commercial farming sector of tsetse reinvading cleared areas is enormous and substantial efforts are made to maintain artificial barriers to tsetse reinvasion. Despite the importance of bovine trypanosomosis in the southern African economy, the actual impact of the disease has hardly been quantified. For example, data on farmer behaviour and performance levels in tsetse-infested areas are not available. The relationship between the disease and livestock production, e.g. its effects on herd structure, herd growth, herd size, calving rates and mortality rates, is not known. This paucity of information on the socio-economic impact of bovine trypanosomosis is not surprising. Since trypanosomosis control was initiated, interventions were guided by entomological and veterinary principles. The impact of the intervention was also measured using entomological and veterinary variables. This type of approach is understandable in the context of large-scale control or eradication. In the context of small-scale sustainable control interventions, the socio-economic impact of the disease and expected socio-economic impact of the interventions become essential variables. Although assumptions can be made on the disease impact, it is often difficult to generalize. Ecological conditions differ between areas and the differences in the epidemiology of the disease may have significant effects on its socio-economic impact. Although the indirect impacts of bovine trypanosomosis may be difficult to quantify, the direct impact on productivity can be assessed easily. Only when these direct impacts are properly understood, can a basis be established to determine if nagana is a constraint to development in a particular area and if disease management practices can be improved in a sustainable way.

1.5 The control of tsetse-transmitted bovine trypanosomosis

Control of tsetse-transmitted bovine trypanosomosis can be based on control of the causal agent, the trypanosome, or control of the vector, the tsetse fly.

1.5.1 The control of the parasite

1.5.1.1 The history of trypanocidal drug development

Chemotherapy is the treatment of disease by use of chemical drugs. Such drugs disrupt or block one of more of the vital processes that are essential to the trypanosome. The first drug used to eliminate *T. congolense* and *T. vivax* infections in cattle was potassium antimony tartrate (Bevan, 1928). The drug had little prophylactic activity and, because it provoked severe tissue reactions, had to be injected intravenously. Despite its high toxicity and in the absence of another less toxic alternative the drug remained in use until the early 1950s. Research into the trypanocidal activity of potassium antimony tartrate led to the development of antimosan. This drug was active against *T. congolense* and *T. vivax* and, to a lesser extent, against *T. brucei*. It could be given intramuscularly or subcutaneously but required repeated doses at four-week intervals (Leach and Roberts, 1981).

Reports on the trypanocidal activity of phenanthridines resulted in the development of a number of trypanocidal drugs. Dimidium bromide was the first phenanthridine with acceptable solubility and was active against *T. congolense* when applied subcutaneously (Carmichael and Bell, 1944). It was used in large-scale mass treatment campaigns in southern, East and Central Africa. However, its use often resulted in photosensitization and severe local reactions.

Davey (1950) was the first to demonstrate the activity of quinapyramine dimethosulphate, a quinoline pyrimidine, against most pathogenic trypanosomes. Subsequently two products were introduced onto the market in the early 1950s, one containing quinapyramine dimethosulphate or quinapyramine sulphate and the other containing the sulphate in combination with quinapyramine chloride known simply as "Antrycide pro-salt". Each formulation had a prophylactic effect of about two months in medium challenge areas and was used widely in Africa between the 1950s and the

1970s (Fiennes, 1953). Because of problems with drug toxicity and the ease with which drug resistance appeared to develop, the drug ceased to be manufactured for use in cattle in 1974 (Ndoutamia *et al.*, 1993).

At about the same time as the development of quinapyramine sulphate, homidium, a new compound belonging to the phenanthridinium class was developed (Watkins and Woolfe, 1952). It was manufactured as both the bromide and chloride salts better know as ethidium bromide and chloride and is still in use. Both are evenly active against *T. congolense* and *T. vivax* but have limited but varying prophylactic activity (Dolan *et al.*, 1990; 1992). The introduction of quinapyramine and homidium in the 1950s meant that for the first time safe mass treatment of cattle was possible. The number of trypanocidal drug treatments administered annually rose dramatically (Ford and Blaser, 1971).

In 1955, a new aromatic diamidine, diminazene aceturate with ultra-short acting trypanocidal activity was developed (Bauer, 1955). The drug had a considerably higher therapeutic index than the other trypanocidal drugs then available. It is still in use today in most countries of the southern African region. At about the same time, Watkins and Woolfe (1956) reported the synthesis of the quinapyramine derivative, pyrithidium bromide. This compound, marketed as Prothidium, had therapeutic and prophylactic action but was less effective than quinapyramine. Resistance rapid developed to the drug when it came into general use. It was withdrawn from the market in 1985. In the late 1950s, Wragg *et al.* (1958) described a new trypanocide, isometamidium chloride, derived from homidium chloride. Isometamidium chloride was marketed in 1961 as both a therapeutic and prophylactic agent. The compound has been used successfully to maintain the productivity of cattle under tsetse challenge both in commercial and communal management systems in most African countries.

Up until the early 1960s several pharmaceutical companies were actively involved in the development of new trypanocidal compounds. However, the trypanocide market is not the most attractive one for large multinational pharmaceutical companies. Hence the drastic reduction in research in trypanocides. More attractive to industry is

the development of improved formulations, and new delivery systems of existing products. Several alternative delivery systems (liposomes, carrier erythrocytes, suramin and dextran complexes, etc.) have been developed for the treatment of cattle using available trypanocides (Peregrine, 1994; Diarra *et al.*, 1998).

1.5.1.2 Resistance of trypanosomes to trypanocides

Chemotherapy for tsetse-transmitted bovine trypanosomosis currently depends upon the salts of 4 compounds, several of which are closely related (Table 1.5.1). Much of the early work on resistance and cross-resistance in trypanosomes infections in cattle was carried out by Whiteside in Kenya during the 1950s (Whiteside, 1960). Since then, (multiple) drug resistant trypanosome strains have been demonstrated for all economically important trypanosome species and over the full range of trypanocidal drugs (Geerts and Holmes, 1998). The factors responsible for the development of resistance to trypanocides are not well known. The exposure of the parasite to subtherapeutic concentrations of the drug (often due to underdosing) has been considered the most important factor (Boyt, 1986). Large-scale drug use and the use of drugs that are eliminated slowly from the body may also contribute to its development (Geerts and Holmes, 1998).

1.5.1.3 History of trypanocidal drug use and drug resistance development in the southern African region

1.5.1.3.1Eastern Zambia

The control of bovine trypanosomosis in eastern Zambia has, for the past 45 years, relied heavily on the use of chemoprophylaxis and chemotherapy. It was only after the discovery of dimidium bromide and antrycide prosalt that the Government gained the upper hand against tsetse in areas of Katete and Petauke Districts that were settled and reinvaded by tsetse in the mid 1950s (Steel and Gledhill, 1955; Vail, 1977). Three-monthly block-treatment with chemoprophylactic drugs was initiated in the mid-1960s and lasted until 1989. The main trypanocide used in those campaigns was isometamidium chloride supplemented by Prothidium between 1970 and 1972 (Leak, 1980). Curative treatments with diminazene aceturate were also administered.

Table 1.5.1: Chemotherapeutic and chemoprophylactic compounds currently used for tsetse-transmitted bovine trypanosomosis.

Compound	Trade Names	Treatment regimen		Activity	Use
		Dose (mg/kg)	Route		
Diminazene aceturate	Berenil® Veriben® Ganaseg® Trypanzen® Trypan®	3.5 - 7.0	i.m.	*T. congolense* *T. vivax* *T. brucei*	Therapeutic
Homidium chloride Homidium bromide	Novidium® Ethidium®	1.0-2.0	i.m.	*T. congolense* *T. vivax* *T. brucei*	Therapeutic/prophylactic at low challenge
Isometamidium chloride	Samorin® Trypamidium®	0.5-1.0	i.m.	*T. congolense* *T. vivax* *T. brucei*	Therapeutic/prophylactic
Quinapyramine dimethylsulphate	Trypacide sulphate®	3.0-5.0	s.c.	*T. congolense* *T. vivax*	Therapeutic/prophylactic
Quinapyramine dimethylsulphate: chloride	Trypacide Pro-salt® Antrycide® Triquin®			*T. brucei*	

i.m = intra muscular
s.c.= subcutaneously

The administration of these campaigns was, however, fraught with difficulties. Lack of transport and frequent shortages of drugs resulted in prolonged treatment intervals. A cost-recovery scheme for trypanocidal drugs (isometamidium chloride and diminazene aceturate) was launched in 1990 and replaced the free-of-charge treatment campaigns. Despite their extensive use, only localised resistance to diminazene aceturate or isometamidium chloride has been reported (Chitambo and Arakawa, 1991; 1992).

1.5.1.3.2 Zimbabwe

Bevan (1928) confirmed the efficacy of potassium antimony tartrate against *T. congolense* and *T. vivax* infection in cattle in Zimbabwe (then Rhodesia). Subsequently, many thousands of head of livestock were treated and saved by its use (Boyt, 1967). It was replaced by dimidium bromide administered subcutaneously and intravenously. Dimidium bromide was used widely until the middle 1950s, when its use was abandoned after disastrous losses due to photosensitization in the eastern districts (Boyt, 1967). It was replaced by the less toxic homidium bromide or chloride. The quinapyramine compounds (Antrycide), the first truly prophylactic trypanocides, were introduced in 1955 (Boyt *et al.*, 1963). It was used extensively in the Sabi Valley (Chipinge District) during the latter half of 1955 (Boyt, 1979). However, in 1962 widespread drug resistance in trypanosomes to this compound was detected (Boyt, 1971). At about the same time, diminazene aceturate was introduced and was quickly taken into general use. It was supplemented, in the mid-1960s, with isometamidium chloride. Despite its large-scale use, resistance of trypanosomes to isometamidium chloride has only been reported sporadically (Boyt, 1971; Lewis and Thomson, 1974). Resistance to diminazene aceturate was only recorded once (Joshua *et al.*, 1995).

1.5.1.3.3 Malawi

In Malawi (then Nyasaland) heavy reliance was initially placed on homidium bromide in the mid-1950s to early 1960s. However, drug resistance to this

compound emerged quickly and campaigns were terminated in 1957 (Matson, 1959). Homidium was replaced by quinapyramine but soon trypanosome strains emerged that were resistant to this compound. This resistance was overcome successfully with diminazene aceturate (Connor, 1989). Since the early 1970s, bovine trypanosomosis has been controlled satisfactorily by chemotherapy using diminazene aceturate and chemoprophylaxis using isometamidium chloride.

1.5.2 The control of the vector

1.5.2.1 Indirect control methods

1.5.2.1.1 Vegetation clearance

The destruction of all trees and shrubs in an area, the oldest tsetse control method, is completely effective. Ruthless vegetation clearing, often combined with settlement, formed the basis of routine tsetse control operations up to the 1930s (Vail, 1977). In south~~w~~estern Zimbabwe, ruthless clearing of vegetation in a ~~patch~~ strip of 15km wide, was undertaken to create a barrier for further advance of tsetse (Robertson and Kluge, 1968). Although vegetation clearing for tsetse control is not practised today, the gradual expansion of the human population has a similar effect. This is the case in many areas in Malawi where, due to the ever-increasing requirement for land for cultivation, most of the tsetse habitat outside national parks, game reserves or forest reserves has been destroyed. Because of its drastic effects on the environment, ruthless vegetation clearing was replaced by a more refined and discriminate approach to tsetse habitat alteration. Discriminative vegetation clearing involved the removal of portions of the vegetation essential for the tsetse's survival. The principle of discriminative clearing was based on the observation that, along fly-round transects, tsetse catches were not distributed randomly but confined to certain parts of the vegetation (Ford *et al.*, 1959). Removal of these sections was expected to suffice to control tsetse (Steel, 1958). Further studies on the distribution of tsetse within its habitat showed that the flies were

more evenly distributed than the fly-round catches suggested (Bursell, 1966; Pilson and Pilson., 1967). Hence, the underlying principle of discriminative clearing as a tsetse control method was based on a bias associated with the sampling method rather than a specific aspect of the tsetse's ecology. Despite its rather doubtful underlying principles, discriminative clearing was highly successful in clearing *G. m. morsitans* in an area in northern Zambia (Glover *et al.*, 1955).

1.5.2.1.2 Game elimination and game fences

At the end of the nineteenth century a severe rinderpest pandemic entered southern Africa from East Africa and killed much of the susceptible game animal populations, many of which were preferred hosts of *G. m. morsitans* and *G. pallidipes*. As a result, tsetse disappeared from large parts of southern Africa. As the animal population in southern Africa recovered from the rinderpest pandemic, surviving tsetse gradually spread from isolated foci in Zimbabwe but especially Zambia and Malawi. The observation during the rinderpest pandemic of the "vital association between the prevalence of big game and the continuance and increase of the fly" (Jack, 1914) led to the development of the concept of game elimination by man as a new tsetse control method. In 1933, a policy of shooting game animals was introduced in Zimbabwe. The Zambian Government recognized the close relationship between tsetse and game by creating, in 1942, the Department of Game and Tsetse Control (Vaughan-Jones, 1948). After initial trials, the method was adopted as a technique for the large-scale control tsetse in Zambia, Zimbabwe, Botswana and South Africa (Du Toit, 1954; Davies, 1980; Evison and Kathuria, 1984). In 1949, for example, 24 871 head of wild animals were destroyed by the Department of Agriculture as part of the tsetse control programme in Zimbabwe alone (Whellan, 1950). The shooting of game, at such a scale, resulted in public opposition. As a result, the method was abolished in 1960. Hunting was reintroduced in 1964 (due to spectacular advances of the fly front following the cessation of hunting), on the basis of selective hunting of tsetse hosts in selected areas defined by fences. The

identification of the preferred host of tsetse was facilitated by the development of immunological methods for identifying the origin of blood meals of tsetse flies (Weitz, 1963). Although game elimination was and still is highly controversial, significant portions of land were reclaimed in Zimbabwe using this method of tsetse control. By 1945, for example, game elimination had contributed significantly to the clearing of approximately 26 000km^2 of tsetse-infested land (Cockbill, 1967).

In the 1950s game fences were introduced in Zimbabwe, Botswana, South Africa and Zambia in an attempt to preclude a wide variety of the preferred hosts of tsetse from reclaimed land and thus reduce the chance of fly re-invasion. Initially, barbed wire fences were used to indicate the start of tsetse control operations areas. Later on, as settled areas moved closer to the operations areas, a cattle fence was used to keep cattle away from operations areas where they could come into contact with tsetse. This approach was improved in the 1950s by replacing barbed wire fences, on wooden poles, by high-tensile steel game fences, originally on wooden poles but eventually on steel supports. In Zimbabwe, these substantial fences were placed, generally, close to the limits of land allocated for settlement-safari areas, game reserves or international boundaries. When game reduction work restarted in 1964, it was done on the selected species basis between an outer game fence and an inner cattle fence. Elephants and buffalo were shot where they were damaging the game fence. The combination of fences with selective elimination of hosts, bush clearing and ground spraying have for long formed the "holding lines" preventing tsetse from re-invading previously cleared areas in Zambia. In 1972, those "holding lines" extended up to 1200km. Such an extensive holding line operation was difficult to maintain and was replaced by aerial spraying in the mid-1970s in Zambia. In Zimbabwe, the system of fences broke down in the war of independence when much fencing material was stolen, and has not been restarted.

1.5.2.2 Direct control methods

The use of insecticides for the control of insects of veterinary and medical importance was practised for many years before it could be used to control tsetse. Only after the discovery of persistent and cheap chlorinated hydrocarbon insecticides was emphasis in the control of tsetse changed from altering the fly's environment to direct attack on the fly using toxic substances. The first extensive use of insecticides for the control of tsetse populations was the campaign carried out in Zululand (South Africa) between 1945-1954 (Du Toit, 1954; Du Toit *et al.*, 1954).

In southern Africa, chemical control of tsetse, can be divided into two phases; (i) the application of insecticides to vegetation and (ii) the use stationary and mobile baits treated with insecticides.

1.5.2.2.1 Application of insecticides to vegetation

Ground spraying

As soon as modern insecticides with sufficient toxicity to tsetse became available, the control of tsetse by application of those compounds to the vegetation became a possibility. In order to be effective in eliminating the tsetse population, the insecticide deposits had to remain toxic for a sufficiently long period to allow the pupae in the ground, present at the start of the operation, to emerge. This could only be achieved with persistent, and highly toxic, chlorinated hydrocarbons such as DDT (Symes *et al.*, 1948; Vanderplank, 1947; Glover, 1961) or dieldrin (Gledhill and Caughey, 1963) and to a lesser extent with synthetic pyrethroids such as deltamethrin (Holloway, 1989). The first of the chlorinated hydrocarbons to become readily available was DDT. This was followed by the isolation of an even more persistent organochlorine compound, dieldrin. Both DDT and dieldrin were the main insecticidal compounds applied from the ground by ground spraying. Ground spraying has undergone little development since it became available in the mid-1950s. However, the method of application has become more selective through a better knowledge of the tsetse's favoured resting sites (Okiwelu, 1976; 1977b).

Ground spraying, using knapsack sprayers, was the main tsetse control method in Zimbabwe between 1960 and 1986 (Hursey and Allsopp, 1984). During this period, over 60 000 km^2 of infested land were reclaimed. Originally, dieldrin was used as insecticide but was replaced by the cheaper DDT in 1967. In the beginning of the 1990s, trials were conducted to assess the feasibility of using deltamethrin in ground spraying operations (Shereni and Pope, 1992). More recently, deltamethrin was used in an attempt to clear flies from an area of approximately 500 km^2 in the north of the country (Shereni, pers. comm.). In the 1950s, ground spraying with DDT or dieldrin was introduced in Zambia (Evison, 1980). The method was used up to the 1970s. In Zambia, a degree of mechanization was achieved by carrying out the less selective ground spraying from four-wheel-drive vehicles (Unimog). In Botswana, DDT and dieldrin ground spraying was used to control *G. m. centralis* in the Okavango Delta until 1972. Dieldrin ground spraying, using knapsack sprayers, was carried out between 1964 and 1985 along the Kwando River in Namibia. From 1985 onwards, dieldrin was replaced by a synthetic pyrethroid, alphacypermethrin (Bingham *et al.*, 1995). Also, in South Africa, combined aerial and DDT ground spraying operations in Zululand resulted in the eradication of *G. pallidipes* in 1954 (Du Toit, 1954; Du Toit *et al.*, 1954; Kappmeier *et al.*, 1998).

Aerial spraying

The aerial application of thermal aerosol has gone through various stages of development since its first use. Early work in Zululand and Zimbabwe used 4% HCH (formerly BHC) as a thermal aerosol or smoke. The insecticide in diesoline was injected into the exhaust pipe of the aircraft emerging as an easily visible white smoke. This smoke enabled the pilots to track the treated area in hours of daylight (late evening and early morning when conditions were suitable). These restrictions limited the size of areas that could be treated.

When ultra low volume formulations of insecticides became available (especially endosulfan and some pyrethroids) and could be applied as cold aerosols, the economics of aerial spraying improved greatly as the load of insecticide could cover much greater areas per aircraft. The technique is based on the application of an aerosol of fine droplets, containing insecticide, over the tsetse's habitat. The droplets are very small so that normally persistent insecticides, such as endosulfan, have no residual action. The correct droplet size is, therefore, fundamental for the success of aerosol application. Since more than 50% of the tsetse population is at any one time in the soil, and is thus not at risk of exposure to the insecticide such applications of non-residual insecticides need to be repeated. Only dieldrin and endosulfan have been used widely in this technique although some trials have been carried out with synthetic pyrethroids (Spielberger *et al.*, 1979).

The first aerial spraying campaign against tsetse was in Zululand (South Africa) between 1945 and 1948 (Du Toit, 1954; Du Toit *et al.*, 1954). Large-scale aerial spraying campaigns, mainly against *G. m. morsitans*, were conducted in Zimbabwe between 1953 and 1988 (Cockbill *et al.*, 1963; Chapman, 1976; Hursey and Allsopp, 1984). In Zambia aerial spraying was initiated in 1968 to hold the gradual re-invasion of tsetse into previously cleared areas. Aerial spraying with endosulfan was conducted between 1968 and 1978 to clear tsetse from extensive areas in the Southern, Western and Eastern Provinces (Evison and Kathuria, 1984). In Botswana, repeated aerial spraying operations have been mounted over 17 years in attempts to eradicate *G. m. centralis* from the Okavango Delta (Bingham *et al.*, 1995).

1.5.2.2.2 Application of insecticides to bait systems

Stationary baits

In the mid-1970s, analyses of the tsetse's behaviour, in the absence of men, suggested that, if the right baits were used, high numbers of tsetse could be attracted (Vale, 1974). Systematic research into the various components of the tsetse's response to baits (Vale, 1982a, 1993b) led to the development of

traps for especially *G. pallidipes* and *G. morsitans* (Vale, 1982b; Flint, 1985, Laveissière *et al.*, 1985). The available traps are sensitive in sampling *G. pallidipes* and have been considered for use in tsetse control (Vale *et al.*, 1986; Hargrove and Langley., 1990). However, since the intention of a tsetse control operation is killing tsetse rather than retaining them, traps to control tsetse have been simplified. This simplification process resulted in the development of "targets" coated with a persistent insecticide. The first target (R-type), developed in Zimbabwe, was three-dimensional and made of black cloth and black mosquito netting (Vale *et al.*, 1986). The original R-type target was much simplified and was replaced by the S-type consisting of a piece of black cotton cloth (0.7 x 1.0m) flanked at both sides by black terylene mosquito netting (0.5 x 1.0m) fastened to a metal frame. Further studies on the attractiveness of targets (Vale, 1993b) and the alighting response of *G. pallidipes* and *G. m. morsitans* resulted in the development of an all-cloth target. It consisted of a central panel of black cloth (1.0 x 1.0m) treated with insecticide and flanked at both sides by untreated panels of blue material (0.5 x 1.0m).

Although some early studies indicated that the presence of animals could improve the catches of tsetse in a trap (Fuller and Mossop, 1929; Swynnerton, 1933; Lloyd, 1935), the role of olfactory components in attracting tsetse to baits was clarified much later. Studies of the true extent of olfactory attraction were facilitated greatly by the introduction of electrocuting capture devices (electric nets) to catch tsetse in the absence of men (Vale, 1974). Using electric nets, Vale (1974) demonstrated that, when man was absent, catches for *G. m. morsitans* and *G. pallidipes* increased significantly. Moreover, catches increased about 20-fold when large doses of cattle odour were added (Hargrove and Vale, 1978; Hargrove *et al.*, 1995). This significant increase in tsetse catches at stationary baits in the presence of oxen was attributed to compounds such carbon dioxide (Vale, 1974, 1980), acetone (Vale and Hall, 1985a) and butanone and 1-octen-3-ol (Hall *et al.*, 1984), present in ox breath. The attractiveness of stationary baits, especially for *G. pallidipes*, was

improved even more after the isolation of 4-methylphenol and 3-*n*-propylphenol from ox urine (Owaga *et al.*, 1988; Vale *et al.*, 1988b). Nevertheless, despite progress made in the identification of artificial odour attractants in the past decade, artificial odours are still less efficient in attracting tsetse than natural ox odour (Torr *et al.*, 1995).

Due to the identification of powerful visual and olfactory attractants the prospect of controlling tsetse with artificial baits became promising. The cost effectiveness of such operations was improved even more by alterations in the design of targets (Vale, 1993a), better target siting (Vale, 1998), more efficient methods of dispensing odour attractants (Torr *et al.*, 1997) and increased persistence of the insecticide (Torr *et al.*, 1992).

Since the initial field trials (Vale *et al.*, 1986; Vale *et al.*, 1988a) and assessment of their effect on the environment (Nagel, 1995), odour-baited, insecticide-treated targets have been used extensively in tsetse control operations in southern Africa and elsewhere (Slingenbergh, 1992). In Zimbabwe, large areas have been and are still being cleared using targets at a density of about 4/km^2 (Shereni, 1990; Lovemore, 1999). In the Western Province of Zambia, approximately 8 000km^2 of land was cleared of *G. m. centralis* (Willemse, 1991; Knols *et al.*, 1993).

Odour-baited, insecticide-treated, targets are also used to prevent tsetse re-invasion into cleared areas. Initially, target barriers were deployed to protect ground-sprayed or aerial-sprayed areas. The combined use of aerial and ground spraying, odour-baited target and target barriers formed the basis of a proposal to eradicate progressively tsetse from the 322 000km^2 fly-belt common to Malawi, Mozambique, Zambia and Zimbabwe (Lovemore, 1986). Research conducted to optimize the design of target barriers indicated that 8km-wide target barriers with a normal target density of 4/km^2 were very effective in preventing tsetse from re-invading previously cleared areas (Hargrove, 1993; Muzari *et al.*, 1996). Such a barrier protects approximately

1 000 000 head of mainly communal cattle from the threat of tsetse in Zimbabwe. In Malawi, odour-baited target barriers along the eastern edge of Nkhotakota Game Reserve and Kasungu National Park suppress the prevalence of bovine and human trypanosomosis. In Namibia, a target barrier along the Kwando River prevents tsetse from spreading into the eastern Caprivi.

Mobile baits

Despite concerted efforts made in their development, artificial tsetse baits have never been able to mimic completely the tsetse's natural host. The attractiveness of hosts to tsetse was exploited as a tsetse control method by researchers in the late 1940s. Experiments conducted in Tanzania resulted in a 95% reduction in the apparent density of *G. pallidipes* five months after DDT-treated oxen were introduced in an area (Whiteside, 1949; Vanderplank, 1947). Similar results were obtained in Zululand (Du Toit, 1954). Less successful experiments were carried out in areas infested by *G. morsitans* and *G. swynnertoni* (Burnett, 1954). Despite initial successes, this promising tsetse control method was abandoned because of the low persistence of the insecticides used.

It took almost 40 years before the method was taken up again. This was a result of the discovery of the persistent and less toxic synthetic pyrethroids. The first controlled study on the persistence of the toxic effect to tsetse of deltamethrin spray, applied to cattle, was conducted in Zimbabwe (Thomson, 1987). Results of the trials indicated a high mortality in *G. pallidipes* and *G. m. morsitans* within the first two weeks of insecticide-treatment followed by a long-lasting knock-down effect.

The promising results of the initial controlled trials were followed by several field trials in the southern African region. A small-scale trial, conducted in the Eastern Province of Zambia, involving the weekly dipping in deltamethrin of 400 head of cattle, resulted in a reduction of the trypanosomosis incidence

from 40%, at the beginning of the trial to 5% eight months later (Chizyuka and Luguru, 1986). Similar effects were observed in other parts of Zambia (Wiersma and Schoonman, 1992) and in Zimbabwe (Thompson *et al.*, 1991).

Despite the successful application of this method in other parts of Africa (Bauer *et al.*, 1988; Bauer *et al.*, 1992b; Fox *et al.*, 1993; Bauer *et al.*, 1995; Leak *et al.*, 1995), it has not been used widely in southern Africa. Since 1986, the treatment of cattle using deltamethrin dip or deltamethrin pour-on formulations constitutes part of the routine tsetse control operations in east/north east Zimbabwe preventing tsetse from re-invading from Mozambique (Shereni, 1990). In South Africa, the weekly dipping of cattle in lambda cyalothrin could control an outbreak of bovine trypanosomosis in Zululand in 1990 (Kappmeier *et al.*, 1998).

1.5.3 The control of tsetse-transmitted bovine trypanosomosis in southern Africa

In most countries of southern Africa, trypanocides are available to farmers at cost. Hence, cattle owners have been able to implement their own disease management strategies using therapeutic and/or prophylactic drugs. The long-term sustainability of such an approach is a function of the probability of trypanosomes developing resistance to those drugs. Trypanocidal drug resistance has been recorded in many countries in West and East Africa (Pinder and Authié, 1984; Dolan *et al.*, 1992). In southern Africa, updated information on the susceptibility of trypanosome strains to trypanocidal drugs is not available. Sensitive methods to determine the susceptibility of trypanosomes to isometamidium chloride have been developed and are being used on a trial basis (Eisler *et al.*, 1996). Unfortunately, these techniques are expensive and cannot be used to assess the susceptibility of trypanosome strains to the most commonly used trypanocide, diminazene aceturate. A first step in determining the probability of drug resistance and, hence, determine the sustainability of drug use in the control of bovine trypanosomosis could be the establishment of the trypanocide drug-use practices by the communal farmer. Information on the frequency with which trypanocides are used, the dose and the mode of application is not

available. Nevertheless, this type of information should be an integral part of decision-making on the control of trypanosomosis in a particular area.

At the moment, the community could apply two tsetse control methods based on bait technology. Stationary baits (odour-baited, insecticide-treated targets (Vale *et al.*, 1986)) have proven to be very effective in controlling tsetse in large, homogenous areas (Vale *et al.*, 1988a). The effectiveness of this method in controlling tsetse in small cultivated areas or in preventing the interaction between tsetse and cattle still has to be determined. The effectiveness of mobile baits (insecticide-treated cattle (Thomson, 1987) in controlling tsetse, under conditions prevailing in southern Africa, still needs to be tested. Moreover, the role that insecticide-treated cattle could play in preventing tsetse from re-invading previously cleared areas and the effect of regular insecticide-treatments on the immunity against tick-borne diseases still has to be assessed.

CHAPTER TWO

THE INTERACTION BETWEEN TSETSE AND CATTLE. THE PLATEAU AREA OF EASTERN ZAMBIA AS AN EXAMPLE

2.1 Introduction

Effective management of a disease requires an understanding of the variables affecting its prevalence and distribution. In tsetse-transmitted bovine trypanosomosis, this involves understanding the dynamics of the vector and host population(s) and the factors affecting the interaction between both.

Tsetse-transmitted bovine trypanosomosis is prevalent in most southern African countries. The economic importance and the prevalence of the disease varies between countries and, within a country, between localities. Nevertheless, one locality of particular importance is the Eastern Province of Zambia.

The Eastern Province of Zambia covers an area of approximately 69 000 km^2. Most of the human population and nearly all its livestock is found on the eastern plateau which follows the international borders with Malawi to the east and Mozambique to the south. The plateau is bounded to the west by the Luangwa Valley. *Glossina m. morsitans*, *G. pallidipes* and *G. brevipalpis* are present in the Luangwa Valley. On the plateau, only *G. m. morsitans* is present. The plateau area of eastern Zambia is one of the few large areas, in southern Africa, were cattle are kept in a tsetse-infested zone. The major source of tsetse is and has always been the Luangwa Valley. Tsetse have been observed in the Luangwa Valley since the nineteenth century. However, after the rinderpest epizootic of the 1890s eastern Zambia was largely free of tsetse. By the end of the nineteenth century, cattle were reared successfully in the Luangwa Valley (Vail, 1977). The quick regeneration of the wildlife population and the protection of game resulted in a concomitant increase in the tsetse population density. At the same time, game and tsetse (*G. m. morsitans*) were spreading out of the Luangwa Valley south and east onto the eastern plateau (Hall, 1910; Neave, 1911) resulting in the first outbreaks of bovine trypanosomosis. During the following decades, both game (mainly elephants) and tsetse spread across the plateau in Lundazi, Chipata, Katete and Petauke Districts. Severe trypanosomosis outbreaks stimulated the Zambian Government into embarking upon an extensive programme of bush clearance and game elimination together with resettlement programmes to induce bush clearing and, hence, reduce the density and spread of tsetse.

Nevertheless, since that period, people and their livestock have lived in the tsetse-infested country of eastern Zambia. Over the years, the tsetse population has clearly adapted to the changed environment and, currently, thrives in a highly cultivated area with few game animals. The encroachment of people into tsetse-infested areas or into potential tsetse habitat is not restricted to eastern Zambia. It has occurred and will continue to occur in, for example, large areas of Mozambique where gradual restocking of cattle and an ever increasing human population will increase the need for land. It occurs, to a certain degree, along Malawi's tsetse-infested national parks and game reserves. Though every situation is different, the plateau of eastern Zambia offers a unique opportunity to study in detail the characteristics of the relationship between tsetse and cattle in an environment resembling the areas of medium to high agricultural potential in southern Africa.

Several analytical models have identified different host-and vector-related variables involved in the epidemiology of tsetse-transmitted (bovine) trypanosomosis (Section 1.2). The host related variables, such as the prevalence of trypanosomal infections in cattle, will be dealt with in the following chapter (Chapter 3). This chapter aims at quantifying the variables that affect the various tsetse-related components of challenge.

In the broadest sense, the density of the tsetse population is probably the most important variable. Several studies have been conducted to determine the dynamics of the density of tsetse populations in Zimbabwe. These studies have concentrated on tsetse populations in the Zambezi Valley under ecological conditions that vary substantially from those prevailing on the Plateau. In the Valley, climatic conditions are more extreme and are likely to affect the tsetse population differently. Moreover, humans and cattle are absent at present and, hence, tsetse rely entirely on game animals as source of food. On the Plateau, progressive clearing of land for cultivation or settlement and the ever increasing human population has resulted in a gradual decrease in the number of game animals making tsetse more dependent on livestock for their survival (Section 2.3). Furthermore, the tsetse's habitat has been altered substantially and, because of the clearing, has become patchy. Although the tsetse's

species-specific behaviour is not affected by changes in the environment, the alterations in the tsetse's habitat and the human interference through livestock management could have a significant effect on the dynamics of the tsetse population. Unfortunately, this information is not available. Therefore a longitudinal study was conducted in Katete District of eastern Zambia. During four consecutive years, the *G. m. morsitans* population was monitored closely. Seasonal patterns in the distribution and abundance of tsetse were determined and reasons for these fluctuations were identified (Sections 2.2 and 2.4).

Another important, tsetse-related, variable determining challenge is the prevalence of trypanosomal infections in tsetse. The proportion of infected flies undergoes substantial spatial and temporal variations. In the context of bovine trypanosomosis management it is important to identify the factors that cause these fluctuations. For this purpose, the monthly infection rate of *G. m. morsitans* in Katete District, eastern Zambia, was determined during four consecutive years (Section 2.5). The role of various variables in affecting the infection rate of the flies was examined.

Finally, the relationship between the abundance of tsetse, the prevalence of trypanosomal infections in tsetse and the prevalence of infection in cattle was established (Section 2.5). This relationship will, to a large extent, determine the appropriateness of various control interventions (Chapter 5).

2.2 Seasonal patterns in the distribution and abundance of *G. m. morsitans* Westwood (Diptera: Glossinidae) on the plateau of the Eastern Province of Zambia

2.2.1 Introduction

In most of the plateau area of eastern Zambia, bovine trypanosomosis is a serious constraint to agricultural development. The prevalence of bovine trypanosomosis, transmitted here by *G. m. morsitans*, is high and significantly reduces cattle productivity (Chapter 4). Despite the importance of the disease and the high agricultural potential of the area little is known of the vector and the relationship between the vector and its environment in this part of Zambia. Studies on the ecology of *G. m. morsitans* have been conducted in the Zambezi and Luangwa Valleys of Zimbabwe and Zambia but may be of little relevance to the conditions prevailing on the plateau (Lloyd, 1912; Pilson and Pilson, 1967). However, an understanding of the ecology of the vector and its relationship with the environment is essential when determining and monitoring the implementation of a strategy for the control of trypanosomosis in an area. In an attempt to clarify the relationship between *G. m. morsitans* and different types of vegetation on the plateau of eastern Zambia, the tsetse population was monitored closely during a period of four consecutive years. Results presented in this section concern seasonal changes in the distribution and abundance of tsetse. The main factors responsible for changes in the distribution and abundance of tsetse are identified.

2.2.2 Materials and methods

2.2.2.1 Study area

The study was carried out in an area of about 20 km^2 situated between 31°47'-31°55' E and between 13°55'-14°12' S in Katete District, Eastern Province, Zambia, at an elevation of approximately 900 m above sea level. It is a highly cultivated area and carries approximately 8-10 head of cattle/km^2 (based on an aerial survey conducted in August 1997) together with goats, pigs, dogs and few game animals (mainly small antelopes). *Glossina m. morsitans* is the only tsetse species present.

The vegetation within the study area can be classified in two main types. Miombo woodland, hereafter referred to as miombo, is a two-storied woodland, with the genera *Brachystegia* and *Julbernardia* dominant. It is mainly found on poorer soils on ridges or slopes. Most of the villages are located in miombo. Munga woodland, hereafter called munga, is a one- or two-storied fairly open woodland where the principal tree genera are *Acacia*, *Combretum* and *Terminalia*. Munga is associated with flat topography following the streams and their smaller tributaries. It is found mainly on better soil types and many areas in this woodland are cleared and cultivated.

The annual climatic cycle comprises three seasons; the warm rainy season (from early November to late April), the cold dry season (from early May to late August) and the hot dry season (from early September to late October). Climatic recordings in miombo and munga do not differ greatly. Throughout the study period, an automatic weather station (Intelligent Sensor SDL 2500 series, Skye Instruments, Ltd., UK) was used to monitor climatic variables continuously. It was located 10 km north of the study area and recorded, on an hourly basis, ambient temperature, ambient relative humidity, solar radiation, soil temperature and rainfall. The vapour pressure deficit was calculated as the difference between the saturation vapour pressure and the actual vapour pressure derived from relative humidity at a given temperature (Rosenberg *et al.*, 1983).

2.2.2.2 Tsetse sampling

Between January 1990 and December 1993, the tsetse population was monitored along the Mkapatile transect. The transect was about 6 km long and had 29 sectors of roughly 200m each. At the end of each sector was a numbered stop, 0 at the start and 29 at the end. Stops 1-6 were situated in miombo and stops 7-29 in munga interspersed with fields. Fly-rounds were conducted along this transect as described by Potts (1930) and Ford *et al.* (1959). Teams of two men traversed them. The teams used a black cloth screen (1.5 x 1m) baited with acetone released at approximately 200 mg/h (Shereni, 1984). The screen hung from a bamboo pole and was kept hanging vertically by weighting with a second bamboo pole at the bottom. The fly-round team remained at each stop for 2 minutes and, using hand nets, captured tsetse alighting on

the screen. All flies were killed immediately after capture using chloroform vapour. Transects were traversed at least twice per week, alternately in opposite directions. The fly-rounds started between 07:00 and 08:00 hours or 15:00 and 16:00 hours in the rainy and hot dry season. During the cold dry season fly-rounds started between 08:00 and 09:00 hours or 14:00 and 15:00 hours. The time fly-rounds were conducted coincided with the diurnal activity peaks of tsetse in the study area (Van den Bossche, unpublished data). Records were kept of the number and sex of the tsetse captured at each stop during each transect. Daily catches per stop were transformed using a square root ($n + 0.5$) transformation (Sokal and Rohlf, 1998). A transformed monthly average index of abundance (IA) of tsetse was calculated as the average number of flies (males and females) captured per stop per fly-round. Averages were detransformed for presentation (Sokal and Rohlf, 1998). All analyses were performed using the statistical package SPSS (SPSS Inc.).

2.2.2.3 Abundance and distribution of cattle

Data on the distribution and abundance of cattle along the transect were collected during each sampling occasion. Cattle counts were made in each sector of the fly-round. Monthly cattle abundance in each vegetation pattern was calculated as the average number of cattle observed per sector per sampling occasion.

2.2.3 Results

2.2.3.1 Tsetse abundance and distribution

A total of 2 900 *G. m. morsitans* were captured on 384 fly-rounds. The number of tsetse captured during each fly-round varied substantially between fly-rounds and between seasons. Overall catches were highest at the end of the hot dry season/beginning of the rainy season and lowest during the cold dry season (Figs.2.2.1, 2.2.2 and 2.2.3). Catches in miombo increased at the beginning of the rainy season, reached their peak at the end of the rainy season and were low during the cold, but especially the hot dry season. The tsetse catches in munga showed a pattern which was the reverse of that in miombo (Figs. 2.2.2 and 2.2.3). The monthly average IA of tsetse in miombo was significantly, negatively, correlated with the monthly average IA in munga ($r = -0.53$, $P<0.001$). In munga the IA of tsetse increased from

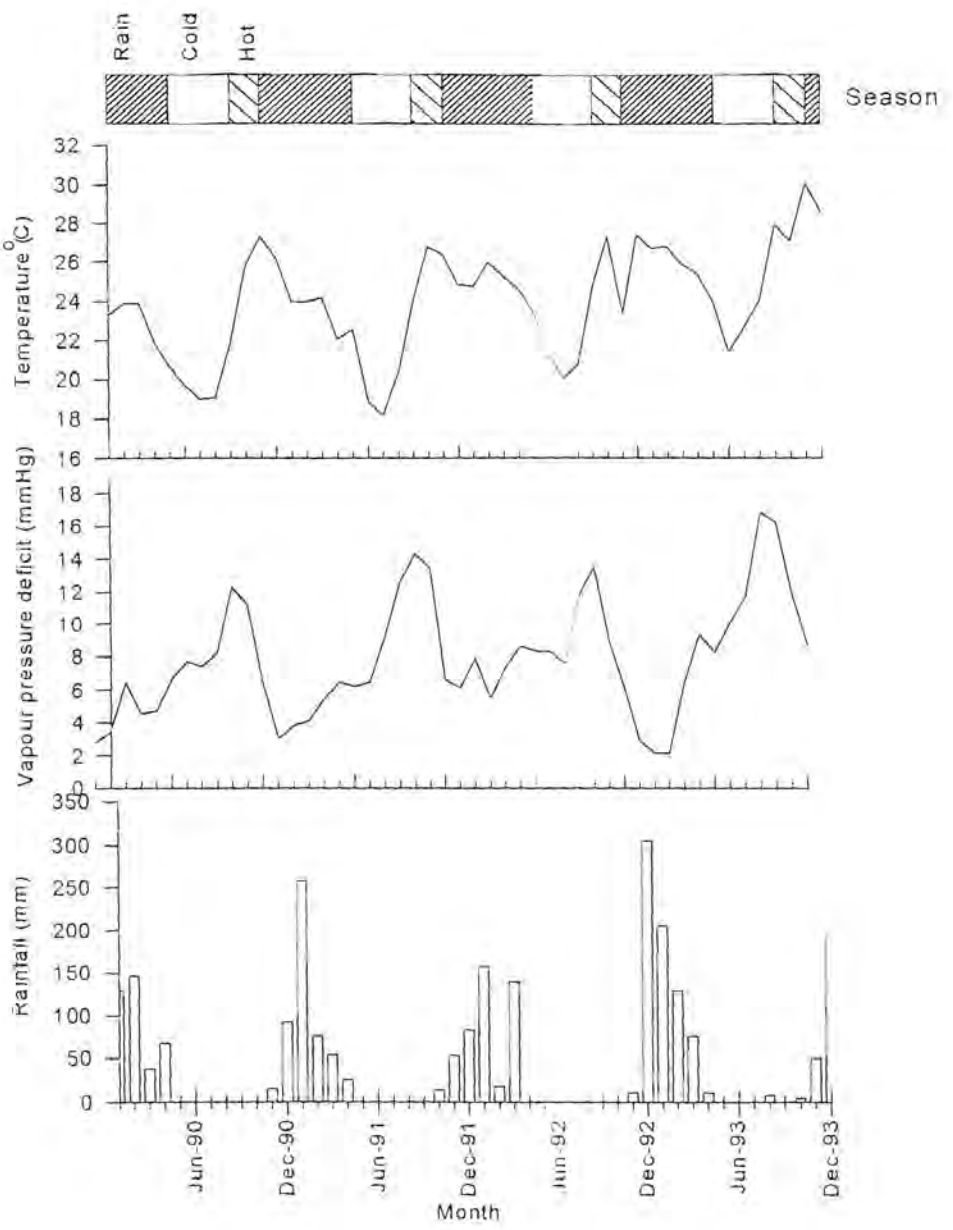

Figure 2.2.1: Monthly average ambient temperature, vapour pressure deficit and rainfall, Katete District, Eastern Province, Zambia.

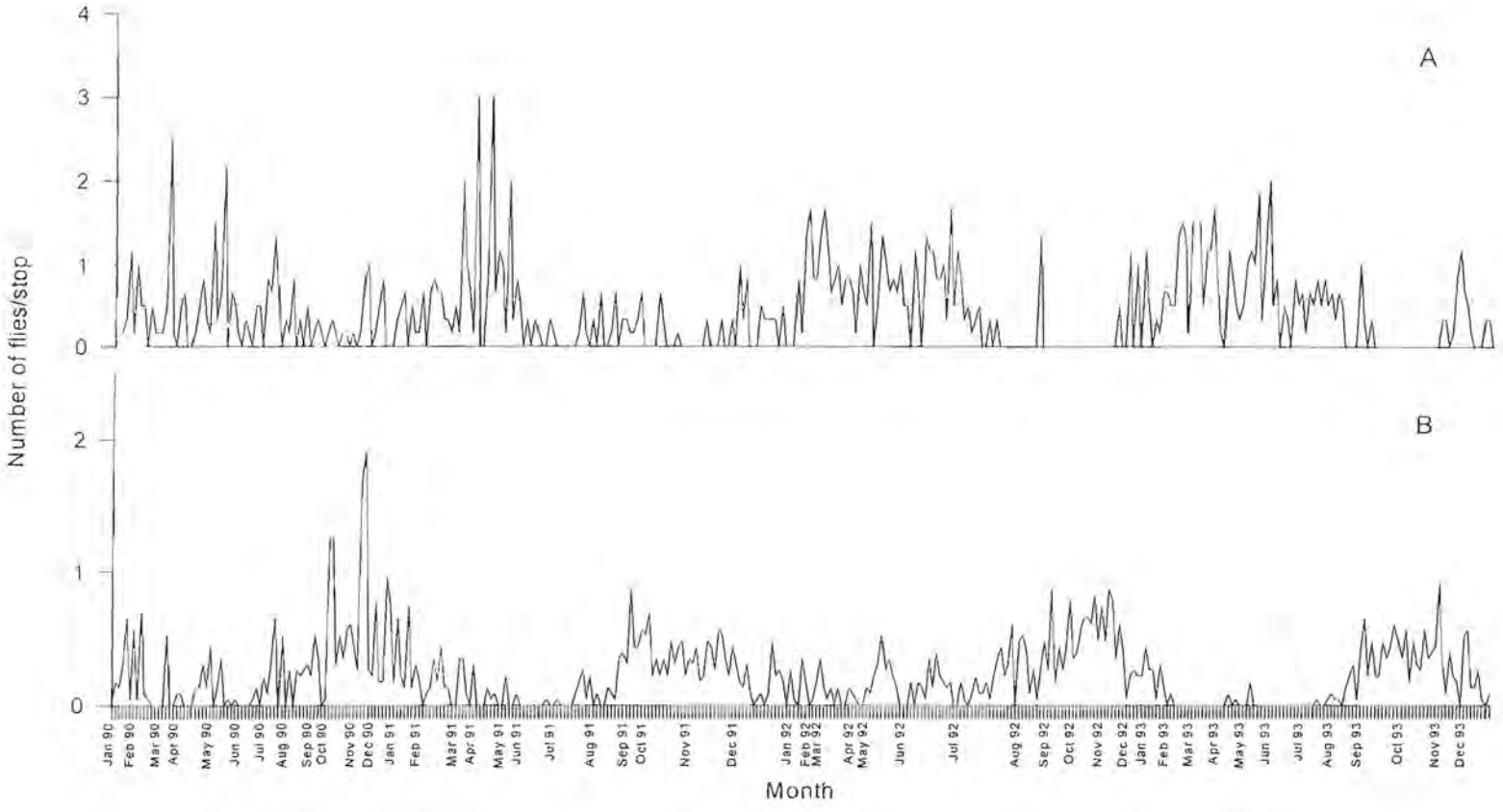

Figure 2.2.2: Number of *G. m. morsitans* captured per stop during each fly-round in miombo (A) and munga (B).

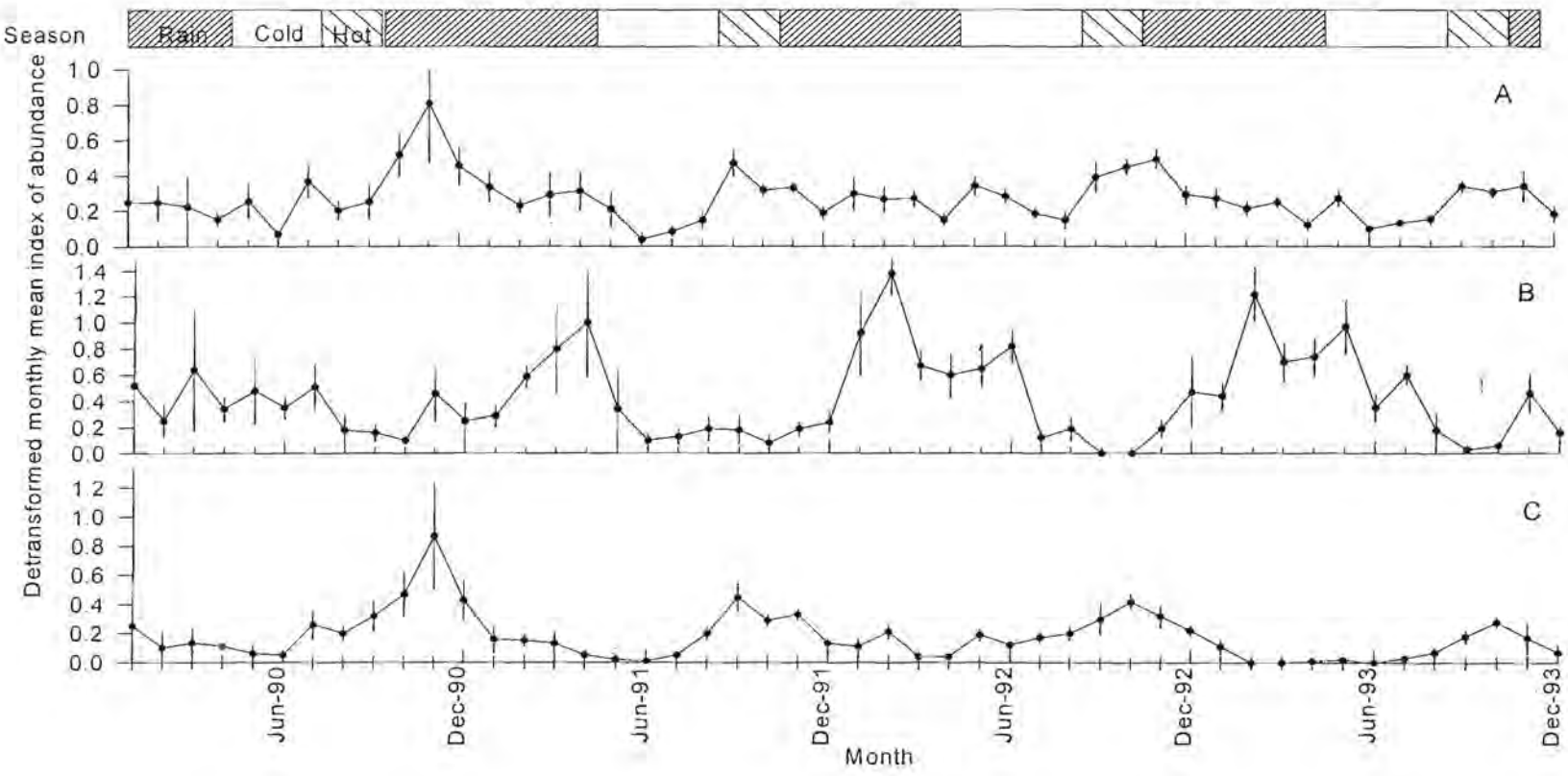

Figure 2.2.3: Detransformed monthly average index of abundance (± 1 s.e.) of *G. m. morsitans* in all vegetation types (A), in miombo (B) and in munga (C).

July/August onwards and reached its maximum at the end of the hot dry season (Figs. 2.2.2 and 2.2.3). Differences within years between minimum and maximum monthly average IA of tsetse varied between 5- and 20-fold and 20- and 100-fold in miombo and munga, respectively, over the four years of records.

Although the majority of tsetse captured (82.1%) were male flies, the monthly average IA of male flies was, in most cases, significantly correlated with the monthly average IA of females and tenerals (Table 2.2.1).

Table 2.2.1: Correlation between monthly average index of abundance of male, female and teneral *G. m. morsitans* in miombo and munga.

		female	teneral
Miombo	male	0.36*	0.35*
	female	-	0.57***
Munga	male	0.38*	0.46**
	female	-	0.66***

*Significantly correlated at the 0.05 (*), 0.01 (**) or 0.001 (***) level of P.

The climatic factor that correlated best with the monthly average IA of tsetse was the vapour pressure deficit. In miombo, the monthly average IA was negatively correlated with the monthly average vapour pressure deficit (r = -0.51, P<0.001). In munga, on the other hand, the correlation was positive (r = 0.49, P<0.001).

2.2.3.2 Cattle abundance and distribution

The distribution and abundance of cattle along the transect also showed a seasonal trend. This was especially the case in munga, during the first three years of observation, where cattle abundance increased gradually from June onwards, reached a maximum at the end of the hot dry season (October-November) and declined steeply at the start of the rainy season (Fig. 2.2.4). Cattle numbers were very low between February and May. In miombo, the seasonal cattle density pattern was less clear and

cattle were observed throughout the year (Fig. 2.2.4). Nevertheless, the abundance of cattle tended to increase from the start of the rainy season, reached its highest peak during the rainy season and decreased at the end of the rainy season. Cattle abundance decreased substantially following the severe drought in the 1991-1992 rainy season.

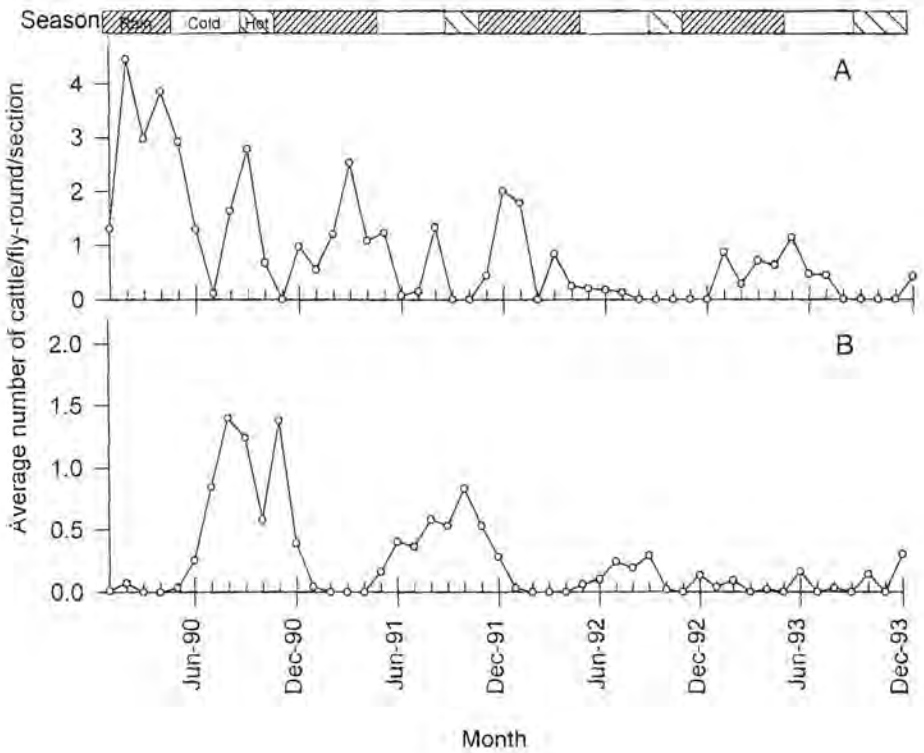

Figure 2.2.4: Monthly average number of cattle seen in each section per fly-round in miombo (A) and munga (B).

The average monthly average IA of *G. m. morsitans* in miombo was negatively correlated with the abundance of cattle in munga in the same and the previous month (Table 2.2.2). It was positively correlated with the abundance of cattle in miombo in the previous month (Table 2.2.2). The average monthly average IA of *G. m. morsitans* in munga, on the other hand, was positively correlated with the abundance of cattle in

munga in the same and the previous month (Table 2.2.2). Stepwise multiple regression was used to investigate the effect of vapour pressure deficit and abundance of cattle in the same and the previous month on the abundance of tsetse in miombo or munga (Sokal and Rohlf, 1998). Variations in the index of abundance of tsetse in miombo were best explained by changes in the vapour pressure

Table 2.2.2: Correlation between monthly average index of abundance of *G. m. morsitans* and average abundance of cattle in miombo and munga in the same ($month_n$) or the previous month ($month_{n-1}$).

			Tsetse ($month_n$)	
			Miombo	Munga
Cattle	Miombo	$Month_n$	0.16	-0.25
	Munga	$Month_n$	-0.41**	0.56**
	Miombo	$Month_{n-1}$	0.33*	-0.16
	Munga	$Month_{n-1}$	-0.39*	0.62**

*Significantly correlated at the 0.05 (*) and 0.001 (**) level of P.

deficit (R^2 = 25.6%, P<0.001). The abundance of cattle in the same of the previous month did not significantly improve the fit of the model (P>0.05). Both vapour pressure deficit and abundance of cattle contributed significantly to the model explaining the variation in the abundance of tsetse in munga. The best fit was obtained when the abundance of cattle in the previous month was used (R^2 = 0.51, P<0.001).

2.2.4 Discussion

Consistent seasonal trends in the IA of tsetse in miombo and munga were observed during the study period. During the rainy season, the IA of tsetse is highest in miombo. It is low during the dry, and especially the hot dry season. In munga, on the other hand, the IA of tsetse is highest during the dry season. Tsetse catches along fly-round transects are heavily biased and may reflect capture probability rather than

population density (Vale, 1974). In the study area the monthly average index of abundance of tsetse explains 74% of the variance in the incidence of bovine trypanosomosis (Section 2.5). Hence, the monthly average index of abundance of tsetse is a good representation of the level of challenge or the density of the tsetse population.

The birth rate, death rate and rate of fly immigration and emigration in a given area determine the density of a tsetse population. Density-independent mortality is often strongly correlated with atmospheric moisture, expressed as saturation deficit (Rogers and Randolph, 1986). Fluctuations in the IA of tsetse in miombo are indeed significantly correlated with the monthly average vapour pressure deficit. In munga, on the other hand, changes in the IA of tsetse cannot be explained by the effect of vapour pressure deficit on the population growth rate. For example, the sudden increase of tsetse abundance in munga in July/August cannot be associated with significant changes in the climatic conditions that would have affected the tsetse's birth and death rate resulting in an increased population growth rate. On the contrary, the tsetse's birth rate is expected to be low during the coldest time of the year when pupal period and inter-larval periods are at their maximum (Phelps and Burrows, 1969). Hence, the sudden increase in the abundance of tsetse in munga is best explained by movement. Tsetse, on average, move randomly in their habitat (Bursell, 1970). Some factors, however, may cause an uneven distribution of tsetse between vegetation types. In Zimbabwe's Zambezi Valley, for example, such an uneven distribution is induced by the extreme climatic conditions during the hot dry season which makes riverine woodland more suitable for tsetse (Hargrove and Vale, 1980). Munga also may be a more suitable vegetation type during the hot dry season. The vegetation is denser than in miombo, possibly offering more suitable microclimatic habitats, and soil humidity is higher compared to the hill slopes covered with miombo. However, the increase in the abundance of tsetse in munga occurs during the cold dry season when climatic conditions in miombo are well within the environmental optimum for *G. m. morsitans* (Rogers, 1979).

Host movements may also affect tsetse movement and distribution. The distribution pattern of cattle along the transect undergoes significant seasonal changes. During the rainy season, cattle are mainly found in miombo whereas from June onwards cattle disperse and are found in both munga and miombo. The observed changes in distribution are in accordance with changes in the management system of communal cattle in eastern Zambia (De Clercq, 1997). During the rainy season cattle are kept near the villages. They are collected from the kraal at approximately 07:30 hours and graze from 8:00 up to 17:00 pm. Hence, the total time available for grazing during the rainy season is about 8 to 9 hours. This coincides with the time the fly-rounds were conducted. To avoid crop damage, cattle graze mainly in miombo where food is abundant during this time of the year. They only enter munga when they are taken there for ploughing. The grazing pattern and management system changes drastically after the crops have been harvested (June-July). Cattle are allowed to roam freely and feed unattended mainly on crop residues. They are not kraaled at night but return to the kraals at regular intervals. In the late dry season (September-October), however, cattle have to move further afield to find grazing but return to their kraals at 3-4 days intervals. At the start of the rainy season, when grass in miombo becomes available, cattle are again kept in miombo and herded away from the germinating crops. Hence, contrary to miombo where cattle are continuously present and seem to play a minor role in the abundance of tsetse, the host availability in munga undergoes abrupt changes. The sudden changes in the grazing pattern and availability of cattle in munga appears to have a significant effect on the abundance of tsetse in this vegetation type. Hence the significant correlation between the abundance of tsetse and the presence of cattle (in the same or the previous month), independent of the climatic conditions, in this vegetation type. During the dry season, tsetse thrive in munga. The microclimate is likely to be favourable and, notwithstanding the gradual decline in the abundance of cattle after 1991-92, host availability is sufficient to maintain tsetse abundance at a level comparable to the preceding years. Such close associations between the apparent distribution of *G. m. morsitans* and the distribution of hosts (game animals) have been reported in the Eastern Province of Zambia (Hall, 1910; Lloyd, 1916). However, despite this abundance of tsetse in munga, a small number of flies remains present in miombo during the hot dry season. This is not surprising since *G. m. morsitans* is

able to withstand the extreme conditions of mopane woodland during the hot dry season in the Zambezi Valley although at a low level (Pilson and Pilson, 1967). This also supports the conclusion that reasons for the movement of tsetse to munga are host rather than climate related. The steep decline in the IA of tsetse in munga during the rainy season is attributed to the sudden decline in host availability. Such an effect of reduced host availability on tsetse abundance has been observed elsewhere and has, for many, years formed the basis of an effective tsetse control strategy (Cockbill *et al.*, 1969). Results from the current longitudinal study suggests that tsetse move between vegetation types and that the direction of movement is induced by the grazing pattern of cattle. A more detailed study in a more confined habitat, using marked flies, will aim at quantifying the movement of tsetse between the rainy and dry season grazing areas of cattle (Section 2.4).

The close relationship between the distribution of tsetse and the distribution of cattle has important repercussions for the epidemiology and control of bovine trypanosomosis in the Eastern Province of Zambia. First, since the distribution of tsetse changes seasonally, figures on the abundance of tsetse should be looked at with caution. Surveys or surveillance operations should cover all available vegetation types in order to obtain a true picture of the abundance of the tsetse population. Second, the close relationship between the distribution of cattle and the distribution of tsetse and the availability of cattle during the activity peaks of tsetse suggests that the challenge cattle undergo will be very much related to the abundance of tsetse. It also implies that insecticide-treatments of cattle should be an effective way in reducing challenge and/or controlling tsetse in Eastern Province (Section 5.7). Such operations should be very effective when implemented at the time of tsetse movement when the tsetse population is likely to be subject to considerable stress. Finally, the concentration of tsetse in miombo during the rainy season could be exploited when utilizing stationary baits to control the fly. The results presented above suggest that the deployment of odour-baited targets in miombo may suffice to control the tsetse population. This irregular deployment of targets, with a concentration of baits in *Brachystegia* woodland, was applied successfully in a tsetse control operation south west of the study area (Section 5.2).

2.3 The importance of cattle as a food source for *G. m. morsitans* Westwood (Diptera: Glossinidae) in Katete District, Eastern Province, Zambia

2.3.1 Introduction

Numerous surveys have been conducted to determine the hosts of various tsetse species (Section 1.2.1.1). These surveys were facilitated greatly by the development of serological methods for the identification of blood meals from haematophagous insects. Work published by Weitz (1963) showed that the feeding pattern of tsetse could be divided into 5 main patterns. Later work proved that this generalization can be misleading in so far that the degree of host preference varies from one locality to another (Staak *et al.*, 1986; Moloo, 1993). The exact knowledge of the main host, however, is one of the major determinants in the ecology and control of tsetse and the epidemiology of African trypanosomosis. Since the work of Clarke (1964) and Okiwelu (1977a) few attempts have been made to identify the hosts of tsetse in Zambia. Moreover, significant changes in the environment and host availability, since the previous surveys were conducted, make the information obtained from these surveys obsolete. To update our knowledge of host preference of tsetse and improve our understanding of the epidemiology of bovine trypanosomosis on the plateau area of eastern Zambia, a survey on the host preference of *G. m. morsitans* was conducted.

2.3.2 Materials and methods

2.3.2.1 Study area

Between November 1989 and December 1991, blood meals were collected in the study area described in Section 2.2.2.1.

2.3.2.2 Tsetse sampling

Most tsetse used in the blood meal work were captured along fly-round transects (Section 2.2.2.2) in the two main vegetation types (Section 2.2.2.1) during the three seasons (Section 2.2.2.1).

2.3.2.3 Bloodmeal collection

Nearly all collected blood meals came from male tsetse with nutritional status varying between hunger stage 1 and hunger stage 2 (or flies containing visible blood) (Jackson, 1933). Immediately after capture, the midgut contents was squashed onto a filter paper disc (Whatman N°1) and each squash labeled. The filter papers were dried in the air and kept in the dark in a plastic bag together with a desiccant. Before mailing the samples were dipped briefly in acetone for sterilisation (Clarke, 1964). The identification of the collected blood meals was carried out by The Robert von Ostertag Institute (Berlin, Germany). Eluted blood meal samples were tested by indirect Enzyme-Linked Immunosorbent Assay (ELISA) using a panel of absorbed and non-absorbed antisera against various animal species (Münstermann, 1984).

2.3.3 Results

Of the 848 blood meals collected, 687 or 81.0% gave positive host identification (Table 2.3.1). No differences in blood meal origin were detected between blood meals collected in different vegetation types or collected during different seasons.

2.3.4 Discussion

The results show that the majority of the meals (75.1%) were taken on cattle, even when other domestic animals (mainly goats, pigs and dogs) were present. This is in contrast to the results of other *G. m. morsitans* host animal surveys in Zambia (Clarke, 1964; Okiwelu, 1977a) and elsewhere (Cockbill *et al.*, 1969; Vale and Cumming, 1976). These surveys showed that suids, particularly warthogs, are the preferred host of *G. m. morsitans*. Reasons for the different feeding habits are obvious.

Table 2.3.1: Origin of blood meals collected from *G. m. morsitans*.

Host-type	Proportion of flies (%)	
	Miombo	Munga
Bovids	86.7	84.9
Cattle	75.1	74.8
Wild ruminant*	10.9	10.1
Duiker	0.7	0.9
Suids	8.4	6.8
Domestic pig	5.8	4.7
Bushpig	1.3	1.0
Warthog	1.3	1.1
Other mammals	2.9	-
Dog	2.0	-
Cat	0.9	-
Birds	0.4	-
Chicken	0.4	-
Reptiles	-	0.3
Crocodile	-	0.3
Primates	1.2	1.5
Man	1.2	1.5

* Wild ruminant which is not bushbuck, buffalo or waterbuck.

Due to extensive cultivation, wild hosts have almost disappeared and have been replaced by livestock. Since tsetse survival largely depends on the regular uptake of blood meals, a gradual adaptation process must have taken place which made *G. m. morsitans* largely reliant on cattle as a food source. In other areas of the Eastern Province where fauna has not been affected by human interference (Luangwa Valley), *G. m. morsitans* still takes the majority of its blood meals from wild Suidae (Sehof, 1975). It is, nevertheless, surprising that in the present study only 5.8% of the blood meals were identified as taken from domestic pigs which are common in the area. This is attributed to the seasonal availability of domestic pigs. Only during the rainy season, when flies are present in greatest number near or around the villages in the miombo (Section 2.2.3.1), domestic pigs become readily available whereas cattle are available throughout the year.

The above described results prove again the danger of generalizing the host preference of *Glossina* and support the observations made by Boyt (1978) that domestic animals, such as cattle, can support tsetse populations. The dependence of tsetse on cattle, as their main food source, is likely to be the reason for the close relationship between the seasonal distribution of tsetse and the grazing pattern of cattle in the study area (Section 2.3.3.2). It also explains the high incidence of bovine trypanosomosis in the study area (Section 2.5.3.4). In view of the observations presented in this paper, useful conclusions can be drawn with regard to the control of tsetse in the study area. Since the tsetse flies in the study area take at least 3 out of 4 feeds on cattle, the treatment of cattle with pyrethroid insecticides must have an immediate and drastic effect on the tsetse population density and consequently trypanosomosis challenge (Section 5.7). This was demonstrated previously when deltamethrin dip (Decatix®, Cooper) was used in an area west of the study area during an eight-month period resulting in an 88% decrease in the trypanosomosis incidence (Chizyuka and Luguru, 1986; Luguru *et al.*, 1993). A large-scale pour-on trial, conducted in the Petauke District of eastern Zambia also resulted in a significant decline in the incidence of bovine trypanosomosis (Section 5.7).

2.4 Movement patterns of *G. m. morsitans* Westwood (Diptera: Glossinidae) between two vegetation types on the plateau of eastern Zambia

2.4.1 Introduction

The longitudinal study of the tsetse population in the two main vegetation types (miombo and munga) of the plateau of eastern Zambia, has shown that the abundance of *G. m. morsitans* varied substantially between seasons. In miombo, changes in the abundance of tsetse correlated well with changes in the climatic conditions. The abundance of tsetse in munga, on the other hand, underwent changes that could not be explained by normal population growth. The seasonal trend in the distribution pattern of tsetse was best explained by movements of the tsetse population resulting from changes in the grazing pattern of the main host, cattle (Section 2.2.4). Despite the correlation between the abundance of tsetse and the abundance of cattle and the high level of coincidence between the changes in the grazing pattern and the changes in the tsetse distribution pattern, movement of tsetse could not be proven unequivocally. To quantify the movement patterns of tsetse between miombo and munga a capture/mark/release/recapture experiment was conducted in a small isolated area where both vegetation types were present. Using the outcome of this experiment the movement patterns are described and the variations in the abundance of tsetse in miombo and munga are explained.

2.4.2 Materials and methods

2.4.2.1 Study area

The tsetse population was monitored in a small area (approximately 6 km^2) of miombo surrounding a patch of munga situated along the edge of a dambo, a seasonally inundated piece of grassland also termed vlei (approximately 0.5 km^2). The munga and dambo together (henceforth referred to as munga) were part of the dry season grazing area of the cattle (approximately 60 in total) from a nearby village (Tundu village). The study area was bordered in the south by a ridge of hills and in the east, west and north by areas cleared of vegetation, some of them being used for cultivation. The area was selected because of these partial barriers restricting the movement of tsetse and, hence, increasing the probability of recapture of marked flies.

Two transects, Chipopela-B and Chipopela-C, were marked out. They were approximately 4 km long and had 20 sections of roughly 200 m each. They both traversed miombo (sections 1-10) and munga (sections 11-20). The transects were traversed at least twice per week, alternately in opposite directions. Fly-rounds were conducted concurrently by two teams.

2.4.2.2 Capture/Mark/Release/Recapture experiment

To investigate the seasonal movement of flies to and from miombo and munga a capture/mark/release/ recapture (CMRR) experiment was conducted along the two transects, between November 1990 and May 1992. All flies captured were marked individually on one or two of six positions recognised on the dorsal part of the thorax (Pollock, 1986). Seven colours (white, green, blue, red, yellow, pink and orange) of artist's oil paint were used, giving a possible total of 1512 individually marked flies. Paint was applied with a thin needle and flies were released, on the spot of capture, immediately after marking. Records were kept of the capture point, sex and the colour code of all marked flies. Upon recapture, notes were made of the fly's colour code and locality of recapture after which the fly was released. Flies recaptured on the day of marking were not considered as recaptures. Since the study aimed at establishing seasonal movement patterns, all flies recaptured in the two rainy seasons, one cold dry and one hot dry season were pooled and the place of marking was compared with the place of recapture.

To facilitate interpretation of the CMRR data, the odds that a fly marked in miombo is recaptured in munga and vice versa, for each of the seasons, were calculated as follows:

$$(N_{ii} \times N_{jj})/(N_{ij} \times N_{ji})$$

Where N is the number of flies marked in a vegetation and recaptured in a vegetation type; i is miombo and j is munga.

The exchange of flies between the two vegetation types was quantified by expressing the flies marked in one vegetation type and recaptured in the other vegetation type, as a percentage of all recaptured flies;

$$R_i \times 100/R_j \times R_i \quad \text{or} \quad R_j \times 100/R_j \times R_i$$

where R is the number of recaptured flies; i is miombo and j is munga (Randolph & Rogers, 1984). Comparison between seasons of the number of flies recaptured in the two vegetation types were made using the Fisher's exact test (Sokal & Rohlf, 1998).

2.4.3 Results

2.4.3.1 Tsetse abundance and distribution

Between November 1990 and May 1992, a total of 2 275 *G. m. morsitans* were captured and marked on 213 fly-rounds along each of the two transects (Table 2.4.1).

Table 2.4.1: Number of *G. m. morsitans* marked in miombo and munga along the Chipopela-B and Chipopela-C transects.

Transect	Vegetation	Male	Female	Teneral
Chipopela-B	Miombo	755	95	52
	Munga	367	46	41
Chipopela-C	Miombo	375	71	72
	Munga	306	49	46

Catches were highest in the miombo section of both transects. They constituted about 63% of the total adult male and adult female and about 59% of the total number of teneral flies captured. The monthly average IA of tsetse underwent seasonal fluctuations comparable to those along the Mkapatila transect (Figs. 2.2.2 and 2.2.3). Fly abundance in miombo was highest during the rainy season, and was lowest during the dry season (Fig. 2.4.1). In munga, on the other hand, catches increased from the cold dry season onwards and reached a maximum at the start of the rainy season (Fig. 2.4.1). The average monthly IAs in miombo and munga along the Chipopela-B

transect were significantly correlated with those along the Chipopela-C transect (r = 0.51, P<0.05 and r = 0.48, P<0.05 for miombo and munga, respectively).

During the cold and the hot dry season, the variance of the fly catches (males plus females) at each of the stops was approximately equal to the average (Kolmogorov-Smirnov test) (Sokal and Rohlf, 1998). This indicates that catches followed a Poisson distribution so were distributed randomly along the transects. In the rainy season, on the other hand, fly catches (males plus females) were higher in miombo and the frequency distribution of catches differed significantly from the Poisson distribution (P<0.001).

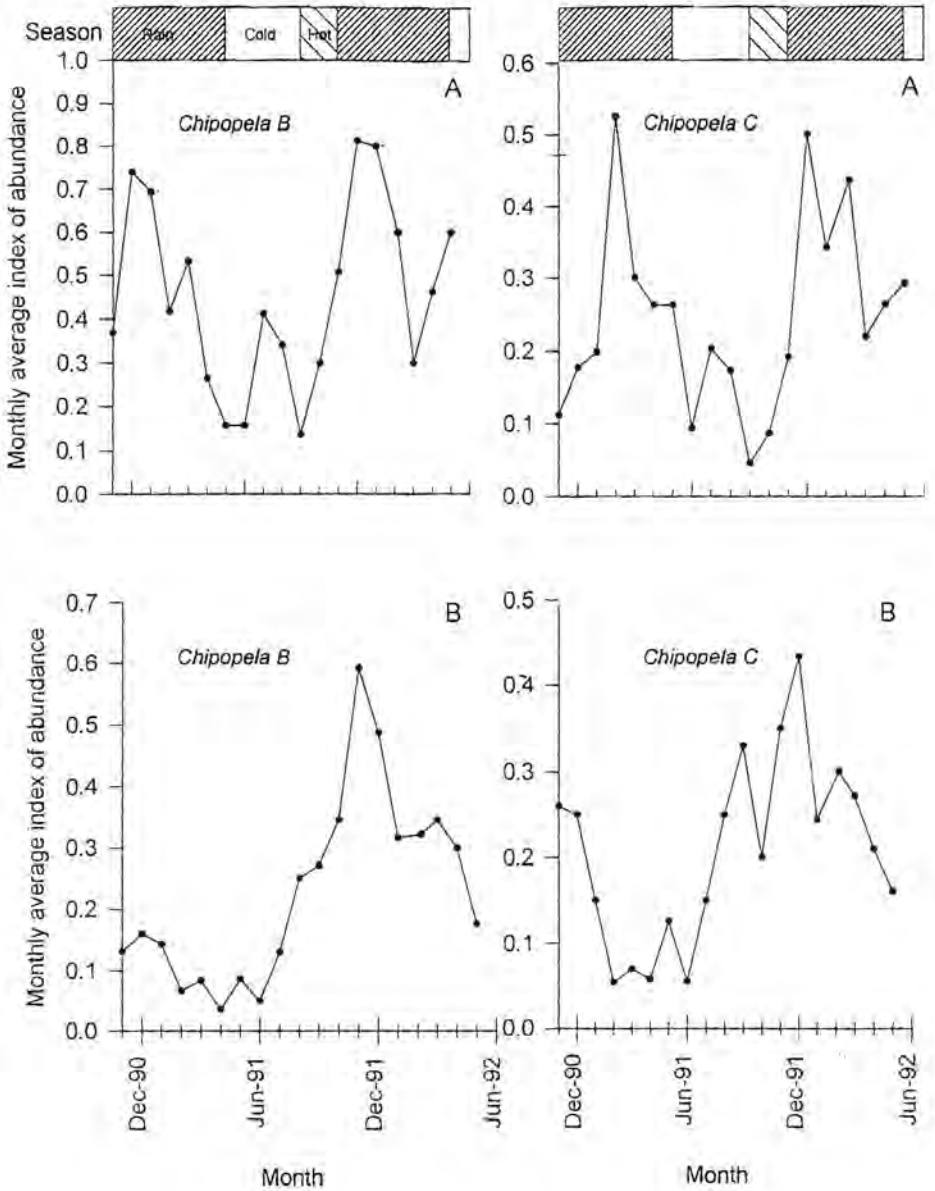

Figure 2.4.1: Monthly average index of abundance of *G. m. morsitans* in miombo (A) and munga (B) along Chipopela-B and Chipopela-C transects.

2.4.3.2 Recapture rates of tsetse

A total of 178 marked flies (7.8% of the total number marked) were recaptured. Approximately 57% and 43% of the recaptured flies were marked in miombo and munga, respectively (Table 2.4.2).

Table 2.4.2: Contingency table showing, for each season, the total number of *G. m. morsitans* (males and females) marked in miombo or munga and recaptured in miombo or munga, the odds of recapture and the odds ratio of the odds.

	Total recaptured	Marked	Recapture		Odds	Odds ratio
			Miombo	Munga		
Rain	96	Miombo	62	2	32.0	16.2
		Munga	21	11	1.9	
Cold	31	Miombo	9	10	0.9	2.7
		Munga	3	9	0.3	
Hot	51	Miombo	8	11	0.8	0.9
		Munga	14	18	0.7	

The majority of the recaptured flies were males (97.8%). No flies were recaptured more than once. The time between marking and recapture differed little between seasons and was on average 10.9 ± 1.2 days. The odds ratios differed substantially between seasons (Table 2.4.2). During the rainy season, a fly marked in miombo was about 16 times more likely be recaptured in miombo than a fly marked in munga. This was reduced significantly in the cold season (odds ratio = 2.7). In the hot dry season, a fly marked in miombo was as likely to be recaptured in miombo as a fly marked in munga (odds ratio = 0.9). The reduction in the odds ratio during the dry season was reflected in the increasing proportion of flies marked in miombo and recaptured in munga during this time of the year (Fig. 2.4.2).

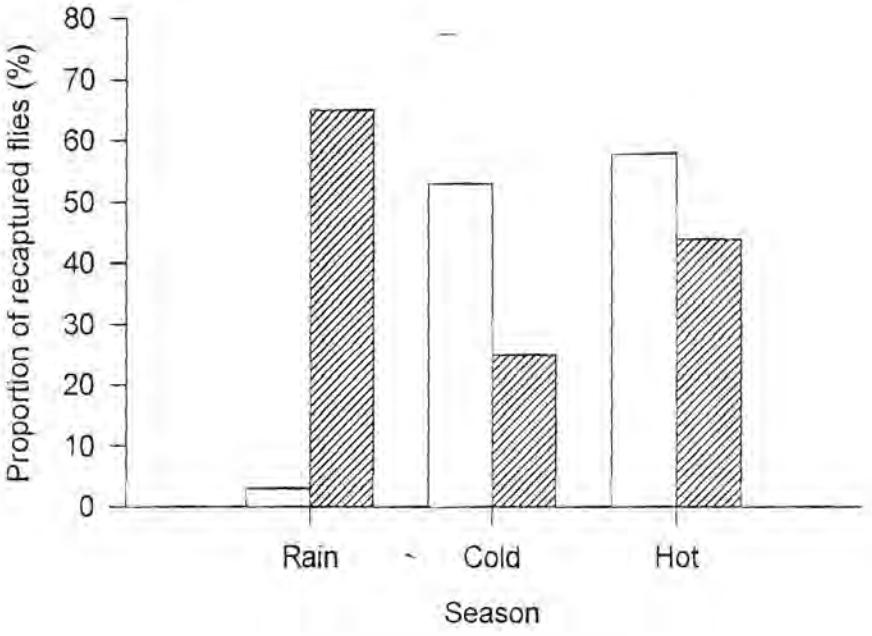

Figure 2.4.2: Proportion of *G. m. morsitans* (males plus females) marked in miombo and recaptured in munga (□) and marked in munga and recaptured in miombo (=) during the rainy, cold and hot dry season.

During the rainy season, 99% of the flies marked in miombo were recaptured in miombo whereas 66% of the flies marked in the munga were recaptured in miombo. During the cold dry season, approximately 50% the flies recaptured in munga were marked in miombo whereas only 25% of the flies marked in munga were recaptured in miombo. Finally, during the hot dry season, there was an equal flow of flies to and from miombo (Fisher's exact test, P = 0.64).

2.4.4 Discussion

The seasonal changes in the distribution pattern and the timing of the changes in the distribution pattern of tsetse along the Chipopela transects are similar to those observed along the Mkapatila transect (Section 2.2.3.1). During the rainy season, the

IA of tsetse is highest in miombo. However, from June onwards, the tsetse abundance in munga increases steeply and reaches a maximum at the end dry/beginning rainy season. During this period, tsetse catches are distributed randomly along both transects.

Those consistent seasonal changes in the distribution of tsetse can be attributed to changes in the movement patterns of tsetse caused by changing grazing patterns of the main host, cattle (Section 2.2.3.2). The results of the C/M/R/R experiment confirm that male tsetse move between miombo and munga and that the direction of the movements varies between seasons. Moreover, the intensity of the exchange of flies between miombo and munga is in line with the observed seasonal changes in the IA of tsetse in both vegetation types. During the rainy season, the majority of tsetse marked in miombo and a high proportion of flies marked in munga are recaptured in miombo. Thus there is an asymmetric ebb and flow of flies between the two vegetation types resulting in a net immigration of tsetse into miombo. This results in the steep increase in the IA of tsetse in miombo (a 2.5-fold and 5-fold increase in the IA of tsetse in the miombo section of Chipopela-B and Chipopela-C, respectively, over a period of two months) and a decline of tsetse abundance in munga. During the cold dry season, on the other hand, a large proportion of miombo male flies move to munga and a substantial proportion tends to stay in munga once they have arrived there. Nevertheless, despite the preference for munga, the movement pattern is less asymmetric and miombo is not completely deserted, hence the random distribution of captures along the transects this time of the year. Finally, during the hot dry season, the cold dry season movement patterns continue although a higher proportion of the munga flies tend to move to miombo. Because of the sampling bias attending the fly-round method, seasonal changes in the movement pattern of female *G. m. morsitans* cannot be determined equivocally. However, the drop in the IA of tsetse in munga during the dry season suggests that female movement patterns do not differ much from those of the male flies.

The observed seasonal movement of tsetse clearly reflects the changes in the grazing pattern of cattle, the major host species at this locality. During the rainy season, cattle

are confined to miombo woodland, hence the aggregation of tsetse in miombo and the movement from munga to miombo. The changes in grazing pattern after the harvest (June-July) result in a concomitant movement of tsetse from miombo to munga. However, since the grazing of cattle is not restricted to munga only, tsetse move between munga and miombo. The C/M/R/R results indicate that, with the exception of the rainy season, movement of tsetse between the two main vegetation types is two directional. Hence, the increase in the abundance of tsetse in munga at the beginning of the cold dry season is not a change in the distribution of tsetse from one vegetation type to another but rather an expansion of the tsetse habitat following the broader grazing pattern of the host.

The concentration of tsetse in miombo during the rainy season could be exploited when utilizing stationary baits to control the fly. The results presented above suggest that the deployment of odour-baited targets in miombo may suffice to control the tsetse population. This irregular deployment of targets, with a concentration of baits in *Brachystegia* woodland, was applied successfully in a tsetse control operation south west of the study area (Section 5.3). Various approaches to the control of *Glossina* spp. have been based on the selective destruction of or the selective application of insecticides to the "essential habitat" of the fly (Jordan, 1986). However, contrary to the observations in the study area, climate and vegetation normally determine the essential habitat of the fly. This often results in a concentration of tsetse in dense woodland along the drainage lines during the hottest and driest times of the year. The finding that tsetse were markedly restricted during the hot dry season became the cornerstone of the discriminative application of insecticides and the subsequent successful clearing of *G. m. morsitans* from Zimbabwe's south-eastern low veld region (Robertson and Kluge, 1968).

2.5 The prevalence of trypanosomal infections in *G. m. morsitans* Westwood (Diptera: Glossinidae) in eastern Zambia

2.5.1 Introduction

An important factor in the complex epidemiology of tsetse-transmitted trypanosomosis is the proportion of infected tsetse flies transmitting the disease (Lambrecht, 1980). The relationship between tsetse and trypanosomes and the temporal variation in the prevalence of metacyclic trypanosomal infections in tsetse has been subject to many investigations (e.g. Harley, 1966; Woolhouse & Hargrove, 1998). More recently, mathematical models have been used to provide estimates of the developmental period of trypanosomes in tsetse and to clarify the age-dependent susceptibility of tsetse to infection (Woolhouse *et al.*, 1993, 1994; Woolhouse & Hargrove, 1998). Despite the economic importance of *Glossina morsitans morsitans* in southern Africa, little is known of the temporal variation in the trypanosome prevalence of this species. An understanding of the relationship between the proportion of infected *G. m. morsitans* and the incidence of bovine trypanosomosis is, however, essential when planning the control of the disease. A study was, therefore, initiated in an area where bovine trypanosomosis, transmitted by *G. m. morsitans*, is one of the major diseases in cattle. Temporal variation in the prevalence of metacyclic and immature infections was determined and age-prevalence relationships were established for both metacyclic and immature infections. The age-prevalence relationship of immature infections was used to clarify changes in the prevalence of metacyclic infections. The temporal variation in the prevalence of infection in tsetse and the factors determining it were used to clarify the incidence of bovine trypanosomosis in the study area.

2.5.2 *Materials and methods*

2.5.2.1 Study area

The study area is described in Section 2.2.2.1.

2.5.2.2 Tsetse sampling

Tsetse were sampled along six fixed fly-round transects. Fly-rounds were conducted along these transects as described in Section 2.2.2.2. Tsetse catches per fly-round were transformed using a square root (n + 0.5) transformation (Sokal and Rohlf, 1998). A transformed monthly average index of abundance (IA) of tsetse was calculated as the average number of flies (males and females) captured per stop per fly-round. Averages were detransformed for presentation (Sokal and Rohlf, 1998). Fifteen epsilon traps (Hargrove & Langley, 1990) were deployed and were operated throughout the day. Flies were harvested twice daily between 10:00 and 12:00 h and between 16:00 and 17:00h.

2.5.2.3 Tsetse dissection

Live flies were dissected within 4 hours of collection. Mouthparts, salivary glands and midgut dissections were performed using the method described by Lloyd & Johnson (1924). Infections in the tsetse flies were identified according to the site of trypanosomal infestation. Infections in the proboscis alone were recorded as *vivax*-type, in the proboscis and the midgut as *congolense*-type and in the midgut alone as immature. When few trypanosomes were present in the midgut, the infection was attributed to trypanosomes from an infective blood meal (Welburn *et al.*, 1989). Those infections were not considered to be immature infections. The salivary glands were examined for mature *brucei*-type infections.

Throughout the analysis it was assumed that midgut or immature infections either mature into *congolense*-type infections or remain immature for the rest of the fly's life. Changes in the age-prevalence relationship of immature infections are thus due either to maturation of immature infections into *congolense*-type infections, acquisition of new midgut infections or a combination of both. The age-prevalence relationship of the sum of immature and *congolense*-type infections, on the other hand, is only

influenced by the acquisition of new midgut infections. Consequently, the age-dependent maturation of midgut infections is represented by the ratio of [*congolense*-type/(immature + *congolense*-type)] infections per ovarian age category.

Between January 1992 and December 1993, infected (mature and immature infections) female flies were aged. Physiological age-determination of females was conducted as described by Saunders (1960) and Challier (1965). Each fly was assigned to an ovarian age category depending on its ovarian configuration. Ovarian age categories 0 and 1 correspond to ages 0 to 8 days and 9 to 16 days respectively; ovarian categories 2 to 7 correspond to additional intervals of 9 days (Woolhouse and Hargrove, 1998). Depending on the content of the uterus, ovarian category 1 to 7 was subdivided into A (egg or first instar larva), B (second instar larva) or C (third instar larva). Ovarian category 0 was subdivided into A (immature egg) and B (mature egg). The ovarian age categories were transformed into days corresponding to the mid-point of each ovarian category.

For male flies the wings were excised and fixed to a microscope slide with sticky tape for analysis of wing fray (Jackson, 1946).

2.5.2.4 Incidence of bovine trypanosomosis

To determine the incidence of bovine trypanosomosis, eight sentinel herds consisting of 20 adult Angoni cattle each were established in the study area. All sentinel animals were kept under traditional village management and were brought for sampling each month during two consecutive years. On each occasion, blood samples were taken from an ear vein directly into heparinized capillary tubes. The packed cell volume of each animal was measured and the blood was examined for the presence of trypanosomes using the buffy-coat, phase-contrast, technique (Murray *et al.*, 1977). Any animal found to be infected with trypanosomes at the monthly samplings was treated with diminazene aceturate (Berénil®, Hoechst) at the dose of 7mg/kg body weight for *T. brucei* or 3.5 mg/kg body weight for *T. congolense* or *T. vivax*, by intramuscular injection. Animals given this dose of diminazene were considered to be protected during the subsequent two weeks and were therefore excluded from the next calculation of incidence.

2.5.2.5 Statistical data analysis

The raw fly data consisted of four dichotomous response variables indicating, for each fly, the presence or absence of *congolense*-type, *vivax*-type, *brucei*-type and immature infections. The explanatory variables were the year and month of fly capture and, for female flies captured between January 1992 and December 1993, the ovarian age of the fly. The data were expressed in the form of monthly prevalence of each of the infection types. Comparisons in the prevalences of infection for each sex between months, between sexes and between years were made using χ^2-tests (Sokal & Rohlf, 1998).

Another part of the analysis investigated the effect of the three explanatory variables (year, month and age) on the prevalence of *congolense*-type, *vivax*-type, *brucei*-type and immature infections. For the purpose of this analysis all midgut infections (immature + those associated with a *congolense*-type infection) were considered. The analysis was restricted to female flies captured between January 1992 and December 1993 for which the ovarian age was known. Logistic regression was used to model the prevalences. Hypothesis testing was done by averages of likelihood ratio χ^2-tests (Sokal & Rohlf, 1998). The significance level was set at 0.05.

The analysis further estimated the *per capita* rate (λ) at which flies become infected and the developmental period (τ) of the trypanosomes in the tsetse fly. The model for age-prevalence data described by Woolhouse (1989) was used. For the mature infections the model had the form:

$$y(a) = 1-\exp[-\lambda(a-\tau)] \quad \text{or} \quad \ln(1-y(a)) = \lambda\tau - \lambda a$$

$$\text{for } a > \tau;\ y(a) = 0 \text{ for } a = \tau$$

where y(a) is the proportion of infected flies at age a (a is the pivotal age of each of the ovarian categories)

The age-prevalence model for immature infections was derived from Woolhouse's model. It had the form:

$$\ln(1-y(a)-z(a)) = \lambda' a$$

where z(a) is the proportion of tsetse with immature infections at age a and λ' is the *per capita* rate at which flies become infected with midgut infections or the slope of a regression equation through the origin.

The log-linear model was fitted to the age-prevalence data using least squares, i.e. with the assumption of approximate normality of ln(1-y) or ln(1-y-z) and estimates of λ, λ' and τ were obtained. Stepwise additions of polynomial terms in age were added when they significantly improved the model fit. Woolhouse (1989) discussed the model's assumptions. The *per capita* rate at which tsetse become infected with immature infections (λ') was compared with the *per capita* rate at which tsetse become infected with mature infections (λ) using a t-test. The GLIM statistical software package was used for all statistical analyses.

2.5.3 Results

2.5.3.1 Temporal variations in the prevalence of trypanosomal infections in tsetse

During the study period (January 1990 to December 1993), a total of 5 701 female and 10 612 male *G. m. morsitans* were captured and screened for the presence of trypanosomal infections. A total of 1 499 males and 747 females had either a *congolense*-type, *vivax*-type, *brucei*-type or an immature infection. *Congolense*-type infections were dominant. They constituted 73.5% of all mature infections. A total of 246 flies (25.5% of all mature infections) had a *vivax*-type infection. Only 10 flies (9 males and 1 female) had salivary gland infections (*brucei*-type). Immature or midgut infections were detected in 7.8% of all dissected flies. For each infection type, the prevalence of infection varied between months (Fig. 2.5.1). Differences between months were only consistently statistically significant ($P<0.01$) for immature and

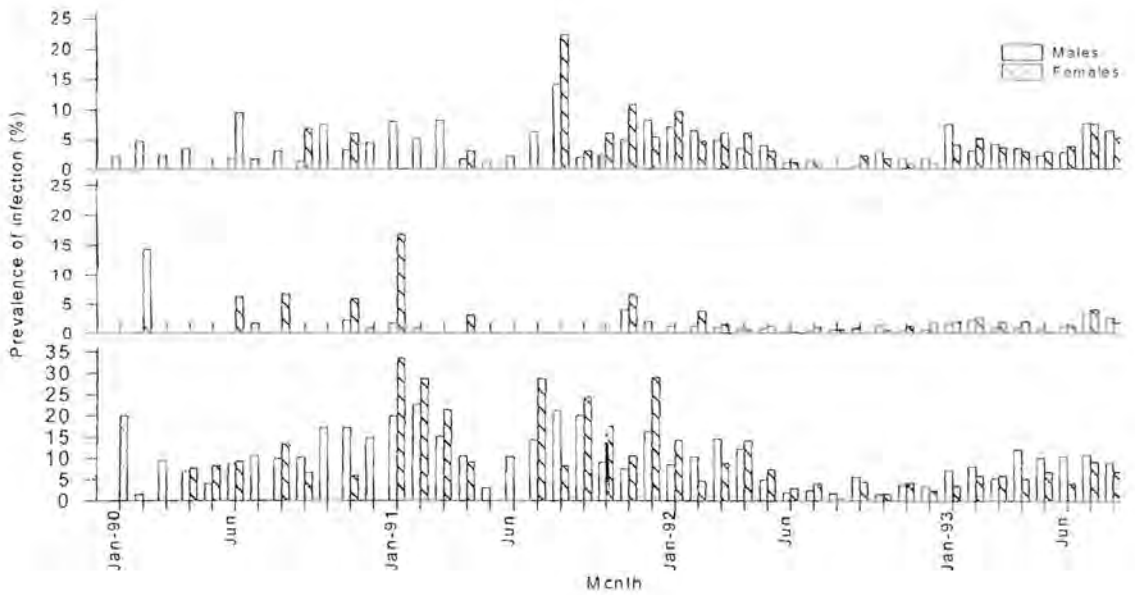

Figure 2.5.1: Monthly proportion of male and female *G. m. morsitans* with (top) *congolense*-type, (middle) *vivax*-type, and (bottom) immature trypanosomal infections.

congolense-type infections in male flies between 1991 and 1993. The monthly proportion of male tsetse with *congolense*-type infections was significantly correlated (r = 0.61, P<0.05) with the monthly proportion of male flies in the higher wing fray categories (wing fray>1). With the exception of *brucei*-type infections in females, annual proportions of infected flies differed between years (P<0.01 for all infection types in males and females) (Table 2.5.1).

The prevalence of *congolense*-type and *vivax*-type was significantly higher in female than in male flies (P<0.05). Immature and *brucei*-type infections were more prevalent in male flies (P<0.05).

2.5.3.2 Effect of year, month and age on the prevalence of infection in female flies

Between January 1992 and December 1993, 4 416 female *G. m. morsitans* were sampled, screened for the presence of trypanosomal infections and aged. The yearly totals were 2162 and 2254 flies for 1992 and 1993, respectively. Monthly sample sizes, pooled over both years, ranged from 175 in January to 599 in May. The effects of year and month on the prevalences, allowing for the effect of the age of the flies, were explored for *congolense*-type, *vivax*-type infections and the sum of immature and *congolense*-type infections (Table 2.5.2).

There was no significant yearly variation in the prevalences of any of the infection-types and no effect of year on the shape of the monthly variation of any of the infection types. For *congolense*-type infections, only age had a significant effect on prevalence. Monthly variations in prevalences of infection were not significant. For *vivax*-type infections both age and month, but not their interaction, were significant factors. For the sum of immature and *congolense*-type infections both age and month, but not their interaction, were significant factors explaining the variation in the prevalence.

Table 2.5.1: Annual number (%) of male and female *G. m. morsitans* harbouring *congolense*-type, *vivax*-type, *brucei*-type and immature trypanosomal infections during four consecutive years.

	Males					Females				
year	n	*congolense* (%)	*vivax* (%)	*brucei* (%)	immature (%)	n	*congolense* (%)	*vivax* (%)	*brucei* (%)	immature (%)
1990	965	30 (3.1)	5 (0.5)	4 (0.4)	101 (10.5)	181	5 (2.8)	5 (2.8)	0 (0)	10 (5.5)
1991	1538	88 (5.7)	20 (1.3)	3 (0.2)	221 (14.4)	256	18 (7.0)	5 (1.9)	0 (0)	44 (17.2)
1992	4096	132 (3.2)	32 (0.8)	1 (0.02)	262 (6.4)	2691	120 (4.5)	43 (1.6)	1 (0.04)	156 (5.8)
1993	4013	182 (4.5)	79 (2.0)	1 (0.02)	338 (8.4)	2573	134 (5.2)	57 (2.2)	0 (0)	149 (5.8)

2.5.3.3 Age-prevalence relationship

Average estimates of prevalences of infection with age were obtained ignoring the effects of month and year. Due to the low prevalence of *brucei*-type infections, they were omitted from the analyses.

The prevalence of *congolense*-type infections ranged from 0% (ovarian age category 0a and 0b) to 9.6% (ovarian age category 5b) (Fig. 2.5.2). For *vivax*-type infections the prevalence varied between 0% (ovarian category 0a) to 6.2% (ovarian category 6a) (Fig. 2.5.2).

Table 2.5.2: Logistic regression analysis of deviance of terms affecting the prevalence of *congolense*-type, *vivax*-type and immature + *congolense*-type infections in female *G. m. morsitans*.

Terms included	Terms added	χ^2-value	d.f.	P-value
congolense-*type infections*				
-	year	3.2	1	0.07
-	month	15.5	11	0.16
-	age	87.1	1	<0.001*
age	year	3.3	1	0.07
age	month	18.1	11	0.07
vivax-*type infections*				
-	year	3.5	1	0.06
-	month	25.7	11	0.007*
month	month.year	13.9	12	0.30
-	age	14.2	1	<0.001*
age	year	3.5	1	0.06
age	month	25.0	11	0.009*
age+month	age.month	11.9	11	0.37
Immature + congolense-*type infections*				
-	year	1.6	1	0.20
-	month	39.9	11	<0.001*
month	month.year	18.3	12	0.10
-	age	19.5	1	<0.001*
age	year	31.7	1	0.19
age	month	36.4	11	<0.001*
age+month	age.month	6.9	11	0.8

* = significant at p=0.01 - = main effect + interaction term . = interaction term only

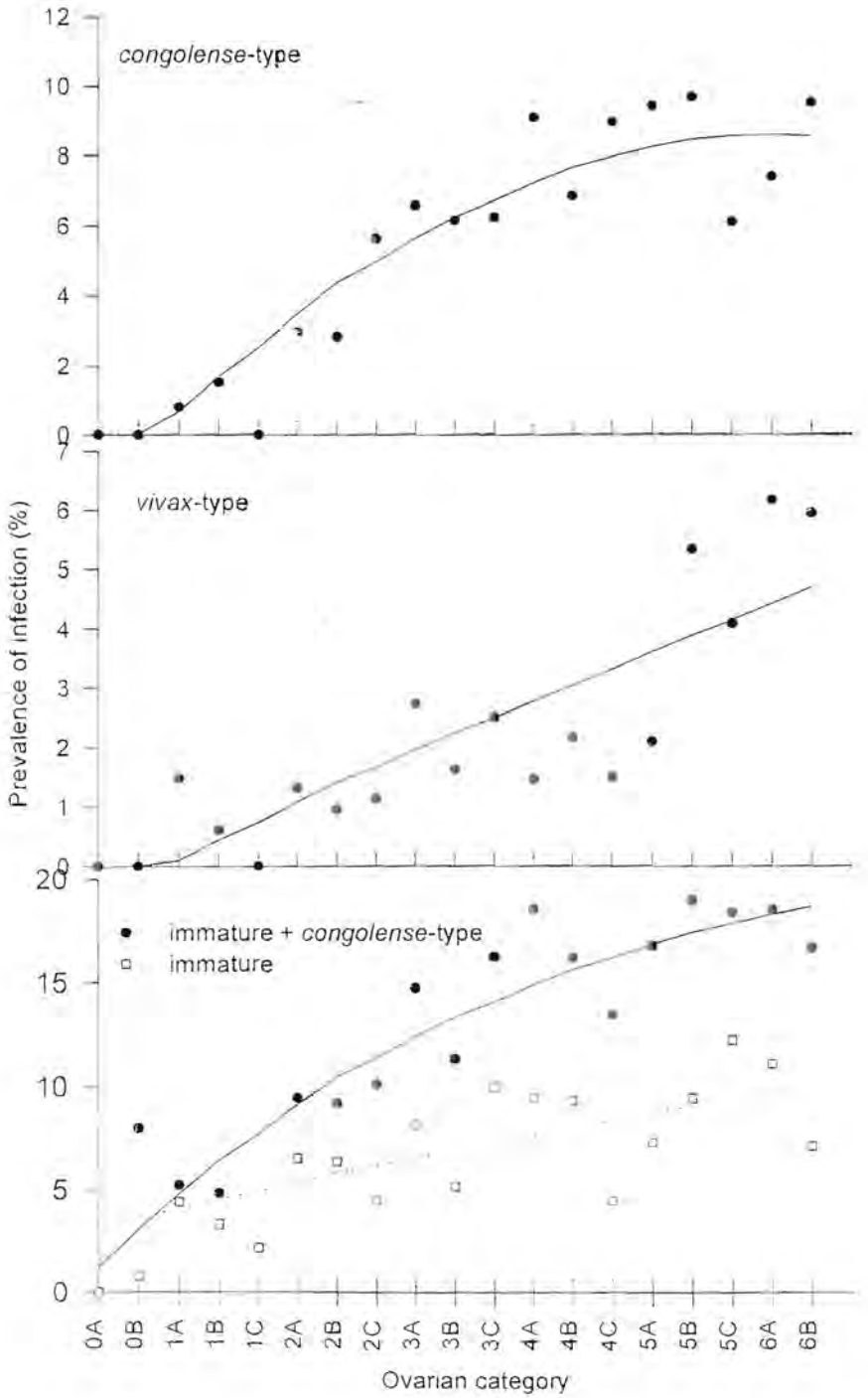

Figure 2.5.2: Variations in prevalence of *congolense*-type, *vivax*-type and *congolense*-type + immature (•) and immature (□) infections of female *G. m. morsitans* with fly age (by ovarian category). The best fit model (line) is ln(1-y) = -0.02107 - 0.002672age + 2.292e-07age^3 for *congolense*-type, ln(1-y) = 0.00795 - 0.0008496age for *vivax*-type and ln(1-y) = -0.00448age + 0.00000032age^3 for *congolense*-type + immature infections.

The model $\ln(1-y(a)) = \lambda\tau - \lambda a$ was fitted to the age prevalence data of the *congolense*-type and *vivax*-type infections to obtain estimates of the *per capita* infection rate (λ) and developmental period (τ). The fitted curves are listed in Table 2.5.3 and plotted, together with the observed prevalences of infection (Fig. 2.5.2). Only the fit of the model for the *congolense*-type infections was significantly improved ($P<0.01$) by adding a cubic term in age. The final model explained 87% and 71% of the variation in $\ln(1-y(a))$ for *congolense*-type and *vivax*-type infections respectively. The parameter estimates ($\pm$ 1 s.e.) for *congolense*-type infections were τ = 7.9 $\pm$ 3.7 days and λ = 0.0026 $\pm$ 0.00043/fly/day for age = τ. For *vivax*-type infections, the parameter estimates ($\pm$ 1 s.e.) were τ = 9.4 $\pm$ 6.5 days and λ = 0.00085 $\pm$ 0.00013 /fly /day for age = τ.

Table 2.5.3: Parameter estimates of age-prevalence models for three trypanosomal infection types in female *G. m. morsitans*.

Estimate	s.e.	parameter
congolense-*type*		
- 0.02107	0.009337	1
- 0.002672	0.0004328	age
2.292 e-07	9.391 e-08	age^3
vivax-*type*		
0.00795	0.005302	1
- 0.0008496	0.0001323	age
Immature + congolense-*type*		
- 0.00448	0.0003929	age
0.00000032	0.00000012	age^3

The prevalence of immature infections varied between 0% (ovarian category 0a) to a maximum of 12.2% (ovarian category 5c) and increased linearly with age (Fig. 2.5.2).

The prevalence of immature + *congolense*-type infections reached a maximum (18.9%) in ovarian category 5b. The model for immature infections was fitted to the age prevalence data of the immature + *congolense*-type infections (Table 2.5.3 and Fig. 2.5.2). A cubic term in age significantly improved the fit ($P<0.05$). This model explains 91.5% of the variation of the prevalence of immature + *congolense*-type infections. The *per capita* rate ($\lambda' \pm 1$ s.e.) at which flies become infected in the midgut was estimated at 0.0045 ± 0.00039/fly/day at day 0. The estimate of λ at age τ for mature infections was significantly lower ($P<0.05$) than the estimate of λ' for immature infections at age 0.

The rate of maturation of midgut infections, expressed by the ratio of *congolense*-type to (immature + *congolense*-type) infections, increased rapidly to a value of about 0.5. at the age of about 30 days after which it declined slightly (Fig. 2.5.3).

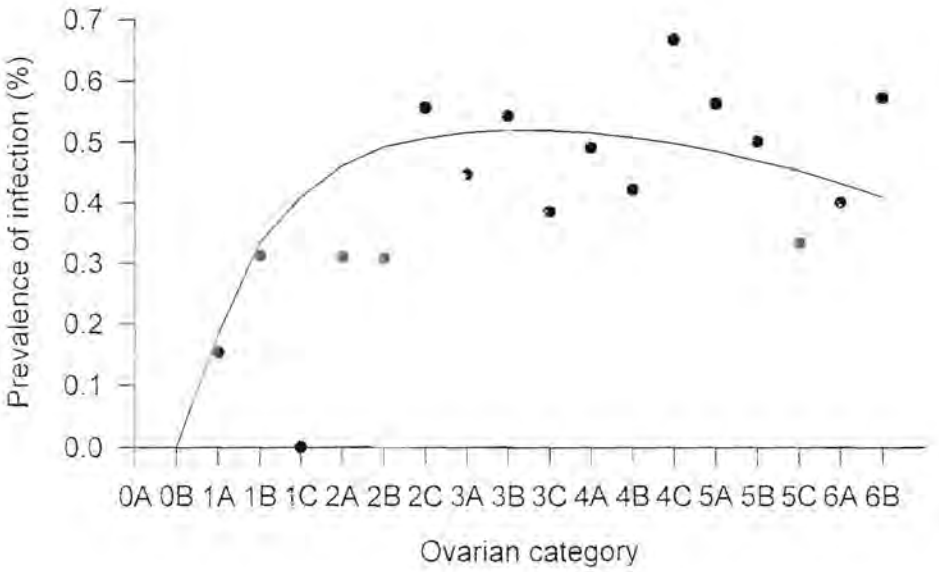

Figure 2.5.3: Variations in the ratio of *congolense*-type infections to (*congolense*-type + immature) infections of female *G. m. morsitans* with fly age (by ovarian category).

2.5.3.4 Incidence of bovine trypanosomosis

The monthly average index of abundance of tsetse was highest at the end of the dry season/beginning of the rainy season (Fig. 2.5.4). The monthly average incidence of trypanosomosis (85% due to *T. congolense*) in the sentinel cattle was 9.3%. It was highest at the beginning of the rainy season (Fig. 2.5.4). The linear regression between monthly average incidence of trypanosomosis (log transformed) and the monthly average index of abundance of tsetse (square root transformed) was highly significant ($P<0.01$) (Fig. 2.5.5). The monthly average index of abundance of tsetse explained 74% of the variance in the monthly average incidence of bovine trypanosomosis.

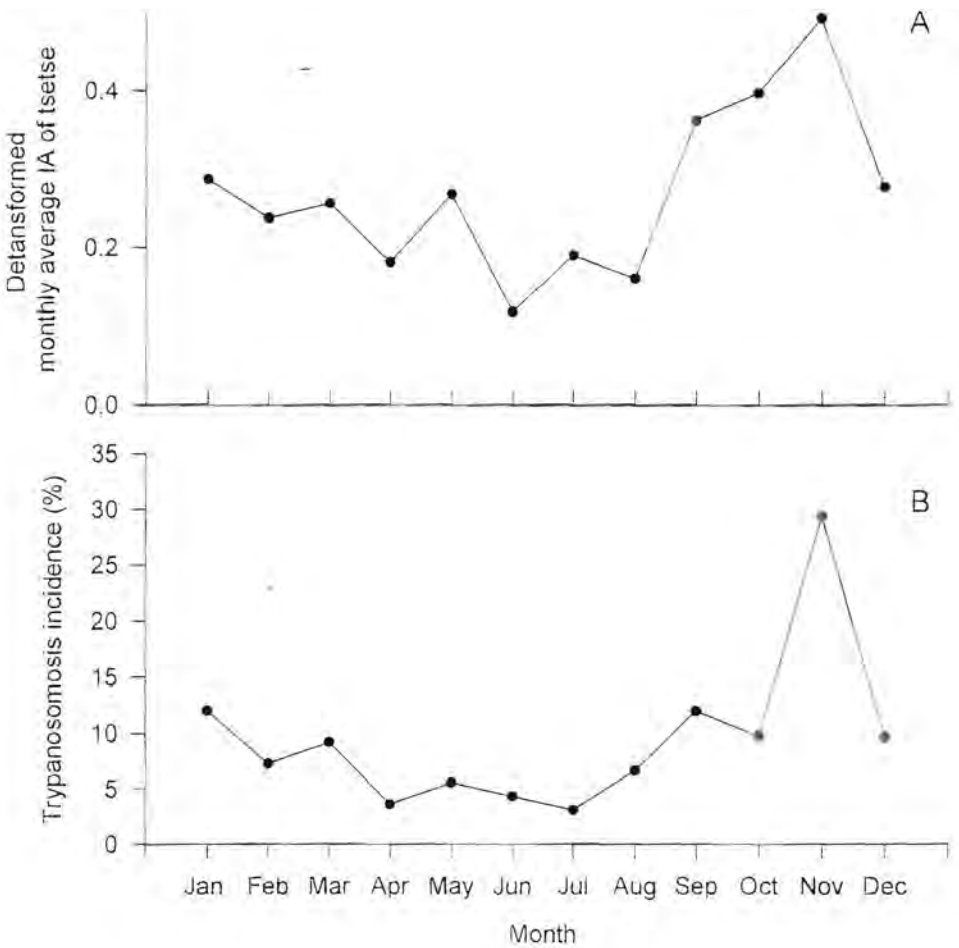

Figure 2.5.4: Detransformed monthly average index of abundance of *G. m. morsitans* (A) and monthly average incidence of trypanosomal infections in sentinel cattle (B).

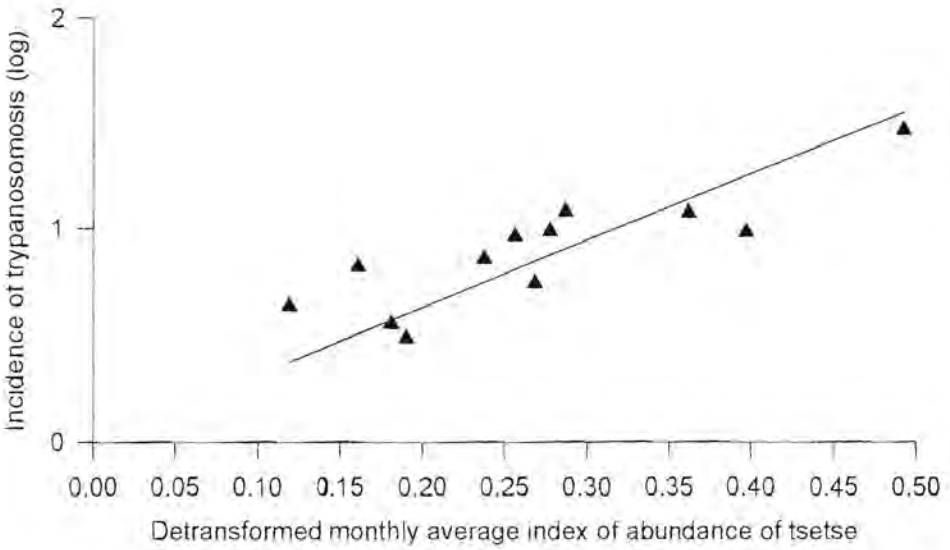

Figure 2.5.5: Relationship between the monthly average incidence of trypanosomal infections in cattle (log transformed) and the detransformed average monthly index of abundance of *G. m. morsitans* in the study area.

2.5.4 Discussion and conclusions

2.5.4.1 Prevalence of trypanosome species in tsetse

Examination of historical data on the prevalence of trypanosomal infections in *G. m. morsitans* in central and eastern Zambia, showed a high prevalence of *vivax*-type infections (Clarke, 1969; Okiwelu, 1977a). *Vivax*-type infections were also dominant in *G. pallidipes* from the Luangwa Valley of eastern Zambia (Woolhouse *et al.*, 1994). The prevalence of trypanosome species in tsetse has been linked to host preference. *Vivax*-type infections are often associated with high percentage of bovid feeds whereas *congolense*-type infections area associated with feeds on suids (Jordan, 1963; Tarimo *et al.*, 1984; Snow *et al.*, 1988). These observations can clearly not be generalised. Notwithstanding the high proportion of cattle feeds (Section 2.3.3), *T. congolense* is the dominant infection-type in tsetse on the eastern plateau of Zambia. *Trypanosoma congolense* is also the main causative agent of bovine trypanosomosis on the eastern plateau and other areas in southern Africa.

2.5.4.2 Temporal variation in the prevalence of trypanosomal infections in tsetse

The monthly prevalence of *congolense*-type infections in male and female flies is low but very similar to those observed in *G. m. morsitans* from other districts of Zambia (Clarke, 1969) and in Zimbabwe (Chorley, 1929; Leggate, 1963). The proportion of *vivax*-type infections in the study area, on the other hand, is substantially lower than in other areas. In the adjacent Luangwa Valley, for example, *vivax*-type infections dominate in tsetse (Woolhouse *et al.*, 1994). The prevalence of *T. vivax* infections is also low in the cattle population in the study area. This change in the trypanosome species prevalence in tsetse is attributed to the, frequently observed, self-cure of *T. vivax* infections in cattle (Gardiner, 1989) and the high dependence of tsetse on cattle as source of food and, hence, source of trypanosomes in the study area (Section 2.3.3).

The correlation between the prevalence of *congolense*-type infections and the proportion of male flies in the higher wing fray categories indicates that the prevalence of trypanosome infections in male flies increases with age indexed by wing fray (Woolhouse *et al.*, 1993, 1994). Hence, temporal variation in the prevalence of *congolense*-type infections in male flies is probably a consequence of changes in the age structure of the flies. In female flies, age is the main factor determining prevalence fluctuations.

The effect of fly sex on the infection rate is complicated by the age distribution of the sample. It is, however, surprising that the prevalence of *brucei*-type infections is higher in males whereas the prevalence of *congolense*- and *vivax*-type infections is higher in females. These results suggest that factors other than the age distribution of the sample may play a role. There is contrasting evidence on the effect of fly sex on the development of *T. congolense* in *G. m. morsitans* (Moloo, 1981; Mwangelwa *et al.*, 1987). Various laboratory studies have, on the other hand, suggested that males

produce a greater proportion of salivary gland infections than females (Burtt, 1946b; Harley, 1971a). Our results confirm the latter observation.

2.5.4.3 Age-prevalence relationship for trypanosomal infections in female tsetse

The estimated developmental period of *congolense*-type infections corresponds well with the average 8 to 9 days observed by Elce (1971). Nantulya *et al.* (1978), however, observed development periods up to 40 days. The estimated developmental period for *vivax*-type infections, agrees with the 5 to 13 days reported by Davies (1977).

For *vivax*-type infections the rise in prevalence was approximately linear with fly age and is consistent with the idea that tsetse flies can readily infect themselves with *T. vivax* throughout their life. This is probably related to the relatively simple developmental cycle of *T. vivax* in the tsetse fly. The prevalence of *congolense*-type infections increases substantially, though not linearly, with increasing age. This age-prevalence model has been observed frequently in other tsetse species (Leak & Rowlands, 1997; Woolhouse & Hargrove, 1998). The shape of the age-prevalence relationship can be explained by (i) variations in the maturation period of trypanosomal infections, (ii) age-dependent decrease in susceptibility to infection or (iii) increased mortality of tsetse infected with a metacyclic *T. congolense* infection (Dale *et al.*, 1995; Woolhouse *et al.*, 1993; Woolhouse & Hargrove, 1998). It is difficult to quantify which of these features contributes most to the model. However, the age-prevalence relationship of immature infections, established during this study, can be used to clarify some of the processes involved in the maturation of *congolense*-type infections. Immature infections must develop immediately after the ingestion of the infected blood meal. Assuming that maturation is restricted to infections obtained during the first blood meal, the prevalence of midgut infections in the youngest age categories cannot be lower than the maximum prevalence of mature, *congolense*-type, infections in the subsequent age categories. This is not the case. The maximum *congolense*-type infection prevalence (9.6 % in ovarian category 5b) is higher than the maximum midgut infection prevalence in the first three age categories (6.5 %).

Furthermore, between ovarian age category 2 and 6 the *congolense*-type infection prevalence increases more than threefold from 2.8% to 9.6%. Hence, despite normal variability in the incubation period of *congolense*-type infections (Dale *et al.* 1995), the observed increase in the prevalence of *congolense*-type infections cannot be due entirely to retarded maturation of trypanosomal infections obtained during the first blood meal. The proportion of immature or midgut infection increases linearly with increasing age. Thus, tsetse acquire midgut infection throughout their lives. These midgut infections give rise to new *congolense*-type infections. The shape of the age-ratio of [*congolense*-type /(immature + *congolense*-type)] infections relationship, however, suggests that the proportion of midgut infections that mature differs with age. Trypanosomal infections obtained early in life contribute more to the *congolense*-type prevalence than those acquired at a later age. Hence, our data suggest that, whereas *G. m. morsitans* can readily acquire midgut infections throughout its life, the proportion of midgut infections that matures decreases with increasing age. The significant difference between the force-of-infection for immature infections (λ') and the force-of-infection for mature *congolense*-type infections (λ) confirms this observation. Obviously, increased mortality of flies with mature infections or loss of infection will also affect the shape of the age-prevalence relationship for *congolense*-type infections. Increased mortality in trypanosome-infected *G. m. morsitans* was observed by Nitcheman (1988) under laboratory conditions. Bursell (1981) suggested that, under field conditions, the effect of trypanosomal infections on life expectancy of tsetse may be a result of the energetic loss associated with the parasite load in the midgut leaving less reserves available for flight and host location. This implies that life expectancy should also be reduced in tsetse with immature infections. The shape of the age-prevalence of immature infections relationship does not suggest any additional mortality of older flies with immature infections. Consequently, the age-prevalence relationships of *congolense*-type infections is best explained by a declining rate of maturation of immature infections with age.

2.5.4.4 The epidemiology of bovine trypanosomosis

The proportion of infected tsetse is an important factor in the epidemiology of bovine trypanosomosis. The *per capita* rate at which female *G. m. morsitans* acquire

congolense-type infections, indicates that 0.26% of the female tsetse, in the study area, acquire mature *T. congolense* infections per day at age τ. It decreases with age > τ. At a feeding interval of 4 days (Rogers, 1988) and a 75% feeding preference for cattle (as is the case in the study area), this corresponds to a successful infection every 72 blood meals on cattle. This is substantially less than the acquisition of midgut infections (one successful infection every 42 blood meals on cattle). The prevalence of *T. congolense* infections in cattle is high in the study area. At an average prevalence of trypanosomosis of 40 %, 5.9% of the feeds on infected cattle develop in a midgut infection in female *G. m. morsitans* at age 0. Only 3.5% of the feeds on infected cattle develop in a mature infection at age τ. The latter figure is slightly higher than the 2.5% obtained by Rogers *et al.* (1973) from an analysis of data on *G. swynnertoni* in Tanzania.

The proportion of infected flies and the population density of tsetse are important components of trypanosomosis challenge. The small proportion of feeds on infected cattle that develop in mature infections in tsetse suggests a high degree of refractoriness of *G. m. morsitans* to infection with *T. congolense* in the study area. Furthermore, our results show that only in male flies the monthly prevalence of *congolense*-type infections undergoes significant but small changes, which are determined largely by the proportion of older flies in the population. Hence, the density of the tsetse population is likely to be an important variable determining trypanosomosis challenge. This is confirmed by the highly significant regression between the incidence of trypanosomal infections in cattle and the index of abundance of tsetse.