Evaluating the impact of a tsetse-repellent technology on the incidence of trypanosomosis in cattle managed under pastoral production systems in selected sites in Kajiado and Narok districts, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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This thesis has been submitted for examination with our approval as the supervisors

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DEDICATION

To my wife Carol,

daughter Anita, and

son Gavin

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ABSTRACT

This study evaluated a tsetse repellent technology developed for the control of tsetse-transmitted trypanosomosis in cattle. The technology comprised a repellent, 2-methoxy 4-methylphenol (or 4-methyl guiaicol), emitted from dispensers attached to collars worn around the necks of cattle. The repellent was synthesized by adding carbon to guiaicol (2-methoxyphenol), a compound that occurs naturally in bovid urine. The dispenser was made up of two aluminium pipes fastened onto the collar using wire strings. Each aluminium pipe measured 10mm in diameter and 10cm long and was joined to corked silicon tygon tube of about 2cm at the distal end (to serve as a point of diffusion). The proximal end of the pipe was sealed with a screw cap. When undamaged, each reservoir was expected to release the repellent at 4.5mg h^{-1} for at least a month. The technology had to be applied to all the animals in a herd for it to be protective. The technology could also be integrated with traps or targets in a strategy referred to as "push-pull" tactic. In this strategy, the repellent technology is expected to 'push' the tsetse flies to traps or targets, while traps or targets 'pull' and hence trap/kill the flies. It has been proposed that this strategy could increase the rates of tsetse suppression beyond the levels that could be achieved by using traps or targets alone.

The study had two components. The first component comprised a field trial carried out in purposefully selected pastoral areas in Nkineji, Narok District and Nkuruman, Kajiado District to evaluate the effectiveness of the technology in a representative situation on-farm. The second component was aimed at developing a mathematical model that would be used to predict the effectiveness of the technology under multiple scenarios. The field trial used a total of 24 randomly selected pastoral cattle herds (with variable number of animals) distributed equally into treatment and control groups. Each study area was allocated equal number of treatment (6 herds) and control (6 herds) herds. The sample size was estimated assuming a type I error (α) of 5%, power (1-type II error [β]) of 80%, intra-herd correlation coefficient of 0.4 and that the repellent technology, if effective, would reduce the incidence of trypanosomosis in treated herds by at least 50% (i.e. one-sided test).

The study was conducted over a period of 12 months preceded by a baseline period of 4 months. All the animals in the recruited herds were screened for trypanosomosis on monthly basis using the buffy coat technique. Tsetse challenge (measured at the village-level), grazing patterns, status of the repellent dispenser and the amount of trypanocides used per herd by the livestock owners were monitored as well. Trypanosomosis incidence was the main outcome indicator of effectiveness. Trypanosomosis incidence was derived at the animal level using both the first infections and all the infections that each subject acquired over the follow-up period. Crude and adjusted effects of the technology were estimated using a marginal model that utilized General Estimation Equations (GEE) to adjust for repeated measures in time. The co-variates considered were age, sex and colour of an animal, area, season and herd size. Descriptive analyses conducted prior to estimating the effectiveness of the technology indicated that tsetse challenge was not unconditionally associated with trypanosomosis incidence. An alternative approach of incorporating the variable in multivariate analyses using dummy variables representing village was developed. The parameter estimates for the dummy variables were, however, treated as nuisance parameters.

The generic mathematical model developed combined tsetse population and trypanosomosis transmission dynamics (referred to as sub-models) with the trypanosomosis transmission sub-model being adapted to capture the effect of the repellent. The repellent technology was assumed to reduce the probability of tsetse feeding on a treated host. Trypanosomosis transmission dynamics in: treated cattle, untreated cattle and game were tracked using coupled differential equations. The impacts of integrated strategies and the synergistic effects that may be expected were evaluated by varying the relevant parameters influenced by the component control methods.

Descriptive analyses of the data from the field trial indicated that, on average, 26.1% (minimum and maximum of 2.1 and 33.8%) of treated animals may have been effectively protected through the sampling intervals as the repellent dispenser experienced frequent damage and leakage. Given the frailties of the repellent dispenser, the analysis of data was conducted in two stages. The first stage used a standard method of analysis of randomized

controlled trials based on the principle of "intention to treat" where the dispenser malfunctions were ignored and all subjects analyzed according to the original treatment assignment. The second stage attempted to account for the dispenser defects by stratifying the analysis by the 'levels of protection' to which each animal could be classified at the time of sampling based on the status of the repellent dispenser. These levels included (i) good – for the animals that had intact dispensers (with the repellent in both reservoirs), (ii) moderate – for the animals that had the repellent in one of the two reservoirs, (iii) poor – for the animals that did not have the repellent in both of the reservoirs, or had dropped its dispenser over the sampling interval, and (iv) the control.

Results obtained from the standard analyses indicated that the technology was associated with a statistically insignificant 14% reduction in trypanosomosis incidence irrespective of the method used in estimating incidence (i.e. first or multiple occurrences of the disease). The decline in incidence in the treatment group did not achieve the threshold set *a priori* (of 50%) to indicate effectiveness. The analyses aimed at accounting for the defects of the repellent dispenser (at stage 2) showed that the frailties of the repellent dispenser, hence the level of protection, was not associated with the incidence of trypanosomosis. Furthermore, animals that had intact dispensers (hence good protection) always belonged to herds that had high proportions of animals with intact dispensers. For most of the sampling interval, such animals, therefore, had 'good protection' related to their individual dispensers as well those of their contemporaries (i.e., herd-effect). The rate of trypanocide use over the study period was 6% higher in the treatment compared to the control group; this finding was also not statistically significant.

The generic model developed was not validated because the values of some of the parameters associated with game could not be authenticated; the estimates for such parameters were obtained from literature. The outputs generated are therefore appropriate for an *ex ante* evaluation of repellent-based competing integrated strategies before they are subjected to a field trial. The outputs show that the effectiveness of the technology declines as the density of herds treated in an area increases, and its use leads to an increase in the transmission potential of the disease as the reproductive number increases as a higher share

of herds are treated. The technology would also need to reduce the probability that tsetse feeds on a treated host by 80% for it to reduce the incidence of trypanosomosis by 50%. Simulation of repellent use combined with traps or targets indicates marginal added value of the repellent to be negligible given the already very effective suppression of fly populations by the bait technologies. Targets are slightly more efficacious than traps – the models developed however considered them as being similar as the purpose of the analyses were to study the trends and not generate exact predictions. These marginal effects, moreover, would be expected only during the first 180 days of a vector suppression intervention program because after this period, tsetse populations would be too low for the repellent technology to provide any apparent benefits. This modelling simulation therefore suggests limited benefits of the tsetse repellent technology, particularly in areas of high tsetse challenge.

It is concluded that the technology in its current form does not yet offer an adequate level of protection to merit further development as a commercial product.

Chapter 1

General introduction

1.1. Background

Agriculture directly influences rural livelihoods by providing incomes, employment and products for home consumption. It indirectly influences prices of commodities and labour markets, amongst other farm-non-farm linkages. In Kenya, this sector accounts for over 27% of Kenya's gross domestic product (GDP), 70% of foreign exchange earnings and up to 60% of export earnings (Ochieng-Odiambo, 1998). The livestock sub-sector contributes up to 10 -12% of the GDP, with the sector being dominated by small producers (FAO, 2005).

In the last two decades, the per capita livestock production and productivity have been stagnant because of (i) poor governance in key agricultural institutions, particularly the cooperative sector and lack of comprehensive legal framework to guide formulation of consistent policies, (ii) lack of capacity by the private sector to take over functions previously performed by the state, incomplete markets and weak marketing systems, (iii) poor or insecure access to land and to farm credit, high cost of farm inputs and heavy taxation of farmers through local authority taxes and other levies, (iv) high prevalence of HIV/AIDS affecting agricultural productivity, (v) low level of public funding and inefficient use of public resources resulting in inefficient rural infrastructure and (vi) inappropriate technology and inadequate funding for research and extension services (FAO, 2005).

The bulk of Kenya's livestock farming is being practised under pastoralism in arid and semi-arid areas. These areas cover about 75% of the total land surface and it has been pointed out that tsetse-borne animal trypanosomosis is the main disease constraint in this

zone. Methods that are being used to control the disease include tsetse control, use of trypanocides or utilization of trypanotolerant cattle. Of these methods, tsetse control has the greatest biological efficacy (McDermott and Coleman, 2001) although it needs to be applied at relatively large spatial scales for it to be cost effective. It is often difficult, however, to effectively suppress tsetse in areas that are prone to re-invasion (Leak, 1999; Vale and Torr, 2005). Moreover, the privatization of veterinary services has promoted the application of tsetse control techniques in small scales that would not be effective in the long run (Torr et al., 2005).

In a bid to increase the number of technologies available for tsetse and trypanosomosis control, the International Centre for Insect Physiology and Ecology (ICIPE) developed a technology that involves the use of tsetse repellents (Saini and Hassanali, 2002). Two types of repellents have been developed. These are: (1) 2-methoxy 4-methylphenol, a synthetic analogue of a mild natural repellent of savannah tsetse species called guaiacol (2-methoxyphenol) found in aged bovid urine; and (2), a blend of odour constituents isolated from waterbuck. The repellents act as olfactory antagonists of a kairomone which tsetse use to locate hosts (International Livestock Research Institute [ILRI]/ICIPE, 2001). Following the development and optimization of the repellents, ICIPE continued to: (1) develop a dispenser; (2) determine an appropriate location where the dispensers could be placed on an animal; and (3) estimate an optimum proportion of cattle in a herd that had to be treated to maximize cost-effectiveness of protection.

The refinement of the synthetic repellent was accomplished much earlier than the blend from waterbuck. Studies conducted by ICIPE showed that the synthetic repellent reduced tsetse challenge by more than 80% and feeding efficiency of the fly by more than 90% (ILRI/ICIPE, 2001). In addition, ICIPE conducted controlled field trials using a small number of animals in Nkuruman, Kajiado District in 1999 and 2003. These indicated that the synthetic repellent significantly reduced trypanosomosis incidence in herds where 75% of cattle were wearing a collar with the repellent dispensers (ILRI/ICIPE, 2001; ILRI/TRC/ICIPE, 2003). The prototype dispenser developed and tested by ICIPE has two reservoirs (Plate 1.1a) made of aluminium pipes (10mm in diameter and 10cm long) and a

corked tube of tygon-silicon tubing as the diffusion point. The reservoirs are attached to a belt using a wire and to treat an animal, the belt is tied around the animal's neck while ensuring that the tygon silicon tube is suspended ventrally (Plate 1.1b). When undamaged, the dispenser releases the repellent at 4.5 mg h^{-1} for at least a month.

Apart from being used to control trypanosomosis, the technology could also be integrated with traps or targets in what has been referred to as "push-pull" tactic to enhance the rates of tsetse suppression. In this strategy, the repellent is expected to "push" tsetse towards a tsetse control device, which could be either baited traps or targets. Compared to traps or targets (which are area-specific), the tsetse repellent technology would suit pastoral production systems because treated animals would be able to move with the technology as they move from one area to another. The effectiveness of the technology when used alone or in integrated versions has, however, not been evaluated. The present study evaluated the effectiveness of the new technology through: (1) an on-farm trial conducted in Nkuruman, Kajiado District and Nkineji, Narok District; and (2), simulation modelling that predicted the impact of the technology under varied scenarios when used alone or with other tsetse and trypanosomosis control methods. The on-farm trial ran for 12 months that was preceded by a baseline phase of four months. The simulation study, on the other hand utilized existing mathematical models on tsetse population dynamics and trypanosomosis transmission which were merged and adapted to capture the effect of the repellent technology.

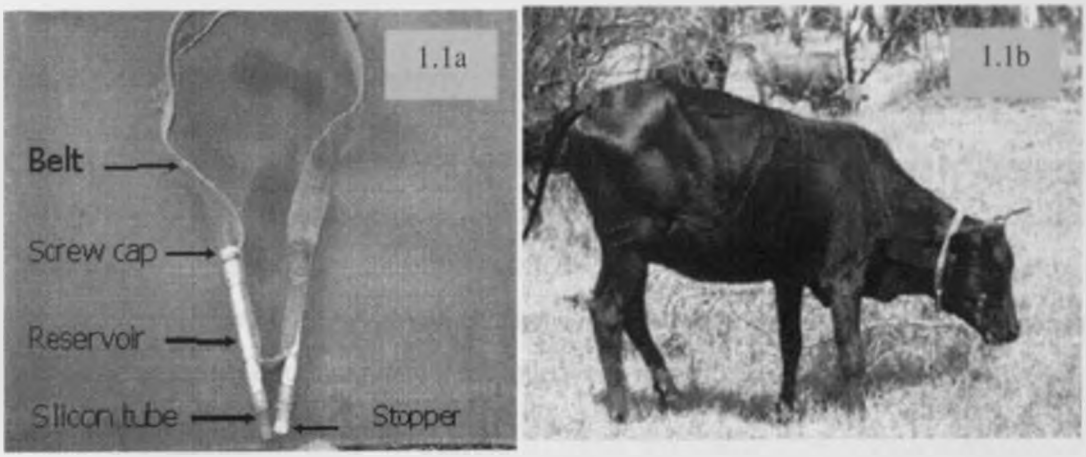


Plate 1.1. The structure of a tsetse-repellent dispenser used in the trial and an animal treated with the repellent

1.2 Overall Objective

The overall objective was to evaluate the effectiveness of the synthetic tsetse repellent technology in reducing the incidence of trypanosomosis in cattle and to identify ways in which the technology could be used with other techniques to control tsetse and trypanosomosis.

1.3 Specific objectives

1.3.1 To identify the factors that influence the effectiveness of the tsetse repellent technology and determine ways of controlling for these effects while estimating the impact of the technology on trypanosomosis incidence,

1.3.2 To estimate crude and adjusted effect of the technology on trypanosomosis incidence in cattle managed under pastoral production systems,

1.3.3 To develop and use a mathematical model to simulate the impact of using the tsetse repellent technology on trypanosomosis transmission when used alone or in integrated versions under varied scenarios.

1.4. Framework of the study

Towards addressing the objectives outlined above, a number of meetings and methodology workshops were held to develop strategies for conducting the study as well as the instruments for collecting data. The incidence of trypanosomosis was taken to be the main outcome variable. The rate of use of trypanocides by the livestock owners was considered to be a secondary outcome since it is related to trypanosomosis incidence.

A systems approach to the analysis of the effectiveness of the technology was attempted through the use of a causal-web model (Figure 1.1). The model guided the randomization of treatment groups, the identification of variables to estimate, and thereafter, the analysis of data with regard to the choice of independent variables whose effects had to be controlled for through multivariate analysis.

The effectiveness of the repellent, for instance, is expected to be lower in areas of high than low tsetse challenge. It is also expected to be more effective against tsetse that have low preference for the target hosts, particularly when there are many alternative hosts which tsetse can feed on in an area (such as game). Meteorological factors, particularly temperature, humidity and wind speed may affect the rate of diffusion and build up of repellent odour plume in immediate surroundings of a treated herd. Similarly, grazing management affects the establishment of an odour plume because sedentary management systems allow for the build up of the repellent odours in the vicinities of treated herds. The size of a herd may also influence the effectiveness of the repellent technology because treating a big herd increases the concentration and stability of a repellent odour plume. This, however, may not always be true because the concentration of animals' own odour increases with an increase with the size of a herd. An interaction between the repellents' and animals' odours would therefore be expected, causing a non-linear relationship between the repellent's effect and the size of a herd. The repellent would also be expected to be more effective when used on young animals than on big ones because young animals are less preferred by tsetse compared to older ones (Torr et al., 2001).

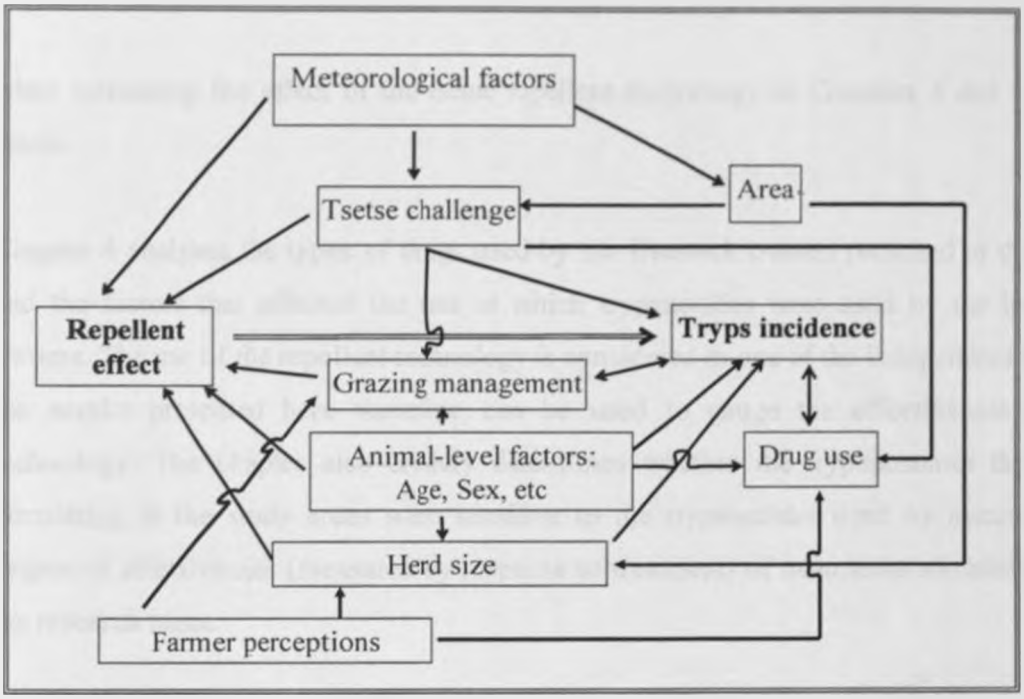


Figure 1.1. A causal-web model showing the factors that could confound trypanosomosis incidence – repellent effect relationship.

1.5. Organization of the thesis

The thesis is divided into eight chapters, the first two of which give a general introduction, including the objectives and framework of the study, and a review of literature. The general introduction outlines some of the previous work that provides the background for the present study. Literature on tsetse and trypanosomosis biology and control continues to increase rapidly. Those presented in Chapter 2 focus on transmission of trypanosomosis and the effectiveness of the available tsetse and trypanosomosis control methods.

Chapter 3 determines the strength and significance of the relationship between tsetse challenge and trypanosomosis incidence in the sites where the trial was being carried out. The parameters that constitute tsetse challenge: blood meal index, infection rates in tsetse and tsetse density are estimated and the factors that influence the estimates discussed. The chapter concludes by suggesting ways in which tsetse challenge could be controlled for

when estimating the effect of the tsetse repellent technology in Chapters 4 and 5 of the thesis.

Chapter 4 analyses the types of drugs used by the livestock owners recruited to the study and the factors that affected the rate at which trypanocides were used by the livestock owners. The use of the repellent technology is considered as one of the independent effects; the results presented here therefore can be used to gauge the effectiveness of the technology. The chapter also crudely determines whether the trypanosomes that were circulating in the study areas were sensitive to the trypanocides used by assessing the degree of effectiveness (measured by response to treatment) of treatments administered by the research team.

The effect of the repellent technology on trypanosomosis incidence and the competence of the repellent dispenser are evaluated in Chapter 5. Both crude and adjusted effects of the technology are estimated using univariable and multivariable analyses. These analyses are conducted in two sets. The first set ignores the frailties of the repellent dispenser and analyses data as per the original treatment allocation whereas the second controls for the dispenser defects.

Chapter 6 illustrates how a generic mathematical model capturing tsetse population and trypanosomosis transmission dynamics was constructed and used in simulating the effect of the technology under varied scenarios. The listed assumptions underlying the simulations are obtained from literature. Predictions generated from the model are discussed and areas for further research identified. This chapter provides outputs that might be considered as being *ex ante* analyses for future re-evaluation of the technology especially if this would be done as a component of integrated control strategies.

Chapter 7 gives general conclusions and recommendations. A list of references is given in Chapter 8.

Chapter 2

Review of Literature

2.1. African trypanosomosis

African trypanosomosis is a tsetse-borne parasitic disease of both man and livestock caused by flagellated protozoans called trypanosomes. The disease is characterized by an acute, subacute or chronic course, fever, anaemia, emaciation and a heavy mortality rate (Radostits et al., 1994). *Trypanosoma vivax*, *T. congolense* and *T. brucei brucei*, *T. simiae*, *T. equinum* and *T.equiperdum* cause animal trypanosomosis while *T. brucei rhodesiense* and *T. b. gambiense* cause acute and chronic human African trypanosomosis, respectively. *T. evansi* is also an important cause of trypanosomosis in camels and horses. This parasite is transmitted mechanically by biting flies and has a broader geographic distribution than those of the other trypanosomes mentioned above as it can infect a variety of mammalian species in Africa, South America and Asia (Radostits et al., 1994).

The diagnostic characteristics of the blood stream forms of the various species of mammalian trypanosomes are the size and shape of the body, the position of the nucleus and kinetoplast and the length and form of the undulating membrane and flagellum as observed in stained slide preparations of the parasites (Jordan, 1986). Based on these morphological characteristics, the salivarian trypanosomes (which undergo a cycle of development in the tsetse fly) can be classified into four main subgenera outlined in Table 2.1.

Table 2.1. Classification of tsetse-transmitted trypanosomes and the site of development in tsetse

Subgenus	Species	Site of development in tsetse
Dutonella [a]	*Trypanosoma vivax*	Proboscis
	T. uniforme	Proboscis
Nannomonas	*T. congolense*	Proboscis and midgut
	T. simiae	Proboscis and midgut
Trypanozoon	*T. brucei brucei*	Salivary glands and midgut
	T. brucei rhodesiense	Salivary glands and midgut
	T. brucei gambiense	Salivary glands and midgut
Pycnomonas	*T. suis*	Salivary gland and midgut

Source: Jordan, 1986, pp. 4

[a] Also mechanically transmitted by biting flies

2.2. Tsetse

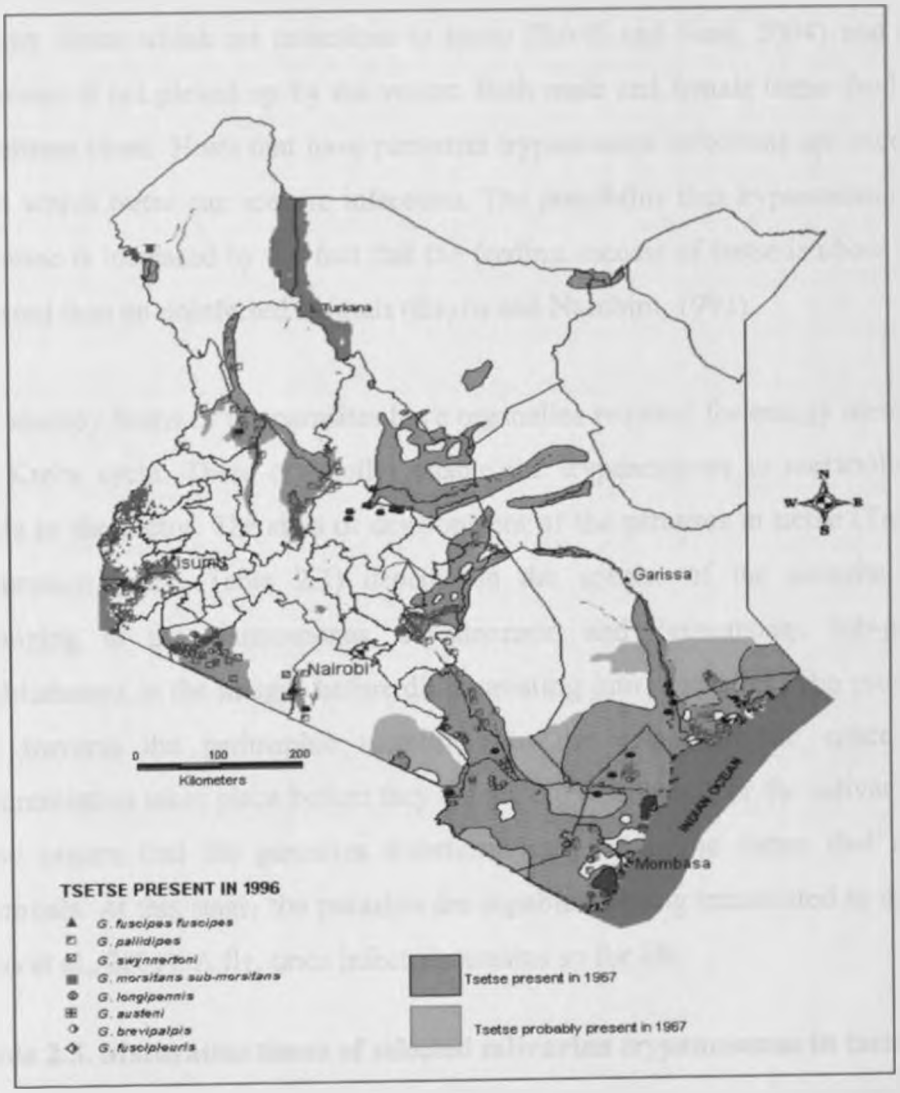
The tsetse fly is an important vector of trypanosomosis. It is placed in the family Glossinidae that has a single genus *Glossina* (Jordan, 1986). The genus is further classified into the sub-genera: *Glossina* (or morsitans group), *Austenina* (or fusca group) and *Nemorhina* (or palpalis group) (Table 2.2).

With regard to geographical distribution, tsetse infests about 8 to11 millionkm^2 of Africa, inhabited by approximately 260 – 300 million people and 45 – 50 million cattle (McDermott and Coleman, 2001). The northern limit corresponds closely to the southern edges of the Sahara and Somali deserts which is about 14 ° N in the northwest and 4 ° N in northeast. The southern limit varies from 10 ° S in the southwest corresponding to the northern edges of the Kalahari and Namibian deserts to between 20-29 ° S in the southeast. Within these general limits, tsetse distribution is not continuous (Jordan, 1986). In Kenya for instance, tsetse is found in arid and semi arid areas, an area that accounts for about 80% of the land surface; the distribution by species is shown in Figure 2.1.

Table 2.2. The sub-genera falling under the genus *Glossina*

Morsitans (Savannah)	Fusca (Austenina)	Palpalis (Nemorhina)
Glossina pallidipes Austen	*G. fusca fusca* Walker	*G. palpalis palpalis* Robineau-Desvoidy
G. morsitans morsitans Westwood	*G. tabaniformis* Westwood	*G. palpalis gambiensis* Vanderplank
G. morsitans centralis Machado	*G. longipennis* Corti	*G. fuscipes fuscipes* Newstead
G. morsitans submorsitans Newstead	*G. brevipalpis* Newstead	*G. fuscipes quanzensis* Pires
G. austeni Newstead	*G. nigrofusca nigrofusca* Newstead	*G. fuscipes martini* Zumpt
G. swynnertoni Austen	*G. fuscipleuris* Austen	*G. tachinoides* Westwood
G. longipalpis Wiedemann	*G. medicorum* Austen	*G. pallicera pallicera* Bigot
	G. severini Newstead	*G. caliginea* Austen
	G. schwetzi Newstead and Evans	
	G. haningtoni Newstead and Evans	
	G. vanhoofi Henrard	
	G. nashi Potts	

Source: Jordan 1986, pp. 14



Source: Socio-Economics Unit, Trypanosomiasis Research Centre, Kenya Agricultural Research Institute

Figure 2.1. Tsetse distribution in Kenya.

2.3. Trypanosomosis transmission

Trypanosomes multiply asexually in the mammalian tissues through binary fission leading to the production of long and slender parasite forms. These differentiate into non-dividing stumpy forms which are infectious to tsetse (Savill and Seed, 2004) and would undergo apoptosis if not picked up by the vector. Both male and female tsetse feed exclusively on vertebrate blood. Hosts that have persistent trypanosome infections are excellent reservoirs from which tsetse can acquire infections. The possibility that trypanosomes are picked up by tsetse is increased by the fact that the feeding success of tsetse is about 75% greater on infected than on uninfected animals (Baylis and Nambiro, 1993).

The stumpy forms of the parasites have organelles required for energy metabolism through the Krebs cycle. These organelles enable the trypanosomes to metabolize their energy needs in the vector. The sites of development of the parasites in tsetse (Table 2.1) and the maturation times (Table 2.3) depend on the species of the parasite. Trypanosomes belonging to the Nannomonas, Trypanozoon and Pycnomonas sub-genera first get establishment in the midgut before differentiating into procyclics. The procyclics replicate and traverse the peritrophic membrane to the ectoperitrophic space where further differentiation takes place before they colonize the proboscis or the salivary glands. It is in these organs that the parasites transform into metacyclic forms that are infective to mammals. At this stage, the parasites are capable of being transmitted to mammalian hosts (Hao et al., 2001). A fly, once infected, remains so for life.

Table 2.3. Maturation times of selected salivarian trypanosomes in tsetse

Trypanosome species	Conditions	Time taken to maturation (days)
T. vivax	*G. palpalis*	
	puparia 26 °C	5
	adults 22 °C	12 - 13
T. congolense	23 – 24 °C	15 - 20
T. simiae	*G. morsitans* 28.3 °C	20
T. b. rhodesiense	*G. m. centralis* 28 °C	23
	G. m. centralis 30 °C	12

Source: Leak, 1999; pp 184

The factors that determine the susceptibility or refractoriness of tsetse to trypanosome infections are poorly understood. Leak (1999) and Welburn and Maudlin (1999) indicate that lectin levels in the gut at the time of parasite uptake, fly species, sex, age, and symbiotic associations between tsetse and gram negative bacteria that infect tsetse are some of the determinants for infection. Welburn and Maudlin (1999) indicate that midgut lectins kill incoming trypanosomes in a process similar to a programmed cell death and that the levels of these lectins increase with the age of the fly, leading to a relationship between age and refractoriness to infection. On the effect of sex of a fly, they point out that males produce significantly more mature trypanosome infections than females due to the operation of a product(s) of a X-linked gene which kills or prevents migrating parasites from maturing. Some tsetse flies also harbour multiple symbionts that contribute to their nutrition and fecundity (Aksoy, 2000). The two gut symbionts that have been identified are *Sodalis glossinidius* and *Wigglesworthia glossinidia*. They are gram-negative bacteria closely related to *Escherichia coli* and are maternally inherited. These symbionts are thought to inhibit the activity of midgut lectins, therefore increasing the susceptibility of tsetse to trypanosome infections (Leak, 1999). Tsetse flies are also capable of mounting an immune response against trypanosomes. This response can discriminate between bacteria and trypanosomes and also between the bloodstream form and procyclic trypanosomes (Hao et al., 2001).

2.4. Pathology

Kitani et al. (2004) and Ngure et al. (1997) have described the processes leading to the induction of acute phase response to a trypanosome infection by a host. This includes fever, leucocytosis, changes in vascular permeability, lipid metabolism and production of acute phase proteins (ceruloplasmin, glycoprotein, creatinine and serum amyloid P). Acute phase proteins have protective roles in the host; they can function as transport molecules, participate in tissue repair, mediate or inhibit inflammatory process and are part of the mechanism that controls homeostasis (Kitani et al., 2004). Trypanosomes, however, evade immunological recognition while proliferating in the mammalian host through antigenic variation of their variant-specific surface glycoprotein. The pathological features that

manifest the infection include anaemia, splenomegally and suppression of T cell proliferation (Magez et al., 2002).

Anaemia is a consistent finding in African trypanosomosis and it is thought to result from either one or a combination of the following processes: haemolysis, haemodilution and dyshaemopoiesis (Amole et al., 1982). Haemolysis results from the deposition of immunological complexes on the surfaces of erythrocytes that triggers the complement fixation cascade. Some of the red blood cells are cleared by the immune system through erythrophagocytosis. Trypanosomes are also known to directly haemolyse red blood cells as they have haemolytic material on their surfaces comprising a mixture of fatty acids and phospholipase A (Anosa, 1988).

Variable effect of the infection on total serum proteins, hence albumin/globulin ratio has been reported (Anosa, 1988). After a *T. congolense* infection, the level of total serum proteins increases in goats (Witola and Lovelace, 1997) and cattle (Wellde et al., 1989). An increase in the level of serum proteins is attributed to the production of immunoglobulin M (Anosa, 1988). However, Adejinmi and Akinboade (2000) reported depressed levels of total serum proteins in *T. brucei* infected goats. Hepatic dysfunction was suspected to have been the cause of the observed decline. The increase in serum proteins could contribute to haemodilution through increased osmotic pressure.

Dyshaemopoiesis, observed in chronic cases, is usually associated with deranged iron metabolism; hence iron deficiency anaemia (Amole et al., 1982). Iron is required for the formation of haemoglobin.

Immunosuppression is another common occurrence in trypanosomosis. This is usually mediated through type 1 cytokines: the gamma interferon and tumour necrosis factor. These cytokines modify T cell and macrophage responsiveness in lymph nodes, spleen and bone marrow (Magez et al., 2002). In addition, impairment of antigenic presentation capacity of macrophages and polyclonal B cell stimulation occurs (Assoku et al., 1977).

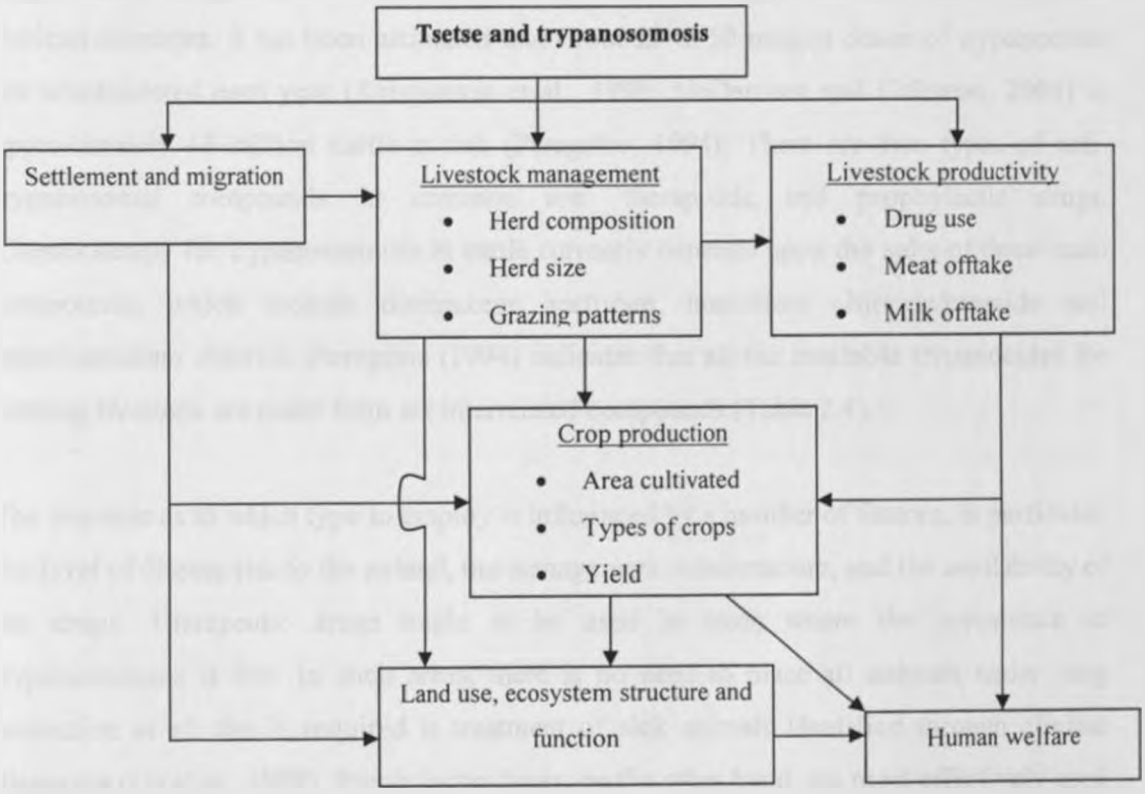
Immunosuppression negatively affects the ability of an animal to control parasitaemia, worsening the prognosis of trypanosomosis in susceptible animals.

2.5. Impact of tsetse and trypanosomosis on agriculture

Swallow (1999) broadly classifies the impacts of tsetse and trypanosomosis on agriculture into direct and indirect effects. The direct effects include impacts on livestock productivity, migration and settlement and livestock management. Indirect impacts affect crop production, land use, ecosystem structure and function and human welfare. These effects are outlined in Figure 2.2. Leak (1999) lists specific impacts of trypansomosis on livestock productivity as increased mortality, abortion, decreased growth rates, stunting, loss of efficiency in animal traction and reduced fertility. He further observes that the presence of tsetse and consequent risk of trypanosomosis reduces the suitability of infested areas for livestock rearing. Kristjanson et al. (1999), on the other hand, describes direct effects as mortality, morbidity, impaired fertility and the costs of implementing and maintaining tsetse fly and trypanosomosis control operations. They identify indirect losses as being the perceived risk of the disease that leads to reduced or total exclusion of livestock from tsetse-infested grazing lands.

The magnitude of the effect of tsetse and trypanosomosis on agriculture is expected to vary with the risk of the disease, production system, and the effectiveness of control strategies employed in an area. The estimates reported in literature vary depending on the methodology used for evaluation, assumptions made, and the type of effect estimated (McDermott and Coleman, 2001). A longitudinal study conducted at Galana ranch, Kenya, showed that untreated Galana Boran lost 14% of their body weight compared with animals maintained on a 3-month Samorin prophylaxis (Wilson et al., 1986). The study predicted that the use of trypanotolerant Orma Boran was likely to lead to a reduction in expenditure on disease control by about USD $ 110, 000 and an increase in the proportion of land used by 5%. A socio-economic study conducted by Kamuanga et al. (2001) in Burkina Faso to test farmers' perception on the effect of tsetse control intervention with insecticide-impregnated targets and cattle treated cattle with deltamethrin 1% as a pour-on formulation

showed that there was an increase in average herd-size by 25%, increase in average number of oxen per household from 0.1 to 1.1, reduction in mortality from 63.1% to 7.1%, reduction in abortion and still births by 55.5% and 51.3%, respectively, increase in live births by 57.6% and an increase in milk yield from 0.2 to 2.2 litres per cow per day in a dry season. In Ghibe Valley, Ethiopia, a suppression of tsetse density using a combination of insecticide-impregnated targets and pour-on treated cattle by 75 and 95% did not result in a significant increase in the body weight of cows, calving rate or the mean body weight of calves below 12 months of age (Rowlands et al., 1999). There was, however, a reduction in calf mortality by 57%, an increase of 45% in the ratio of live calves over 12 months of age to cows over 36 months and an increase in body weight of males by 8%.



Source: Swallow, 1999

Figure 2.2. Direct and indirect effect of tsetse and trypanosomosis on agriculture.

2.6. Tsetse and trypanosomosis control

Currently, there are three principal methods of tsetse and trypanosomosis control, namely the use of trypanocidal drugs, use of trypanotolerant animals and tsetse control. There are a number of options of controlling tsetse - these are discussed in Section 2.6.3. Farmers also manage their grazing patterns in such a way as to avoid densely infested areas.

2.6.1. Trypanocides

Trypanocidal drugs remain the principal method of animal trypanosomosis control in most African countries. It has been estimated that about 25 to 50 million doses of trypanocides are administered each year (Kristjanson et al., 1999; McDermott and Coleman, 2001) to approximately 44 million cattle at-risk (Peregrine, 1994). There are two types of anti-trypanosomal compounds in common use: therapeutic and prophylactic drugs. Chemotherapy for trypanosomosis in cattle currently depends upon the salts of three main compounds, which include diminazene aceturate, homidium chloride/bromide and isometamidium chloride. Peregrine (1994) indicates that all the available trypanocides for treating livestock are made from six interrelated compounds (Table 2.4).

The decision as to which type to employ is influenced by a number of factors, in particular, the level of disease risk to the animal, the management infrastructure, and the availability of the drugs. Therapeutic drugs ought to be used in areas where the prevalence of trypanosomosis is low. In such areas, there is no need to place all animals under drug protection as all that is required is treatment of sick animals identified through clinical diagnosis (Gu et al., 1999). Prophylactic drugs, on the other hand, are most effectively used in areas of medium-to-high challenge (Connor, 1989). In these areas, cattle are constantly under threat of trypanosomosis and it is likely that all will become repeatedly infected, and therefore must be placed under drug protection (Gu et al., 1999). Table 2.4 gives a list of trypanocides commonly used, their spectra of activity, the animal species in which the compounds should be used and the routes of administration.

The therapeutic and prophylactic use of trypanocides is beset by numerous limitations, including toxicity and development of resistance by the parasites. The emergence of drug-resistant trypanosome strains is considered a very serious problem in trypanosomosis control, especially for the resource-poor at-risk populations and farmers in Africa and in the context of sustainable parasite control (Anene et al., 2001). Drug resistance most probably arises from under-dosing as the use of trypanocides is increasingly not being supervised due to the demise of state-funded veterinary services across Africa (Holmes, 1997). Most cattle owners obtain these products from drug retail outlets that are manned by salespersons with no qualifications necessary for drug dispensation and advice (Bett et al., 2004a). Moreover, about 60% of the drugs that are available in such markets are fakes with little or no therapeutic activity (Broussard, 1996).

2.6.2. Trypanotolerant livestock

Trypanotolerance, the ability of some livestock breeds to survive, reproduce and remain productive under trypanosomosis risk without aid of trypanocidal drugs was recognized and exploited by farmers long before research on trypanotolerance began. Cattle breeds that have been found to have some degree of natural resistance to trypanosomosis include the humpless longhorn N'Dama and Baoulé and the short-horn Muturu in west Africa (Murray et al., 1982) and Orma Boran in East Africa (Dolan, 1998). The exploitation of trypanotolerant breeds is practiced as a major option for sustainable livestock production in nineteen countries in the most humid parts of west and central Africa (d'Ieteren et al., 1998) and less so in other tsetse infested areas because:

(i) Farmers are conservative in their breed preferences particularly for multipurpose breeds;

(ii) There have been policy restrictions for the importation of tolerant livestock into some countries;

(iii) Breeding culture tends to favour local breeds, and hence resistance to the importation of trypanotolerant breeds; and

(iv) Lack of incentives to invest in low input production systems, where, any local breed will probably suffice even if losses due to trypanosomosis are incurred (d'Iteren, 1993).

Table 2.4. Chemotherapeutic and chemoprophylactic compounds used for animal trypanosomosis

Compound	Trade name(s)	Treatment regimen		Use	Activity in the field	Animal
		Dose mg/kg	Route			
Diminazene aceturate	Berenil Veriben	3.5-7.0	i. m.	T	*T. congolense* *T. vivax* (*T. brucei*) (*T. evansi*)	Cattle Small ruminants [Dogs] [Equidae]
Homidium chloride	Novidium	1.0	i. m.	T/P	*T. congolense* *T. vivax*	Cattle Small ruminants
Homidium bromide	Ethidium	1.0	i. m.	T/P		Pigs [Equidae]
Isometamidium chloride	Samorin	0.25-0.5	i. m.	T/P	*T. congolense* *T. vivax*	Cattle Small ruminants
	Trypamidium	0.5-1.0	i. m.		*T. brucei* *T. evansi*	Equidae Camels
Quinapyramine dimethylsulphate	Trypacide sulphate	3.0-5.0	s. c.	T	*T. congolense* *T. vivax*	Camels Pigs Equidae
Quinapyramine dimethylsulphate:chloride (3:2 W/W)	Trypacide prosalt	3.0-5.0	s. c.	P	*T. brucei* *T. evansi* *T. simiae*	Dogs
Suramin	Naganol	7.0-10.0	i. v.	T(P)	*T. evansi*	Camels Equidae
Melarsomine	Cymelarsan	0.25	s. c./i. m	T	*T.evansi*	Camels

Source: Peregrine, 1994

Key

( ) Limited activity [ ] Small therapeutic index i. m. intramuscularly

s. c. Subcutaneous i. v. Intravenously T Therapeutic P Prophylactic

While it is generally accepted that trypanotolerance as a breed characteristic is under genetic control, there is evidence that the stability of trypanotolerance can be affected by environmental factors, such as overwork, inter-current disease and repeated breeding, pregnancy, parturition, suckling and lactation (d'Ieteren et al., 1998). Probably the single most important factor is nutrition. In the first large-scale attempt to evaluate the effect of trypanosomosis risk on performance of N'Dama and West African Shorthorn cattle at 30 different locations, research performed by the International Livestock Centre for Africa (ILCA), Food and Agriculture Organisation (FAO) and the United Nations Environment Programme (UNEP) demonstrated that although these breeds remain productive under trypanosomosis risk, their outputs were affected by increasing risk (International Livestock Centre for Africa [ILCA], 1979). A recent analysis of data collected from an Orma Boran breeding program indicates that resistance to trypanosomosis was negatively associated with increased tsetse challenge, increased number of previous infections and infection with *Trypanosoma vivax* as compared to *T. congolense* (Bett et al., 2004b).

2.6.3. Tsetse control

The approaches to controlling tsetse and the attendant strengths and weaknesses are listed in Table 2.5. In the past, there were widespread attempts to control tsetse by clearing the vegetation where the flies rested and shooting game which the flies fed on. Such methods are no longer tolerated although they reduced tsetse density in some areas (Schofield and Maudlin, 2001; Schofield and Kuzoe, 2004).

Table 2.5. Current methods of tsetse control

Method	Comments
Ground spraying	Effective; labour and management intensive; excessive use of insecticides
Aerial spraying	Effective; expensive; excess use of insecticides
Odour-baited traps or targets impregnated with insecticides	Effective; labour and management intensive but community may be able to handle technology independently; limits use of insecticides
Pour-on (application of insecticides to the animals on which tsetse feed)	Act as mobile targets with built-in odour attractants; effective if sufficient animals are treated relative to the desired level of control; gives bonus of tick control on treated animals; may be beyond budget of poor farmers
Sterile insect technique (liberation of sterilized flies to compete with wild flies)	Effective when the population is first suppressed by, for example, targets; very expensive at outset, but if successful, the recurrent costs decline sharply; environmentally friendly

Source: Schofield and Maudlin, 2001

2.6.3.1. Ground and aerial spraying

The use of ground spraying was commenced in 1945 when synthetic insecticides, Dichloro-Diphenyl-Trichloroethane (DDT) and benzene hexachloride BHC (HCH) were made available (Schofield and Kuzoe, 2004). The residual effects of these chemicals on non-target organisms discouraged their continued use. Ground spraying has now been supplanted by sequential aerial technique (SAT) that uses ultra low dosages of biodegradable insecticides. Aerial spraying has been used previously in Botswana, Uganda and Zimbabwe (Jordan, 1986). The SAT was recently used in Okavango delta, Botswana, as part of a tsetse eradication campaign (Allsopp, 2002). In this operation, aircrafts fitted with Satloc guidance equipment based on global positioning systems (GPS) were used to deliver insecticides (0.35% deltamethrin). This allowed the aircrafts to have an independent navigation without ground marker support system. Environmental impact assessment showed that the operation did not have deleterious effects on aquatic and terrestrial communities. Preliminary reports indicate that this intervention was very successful as no tsetse was captured in the first five months. Past insecticide control techniques did not,

however, eradicate tsetse probably due to the seasonal nature of spraying operations (Schofield and Maudlin, 2001).

2.6.3.2. Traps and targets

Traps or targets have been used to control tsetse in Nkuruman, Kenya, Antelope Island, Zimbabwe, Rifa triangle, Zimbabwe, Sioma campaign, Zambia (Hargrove, 2003), Mali, Nigeria and Côte d'Ivoire (Schofield and Kuzoe, 2004). The first successful tsetse control using traps (Harris traps) was conducted in Zululand between 1920 and 1940 (Leak, 1999). The technology was subsequently improved by Swynnerton (1933), Moloo (1973) and Langridge (1975). The improvement of trap designs was accelerated by the development of electric screens by Vale (1974); electric screens allow for the assessment of the efficiencies of the various trap models. Advances were also made in the production of synthetic attractants when Vale (1980) found out that tsetse could respond to olfactory cues. Presently, the available odours are more effective for *morsitans* group of tsetse than the *palpalis* group (Leak, 1999). The biconical trap developed by Challier and Laveissiere, (1977) and NG2G developed by Brightwell et al. (1987) were used in the present study.

The principle behind the use of traps or targets is that tsetse flies locate and encounter suitable hosts on which to feed using visual and olfactory cues. The design of traps and targets mimics key features of host animals, attracting tsetse such that they are captured and killed (Schofield and Kuzoe, 2004). The colour of the trap or target and type of bait used are very important in influencing the number of tsetse attracted or entering the device. For all tsetse species studied so far, blue and white are the most attractive colours, with pthalogen blue being considered as the best (FAO, 1982b). In case of traps, captured flies can be identified and counted for monitoring purposes. This is not possible with the use of targets because flies that pick up lethal dose of an insecticide drop off.

Synthetic pyrethroids, commonly deltamethrin, are usually impregnated on traps and targets and either kill the flies that come into contact with treated surfaces directly or indirectly through a knock-down effect (Leak, 1999). The knock-down effect results from

paralysis caused by binding of pyrethrins to lipids in the nervous tissues of the flies. For targets, knock-down may be a highly significant mode of effect compared to direct mortality because most of the flies that come in contact with it are knocked-down and at least 85% of flies knocked-down will die mainly through predation. In traps, tsetse are exposed to the insecticides for a prolonged length of time, hence mortality may be an important effect (Leak, 1999).

Efficient trap and target deployment represents a balance between high trap (or target) density to maximize the likelihood that the device is encountered by tsetse and low density to minimize on costs. Field experiments have shown that *morsitans* group tsetse respond to odours from a cow up to 50-100m downwind and once flies encounter a suitable odour plume, they tend to fly upwind. Williams et al. (1992) argue that 100m is the probable limit of attractiveness of most odour-baited traps and targets and show that a density of traps or targets of four per square kilometre would be sufficient for the control of *morsitans* group tsetse. However, small scale operations involving traps or targets are liable to fail as a consequence of invasion pressure (Hargrove, 2003). This requires the setting up of barriers using traps or targets between the treated and un-treated areas along the re-invasion points (Schofield and Kuzoe, 2004). The effectiveness of the technology might also be affected by the density of the vertebrate hosts that tsetse can feed on in an area (FAO, 1982b), the activity of the flies, how they move in their active state, whether they move into the vicinity of a trap or target and finally, whether they are trapped or killed (Williams et al., 1992).

Sustainability of tsetse control using traps or targets usually requires community participation and motivation to contribute towards the success of the intervention. Difficulties emerge in maintaining the motivation of these communities to provide and service traps or targets as those living in the centres of controlled areas may receive greater benefits yet be less willing to contribute to the maintenance of the control than those living at the periphery (Holmes, 1997). The participation of each community member is also dependent on whether or not he/she has livestock that he/she perceives to be at risk from trypanosomosis.

2.6.3.3. Insecticide-treated livestock

Insecticide-treated livestock was developed as a method of tsetse control from the concept of baited traps and targets (Baylis and Stevenson, 1998). This involves treating cattle with appropriate insecticides by means of cattle dips, pour-ons, spot-on or spray on veterinary formulations (Schofield and Kuzoe, 2004). Tsetse coming to feed on cattle or other treated domestic livestock will be killed by picking up a lethal deposit of insecticide on the ventral tarsal spines and on pre-tarsi while feeding. The technology is widely accepted by a majority of livestock owners in Africa (Thomson et al., 1991) and also expected to be more sustainable as it can be financed by the livestock owners themselves (Hargrove et al., 2000). Some of the studies that have reported the use of the technology to control tsetse and trypanosomosis, with different degrees of success are Lohr et al. (1991) and Stevenson et al. (1991) in Kenya, Fox et al. (1993) in Tanzania, Chizyuka and Liguru (1986) in Zambia, Thomson et al. (1991) in Zimbabwe, Bauer et al. (1999) in Burkina Faso, Leak et al. (1995) in Ethiopia and Okello-Onen et al. (1994) in Uganda.

Of the commonly used synthetic pyrethroids, deltamethrin appears to be the most potent, photo-stable and persistent at very low dosages (Hadaway et al., 1976; Van den Bossche and Van Hees, 1987; Thomson et al., 1991). It also has low mammalian toxicity and minimal environmental impact (Thomson et al., 1991). These advantages make it a preferred ingredient in a majority of formulations of synthetic pyrethroids. The other pyrethroids that have also been used include cypermethrin (Kamau et al., 2000) and alphacypermethrin, beta-cyfluthrin and lambdacyhalothrin (Mangwiro et al., 1999).

The effect of this technology on tsetse control, however, depends on: a relatively high proportion of feeds being taken from cattle; a sufficient proportion of cattle population being treated; and a sufficiently low level of reinvasion (Leak, 1999). In some countries, the use of pyrethroids on cattle has been banned because of tick resistance (d'leteren et al., 1998).

2.6.3.4. Sterile insect technique (SIT)

SIT involves breeding a large number of males of the target vector species and sterilizing them (using irradiation) before being released by air to infested areas. For the technique to be effective in eradicating the target species, sterilized males must outnumber the wild ones by a ratio of at least 10:1 (FAO, http://www.fao.org/NEWS/1998/sit-e.htm). SIT is usually applied once the wild tsetse population has been effectively suppressed using traps, targets or insecticide-treated livestock so as to achieve the ratios stated above.

SIT combined with other vector control methods (in integrated pest management) has been successfully used to eradicate the tsetse fly *Glossina austeni* in Zanzibar and New World screwworm (NWS), *Cochliomyia hominivorax*, in North America (Reichard, 2002). In the Zanzibar case, the ratio of sterilized:wild males attained was 50:1 (FAO, http://www.fao.org/NEWS/1998/sit-e.htm). Vale and Torr (2005) have, however, demonstrated that reducing tsetse flies' birth rate through the use of SIT is less effective in controlling tsetse flies compared to increasing the mortality rate by using traps, targets or insecticide treated livestock.

2.7. Development and evaluation of new methods or strategies for tsetse and trypanosomosis control

2.7.1. Limitations of the available tsetse and trypanosomosis control methods

Holmes (1997) and McDermott and Coleman (2001) describe some of the limitations of the available tsetse and trypanosomosis control methods. The lack of adequate extension services in tsetse-infested areas further curtails the provision of professional guidance and supervision in the application of these technologies. The community-based control operations, especially deployment of traps or targets, are dependent on pooling private resources to achieve a public good (Torr et al., 2005). Problems inherent in any collective action, allied to a lack of technical advice and the economic constraints faced by poor communities in rural areas, mean that effective baits are seldom optimally deployed

(Dransfield and Brightwell, 2004). With regard to the use of trypanocides, resistance to one or more of the three compounds currently used for the treatment of trypanosomosis in cattle (diminazene aceturate, homidium chloride/bromide and isometamidium chloride) is known to be present in at least 13 sub-Saharan countries (Geerts and Holmes, 1998). These limitations necessitate: (a) judicious application of the available technologies; (b) development of new technologies; and (c), development of integrated control strategies.

The combination of pour-on and strategic use of trypanocidal drugs is considered the most likely to be delivered since these control methods are largely private in nature (all benefits accrue to the person paying for the service/goods) and can be delivered by the private sector (McDermott and Coleman, 2001). The challenges associated with the delivery of the other tsetse control methods/integrated approaches could be addressed by blending them with rural development initiatives or other disease control measures (Holmes, 1997). Combining tsetse/trypanosomosis control with other rural development initiatives has, however, not been tested or used before.

2.7.2. Development of new technologies

The development of new tsetse and trypanosomosis control technologies has been very slow. A range of livestock keeper-based tsetse control technologies have been developed in the recent past. Some of the new methods, including the tsetse repellent technology, are highlighted below.

2.7.2.1. Trypanocides, pour-ons and vaccines

Pharmaceutical companies have substantially reduced their support for the development of new trypanocidal drugs because they perceive the market for such drugs to be relatively unprofitable (Holmes, 1997). There are, however, numerous but isolated efforts aimed at evaluating new compounds for their antitrypanosomal activity. Examples are tyropeptin A (against *T. brucei*) (Steverding et al., 2006) and furamidine (against *T. cruzi*) (de Souza et al., 2006). The identification of pour-ons as a technique to control tsetse came long after the

pyrethroids had been registered as acaricides (d'Ieteren et al., 1998). This shows that pyrethroids were not originally developed for tsetse and trypanosomosis control. Nonetheless, the technology is being adapted for tsetse control by replacing 'whole-body' treatments with restricted application of pyrethroids on the belly and the legs where tsetse (particularly *G. pallidipes*) likes alighting on (DFID-AHP, 2004). Insecticide-treated nets have also been used in small scales to control tsetse in zero grazing units in western Kenya (DFID-AHP, 2004). The insecticide-treated nets were adapted from those used to control mosquitoes. The development of an anti-infection vaccine has been frustrated by the antigenic variation of the trypanosomes (d'Ieteren et al., 1998). This was recently replaced by anti-disease vaccine (Authié et al., 1993) and studies to validate this new concept are yet to be scaled up.

2.7.2.2. Tsetse repellent technology

Tsetse show approaching or avoiding behaviour to different odour stimuli (Voskamp et al., 1999). This behaviour has been used in the identification of potent tsetse allomones (repellents) (Saini and Hassanali, 2002). Davis (1985) described five sensory mechanisms by which repellents may evoke avoidance behaviour by mosquitoes; these may also explain how tsetse flies react to repellents. Repellents may: (i) activate a receptor system that mediates a competing or inappropriate behaviour pattern; (ii) interact with and inhibit the response of a cell which is sensitive to an attractive compound; (iii) interact with their own specific receptors and be attractive at low intensities and repellent at higher doses; (iv) activate specific noxious odour receptors; and (v), simultaneously activate many receptor types so that any olfactory information specific to host-finding is lost in the resulting barrage of sensory input.

Gikonyo et al. (2002) demonstrated the differences in the odor composition of animals preferred (buffalo and ox) and non-preferred (waterbuck) by tsetse which propelled the development of the tsetse repellent technology. They found out that waterbuck odor had δ-octalactone, 2-methoxyphenol (guaiacol), 3-isoprophyl-6-methylphenol, and a series of C_8-

C_{13} methyl ketones that were not detected, or present in trace amounts in preferred hosts. Moderate amounts of $C_5 - C_9$ straight chain fatty acids were also identified.

Previously, controlled studies had been carried out by Torr et al. (1996) to test the efficacy of pentanoic acid, 2-methoxyphenol, hexanoic acid and aceto-phenone. They showed that these compounds caused a small (insignificant) reduction in feeding efficiency by tsetse on treated hosts. This effect was not expected to reduce trypanosomosis transmission, especially in areas of high tsetse challenge (Torr et al., 1996). The tsetse repellent used in this study, that is 2-methoxy 4-methylphenol was synthesized by adding carbon to 2-mehoxyphenol (Saini and Hassanali, 2007). The compound was shown through lab and field experiments (using *G. morsitans morsitans* and *G. pallidipes*) to be more potent than the 2-methoxyphenol (Saini and Hassanali, 2007). It was also more potent than six other analogues of 2-methoxyphenol including 2 methoxyfuran, 2, 4 dimethylphenol, 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4-allyl-2-methoxyphenol (4-allylguaiacol, eugenol), 3, 4 methylenedioxytoluene and 3, 4 dimethoxystyrene. At the same time, ICIPE did a series of studies to develop an appropriate dispenser as well as determine the site on an animal where the dispenser could be placed. Different sites were the neck, tail, feet and back of an animal (ICIPE/ILRI, 2001). These studies recommended that the dispenser should be placed on the neck as this was the most convenient site even though the technology was very effective when tied to the feet.

2.7.3. Evaluation of new tsetse and trypanosomosis control technologies

The effectiveness of new tsetse and trypanosomosis control technologies has traditionally been assessed using field trials. Such studies, however, need to be integrated with theoretical analyses (using simulation/mathematical models) (McDermott and Coleman, 1999). In this system, field studies would provide data for estimating the effectiveness of a technology and at the same time, the values of parameters used in building the models. Mathematical models, in turn, provide a framework for evaluating the expected impact of a technology when used alone or in integrated versions in multiple settings.

2.7.3.1. Field trials

Field trials (also called controlled or clinical trials) are planned experiments designed to assess the efficacy of a treatment by comparing the outcomes in group of animals treated with the test treatment with those observed in a comparable group of animals receiving a control treatment. Both groups of animals are followed over the same time period (Meinert and Tonascia, 1986). It is the most appropriate way for evaluating animal-health interventions because they allow much better control of potential confounders than observational studies as well as reducing bias due to selection and misinformation (Dohoo et al., 2003).

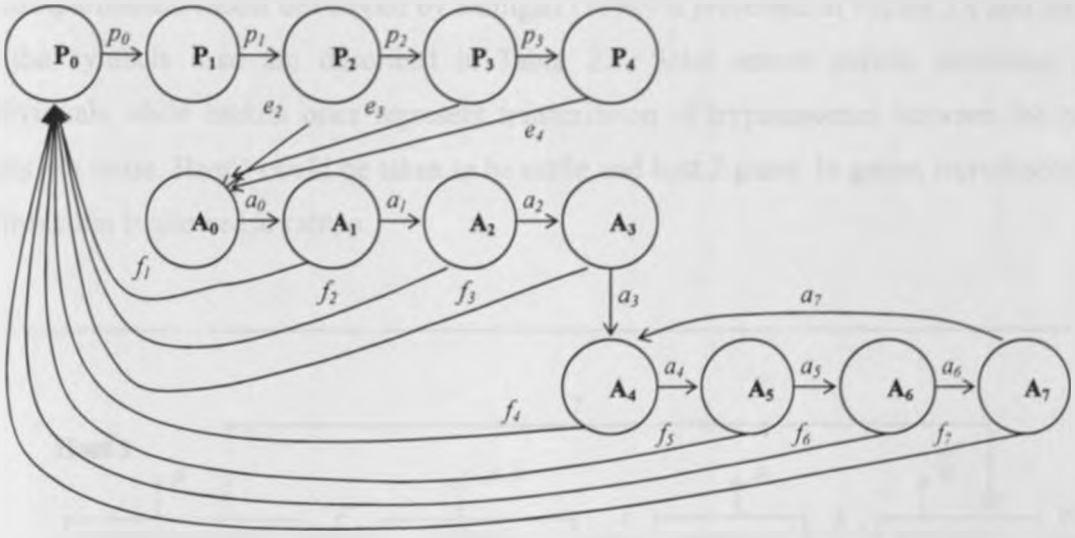
2.7.3.2. Mathematical modeling

2.7.3.2.1. Tsetse population dynamics models

The rate of change of tsetse population in a defined area is determined by the intrinsic growth rate and the balance between the rates of immigration and emigration.

Hargrove (1988) describes a model that estimates intrinsic growth rate of tsetse based on key stages of tsetse's life cycle using Leslie matrix (also called population projection matrix). This was an original attempt to use a population projection matrix to analyze tsetse population dynamics. Jarry et al. (1996) modified this model by collapsing the ages of tsetse used by Hargrove (1988) into eight physiological age groups (Figure 2.3). Williams et al. (1992) later developed a model that describes the spatio-temporal dynamics of a tsetse population incorporating both the growth and dispersal rates.

Few studies have compared the outputs of controlled field trials with those of mathematical models. Warnes et al. (1999) found out that the performance of target barriers in preventing the reinvasion of tsetse to cleared areas was comparable to predictions derived from mathematical models.



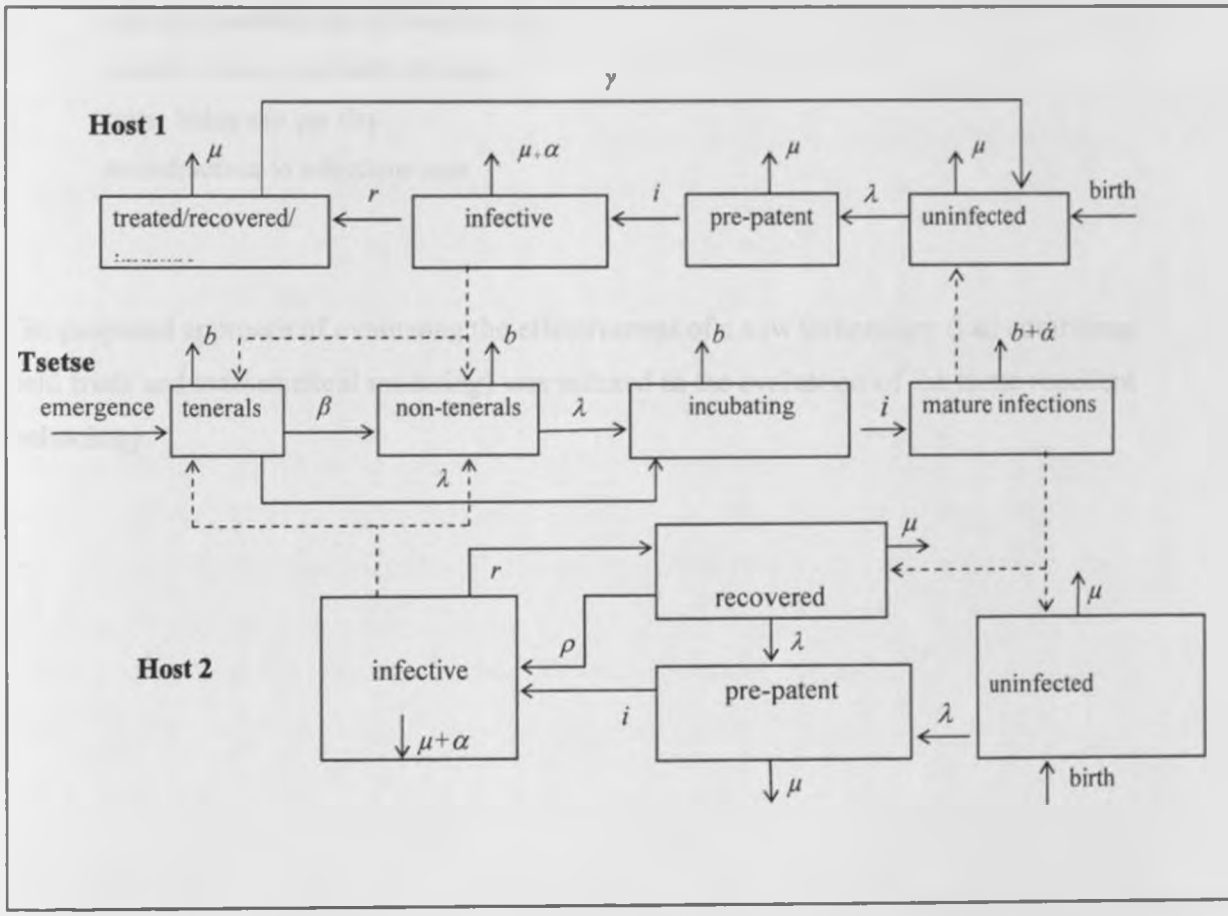
Source: Jarry et al., 1996

Figure 2.3. Graph of the life cycle of Glossinae showing 5 pupal stages P_0, P_1, P_2, P_3, P_4 and 8 adult stages A_0, A_1, A_2, A_3, A_4, A_5, A_6, A_7 corresponding to the physiological ages. The coefficients p_i represent the survival rate of pupae in class P_i and the coefficients a_i the survival rates of adults in class A_i over a period of h days. e_i is the emergence rate of pupae in stage P_i and f_i is the average fecundity of females in class A_i.

2.7.3.2.2. Trypanosomosis transmission models

The involvement of several species of trypanosomes, tsetse and vertebrate hosts in trypanosomosis has discouraged the development of mathematical models of the sort that have been available for other vector-borne diseases (Rogers, 1988). Compartmental models (with state variables: Susceptible, Exposed, Patent, Recovered individuals) involving two vertebrate, one vector and multiple trypanosome species have been developed by Rogers (1988), Milligan and Baker (1988), Milligan (1990) and Artzrouni and Gouteux (2001). These models vary by the vertebrate hosts used. Rogers (1988) used pigs and humans; Milligan and Baker (1988) used cattle and wild hosts and evaluated the impact of using trypanocides on disease transmission, while Artzrouni and Gouteux (2001) used humans versus other pool of hosts. Milligan (1990) developed a generic model without explicitly identifying particular host species. Baker (1992) extended these models by classifying tsetse into those that are RLO (rickettsia-like organisms)-positive versus RLO-negative.

A compartmental model developed by Milligan (1990) is presented in Figure 2.4 and some of the symbols used are described in Table 2.7. Solid arrows denote movement of individuals while broken ones represent transmission of trypanosomes between the two hosts via tsetse. Host 1 could be taken to be cattle and host 2 game. In game, recrudescence of infection is allowed at rate, ρ.



Source: Milligan, 1990

Figure 2.4. Compartmental model of animal trypanosomosis.

The parameters used to build the mathematical model shown in Figure 2.4 include:

μ	baseline mortality rate of vertebrate host per day
r	recovery rate per day
α	parasite induced mortality of vertebrate host per day
i	rate of progression to infectiousness per day
λ	force of infection per day
γ	rate of loss of immunity/resistance per day
b	baseline mortality rate of tsetse per day
$\bar{\alpha}$	parasite induced mortality of tsetse
β	tsetse biting rate per day
ρ	recrudescence to infectious state

The proposed approach of evaluating the effectiveness of a new technology (i.e., combining field trials and mathematical modeling) was utilized in the evaluation of the tsetse repellent technology.

Chapter 3

Estimation of tsetse challenge and its association with trypanosomosis incidence in cattle

3.1. Introduction

Tsetse challenge is a strong predictor for trypanosomosis and it influences the effectiveness of tsetse and trypanosomosis control techniques. It is a composite variable given by the product of tsetse density, trypanosomosis prevalence in tsetse and the proportion of meals obtained by tsetse from livestock (Leak et al., 1988). Studies that have evaluated its association with trypanosomosis prevalence in livestock have produced inconsistent results. Fall et al. (1999) observed that a significant association between the variables could be obtained when data collected over a 4-year period were analyzed as an aggregated data set but not when the same data were analyzed as monthly infection rates and tsetse challenges. Leak et al. (1993) observed a significant association between the two variables in one of the two sites used in the study (Ghibe) but not at the other (Tolley). Similarly, Karanja (2005) did not find a significant association between these variables in a study carried out in Busia District, Kenya.

To demonstrate a statistically significant relationship between these variables, their estimates ought to be accurately determined using efficient sampling and diagnostic techniques (Jordan, 1986). Some of the reliable techniques that could be used include polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) tests to determine infection rates in tsetse (Ouma et al., 2000; Njiru et al., 2004) and livestock (Greiner et al., 1997; Mattioli et al., 2001) as well as PCR-RFLP (restriction fragment length polymorphism) technique to identify the sources of blood meals in tsetse (Steuber et al., 2005). Most studies, however, find it costly to use the highly sensitive tests for routine epidemiological surveillance. Moreover, apparent tsetse density is usually the most variable

component of tsetse challenge (Rogers, 1985), implying that reliable estimates of challenge could still be obtained by efficiently measuring tsetse density.

This chapter evaluates the association between tsetse challenge and trypanosomosis incidence using the data collected from the trial for the purposes of determining ways of incorporating tsetse challenge as a prognostic factor. Furthermore, values for some of the parameters used in building mathematical models described in Chapter 6 are estimated.

3.2. Materials and methods

3.2.1. Study areas

3.2.1.1. Location and physical features

The study was conducted at Nkuruman located in Nkuruman sub-location, Kajiado District and Nkineji, an area that straddles Megwara and Maji Moto sub-locations in Narok District. These districts lie in the southwestern part of the Rift Valley Province and border Tanzania to the south (Figure 3.1).

The southwestern border of Nkuruman is formed by an escarpment, which also forms the southwestern wall of the Rift Valley. The escarpment rises steeply in a series of stepped rocky faults, with the crest being about 2 300m above sea level. The southern end of Ewaso Ngiro River crosses the floor of the valley where settlements are found. The floor is about 900m above sea level. The area is semi-arid and it is classified into eco-climatic zone V (Bekure et al., 1991) with a mean annual rainfall of 450mm on the floor, rising to about 750mm on the forested ridges and at the peak of the escarpment. The vegetation changes from open savannah grasslands with scattered shrubs on the plain, to open *Acacia tortilis* woodland towards the foot of the escarpment, to dense *Acacia-Commiphora* bush on the lower slopes and, finally, to *Tarchonanthus* thicket and submontane forest at the cliff. Clear, fast-running, rocky streams flow down the escarpment, fringed on their lower reaches with tall riparian forest of figs (*Ficus* spp.) (Birdlife International, 2005). The tsetse

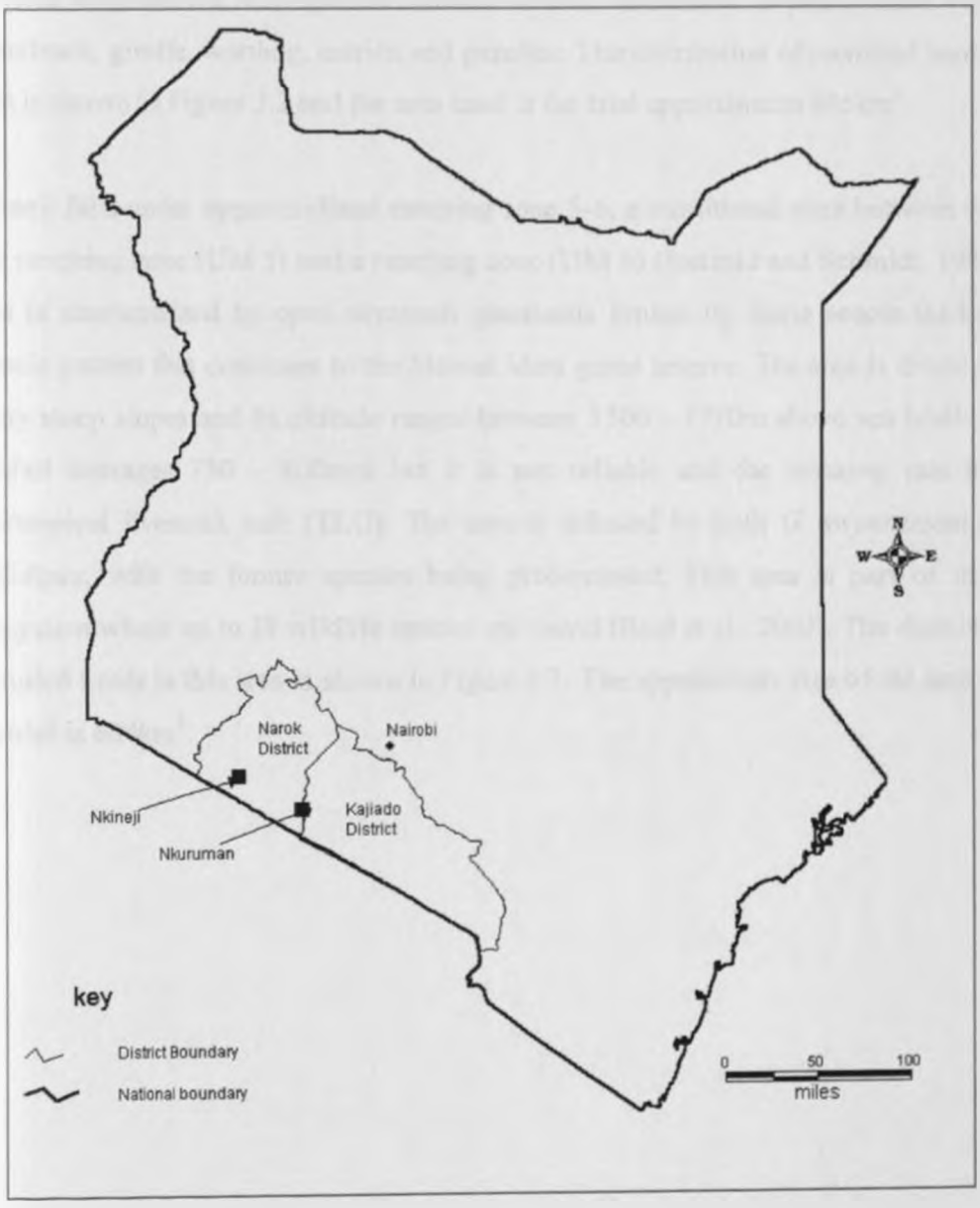


Figure 3.1. Map of Kenya showing the location of the two study sites: Nkuruman and Nkineji.

species found in the area are *Glossina pallidipes* and *G. longipennis*. *G. pallidipes* is widely distributed but *G. longipennis* is found mainly in dense thickets. The stocking rate is about 3 - 7ha/tropical livestock unit (Bekure et al., 1991). Game is abundant especially in the forested areas and the main species included buffalo, wildebeest, elephant, eland, bushbuck, waterbuck, giraffe, warthog, ostrich and gazelles. The distribution of recruited herds in the area is shown in Figure 3.2 and the area used in the trial approximates 655km^2.

Nkineji falls under upper midland ranching zone 5-6, a transitional zone between sorghum and ranching zone (UM 5) and a ranching zone (UM 6) (Jaetzold and Schmidt, 1983). The area is characterized by open savannah grasslands broken by dense acacia thickets in a mosaic pattern that continues to the Maasai Mara game reserve. The area is dissected with many steep slopes and its altitude ranges between 1500 – 1770m above sea level. Annual rainfall averages 730 – 800mm but it is not reliable and the stocking rate is about 3ha/tropical livestock unit (TLU). The area is infested by both *G. swynnertoni* and *G. pallidipes*, with the former species being predominant. This area is part of the Mara ecosystem where up to 38 wildlife species are found (Reid et al., 2003). The distribution of recruited herds in this area is shown in Figure 3.3. The approximate size of the area used in the trial is 684km^2.

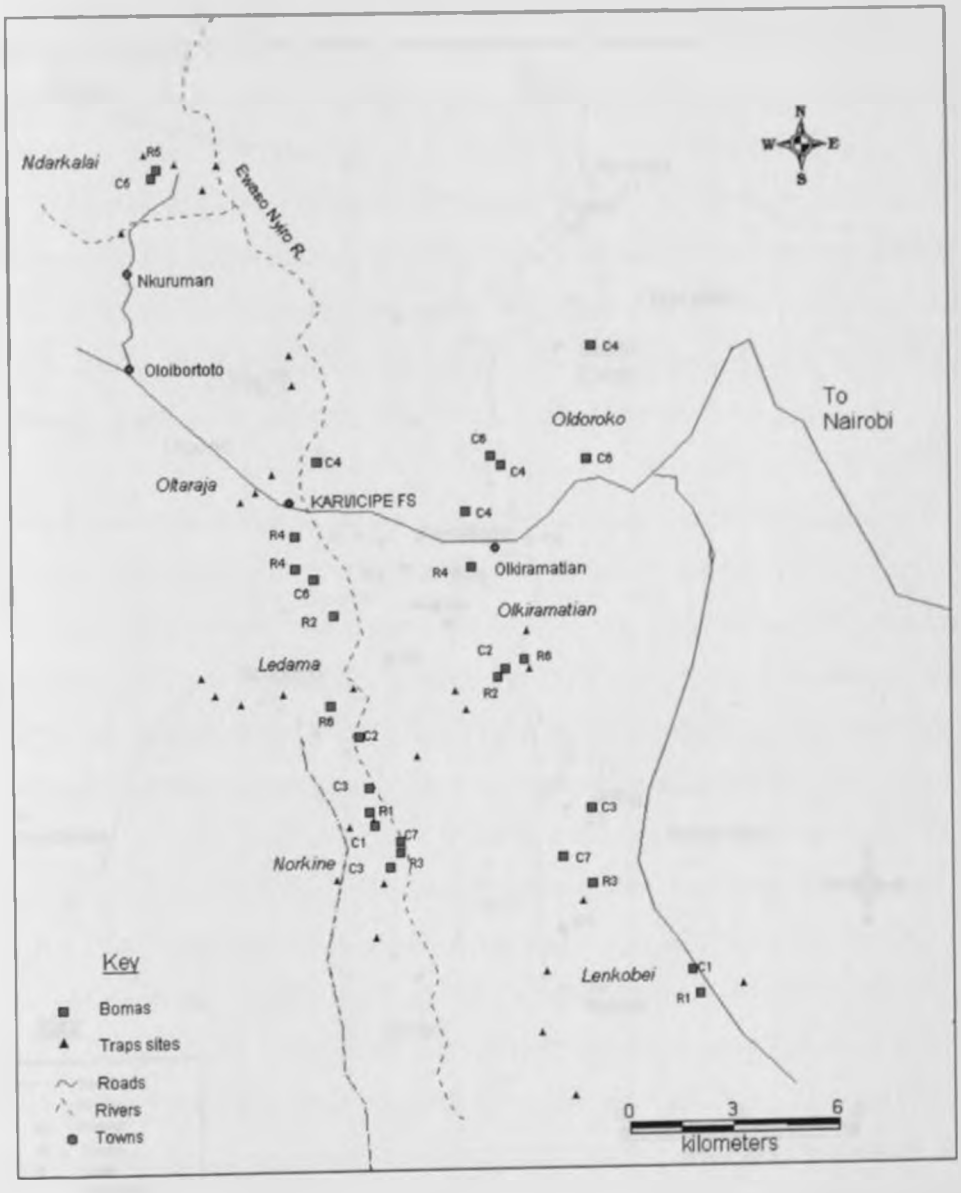


Figure 3.2. Map of Nkuruman study site showing the distribution of *bomas* (cluster of households) and tsetse trapping sites.

Figure 3.3. Map of Nkineji showing the distribution of *homas* (cluster of households) and tsetse trap sites.

3.2.1.2. Socio-economic activities of the local inhabitants

The inhabitants of the areas are Maasai pastoralists who keep cattle, sheep, goats and donkeys. Zebu cattle and the small East African goat are the predominant domestic animal species raised at Nkuruman, while zebu cattle and black head Persian/Maasai sheep comprised the majority of the animals kept at Nkineji. The cattle herd sizes average 50 and 80 at Nkuruman and Nkineji, respectively. The size of family units, to a large extent, is determined by the labour needed to manage the herds and flocks as these animals formed the main source of livelihoods for the pastoral households. A man with many animals can afford to marry many wives and have more children. He could also take impoverished friends or relatives as dependents, adding to his prestige and labour force.

A primary goal of livestock production in these societies is to produce milk for subsistence; animals are sold for cash primarily to buy subsistence goods, services and production inputs. The government had sought to change the orientation of production from subsistence to commercial farming through group ranching in 1965 (Bekure et al., 1991). It was believed that security of land tenure through organized groups would reduce the pastoralists' tendency to overstock the ranges, increase their incentive to invest in range improvement and act as collaterals for loans to invest in their developments. The original concept of group ranches was, however, undermined by the failure of the members of the group ranches to adopt the grazing quotas that had been legislated. They probably espoused the concept of group ranches to stem the encroachment on their land by the farmers from the other ethnic groups (Bekure et al., 1991). Even though the legislations that were to define grazing quotas were not adopted, the group ranches developed less strict policies that guide seasonal grazing patterns. The group ranches used in the present study were Olkiramatian and Shompole in Nkuruman and Koiyaki in Nkineji.

Horticultural farming has also been developing in these regions over the years. Each member of Olkiramatian and Shompole group ranches was expected to have a piece of land at irrigable areas for growing vegetables such as okra, egg plant, pepper, tomatoes, onions, etc. These vegetables had ready markets at Nairobi, with some being exported. At Nkineji,

few farmers grew maize for subsistence. In this area, farmers relied on rain as there were no reliable water masses for large-scale irrigation.

Tsetse-transmitted cattle trypanosomosis has been identified as one of the constraints to livestock production in Nkuruman (Dransfield et al., 1990; Mwangi et al., 1998) and Nkineji (Mukiria, 2002). The prevalence of trypanosomosis in cattle varied between 5 – 20% in both areas with a higher prevalence during wet than dry seasons (Mwangi et al., 1998). East Coast fever also occurred periodically at Nkuruman, especially in the forested areas. Community-based tsetse control projects had been initiated in Nkuruman with variable outcomes. Dransfield et al. (1990) introduced traps in Nkuruman but the project was not sustained. The Kenya Pastoralist Development Association (KEPDA) had also been involved in tsetse control activities in Nkuruman by helping the community to make, deploy and service NGU traps.

The Kenya Agricultural Research Institute (KARI) and the International Center for Insect Physiology and Ecology (ICIPE) have field stations in Nkuruman from where linkages between the community and the research institutes are established for tsetse and trypanosomosis research programs. The community at Nkuruman has, therefore, had a fairly good training from various government agencies and non-government organizations (NGOs) on various aspects of tsetse and trypanosomosis. In contrast, livestock owners at Nkineji had not been exposed to the same degree of awareness with regard to tsetse and trypanosomosis control compared to the livestock owners from Nkuruman. There were also no reports of organized interventions against tsetse and trypanosomosis in the area except a field trial that had been conducted by Kenya Trypanosomiasis Research Institute (KETRI [now Trypanosomiasis Research Centre-Kenya Agricultural Research Institute, TRC-KARI]) to test a dip formulation of a pyrethroid insecticide introduced into the country by Coopers Kenya Limited.

3.2.2. Data collection

3.2.2.1. Phases of the study

The two phases of the study were: (a) a 4-month baseline data collection phase that ran between April and August 2004, and (b), a 12-month intervention phase carried out between August 2004 and August 2005 (Table 3.1).

Table 0.1. Phases of the field trial conducted at Nkineji, Narok and Nkuruman, Kajiado to evaluate the effectiveness of the tsetse repellent technology (April 2004 – August 2005)

Area	Time in months																
	April 04				Aug 04												Aug 05
	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
Narok	Baseline phase				Intervention phase												
Kajiado							*										

*A round of data collection was missed in October 2004 in Nkuruman, Kajiado District. The gap in data was accounted for in the analysis by censoring the interval and recalculating the animal-time at risk.

3.2.2.2. Trypanosomosis incidence in cattle

3.2.2.2.1. Sample size determination

The number of animals that could be treated with the repellent at any one time was constrained by the amount of repellent supplies and the rate of synthesis of the repellent to 1000. Sample size estimation was therefore limited to determining the number of herds to which the animals could be distributed. A uniform allocation ratio[1] of herds to the treatment group was preferred. This means that the probability of assigning a herd to a treatment group, for instance, was similar to that of the control group.

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[1] A uniform allocation ratio ensures that an equal number of herds (and animals) are recruited to the treatment groups. A uniform allocation ratio is often preferred unless there are valid reasons to allocate disproportionately larger number of subjects to one treatment than to another (Meinert and Tonascia, 1986).

An initial methodology workshop convened at ILRI set the parameters that were used to estimate the number of herds. Trypanosomosis incidence was taken to be the primary outcome measure[2] for the study. The baseline trypanosomosis incidence, p was assumed to be 10%. The detectable level of treatment effect was set at 50% reduction in trypanosomosis incidence through wide consultations with various scientists. Other parameters used, and required for the estimation of the sample size include the level of confidence of a one-tailed test (1- α) of 95%, a power (1- β) of 80% and an intra-herd correlation coefficient, ρ, of 0.4. The choice of the value of the correlation coefficient was guided by an observation made by Otte and Gumm (1997) who reported that most infectious diseases have intra-herd correlation coefficients ranging between 0.04 and 0.42. It was presumed that the requirement for the treatment of all the animals in a herd with the repellent would enhance intra-herd clustering, hence the use of a conservative estimate of 0.4.

The formulae used to estimate the sample size (Equations 3.1 and 3.2 (Dohoo et al., 2003)) were ran in Microsoft Excel with the outputs being compared to those obtained from STATA 8 using the commands *sampsi* and *sampclus*[3]. The first step used the formula:

$$n = \frac{[Z_\alpha \sqrt{(2pq)} - Z_\beta \sqrt{p_1 q_1 + p_2 q_2}]^2}{(p_1 - p_2)^2} \qquad (3.1)$$

where: p a priori estimate of the baseline trypanosomosis prevalence (p_1, p_2 – estimates in the two treatment groups. p is an average of p_1 and $p2$)

q 1-p ($q_1 = 1 - p_1$ and $q_2 = 1 - p_2$)

Z_α the value of Z_α required for 95% confidence from the standard normal distribution

[2] The primary outcome measure should be (i) easy to diagnose or observe, (ii) free of measurement or ascertainment errors, (iii) capable of being observed independent of treatment assignment, (iv) clinically relevant, and (v), chosen before the start of data collection (Meinert and Tonascia, 1986).

Z_β the value of Z_β required for power of 80% (1-tailed test), i.e. –0.84.

The sample size obtained from Equation 3.1, n, was adjusted based on the level of clustering within herds using:

$$n' = n(1 + \rho(m - 1)) \quad (3.2)$$

where:

n' is the adjusted sample size

ρ intra-herd correlation coefficient i.e. 0.4

m average herd size

The sample size estimated using the input parameters described above (shown in Table 3.2) was very huge and could not be used in the study given the limited resources that were available. The estimates in the table (Table 3.2) show that the number of animals that had to be used to test a reduction of incidence by 50% from 10 to 5% with a power of 80% was at least 7010 per treatment group assuming an average herd size of 50 and a uniform allocation of animals to the experimental groups. An alternative approach of treating the *priori* incidence as a 12-month cumulative incidence instead of monthly incidence gave a reasonable sample size which was then used in the trial. The *priori* cumulative trypanosomosis incidences were derived using the formula:

$$CI = 1 - (1 - p)^t \quad (3.3)$$

where:

CI – cumulative incidence

p – monthly incidence of trypanosomosis in each treatment group

t – time in months that the trial was to be conducted

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[3] *sampclus* calculates sample size and number of clusters for cluster sampled studies, correcting for any intraclass correlation using the estimates generated from *sampsi*, which must precede it

With a one-tailed test being deemed valid for the study, a sample size of 12 herds was estimated from this calculation. This is because the technology was always expected to reduce the incidence on trypanosomosis (i.e. the incidence of the disease in the treated group of animals was always expected to be lower than that of the control).

Table 3.2. The number of herds (and corresponding number of animals) that would be required to detect a 50% reduction of trypanosomosis incidence with 80% power at the various levels of input parameters, i.e. a *priori* outcome incidence, statistical test for the 95% level of confidence and the expected average herd size, *m*

Priori tryps incidence (outcome measure)	Test for 95% CI	Average herd size, *m*			
		50	100	200	400
Monthly incidence (10 vs. 5%) [a]	One-tailed [b]	140 (7 010)	138 (13 816)	137 (27 429)	137 (54 653)
	Two-tailed [c]	179 (8 939)	176 (17 618)	175 (34 976)	174 (69 691)
One-year cumulative incidence (72 vs. 46%) [d]	One-tailed	12 (594)	12 (1 170)	12 (2 322)	12 (4 627)
	Two-tailed	16 (798)	16 (1 573)	16 (3 122)	16 (6 221)

[a] *Priori* trypanosomosis incidences in the control and experimental groups are 10 and 5%, respectively
[b] The value of the Z_α required for 95% confidence while using a one-tailed test is 1.64
[c] The value of the Z_α required for 95% confidence while using a two-tailed test is 1.96
[d] *Priori* trypanosomosis incidences in the control and experimental groups are 72 and 46%, respectively

3.2.2.2.2 Selection of villages and study herds

Reconnaissance surveys were conducted in the study areas to obtain background information on the distribution of households, herd sizes, grazing patterns, tsetse density and trypanosomosis prevalence which was used to develop a sampling frame. The sampling frame listed cattle herds; this list was stratified by herd size and village. Villages were selected purposively to ensure independence in grazing patterns and effective management of the study with regard to convenience in surveillance. The villages selected in Nkuruman were Lenkobei, Oloisinyai, Olkiramatian, Oltaraja, Ledama, Norkine and Ndarkalali (Figure 3.2), while those selected in Nkineji were Irpoori, Iloongishu, Parketapo, Songoyo, Olooriri and Nkoswani (Figure 3.3). The number of villages used in Nkuruman was more than the number of herds because of seasonal grazing patterns. In Olkiramatian group ranch for example, Oloisinyai and Olkiramatian were used for grazing in the wet season while Ledama and Oltaraja were used in the dry season. The villages falling under Shompole group ranch included Lenkobei and Norkine and were used in the wet and dry season.

respectively. Cattle owners at Ndarkalali, a village in Olkiramatian group ranch, had a sedentary lifestyle. Grazing patterns at Koiyaki group ranch at Nkineji were less affected by season as pastures were always available. However, transhumance was practised by some farmers in the dry season. Animals were driven towards the Maasai Mara game reserve during dry season and brought back as soon as wet season set in.

Herds were grouped into three classes depending on size, i.e. <50, 50 – 99 and ≥100. Within each class and village, treatment herds were selected randomly without replacement using computer-generated random numbers. This ensured the selection of herds of different sizes. Herds that had >200 animals were removed from the sampling frame to limit the trial to those herds that had manageable numbers of animals for repellent application and trypanosomosis surveillance. Control herds were selected purposively to match treatment herds in size and areas used for grazing over the seasons while ensuring that the selected herds were always managed independently. This process ensured that a pair of treatment and control herds was chosen from every village.

3.2.2.2.3. Animal sampling

All the animals in the selected cattle herds were ear-tagged at the beginning of the study except the very young calves that had not been taken out for grazing by the time the study started. But as soon as the calves attained about 50kg body weight, they were ear-tagged together with any new animal that was brought into a herd. At the time of ear-tagging, sex, weight, age, colour and owner of each animal were recorded and a blood sample obtained for the determination of the infection status.

All the ear tagged animals were sampled at monthly intervals. They were restrained in a crush and blood collected from the ear veins using a pair of heparinized capillary tubes. The samples were screened for the presence of trypanosomes using haematocrit centrifugation and examination of the buffy coat using phase contrast microscopy (BCT) (Murray et al., 1977). The degree of anaemia was estimated by measuring packed cell volume % (PCV). The intensity of infection was quantified as a parasitaemia score. Trypanosomes were

characterized by species depending on their motility characteristics observed on wet preparations of the buffy coat. This was always confirmed through thin blood smears prepared from the buffy coat and stained with Giemsa. The stained thin blood smears were prepared from the buffy coat to increase the probability of picking the parasites. The animals were also weighed using a weighband at the time of sampling and body condition scoring done for weaners and adults using a nine-point scoring system developed by ILCA (ILRI, 2001). All the animals that were found positive and those that had a PCV of < 22 were treated with diminazene aceturate at 7 $mgkg^{-1}$ body weight. Other conditions that were observed or presented by the cattle owners were also managed appropriately. Calves, weaners and lean animals were also given broad spectrum anthelmintics every four months.

3.2.2.2.4. Volume of drugs used by livestock owners

At the beginning of the study, all the recruited cattle owners were shown how to make records of the drugs used, animals sold or bought and the ones that died. One enumerator in each study site was identified and trained to assist the cattle owners in making the records. The enumerators visited each cattle owner at least once in three days to check the records made and if necessary, correct errors that might have been made. The enumerators were also expected to inquire and record any event that might have occurred in the visit interval but not recorded by the livestock owners. These records were checked by the research team at time of sampling and recording errors pointed out to both the enumerators and the cattle owners.

3.2.2.3. Estimation of tsetse challenge

Factors that may influence tsetse challenge were identified through a causal web model (Figure 3.4). As expected, meteorological factors (rainfall density, humidity, temperature) are the main drivers of the system. These factors influence tsetse's ecology and population dynamics. The model helped to visualize the relationships between the different variables that were being measured.

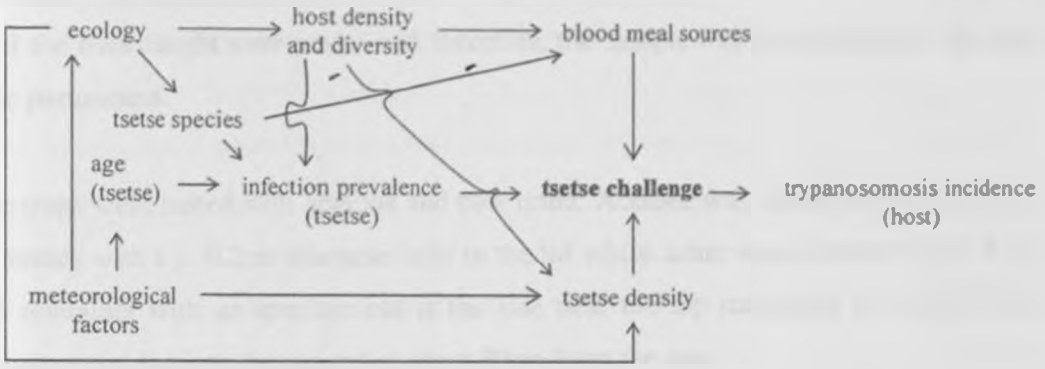


Figure 3.4. Causal web model showing the variables that may influence tsetse challenge, hence trypanosomosis incidence in mammalian hosts.

3.2.2.3.1 Tsetse density

Tsetse flies were sampled once every month in each village using stationary traps. Each trapping session ran for 72 hours and harvests made every 24 hours. A total of 30 traps were used per area and were distributed such that each village had five traps. The choice of a trap used in an area depended on its efficiency in sampling the prevalent tsetse species in that area. At Nkuruman for instance, NG2G trap was used because it is efficient in trapping *G. pallidipes* (FAO, 1982a; Kasilagila, 2003) whereas biconical trap was used at Nkineji (Plate 3.1). The choice of the trap used in Nkineji was made through trap efficiency experiments that were carried out in the area before the commencement of the study. These experiments recommended the use of biconical trap as it caught the highest number of flies compared to NG2G, NZI and pyramidal traps (ILRI/TRC/ICIPE, 2003). The trap was also used by Ndegwa and Mihok (1999) as a standard trap when they were evaluating new model traps for *G. swynnertoni* in Kenya and Tanzania. An attempt was also made to supplement traps catches of *G. swynnertoni* with "man fly-rounds[4]" because it was known

[4] "Man fly-rounds" is a system of determining the apparent density of tsetse flies where a team of about three people traverse a target area along pre-determined transects while carrying a 1.0 x 1.3 m black screen that attracts the flies. The team would stop after every 200 m to trap and count flies that have alighted on the screen. This technique was in use before efficient traps were developed. It is also recommended for use in trapping flies that cannot be efficiently trapped using the standard trapping devices, e.g. *Glossina swynnertoni* and *G. morsitans morsitans*.

beforehand that trapping *G. swynnertoni* would be difficult. This was discontinued because most of the flies caught were males and, therefore, the sample was not expected to provide reliable parameters.

All the traps were baited with acetone and cow urine. Acetone was dispensed from 300-ml glass bottles with a *c.* 0.2cm diameter hole in the lid whilst urine was dispensed from a 2L plastic container with an aperture cut at the side near the top measuring 2 x 4cm. Both containers were dug into the ground at about 30cm from the trap.

All flies caught were identified and recorded according to species, sex and teneral/non-teneral status. Records were made for each day but the apparent density estimates represented by fly per trap per day (FTD) utilized the total number of flies caught over the sampling period. Daily maximum and minimum temperatures, humidity and rainfall were also measured at the study sites.

Plate 3.1. Traps used for sampling tsetse in the tsetse repellent evaluation trial conducted at Nkuruman, Kajiado District (NG2G) and Nkineji, Narok District (biconical) (2004 – 2005).

3.2.2.3.2 Trypanosome ifection prevalence in tsetse

A constant proportion (10%) of tsetse flies caught in each trap was randomly selected and dissected to determine trypanosome infection prevalence. Dissections were carried out in 0.9% normal saline. The trypanosome parasites that were prevalent in the two areas were:

Trypanosoma congolense and *T. vivax*. The determination of a fly's infection status was therefore geared towards examining midgut and mouth parts - labrum and hypopharynx for the presence of these trypanosomes using phase-contrast microscopy. Depending on the organs infected, the possible diagnostic outcomes for positive infections included: (1) "Congolense-type infection" – when both midgut and mouthparts were found infected; (2) "Vivax-type infection" – when only the mouth parts were found infected; and (3) immature infections – when only the midgut was found infected.

3.2.2.3.3. Aging of tsetse

The age distribution of the sampled female tsetse was determined through ovarian dissection (FAO, 1982b). An attempt was made to simultaneously determine the infection status and age of each female tsetse. Since this procedure required more concentration and time, it was discontinued because it limited the number of flies that were being dissected per village per session. Throughout the study, the dissections were always done by two technicians.

3.2.2.3.4. Blood meal sources

Gut contents from tsetse that were judged by external appearance to contain fresh blood meals were expressed onto a Whatman filer paper (Whatman International Ltd). The abdominal wall was cut and gut pulled out using a clean forceps that was always cleaned with ethanol before being re-used. Each filter paper was pretreated with 0.2% sodium azide, a preservative. Filter papers carrying the blood meal samples were always kept in a dessicator but separated using grease proof paper. A data record sheet was completed with date and details of the sex and species of each fly and the collection site. The samples were analyzed at International Centre for Insect Science and Physiology (ICIPE) using enzyme linked immunosorbent assay (Rurangirwa et al., 1986).

3.2.3. Data analysis

Data were stored in a relational database designed using Microsoft® Access and statistical analyses conducted in STATA 8.2 (StataCorp., 2003). The level of significance for all the statistical tests was set at 5%.

Monthly averages of rainfall density, maximum and minimum temperatures and flies per trap per day (FTD) were calculated and the correlation between rainfall density and FTD determined by area.

Tsetse challenge was estimated as the product of apparent tsetse density, the prevalence of mature trypanosome infection in dissected flies and the proportion of blood meals obtained from cattle. The age structure of captured tsetse was assumed to have an exponential distribution with young flies constituting the majority. The hazard rate of tsetse, λ was also assumed to be constant. The maximum likelihood function (Equation 3.3) of the exponential probability distribution was used to estimate λ (Equation 3.7) through the steps represented by Equations 3.4, 3.5 and 3.6 (*NIST/SEMATECH e-Handbook of Statistical Methods*, http://www.itl.nist.gov/div898/handbook/).

$$L = C(\prod_{i=1}^{r} f(t))(1 - F(T)^{n-r} \tag{3.3}$$

$$L = C\lambda^{r} e^{-\lambda\sum_{i=1}^{r} t_i}(e^{-\lambda(n-r)T}) \tag{3.4}$$

$$\ln L = \ln C + r\ln\lambda - \lambda\sum_{i=1}^{r} t_i - \lambda(n-r)T \tag{3.5}$$

$$\frac{\partial \ln L}{\partial \lambda} = \frac{r}{\lambda} - \sum_{i=1}^{r} t_i - (n-r)T = 0 \tag{3.6}$$

$$\lambda = \frac{r}{\sum_{i=1}^{r} t_i + (n-r)T} \tag{3.7}$$

where:

L – Likelihood function

C - constant

r – the number of female tsetse dissected

$f(t)$ – probability density function

$F(T)$ – cumulative density function

T – maximum age in days that could be allowed by aging system

n – total number of female tsetse caught

Area- and village-level estimates of challenge were estimated. Due to the small number of blood meal samples successfully identified, an average area-level proportion of meals obtained from cattle were used in the estimation of tsetse challenge. The challenge variable was further log-transformed as $\log_{10}$ (*tsetse challenge* + 1) and used as an independent variable in a Poisson model that analyzed its unconditional relationship with trypanosome incidence in cattle. The outcome variable – trypanosomosis incidence in cattle – was derived from the data collected from the control groups because it was expected that the use of the repellent would reduce the incidence of trypanosomosis in treated cattle hence bias tsetse challenge-trypanosomosis incidence association. Analyses were carried separately for each site at both area- and village-levels. At each level, two models were fitted to the data, one correlated monthly estimates of tsetse challenge and trypanosomosis prevalence, whereas the other used tsetse challenge lagged by one month.

To investigate whether the use of trypanocides by cattle owners over the inter-sampling interval could have biased the association between tsetse challenge and trypanosomosis incidence, an additional analysis was conducted at the village level where the number of animals treated in a month was added to number found infected at the end of that month and used as a dependent variable. Tsetse challenge was used as an independent factor. The assumption made here is that treated animals were always infected until given the treatment. For the analyses conducted at the village-level, clustering (from repeated sampling of herds) was accounted for using Huber-White-Sandwich estimator of variance (Caroll et al., 1998).

3.3. Results

3.3.1. Rainfall density

The total amount of rainfall recorded over the 12-month period in Nkuruman and Nkineji was 605.2mm and 921.1mm, respectively. Nkuruman had two wet seasons with varying rainfall densities. The first, characterized by low rainfall density, commenced in November and ended in January while the second started in March and ended in May. Nkineji had one long rainy season that gradually built up as from February and had not ended by the time the study was terminated in August (Figure 3.5). At Nkineji, temperature records made before month 4 were not reliable, therefore, were not analyzed. Nevertheless, the records made as from month 4 indicate that the minimum temperatures were lower than those observed at Nkuruman. Furthermore, both minimum and maximum temperatures slightly dropped at both areas over the wet season.

3.3.2. Tsetse species and apparent density

In each area, two tsetse species were identified, namely, *Glossina pallidipes* and *G. longipennis* in Nkuruman and *G. pallidipes* and *G. swynnertoni* in Nkineji. The predominant species in Nkuruman was *G. pallidipes*; the ratio of the apparent densities of *G. pallidipes* and *G. longipennis* was 735:1. In Nkineji, the predominant tsetse species was *G. swynnertoni* although the ratio between the apparent densities (from the FTDs) of *G. swynnertoni* and *G. pallidipes* was high (1:1). Trap efficiency for *G. swynnertoni* was poor as the FTD values were low even when the number of flies caught in the vehicle while traversing the villages in the area was high. Nonetheless, the variation of the FTD with time, and its relationship with rainfall density is depicted in Figure 3.5. The trends in maximum and minimum temperatures are given by Figure 3.6.

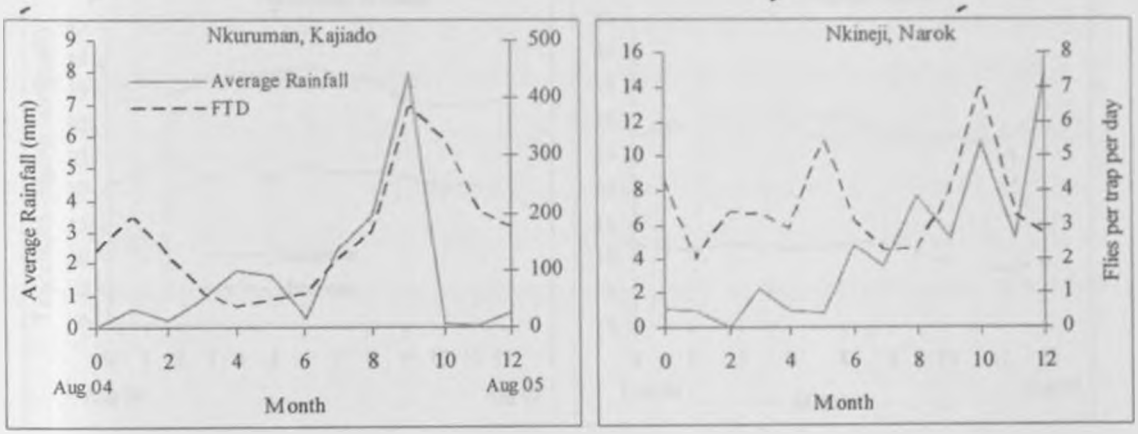


Figure 3.5. The relationship between monthly average rainfall density and the number of flies per trap per day over the intervention phase of the tsetse repellent evaluation trial conducted at Nkuruman, Kajiado and Nkineji, Narok (2004 – 2005).

In Nkuruman, the apparent tsetse density varied with season and the correlation between average rainfall density and the log transformed FTD was 0.31 (P = 0.32). In Nkineji, the apparent tsetse density was more or less constant, though with occasional peaks. The correlation coefficient between the apparent and rainfall densities was 0.14 (P = 0.66). Lagging rainfall by a month resulted in a stronger correlation between log FTD and rainfall in Nkuruman (ρ = 0.5, P = 0.11) but not in Nkineji (ρ = 0.00, P = 0.99). The apparent densities also varied by village, more so in Nkuruman than in Nkineji (Table 3.3).

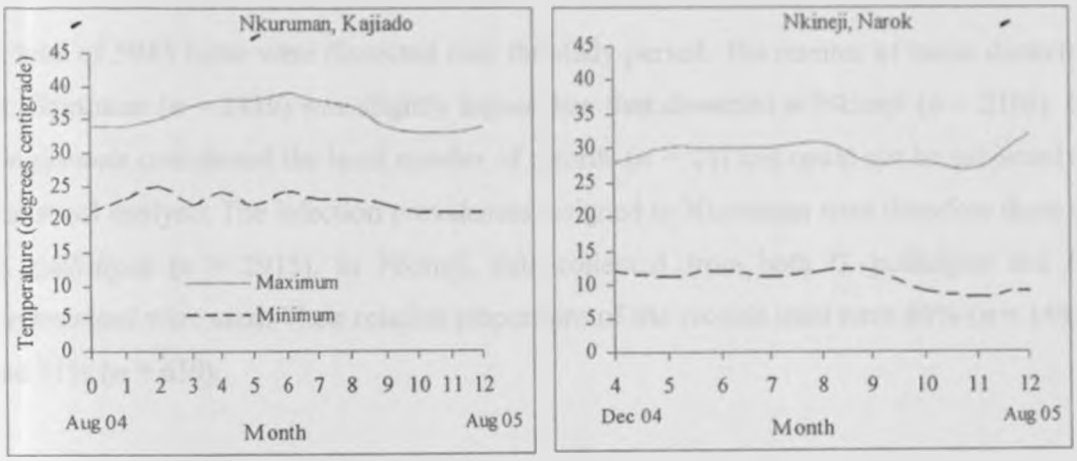


Figure 3.6. The average daily minimum and maximum temperatures (°C) recorded at the study sites used for the tsetse repellent evaluation trial (2004 – 2005).

Table 3.3. The average number of tsetse flies per trap per day (FTD) and the corresponding minimum and maximum values, by village, over the intervention period of the tsetse repellent evaluation trial carried out in Nkuruman, Kajiado and Nkineji, Narok (2004 – 2005)

Area	Village	FTD	Range
Nkineji	Irpoori	6.5	0.9 - 16.9
	Iloongishu	0.5	0.0 -1.1
	Songoyo	4.9	1.5 – 14.4
	Parketapo	2.5	0.8 – 5.5
	Olooriri	5.6	0.8 – 12.3
	Nkoswani	1.9	0.0 - 4.6
Nkuruman	Lenkobei	18.3	0.1 - 122.1
	Oloisinyai	0.5	0.0 - 2.8
	Ledama	266.8	12.1 - 806.5
	Oltaraja	494.9	129.7 - 912.6
	Ndarkalali	17.6	5.9 - 45.5
	Norkine	89.6	6.7 - 241.5

3.3.3. Trypanosome infection prevalence in tsetse

A total of 5045 tsetse were dissected over the study period. The number of tsetse dissected at Nkuruman (n = 2939) was slightly higher than that dissected at Nkineji (n = 2106). *G. longipennis* contributed the least number of records (n = 24) and could not be subjected to statistical analysis. The infection prevalences assigned to Nkuruman were therefore those of *G. pallidipes* (n = 2915). In Nkineji, data collected from both *G. pallidipes* and *G. swynnertoni* were used. Their relative proportions of the records used were 69% (n = 1447) and 31% (n = 659).

There was a significant variation in the proportions of tsetse that had mature trypanosome infections between Nkineji (4.7%, [99/2106]) and Nkuruman (0.6%, [17/2939]) (χ^2 = 92.81, P = 0.00) but not between villages within an area (Table 3.4). A similar trend was maintained when immature and mature infections were combined; the respective proportions were 5.7% (120/2106) and 1.5% (45/2939) in Nkineji and Nkuruman. At Nkineji, the infection prevalence could be stratified further by tsetse species. This analysis showed that *G. swynnertoni* had a higher prevalence (5.8%, [38/659]) of mature infections than *G. pallidipes* (4.2%, [61/1447]). This variation was, however, not significant (χ^2 = 2.43, P = 0.12). In the same area, the proportion of tsetse that had mature *T. congolense* type infections was 1.4% (29/2106). This proportion did not vary with species (i.e. 20/1447 in *G. pallidipes* compared to 9/659 in *G. swynnertoni*) unlike the pattern observed with *T. vivax* infections where a higher proportion (4.4%, [29/659]) of *G. swynnertoni* were infected compared to *G. pallidipes* (2.8%, [41/1447]). These ratios are barely insignificantly different (χ^2 =3.46, P = 0.06).

There was no significant variation in the infection prevalences with sex of the flies (overall, 2.1% (39/1858) males and 2.4% (77/3187) females were infected (χ^2 = 0.52, P = 0.47)). In Nkineji, 4.9% (35/715) and 4.6% (64/1391) (χ^2 = 0.09, P = 0.76) of male and female tsetse had mature infections, whereas, in Nkuruman, the respective proportions were 0.4% (4/1143) and 0.7% (13/1796) (χ^2 = 1.69, P = 0.19). The effect of season on trypanosomosis prevalence in tsetse is demonstrated in Table 3.5. In Nkineji, the prevalence increased over

the dry season (barely insignificant) unlike in Nkuruman where the prevalence was significantly higher during the wet than dry season.

Table 3.4. The percentage of tsetse infected by trypanosomes, stratified by area and village, determined over the intervention phase of the tsetse repellent evaluation trial (2004 – 2005)

Area	Village	*n*	% trypanosomosis prevalence (number of flies positive)				χ^2; $P > \|\chi 2\|$ [a]
			Congolense-type	*Vivax-type*	Immature	overall [b]	
Nkineji	Irpoori	632	0.8 (5)	3.5 (22)	0.8 (5)	4.3 (27)	7.02 (5 df);
	Iloongishu	68	2.9 (2)	7.4 (5)	0.0	10.3 (7)	$P = 0.21$
	Songoyo	429	2.3 (10)	1.2 (5)	0.9 (4)	3.5 (15)	
	Parketapo	272	1.5 (4)	4.0 (11)	1.5 (4)	5.5 (15)	
	Olooriri	481	0.8 (4)	4.0 (19)	1.0 (5)	4.8 (23)	
	Nkoswani	224	1.8 (4)	3.6 (8)	1.3 (3)	5.4 (12)	
Nkuruman	Lenkobei	108	0.0	0.0	0.0	0.0	3.58 (5 df);
	Oloisinyai	18	0.0	0.0	0.0	0.0	$P = 0.61$
	Ledama	834	0.1 (1)	0.5 (4)	0.5 (4)	0.6 (5)	
	Oltaraja	927	0.4 (4)	0.1 (1)	1.1 (10)	0.5 (5)	
	Ndarkalali	322	0.6 (2)	0.6 (2)	0.9 (3)	1.2 (4)	
	Norkine	730	0.0	0.4 (3)	1.5 (11)	0.4 (3)	

[a] $\chi 2$ test assessing the significance of the variation of the overall trypanosomosis prevalence by village
[b] refers to the proportion of the total number of cases caused by *congolense-type* and *vivax-type* trypanosomes, excluding immature infections

Table 3.5. Variation by season in the percentage of tsetse infected with trypanosomes in the course of tsetse repellent evaluation trial (2004 – 2005)

Area	Season	*n*	% trypanosomosis prevalence				χ^2; $P > \|\chi^2\|$ [a]
			Congolense-type	*Vivax-type*	Immature	overall [b]	
Nkineji	Dry	1021	1.2 (12)	4.4 (45)	1.0 (10)	5.6 (57)	3.44 (1 df);
	Wet	1085	1.6 (17)	2.3 (25)	1.0 (11)	3.9 (42)	0.06
Nkuruman	Dry	1796	0.1 (1)	0.1 (2)	1.0 (17)	0.2 (3)	13.59 (1 df);
	Wet	1143	0.5 (6)	0.7 (8)	1.0 (11)	1.2 (14)	0.00

[a] χ^2 test assessing the significance of the variation of the overall trypanosomosis prevalence by season
[b] refers to the proportion of the total number of cases caused by *congolense*-type and *vivax*-type trypanosomes, excluding immature infections

3.3.4 Blood meal analysis

Gut contents from a total of 312 tsetse, 221 from Nkuruman and 91 from Narok were sampled. Only 139 samples had identifiable blood meals. These results are tabulated in Table 3.6. The table indicates that the major sources of blood meals varied with the area. In Nkuruman, most of the meals were obtained from warthog and elephant whilst in Nkineji, the bovidae group (buffalo, cattle and other bovids) comprised the major blood meal source.

Table 3.6. Feeding patterns of *G. pallidipes* determined in the course of the tsetse repellent evaluation trial in Nkuruman, Kajiado and Nkineji, Narok (2004 – 2005)

Host species	Nkuruman		Nkineji	
	n	%	*n*	%
Bovine	9	8.0	8	30.8
Goat	7	6.2	3	11.5
Sheep	2	1.8	4	15.4
Thomsons gazelle	-	-	1	3.8
Buffalo & other bovidae	10	8.8	6	23.1
Baboon	-	-	1	3.8
Impala	2	1.8	1	3.8
Kudu	7	6.2	1	3.8
Kongoni	1	0.9	1	3.8
Elephant	15	13.3	-	-
Warthog	32	28.3	-	-
Giraffe	7	6.2	-	-
Zebra	13	11.5	-	-
Wildbeast	1	0.9	-	-
Ostrich	6	5.3	-	-
Man	1	0.9	-	-
Total	113		26	

Up to 23 blood meal samples, including 20 from Nkuruman, were of mixed source. Twenty of these were obtained from two hosts, while three were from three hosts.

3.3.5 Age structure

One-thousand-four-hundred and eighty-three female tsetse were dissected for ovarian aging. The total median age in days translate to 52572. Eight-hundred and eighty were aged at Nkuruman while 603 were aged at Nkineji. All the flies aged at Nkuruman were *G.*

pallidipes. Those aged at Nkineji comprised 477 *G. pallidipes* and 126 *G. swynnertoni*. The overall baseline hazards did not vary significantly by area (Log Rank $\chi^2 = 0.72$, $P = 0.40$). The respective hazard estimates of tsetse sampled at Nkuruman and Nkineji were 0.029 (95% CI: 0.027, 0.031) and 0.028 (95% CI: 0.025, 0.029). In Nkineji, the sampled *G. swynnertoni* had a significantly lower baseline hazard (mortality rate) estimate than *G. pallidipes* (Log Rank $\chi^2 = 4.76$, $P = 0.03$). The estimates were 0.028 (95% CI: 0.025, 0.031) and 0.026 (95% CI: 0.022, 0.031) for *G. pallidipes* and *G. swynnertoni*, respectively. Table 3.7 shows the estimated baseline hazard rates by village. In Nkuruman, Lenkobei and Oloibortoto had lower hazards than those of the other villages. All the villages in Nkineji had similar baseline hazards.

In Nkuruman, 820 tsetse were aged in the dry season and only 60 were aged in the wet season. The respective hazard rates for the dry and wet season were 0.030 (95% CI: 0.027, 0.031) and 0.019 (95% CI: 0.015, 0.025). These rates were significantly different (Log Rank $\chi^2 = 32.51$, $P = 0.00$), with tsetse sampled over the wet season having lower baseline hazard compared to that of the dry season. It was not possible to compare seasonal effects in Nkineji because all the dissections were carried out in the wet season.

Table 3.7. Estimates of the baseline hazard rates of tsetse trapped in Nkineji, Narok and Nkuruman in the course of the tsetse repellent evaluation trial (2004 – 2005)

Area	Village	Number aged	Total time (days)	Baseline hazard	
				Estimate	95% CI
Nkineji	*G. pallidipes*				
	Irpoori	187	6586	0.028	0.024, 0.033
	Songoyo	207	7676	0.027	0.023, 0.031
	Olooriri	19	712	0.026	0.016, 0.042
	Nkoswani	64	2130	0.030	0.023, 0.038
	G. swynnertoni				
	Irpoori	1	38	-	-
	Songoyo	31	1326	0.023	0.016, 0.033
	Olooriri	35	1352	0.026	0.018, 0.036
	Nkoswani	59	2098	0.028	0.021, 0.036
Nkuruman	Lenkobei	30	1216	0.025	0.017, 0.035
	Ledama	121	3766	0.032	0.027, 0.038
	Oltaraja	196	5560	0.035	0.030, 0.041
	Ndarkalali	50	1352	0.037	0.027, 0.049
	Norkine	183	5392	0.034	0.029, 0.039
	Oloibortoto	300	13368	0.022	0.019, 0.025

3.3.6 Trypanosomosis incidence in cattle

3.3.6.1 Baseline phase

Figure 3.7 summarizes the overall trypanosomosis incidence over the baseline study period by village. There was a village-to-village variation in incidence with Lenkobei, Songoyo, Parketapo, Olooriri and Nkoswani having relatively high incidences. Ndarkalali, Norkine, Irpoori and Iloongishu had moderate incidences, while Oloisinyai, Ledama, Oltaraja and Olkiramatian had low incidences.

3.3.6.2 Longitudinal phase

The average trypanosomosis incidence varied significantly by area. In Nkuruman, the incidence was 7.2% (296 cases out of 4094 animal-months; 95% CI: 6.4, 8.1%) while in Nkineji, the prevalence was 10.2% (899 cases out of 8805 animal-months; 95% CI: 9.5,

10.9%). Eighty-three percent (95% CI: 78.3, 87.2) of the cases in Nkuruman were caused by *T. congolense* alone (i.e. 246/296) with the remaining 17% (95 % CI: 12.8, 21.7%) being caused by *T. vivax* alone (i.e. 50/296). In Nkineji, the relative proportions of cases attributed to *T. congolense* alone of 51.4% (462/899, 95% CI: 48.1, 54.7%) and *T. vivax* alone of 48.1% (432/899, 95% CI: 44.7, 51.4%) were not significantly different. The remaining few cases ($n = 5$) was attributed to mixed infections.

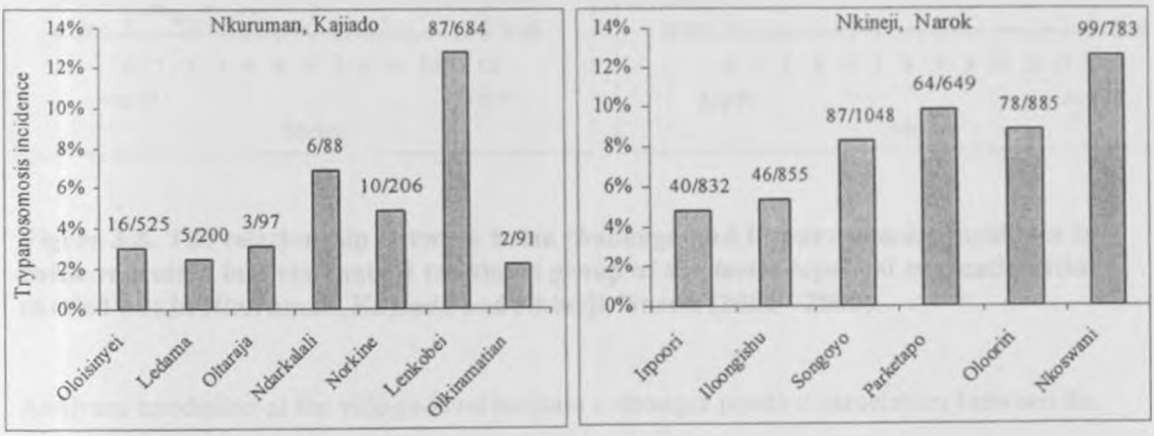
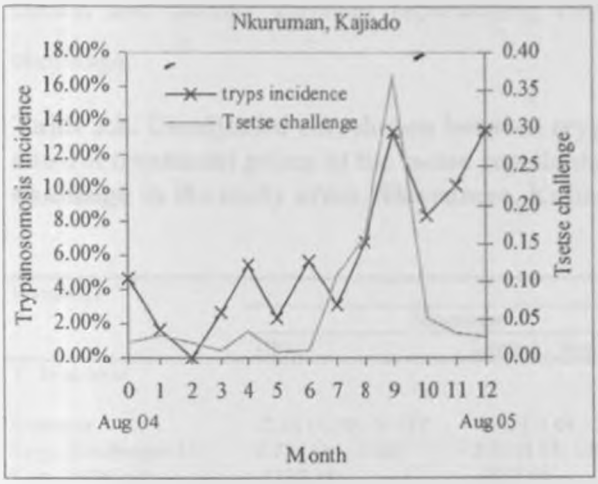


Figure 3.7. Mean incidence of trypanosomosis in cattle during the baseline period of the tsetse repellent evaluation trial carried out in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005). Absolute number of cases and animal-months are given on the body of the graphs.

Figure 3.8 shows the relationship between tsetse challenge and trypanosomosis prevalence in cattle, by area. When data aggregated at the area level were analyzed using a group-level Poisson regression model, trypanosomosis incidence and $\log_{10}$ transformed tsetse challenge were significantly associated at Nkuruman but not at Nkineji (Table 3.8). The strength and significance of the association observed at Nkuruman was not affected by lagging the tsetse challenge variable by one month. At Nkineji, lagging the variable by a month led to an increase in the value of the parameter although both forms of the variable (direct and lagged by one month) were not significant.



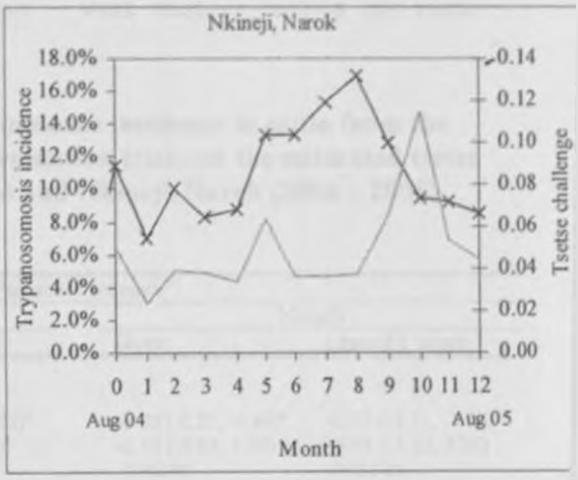


Figure 3.8. The relationship between tsetse challenge and trypanosomosis incidence in cattle recruited into the control treatment group of the tsetse repellent evaluation trial carried out in Nkuruman, Kajiado and Nkineji, Narok (2004 – 2005).

Analyses conducted at the village-level indicate a stronger positive association between the trypanosomosis incidence and tsetse challenge when the later variable was lagged by a month compared to when lagging was ignored. At Nkineji, none of the models produced statistically significant association irrespective of the level of analysis (area- or village-level) (Table 3.8). The log likelihood estimates further indicate that the model fitted data collected at Nkuruman better than those collected at Nkineji. Reducing the scale of analysis from the area to the village improved the fit of the model. A stronger correlation between tsetse challenge and trypanosomosis incidence was obtained when the dependent variable included the number of animals treated by the owners over a sampling interval. The fit of the model was, however, lower than those that used only on the infected animals to estimate disease prevalence.

To achieve the desired power of the trial (80%), data collected at the two areas, Nkuruman and Nkineji had to be combined in one analysis. A universal approach to accounting for tsetse challenge across areas was therefore required. Given that tsetse challenge estimated here (using tsetse density, proportion of tsetse infected by trypanosomes and blood meal index) was found not to be significantly associated with the outcome, two variables -

season and dummy variables representing villages – were used as proxies for tsetse challenge.

Table 3.8. Unadjusted correlation between trypanosome incidence in cattle from the control treatment group of the tsetse repellent evaluation trial and the estimated tsetse challenge in the study areas, Nkuruman, Kajiado and Nkineji, Narok (2004 – 2005)

Parameter	Parameter estimate			
	Nkuruman		Nkineji	
	Direct	Lagged 1 month	Direct	Lagged 1 month
1. Area-level				
Constant	-2.83 (-2.98, -2.69)[a]	-2.89 (-3.04, -2.73)[a]	-2.05 (-2.23, -1.88)[a]	-2.13 (-2.31, -1.96)[a]
Log_{10} (challenge+1)	2.73 (1.61, 3.86)[a]	2.72 (1.54, 3.89)[a]	-2.12 (-5.51, 1.27)	-0.01 (-3.27, 3.26)
Log likelihood	-1137.19	-1033.00	-2696.91	-2550.21
2. Village-level (trypanosomosis prevalence as outcome)				
Constant	-2.42 (-3.02,-1.83)[a]	-2.64 (-2.99, -2.29)[a]	-2.01 (-2.46, -1.56)[a]	-1.96 (-2.34, -1.57)[a]
Log_{10} (challenge+1)	-0.98 (-6.06, 4.09)	3.59 (-4.54, 11.72)	-1.54 (-7.91, 4.82)	-2.75 (-6.65, 1.15)
Log pseudolikelihood[b]	-784.85	-872.21	-2230.12	-2227.24
3. Village-level (trypanosomosis prevalence and number of animals treated with trypanocides as outcome)				
Constant	-1.29 (-1.72, -0.86)[a]	-1.57 (-2.11, -1.03)[a]	-0.43 (-1.11, 0.25)	-0.37 (-1.01, 0.27)
Log_{10} (challenge+1)	1.27 (-1.76, 4.30)	6.84, (-3.39, 17.07)	-3.65 (-10.32, 3.01)	-4.72 (-10.66, 1.23)
Log pseudolikelihood[b]	-1519.28	-1667.74	-4166.02	-4153.77

[a] Significant at α=0.05

[b] Log likelihood generated from a model where clustering at the village-level has been accounted for using Huber-White variance estimator

3.4 Discussion

A positive correlation between tsetse challenge and trypanosomosis incidence was observed at Nkuruman and not in Nkineji because *G. pallidipes*, unlike *G. swynnertoni*, can be efficiently sampled. It is difficult to sample *G. swynnertoni* with the existing traps because it is known to circle around artificial objects without alighting on them (Williams et al., 1992). Previous attempts to develop effective trapping devices by Ndegwa and Mihok (1999) and Swynnerton (1933) were not successful. Similar challenges involving sampling of *G. morsitans morsitans* have been reported by Van den Bossche et al. (2004). In that study, the density of *G. m. morsitans* was indirectly estimated using sentinel cattle herds. The low efficiency of sampling tsetse at Nkineji also lowered the success rate of capturing sated flies. Artificial refuges have been successfully used elsewhere to sample fed

flies but this system requires temperatures of more than 32° C because at this level, tsetse would seek shelters that are cooler than the outside air (Torr and Hargrove, 1999)

The number of flies caught by traps is conditioned by environmental and physiological factors. Rainfall, humidity and temperature are all closely correlated, with periods of rainfall being followed by lower temperatures and high relative humidity. Given that the average temperatures were generally lower at Nkineji than at Nkuruman, environmental effects were likely to be manifested more at Nkineji than at Nkuruman. It is also thought that emigration of tsetse to new habitats that develop in the wet season may also cause an apparent localized decline in tsetse density (Leak et al., 1993). At Nkineji, tsetse habitats were evenly distributed throughout the study area, implying that fly dispersal was unlikely to have contributed to the apparent decline of FTD observed in the wet season.

At Nkuruman, temporal variation in tsetse density was positively correlated with rainfall density lagged by one month. Positive correlation between rainfall density and tsetse density has been reported by Makumi et al. (2000), Kamau et al. (2000) and Karanja (2005). The high temperatures observed in dry season negatively affect tsetse's population growth rates by reducing fecundity and increasing mortality rates. High temperatures also cause tsetse to increase the rate of feeding, which would lead to increased rate of feeding-related mortality (Randolph et al., 1992) if they successfully find hosts. Flies that emerge in hot seasons also have low levels of fat. Given the low fat levels and the poorly developed flight musculature, teneral tsetse would be particularly exposed to the risk of starvation (Torr and Hargrove, 1999). Inter-larval period usually take 8 – 9 days in the cooler times of the year and typically, tsetse feed 2 – 3 times before producing a pupa (Gaston and Randolph, 1993). In hot seasons, the inter-larval period shortens implying that either tsetse must feed more frequently or their pupae are produced with fewer meals; either of these processes could increase mortality (Torr and Hargrove, 1999). The influence of seasonal events on spatial distribution of both cattle and game in a semi arid area like Nkuruman could indirectly affect the spatial distribution of tsetse as flies will be able to survive starvation in areas where vertebrate hosts congregate.

All the blood meal samples analyzed were obtained from *G. pallidipes*. Samples obtained from *G. swynnertoni* could not be analysed as they were always found to have been digested. This is related to the lack of efficient trapping device for the fly. At Nkuruman, only about 8% of the meals were obtained from cattle. The main blood meal sources were warthog, zebra and elephant. These findings are similar to those obtained by Sasaki et al. (1995) who concluded that few samples were obtained from cattle with the main sources being bushbuck, ostrich, elephant, buffalo and warthog. At Nkineji, most of the meals were obtained from bovids. Clausen et al. (1998) identified the main blood meal sources for *G. pallidipes* as being ruminants, bushpig and warthog and pointed out that feeding patterns vary by area depending on the presence of different host populations. The differences in the principal sources of blood meals by area reported in this study most probably mirrored the differences in host composition by area and habitats that influenced tsetse-host interaction.

Although the number of blood meals analyzed was small, the results could explain the observed variation in the composition of prevalent trypanosome parasites by area because there is a correlation between the proportion of bovid feeds and *T. vivax* infection (Snow et al., 1988). The low proportion of meals obtained from bovids at Nkuruman negatively influenced the prevalence of *T. vivax*, whereas at Nkineji, *T. congolense* and *T. vivax* were both prevalent as bovids could maintain the transmission of both parasites. Tarimo et al. (1981) also observed that the prevalence of *T. vivax* at Mkwaja Ranch, Tanzania was 1/10 that of *T. congolense* since about 75% of the meals were taken from warthog and bushpig. Snow et al. (1988) correlated the high *T. vivax* infection rates in southeast Uganda and western Kenya to bovid-feeding *G. pallidipes* and the low *T. vivax* infection rates to suid-feeding tsetse in coastal Kenya.

A few blood meals were characterized as being from more than one host species. These could have been obtained from flies that failed to fully engorge on one host, most probably due to interrupted feeding. Given that the ELISA test used to identify the blood meals characterized hosts to the species level, it is probable that there were more blood meals obtained from more than one animal at a host species level. Torr et al. (2001), however,

observed that when interrupted, tsetse does not attempt to complete its meal on a neighbouring animal but returned to the original host or left the vicinity entirely. Irrespective of the type of a host chosen by tsetse to complete its meal, interrupted feeding boosts tsetse's vectorial capacity. Moreover, infected flies significantly probe more times and they tend to take more time to engorge than non-infected flies and that parasite transmission rates are often higher in the first and second probings than those for subsequent feedings (Roberts, 1981). This is of particular significance to epidemiological studies because it is usually assumed that trypanosome-infected flies could transmit the parasites as they feed once in 3 - 4 days (Rogers, 1988; McDermott and Coleman, 2001). It is also probable that parasites could be transmitted while probing even if feeding attempts were unsuccessful. This phenomenon has been observed with the transmission of leishmania by sandflies (Hurd, 2003) and could explain the inordinately high prevalence of trypanosomosis in some areas where tsetse population is low.

Apart from the observed differences in the preferred sources of blood meals, flies sampled at Nkuruman had significantly lower rates of trypanosome infection than those from Nkineji. The different habitats might have influenced the differences in the observed rates of infection because the tsetse sampled at Nkineji had more or less similar levels of infection. It has also been recently confirmed that tsetse at Nkuruman are more refractory to infections by *T. b. rhodesiense* than those from Busia (Okoth et al., 2006). Tsetse susceptible or refractory to infection by one stock of *T. congolense* is also known to be susceptible or refractory to infections by different stocks of *T. congolense*, *T. b. brucei* and *T. b. gambiense* and that susceptible flies mature a significantly greater proportion of midgut infections than refractory ones (Maudlin et al., 1986). It is, therefore, probable that the differences observed in infection rates between areas could be attributed to differences in tsetse's susceptibility to trypanosome infection.

Evidence for tsetse movements between villages was obtained by Brightwell et al. (1997) through mark-release-recapture experiments at Nkuruman. This shows that there was mixing of tsetse between villages, hence the minimal variation in infection rates between villages. More information on the rates of tsetse dispersal is provided by Hargrove (2000)

who showed that tsetse would re-colonize a cleared area of about 100Km^2 if no barriers are used to check re-invasion. If the infection rates had been determined using a more sensitive test than the dissection technique, it would have been even more difficult to find any differences in infection rates between villages.

The other variables that could influence trypanosome infection rates in tsetse are sex, species and age of the fly. This study did not find the effect of sex to be significant. Similar findings have been reported by Moloo et al. (1992). Njiru et al. (2004), however, found significantly higher infection prevalence in female *G. pallidipes* compared to males. Although not statistically significant, *G. swynnertoni* had higher infection prevalence than *G. pallidipes* at Nkineji. Studies carried out by Tarimo et al. (1985) and Rogers and Boreham (1973) showed that *G. pallidipes* is less likely to acquire a trypanosome infection from an infected blood meal than *G. swynnertoni*. The proportion of blood meals that could give rise to an infection in *G. pallidipes* is 0.033, while for *G. swynnertoni*, the probabilities are 0.35 for *T. vivax*, 0.05 for *T. congolense* and 0.08 for *T. brucei*.

Sampling bias could contribute to variation in the infection rates between species of tsetse. Ovarian aging showed that sampled *G. swynnertoni* had, on average, lower mortality rates compared to *G. pallidipes*. The prevalence of mature trypanosome infections of tsetse flies increases with age (Woolhouse and Hargrove, 1998). *G. pallidipes* sampled in all the villages (except Lenkobei) had comparable age structure indicating that there was no sampling bias for this species across villages. Sampling bias that manifests in varied infection rates can be controlled for if age and infection levels of individual are simultaneously determined. This procedure requires the availability of adequate resources such that a representative number of flies are dissected at any one time. An increase in the number of dissectors could, however, increase the magnitude of inter-operator error. Torr and Hargrove (1999) noted that operator error should be considered as an important factor in such a study. It is difficult to determine the age structure of a tsetse population using ovarian aging because this technique assumes that the population is stationary (Jarry et al., 1999). Moreover, tsetse catches obtained using a stationary trap are biased to females that would have deposited their larvae or those requiring a blood meal to nourish the larvae at

the stage of their most rapid growth (Torr and Hargrove, 1999). Against this background, the ovarian dissection data collected in this study compares the mean age of the sampled tsetse as sampling bias (between traps and villages) with respect to age could affect the estimates of other parameters estimated from these samples, for example the infection rates. The higher trypanosomosis prevalence in *G. swynnertoni* compared to *G. pallidipes* for example, could be attributed to the sampling devices capturing older *G. swynnertoni* than *G. pallidipes*. The comparison of tsetse challenge across village is however not affected by this sampling bias because there was no variation in the ages of sampled tsetse. A similar approach was used by Torr and Hargrove (1999) to determine the sampling biases of artificial refuges, traps and mobile baits in a hot season in Zimbabwe.

Leak et al. (1988) and Leak et al. (1993) indicate that the use of trypanocides by farmers, which at times is not recorded, masks the association between tsetse challenge and trypanosomosis prevalence. This appears to be the case in this study because when the outcome includes the number of animals treated by farmers over a sampling interval, the strength of association between the variables increases. This approach does not, however, eliminate the "background noise" because some of the treatments made by cattle owners were inappropriate. This "noise" is manifested by a reduction in the log likelihood estimates and the lack of statistical significance for the association between the two variables.

The difficulties associated with measuring tsetse challenge and its inconsistent association with the disease incidence encourages the use of apparent tsetse density as a proxy for challenge in most epidemiological studies. Tsetse density alone does not, however, indicate trypanosomosis risk (Moloo et al., 1980). That is why in some places, for instance Mafia Island, Tanzania, the low risk of trypanosomosis could not be predicted by the abundance of *G. brevipalpis* (Goossens et al., 2006). Similar limitations were observed at Narok where the estimated apparent tsetse density was too low to predict the prevalence of the disease. This affected the estimation of tsetse challenge. To overcome this problem, season and village were included as fixed effect variables to account for variation between areas in

exposure to the disease. More efficient devices for sampling tsetse, especially *G. swynnertoni*, need to be developed.

Chapter 4

The rate of use and effectiveness of trypanocides administered by the recruited cattle owners

4.1. Introduction

Livestock owners use trypanocidal drugs as the principal method of animal trypanosomosis control despite the fact that trypanocides have relatively smaller impact on reducing the transmission of the disease compared to tsetse control methods (McDermott and Coleman, 2001). Trypanocides are widely used because their benefits can be captured by a person paying, unlike tsetse control methods which require collective action of many people to be effective and payers and non-payers benefit equally (McDermott and Coleman, 2001; Machila et al., 2003).

Livestock owners usually treat their animals without any supervision from animal health workers even though most of them do not have the facilities or the expertise to determine and administer the right dosages. This practice has been reported to reduce the effectiveness of trypanocides because the use of under-strength drug preparations fails to clear infections and also contributes to the development of drug resistance (Geerts and Holmes, 1998). In some areas like Nkuruman, Kenya, livestock owners give drugs at dosage rates that are higher than the recommended standard dose or treat animals inappropriately, with productive animals in a herd being preferentially treated even though they may not have trypanosomosis (Roderick et al., 2000). Inappropriate use of trypanocides has also been reported in Busia District, Kenya (Machila et al., 2003) and the Eastern Province of Zambia (Van den Bossche et al., 2000). The decentralization of the drug distribution system has also promoted the sale of generic drugs (McDermott and Coleman, 2001). Most of the sales are conducted by traders and shopkeepers who do not have professional expertise to advise on the use of these products (Bett et al., 2004a).

Data on the amount and type of trypanocides used per household were collected in the trial to determine: (i) the rate and factors that influenced the use of trypanocides in the recruited herds and (ii) whether trypanocidal drug use influenced trypanosomosis incidence; therefore requiring an analytical means of controlling for their effect.

4.2. Materials and methods

4.2.1. Study areas and data collection

The study areas are as described in Section 3.2.1.

4.2.2. Data collection

The procedures used to collect data (selection of animals, animal sampling and recording the volume of drugs used by livestock owners) have been described in Section 3.2.2.

While collecting blood samples, livestock owners were always prompted to identify animals that they perceived as having had trypanosomosis. The livestock owners' diagnoses were later compared to those of the technical team.

4.2.3. Data analysis

4.2.3.1. Estimating response to trypanocidal treatments administered by the research team

Indices of effectiveness of treatments administered by the technical team at the time of sampling were estimated by generating conditional probabilities of an animal being found negative for trypanosomosis in the current month (month *i*) given that it was found positive on buffy coat technique (BCT) and therefore treated in the previous month (month *i*-1). Two types of analyses corresponding to how an animal was classified as having recovered from an infection were carried out. The first analysis used BCT test results as the only

determinant, while the second used both BCT and packed cell volume (expressed in percentage) in series, with a PCV % cut-off point being set at 26%. With series interpretation[5], only animals that test positive to both tests (BCT and PCV) are considered test positive. This approach increases specificity of the test system but decreases sensitivity. The choice of the PCV % cut-off point was based on the descriptive analyses that showed that infected animals had a mean PCV % of 24.9 with a 95% confidence interval ranging between 24.5 and 25.5. Rowlands et al. (2001) also used the same level as a cut-off point when analyzing data collected from Ghibe Valley, Ethiopia. Animals that were treated by the owners in the intervening period were excluded from the analysis.

4.2.3.2. Treatments administered by the cattle owners

4.2.3.2.1. Factors that affected the rate of treatment

Time intervals in days between successive treatments made by the owners at the animal-level were obtained and the rate of treatment was calculated as a time-to-event incidence described by the length of time between treatments. Two or more treatments could be administered to the same subject, necessitating the use of multiple failure survival analysis to identify the factors that influenced the rate of treatment. A conditional risk set model was therefore selected for this analysis with successive treatments (events) being assumed to be ordered. The baseline hazard was not constant but increased exponentially with time. Survival times were therefore assumed to follow a Weibull distribution, as this fitted the data better than an exponential distribution. In a Weibull model, the baseline hazard function has a shape that gives rise to a Weibull distribution of survival times defined by two non-negative parameters: a scale parameter,λ, and a shape parameter, p (Dohoo et al., 2003). The baseline hazard is given by:

$$h(t) = \lambda p(t)^{p-1} \qquad (4.1)$$

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[5] An alternative interpretation is referred parallel testing. It considers animals that test positive to any of the test or to both tests as test positive. Parallel interpretation increases sensitivity and decreases specificity.

The shape of the hazard function depends on the value of p. If $p = 1$, the function is flat and reduces to an exponential distribution[6]. If $p < 1$, the hazard function declines monotonically. If $p > 1$, the function increases monotonically with a value between 1 and 2 producing a curve that increases at a decreasing rate. If $p = 2$, the hazard function increases linearly with time and if $p > 2$, the function increases at an ever-increasing rate.

Factors that were expected to influence the rate of treatment are listed in Table 4.1. A variable (strata) was generated to identify failure risk groups for each treatment number and used in the model as a fixed effect to stratify the analysis as per the number of previous treatments. This variable was treated as a nuisance factor. Since observations were deemed to cluster at a herd level, a shared frailty term was included in the model to adjust for the lack of independence. The frailty term was assumed to follow a gamma distribution with a mean of 1 a variance of θ. Gamma distribution represents the sum of n exponentially distributed random variables typically defined by the shape and scale parameters that can have non-integer values. The parameters scale and shape represent the practical range and the profile of the distribution, respectively. The properties of the gamma distribution[7] include mean and variance. Mean is estimated by *scale* x *shape* and variance is given by $scale^2$ x *shape*.

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[6] An exponential model is a parametric function that assumes that the baseline hazard, $h_0(t)$ is constant over time. The model is expressed as: $h(t) = c(e^{\beta x})$, where C is the constant baseline hazard and β represent parameter values for a set of predictors. The survival probability for any individual will have a decreasing exponential distribution.

[7] Other properties of the gamma distribution include skewness given by: $2/\sqrt{shape}$ and kurtosis given by: $3 + 6/shape$

Table 4.1. Mean interval, in days, of treatments involving trypanocidal drugs administered by livestock owners recruited for the repellent evaluation trial to their own cattle in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005). The intervals are stratified by area and predictor variables

Variable	Level	Area			
		Nkineji		Nkuruman	
		n	Mean treatment interval (days)	*n*	Mean treatment interval (days)
Age	Adult	3980	123.1	2646	108.2
	Calf	1719	125.3	840	108.7
Sex	Male	1580	123.0	812	97.0
	Female	4119	125.2	2674	112.1
Body condition [a]	Lean	565	96.3	674	89.9
	Medium	3820	126.0	2094	113.0
	Fat	40	129.1	4	154.5
Group	Treatment	2881	132.7	3486	104.4
	Control	2818	116.4	2098	111.3
Season	Dry	2817	83.5	1856	121.9
	Wet	2882	164.8	1630	93.4

[a] Body condition scoring was not done for young animals

4.2.3.2.2 Proportion of positive rating by the livestock owners

Given that at least some trypanocidal treatments administered by livestock owners are inappropriate (Van den Bossche et al., 2000; Machila et al., 2003) a number of animals treated by the livestock owners might not have had trypanosomosis. The degree of agreement between the livestock owners' and the technical personnels' diagnoses was estimated using a method described by Fleiss (1981). This analysis focussed on those animals which had both the livestock owners' and the technical teams' diagnoses generated at the time of sampling. The livestock owners often identified more than one animal in a herd as being sick; each livestock owner's ratings would therefore be considered as being clustered at the herd level. The method described by Fleiss (1981) regard such outcomes as being multiple ratings, m_i, clustered per subject, n_i. Positive ratings per subject, x_i

comprised the livestock owners' diagnoses that were confirmed as being truly positive by the technical team.

The formula used to estimate the overall proportion of positive rating is:

$$\bar{p} = \frac{\sum_{i=1}^{n} x_i}{nm} \qquad (4.1)$$

where the mean number of rating per subject is given by:

$$\bar{m} = \frac{\sum_{i=1}^{n} m_i}{n} \qquad (4.2)$$

A confidence interval for the proportion was also derived as described in the text (Fleiss, 1981).

4.2.3.3. Effectiveness of treatments administered by livestock owners

The effectiveness of individual treatments administered by the recruited livestock owners could not be accurately determined because some of the treatments may have been administered to non-infected animals. Such incorrectly treated animals could have, nonetheless, given a negative test result at the time of screening. An index of effectiveness of such treatments was approximated by assessing the relationship between the number of treatments administered per herd per month and the corresponding trypanosomosis incidence assuming that a negative relationship would indicate a degree of effectiveness. A kernel smoothing function[8] was used for this purpose. Kernel estimators smooth out the

[8] Kernel smoothing function is of the form: $f(x) = \frac{1}{n}\sum_{i=1}^{n} K(\frac{x - x(i)}{h})$ where K is one of the Kernel functions that include uniform, triangle, epanechnikov, quartic, triweight, gaussian or cosinus and h is the

contribution of each observed data point over a local neighborhood of that data point. The function is a weighted average of all the data points where weights are specified using a Kernel function, *K*, and bandwidth, *h*. It gives valuable insight into the average behavior of the population over time. In this analysis, the smoothing function involved the regression of the incidence of trypanosomosis incidence on the number of treatments administered by the cattle owners. The bandwidth was 0.8 units.

4.3. Results

4.3.1. Response to treatments administered by the technical team

Table 4.2 shows the results of an assessment of the success of treatments administered by the research team at sampling time. Generally, the effectiveness of the treatments was higher in Nkuruman than in Nkineji. There was a slight general improvement of the indices of effectiveness (by not more than 5%) when BCT and PCV % were used in series to determine response to treatment. There was also insignificant variation in the effectiveness of treatments between trypanosome species.

4.3.2. Treatments administered by the cattle owners

4.3.2.1. The number of treatments and drugs used

A total of 2169 treatments were recorded by the livestock owners with 1607 being administered in Nkineji and 562 in Nkuruman. Curative treatments accounted for the majority (87.7%) of the treatments administered. Table 4.3 gives a summary of the type of the drugs used by the cattle owners over the study period.

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bandwidth. Small values of *h* give spiky estimates while large values lead to oversmoothing. Gaussian Kernel used in this analysis is of the form $\frac{1}{\sqrt{2\pi}}\exp(-\frac{1}{2}u^2)$.

Table 4.2. The number of animals found infected through two successive months (*i*, *i*+1) stratified by area and trypanosome parasite and the levels of success achieved by the treatments administered by the research team (2004 – 2005)

Area	BCT test result					Total	Crude treatment Success % [a]	Treatment Success % [b]
	Month *i*	Month *i*+1						
		T. c	T. v	Mixed	- ve			
Nkineji, Narok	T. c	46	31	0	429	506	89.5	89.5
	T. v	19	62	2	410	493	83.2	90.5
	Mixed	0	2	0	6	8	75.0	66.7
	- ve	453	391	7	7248	8099		
	Total	518	486	9	8093	9106	88.9	93.6
Nkuruman, Kajiado	T. c	6	3	0	136	145	93.8	98.9
	T. v	0	0	0	17	17	100.0	100.0
	Mixed	0	0	0	1	1		
	- ve	156	27	0	2759	2942		
	Total	162	30	0	2913	3105	93.8	97.3

T. c – *Trypanosoma congolense*
T. v – *Trypanosoma vivax*
Mixed – *T. congolense-T. vivax* co-infection
[a] Estimated treated success using BCT test to ascertain infection status
[b] Estimated treatment success using BCT test and PCV (> 26) in series to ascertain infection status
Example – in Nkineji, 46 animals were found infected with T. c in Month *i* and Month *i*+1; most likely, these animals did not respond to treatments given in Month *i*

Table 4.3. The types of drugs used by the livestock owners that participated in the tsetse repellent evaluation trial in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)

Area	Drug	Trade name	Frequency (by area)	
			n	%
Nkineji, Narok	Diminazene aceturate	Veriben®,Berenil®, Dimaze®	2, 057	91.4
	Tetracycline	Adamycin®, Alamycin®, Oxytetracyclin®	73	3.2
	Homidium chloride	Novidium®	121	5.4
		Total	2, 251	
Nkuruman, Kajiado	Diminazene aceturate	Veriben®, Diminasan®	232	36.5
	Tetracycline	Adamycin®, Alamycin®, Oxytetracyclin®	153	24.1
	Homidium chloride	Novidium®	229	36.1
	Penicillin streptomycin	PenStrept®	12	1.9
	Isometamidium chloride	Samorin®	5	0.8
		Others	4	0.6
		Total	635	

The number of treatments administered by the livestock owners varied with the day of the sampling interval (Figure 4.1). The number of treatments rose steadily after sampling but declined in about 14 – 12 days before the sampling that followed. The small number of treatments observed after screening could be related to the fact that most of the animals that were clinically sick or perceived so by cattle owners were treated at the time of screening. Towards the end of an inter-sampling interval, cattle owners seemed to reduce the rate of treatment as they were willing to wait for screening and treatments that were to be administered by the trial. This pattern did not vary with the treatment group.

The number and proportion of animals that were treated at least once by the owners by the end of the trial are shown in Table 4.4. A large proportion 60% (987/1644) of treatments was administered by the livestock owners in Nkineji than in Nkuruman where 42.5% (397/934) of the animals were treated. Most of the treatments involved the use of trypanocides.

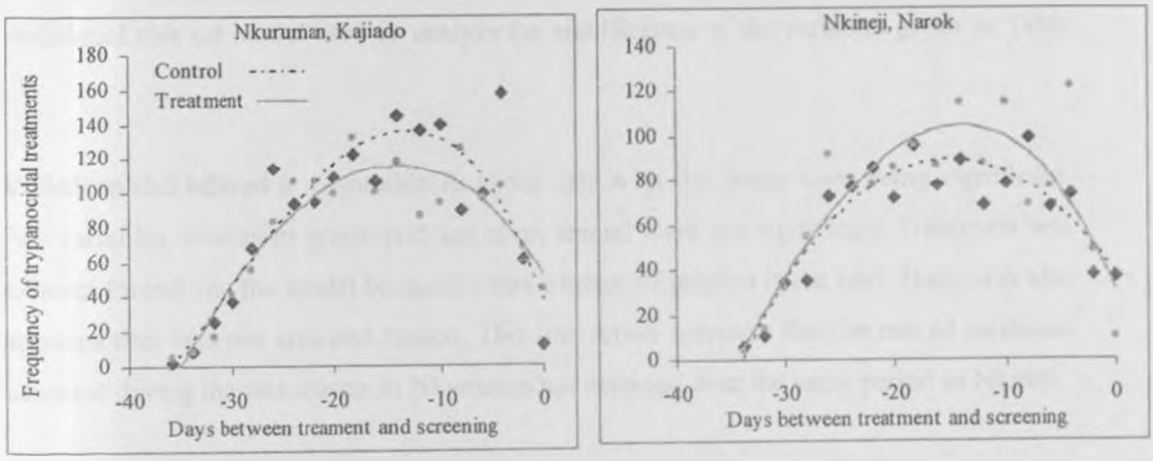


Figure 4.1. The frequency of the use of trypanocides by livestock owners recruited for the tsetse repellent evaluation trial on cattle over all the sampling intervals in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)[9].

[9] Sampling intervals approximated 30 days but livestock owners continued treating their animals through the sampling intervals. Records were always collected on the sampling day, which is why the *X*- axis indicates the number of days between treatment and sampling time (when records were collected).

Table 4.4. The proportion of cattle used in the tsetse repellent evaluation trial that were treated over a period of one year with a trypanocide or any veterinary drug in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)

Drug used	Treatment group	Area					
		Nkineji			Nkuruman		
		n	No. treated	%	*n*	No treated	%
Trypanocide	Repellent	829	529	64.0	384	159	41.4
	Control	815	458	56.2	550	238	43.3
Any vet drug	Repellent	829	547	66.0	384	179	46.6
	Control	815	467	57.3	550	271	49.3

4.3.2.2 Factors that affected the rates of treatment

The overall mean treatment interval with a trypanocidal drug was 118.5 (SD 78.7) days. The mean treatment intervals by area were 108.6 (SD 69.9) and 124.5 (SD 83.0) days in Nkuruman and Nkineji, respectively. Table 4.5 gives an output of a multivariable conditional risk set model used to analyze the significance of the variables given in Table 4.1.

Weibull model offered a reasonable fit to the data with the frailty term being significant. Two variables: treatment group and age of an animal were not significant. Treatment was however forced into the model because it was a factor of interest in the trial. There was also an interaction between area and season. This interaction indicates that the rate of treatment increased during the wet season in Nkuruman but declined over the same period in Nkineji.

Cattle owners tended to treat males more than females. They also treated lean animals more than the moderate or fat ones. There was no significant difference between the rates of treatment between moderate and fat animals.

Table 4.5. The factors that influenced the rate of trypanocide use by livestock owners recruited for tsetse repellent evaluation trial in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)

Variable	Levels	Hazard Ratio	Std Error	95% CI	*P*
Group	Treatment	1.06	0.14	0.81, 1.37	0.67
	Control	1.00			
Area	Nkuruman	0.31	0.15	0.12, 0.81	0.02
	Nkineji	1.00			
Season	Wet	0.27	0.02	0.24, 0.30	0.00
	Dry	1.00			
Sex	Male	1.12	0.07	0.99, 1.26	0.07
	Female	1.00			
Body condition	Lean	1.65	0.11	1.46, 1.87	0.00
	Medium	1.00			
	Fat	0.53	0.24	0.22, 1.29	0.16
Area*Season	Nkuruman*Wet	5.67	0.63	4.54, 7.07	0.00
Ln ρ		0.57	0.02	0.53, 0.61	0.00
Ln θ		-2.22	0.36	-2.92, -1.51	0.00
ρ		1.78		1.72, 1.83	
$1/\rho$		0.56		0.54, 0.58	
θ		0.11		0.05, 0.22	

Log likelihood =-4336.4, Number of observations: 7195, Number of failures: 1824, time at risk: 848624 days. Likelihood-ratio test of theta=0: $\chi 2$ =61.77, P = 0.000. Strata fitted as fixed effects but treated as nuisance factors.

4.3.3 Rating of the diagnoses made by the livestock owners

Diagnoses made by a total of 20 farmers were compared to those of the technical team. Out of 247 animals that the owners perceived as having had trypanosomosis, 59 were identified by the technical team as being positive. The overall proportion of positive ratings was 23.9% (95% CI: 18.6, 29.2%). Few ratings were carried out at Nkuruman, Kajiado. Only 9 subjects participated in the exercise giving a total of 45 ratings. Nine of these were positive on BCT. The estimated proportions of positive ratings per area were 20.0% and 24.8% in Nkuruman and Nkineji, respectively. These proportions were not significantly different ($P > 0.05$). Because of the small number of subjects graded at Nkuruman, this analysis

assumes the overall proportion of positive rating as being indicative of the agreement between livestock owner and the technical team's diagnoses of trypanosomosis.

4.3.4 The effectiveness of treatments administered by the cattle owners

Figure 4.2 shows the number of cases that did not respond to the treatments given by the livestock owners. The time when the treatments were administered, relative to the time of sampling is shown on the X-axis. Most of the cases were observed in Nkineji. The number of cases that failed to respond to treatment increased with the number of treatments made by cattle owners. In both areas, most of the cases that relapsed were attributed to *T. congolense* than to *T. vivax*, although in Nkuruman, *T. congolense* was the predominant causative agent. The kernel smoothing functions shown in Figure 4.3 does not show the expected negative correlation between drug use and trypanosomosis incidence. On the contrary, the curve indicates a positive relationship, implying that the rate of use of trypanocides increased with the incidence of the disease.

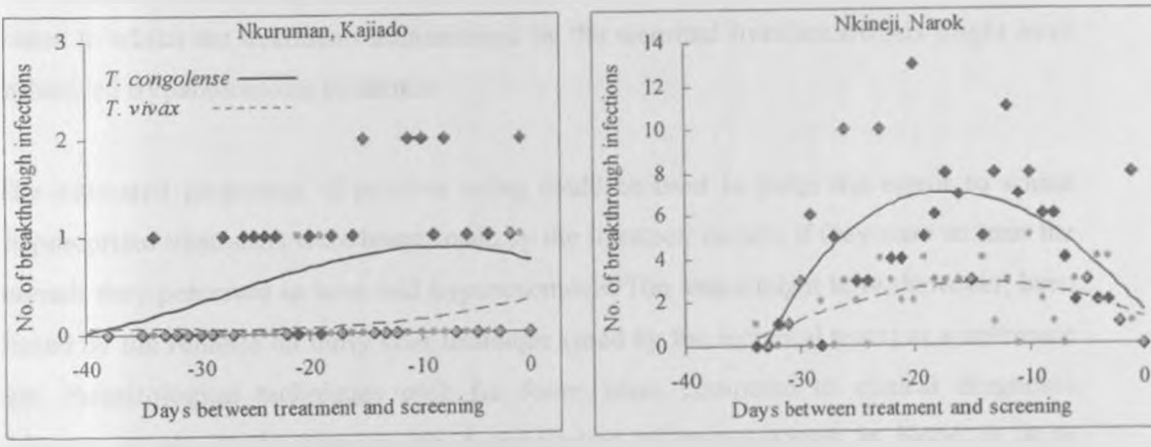


Figure 4.2. The number and type of trypanosome infections that failed to respond to trypanocidal treatments administered by the livestock owners who participated in the tsetse repellent evaluation trial (2004 – 2005)

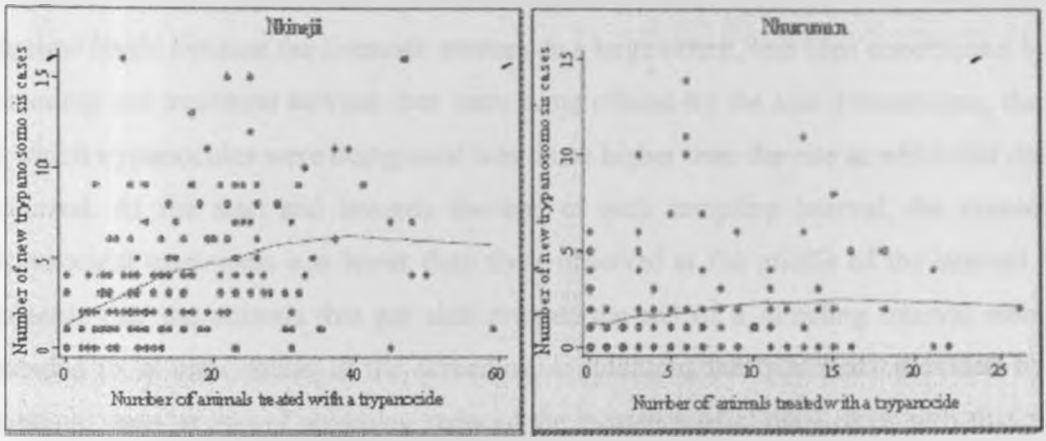


Figure 4.3. A Kernel smoothing function showing the relationship between the incidence of trypanosomosis in cattle used in the tsetse repellent evaluation trial and the number of treatments administered by the livestock owners per herd per month in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005).

4.4 Discussion

This chapter analysed the factors that influenced the rate of use of trypanocides and the extent to which the treatments administered by the recruited livestock owners might have influenced trypanosomosis incidence.

The estimated proportion of positive rating could be used to judge the extent to which inappropriate treatments were being made by the livestock owners if they were to treat the animals they perceived to have had trypanosomosis. The index might have, however, been biased by the reliance on buffy coat technique (used by the technical team) as a reference test. Parasitological techniques pick far fewer cases compared to clinical diagnoses (Magona et al., 2003) because the demonstration of trypanosomes in blood is quite unreliable as large proportions of the infections (50 – 80%) do not develop detectable levels of parasitaemia in the field (Killick-Kendrick, 1968). A study conducted by Catley et al. (2002) in Tana River, Kenya, confirmed that pastoralists' characterization of trypanosomosis is highly correlated with the standard clinical picture of the disease.

The rate of use of trypanocides estimated in the study were expected to be lower than the baseline levels because the livestock owners, to a large extent, had been conditioned by the screening and treatment services that were being offered by the trial. Nonetheless, the rate at which trypanocides were being used was much higher than the rate at which the disease occurred. At the start and towards the end of each sampling interval, the number of trypanocidal treatments was lower than those observed at the middle of the interval. It is probable that the animals that got sick towards the end of a sampling interval were not attended to in anticipation of the screening. In addition, the treatments provided by the technical team as part of screening reduced the incidence of clinical cases such that at the beginning of a screening interval, very few animals needed to be treated. The commonly used drug was diminazene aceturate; most cattle owners thought that prophylactic drugs caused negative side effects like swelling at injection sites. Prophylactic drugs were therefore used sparingly, particularly in dry seasons when livestock owners regarded their animals as being weak to withstand the side effects of the drugs.

Factors that significantly affected the rate of treatment include body condition and sex of an animal and an interaction between area and season. Lean animals were treated more frequently than those that were in better body condition, probably because they were perceived to be sick. It is also probable that the lean animals that had trypanosomosis were repeatedly treated over time because most livestock owners administer trypanocides without supportive therapy. Lean animals would particularly take more time to respond to such treatments, prompting the owners to administer more trypanocides based on the false belief that such cases would still have trypanosomosis. Even though this practice can be judged to be inappropriate, treating clinically sick animals may be an effective way of combating trypanosomosis in absence of alternative methods of diagnosis in trypanosomosis endemic areas (Van den Bossche et al., 2000). It would, however, require the sensitization of livestock owners on the need to include supportive therapies such as multivitamins on such treatments. Males were also treated at a significantly higher rate than females. Although males are more susceptible to trypanosomosis than females (Rowlands, et al. 2001), some of the treatments could have been inappropriate because the livestock owners highly regarded male animals, especially the ones that were being used for

breeding. Van den Bossche et al. (2000) found a similar practice in Eastern Zambia where farmers preferred treating productive animals in a herd.

Controlling for the area, the differences in tsetse challenge between villages did not affect the rate of drug use. This is in tandem with the practice of treating lean or clinically sick animals rather than those infected with trypanosomes. This is more or less similar to the findings published by Van den Bossche et al. (2000) where there was no difference in the amount of trypanocides used between a tsetse-infested and a tsetse-controlled area. From the significant interaction between area and season, it can be inferred that cattle owners reacted to seasonal variation in disease risk. At Nkuruman, tsetse population and hence trypanosomosis incidence, increases in the wet season and declines in the dry season. At Nkineji, there are no major changes in tsetse density with season but the incidence of the disease increases during the dry season because livestock are driven towards high challenge areas in search of pastures.

The high indices of effectiveness of treatments administered by the technical team demonstrates that most of the trypanosome parasites found in the study areas were sensitive to the trypanocides that were being used. The effectiveness of the treatments was negatively correlated with tsetse challenge because these treatments were more effective at Nkuruman (where the tsetse challenge was low) than at Nkineji (where the tsetse challenge was high). High tsetse challenge increases the rates of re-infection. It has been suggested that diminazene aceturate has a prophylactic period of about 21 days but this depends on the sensitivity of the parasites to diminazene and the dose of drug used (Peregrine and Mamman, 1993).

The positive association between livestock owner treatments and trypanosomosis incidence implies that treatments administered by the livestock owners might not have significantly influenced trypanosomosis incidence. A small proportion of treatments made by the cattle owners were also unable to clear the infection. Relapses were mostly caused by *T. congolense*, a parasite that has been incriminated in many cases of drug resistance, for instance in Ghibe valley (Mulugeta et al., 1997). This implies that *T. congelense* has a

lower sensitivity to trypanocides that *T. vivax*. Such infections re-established as soon as the drug levels declined. Given the high indices of effectiveness of treatments administered by the technical team, it can be observed that the low levels of effectiveness of treatments administered by the stock owners might not have been due to re-infection. This is because the treatments made by the stock owners had lasted shorted than those of the technical team.

This analysis confirms that the rate of use of trypanocides by the stock owners did not vary by the treatment group. It also shows that some of these treatments were not effective. This implies that including the rate of use of trypanocides by the stock owners in the analysis of the effectiveness of the tsetse repellent technology may not be beneficial.

Chapter 5

Assessing the effectiveness of the synthetic tsetse repellent technology on trypanosomosis incidence in cattle

5.1. Introduction

A new therapeutic or prophylactic compound undergoes four phases of research before its effectiveness can be ascertained. These requirements may, however, vary with the country (Dohoo et al., 2003). The development and evaluation of the synthetic repellent technology followed this pathway. The present study, classified as Phase III trials, focused on the determination of the effectiveness of the technology in a representative situation on farm. It was initiated after the completion of Phase I and II studies that successively assessed the safety of the repellent (toxicological studies) and documented its effect in a small number of animals from the target population. Toxicological assessment showed that the repellent does not have adverse effects on the exposed animals (ILRI/TRC/ICIPE, 2004) while controlled trials conducted by ICIPE at Nkuruman, Kenya showed that the synthetic tsetse repellent technology significantly reduced tsetse challenge by more than 80% (ILRI/ICIPE, 2001; ILRI/TRC/ICIPE, 2003).

Apart from assessing the effectiveness of the technology, the present trial also assessed a new treatment strategy that had not been tested before of treating all the animals in a herd. In the previous trials, up to 75% of the animals in a herd had been treated.

5.2. Materials and methods

5.2.1. Study area and sample size estimation

A description of the study area, methods used in the estimation of the sample size, selection of villages and study herds and collection of blood samples are described in Sections 3.2.1 – 3.2.2.

5.2.2. Examination and refilling of the repellent dispensers

Although it was expected that the prototype repellent dispenser (Plate 5.1) would hold the repellent for at least a month after being filled, it was realized early in the trial that the dispenser design was sensitive to abrasions and tension, especially at the point of diffusion. Most of the damage recorded involved the loss of the silicon-tygon tube, stoppers and at times the loss of the whole collar. At the time of sampling, the state of the repellent dispensers and the amount of the repellent that it contained were recorded. Various levels of dispenser damage or loss were recorded as: loss of whole collar, loss of aluminium reservoir, cut silicon-tygon tube or loss of a stopper. Depending on the level of damage of the repellent dispenser observed at the end of every sampling interval, each animal was put into different protection levels listed in Table 5.2. The damaged or lost dispensers were replaced or repaired *in situ* depending on the extent of damage.

Table 5.1. A scheme used to classify the levels of protection of each animal treated with the repellent technology as at the end of a sampling interval based on the state of the repellent dispenser (2004 – 2005)

Level of protection	Description
Good	Includes animals that had intact dispensers with the two reservoirs having the repellent at the time of sampling. This classification ignores the variation in the amount of the repellent found in the reservoir since the diffusion parameters of the repellent were shown to follow first order kinetics (ILRI/ICIPE, 2004). It has, however, been suggested that the diffusion of the repellent under field conditions may be faster due to animal agitation than that measured in the lab (Andoke, per. comm.). No data are currently available to support this hypothesis.
Moderate (one reservoir empty)	Includes animals whose dispensers had the repellent in one of the two reservoirs at the time of sampling. The other reservoir could be intact but empty or totally damaged. This damage could be in any part of the reservoir that would allow leakage of the repellent.
Poor (Both reservoirs empty)	Includes animals whose dispensers had no repellent in both reservoirs at the time of sampling. Two types of reservoir defects could be put into this category: (1) the dispensers that had reservoirs damaged (usually at the diffusion point) therefore allowing the repellent to leak, or (2) those that were intact but empty (could be due to faster rate of release of the repellent). The proportion of animals with dispensers found intact but empty ranged between 13 and 25% of the total found empty. Leakage of the repellent (following damage of the reservoir) therefore accounted for a large proportion of those classified into this category.
Poor (Lost/stolen dispensers)	Includes animals whose dispensers got lost or stolen over the sampling interval The dispensers were replaced at the time of sampling.

5.2.3 Data analysis

Given the frailties of the repellent dispenser, the analysis was conducted in two stages. In the first stage, analyses were conducted without controlling for the defects of the dispenser. The subsequent analyses conducted (stage 2) were geared towards evaluating whether the defects of the dispenser could have introduced some bias in the estimation of the repellent effect.

5.2.3.1. The outcome of interest

Trypanosomosis incidence rate[10] was used as the main outcome of interest. The incidence was derived at the animal-level using both the first positive cases and all the infections identified over the follow up period using the buffy coat technique (BCT). The later case included cumulative number of positive cases observed over the follow up period was obtained. The estimates of trypanosomosis incidences were derived utilizing the animal-years-at-risk as an offset, with no distinction being made between cases caused by the different species of trypanosomes. Incidence rate (I) was derived using the formula (Dohoo et al., 2003):

$$I = \frac{\text{number of cases of disease (in this case tryps) in a defined time period}}{\text{number of animal-time units at risk during the time period}} \quad (5.1)$$

For the derivation of the animal-time at risk, the time difference (in years) between the dates when each subject was recruited and when it was (i) found positive for trypanosomosis, (ii) withdrawn from the study, or (iii) when the study was terminated (whichever of the events was achieved earlier) was calculated. While estimating the incidence using the first occurrences of the disease, the upper limit of the animal-time at risk was marked by the time when an animal was found infected for the first time. For the incidence derived using multiple infections, the animal-time at risk was terminated at a time when an animal was found with the final infection. A possible reduction in the risk of subsequent infections following treatment of the earlier cases was ignored when deriving animal-time at risk for repeated infections because diminazene aceturate, the trypanocide used in the trial, exhibits a prophylactic activity in cattle for periods varying from a few days to a few weeks (Peregrine and Mamman, 1993). Data collected in the first month of the trial were also excluded from the analysis because the observed level of damage of the

[10] An incidence rate is the number of new cases of disease in a population per unit of animal-time during a given time period (Dohoo et al., 2003). It a positive measure without an upper bound. Incidence rates are sometimes referred to as incidence density.

dispensers was very high during this time compared to those observed at subsequent sampling periods.

5.2.3.2. Analyzing the effect of the repellent while ignoring the defects of the dispenser

5.2.3.2.1. Descriptive statistics

Descriptive statistics showing the number and sizes of the herds used in the trial are shown in Table 5.3. In the course of the trial, it was found out that a total of six herds (three at each site) that had been presented by the owners as being single units were made up of at least two subunits that were always being managed separately. These subunits were treated as independent herds, making the total number of herds used to be 30.

Table 5.2. Baseline characteristics of the herds used in the tsetse repellent evaluation trial conducted in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)

Characteristic		Area			
		Nkuruman, Kajiado		Nkineji, Narok	
		Treatment	Control	Treatment	Control
Number of herds		7	8	7	8
Herd size [a]	< 50	6 (31)	5 (27)	1 (34)	2 (25)
	50 – 99	1 (51)	3 (76)	3 (66)	5 (79)
	≥ 100	-	-	3 (134)	1 (101)
Baseline trypanosomosis incidence [b]		6.5 (4.9, 8.7)	6.1 (4.8, 7.8)	8.0 (6.8, 9.3)	8.1 (7.1, 9.4)

[a] Herd size indicated by number of animals within herds. Average herd size enclosed by the parentheses
[b] % incidence and 95% Confidence interval estimated over the baseline phase of the trial

5.2.3.2.2. Multivariable analysis

Multivariable analysis was conducted using a Poisson regression model. The model falls in the class of generalized linear models that allows the linearization of counts of outcome through a log link, therefore connecting a linear predictor, η_{ij} to the response, Y_{ij} that has a

mean μ_{ij}. In this group of models, the mean and the variance of the outcome are related, and could be represented as:

$$\text{var}(Y_{ij}) = \phi\mu_{ij} \qquad (5.2)$$

where ϕ is a dispersion parameter (Dohoo et al., 2003). For Y_{ij} to fit the Poisson distribution, ϕ must be equal to one. The data are, however, said to be overdispersed if ϕ is greater than one or underdispersed if ϕ is less than one. The dependent variable captured multiple infections over time.

A naïve Poisson model was used to explore the data using the predictors listed in Table 5.4. This model showed that the data (for both the first and all cases of trypanosomosis as outcomes) were overdispersed because the unadjusted variance of counts of cases was much higher than the mean. The analysis, therefore, proceeded to utilize a marginal model that has been developed for analyzing data with extra-Poisson variation. Both unadjusted and adjusted incidence rate ratios were derived using this model with unadjusted analyses being run with a single independent variable at a time. The models used general estimation equations (GEE) that incorporate a working correlation matrix to adjust for repeated measures in time. An auto-regressive correlation matrix was deemed appropriate for the data. The standard errors were adjusted using Huber White Sandwich variance estimator (Caroll et al., 1998). Following this approach, the parameter values would give reliable estimates of population effects even if the correlation and (or) variance components are wrongly specified.

Table 5.3. Descriptive statistics and crude incidence rates at the levels of covariates that were considered for multivariate analysis of the effect of the repellent technology on trypanosomosis incidence in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)

Variable	Levels	Method of estimating trypanosomosis incidence					
		Using the first cases only			Using all the cases observed		
		Cases	Animal -years	Incidence (95% CI)	Cases	Animal-years	Incidence (95% CI)
Treatment	Repellent	403	506	0.80 (0.72, 0.88)	546	557	0.98 (0.90, 1.07)
	Control	473	458	1.03 (0.94, 1.13)	647	512	1.26 (1.17, 1.30)
Age [a]	Calf	258	279	0.92 (0.82, 1.04)	345	305	1.13 (1.01, 1.26)
	Adult	618	685	0.90 (0.83, 0.98)	848	764	1.11 (1.04, 1.19)
Color [b]	Light	186	175	1.06 (0.92, 1.23)	258	196	1.31 (1.16, 1.49)
	Dark	690	789	0.87 (0.81, 0.94)	935	873	1.07 (1.00, 1.14)
Sex	Male	248	243	1.02 (0.90, 1.16)	343	272	1.26 (1.13, 1.40)
	Female	628	721	0.87 (0.80, 0.94)	850	798	1.07 (0.99, 1.14)
Season	Wet	387	433	0.89 (0.81, 0.99)	616	509	1.21 (1.12, 1.31)
	Dry	489	531	0.92 (0.84, 1.01)	577	561	1.03 (0.95, 1.12)
Area	Nkuruman	246	322	0.76 (0.67, 0.87)	294	338	0.87 (0.77, 0.96)
	Nkineji	630	642	0.98 (0.91, 1.06)	899	731	1.22 (1.15, 1.31)

[a] Age is categorized into two depending on feeding management. Adults include animals which were always being taken out to distant fields for grazing while calves were always confined around homesteads.

[b] Dark animals include those which had complete black or brown complexion, while light include all the other colours, e.g. white, roan, etc.

The independent factors studied were treatment, age, sex, colour, herd size, season, and area. Treatment was specified as repellent or control, age as calf or adult, colour of an animal as either dark or light, herd size as a continuous variable and season as either wet or dry, depending on rainfall density. Age was classified depending on the feeding management of animals. Adults included the animals that were always grazed out in the communal grazing fields while calves included all the young animals that were always confined around homesteads. The different colours of animals were first analyzed as a four-level variable (brown, black, white and others) but afterwards, it was collapsed into dark

(complete brown or black or shades of brown or black) and light (white, dirty white, roan, multiple colours which include white/dirty white) when the original form of the variable was found to be insignificant. Wet season included the months of March, April, May, June, July and August in Nkineji and December, March, April and May in Nkuruman. Disease incidence is expected to be higher during the wet than dry season because in the wet season, tsetse density increases due to improved survival rates of the flies.

Village was included as a fixed effect (as discussed in Chapter 3) to adjust for clustering within villages. Area – i.e. Nkuruman versus Nkineji - represented environmental or management factors that may not have been explicitly captured by the other variables, e.g. variation in the composition of tsetse and trypanosome parasite species. Using the village as a fixed effect made area to be insignificant in the models. Area was, however, retained in both models to control for ecological differences between Nkineji, Narok and Nkuruman, Kajiado. The parameter estimates generated for the levels of village were treated as nuisance[11] factors. Herd size was fitted as a continuous variable in the models. Its relationships with the dependent variables were tested by fitting quadratic terms involving the variable in each model. These quadratic terms were not significant.

5.2.3.3. Analyzing the effect of the repellent while accounting for dispenser defects

5.2.3.3.1. The defects of the repellent dispenser

The distribution of the protection levels described in Table 5.2 was stratified by area and plotted over time.

5.2.3.3.2. Descriptive analysis on the association between protection levels and trypanosomosis risk

[11] Nuisance parameters include estimates that are not the primary focus of the study even though they have important effects. The variable is specified correctly but the estimates may not be shown in the output table.

Given that following an infective tsetse bite, an animal would take a period of about two weeks to manifest trypanosomosis, it is likely that infections diagnosed in any one sampling period could have been acquired in a period of not more than two months before sampling, especially between 15 and 45 days before the monitoring event. Moreover, the high rate of use of trypanocides by the cattle owners (discussed in Chapter 4) might have also reduced the length of time an animal harboured a patent infection.

Repellent-treated animals were classified into nine categories depending on the state of the dispenser through any two months, current (month *i*) and previous (month *i*-1). Follow-up periods were not allowed to overlap (i.e., the periods considered were 1-2, 3-4, 5-6, 7-8, 9-10, 11-12 were considered). The trypanosomosis risk associated with each category was derived as the proportion of animals found positive for trypanosomosis at the monitoring event at the end of month *i*, of the total animals classified into that category. This analysis was also stratified by village. Baseline trypanosomosis risk was derived from the control group and used as a reference for conducting a χ^2 test to explore whether the disease incidence among the animals in each category of dispenser status was significantly different from the disease incidence among the control animals.

The effectiveness of the technology in protecting a treated animal may depend on the strength of the repellent odour plume created at the herd-level (Hassanali, personal communication). To assess whether this principle could have influenced the results obtained from this analysis, the correlation between herd- and animal-level protection levels was investigated using box and whisker plots. Herd-level protection was represented by the proportion of animals, P_R, that had the repellent at the end of the month, taking into consideration the number that had good dispensers and those that had ones with one reservoir empty. The relationship between protection level and trypanosomosis risk at the animal level was further stratified by the proportion of animals that had the repellent at the herd level.

The proportion of animals that had the repellent at the end of a sampling interval was weighted using:

$$P_R = \frac{GD + (0.5 \cdot OE)}{TD} \qquad (5.3)$$

where:

GD - refers to the number of animals that had good dispensers at the end of month *i*

OE – the number of animals that had dispensers with one reservoir empty at the end of month *i*

TD – Total number of animals in a herd that were treated at the start of month *i*.

5.2.3.3.3. Multivariable analysis

Survival analysis was used to analyze the effect of the repellent on trypanosomosis incidence at animal level while: (i) accounting for the defects of the dispenser, and (ii), adjusting for other independent variables. This analysis was simplified through the use of the first cases only to estimate incidence.

Survival analysis takes into account the amount of time a subject contributes to a study before it realizes an event of interest (hazard, h_o) or is lost to follow-up (Dohoo et al., 2003). A proportional hazard's form of the Weibull model was used for this analysis because it fitted the data better than the Cox proportional hazards model that ignores the functional form of the baseline hazard. The exponential model could not also be used because the parametric distribution of the baseline hazard (dependent on time but independent of predictors) increased with time (Figure 5.1). This distribution was generated by estimating piece-wise baseline hazards using an exponential model at time intervals: 1-2, 3-4, 5-6, 7-8, 9-10 and 11-12 months and plotting them against time. The distribution of the baseline hazard is similar to the pattern displayed in Figure 5.1 where trypanosomosis incidence increased with time, more so at Nkuruman than Nkineji. Only the first occurrences of infections were used in the derivation of outcome.

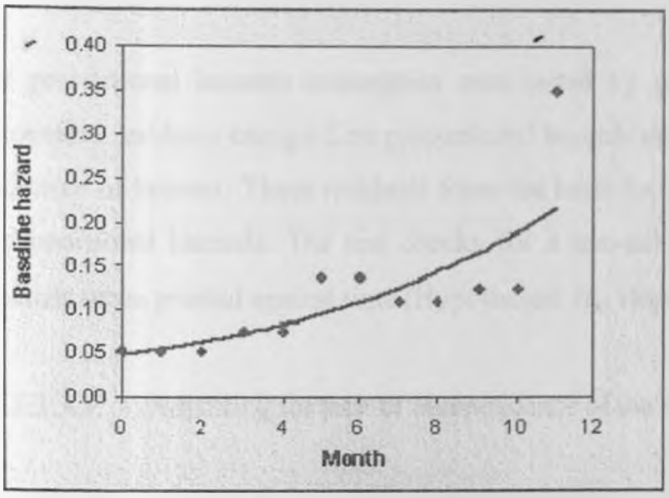


Figure 5.1. The distribution of the baseline hazard of trypanosomosis in cattle used in the tsetse repellent evaluation trial conducted in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005).

5.2.3.3.3.1 Predictors

The factors considered in the analysis include repellent treatment, sex, age and colour of an animal, season, herd size and area. Village was also included as a fixed effect and its parameters treated as nuisance factors. Crude hazard ratios for the likelihood of new trypanosomosis infections were generated. For categorical variables, the levels that had most records served as the reference (reference cell coding).

Treatment variable had four levels. One of these was the control while the repellent treatment group was classified into: good, one reservoir empty and both reservoir empty depending on the status of the dispenser at the time of sampling. Larger animals attract more tsetse flies (Torr et al., 2001) and so males and older animals are expected to experience relatively higher risk of the disease than females or younger animals. Likelihood ratio tests were used to select variables that were significant at an alpha level of 0.05.

5.2.3.3.3.2. Proportional hazards assumption

The proportional hazards assumption was tested by generating Schoenfeld and scaled Schoenfeld residuals using a Cox proportional hazards model (Dohoo et. al., 2003) that had predictors of interest. These residuals form the basis for a statistical test of the assumption of proportional hazards. The test checks for a non-zero slope of the scaled Schoenfeld residuals when plotted against time (Hypotheses: H_a: slope $\neq 0$ and H_o: slope $= 0$).

5.2.3.3.3.3. Adjusting for lack of independence of the survival times

Due to the fact that all animals in a herd had to be treated (i.e., carry the repellent collar) to maximize the repellent effect, the survival times for subjects within treated herds were not independently distributed. Other management practices such as grazing patterns also contribute to within-herd correlation between animals. This correlation was accounted for by adding a shared gamma distributed frailty term, α, to the Weibull model that is assumed to have a mean of 1 and variance of θ. Hazards at any point in time were therefore products of α since in a frailty model, an unmeasured latent effect (random effect) is assumed to act multiplicatively on the hazard (Dohoo et. al., 2003).

5.2.4. Potential sources of bias

5.2.4.1. Lack of blinding

No attempt was made to blind the treatments from the farmers, the research technicians, or the researchers themselves. The need for blinding had been considered during the field trial design, but had been rejected for two main practical reasons:

a) The repellent odour is quite distinct, and it was not considered possible to create a placebo liquid that would be indistinguishable in odour from the repellent;

b) There were insufficient collar and dispenser equipment supplies to permit treating control herds with collars containing a placebo liquid without having to reduce the sample size substantially.

The lack of blinding could contribute potential bias in several ways, including:

a) Researchers and research technicians might have been more rigorous in their evaluation of the treatment animals, thereby detecting more disease than in the control herds (negative bias);

b) Researchers and research technicians might have been expecting (and wanting) to find significant difference in the treatment animals, and err in measurements in favour of that difference (positive);

c) Cattle owners might pay more careful attention to treated animals, benefiting the herd health (positive); and

d) Cattle owners might have pushed treated herds to high challenge areas reducing the expected effectiveness of the repellent (negative).

The epidemiological team that implemented field activities was independent from the one that developed the technology, the risk of bias was therefore considered to be low.

5.2.4.2. Shared grazing fields

Under traditional management practices, individual herds are sometimes mixed with other herds during grazing or watering. There was concern that this practice might influence the results by offering tsetse flies immediate alternative targets when faced with treated animals, or by untreated animals attracting flies to the treated herd. Treated herds that were more likely to be mixed with others were identified and in Narok, a control herd that was always found mixed with a treatment herd was dropped from the study. The effect of mixing treated and untreated herds on the efficacy of the repellent was assessed through sensitivity analyses where one set of analyses were conducted using all the records from all the herds and the other used the records from only those herds that were always found grazing independently. The outputs of these two analyses were then compared to gauge the extent to which mixing of herds biased the results.

5.2.4.3. Missing data

Missing or incomplete data is a common problem in longitudinal studies. Peduzzi et al. (2002) defined three types of missing data: missing completely at random (MCAR); missing at random (MAR); and missing not at random (MNAR). The MCAR occurs when "missingness" is independent of the outcome of interest, unlike MAR where subjects are censored depending on observed data, for example eliminating subjects based on observed criteria. MNAR, on the other hand, depends on both the observed and missing data. This occurs when a subject drops out of a study because of deterioration of its condition. Most of the missing data observed in this study can be classified under MCAR. Such data were excluded from the analysis. Dropping such data from analysis does not affect validity, unlike eliminating MAR or MNAR data (Peduzzi at al., 2002).

5.2.4.4. Information bias

Information bias relates to the effects of incorrectly classifying or measuring the study subjects' exposure, extraneous factors and/or outcome status. For categorical outcomes such as the disease status, the resultant bias is called misclassification bias. In this study, the use of BCT as a diagnostic test to determine the trypanosomosis status of an animal could have introduced non-differential misclassification because the test has a low sensitivity (Killick-Kendrick, 1968). However, the validity of the results obtained from the test was ascertained by evaluating the consistency with known biological knowledge of the relationship between the other independent variables and trypanosomosis incidence.

5.3. Results

5.3.1. Trypanosomosis incidence

Considering only the first cases, a total of 876 new cases were identified over a period of 964 animal-years. From this, the overall trypanosomosis incidence was 0.91 (95% CI: 0.86, 0.96) per animal per year. The overall incidences in the treatment and control groups are given in Table 5.4. In Nkineji, the respective incidences in the treatment and control groups were 0.85 (322 cases/378 animal-years [95% CI: 0.76, 0.95]) and 1.17 (308 cases/264 animal-years [95% CI: 1.04, 1.30]), while in Nkuruman, they were 0.63 (81 cases/128 animal-years [95% CI: 0.50, 0.79]) and 0.85 (165 cases/194 animal-years [95% CI: 0.73, 0.99]).

When multiple infections were considered, a total of 1193 cases and 1069 animal-years were obtained giving an overall incidence of 1.12 (95% CI: 1.05, 1.18). In Nkineji, the incidences in the treatment and control group were 1.06 (452 cases/425 animal-years [95% CI: 1.06, 1.17]) and 1.46 (447 cases/306 animal-years [95% CI: 1.33, 1.60]), respectively. In Nkuruman, the incidences were 0.71 (94 cases/133 animal-years [95% CI: 0.57, 0.86]) and 0.97 (200 cases/206 animal-years [95% CI: 0.84, 1.12]) in the treatment and control herds, respectively.

Figure 5.2 shows the trend in disease incidence in treatment and control groups at the two study areas. In the figure, trypanosomosis incidence is derived using the first cases only. Also shown by the light-coloured broken curve is the transformed (using log (n+1)) average number of tsetse per trap per day (FTD). The incidence of the disease in the two groups of animals was fairly comparable over the baseline period and the blanket treatment of animals with diminazene aceturate in Month 0 did not substantially affect the incidence of the disease in Month 1. Thereafter, trypanosomosis incidence was generally lower in the treatment than in the control group for most of the period. In Nkuruman, the changes in disease incidence closely followed that of the fly density whereas in Nkineji, the disease incidence was more or less constant, except the spike observed in the control group in the Months 5-9 despite a decline in FTD over the same period. The figure shown in Appendix I gives the incidence at the village level. This figure shows that there was no village-to-village variation in the difference in disease incidence between the treatment groups.

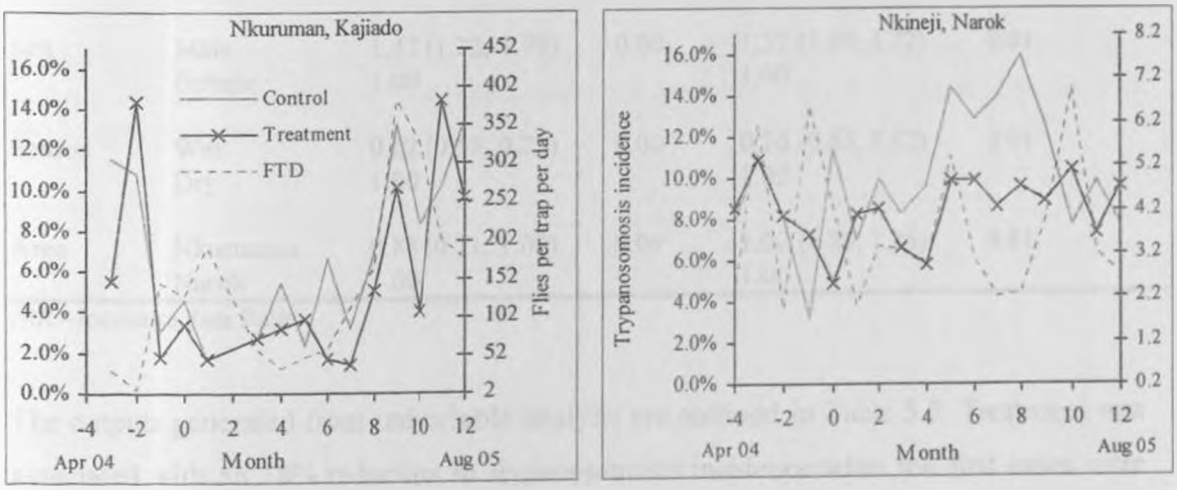


Figure 5.2. Monthly incidence of trypanosomosis in cattle used for tsetse repellent evaluation trial and transformed apparent tsetse density (Flies per trap per day) in the areas where the study was being carried out in Narok (Nkineji) and Kajiado (Nkuruman) Districts (2004 – 2005).

5.3.1.1. Analysis of trypanosomosis incidence while ignoring the defects of the dispenser

Table 5.5 shows unadjusted effect of treatment, together with those of the other variables used in the analysis.

Table 5.4. Crude incidence rate ratios at the levels of categorical variables measured in the tsetse repellent evaluation trial carried out in Nkineji, Narok and Nkuruman, Kajiado estimated using the GEE model while considering one variable at a time

Variables	Level	Method of estimating trypanosomosis incidence			
		Using first cases only		Using all the cases acquired	
		IRR (95% CI)	*P*>|Z|	*IRR* (95% CI)	*P*>|Z|
Treatment	Repellent	0.82 (0.69, 0.97)	0.02	0.86 (0.71, 1.06)	0.16
	Control	1.00		1.00	
Colour	Light	1.12 (0.90, 1.38)	0.31	1.10 (0.86, 1.40)	0.44
	Dark	1.00		1.00	
Age	Calf	1.34 (1.11, 1.62)	0.00	1.14 (0.91, 1.43)	0.26
	Adult	1.00		1.00	
Sex	Male	1.47 (1.22, 1.79)	0.00	1.37 (1.09, 1.72)	0.01
	Female	1.00		1.00	
Season	Wet	0.62 (0.53, 0.72)	0.00	0.76 (0.63, 0.92)	0.01
	Dry	1.00		1.00	
Area	Nkuruman	0.85 (0.71, 1.02)	0.09	1.02 (0.83, 1.26)	0.81
	Narok	1.00		1.00	

IRR – Incidence Rate Ratio

The outputs generated from univariable analysis are outlined in Table 5.5. Treatment was associated with an 18% reduction in trypanosomosis incidence when the first cases were used to estimate the incidence of the disease. This is slightly better than the 14% reduction in disease incidence when the outcome included multiple cases observed over the follow-up period. This effect (i.e., unadjusted for covariates and auto-correlation in the data) was significant only for the case where the outcome comprised the first cases of trypanosomosis. The covariates whose effects were significant while using the first cases as an outcome were males compared females, dry season versus wet season, and being a calf

as opposed to being an adult (Table 5.5). Only two variables – sex and season – were significant when the outcome considered all the infections each subject got over the follow up period.

Tables 5.6 and 5.7 show the adjusted effect of the repellent estimated using a GEE model with the outcomes being the first and all the cases of trypanosomosis acquired by each subject over the follow-up period, respectively. The variables retained in the model based on their significance include sex, season, area and an interaction between area and season. As described above, village was included as a fixed effect in both models but the parameters estimated were treated as nuisance parameters. Irrespective of the outcome variable used, the adjusted effect of the technology was not significant. Moreover, the adjusted effects were approximately equal, at about 14% reduction in disease incidence. The models further show that while controlling for the effect of other covariates, males had a higher incidence of the disease than females. The interaction between area and season indicates that trypanosomosis incidence declined over the wet compared to the dry season in Narok but remained almost at the same level at Nkuruman.

Table 5.5. Adjusted incidence rate ratios obtained from a GEE model with the outcome being the incidence of trypanosomosis derived from first cases observed in the tsetse repellent evaluation trial (2004 – 2005)

Variable	Level	Incidence Rate Ratio (IRR)			$P>\|Z\|$
		Estimate	SE	95% CI	
Treatment	Repellent	0.87	0.06	0.72, 1.04	0.13
	Control	1.00			
Sex	Male	1.48	0.14	1.24, 1.78	0.00
	Female	1.00			
Season	Wet	0.51	0.05	0.42, 0.62	0.00
	Dry	1.00			
Area	Kajiado	1.01	0.54	0.36, 2.85	0.98
	Narok	1.00			
Area x Season	Kajiado x Wet season	1.97	0.37	1.36, 2.83	0.00

Scale parameter = 1; Wald $\chi2(16) = 199.4$, $P = 0.00$, Standard errors scaled using robust variance estimator

Table 5.6. Adjusted incidence rate ratios obtained from a GEE model with the outcome being the incidence of trypanosomosis derived from multiple cases observed in the tsetse repellent evaluation trial (2004 – 2005)

Variable	Level	Incidence Rate Ratio			*P*>|Z|
		Estimate	SE	95% CI	
Treatment	Repellent	0.86	0.10	0.69, 1.08	0.20
	Control	1.00			
Sex	Male	1.42	0.17	1.13, 1.78	0.00
	Female	1.00			
Season	Wet	0.67	0.09	0.53, 0.86	0.00
	Dry	1.00			
Area	Kajiado	1.51	0.84	0.51, 4.46	0.45
	Narok	1.00			
Area x Season	Kajiado x Wet season	1.49	0.33	0.97, 2.30	0.07

Scale parameter = 1; Wald $\chi 2(16) = 83.3$, $P = 0.00$, Standard errors scaled using robust variance estimator

5.3.2 Analysis of the defects of the repellent dispenser

5.3.2.1 Distribution of the dispenser defects

The distribution of dispenser defects by area over the study period is shown in Figure 5.3. The proportion of animals that had good collars never exceeded the 30% level observed in Nkineji between months 3 and 6. In the first month of the trial, the level of damage was very high in both areas because at the time of sampling as none of the animals had good protection. Only 2.1% of the animals had moderate protection, 79.7% had poor protection related to damaged reservoirs and the rest 18.2% had poor protection related to lost dispensers.

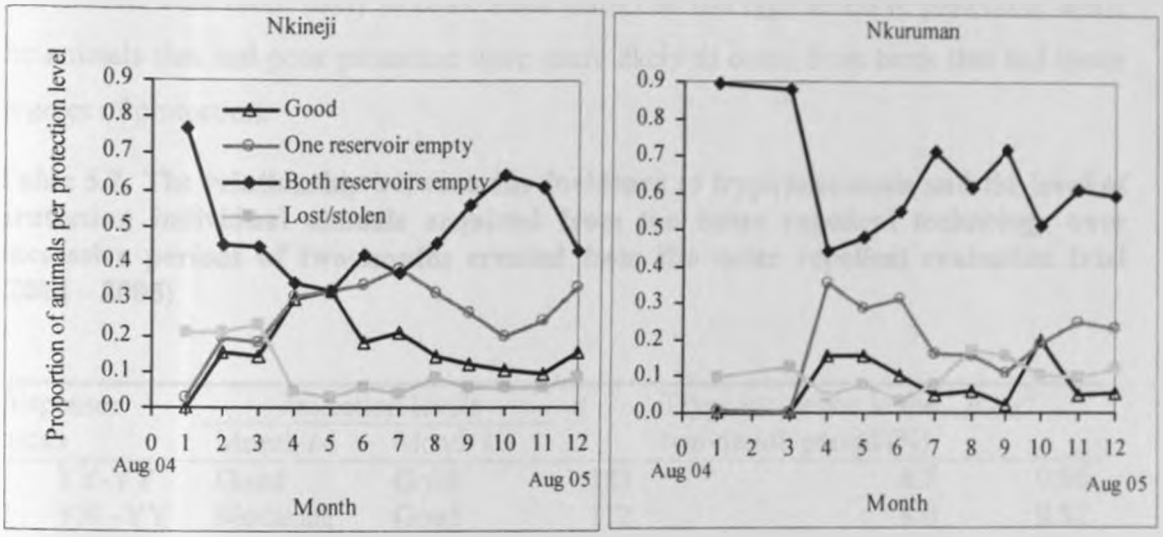


Figure 5.3. The distribution of the states of the repellent dispenser at each monthly monitoring event over the longitudinal phase of the tsetse repellent evaluation trial carried out in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005).

5.3.2.2. Association between the level of protection and trypanosomosis risk at the animal level

The distribution of the repellent-treated animals into the various levels of protection over two-month periods (Table 5.8) reveals that few animals were found having a good level of protection (dispenser classified as YY-YY) through the two inspection times. Such animals did not, however, have a lower risk of trypanosomosis compared to those that had damaged dispensers (classified under moderate and poor levels of protection). There was also no inverse relationship between the presumed varying levels of protection associated with the different dispenser states and trypanosomosis risk. Four categories (YY-NN, YN-YN, YN-NN and NN-YN) had significantly lower trypanosomosis risk compared to the control group. These categories, however, included animals that had defective dispensers at some stage of the risk period and so were considered to have had moderate protection. Multiple comparisons using the χ^2 test might, however, increase the error margin.

There was a positive correlation between the levels of protection at the herd- and the animal-levels (Figure 5.4). This shows that animals that had good protection through any two months were more likely to come from herds that had high levels of protection, while the animals that had poor protection were more likely to come from herds that had lower degrees of protection.

Table 5.7. The relationship between the incidence of trypanosomosis and the level of protection individual animals acquired from the tsetse repellent technology over successive periods of two-months created from the tsetse repellent evaluation trial (2004 – 2005)

Dispenser status		Protection levels		*n*	Tryps incidence in the two-month period (%)	$p > \chi^2$
		Month *i*-1	Month *i*			
1	YY-YY	Good	Good	183	8.7	0.96
2	YN –YY	Moderate	Good	112	8.0	0.82
3	YY-YN	Good	Moderate	156	5.1	0.13
4	NN-YY	Poor	Good	125	4.0	0.07
5	YY-NN	Good	Poor	69	5.7	0.41
6	YN-YN	Moderate	Moderate	295	5.6	0.16
7	YN-NN	Moderate	Moderate	225	6.0	0.16
8	NN-YN	Poor	Moderate	299	4.7	0.02
9	NN-NN	Poor	Poor	838	6.9	0.11
10	Control	N/A	N/A	2764	8.6	reference

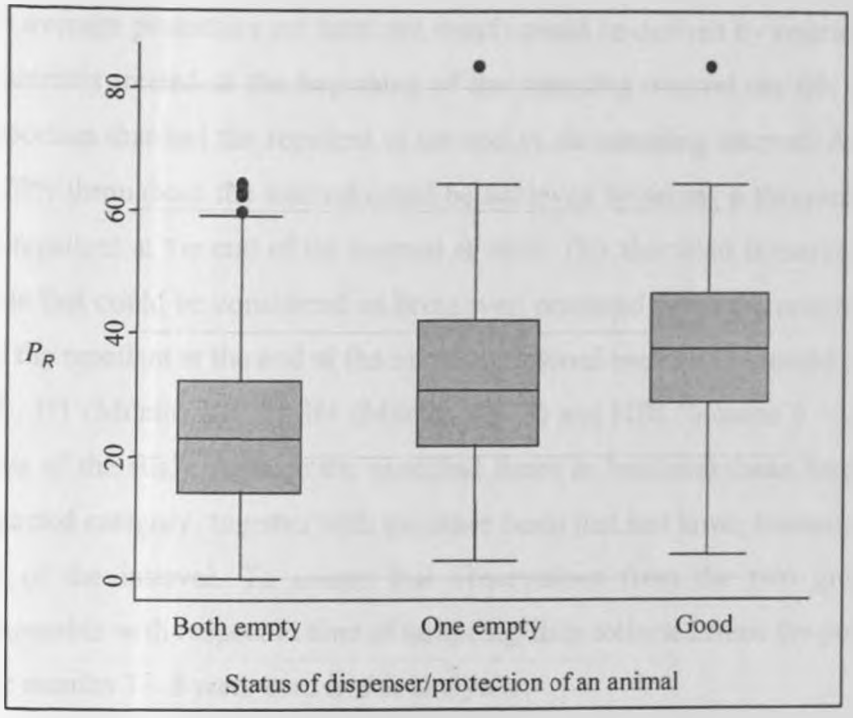


Figure 5.4. The relationship between the estimated levels of protection provided by the repellent technology at a herd- (*y*-axis) and at an animal-level (*x*-axis) throughout the longitudinal phase of the trial at Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005).

5.3.2.3. Stratifying the relationship between protection level and trypanosomosis risk at the animal level by an estimated degree of protection at the herd-level

Figure 5.5 shows the proportion of animals in each of the treated herds that had good dispensers, hence the repellent in both reservoirs, at the end of each sampling interval. The equation (Equation 5.3) used in estimating the degrees of protection expected at the herd level (used in Figure 5.4) underestimates the amount of protection because it is known that reducing the dose of the repellent by half does not lead to a proportionate reduction in repellent effect (G. Vale, personal communication). The stratification conducted here, apart

from providing conservative results, serves to determine whether the degree of protection at the herd level influenced the outputs presented in Table 5.8.

The average protection per herd per month could be derived by considering the proportion of animals treated at the beginning of the sampling interval (in this case 100%) and the proportion that had the repellent at the end of the sampling interval. An average protection of 70% throughout the interval could be achieved by setting a threshold proportion having the repellent at the end of the interval at 40%. This threshold is marked in Figure 5.5. The herds that could be considered as being well protected given the proportion of animals that had the repellent at the end of the sampling interval were: H1 (Months 3 - 8), H2 (Months 3 – 7), H3 (Months 3 – 5), H4 (Months 4 – 7) and H10 (Months 6 – 7). During the other times of the study (outside the specified times in brackets) these herds fell in the poorly protected category, together with the other herds that had lower treatment coverage as at the end of the interval. To ensure that observations from the two groups of herds were comparable with respect to time of sampling, data collected from the poorly protected herds over months 3 – 8 were used in this analysis.

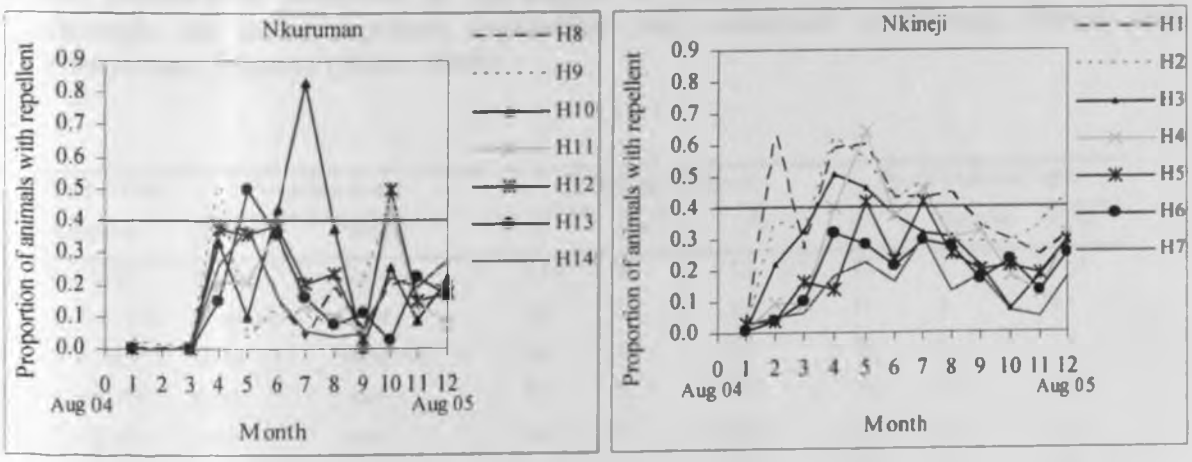


Figure 5.5. The proportion of animals in each of the herds treated with the repellent technology (Herd 1 to 14, given in the legend as H1-H14) that had intact dispensers, hence the repellent, at the end of each month of the trial.

The results given in Table 5.9 indicate that the incidence of trypanosomosis in the animals that had good protection (category YY-YY) did not significantly vary with the level of protection at the herd level ($\chi^2 = 0.43$, $P = 0.51$). Merging the categories with dispensers having at least one reservoir having the repellent (categories 2 – 8) gave incidences of 6.3% (20/319) and 5.3% (20/375) in the well- and poorly-protected categories of herds, respectively. These incidences were not significantly different ($\chi^2 = 0.28$, $P = 0.60$). Similarly, the incidence of the disease in category NN-NN did not vary with the level of protection of the herds ($\chi^2 = 1.45$, $P = 0.23$). This analysis, although limited by the number of records, generally showed that the level of protection at a herd-level did not influence the risk of an animal getting infected. The distribution of the animals in each protection category are similar to that shown in Figure 5.4 because in the well protected category of herds, most of the animals had dispensers with the repellent in both reservoirs through the two-month intervals. In the same vein, the majority of animals that came from poorly protected herds had empty dispensers at the time of sampling.

Table 5.8. The relationship between the level of protection and trypanosomosis risk at the animal-level stratified by the degree of protection expected at the herd level through the tsetse repellent evaluation trial conducted at Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005)

States of the dispenser	Protection levels		Well protected herds			Poorly protected herds		
	Month i-1	Month i	n	No. + ve	I (%)	n	No. +ve	I (%)
1. YY-YY	Good	Good	110	8	7.3	27	3	11.1
2. YN -YY	Moderate	Good	61	1	1.6	11	1	9.1
3. YY-YN	Good	Moderate	45	2	1.0	42	3	7.1
4. NN-YY	Poor	Good	37	5	13.5	46	2	4.3
5. YY-NN	Good	Poor	20	3	15.0	24	0	0.0
6. YN-YN	Moderate	Moderate	39	2	5.1	57	3	5.3
7. YN-NN	Moderate	Poor	52	2	3.8	91	7	7.7
8. NN-YN	Poor	Moderate	65	5	7.7	104	4	3.8
9. NN-NN	Poor	Poor	105	4	3.8	354	25	7.1

I – Trypanosomosis incidence

5.3.2.4. Analyzing the effect of the repellent while capturing the defects of the dispenser

5.3.2.4.1. Univariable analysis

Table 5.10 shows descriptive statistics of the factors used in building the Weibull model. The hazard of the infection was not associated with the level of protection that an animal had because those animals that had good dispensers had higher hazards of the diseases compared to those that had faulty dispensers. Animals that had good dispensers did not have significantly lower hazard compared to the controls. For the other variables, the crude hazard rate ratios portray a similar effect as had been indicated by the crude incidence rate ratios.

Table 5.9. Descriptive statistics and crude hazard ratios of the independent variables used in building the Weibull model used to analyze data collected from the tsetse repellent evaluation trial conducted in Nkineji and Nkuruman (2004 – 2005)

Variable	Levels	Incidence count	Risk time (months)	Crude Hazard Rate Ratio		*P*
				Estimate	95%	
Protection levels	Good	65	993	0.89	0.54, 1.45	0.63
	Moderate	84	1 648	0.63	0.39, 1.02	0.06
	Poor	231	3 160	0.86	0.55, 1.35	0.52
	Control	477	5 723	1.00		
Sex	Male	239	2 862	1.21	1.04, 1.41	0.01
	Female	618	8 662	1.00		
Colour	Light	182	2 122	1.17	0.99, 1.39	0.06
	Dark	675	9 402	1.00		
Age	Calf	251	3 219	1.13	0.98, 1.32	0.09
	Adult	606	8 305	1.00		
Herd size				1.00	0.99, 1.00	0.46
Season	Wet	382	5 162	0.78	0.68, 0.90	0.00
	Dry	475	6 362	1.00		
Area	Nkuruman	239	3 890	0.64	0.42, 0.97	0.04
	Nkineji	618	7 634	1.00		

5.3.2.4.2. Multivariable analysis

Table 5.11 presents the final model obtained from the survival analysis. The shape parameter of the Weibull distribution ρ =1.45 confirms that the baseline hazard increased with time. The variance of the gamma frailty estimated to be 0.07 (0.03, 0.17) was significantly different from zero, implying that observations clustered at the herd level. All the variables except season and interaction between season and area satisfied the proportional hazards assumption (Table 5.12). Fitting season as time varying covariate did not change the effect of treatment. It was therefore ignored because this could have complicated the interpretation of the model.

Table 5.10. Adjusted hazard rate ratios (HRR) at the levels of protection of the tsetse repellent technology estimated using a Weibull model with the outcome being trypanosomosis incidence in cattle over the longitudinal phase of the trial (2004 – 2005)

Variable	Level	*HRR*		
		Estimate	95% CI	*P*
Protection levels	Good	0.85	0.60, 1.21	0.37
	Moderate	0.62	0.44, 0.86	0.00
	Poor	0.82	0.62, 1.09	0.18
	Control	1.00		
Sex	Male	1.24	1.06, 1.44	0.01
	Female	1.00		
Season	Wet	0.65	0.55, 0.77	0.00
	Dry	1.00		
Area	Nkuruman	1.17	0.44, 3.10	0.75
	Nkineji	1.00		
Area*Season	Nkuruman*Wet season	1.69	1.24, 2.30	0.00
Other parameters				
ln ρ a		0.37	0.31, 0.43	0.00
ln θ b		-2.57	-3.45, -1.69	0.00
ρ b		1.45	1.37, 1.54	
1/ρ a		0.69	0.65, 0.73	
θ b		0.08	0.03, 0.19	

Number of subjects = 2058, Number of failures = 856, Log likelihood = -1791.5, Time at risk 11856 months
a ρ is the shape parameter defined by Weibull distribution indicating that the hazard increases with time
b θ is the variance of the gamma distribution that accounts for shared frailty at herd level. Likelihood-ratio test of θ =0: $\overline{\chi^2}$ (1) = 18.71, P = 0.00

Table 5.11 shows the variables that were retained in the Weibull model. Village was fitted as a fixed effect but the estimated parameters were treated as nuisance parameters. The animals that had good protection had a hazard rate ratio that was not significantly different from the null. Animals that had one reservoir empty, however, had significantly lower hazard rate ratio (by 38%) compared to the controls. This is similar to the pattern that is described in 5.3.3.2 where animals that had good protection over two month intervals had high trypanosomosis risk and those that had lower levels of protection had lower disease risk. There was a significant interaction between area and season. Using the dry season as the reference, the interaction term reveals that the incidence of the disease increased in the wet season in Nkuruman but declined over the same period in Nkineji.

Table 5.11. Output of a statistical test that uses Schoenfeld and scaled Schoenfeld residuals testing the validity of the proportional hazards assumption of the variables fitted in the Weibull model used to analyze the effect of the tsetse repellent technology

Variable	Level	ρ	χ^2	P
Protection levels	Good	0.06	3.08	0.08
	Moderate	0.03	0.81	0.37
	Poor	-0.03	0.97	0.32
Sex	Male	0.02	0.44	0.51
Age	Calf	0.04	1.55	0.21
Color	Light	0.03	1.03	0.31
Herd size		0.01	0.05	0.82
Season	Wet	-0.18	51.90	0.00
Area	Nkuruman	0.03	0.43	0.51
Season*Area		0.13	23.37	0.00

5.4. Discussion

The effect of the technology was analyzed in two subsequent stages with an attempt to account for the defects of the repellent dispenser. The models used in the analyses – GEE and Weibull – accounted for correlated observations in time. The GEE model allows for the adjustment of variance estimates by using empirical estimates of within cluster correlations (α) captured in a working correlation matrix (Schukken et al., 2003). Overdispersion occurs quite frequently in discrete data and Lambert and Roeder (1995) and Boyle et al. (1997) pointed out that this problem is particularly common in Poisson type data. Some of the extra-variance can be accounted for by fitting significant predictors in a naïve model. Dohoo et al. (2003) indicates that residual overdispersion exists if unadjusted variance of number of cases is greater than twice the unadjusted mean. The additional analyses that utilized Weibull model were aimed at evaluating whether the observed frailties of the repellent dispenser might have introduced some bias in the estimation of the treatment effect.

The descriptive statistics and the Weibull model show that animals that had good protection had a higher risk of trypanosomosis than those that had lower levels of protection. The well protected animals were well distributed in all the treatment herds and sampling times, ruling out the chance that animals classified to this level could have come from a particular sub-population of treated animals. Even though the observed variation in disease risk was not significant, it is probable that there could have been an unmeasured antecedent factor, for example an animal's temperament, which allowed the hypoactive animals to maintain a good state of the dispenser for an extended period of time. Such animals would also be expected to be more tolerant to tsetse bites than the hyperactive ones. This is supported by the fact that close-range orientation of tsetse towards a host is made without regard to its smell such that in a herd, the probability of an animal being bitten is determined by an individual's defensive behaviour rather than its smell (Torr et al., 2006). Such assumptions would, however, require more information on the hunger status of the flies that succeed to feed on the repellent-protected animals since it cannot be ruled out that the flies that fed on

treated hosts were making desperate attempts to obtain a blood meal after failing to feed on defensive hosts.

Males were identified as having had a higher risk of the disease than females. Similar findings have been reported by Dolan (1998) and Rowlands et al. (2001). Rowlands et al. (2001) suggested that adult male cattle appear to be more susceptible to trypanosome infection than adult female cattle and that there may be a physiological difference between males and females in their attraction to tsetse flies or susceptibility to trypanosome infection. Some of the physiological differences alluded to by Rowlands et al. (2001) have been described by Torr et al. (2006) who further ranked tsetse's attraction to different types of cattle in the order ox>cow>heifer>calf. The present analysis, however, has not specifically associated the increased susceptibility of male animals to trypanosomosis to their advanced age because the variable used in the analysis (sex) captured males of all age groups and weights. Rowlands et al. (2001) did not find significant differences between sexes up to 24 months of age.

The interaction between season and area obtained from the survival analysis relates to the seasonal variation in the density of the flies and grazing patterns. In Nkuruman, the population of *G. pallidipes* closely follows seasonal trends, increasing in wet and declining in dry season (as discussed in Chapter 3). Trypanosomosis incidence closely followed these trends. In Nkineji, tsetse density was more or less uniform throughout the year. The disease incidence, however, increased in the dry season because animals were driven to high tsetse challenge areas in search of pasture. These areas are usually avoided in wet seasons when pastures are available in open plains. The distances between dry and wet grazing areas in Nkuruman were very short, making the changes in tsetse challenge between areas to be insignificant. The effects of the other variables, i.e., herd size and tsetse challenge was as expected.

The analyses suggest that applying the repellent collar to all cattle in a herd did not significantly reduce the incidence of trypanosomosis at both animal and herd level. This

effect also failed to achieve the threshold of a 50% reduction set *a priori* to indicate sufficient effectiveness.

It is difficult to explain why so many problems with the dispenser design were experienced during the trial given that the same dispenser technology had provided such positive results in earlier trials reported by ICIPE. It would be tempting to attribute the negative technical evaluation of the repellent technology found by the present field trial to the problems with the dispenser design; however, by disaggregating the data by condition of the dispenser and its presumed impact on protection levels, this explanation has been effectively eliminated.

Given that the trial design provided for maximum protection of treated herds (i.e., all animals in the herd were provided the repellent collar), the results clearly indicate that the repellent technology in its current form does not yet offer an adequate level of protection to merit further development for commercial uptake.

Chapter 6

Using a mathematical model to evaluate the expected impact of the repellent technology on trypanosomosis transmission when used alone or in integrated versions

6.1. Introduction

Mathematical models are increasingly being used in population biology and epidemiology to study vector population and disease transmission dynamics. They offer a framework for evaluating the expected impact of competing intervention strategies in multiple settings. Many real-life modeling problems are, however, sufficiently simple that they can be satisfactorily approximated by a set of analytical equations. These models provide insights on the relative efficacies of the strategies being evaluated and may help in the identification of areas that need further research.

Unlike the case with most insects and diseases of medical and veterinary importance, strategies for controlling tsetse and trypanosomosis are seldom based on mathematical models (Vale and Torr, 2005). This is inconsistent with the fact that numerous mathematical models describing tsetse population dynamics (Hargrove, 1988; Williams et al., 1992; Jarry et al., 1996; Hargrove, 2000; Artzrouni and Gouteux, 2003) and trypanosomosis transmission (Milligan and Baker, 1988; Milligan, 1990; Baker, 1992; Rogers, 1998; McDermott and Coleman, 2001) have been developed.

The existing models were, however, developed to specifically study either the vector population or disease transmission dynamics. Although they provide an in-depth analysis of the respective components of the disease system, they are not amenable for use in the evaluation of integrated control strategies that simultaneously influence the dynamics of tsetse population and trypanosomosis transmission. This study developed a generic model that could be used in evaluating integrated tsetse and trypanosomosis control strategies by

merging the two types of models. The trypanosomosis transmission sub-model was further adapted to capture the effect of the repellent. The simulations carried out throughout the chapter are limited to *G. pallidipes* because the tsetse fly species has been well studied, therefore, there are numerous publications that provide model parameters for the species.

6.2. Methodology

6.2.1. Description of the basic vector-host trypanosomosis model

Trypanosomoses involve several parasite species transmitted among many different types of vertebrate hosts by 20 to 30 species of tsetse (Rogers, 1988). The basic trypanosomosis model developed by Rogers (1988) and Milligan and Baker (1988) involves two vertebrate- and one vector-host species but can be expanded to include more hosts and parasites. The model was developed by modifying a malaria transmission model developed by Ross and MacDonald (Ross, 1911; McDonald, 1957) to capture the basic features of trypanosomosis transmission.

Depending on the infection status, vertebrate hosts are assumed to fall into one of the four possible states: Susceptible, Pre-patent, Patent and Recovered/Removed. The vector can be classified into three states: Susceptible, Incubating and Infectious because once infected, it remains so for life. Assuming a two-host model [1..2], infections are acquired by a susceptible host i from the vector at a rate dependent on the rate of host-biting by each individual vector (a bites/unit time), the proportion of vectors infected, y, the proportion of infected bites that gives rise to an infection, b, and the vector:host ratio, m. The host [i]'s per capita rate of acquiring an infection, λ, would be given by:

$$\lambda_{[i]} = a \cdot b \cdot m \cdot y \cdot S \qquad (6.1)$$

The rate of host-biting per day by a vector, a, is derived as p/d, where p is the probability that a fly will obtain its blood meal from host i. The host incubates the infection for a period $1/l$ (where l is the rate of progression to the patent class) and patent infections are

lost through recovery after an infectious period of $1/r$ (r is the rate of recovery). Once a vertebrate host recovers or is cured of the disease, it remains immune to re-infection for a period $1/\gamma$ such that the rate of loss of immunity is γ. Each of the states experiences a baseline mortality rate, μ_h, with patent hosts experiencing a case fatality rate of φ.

The rate at which the vector acquires an infection depends upon the prevalence of the disease in the vertebrate hosts, x, the rate of feeding on each of the host, a and the probability that an infected blood meal will produce a mature infection, f. The vector's per capita rate of acquiring an infection, λ_v, would, hence, be estimated as:

$$\lambda_v = f \cdot (a_1 x_1 + a_2 x_2) \tag{6.2}$$

In the case of *T. brucei* infections, Equation 6.2 applies only to newly emerged flies for a period t days during which they are susceptible to infection whilst taking their blood meal (Rogers, 1988). The proportion of flies, E, which will not have fed or died in t days given the emergence rate u, is given by:

$$E = \frac{u}{(a_1 + a_2 + \mu_v)} \cdot 1 - e^{-(a_1 + a_2 + \mu_v)t} \tag{6.3}$$

Equation 6.2 then becomes:

$$\lambda_v = f \cdot (a_1 x_1 + a_2 x_2) \cdot E \tag{6.4}$$

Rogers (1988) indicates that t and f tend to compensate for each other in that a large value of t is associated with small value of f.

The fly must incubate the disease for T days before being infectious. These infections are lost through the natural mortality of the vector, μ_v, which applies equally to the other two states. Whether or not infectious flies suffer an increased mortality rate consequent to the infection is debatable. Throughout this chapter, this event is ignored. The number of new

infections that will arise from a single current infection (the basic reproductive number, R_0) assuming one vector species is given by (Rogers, 1988):

$$R_0 = \frac{f \cdot e^{-\mu_v T}}{\mu_v} \cdot D \cdot \left(\frac{a_1 b_1 m_1}{r_1} + \frac{a_2 b_2 m_2}{r_2} \right) \tag{6.5}$$

where:

r_1 and r_2 – are the rates of recovery in hosts 1 and 2,

D – a constant whose value depends on the trypanosome species. For *T. vivax* and *T. congolense*, D=1 while for *T. brucei*, D is given by:

$$\frac{u}{(a_1 + a_2 + \mu_v)} \cdot 1 - e^{-(a_1 + a_2 + \mu_v)} \tag{6.6}$$

The transmission dynamics of the different trypanosomes are generated independently such that the model does not allow for mixed infections.

6.2.2. Adaptation of the basic model to capture the effects of the repellent

In reference to the two-host-one-vector model described above and assuming that the host groups 1 and 2 represent cattle and game respectively, treatment of cattle with a repellent introduces a subpopulation of cattle that is less preferred by tsetse compared to untreated cattle. It can be assumed that there are three groups of vertebrate hosts made up of repellent-treated, untreated cattle and game. The probability that a fly will feed on any animal from any of the host groups will depend on the fly's feeding preference, ω, and the relative abundance of the host group (Artzrouni and Gouteux, 2001; Milligan and Baker, 1988). The repellent is expected to reduce the fly's preference for treated cattle by a factor τ (assumed to depend on the efficacy) such that the effective preference would become $\tau.\omega$.

Representing the population of the repellent treated cattle by N_1, untreated ones by N_2 and game by N_3, the respective weighted probabilities, p_1, p_2 and p_3, that tsetse will feed on a host in the groups are given by:

$$p_1 = \frac{N_1 \cdot \tau \cdot \omega}{N_1 \cdot \tau \cdot \omega + N_2 \cdot \omega + N_3 \cdot (1-\omega)} \tag{6.7}$$

$$p_2 = \frac{N_2 \cdot \omega}{N_1 \cdot \tau \cdot \omega + N_2 \cdot \omega + N_3 \cdot (1-\omega)} \tag{6.8}$$

$$p_3 = \frac{N_3 \cdot (1-\omega)}{N_1 \cdot \tau \cdot \omega + N_2 \cdot \omega + N_3 \cdot (1-\omega)} \tag{6.9}$$

These probabilities are derived using a model adapted from Artzrouni and Gouteux (2001). Game represent a pool of animals, apart from cattle, which tsetse can obtain meals from. The repellent, depending on its efficacy, reduces the probability of tsetse biting on treated hosts. Because the total probability of a fly obtaining a blood meal from any host is constrained to one, any reduction in the chance that a fly will feed on a repellent-treated host will be compensated by a proportionate increase in the probability of feeding on the other hosts.

The estimation of the force of infection in the fly and the reproductive number changes accordingly. The force of infection becomes:

$$\lambda_v = f \cdot (a_1 x_1 + a_2 x_2 + a_3 x_3) \tag{6.10}$$

and the R_0 becomes:

$$\frac{f \cdot e^{-\mu_v T}}{\mu_v} \cdot D \cdot \left(\frac{a_1 b_1 m_1}{r_1} + \frac{a_2 b_2 m_2}{r_2} + \frac{a_3 b_3 m_3}{r_3} \right) \tag{6.11}$$

The formulation of the force of infection in the vertebrate hosts is as described by equation 6.1. The rate of host biting by the vector changes proportionately to the probabilities described in equations 6.7 – 6.9.

Table 6.1. Notation employed in the generic model of trypanosomosis transmission dynamics

Parameter	Description	Typical values		Source
General				
A_T	Size of the target area (km^2)	528		Model assumption (Figure 6.1). Targeted for tsetse control using traps/targets
A_R	Size of a spatial unit where repellent-treated cattle are confined (km^2)	4		Pastoral cattle graze ≤ 4 km day^{-1}. Similar observations made by Hargrove et al (2003)
C	Stocking rate (ha/Total Livestock Unit)	2 4		Model assumption
W	Cattle:game ratio	3		Model assumption
Q	Tsetse preference for cattle	0 5		Estimated from the study
τ	Efficacy of the repellent	0 - 1		Varied to study proportional increase in the efficacy of the repellent
Φ	Ratio of repellent treated herds	0 - 1		Varied to study the effect of treatment coverage
M	Tsetse:cattle ratio (determines the carrying capacity of an area for tsetse)	10		Estimated from the trial
D	Duration of feeding cycle in flies (days)	3		Hargrove, 2003
G	Growth rate of tsetse population (day^{-1})	0.0075 - 0 015		Hargrove, 2003
μ_v	Baseline mortality rate of tsetse (day^{-1})	0.03		McDermott and Coleman, 2001; Baker, 1992; Williams et al., 1990; Dransfield et al., 1985; Rogers, 1984
A	Root mean square daily displacement of tsetse (*G. pallidipes*) (m)	200 - 800		Hargrove, 2000; Vale et al., 1984
Disease-specific		***T.congolense***	***T. vivax***	
Tsetse				
T	Incubation period (days)	20	10	Rogers, 1988
F	Probability of an infected blood meal giving rise to infection	0.025	0.177	Rogers, 1988
Host 1 (Repellent-treated cattle)				
$1/l_1$	Incubation period (days)	15	15	Milligan and Baker, 1988
$1/r_1$	Duration of infection (days)	20	20	
$1/\gamma_1$	Duration immunity (days)	22	22	Rogers, 1985
M_1	Baseline mortality rate (day^{-1})	0 0005	0.0005	Milligan and Baker, 1988
B_1	Probability of infected fly bite producing infection	0.46	0 29	Rogers, 1988
Φ_1	Case fatality rate (day^{-1})	0 002	0 002	Milligan and Baker, 1988
Host 2 (Untreated cattle)				
$1/l_2$	Incubation period (days)	15	15	Milligan and Baker, 1988
$1/r_2$	Duration of infection (days)	20	20	
$1/\gamma_2$	Duration immunity (days)	22	22	Rogers, 1985
M_2	Baseline mortality rate (day^{-1})	0.0005	0 0005	Milligan and Baker, 1988
B_2	Probability of infected fly bite producing infection	0 46	0 29	Rogers, 1988
Φ_2	Case fatality rate (day^{-1})	0.002	0 002	Milligan and Baker, 1988
Host 3 (Game)				
$1/l_3$	Incubation period (days)	15	15	Milligan and Baker, 1988
$1/r_3$	Duration of infection (days)	50	50	Milligan and Baker, 1988
$1/\gamma_3$	Duration immunity (days)	100	100	Model assumption
M_3	Baseline mortality rate (day^{-1})	0.0006	0.0006	Milligan and Baker, 1988
B_3	Probability of infected fly bite producing infection	0.46	0.29	Rogers, 1988
Φ_3	Case fatality rate (day^{-1})	0 001	0.001	Milligan and Baker, 1988

6.2.3. Tsetse population dynamics model

William et al. (1992) suggests that a Fisher equation (6.12) provides a suitable model of spatio-temporal dynamics in tsetse population. Population growth is captured using a logistic function whereas dispersal follows diffusion process. The model is written as:

$$\frac{\partial V(x,t)}{\partial t} = \alpha \nabla^2 \rho(x,t) + g \cdot \rho(x,t)[1 - \frac{\rho(x,t)}{K}] \qquad (6.12)$$

where: V - is the tsetse population at time t at location x,

α - diffusion coefficient per day,

ρ - tsetse population at time t-1,

g – growth rate of tsetse,

K - is the carrying capacity of a target area.

The operator $\nabla^2 \int \frac{\delta^2}{\delta x^2} + \frac{\delta^2}{\delta y^2}, V(x,t)$ defines the population density at position x in the x, y plane at time $t \geq 0$, and α is the diffusion coefficient (km^2 per day), which can be derived from the root-mean-square displacement in one day,λ as (Hargrove 2003):

$$\alpha = \frac{\lambda^2}{4} \qquad (6.13)$$

The model ignores density dependence in diffusion. There is, however, no evidence to support this phenomenon or its quantitative importance

The Fisher equation does not have a known analytical solution. Hargrove (2003) suggests the subdivision of the target area into n x n lattice of square cells with lengths of each side being defined equivalently to some arbitrary length on the ground (i.e. grid). An example is outlined in Figure 6.1 where the target area (referred throughout this chapter) is made up of 12 x 11 lattices of square cells. The area of each spatial unit $X(i,j)$ (i=1,n; j=1,n) is assumed to be $4km^2$ to capture an approximate daily distance covered by pastoral herds while grazing. The change in population within each lattice will depend on the growth rate and

the difference between the number of flies that move in and those that move out to contiguous lattices. Fly movement can be defined in the instantaneous case by the operator.∇ and its rate depends on the diffusion coefficient, α. The numerical solution to the Fisher equation can then be approximated by:

$$\frac{\partial V}{\partial t} = V \cdot g \cdot (1 - (\frac{V}{K})) + \alpha X \qquad (6.14)$$

where:

$$X = V(i, j-1) + V(i-1, j) + V(i, j+1) + V(i+1, j) - 4 \cdot V(i, j) \qquad (6.15)$$

Tsetse control technologies including insecticide-treated cattle, traps or targets, impose an extra mortality rate, η, on tsetse population, effectively reducing both the growth rate and carrying capacity of the target area. The growth rate, g, and carrying capacity, K, in equation 6.14 are then replaced by:

$$g^* = g \cdot (1 - \frac{\eta}{r}) \text{ and } K^* = (1 - \frac{\eta}{g}) \qquad (6.16)$$

Hargrove et al. (2003) summarized the steps that can be followed in estimating the extra mortality rate, η, which would be imposed on tsetse population by insecticide-treated cattle of a given size as well as traps or targets at a given density. They showed that the probability, p_M that a given fly in 1km^2 around a herd of mass M (in tons) visits that herd on a particular day is given by:

$$p_M = 0.02(M^{0.475})/(0.4^{0.475}) \qquad (6.17)$$

If mortality is a Poisson process, η from insecticide treated cattle is estimated using:

$$\eta_{ITC} = -\log_e(1 - p_M) \qquad (6.18)$$

Hargrove et al. (2003) further show that if there are n such herds of masses, $M(1)$, $M(2)$ .. $M(n)$ and each of these herds is assumed to act independently in the area, the total imposed instantaneous mortality, η_G, due to all n herds becomes:

$$\eta_G = \sum_{i=1}^{n} -\log_e(1 - p_{M(i)}) \tag{6.19}$$

For traps and targets, if the probability that a fly contacts a trap or target on a given day is p_T and the target density is s km^{-2}, the instantaneous mortality η_T is given by:

$$\eta_T = -s\log_e(1 - p_T) \tag{6.20}$$

Throughout this modelling simulation, it is assumed that traps and targets suppress tsetse at the same rate. Hargrove (2003) and Vale et al. (1986) have, however, shown that the kill rate of *G. pallidipes* by a target is an order of magnitude greater than that of a trap because 50% of tsetse flies approaching mechanical traps are actually captured, whereas all flies contacting insecticide-treated targets are killed. Moreover, this analysis is intended to demonstrate relative impacts of integrated control strategies because traps or targets do not always operate at optimal efficiency.

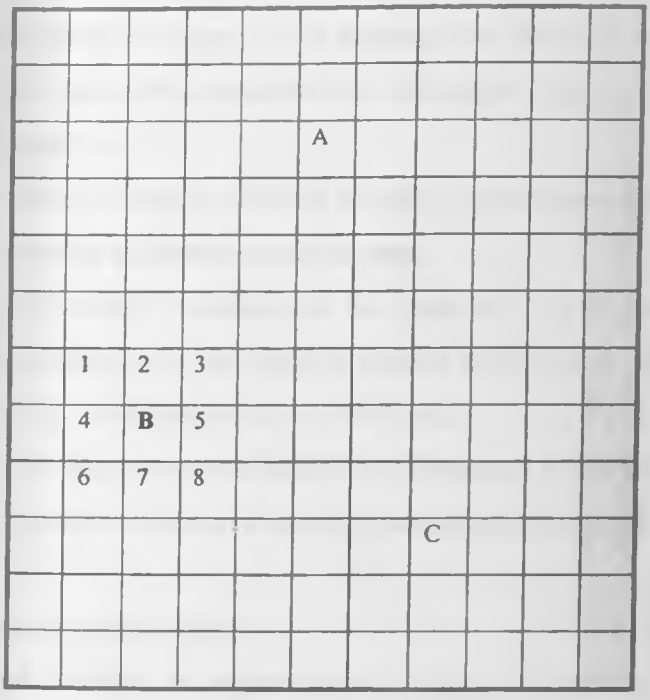


Figure 6.1. A schematic representation of the area targeted for tsetse control. Repellent treated cattle are assumed to be confined to areas A, B and C. Tsetse is not suppressed within the grey shaded band on the right to allow for reinvasion.

6.2.4 Push-pull tactic – developing hypotheses on the possible ways of integrating the repellent technology with traps or targets in tsetse suppression

Saini and Hassanali (2002) have opined that the rates of tsetse suppression could be enhanced if the repellent technology is integrated with tsetse control technologies in what is referred to as "push-pull" strategy. This involves the use of the repellent technology ("push" component) in areas where tsetse is being suppressed using traps or targets ("pull" component). A similar strategy involving the use of insecticide treated cattle as a pull component had been proposed but was not developed because of the difficulties in determining the proportion of animals in a herd that have to be covered with either of the

treatments. Animals treated with insecticides usually rub off with untreated ones making it difficult to isolate the treated versus untreated particularly in areas where communal grazing is practiced. The present analysis evaluates an integrated strategy involving the repellent and traps or targets. This strategy has, however, not been validated. Theoretical analyses are therefore conducted to investigate impact of the strategy under varied scenarios related to:

(i) the distribution, size and number of herds treated with the repellent vis-à-vis the density of other hosts in an area;

(ii) the relative location of the traps or targets and repellent treated herds as influenced by the grazing system under which the treated herds are managed (e.g. sedentary versus mobile); and

(iii) the parameters that define the dynamics of the tsetse population especially the growth rate and root mean square displacement per day.

The assumptions made include:

1. Since it might be impractical to apply the repellent treatment to all the animals within a tsetse control area, the effects of "push-pull" strategy would be limited to a zone within the target area where repellent-treated animals will be confined.
2. Traps or targets are deployed at a uniform grid with mean spacing being greater than 200m (Figure 6.2).
3. The interaction between the repellent technology and traps or targets within the defined zone can be captured by increasing the probability that a fly, in a $1km^2$ neighbourhood, is caught by a trap or target from the baseline of 2% (Hargrove et al., 2003) to a maximum of 4%. It is further assumed that it will be difficult to increase the efficiency of a trap or target beyond 4% without a corresponding improvement in trap or target design and potency of attractants because when a fly finds a trap or target, it may circle it without entering/landing on it (William et al., 1992). This level of efficiency (kill rate of 4% per trap per day) is in fact conservative given that traps or targets rarely work at optimum level of 2%.
4. Increasing levels of trap or target effectiveness (from 2 to 4%) represent increasing efficacies of the repellent odour plume, which might be more stable and efficacious

when used in bigger than smaller herds. This analysis, however, assumes a stable herd size of 20 animals. Scenario analyses predicting the effect of treating one or many of such herds within the target zone are conducted, with the number of available herds being constrained by the size (hence carrying capacity) of the zone.

5. The synergistic effect between the repellent technology and traps or target described above may depend on the relative locations of treated herd(s) and traps or targets. Two distinct areas are defined based on the radius of attraction of tsetse of a baited trap or target (100m) (Williams et al., 1992) and used to weight the expected increase in trap or target effectiveness, assuming that tsetse and treated herds randomly move within the target zone. When a treated herd is within this circle of attraction, tsetse will always be "pushed" towards a trap or target, otherwise, tsetse will be "pushed" towards any of the four directions that are possible in an x, y plane assuming an orthogonal fly movement. Orthogonal tsetse movement was adopted from Hargrove (2000), Hargrove (2003) and Hargrove et al. (2003) and as it simplifies modeling dispersal rates of the vector in space and time. The probability, therefore, that the effectiveness of a trap or target will be influenced by a treated herd outside the circle of attraction will reduce to 0.25 of that expected if the herd was within the circle of attraction of a trap or target.
6. The distance that tsetse can cover per burst of activity as a result of being agitated at point x can be derived from flight speed, approximated to be between 6 -10ms^{-1} and the duration of such bursts, lasting for 30 – 50s irrespective of hunger state (Brady, 1988). A repellent treated herd can therefore cause a fly to get to the circle of trap's attraction as long as it is located at about 500m away from a trap. This implies that a mobile herd confined within zone B (Figure 6.1) can as well influence the activity of traps or targets located in contiguous cells (1 – 8) so long as such a herd is located $\leq$ 500m from the trap or target.

6.2.4.1. Implementation

To increase the accuracy of predictions, a spatial model allowing for lattices of 1km^2 is used in evaluating "push-pull" strategy. The relative locations of treated herds and traps or

targets could be mapped by further demarcating a 1 x 1km^2 lattice into smaller spatial units taking into consideration the circle of attraction of a trap or target. A schematic representation of this model is demonstrated in Figure 6.2 where 100 spatial units of 100 x 100m are generated from a 1 x 1km^2 lattice (B). If a repellent-treated herd is randomly grazed within the zone, the traps or targets whose effectiveness would be influenced are those marked in Figure 6.2 with light up-diagonal lines. Some of these targets are located in contiguous areas 1 - 8. The specific locations from where such a herd will influence the traps or targets given orthogonal fly movement are marked with an asterisk. Assuming random movement of animals, the probability that a treated herd will be in any of the location is equivalent to the inverse of the total number of cells within the zone [B] and as the number of treated herds increase, the probability that any of the treated herds will be in a cell will increase.

Williams et al. (1992) and Hargrove (2000) have shown that the rate at which tsetse invade a controlled area from an adjacent untreated area is proportional to root mean square displacement per day and the square root of the growth rate, *g*. In this analysis, the sensitivity of the outputs to the variation in the values of these two parameters is evaluated.

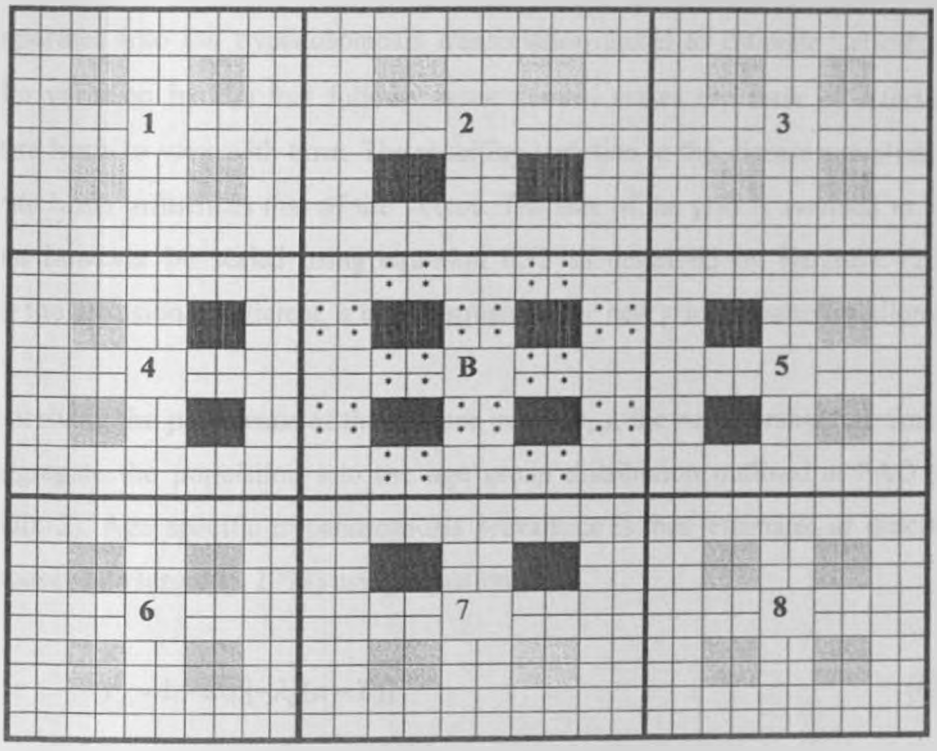


Figure 6.2. Schematic diagram of an array of traps or targets deployed at 4 km^{-2} to indicate the relative location of the traps or targets and repellent treated herds. Shaded regions are areas of attraction of traps or targets deployed at a uniform grid. Areas marked with an asterisk represent the locations where a repellent-treated herd will influence the effect of traps or target. Traps or targets marked with up-diagonal lines are those whose efficiencies will be influenced by repellent treated herds confined in area B.

6.2.5. Melding population and disease transmission models

The site-specific tsetse population, $V(t)$, predicted by the tsetse population dynamics model is incorporated into the trypanosomosis transmission model to estimate tsetse:host ratio, $m_{[i]}$. The variation in $V(t)$ that follows tsetse control makes the force of infection λ in vertebrate hosts to vary with time. The resulting variation in the disease prevalence in the vertebrate hosts influences that of the vector. The size of the grid is assumed to be $4km^2$ (this can however be scaled using equation 6.13 as described by Hargrove (2003) by dividing the diffusion coefficient, α by the square of the new grid measured in kilometres).

Before deriving the prevalence of the disease in tsetse, y, the survivorship function is used to disaggregate the population into the age group distribution outlined in FAO (1982a) (Appendix 2). Age specific trypanosomosis prevalence is then estimated as described by (Woolhouse and Hargrove, 1998) using equation:

$$y_t = 1 - \exp[-\lambda_v (a - T)] \qquad (6.21)$$

Where:

λ_v – is the force of trypanosomosis infection in tsetse,

a – median age of the flies in an age group,

T – incubation period of the disease in tsetse.

Fly age, a, is taken as the mid point of the range represented by the ovarian categories. The overall prevalence is obtained by aggregating the age-specific prevalences. This represents a weighted average because the population is usually made up of many young flies that do not have patent infections. The maximum age of tsetse was taken to be 90 instead of 160 days (Appendix 2) because this improved the speed at which iterations were being ran without any loss on the accuracy of the predictions.

The trypanosomosis transmission dynamics in the vertebrate hosts may be mirrored by coupled first-order differential equations as described by Rogers (1988), Milligan and Baker (1988) and Milligan (1990) for the SEIR compartmental model. The equations are:

Repellent-treated cattle (N_1):

$$\partial S_1/\partial t = \mu_1 N_1 + \gamma_1 R_1 - S_1((p_1/d \cdot b_1 \cdot V_t/N_1 \cdot y_t) + \mu_1) \quad (6.22)$$

$$\partial E_1/\partial t = S_1(p_1/d \cdot b_1 \cdot V_t/N_1 \cdot y_t) - E_1(l_1 + \mu_1)$$

$$\partial I_1/\partial t = l_1 E_1 - I_1(r_1 + \varphi_1 + \mu_1)$$

$$\partial R_1/\partial t = r_1 I_1 - R_1(\gamma_1 + \mu_1)$$

$$N_1 = S_1 + E_1 + I_1 + R_1$$

The initial values for the variables were: $S_1(0)=N_1$, $E_1(0)=I_1(0)=R_1(0)=0$

Untreated cattle (N_2):

$$\partial S_2/\partial t = \mu_2 N_2 + \gamma_2 R_2 - S_2((p_2/d \cdot b_2 \cdot V_t/N_2 \cdot y_t) + \mu_2) \quad (6.23)$$

$$\partial E_2/\partial t = S_2(p_2/d \cdot b_2 \cdot V_t/N_2 \cdot y_t) - E_2(l_2 + \mu_2)$$

$$\partial I_2/\partial t = l_2 E_2 - I_2(r_2 + \varphi_2 + \mu_2)$$

$$\partial R_2/\partial t = r_2 I_2 - R_2(\gamma_2 + \mu_2)$$

$$N_2 = S_2 + E_2 + I_2 + R_2$$

The initial values for the variables were: $S_2(0)=N_2$, $E_2(0)=I_2(0)=R_2(0)=0$

Game (N_3):

$$\partial S_3/\partial t = \mu_3 N_3 + \gamma_3 R_3 - S_3((p_3/d \cdot b_3 \cdot V_t/N_3 \cdot y_t) + \mu_3) \quad (6.24)$$

$$\partial E_3/\partial t = S_3(p_3/d \cdot b_3 \cdot V_t/N_3 \cdot y_t) - E_3(l_3 + \mu_3)$$

$$\partial I_3/\partial t = l_3 E_3 - I_3(r_3 + \varphi_3 + \mu_3)$$

$$\partial R_3/\partial t = r_3 I_3 - R_3(\gamma_3 + \mu_3)$$

$$N_3 = S_3 + E_3 + I_3 + R_3$$

The initial values for the variables were: $S_3(0)=N_3$, $E_3(0)=I_3(0)=R_3(0)=0$

Given that the size of a cattle herd is assumed to be constant (i.e. 20 animals), the proportion of repellent treated herds,Φ is directly equivalent to the proportion of animals treated in an area such that the population of cattle in a unit area (of $4km^2$) is given by:

$$\Phi N_1 + (1-\Phi)\cdot N_2 \qquad (6.25)$$

The model assumes that vertebrate host populations are constant in size N_1, N_2 and N_3 so that births balance deaths. Refinements to allow for variation in these populations make little difference to the transmission dynamics. In this analysis, cattle are assumed to have no natural immunity or trypanotolerance. After becoming infected, cattle remain parasitaemic until death unless they are treated with trypanocidal drugs. This treatment moves the hosts to the "immune" or removed class lasting $1/\gamma_1$ and $1/\gamma_2$ in treated and untreated groups, depending on the type of the trypanocide used.

The assumptions relating to game are more tentative, as information on the infectivity, duration of parasitaemia and pathogenicity of trypanosomes in wild animals is scarce (Milligan and Baker, 1988).

6.2.6. The use of prophylactic drugs

There are two general strategies for treating cattle: mass treatment, with all animals being treated at a certain rate, and targeted or selective treatment, when only those cattle showing symptoms are treated (Milligan and Baker, 1988). To investigate whether the repellent technology could be successfully integrated with mass chemotherapy, the model is adapted to capture the effect of using prophylactic drugs following Milligan and Baker (1988). It is assumed that only the repellent-treated group of cattle will, in addition, be treated with prophylactic drugs at the end of each three-month "immunity" period provided by the drug. Following treatment, the compartments: S_1, E_1 and I_1 lose individuals instantaneously to

the class R_1. These events are captured as pulses, starting on day 300 when the system will have equilibrated and repeated at 90-day intervals. The rate of loss of "immunity" γ_1 is therefore varied accordingly. Animals that get infected in the intervening period are strategically treated.

6.2.7. Software

The model was constructed in Berkeley Madonna modeling software using a series of flowcharts and sub-models. Runge-Kutta 4 integration method with a time step of 0.02 days was preferred because the integration method treats every step in a sequence of integration steps in identical manner. Any point, therefore, along the trajectory of differential equations could be used as reference.

6.2.8. Sensitivity analyses

Sensitivity analyses were conducted to evaluate the relative importance of each parameter used in building the model. For each parameter, minimum and maximum values were respectively defined by halving and doubling the baseline value. Within these limits, 100 simulations utilizing an arithmetic series of parameter values were produced using batch runs option in Berkeley Madonna. From these simulations, variability of reproductive number and equilibrium trypanosomosis prevalence in tsetse, cattle and game was estimated using coefficient of variation. The sensitivity analyses were conducted separately for each trypanosome parasite *T. congolense* and *T. vivax*.

6.3. Results

6.3.1. Sensitivity analyses

The results outlined in Table 6.2 show the variation in the equilibrium prevalences of *T. congolense* and *T. vivax* when the values of the model parameters are varied. Since each

parameter is varied independently, these results reflect the sensitivity of the model to each parameter, and therefore the relative accuracy which they need to be measured. Generally, the equilibrium prevalences of *T. congolense* are more sensitive to the model parameters than those of *T. vivax*. This is because *T. congolense* has a higher threshold for transmission compared to *T. vivax*. Irrespective of the parasite, the parameters that were identified as being important include length of feeding interval of tsetse (d), tsetse:host ratio (m), probability that tsetse gets infected from a single infected meal (f), latent period of the disease in tsetse (T), host preference by tsetse (ω) and the recovery rate of cattle (r). Varying the preference of tsetse for cattle has a marked effect on the equilibrium prevalence of the other hosts and R_0 but not so much on the equilibrium prevalence of cattle.

Table 6.2. Outputs of a sensitivity analysis showing the coefficient of variation in trypanosomosis prevalence of *T. congolense* and *T. vivax* in tsetse, cattle and game and reproductive numbers associated with the variation in the values of the model parameters

Parameter	*Trypanosoma congolense*				*Trypanosoma vivax*			
	Equilibrium prevalence			R_o	Equilibrium prevalence			R_o
	Tsetse	Cattle	Game		Tsetse	Cattle	Game	
d	83.9	60.6	59.0	88.4	28.8	5.7	5.3	88.4
m	16.7	16.7	15.6	35.2	1.5	2.6	2.3	35.2
f	43.5	14.9	13.9	35.2	21.6	1.5	1.5	34.1
ω	21.2	0.6	45.6	27.7	9.2	0.6	23.1	27.6
T	80.0	58.4	57.3	26.2	19.2	1.5	1.3	13.2
r_{cattle}	22.7	38.2	6.3	22.4	9.8	28.2	0.6	22.4
b_{cattle}	8.3	13.4	2.4	21.3	1.0	2.6	0.1	21.3
r_{game}	10.0	3.1	21.4	18.2	3.0	0.2	11.7	18.2
b_{game}	5.8	1.6	11.7	17.8	0.7	0.0	2.3	17.8
W	4.4	1.2	1.1	1.9	3.9	0.3	0.4	1.8
γ_{cattle}	8.0	12.8	2.4	0.1	5.2	14.3	0.3	0.1
l_{cattle}	5.5	8.9	1.5	0.1	3.7	10.0	0.2	0.1
l_{game}	3.8	1.2	7.8	0.1	2.4	0.2	8.8	0.1
γ_{game}	0.1	0.0	0.2	0.1	0.0	0.0	0.0	0.1

For each parameter separately, maximum and minimum values were obtained by doubling and halving the baseline values. Up to 100 batch runs were then generated using arithmetic series of the parameter values bounded by the limits. The coefficients of variation in the equilibrium trypanosomosis prevalence and reproductive numbers were then computed for each parasite from the outputs.

6.3.2 Equilibrium trypanosomosis prevalence

The predicted equilibrium trypanosomosis prevalence in cattle, game and tsetse and reproductive numbers of the respective parasites are presented in Table 6.3. The transmission of *T. brucei* could not be maintained in the system ($R_0 < 1$). In Kenya, *T. brucei* is usually limited in its geographical distribution since it requires relatively high threshold for transmission compared to the other parasites. The predictions presented in Table 6.3 (particularly of *T. congolense*) could be considered as being typical of those observed in Narok where the apparent prevalence in cattle averaged 10% (using buffy coat technique whose sensitivity ranges between 50 and 80%). The model provides true prevalence that may not be obtained using imperfect tests.

Table 6.3. Predictions from the generic trypanosomosis transmission model using parameters and variables in Table 6.1

	T. congolense	*T. vivax*	*T. brucei*[a]
Equilibrium trypanosomosis prevalence in cattle	24.5%	32.8%	-
Equilibrium trypanosomosis prevalence in game	55.4%	72.4%	-
Equilibrium trypanosomosis prevalence in tsetse	3.5%	35.0%	-
Reproductive number (R_0)	4.8	28.6	0.5

[a] Equilibrium trypanosomosis prevalence not given because the transmission of the parasite could not be sustained in the system, i.e. $R_0 < 1$. R_0 was estimated by replacing $T = 25$; $f = 0.065$; b_1, b_2, $b_3 = 0.62$ (Rogers, 1988). The other parameters were similar to those of *T. congolense* and *T. vivax*.

6.3.3 The effect of the repellent technology on trypanosomosis prevalence

The results of scenario analyses showing the relationship between the efficacy of the repellent and equilibrium trypanosomosis prevalence when the number of herds treated is varied are shown in Appendix 3. Each spatial unit ($4km^2$) is assumed to accommodate eight cattle herds of 20 animals each. Appendix 3 (a) shows that the expected impact of the repellent technology declines with an increase in the number of herds treated in an area. It

is predicted that with an efficacy of 80%, the technology will reduce the equilibrium trypanosomosis prevalence by 50% when only one herd is treated. The use of the repellent is also expected to increase the risk of untreated herds, tsetse and game acquiring trypanosome infections and as the number of repellent treated herds and (or) the efficacy of the repellent increase(s), the risk of the disease in the untreated hosts increases. Similarly, the R_0 is predicted to increase with an increase in the efficacy and (or) the number of animals treated. The effect of the technology on R_0 and trypanosome prevalence in tsetse increases exponentially with efficacy, whereas in untreated hosts, the effect increases linearly with efficacy.

Assuming that the equilibrium disease prevalence presented in Table 6.3 is within the levels that stock owners are willing to accommodate, the extra risk that will be borne by the untreated cattle following the use of the repellent (Appendix 3(b)) will require an increased rate of treatment (Appendix 3(f)) for the original (baseline) levels of disease burden to be maintained. Appendix 3 (b) and (f) show that there is a linear relationship between the extra risk and rate of treatment required for the baseline levels of the disease to be maintained. The ability of the model to capture the "spill-over" effects provides an opportunity for predicting the societal benefits/costs of the intervention.

Appendix 4 evaluates the effect of the technology under varied levels of tsetse challenge. In this scenario, four herds are assumed to be treated with the repellent technology. The effect of the technology is predicted to have a relatively higher effect in areas of low than high challenge. Similar to the trends demonstrated above, untreated animals and tsetse experience an increased risk of getting infected. The increased risk in untreated hosts and tsetse is however more variable at low than high levels of tsetse challenge. This can be explained by the pattern predicted in Appendix 4(d) where at low tsetse:host ratio, trypanosomosis prevalence is highly variable compared to a more stable trajectory expected at high tsetse:host ratios. Appendix 4(d) also shows that prevalence of *T. vivax* increases more rapidly with an increase in tsetse:host ratio that that of *T. congolense*.

The patterns shown by the figures in Appendix 4 could be generalized to a number of scenarios that influence the force of infection in a similar manner as the tsetse:host ratio. These are defined by the differences in tsetse's blood meal index and infection rates. The effectiveness of the technology is expected to be higher when used on animals that are less preferred by tsetse for blood meal compared to those that are highly preferred. Likewise, it will be more efficacious in areas where the infection prevalence in flies is low compared to areas where the prevalence is high. Such variations, hence the effectiveness of the technology, is likely to vary by tsetse species.

6.3.4 Effect of the repellent on the transmission of *T. brucei*

The impact of the repellent technology on the transmission potential of *T. brucei* could depend on the proportion of cattle treated and the expected efficacy of the repellent (Figure 6.3). The model predicts that the use of the repellent might enable the transmission of the disease ($R_0 \geq 1$ [marked with dotted line in Figure 6.3]) if: (i) all the cattle are treated with a repellent having an efficacy of $\geq$ 50%, (ii) 75% of the cattle are treated with a repellent having an efficacy of $\geq$ 70%, or (iii) if 50% of cattle are treated with a repellent having $\geq$ 95% efficacy (Figure 6.5). It is also predicted that treating < 50% of the animals will not have a substantial impact on *T. brucei* transmission. More analyses are, however, required to test the stability of the system at the threshold level where the R_0 is 1. The ratio of cattle:game might also affect the accuracy of the predictions made.

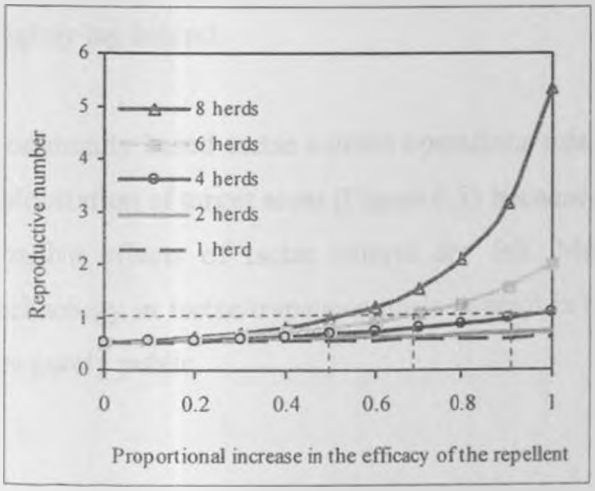


Figure 6.3. Changes in the reproductive number of *T. brucei* with the efficacy of the repellent and the number of herds treated in an area of 4km^2. Each herd is made up of 20 animals.

6.3.5. Integrating the repellent with traps or targets in "push-pull" strategies

6.3.5.1. Tsetse control using traps or targets

The change in the population density of tsetse following the use of traps or targets at varying effectiveness in the target area (of 480km^2) is shown in Figure 6.4(a). The density of the traps/targets is assumed to be 4 km^{-2} but the effectiveness (kill rate/trap or target/day) of each device is varied from 0.5 to 2% in a bid to simulate the challenges that usually accompany the servicing of traps or targets in community-based interventions. Tsetse control is instituted after day 400 to allow the system to equilibrate. The predicted trypanosomosis prevalence in cattle is shown in Figure 6.4(b). These predictions show that at optimum effectiveness (i.e. kill rate of 2%/trap or target/day), traps or targets effectively suppress tsetse populations over a period of about 180 days. At lower trap or target effectiveness, tsetse would not be effectively controlled over a period of 200 days. The rates of decline in the prevalence of the disease mirror that of tsetse, though they tend to slightly lag behind.

Community based tsetse control operations usually undergo cycles of suppression and re-colonization of target areas (Figure 6.5) because complacency usually sets in as soon as the positive effects of tsetse control are felt. More importantly, the sustainability of the technology in tsetse/trypanosomosis control is usually limited by the fact that its benefits are purely public.

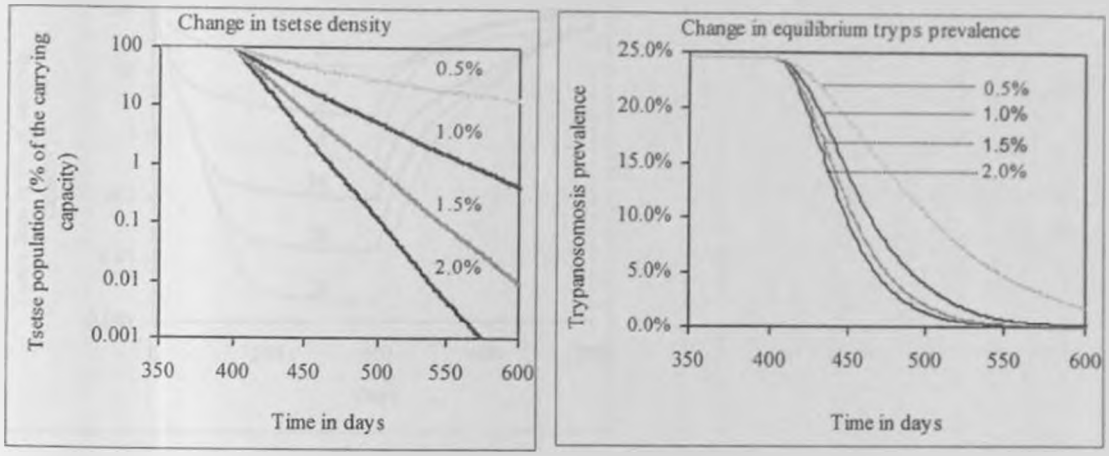


Figure 6.4. Predicted rates of decline of the population of tsetse and trypanosomosis prevalence following the use of traps or targets at various effectiveness (kill rate/trap or target/day) illustrated on the body of the graphs. The density of traps or targets is assumed to be 4 km^{-2}.

Figure 6.5 demonstrates the importance of re-invasion in frustrating effective tsetse control. The kill rate/trap or target/day is set at 2%. The outputs reveal that the effectiveness of the intervention increases with the distance from the source of re-invasion. But as soon as the intervention is terminated, re-colonization ensues. The rate of re-infestation is expected to be faster in areas close to the uncontrolled zones compared to those further away because of the relatively higher density of the residual tsetse in the area that will start reproducing as soon as the growth constraint is removed. Flies emigrating from uncontrolled zones will also colonize areas at the periphery at a faster rate than those further away. Figure 6.5 also demonstrates a phenomenon that has been described by Hargrove (2003) where tsetse population slightly declines in an uncontrolled area adjacent to the control area (distance 0) when tsetse suppression is being instituted.

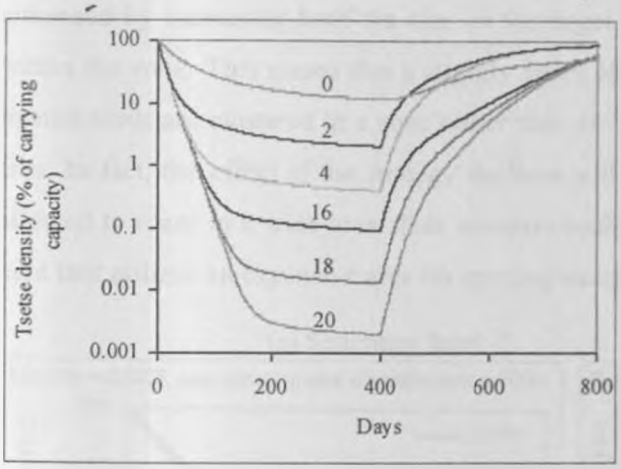


Figure 6.5. Predicted rates of decline of tsetse population following the use of traps or targets with a kill rate of 2%/trap or target/day and subsequent re-infestation after the termination of control (on day 400) in specific areas along a transect running perpendicularly to the source of re-invasion. Distances (in km) are shown against each trajectory representing an area of 1km^2. Mean square displacement = 400m and growth rate = 1.5%.

6.3.5.2 Can the rate of tsetse suppression be enhanced by integrating traps or targets with the repellent technology?

Outputs given in Figures 6.6 and 6.7 predict the effectiveness of "push-pull" strategy in tsetse suppression. Figure 6.6 compares the effectiveness of the strategy when it involves a sedentary herd confined within the circle of attraction of a trap or target vis-à-vis a mobile herd, both in a zone of 1km^2, whereas Figure 6.7 evaluates the effect of varying both the size of the target zone and the number of herds treated within the zone. The trajectories in Figure 6.7 represent the expected baseline scenario, i.e. using traps or targets alone and "push-pull" strategies involving one herd and all the available herds in the target zone. Generally, "push-pull" strategy is expected to be more efficacious when used on a tsetse population that has lower rates of growth and dispersal compared to those with higher rates. It is predicted that a sedentary herd confined within the circle of attraction of traps or targets would provide a slightly better effect than a mobile herd because the former scenario allows for the maintenance of a critical distance between a trap or target and the

repellent treated herd. The effectiveness of "push-pull" strategy (Figure 6.7) could be enhanced by increasing both the size of the target zone and the number of herds treated within the zone. This means that a slightly better effect could be observed when repellent-treated herds are clustered in a zone rather than distributed diffusely within a tsetse control area. In fact, the effect of the strategy declines with the treatment of only one herd that is allowed to roam in a wide area. This scenario could be associated with treating a pastoral herd that utilizes an expansive area for grazing/watering.

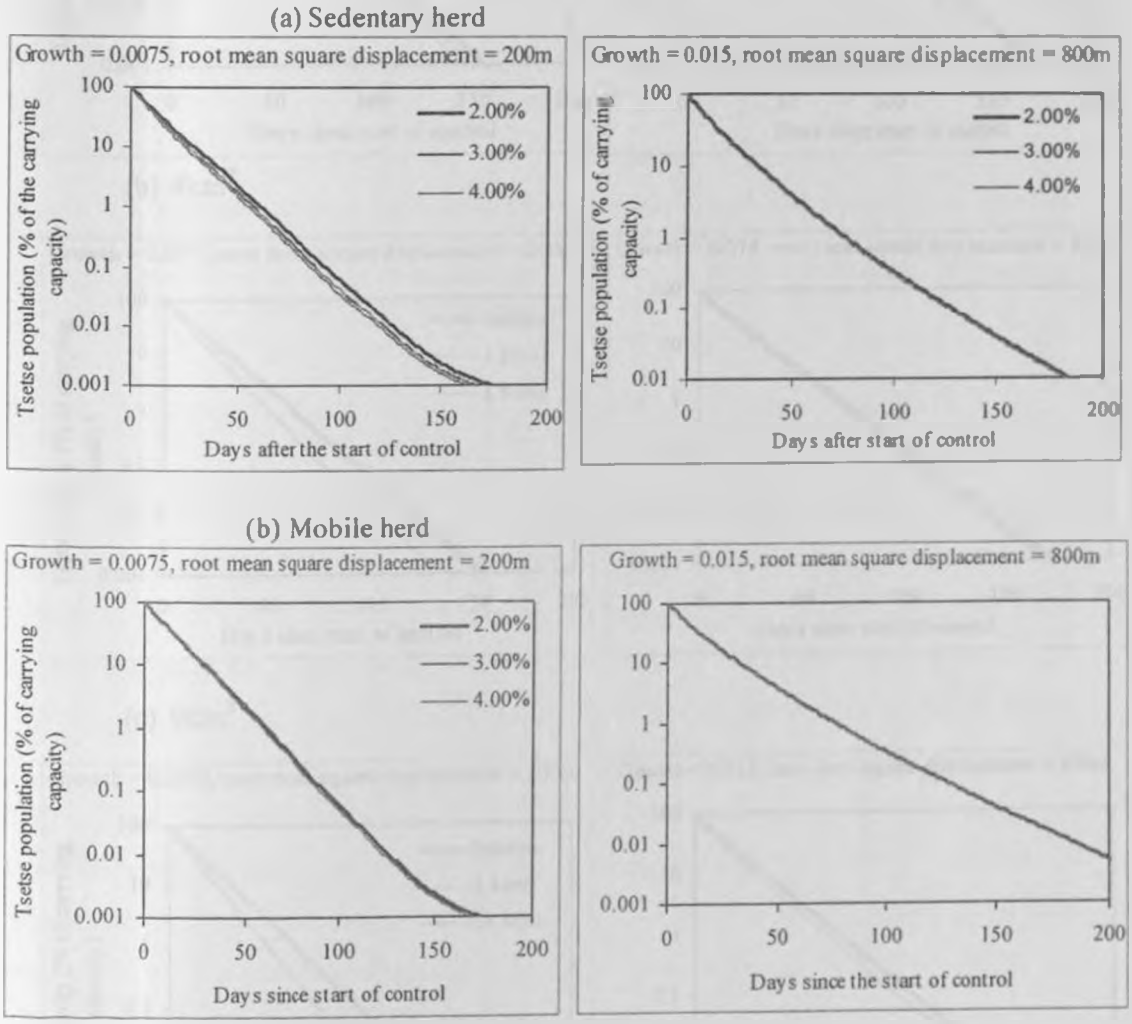
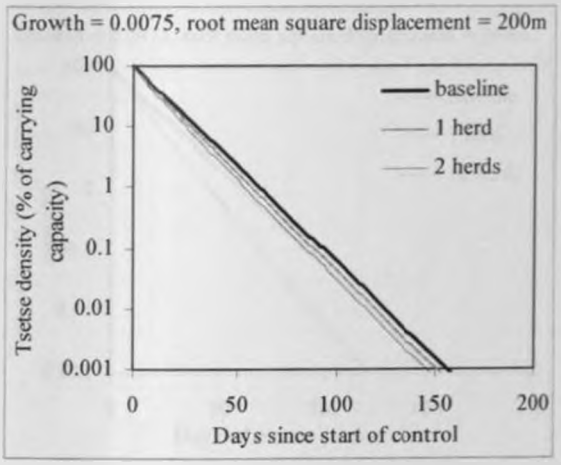
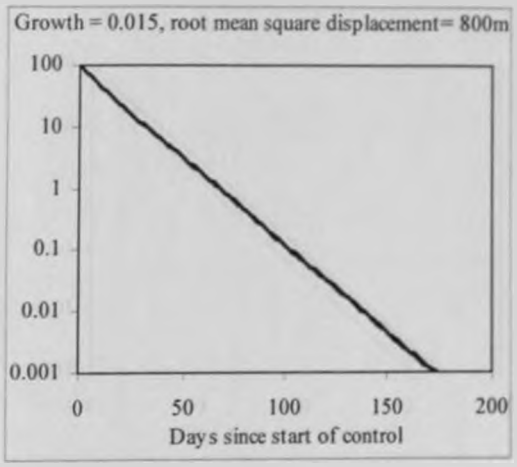


Figure 6.6. Comparing the expected rates of decline of population of tsetse within a zone of 1km^2 following the use of "push-pull" strategy involving either a sedentary or mobile herd. The effectiveness of traps or targets is assumed to increase from 2 to 4%.

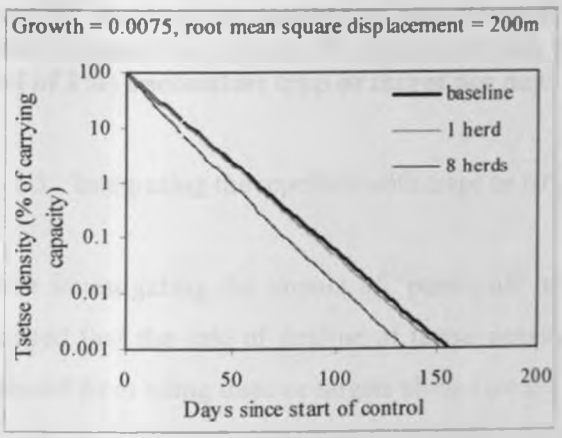
Figures 6.7a to 6.7d – title below Figure 6.7d

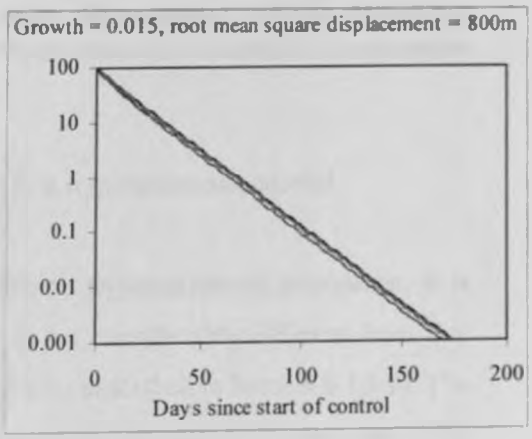
(a) 1km^2



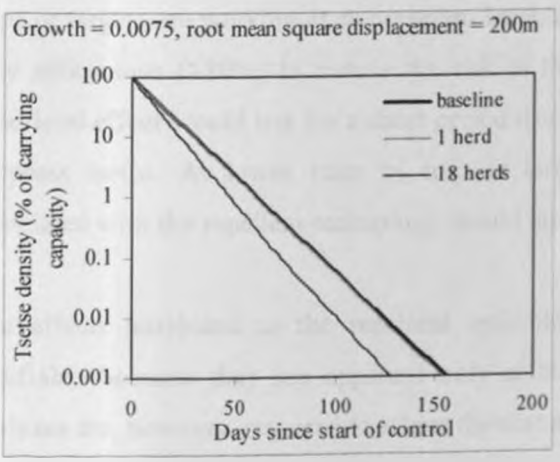


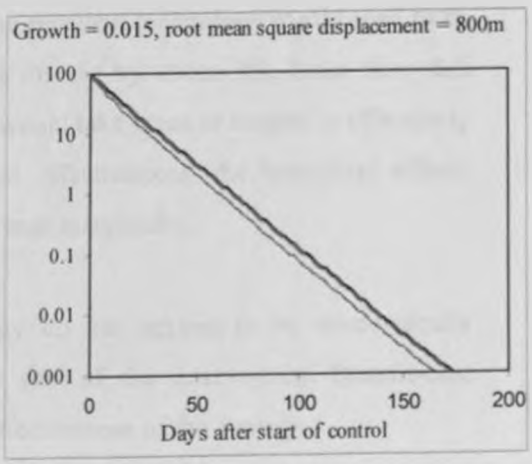
(b) 4km^2





(c) 9km^2





(d) 25km^2

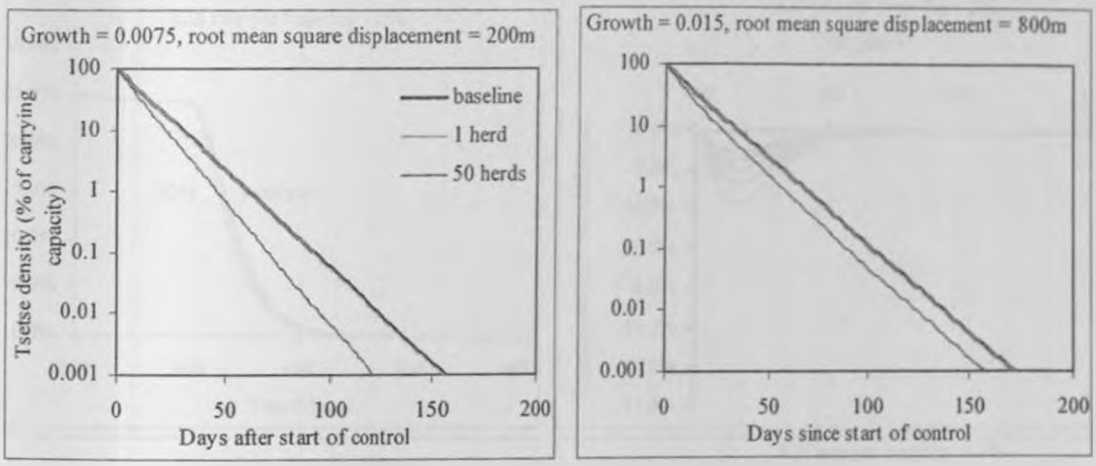


Figure 6.7. Comparing the expected effects of using "push-pull" strategy to control tsetse by varying the number of herds treated with the repellent and the size of the zones targeted for control. It is assumed that 4% of tsetse (i.e. double of the baseline level of 2%) encounters trap or target per day.

6.3.5.3 Integrating the repellent with traps or targets in trypanosomosis control

While investigating the impact of "push-pull" tactic on trypanosomosis prevalence, it is assumed that the rate of decline of tsetse density is not considerably different from that expected from using traps or targets alone (see the results described in Section 6.3.5.1). The outputs in Figure 6.8 show that the benefits that could be associated with the use of the repellent technology decline with an increase in the effectiveness of traps or targets. When traps or targets are working at an optimum level, the repellent technology would need to be very efficacious (>80%) to reduce the risk of the disease by about 2%. Even then, this beneficial effect would last for a short period that would take traps or targets to effectively suppress tsetse. At lower rates of trap or target effectiveness, the beneficial effects associated with the repellent technology would increase marginally.

The effects attributed to the repellent technology do not appear to be economically justifiable because they are apparent only at the start of the intervention. Benefit-cost analyses are, however, required to assess the cost effectiveness of the strategy.

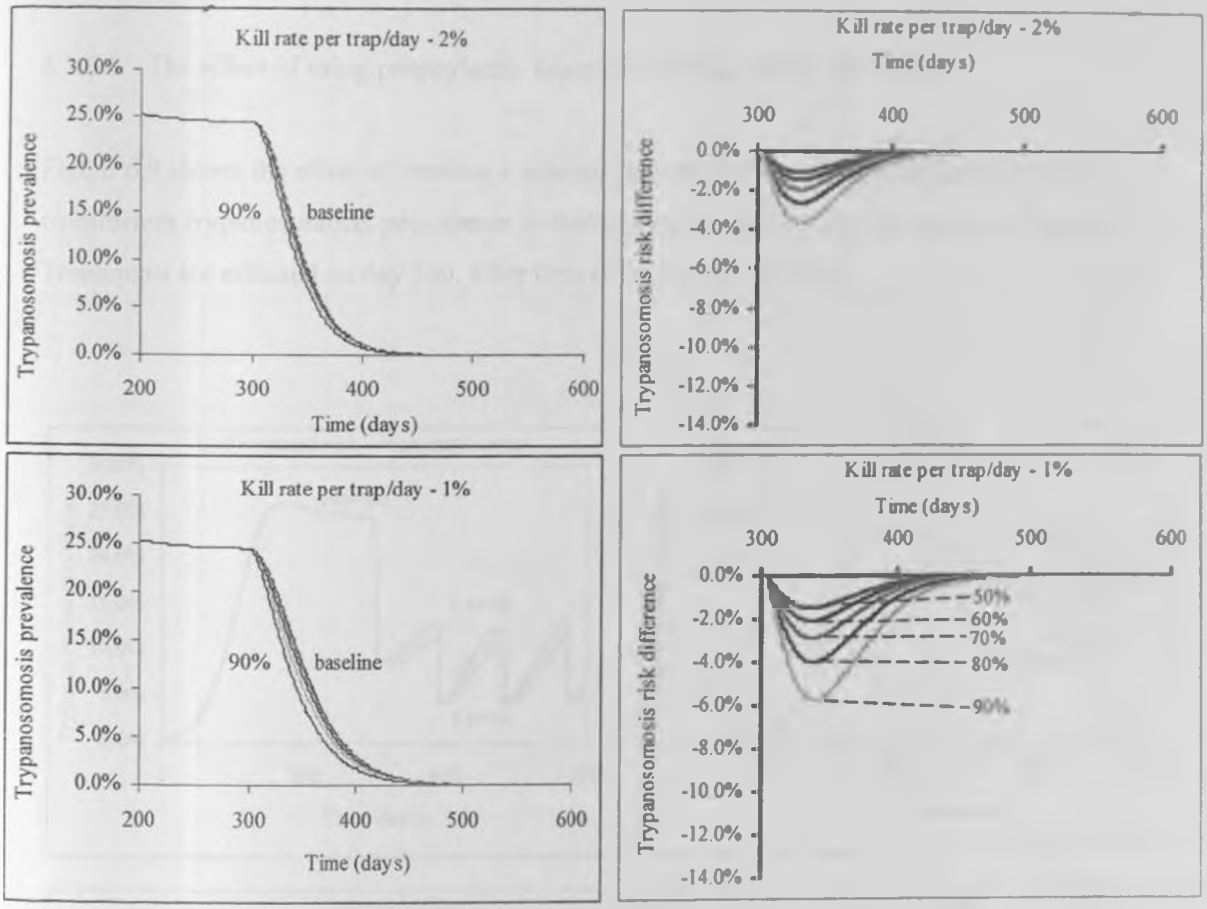


Figure 6.8. Predicted effect of "push-pull" strategy on the prevalence of trypanosomosis. The kill rate of tsetse per trap or target/day is varied from 1 – 2% per day and the effectiveness of the repellent technology ranges from 50 – 90%. One set of graphs show the rates of decline of the disease whereas the gives risk difference that could be attributed to the repellent technology. In this case, the reference is the prevalence that could be expected from using traps or targets alone.

6.3.6 Mass chemotherapy with prophylactic trypanocidal drugs

6.3.6.1 The effect of using prophylactic drugs on trypanosomosis prevalence

Figure 6.9 shows the effect of treating a sub-population of cattle with prophylactic drugs on equilibrium trypanosomosis prevalence in tsetse, treated cattle, untreated cattle and game. Treatments are effected on day 300, after the system has equilibrated.

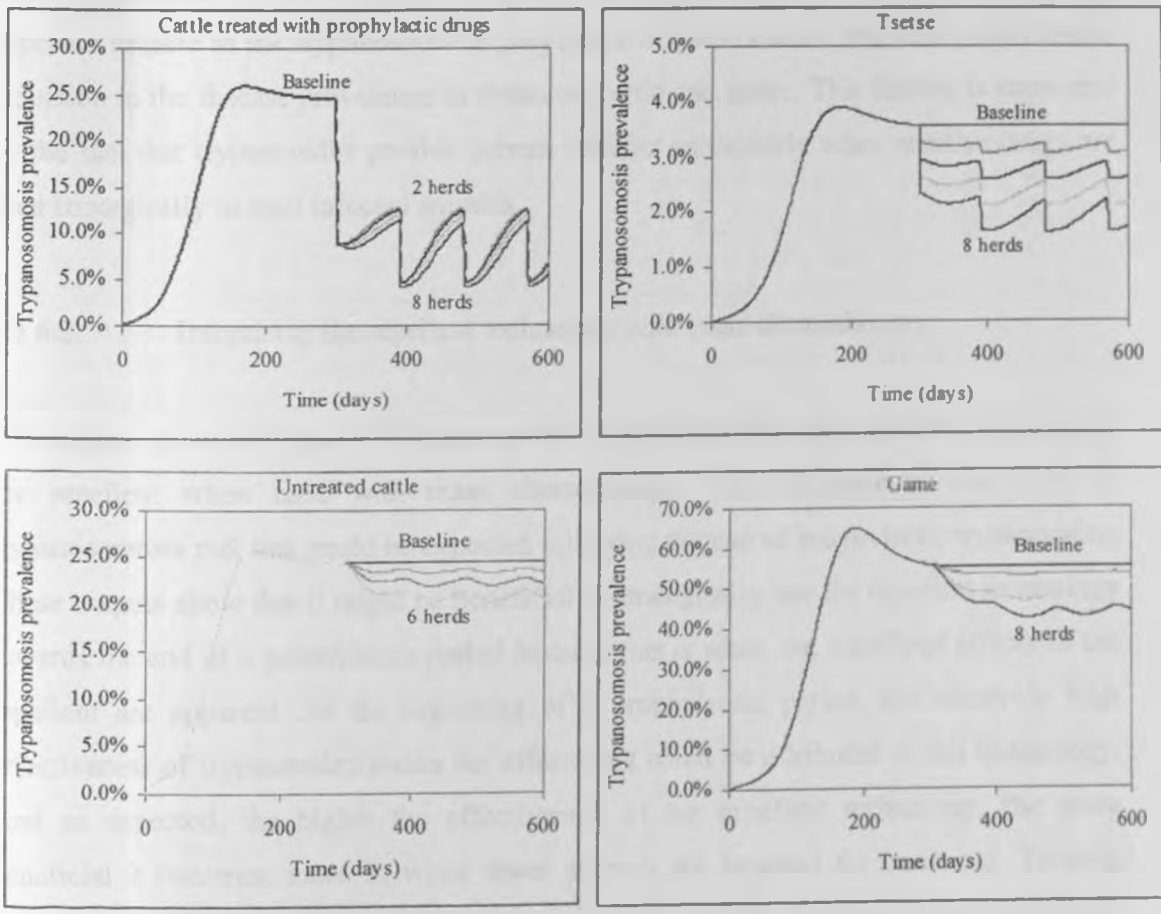


Figure 6.9. Predicted effect of using prophylactic trypanocidal drugs on varied number of herds -2, 4, 6 and 8 - on equilibrium trypanosomosis prevalence in tsetse, treated cattle, untreated cattle and game. The target area can accommodate a total of 8 herds and mass treatments are administered at 90 day intervals. The average protection period is assumed to be 90 days.

The outputs (Figure 6.9) show that mass chemotherapy substantially reduces equilibrium trypanosomosis prevalence by more than 50% from the baseline level in the treated group of animals. This effect is expected to be more apparent at the early than late stages of the prophylactic period because the drug will be metabolized over time. Varying the number of herds treated in an area is not expected to result in much change in the effectiveness of chemoprophylaxis.

The equilibrium prevalence in tsetse is also expected to decline with the use of prophylactic drugs. The magnitude of this effect is predicted to be linearly related to the number of herds treated. This could be due to an expected increase in the probability of the vector feeding on a treated, therefore, uninfected host. It is also probable that parasites ingested by tsetse with trypanocides in blood meal may fail to establish, leading to aborted infections. The expected decline in the trypanosomosis prevalence in tsetse causes less than proportionate reduction in the disease prevalence in untreated cattle and game. This finding is supported by the fact that trypanocides provide private benefits particularly when curative drugs are used strategically to treat infected animals.

6.3.6.2. Integrating the repellent technology with mass chemotherapy

The outputs given in Figure 6.10 illustrate the expected benefits that could be attributed to the repellent when used with mass chemotherapy. The reference is the level of trypanosomosis risk that could be expected following the use of prophylactic trypanocides. These outputs show that it might be beneficial to strategically use the repellent technology towards the end of a prophylactic period because this is when the beneficial effects of the repellent are apparent. At the beginning of a prophylactic period, the relatively high effectiveness of trypanocides masks the effects that could be attributed to this technology. And as expected, the higher the effectiveness of the repellent technology, the more beneficial it becomes, more so when fewer animals are targeted for treatment. Treating many herds (75% compared to 25%) reduces the effectiveness of the repellent technology, therefore the beneficial effects that could be attributed to it. Under this scenario, only the

very efficacious repellent technology (> 70%) would manifest its effects although the strategy may not be economically justifiable.

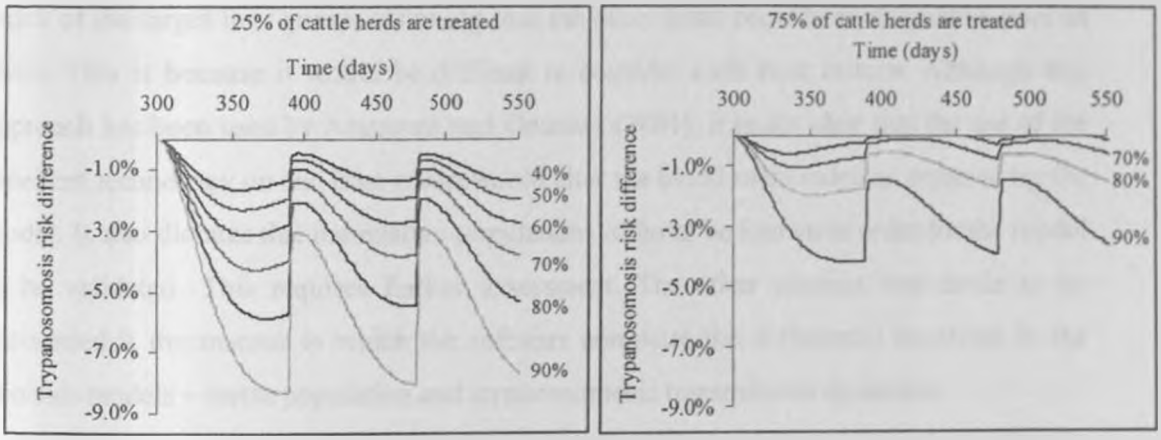


Figure 6.10. Predicted effect of combining the repellent technology and mass chemotherapy on equilibrium trypanosomosis prevalence. The intervention starts on day 300 after achieving equilibrium prevalence and the outcome is the extra reduction in disease prevalence over the level which could be realized by using prophylactic drugs alone. The trajectories represent various levels of effectiveness of the repellent technology.

6.4 Discussion

The generic model developed allows for the evaluation of various tsetse and trypanosomosis control methods when used singly or in integrated versions. The model has the potential to simulate disease dynamics both in the treated and untreated cattle kept in the same locale. It also offers an opportunity for the quantification of "spill over" effects that have been associated with most of the tsetse and trypanosomosis control methods. The use of the model, at the moment, is limited to *ex ante* analysis (preceding a field trial) because it has not been validated. *Ex ante* analysis would formulate best bet strategies expected to have a relatively higher impact on disease transmission for field evaluation. The field trial, on the other hand, would generate data that can be used for validating the model. Nonetheless, the model behaves as those described by Rogers (1988) and Milligan

and Baker (1988) since the predicted equilibrium prevalences are sensitive to the values of the parameters that have been described by these workers as being important.

The weakness of the model resides in the manner in which the probability of a fly biting an animal is estimated. It uses both the total population of hosts in an area and the blood meal index of the target host group, assuming that the other hosts provide an alternative pool of hosts. This is because it would be difficult to consider each host in turn. Although this approach has been used by Artzrouni and Gouteux (2001), it is not clear that the use of the repellent technology on one host group would alter the blood meal index as assumed by the model. It also dictates that the relative populations of hosts be known in order for the model to be validated. This requires further assessment. The other element that needs to be reassessed is the manner in which the software computes the differential equations in the two sub-models – tsetse population and trypanosomosis transmission dynamics.

Nonetheless, the outputs show that the equilibrium prevalences of *T. congolense* are more sensitive to the changes in the parameter values than those of *T. vivax*. This is because *T. congolense* has a higher threshold for transmission, and therefore, will require a longer time to attain a stable equilibrium compared to *T. vivax*. Accordingly, it is expected that the use of the repellent will have a greater impact on the transmission of *T. congolense* than *T. vivax*.

As stated above, the model captures the effect of the repellent by reducing tsetse's preference for a treated host, therefore reducing the probability that such a host is bitten. The vector is allowed to switch its diet depending on the efficacy of the repellent, the number of animals treated and the availability of alternative (untreated) hosts within the target area. The switch in tsetse's diet has been demonstrated by Vale and Cumming (1976) in an experiment where the preferred hosts, warthogs and elephants, were successively removed in an area to investigate whether their removal would lead to tsetse eradication through starvation. The removal of warthogs led to a switch in tsetse diet from 80% warthog to 40 – 80% bovids (mainly kudu), 20 – 50% elephant and 0 – 20% warthog. The removal of elephants made bovids to be the main source of blood meal. These changes did

not have any drastic effect on the numbers and nutritional state of tsetse. In this model, the extra mortality that may be attributed to starvation has been ignored because it incorporates alternative hosts. It is also expected that starving flies will still feed on treated hosts as the repellent does not offer total protection that could be compared to host elimination.

The variation in the proportion of animals treated and the subsequent changes in the main sources of tsetse's diet affect the transmission dynamics of the disease. The impact of the repellent is expected to be higher when used on a small than large number of herds. Treating a large proportion of animals with the repellent reduces the number of alternative hosts available to tsetse. The increased risk of the disease in untreated hosts follows the expected switch in tsetse's diet. The risk is expected to increase linearly with the efficacy of the repellent. In the vector, the risk rises exponentially with an increase in the efficacy of the repellent. The predicted patterns of increased trypanosomosis risk in untreated hosts and tsetse reflect the differences in the manner in which the forces of infection are defined for each host. All the vectors can theoretically get infected from one host whereas the ratio of vectors:hosts determines the rate of infection in vertebrate hosts.

The rise in the transmission potential of the disease (estimated by R_0) following the use of the repellent suggests that the technology should always be integrated with other tsetse and trypanosomosis control methods. For the zoonotic parasite, *T. brucei*, an increase in its transmission potential does not necessarily result in a significant increase in the risk of the disease in humans because the removal of preferred hosts will not make tsetse to switch their meals to humans especially if there are other animals within the target area. A study conducted by Grebaut et al. (2004) in southern Cameroun showed that the proportion of blood meals taken on humans did not significantly increase when the preferred hosts (domestic pigs) were absent. Given that naturally humans are not preferred by tsetse, the proportion of meals obtained by tsetse from humans could be used to set the upper limit of the repellents' efficacy. This proportion is always considered to approximate 28 - 30% (Rogers, 1988; Diallo et al., 1997). From this generalization, the effect of the repellent is not expected to exceed a 70% efficacy level.

An integrated strategy that has been proposed involves a combination of traps or targets and the repellent treated animals. Traps or targets are known to be more efficacious because they give a regular shape of a treated area unlike insecticide treated cattle that can leave untreated 'islands' depending on their distribution within the treated area (Hargrove, 2003; Hargrove at al., 2003). Although a benefit cost analysis is required to test the cost effectiveness of "push-pull" tactic, the expected impact of this strategy on tsetse suppression and disease prevalence is minimal. This is because on their own, traps or targets are very effective in suppressing tsetse. This analysis predicts that it will take about six to seven months to effectively suppress tsetse. A slightly longer time would be required for eradication; Hargrove (1993) indicates that a density of four targets/km^2 is sufficient to eradicate tsetse population in nine to twelve months if targets are in good working order for the whole of the period.

Assuming that the "push pull" tactic will improve the effectiveness of traps or targets, the predicted rates of tsetse suppression suggest that clustering repellent treated herds in a zone within a tsetse control area would result in an enhanced tsetse suppression within the zone whereas distributing treated herds randomly in the area will not have an impact of the rates of suppression. The philosophy behind this strategy was conceived from the one that has been proposed for the control of lepidopteran stem borers affecting cereal crops in Kenya and Uganda (Khan et al., 1997). In this strategy, the main plant (targeted by the pest), for example maize, is intercropped with a leguminous plant that repels the pest, that is desmodium. The attractant, either Napier grass or Sudan grass is planted at the edges of a maize farm. The success of this system depends on desmodium plants being intercropped with the main crop implying that if only one repellent plant is put into the maize farm, the "push-pull" effect would be substantially diluted. The use of "push-pull" strategy to control tsetse will, however, be limited by the number of animals that can be treated in the target zone because in tsetse infested areas, cattle usually co-exist with game. With regard to the use of prophylactic drugs, it can be concluded that the repellent technology may be used strategically towards the end of the prophylactic period, unlike the case with traps or targets where the effects of the repellent are apparent at the beginning of the intervention. The

effects captured by the model are, however, marginal and would require benefit-cost analysis to gauge their cost-effectiveness.

Chapter 7

General conclusions and recommendations

The overall objective of the study was to evaluate the effectiveness of the tsetse repellent technology in reducing the incidence of trypanosomosis in cattle and identify ways of integrating it with other tsetse and trypanosomosis control methods. The general conclusions and recommendations that can be drawn from the different components of the study, starting with the field trial are given in this chapter.

7.1. The field trial

The study has shown that the repellent technology, as it is currently formulated, does not sufficiently reduce the incidence of trypanosomosis in cattle. The analyses conducted in Chapter 5 show that although the defects of the dispenser might have reduced the treatment coverage, the incidence of the disease was independent of the status of the dispenser. The effective sample size used in the analyses that adjust for the defects of the repellent dispenser was, however, small; therefore, the conclusions drawn (and hence the evaluation carried out in the study) focus on the technology and not the repellent per se. The study thus sought to analyse both the disease incidence (impact indicator) and the treatment coverage (process indicator). Although more field trials may be required to confirm the results obtained from this study, it is apparent that the technology does not merit further development into a commercial product before its usefulness can be ascertained.

There was not a substantial difference between the estimated crude and adjusted effects of the repellent technology. This implies that the process used in choosing herds and allocating treatments was relatively sufficient in controlling for extraneous factors. Nonetheless, it was important to adjust for residual confounding through multivariable analysis. The effects of tsetse challenge and trypanocide had to be ascertained through a more rigorous analysis before being included in the analysis, unlike the other covariates – sex, age, season, area, herd size and colour – that could be easily be fitted in the model. For

tsetse challenge, it was found out that out of the three variables that constitute challenge (flies per trap per day (FTD), blood meal index and infection rates in flies), FTD is the most variable and needs to be estimated accurately. The study established that the relationship between tsetse challenge and trypanosomosis incidence in cattle was apparent in Nkuruman, where FTD could be reliably estimated than at Nkineji, where the prevalent tsetse species could not be efficiently trapped. It is recommended thus that efficient trapping devices for *G. swynnertoni*, and for that matter, important vectors for trypanosomosis, be developed.

The rate of use of trypanocides by the livestock owners was quite high: the daily rate of treatment was about 1 treatment per 100 animals per day. The effectiveness of the treatments offered by the technical team was good (over 80%), implying that the parasites that were circulating in the area were very sensitive to trypanocides. On the contrary, some of the treatments administered by the cattle owners were not effective despite the fact that the period between treatment (by the pastoralists) and screening was short. This is why it was assumed that the use of the trypanocides by the livestock owners was unlikely to have influenced the estimation of the effect of the technology. A related study showed that most of the trypanocides were being misused. Although the main focus of this analysis was to find ways of accounting for drug use in the estimation of the repellent effect, it is recommended that livestock owners should be sensitized on the appropriate ways of using the drugs. The current policy on the use of veterinary drugs, however, assumes that these drugs will always be administered by professionals. This should also be re-evaluated based on the fact that animal health service providers are not always available in most arid and semi arid areas. Lastly, the estimation of amount of drugs used by livestock owners should always be done in field trials and epidemiological studies as this allows for an indirect way of estimating disease burden, although some of the treatments are inappropriate.

7.2. Mathematical modelling

The model developed combines both tsetse population and trypanosomosis transmission dynamics and has the potential to predict the risk of the disease in treated and untreated animals. The outputs from the model show that the effectiveness of the technology declines as the density of herds treated in an area increases, and its use leads to an increase in the transmission potential of the disease as the reproductive number increases as a higher share of herds are treated. Simulation of repellent use combined with traps or targets indicates marginal added value of the repellent to be negligible given the already very effective suppression of fly populations by the bait technologies. This modelling simulation therefore suggests limited benefits of the tsetse repellent technology, particularly in areas of high tsetse challenge.

Given the fact that there was no sufficient data to validate the model, its use is limited to *ex ante* analysis. It is therefore recommended that future studies aimed at evaluating a tsetse repellent technology consider the scenario analyses presented and adapt the model based on any new knowledge that may be generated. The scenario analyses conducted here are limited by lack of information relating to the effectiveness of the repellent at varying herd sizes, the behaviour of tsetse in its habitat following the use of the repellent, the hunger states of the flies that manage to feed on treated hosts, the magnitude and nature of interaction between the repellent and traps or targets in "push-pull" tactic, the critical distance over which the repellent would have an affect, etc. Related to this is the need to estimate the values of the sensitive parameters identified in Chapter 6. This study recommends that this information should be derived from controlled experiments as they may help explain the results obtained from the field trial. Some of the model parameters, e.g. rate of recovery of treated animals, ratio of hosts in an area could be estimated through carefully designed participatory epidemiological techniques involving livestock owners. The model should also be integrated to socio-economic studies as it may provide a framework for carrying out economic impact assessments.

Chapter 8

List of References

Adejinmi, J. O., Akinboade, O. A. 2000. Serum biochemical changes in WAD goats with experimental mixed *T. brucei* and *Cowdria ruminantum* infections. *Tropical Veterinarian*. 18(1-2), 111 – 120.

Aksoy, S. 2000. Tsetse – A haven for micro-organisms. *Parasitology Today*. 16(3), 114 – 118.

Allsopp, R. 2002. Tsetse control in Botswana – a reversal in strategy. *Pesticide outlook*. 73 – 76.

Amole, B. O., Allen, B., Clarkson, J. R., Hannan, L. S. 1982. Pathogenesis of anaemia in *Trypanosoma brucei*-infected mice. *Infection and Immunity*. 36(3), 1060 – 1068.

Anene, B. M., Onah, D. N., Nawa, Y. 2001. Drug resistance in pathogenic African trypanosomes: what hopes for the future? *Veterinary Parasitology*. 96(2), 83 – 100.

Anosa, V. O. 1988. Haematological and biochemical changes in human and animal trypanosomiasis. Part II. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux*. 41(1), 151 – 164.

Artzrouni, M., Gouteux, J-P. 2001. A model of Gambian sleeping sickness with open vector populations. *IMA Journal of Mathematics Applied in Medicine and Biology*. 18(2), 99 – 117.

Artzrouni, M., Gouteux, J–P. 2003. Estimating tsetse population parameters: application of a mathematical model with density dependence. *Medical and Veterinary Entomology*. 17, 272 – 279.

Assoku, R., Tizard, I., Neilsen, K. 1977. Free fatty acids complement activation and polyclonal B cell stimulation as factors in the immunopathogenesis of African trypanosomiasis. *Lancet*. 2, 956 – 959.

Authié, E., Duvallet, G., Robertson, C., Williams, D. J. 1993. Antibody responses to a 33 kDa cystein protease of *Trypanosoma congolense*: relationship to 'trypanotolerance' in cattle. *Parasite Immunology*. 15, 465 – 474.

Baker, R. D. 1992. Modelling trypanosomiasis prevalence and periodic epidemics and epizootics. *IMA Journal of Mathematics Applied in Medicine and Biology*. 9, 269 – 287.

Bauer, B., Amsler-Delafosse, S., Kabore, I., Kamuanga, M. 1999. Improvement of cattle productivity through rapid alleviation of African trypanosomosis by integrated disease

management practices in the agro-pastoral zone of Yalé, Burkina Faso. *Tropical Animal Health and Production*. 31, 89 – 102.

Baylis, M., Nambiro, C. O. 1993. The effect of cattle infection by *Trypanosoma congolense* on attraction and feeding success of the tsetse fly *Glossina pallidipes*. *Parasitology*. 106, 357 – 361.

Baylis, M., Stevenson, P. 1998. Trypanosomiasis and tsetse control with insecticidal pour ons – fact and fiction. *Parasitology Today*. 14, 77 – 82.

Bekure, S., de Leeuw, P. N., Grandin, B. E., Neate, P. J. H. 1991. An analysis of the livestock production system of Maasai pastoralists in eastern Kajiado district, Kenya. Technical Report, Nairobi, Kenya.

Bett, B., Machila, N., Gathura, P. B., McDermott, J. J., Eisler, M. C. 2004a. Characteristics of shops selling veterinary medicines in a tsetse-infested area of Kenya. *Preventive Veterinary Medicine*. 63, 29 – 38.

Bett, B., Orenge, C., Irungu, P., Munga, L. K. 2004b. Epidemiological factors that influence time-to-treatment of trypanosomosis in Orma Boran cattle raised at Galana ranch, Kenya. *Veterinary Parasitology*. 120, 43 – 53.

BirdLife International. 2005 BirdLife's online World Bird Database: the site for bird conservation. Version 2.0. Cambridge, UK: BirdLife International. http://www.birdlife.org.

Boyle, P., Flowerdew, R., Williams, A. 1997. Evaluating the goodness of fit in models of sparse medical data: a simulation approach. *International Journal of Epidemiology*. 26, 651 – 656.

Brady, J. 1988. The circadian organization of behaviour: time keeping in the tsetse fly, a model system. *Advances in the study of behaviour*. 18, 153 – 159.

Brightwell, R., Dransfield, R. D., Kyorku, C., Golden, T. K., Tarimo, S. A., Mungai, D. 1987. A new trap for *Glossina pallidipes*. *Tropical Pest Management*. 33(2), 151 – 159.

Brightwell, R., Dransfield, R. D., Stevenson, P., Williams, B. 1997. Changes over twelve years in populations of *Glossina pallidipes* and *Glossina longipennis* (Diptera: Glossinidae) subject to varying trapping pressure at Nkuruman, south-west Kenya. *Bulletin of Entomological Research*. 87, 349 – 370.

Broussard, P. 1996. Third world hit by traffic in fake drugs. Guardian Weekly, 10 November, 1996.

Caroll R. J., Wang, S., Simpson, D. G., Stromberg, A. J., Ruppert, D. 1998. The sandwich (robust covariance matrix) estimator. Technical Report. Preprint available at http://stat.tamu.edu/ftp/pub/rjcarroll/sandwich.pdf.

Catley, A., Irungu, P., Simiyu, K., Dadye, J., Mwakio, W., Kiragu, J., Nyamwaro, S. O. 2002. Participatory investigations of bovine trypanosomiasis in Tana River district, Kenya. *Journal of Medical and Veterinary Entomology*. 16(1), 55-66.

Challier, A., Laveissière, C. 1973. Un nouveau piege pour la capture des glossines (*Glossina*, Diptera). Description et essais sur le terrain, Cahiers ORSTOM. *Series Entomologie Medicale et Parasitologie*. 11, 251–262.

Chizyuka, H. G. B., Liguru, S. M. K. 1986. Dipping to control vectors of cattle parasites. *Parasitology Today*. 2, 123.

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

Connor, R. J. 1989. Final report of the regional trypanosomosis expert. Regional tsetse and trypanosomosis control programme, Malawi, Mozambique, Zambia and Zimbabwe. FGU-Kronberg Consulting and Engineering GmbH, Germany.

Davis, E. E. 1985. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes (Diptera: Cilicidae). *Journal of Medical Entomology*. 22, 237 – 243.

de Souza, E. M., Oliveira, G. M., Boykin, D. W., Kumar, A., Hu, Q., De Nazare, C., Soeiro, M. 2006. Trypanocidal activity of the phenyl-substituted analogue of furamidine DB569 against *Trypanosoma cruzi* infection in vivo. *Journal Antimicrobial Chemotherapy*. 58(3), 610 – 614.

DFID-AHP, 2004. Recent advances in livestock keeper-based tsetse control: the way forward. Report of a workshop organized by the DFID Animal Health Program, held in Nairobi, Kenya, 21 -23 October 2003. DFID Animal Health Program, Centre for Tropical Veterinary Medicine, Edinburgh, Scotland. 68 pp.

Diallo, B. P., Truc, P., Laveissière, C. 1997. A new method for identifying blood meals of human origin in tsetse flies. *Acta Tropica*. 63, 61 – 64.

d'Ieteren, G. D. M., Authié, E., Wissocq, N., Murray, M. 1998. Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. *Review of Science and Technology, Office of International Epizootics*. 17(1), 2 – 34.

Dohoo, I., Martin, W., Stryhn, H. 2003. Controlled trials. In: Veterinary Epidemiologic Research, AVC Inc, PEI, Canada. pp 185 – 205.

Dolan, R. B. 1998. The Orma Boran: a trypanotolerant East African breed. Fifteen years of research on Galana Ranch in Kenya. Kenya Trypanosomiasis Research Institute, Kikuyu, Kenya.

Dransfield, R. D., Brightwell, R. 2004. Community participation in tsetse control: the principles, potential and practice. In: The Trypanosomiases (Maudlin, I. et al., eds), pp. 509 – 523. CABI Publishing.

Dransfield, R. D., Brightwell, R., Kyorku, C., Williams, B. 1990. Control of tsetse fly (Diptera: Glossinidae) populations using traps at Nkuruman, South-West Kenya. *Bulletin of Entomological Research.* 80, 265 – 276.

Dransfield, R. D., Chaudhury, M. F. B., Tarimo, S. A., Golder, T. K., Brightwell, R. 1985. Population dynamics of *Glossina pallidipes* under draught conditions at Nkuruman, Kenya. 18th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Harare, Zimbabwe, 1985, pp 284 – 292. OAU/STRC, Nairobi.

Fall, A., Diack, A., Diaité, A., Seye, M., d'Ieteren, G. D. M. 1999. Tsetse challenge, trypanosome and helminth infection in relation to productivity of village Ndama cattle in Senegal. *Veterinary Parasitology.* 81, 235 – 247.

FAO, 1982a. Training manual for tsetse control personnel. Volume 1. Eds. J. N. Pollock. Food and Agriculture Organization of the United Nations, Rome.

FAO, 1982b. Training manual for tsetse control personnel, Volume 4. Food and Agriculture Organization of the United Nations, Rome.

FAO, 2005. Livestock Sector Brief. Food and Agriculture Organization of the United Nations, FAO and Livestock Information, Sector Analysis and Policy Branch, AGAL.

Fleiss J. L. 1981. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley, pp 225-232.

Fox, R. G. R., Mmbando, S. O., Fox, M. S., Wilson, A. 1993. Effect on herd health and productivity of controlling tsetse and trypanosomiasis by applying deltamethrin on cattle. *Tropical Animal Health and Production.* 25, 203 – 214.

Gaston, K. A., Randolph, S. E. 1993. Reproductive underperformance of tsetse flies in the laboratory, related to feeding frequency. *Physiological Entomology.* 18, 130 – 136.

Geerts, S., Holmes, P. H. 1998. Drug management and parasite resistance in bovine trypanosomiasis in Africa. PAAT Technical Series, No. 1, FAO Rome, pp 31.

Gikonyo, N., Hassanali, A., Njagi, P. G. N., Gitu, P. M., Midiwo, J. O. 2002. Odour composition of preferred (buffalo and ox) and non-preferred (waterbuck) hosts of some savannah tsetse flies. *Journal of Chemical Ecology.* 28, 969 – 981.

Goossens, B., Mbwambo, H., Msangi, A., Geysen, D., Vreysen, M. 2006. Trypanosomosis prevalence in cattle on Mafia Island (Tanzania). *Veterinary Parasitology.* 139(1 – 3), 74 – 83.

Grebaut, P., Mbida, J. A., Kondjio, C. A., Njiokou, F., Penchenier, L., Laveissière, C. 2004. Spatial and temporal patterns of human African trypanosomosis (HAT) transmision risk in the Bipindi focus in the southern Cameroun. *Vector borne Zoonotic Diseases*. 4(3), 230 – 238.

Greiner, M., Kumar, S., Kyeswa, C. 1997. Evaluation and comparison of antibody ELISAs for serodiagnosis of bovine trypanosomosis. *Veterinary Parasitology*. 73(3-4), 197 – 205.

Gu, Y., Gettinby, G., McKendrick, I., Murray, M., Peregrine, A. S., Revie, C. 1999. Development of a decision support system for trypanocidal drug control of bovine trypanosomosis in Africa. *Veterinary Parasitology*. 87, 9 – 23.

Hadaway, A.B., Barlow, F., Turner, C.R. 1976. The susceptibility of different species of tsetse flies to some insecticides. Centre for Overseas Pest Research. Miscellaneous Report No. 234. pp. 4.

Hao, Z., Kasumba, I., Lehane, M., Gibson, W., Kwon, J., Aksoy, S. 2001. Tsetse immune responses and trypanosome transmission: implication for the development of tsetse-based strategies to reduce trypanosomiasis. *Proceedings of the National Academy of Sciences of the United States of America*. 98(22), 12648 – 12653.

Hargrove, J. W. 1988. Tsetse: the limits to population growth. *Medical and Veterinary Entomology*. 2, 203 – 217.

Hargrove, J. W. 1993. Target barriers for tsetse flies (*Glossina* spp) (Diptera: Glossinidae): quick estimates of optimal target densities and barrier widths. *Bulletin of Entomological Research*. 83, 197 – 200.

Hargrove, J. W. 2000. A theoretical study of the invasion of cleared areas by tsetse flies (Diptera: Glossinidae). *Bulletin of Entomological Research*. 90, 201 – 209.

Hargrove, J. W. 2003. Tsetse eradication: sufficiency, necessity and desirability. Research Report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.

Hargrove, J. W., Torr, S. J., Kindness, H. M. 2003. Insecticide-treated cattle against tsetse (Diptera: Glossinidae): what governs success? *Bulletin of Entomological Research*. 93, 203 – 217.

Hargrove, J. W., Williams, B. G. 1998. Optimized simulation as an aid to modelling, with an application to the study of a population of tsetse flies, *Glossina morsitans morsitans* (Diptera: Glossinidae). *Bulletin of Entomological Research*. 88, 425 – 435.

Hargrove, J.W., Omolo, S., Msalilwa, J.S.I. and Fox, B. 2000. Insecticide treated cattle for tsetse control: the power and problems. *Medical and Veterinary Entomology*. 14, 123 – 130.

Holmes, P. H. 1997. New approaches to the integrated control of trypanosomosis. *Veterinary Parasitology*. 72(2-3), 121 – 135.

Hurd, H. 2003. Manipulation of medically important insect vectors by their parasites. *Annual Review of Entomology*. 48, 141 – 161.

International Livestock Centre for Africa (ILCA) (1979). – Trypanotolerant livestock in West and Central Africa. Vol. 1, Monograph No. 2. ILCA, Addis Ababa, Ethiopia.

ILRI (International Livestock Research Institute). 2001. ILRI on a disc. Version 3. [Multi-document CD ROM]. ILRI. Nairobi, Kenya.

ILRI/ICIPE, 2001. Getting tsetse repellent out of the laboratory and to the farmer: enhancing the transfer, delivery and adoption of new control technology for improved livestock health and productivity. Tsetse Repellent Project Proposal, Nairobi.

ILRI/TRC/ICIPE, 2003. Enhancing the diffusion of new tsetse control technologies for improved livestock health and productivity in smallholder indigenous communities of sub-Saharan Africa. Implementation Progress Report No. 2, October 1, 2002 – September 30, 2003.

ILRI/TRC/ICIPE, 2004. Enhancing the diffusion of new tsetse control technologies for improved livestock health and productivity in smallholder indigenous communities of sub-Saharan Africa. Implementation Progress Report No. 3 October 1, 2003 – September 30, 2004. Nairobi.

Jaetzold, R., Schmidt, H. 1983. Farm management handbook of Kenya. Vol. II. Natural conditions and farm management information, Part B, Central Kenya (Rift Valley and Central Provinces). Ministry of Agriculture and German Agricultural Team, Nairobi.

Jarry, M., Gouteux, J. P., Khaladi, M. 1999. Estimation of age-dependent survival rates of female tsetse flies (Diptera: Glossinidae) from ovarian age distributions. *Bulletin of Entomological Research*. 89, 515 – 521.

Jarry, M., Khalandi, M., Gouteux, J.-P. 1996. A matrix model for studying tsetse fly populations. *Entomologia Experimentalis et Applicata*. 78, 51 – 60.

Jordan, A. 1986. Trypanosomiasis control and African rural development. Longman, London.

Kamau, S. W., Omukuba, J., Kiragu, J., Masika, P., Ndungu, J. M., Wachira, P., Mehlitz, D. 2000. Financial analysis of animal trypanosomosis control using cypermethrin pour-on in Kenya. *Preventive Veterinary Medicine*. 44, 231 – 246.

Kamuanga, M., Sigué, H., Swallow, B., Bauer, B., d'Ieteren, G. 2001. Farmers perception of the impacts of tsetse and trypanosomosis control on livestock production: evidence from southern Burkina Faso. *Tropical Animal Health and Production*. 33(2), 141 – 153.

Karanja, S. M. 2005. Epidemiology and importance of trypanosomosis, helminthosis and tick-borne diseases on the performance of cattle in Busia district, Kenya. PhD Thesis, Institute for Parasitology and International Animal Health, Faculty of Veterinary Medicine, Freie Universität Berlin.

Kasilagila, G. 2003. Studies on trap effectiveness of tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) in the Tanga Region of north eastern Tanzania. *Acta Tropica*. 87, 385 – 392.

Khan, Z. R., Ampong-Nyarko, K., Chiliswa, P., Hassanali, A., Kimani, S., Lwande, W., Overholt, W. A., Pickett, J. A., Smart, Wadhams, L. J., Woodcock, C. M. 1997. Intercropping increases parasitism of pests. *Nature*. 388, 631 – 632.

Killick-Kendrick, R. 1968. The diagnosis of trypanosomiasis of livestock – a review of current techniques. *Veterinary Bulletin*. 38, 191 – 199.

Kitani, H., Yagi, Y., Naessens, J., Sekikawa, K., Iraqi, F. 2004. The secretion of acute phase proteins and inflammatory cytokines during *Trypanosoma congolense* infection is not affected by the absence of the TNF-α gene. *Acta Tropica*. 92, 35 – 42.

Kristjanson, P. M., Swallow, B. M., Rowlands, G. J., Kruska, R. L., de Leeuw, P. N. 1999. Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agricultural Systems*. 59, 79 – 98.

Lambert, D., Roeder, K. 1995. Overdispersion diagnostics for generalized linear models. *Journal of American Statistical Association*. 90, 1225 – 1235.

Langridge, W. P. 1975. Design and operation pf the "Langridge" tsetse fly trap. In: *The 14th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISTRC)*, OAU/ISTRC. Dakar, Senegal, publication no. 109, 277 – 281.

Leak, S. G. A. 1999. Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis. CABI/ILRI, Wallingford, UK.

Leak, S. G. A.,. Awoume, K, Colardelle, C., Duffera, W., Feron, A., Mahamat, B., Mawuena, K., Minengu, M., Mulungo, M., Nankodaba, C., Ordner, G., Pelo, M., Sheria, M., Tikubet, G., Toure, M., Yangari. G. 1988. Determination of tsetse challenge and its relationship with trypanosome prevalence in trypanotolerant livestock at sites of the African trypanotolerant livestock network. In: Livestock Production in Tsetse Affected

Areas of Africa. The African trypanotolerant livestock network, proceedings of a meeting held 23-27 November 1987 Nairobi, Kenya

Leak, S. G., Mulâtu, W., Authié, E., d'Ieteren, G. D., Peregrine, A. S., Rowlands, G. J., Trail, J. C. 1993. Epidemiology of bovine trypanosomosis in the Ghibe valley, southwest Ethiopia. 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropica*. 53(2), 121 – 134.

Leak, S.G.A., Woudyalew, M., d'Ieteren, G.D.M. 1995. A trial of cypermethrin pour on to control *G. fuscipes fuscipes* and *G. morsitans submorsitans* (Diptera: Glossinidae) in South West Ethiopia. *Bulletin of Entomological Research*. 85, 241 – 251.

Lohr, K.F., Omukuba, J.N., Njogu, A.R., Maloo, S.H., Gisemba, F., Okedi, T., Mwongela, S. 1991. Investigations of the efficacy of flumethrin pour on for the control of high tsetse and trypanosomosis challenge in Kenya. *Tropical Medicine and Parasitology*. 42, 131 - 134.

Machila, N., Wanyangu, S. W., McDermott, J., Welburn, S. C., Maudlin, I., Eisler, M. C. 2003. Cattle owners' perceptions of African bovine Trypanosomiasis and its control in Busia and Kwale districts of Kenya. *Acta Tropica*. 86, 25 – 34.

Magez, S., Stijlemans, B., Caljon, G., Eugster, H. P., De Baetselier, P. 2002. Control of experimental *Trypanosoma brucei* infections occur independently of lymphotoxin-alpha induction. *Infection and Immunity*. 70(3), 1342 – 1351.

Magona, J. W., Mayende, J. S., Olaho-Mukani, W., Coleman, P. G., Jonson, N. N., Welburn, S. C., Eisler, M. C. 2003. A comparative study on the clinical, parasitological and molecular diagnosis of bovine trypanosomosis in Uganda. *Onderstepoort Journal of Veterinary Science*. 70(3), 213 – 218.

Makumi, J. N., Stevenson, P., Green, C. H. 2000. Control of *Glossina longipennis* (Diptera: Glossinidae) by insecticide-treated targets at Galana Ranch, Kenya, and confirmation of the role of *G. longipennis* as a vector of cattle trypanosomiasis. *Bulletin of Entomological Research*. 90, 397 – 406.

Mangwiro, T. N., Torr, S. J., Cox, J. R., Holloway, M. T. 1999. The efficacy of various pyrethroid insecticides for use on odour-baited targets to control tsetse. *Medical and Veterinary Entomology*. 13(3), 315 – 323.

Mattioli, R. C., Faye, J. A. Jaitner, J. 2001. Estimation of trypanosome status by the buffy coat technique and an antibody ELISA for assessment of the impact of trypanosomosis on health and productivity of N'Dama cattle in the Gambia. *Veterinary Parasitology*. 95(1), 25 – 35.

Maudlin, I., Dukes, P., Luckins, A. G., Hudson, K. M. 1986. Extrachromosomal inheritance of susceptibility to trypanosome infection in tsetse flies. II. Susceptibility of selected lines

of *Glossina morsitans morsitans* to different stocks and species of trypanosome. *Annals of Tropical Medicine and Parasitology.* 80(1), 97 – 105.

McDermott, J. J., Coleman, P. G. 2001. Comparing apples and oranges – model-based assessment of different tsetse-transmitted trypansomosis control strategies. *International Journal for Parasitology.* 31, 603 – 609.

McDermott, J., Coleman, P. 1999. Research into trypanosomosis epidemiology – the essential contributions of theory, models, diagnostics and field studies. In: Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vector (ICPTV), No. 1. Ed. M. Eisler and P. Holmes, FAO, Edinburgh.

McDonald, G. 1957. The epidemiology and control of malaria. Oxford University Press. Oxford, UK.

Meinert, C. L., Tonascia, S. 1986. Clinical trials, Design, Conduct, and Analysis. Oxford University Press, New York. Pp. 469.

Milligan, P. 1990. Modelling trypanosomiasis transmission. *Insect Science and its Application.* 11, 301 – 307.

Milligan, P. J. M., Baker, R. D. 1988. A model of tsetse-transmitted animal trypanosomiasis. *Parasitology.* 96, 211 – 239.

Moloo, S. K. 1973. A new trap for *Glossina pallidipes* Austen and *G. fuscipes* Newstead (Diptera: Glossinidae). *Bulletin of Entomological Research.* 63, 231 – 236.

Moloo, S. K., Kutuza, S. B., Boreham, P. F. L. 1980. Studies on *Glossina pallidipes*, *G. fuscipes fuscipes* and *G. brevipalpis* in terms of epidemiology and epizootiology of trypanosomiasis in south-eastern Uganda. *Annals of Tropical Medicine and Parasitology.* 74, 219 – 237.

Moloo, S. K., Sabwa, C. L., Kabata, J. M. 1992. Vector competence of *Glossina pallidipes* and *G. morsitans* centralis for *Trypanosoma vivax*, *T. congolense* and *T. b. brucei*. *Acta Tropica.* 51(3-4), 271 – 280.

Mukiria, P. 2002. Back to office report on tsetse and trypanosomosis survey in Narok. KETRI.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P. A., Masake, P. R., Peregrine, A. S. 1997. Long-term occurrence of *Trypanosoma congolense* resistance to diminazene, isometamidium and homidium in cattle at Ghibe, Ethiopia. *Acta Tropica.* 64(3-4), 205 – 217.

Murray, M., Morrison, W. I., Whitelaw, D. D. 1982. Host susceptibility to African trypanosomiasis: trypanotolerance. *Advances in Parasitology.* 21, 1- 68.

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

Mwangi, E. K., Stevenson, P., Gettinby, G., Reid, S. W. and Murray, M. (1998). Susceptibility to trypanosomosis of three *Bos indicus* cattle breeds in areas of differing tsetse fly challenge. *Veterinary Parasitology*. 79, 1-17

Ndegwa, P. N., Mihok, S. 1999. Development of odour-baited traps for *Glossina swynnertoni* (Diptera: Glossinidae). *Bulletin of Entomological Research*. 89, 255 – 261.

Ngure, R. M., Eckersall, P. D., Jennings, F. W., Burke, J. M., Stear, M. J., Kennedy, P. G. E., Murray, M. 1997. Major acute phase response of haptoglobulin and serum amyloid-P following experimental infection of mice with *Trypanosoma brucei brucei*. *International Parasitology*. 46, 247 – 254.

Njiru, Z. K., Makumi, J. N., Okoth, S., Ndungu, J. M., Gibson, W. C. 2004. Identification of trypanosomes in *Glossina pallidipes* and *G. longipennis* in Kenya. *Infection Genetics Evolution*. 4(1), 29 – 35.

Ochieng-Odiambo, M. 1998. Liberalisation, law and management of public land and forests in Kenya. In: Common Poverty Resource Management in East Africa, Ed. Gibson Clark, Banana Abwooli and Ntarmbirweki J. Proceedings of the Regional Symposium on Common Property Resource Management in East Africa held in Kampala, March 26 – 28 1996 under auspices of Makerere Institute of Social Research (MISR) sponsored by Ford foundation of the United Nations. Pp 1 -27.

Okello-Onen, J., Heinonen, R., Ssekitto, C.M., Mwayi, W.T., Kakaire, D. and Kabarema, M. 1994. Control of tsetse flies in Uganda by dipping cattle in deltamethrin. *Tropical Animal Health and Production*. 26, 21 - 27.

Okoth, S. O., Kokwaro, E. D., Kiragu, J. M., Murilla, G. A. 2006. Susceptibility and transmission capacity of sub-populations of *Glossina pallidipes* to human infective *Trypanosoma brucei rhodesiense*. *Trends in Medical Research*. 1(1), 75 – 85.

Otte, M. J., Gumm, I. D. 1997. Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. *Preventive Veterinary Medicine*. 31, 147 – 150.

Ouma, J. O., Masake, R. A., Masiga, D. K., Moloo, S. K., Njuguna, J. T., Ndungu, J. M. 2000. Comparative sensitivity of dot-ELISA, PCR and dissection method for the detection of trypanosome infections in tsetse flies (Diptera: Glossinidae). *Acta Tropica*. 75(3), 315 – 321.

Peduzzi, P., Henderson, W., Hartigan, P., Lavori, P. 2002. Analysis of randomized control trials. *Epidemiologic Reviews*. 24(1), 26-38.

Peregrine, A. S. 1994. Chemotherapy and delivery systems: heamoparasites. *Veterinary Parasitology*. 54, 223 – 248.

Peregrine, A. S., Mamman, M. 1993. Pharmacology of diminazene: a review. *Acta Tropica*. 54, 185 – 203.

Radostits, O. M., Blood, D. C., Gay, C. C. 1994. Diseases caused by trypanosomes. In: Veterinary Medicine, a textbook of the diseases of cattle sheep, pigs, goats and horses, 8th Edition. Baillière Tindall, London pp 1212.

Randolph, S. E., Williams, B., Rogers, D.J., Connor, H. 1992. Modelling the effect of feeding-related mortality on the feeding strategy of tsetse (Diptera: Glossinidae). *Medical and Veterinary Entomology*. 6, 231 – 240.

Reichard, R. E. 2002. Area-wide biological control of disease vectors and agents affecting wildlife. *Review of Science and Technology*. 21(1), 179 – 185.

Reid, R. S., Rainy, M., Ogutu, J., Kruska, R. L., Kimani, K., Nyabenge, M., McCarthy, M., Kshatriya, M., Worden, J., Nganga, L., Owuor, J., Kinoti, J., Njuguna, E., Wilson, C. J., Lamprey, R. 2003. People, wildlife and livestock in the Mara ecosystem: the Mara count 2002. Report, Mara count 2002, International Livestock Research Institute, Nairobi, Kenya.

Roberts, L. W. 1981. Probing by *Glossina morsitans morsitans* and transmission of Trypanosoma (Nannomonas) congolense. *American Journal of Tropical Medicine and Hygiene*. 30(5), 948 – 951.

Roderick, S., Stevenson, P., Mwendia, C., Okech, G. 2000. The use of trypanocides and antibiotics by Maasai pastoralists. *Tropical Animal Health and Production*. 32, 361 – 374.

Rogers, D. J. 1984. The estimation of sampling biases for male tsetse. *Insect Science and its Application*. 5, 369 – 373.

Rogers, D. J. 1985. Trypanosomiasis "risk" or "challenge": a review. *Acta Tropica*. 42(1), 5 – 23.

Rogers, D. J. 1988. A general model for the African trypanosomiases. *Parasitology*. 97, 193 – 212.

Rogers, D. J., Boreham, P. F. L. 1973. Sleeping sickness survey in the Serengeti area (Tanzania) 1971. II. The vector role of *Glossina swynnertoni* Austen. *Acta Tropica*. 30, 24 – 35.

Ross, R. 1911. The prevention of malaria. Murray. London.

Rowlands, C. J., Mulatu, W., Leak, S. G., Nagda, S. M., d'Ieteren, G. D. 1999. Estimating the effects of tsetse control on livestock production – a case study in the southwest Ethiopia. *Tropical Animal Health and Production*. 31(5), 279 – 294.

Rowlands, G. J., Leak, S. G. A., Peregrine, A. S., Nagda, S. M., Woudyalew Mulatu, d'Ieteren, G. D. M. 2001. The incidence of new and the prevalence and persistence of recurrent trypanosome infections in cattle in southwest Ethiopia exposed to a high challenge with drug-resistant parasites. *Acta Tropica*. 79, 149 – 163.

Rurangirwa, F. R., Minja, S. H., Musoke, A. J., Nantulya, V. M., Grootenhuis, J., Moloo, S. K. 1986. Production and evaluation of specific antisera against sera of various vertebrate species for identification of bloodmeals of *Glossina morsitans centralis*. *Acta Tropica*. 43, 379 – 389.

Saini, R. K., Hassanali, A. 2002. Attractants and repellents for tsetse – where do we go from here? In: Proceedings of the joint ICTTD-2/ICPTV Workshop on Integrated Vector Control including synergistic use of drugs and bait technologies for the control of trypanosomiasis and tick borne diseases, held 10-12th April 2002 at the Institute of Tropical Medicine, Antwerp, Belgium. Pg 31-32.

Saini, R. K., Hassanali, A. 2007. A 4-alkyl-substituted analogue of guaiacol shows greater repellency to savannah tsetse (*Glossina* spp.). *Journal of Chemical Ecology*. 33(5), 985 – 995.

Sasaki, H., Kang'ethe, E. K., Kaburia, H. F. 1995. Blood meal sources of *Glossina pallidipes* and *G. longipennis* (Diptera: Glossinidae) in Nkuruman, southwest Kenya. *Journal of Medical Entomology*. 32(3), 390 – 393.

Savill, N. J., Seed, J. R. 2004. Mathematical and statistical analysis of the *Trypanosoma brucei* slender to stumpy transition. *Parasitology*. 128, 53 – 67.

Schofield, C. J., Kuzoe, F. A. S. 2004. Strategic review of traps and targets for tsetse and African trypanosomiasis control. UNICEF/UNDP/World Bank/WHO.

Schofield, C. J., Maudlin, I. 2001. Trypanosomosis control. *International Journal for Parasitology*. 31, 615 – 620.

Schukken, Y. H., Grohn, Y. T., McDermott, B., McDermott, J. J. 2003. Analysis of correlated discrete observations: background, examples and solutions. *Preventive Veterinary Medicine*. 59(4), 223 – 240.

Snow, W. F., Tarimo, S. A., Staak, C., Butler, L. 1988. The feeding habits of the tsetse, *Glossina pallidipes* Austen on the south Kenya coast, in the context of its host range and trypanosome infection rates in other parts of East Africa. *Acta Tropica* 45(4), 339 – 349.

Steuber, S., Abdel-Rady, A., Clausen, P. H. 2005. PCR-RFLP analysis: a promising technique for host species identification of blood meals from tsetse flies (Diptera: Glossinidae). *Parasitology Research*. 97(3), 247 – 254.

Stevenson, P., Munga, L., Makumi, L., Baylis, M., Alushula, L. 1991. The control of tsetse and trypanosomiasis by deltamethrin treatment of ranch cattle in Kenya. Proceedings of 21st ISCTRC Meeting at Yamoussoukro, Ivory Coast. pp. 201. OAU/STRC, Nairobi.

Steverding, D., Pemberton, A. J., Royle, H., Spackman, R. W., Rivett, A. J. 2006. Evaluation of the antitrypanosomal activity of tyropeptin A. *Planta Medica*. 72(8), 761 – 763.

Swallow, B. 1999. Impact of trypanosomosis on African agriculture. FAO. http://www.fao.org/AG/againfo/programmes/es/paat/documents/papers/Pos2.pdf#search='swallow%20trypanosomiasis'

Swynnerton, C. F. M. 1933. Some traps for tsetse flies. *Bulletin of Entomological Research*. 24, 69 – 102.

Tarimo, C. S., Gates, B. D., Williamson, D. L. 1981. Feeding preference of Glossina in North-eastern Tanzania. In the 17th Meeting of ISCTRC, Arusha, Tanzania, pp 415 – 418. Nairobi, Kenya, OAU/STRC.

Tarimo, S. A., Snow, W. F., Butler, L., Dransfield, R. 1985. The probability of tsetse acquiring trypanosome infection from single blood meal in different localities in Kenya. *Acta Tropica*. 42, 199 – 207.

Thompson, J.W., Mitchell, M., Rees, R.B., Shereni, W., Schoenfeld, A. H., Wilson, A. 1991. Studies on the efficacy of deltamethrin applied to cattle for the control of tsetse flies (*Glossina* spp.) in Southern Africa. *Tropical Animal Health and Production*. 23, 221 – 226.

Torr, S. J., Hargrove, J. W. 1999. Behaviour of tsetse (Diptera: Glossinidae) during the hot season in Zimbabwe: the interaction of micro-climate and reproductive status. *Bulletin of Entomological Research*. 89, 365 – 379.

Torr, S. J., Hargrove, J. W., Vale, G. A. 2005. Towards a rational policy for dealing with tsetse. *Trends in Parasitology*. 21(11), 537 – 541.

Torr, S. J., Mangwiro, T. N. C., Hall, D. R. 2006. The effects of host physiology on the attaction of tsetse (Diptera: Glossinidae) and *Stomoxys* (Diptera: Muscidae) to cattle. *Bulletin of Entomological Research*. 96, 71 – 84.

Torr, S. J., Wilson, P. J., Schofield, S., Mangwiro, T. N. C., Akber, S., White, B. N. 2001. Application of DNA markers to identify the individual-specific hosts of tsetse feeding on cattle. *Medical and Veterinary Entomology*. 15, 78 – 86.

Torr, S.J., Mangwiro, T.N.C., Hall, D.R. 1996. Responses of tsetse flies (Diptera: Glossinidae) to synthetic repellents in the field. *Bulletin of Entomological Research*. 86, 609-616.

Vale, G. A. 1974. The responses of tsetse flies (Diptera: Glossinidae) to stationary and mobile baits. *Bulletin of Entomological Research*. 64, 545 – 588.

Vale, G. A. 1980. Field studies of the responses of tsetse flies (Glossinidae) and other Diptera to carbon dioxide, acetone and other chemicals. *Bulletin of Entomological Research*. 70, 563 – 570.

Vale, G. A., Cumming, D. H. M. 1976. The effect of selective elimination of hosts on a population of tsetse flies (*Glossina morsitans morsitans* Westwood (Diptera, Glossinidae). *Bulletin of Entomological Research*. 66, 713 – 729.

Vale, G. A., Hargrove, J. W., Cockbill, G. F., Phelps, R. J. 1986. Field trials of baits to control populations of *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen (Diptera: Glossinidae). *Bulletin of Entomological Research*. 76, 179 – 193.

Vale, G. A., Hursey, B. S., Hargrove, J. W., Torr, S. J., Allsopp, R. 1984. The use of small plots to study populations of tsetse (Diptera: Glossinidae). *Insect Science and its Application*. 5, 403 – 410.

Vale, G. A., Torr, J. 2005. User-friendly models of costs and efficacy of tsetse control: application to sterilizing and insecticidal techniques. *Medical and Veterinary Entomology*. 19, 293 – 305.

Van den Bossche, P., Doran, M., Connor, R. J. 2000. An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Tropica*. 75, 247 – 258.

Van den Bossche, P., Munsimbwe, L., Mubanga, J., Jooste, R., Lumamba, D. 2004. A large-scale trial to evaluate the efficacy of a 1% pour-on formulation of cyfluthrin (Cylence, Bayer) in controlling bovine trypanosomosis in Eastern Zambia. *Tropical Animal Health and Production*. 36, 33 – 43.

Van den Bossche, P., Van Hees, J. 1987. Observations on the remnant effect of deltamethrin acaricide liquid on tsetse flies under laboratory conditions. Proceedings of the 19th ISCTRC Conference held at Lome, Togo. OAU/STRC, Nairobi.

Voskamp, K. E., Everaarts, E., Den Otter, C. J. 1999. Olfactory responses to attractants and repellents in tsetse. *Medical and Veterinary Entomology*. 13, 386 – 392.

Warnes, M. L., Van den Bossche, P., Chihiya, J., Mudenge, D., Robinson, T. P., Shereni, W., Chadenga, V. 1999. Evaluation of insecticide-treated cattle as a barrier to re-invasion of tsetse to cleared areas in north-eastern Zimbabwe. *Medical and Veterinary Entomology*. 13(2), 177 – 184.

Welburn, S., Maudlin, I. 1999. Tsetse – Trypanosome interactions: Rites of passage. *Parasitology Today*. 15(10), 399 – 403.

Wellde, B. T., Reardon, M. J., Kovatch, R. M., Chumo, D. A., Williams, J. S., Boyce, W. L., Hockmeyer, W. T., Wykff, D. E. 1989. Experimental infection of cattle with *T. brucei rhodesiense*. *Annals of Tropical Medicine and Parasitology*. 83, 133 – 150.

Williams, B. G., Dransfield, R. D., Brightwell, R. 1990. Tsetse fly (Diptera: Glossinidae) population dynamics and the estimation of mortality rates from life table data. *Bulletin of Entomological Research*. 80, 479 – 485.

Williams, B., Dransfield, R., Brightwell, R. 1992. The control of tsetse flies in relation to fly movement and trapping efficiency. *Journal of Applied Ecology*. 29, 163 – 179.

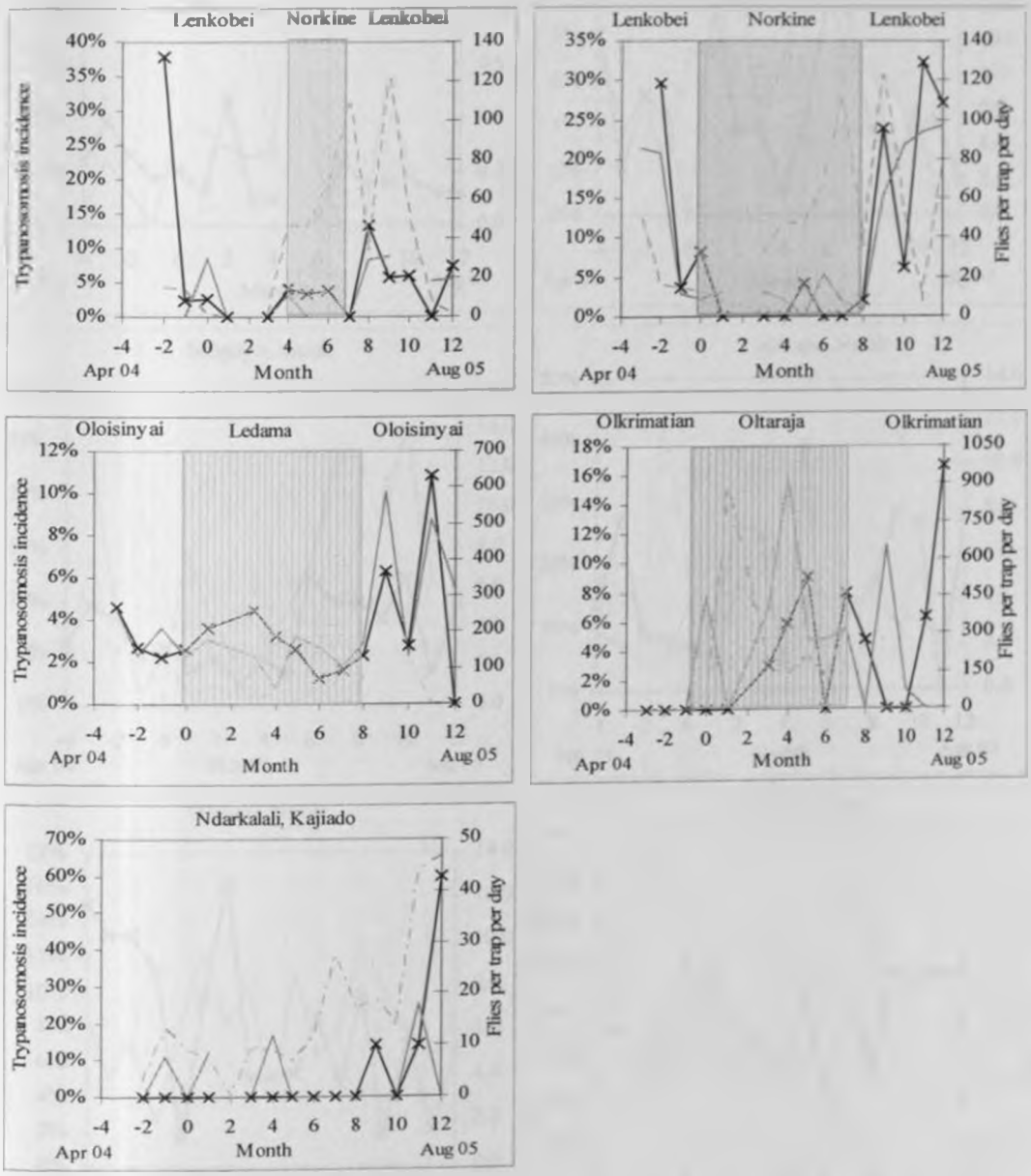
Wilson, A. J., Gatuta, G. M., Njogu, A. R., Mgutu, S. P., Alushula, H. 1986. A simple epidemiological method for animal trypanosomiasis to provide relevant data for effective financial decision-making. *Veterinary Parasitology*. 20(4), 261 – 274.

Witola, W. H., Lovelace, C. E. A. 1997. Serum proteins in indigenous Zambian goats with trypanosomosis. *Journal of the Federation of American Societies for Experimental Biology (FASEB)*. 11(9), A 1257.

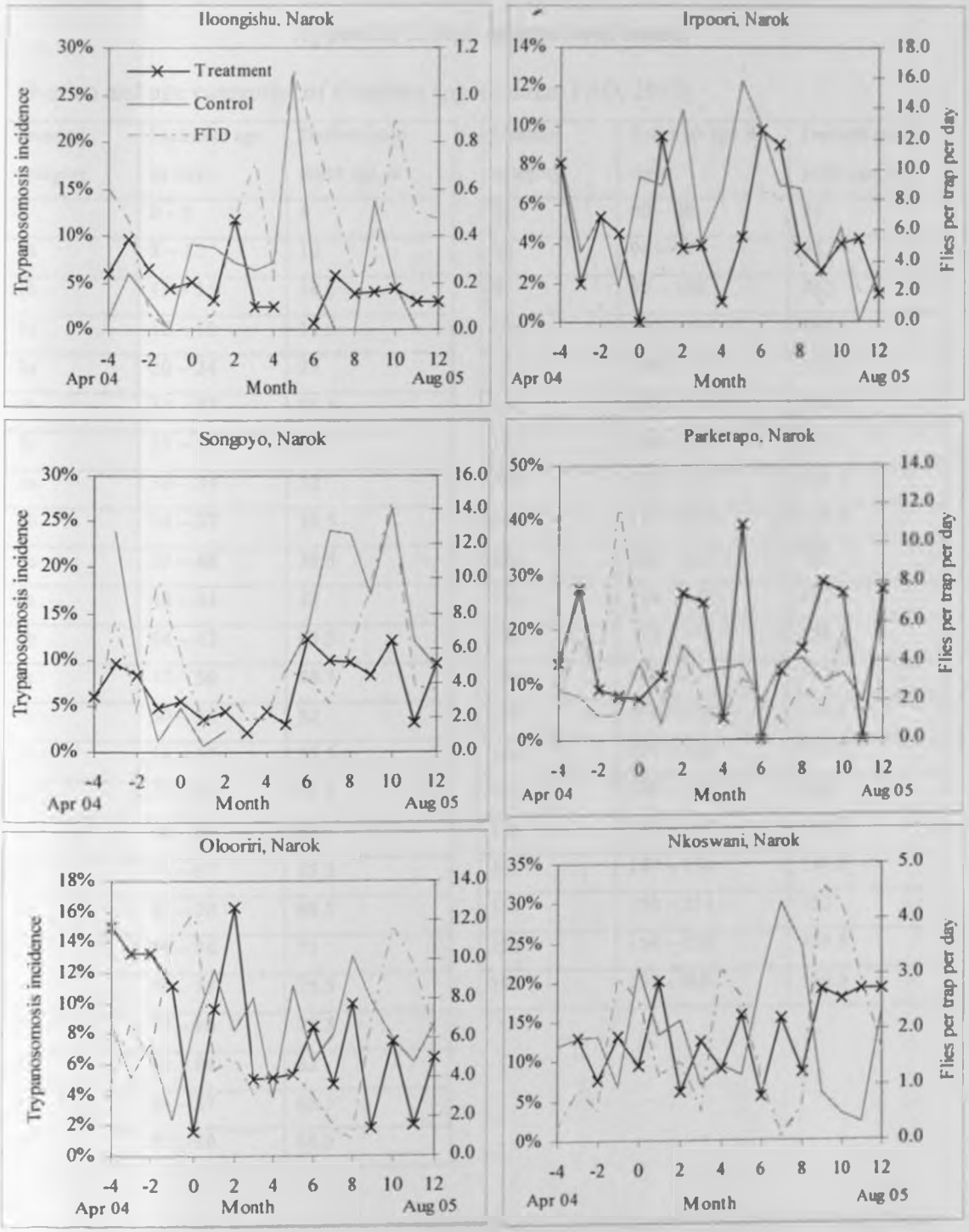
Woolhouse, M. E. J., Hargrove, J. W. 1998. On the interpretation of age-prevalence curves for trypanosome infections of tsetse flies. *Parasitology*. 166, 149 – 156.

Appendices

Appendix 1. Monthly incidence of trypanosomosis in cattle used in the tsetse repellent evaluation trial and transformed apparent tsetse density (FTD) in the villages where the study was carried out in Nkineji, Narok and Nkuruman, Kajiado (2004 – 2005).



(a) Kajiado - Herds changed grazing sites (villages) depending on season except in a village called Ndarkalali (last graph). The villages where the herds were located at various time points have been indicated by the headings in the graphs and bands in the body of the graphs. Grey bands correspond with dry season grazing areas and white bands were the wet season grazing areas. There were two treatment and control herds in Oloisinyai-Ledama-Oloisinyai villages that were managed independently. Their incidences have been averaged by treatment group.



(b) Narok - herds did not change their grazing areas except few instances during drought. Control and treatment herds are compared based on trypanosomosis incidence

Appendix 2. Age categories of tsetse.

Ovarian and age categories of *Glossina* spp. (Source: FAO, 1982)

Ovarian category	Estimate age in days	Derived mid point age, *a*
0	0 - 8	4
1a	8 – 12	10
1b	13 – 16	14.5
1c	16 – 19	17.5
2a	20 – 24	22
2b	24 – 27	25.5
2c	27 – 30	28.5
3a	30 – 34	32
3b	34 – 37	35.5
3c	37 – 40	38.5
4a	40 – 44	42
4b	44 – 47	45.5
4c	47 – 50	48.5
5a	50 – 54	52
5b	54 – 57	55.5
5c	57 – 60	58.5
6a	60 – 64	62
6b	64 – 67	65.5
6c	67 – 70	68.5
7a	70 – 74	72
7b	74 – 77	75.5
7c	77 – 80	78.5
8a	80 – 84	82
8b	84 – 87	85.5
8c	87 – 90	88.5

Ovarian category	Estimate age in days	Derived mid point age, *a*
9a	90 – 94	92
9b	94 – 97	95.5
9c	97 – 100	98.5
10a	100 – 104	102
10b	104 – 107	105.5
10c	107 – 110	108.5
11a	110 – 114	112
11b	114 – 117	114.5
11c	117 – 120	118.5
12a	120 – 124	122
12b	124 – 127	125.5
12c	127 – 130	128.5
13a	130 – 134	132
13b	134 – 137	135.5
13c	137 – 140	138.5
14a	140 – 144	142
14b	144 – 147	145.5
14c	147 – 150	148.5
15a	150 – 154	152
15b	154 – 157	155.5
15c	157 - 160	158.5

To give an example, the proportion of flies that will have gotten infected by the time they are about 47-50 days old is obtained by:

$$y_{47-50} = 1 - \exp[-\lambda_v(48.5 - T)]$$

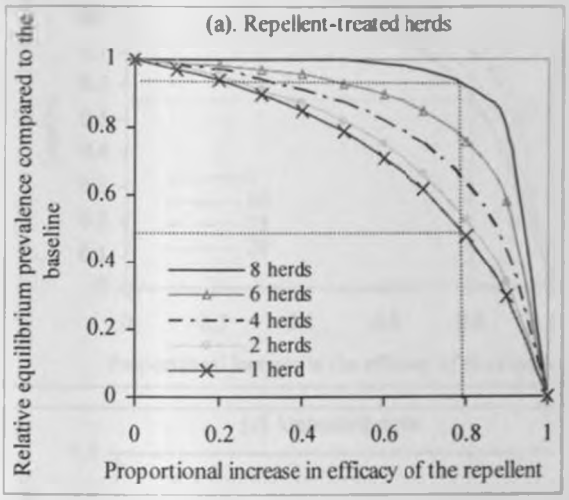
where:

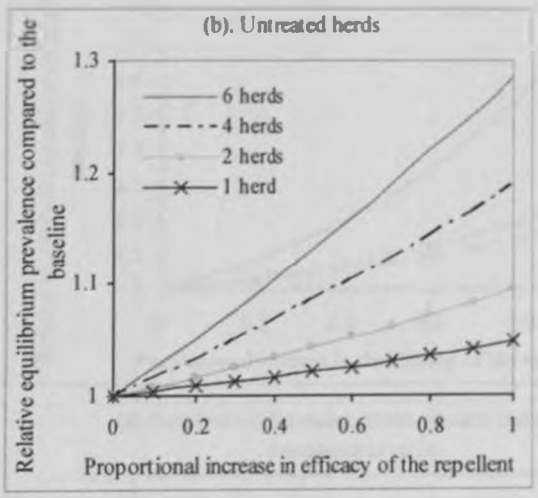
$$\lambda_v = f \cdot (p_1/d \cdot x_1 + p_2/d \cdot x_2 + p_3/d \cdot x_3)$$

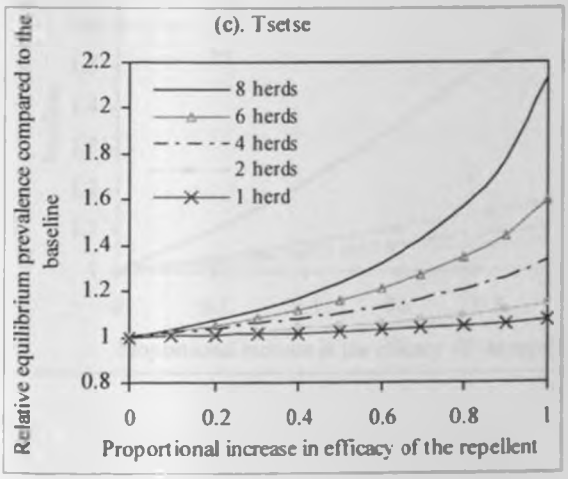
T – determined whether the formula returns the proportion of flies infected by *T. vivax* (if T = 10) or *T. congolense* (if T = 20).

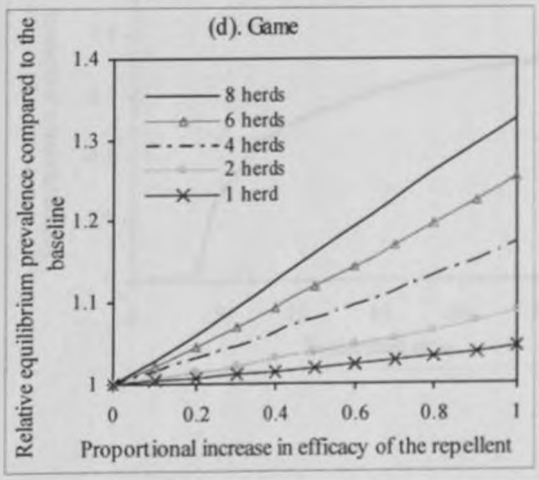
The overall prevalence is obtained by aggregating the number infected across all ages.

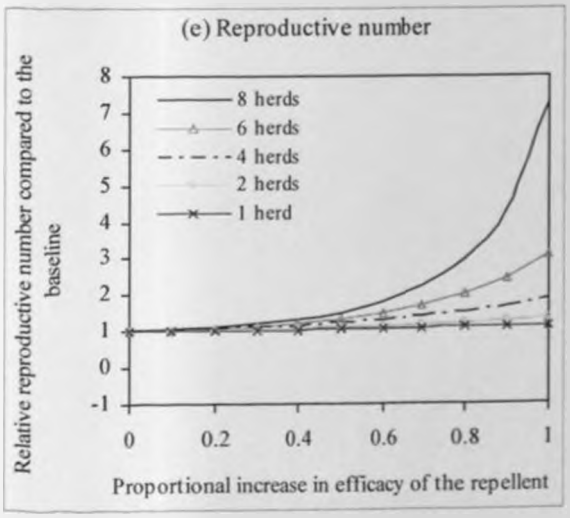
Appendix 3. The expected effect of the repellent technology on equilibrium trypanosomosis prevalence in: (a) repellent-treated herds, (b) untreated herds, (c) tsetse and (d) game under varied levels of treatment coverage. The effect of treatment on reproductive number is illustrated in (e). Figure (f) shows the relative increase in the rate of treatment in untreated herds that is expected to result in the maintenance of the baseline equilibrium prevalence.

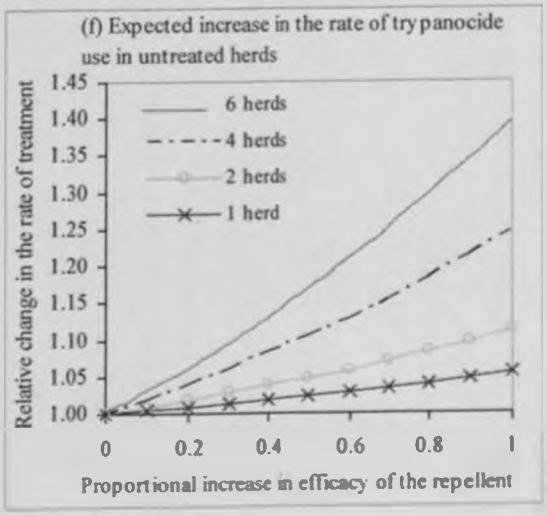




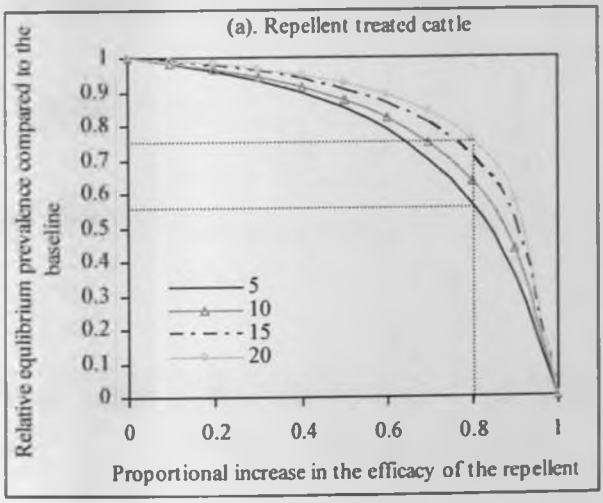


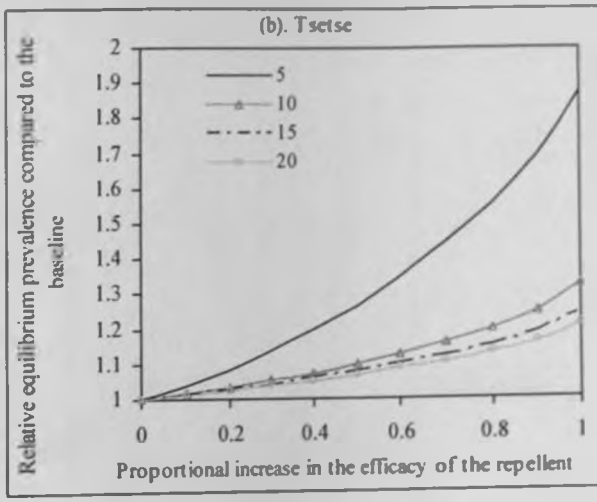


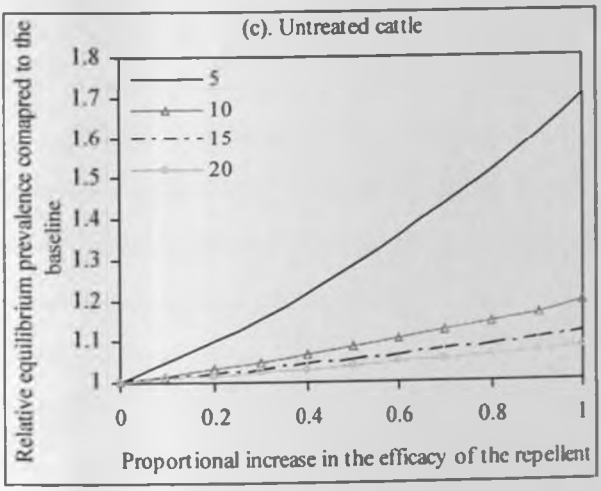


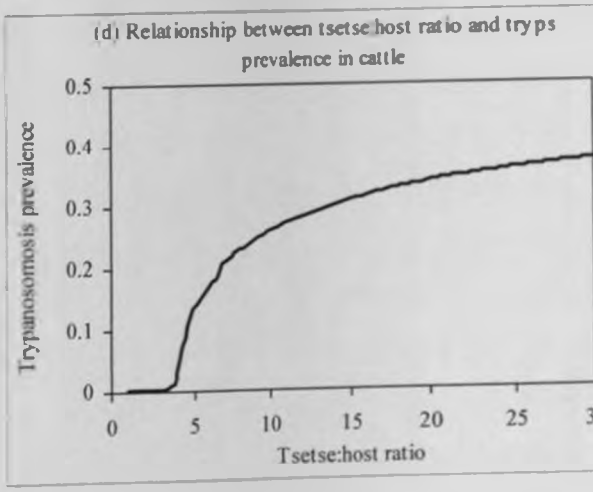


Appendix 4. The expected effect of the repellent technology on equilibrium trypanosomosis prevalence under varying levels of tsetse challenge represented by tsetse:host ratio. Four out of a total of eight herds assumed to be confined within an area of 4km^2 are treated with the repellent









Appendix 5. Data sheets

1A. Data sheet used for recording the number and species of tsetse caught in traps

Location _____

Date traps set _____ Date traps emptied _____

Trap No.	Spp.	No. Non-Tenerals		No. Tenerals		Total	No./spp. Other biting flies	Remarks
		Male	Female	Male	Female			

1B. Data sheet used for identifying blood meal samples

A. Data sheet for collection of blood meal

Sheet No._____ Smear No.____ Date of collection_____ Tsetse species____ Sex____

Degree of engorgement_____ other remarks_____

B. Record sheet for identification of blood meal

Collected by_____ Date_____

C. Details of collection area

Possible hosts_____

Exact locality_____

Tsetse species present_____

1C. Tsetse dissection form (for recording trypanosome infection status)

Village: _____ Date set: _____ Date emptied _____

Date dissected _____

Tsetse No	Trap No	Species	Sex	Labrum	Hypopharynx	Midgut	Tryps spp

1D. Ovarian aging data sheet

Date of collection _____ District _____ Village _____

Trap No.	Spp	NT Male	NT Female	T Male	T Female	Total

No.	Tsetse Spp.	Trap No.	Uterine content	Large ovary (L/R)	Large ovariole (I/O)	Fly age (days)

1E. Wing fray aging form

Date of collection _____ Village_____ District _____

Spp	TM	NTM	TF	NTF	TOTAL	COMMENTS

Fray Category	Frequency	Factor	Product
1		1	
2		2	
3		3	
4		4.4	
5		5.5	
6		6.9	
Sum			

MWFV = $\frac{\text{Sum of products}}{\text{Sum of frequency}}$

Mean age _____

1F. Data sheet for recording the status of the repellent and dispenser

Farmer _____ Village _____ Date _____

IDNO.	Collar	Reservoirs	Silicon tube		Stopper		Repellent		Remarks
			1	2	1	2	1	2	

1G. Data sheet for thin blood smears

Area: (Nkuruman/Narok) _____ Farmer's name_____

Date of preparation _____ Date of examination_____

ID No.	*T. vivax*	*T. congolense*	*A. marginale*	Piroplasms [only]	Remarks

1H. Data sheet used for recording trypanocide use, purchases, sales and births

Farmer _____ Location [Nkuruman/Narok] _____

Village _____

ID NO	DATE	TREATMENTS		REMOVAL		ADDITION		BIRTHS
		Drug 1	Drug 2		Reason		Reason	

1I. Data sheet used for trypanosomosis surveillance

Farmer _____ Village _____ Date _____

ID No.	Weight	Condition score	PCV	BCT	Treatment	Comments