A FIELD EVALUATION OF THREE TRYPANOSOMOSIS CONTROL STRATEGIES, IN KWAZULU-NATAL, SOUTH AFRICA

By

FORBES RICHARD EMSLIE

Promoter: Prof. B. Gummow

Co-promoter: Prof. B. L. Penzhorn

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Rural subsistence farming practices are the primary agricultural activity in northeastern KwaZulu-Natal, South Africa. Cattle in this area have long been affected by tsetse-borne trypanosome infections. The causative organism, *Trypanosoma brucei brucei* was first identified by Bruce in the late 1800's. Approximately 120000 cattle fall within a tsetse (*Glossina austeni* and *Glossina brevipalpis*) belt common to Mozambique and South Africa.

Between 1991 and 1994 cattle in this area were treated with homidium bromide, and dipped with cyhalothrin, in an attempt to control trypanosomosis. No control measures have been implemented since 1994, however, and trypanosomosis re-emerged as a threat to animal health.

In order to determine the optimum control measure available, a longitudinal incidence study was conducted to evaluate three possible control options.

Four sentinel herds were selected from populations exposed to similar trypanosome challenges. The baseline trypanosome

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incidence rate was determined for each herd, after which each herd was subjected to a different trypanosome control measure.

Two of the herds were subjected to topical pyrethroid treatment (Cyfluthrin pour-on and Flumethrin plunge-dip) as vector-control measures, one herd was treated 6 weekly with an injectable trypanocidal drug (isometamidium hydrochloride), and one herd served as an untreated control group.

Monthly incidence rates were determined using the 'dark-ground buffy smear technique'.

The monthly incidence rates were standardized in order to account for variation in trypanosomosis challenge between the 4 herds. The standardized rates were then compared and the impact of the control strategies was quantified using the Area Under The Curve method.

The cost efficacy of each control strategy was evaluated based on a partial budget system.

Both the cyfluthrin pour-on and the injectable trypanocide were cost effective and had a dramatic trypanosomosis control effect with the pour-on having the greater impact/ control.

The flumethrin plunge-dip displayed moderate trypanosomosis control properties, but proved not to be cost effective.

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CHAPTER 1

1   <u>INTRODUCTION</u>

In 1836 pioneers reported losses of cattle to a disease known as "fly-disease" as they moved northward into the areas we now know as Limpopo and Mpumalanga provinces, whilst from 1840 to 1872 Zulu cattle owners described a disease which they knew as 'unakane' or nagana, meaning "tsetse fly disease", in "Zululand" which is the low-lying north-eastern part of the present KwaZulu-Natal Province from the Tugela River to the Mozambique border (Connor 1994). Bruce (1895) linked tsetse flies with this disease in cattle in Zululand and also identified one of the causative pathogenic trypanosomes, *Trypanosoma brucei*.

As wild animals were hunted and land was settled and populated with cattle in the northwestern, northeastern and eastern parts of South Africa so the area inhabited by tsetse contracted. Then Rinderpest crossed into South Africa and between 1896 and 1897 large numbers of cattle and antelope died. As a result there were insufficient hosts and one of the tsetse species (*Glossina morsitans*) disappeared from South Africa leaving only Zululand with endemic tsetse populations, albeit over a smaller distribution and in much reduced numbers

(Fuller 1923; Henning 1956; Phelps & Lovemore 1994; Kappmeier *et al.* 1998).

In 1897 Zululand was annexed by the British and the first game reserves were proclaimed as part of new game preservation laws. Wild animal populations started to increase before being decimated by the Rinderpest epidemic, and up until 1904 no cases of nagana were reported, with only small isolated pockets of tsetse surviving in the presence of game animals (Fuller 1923; Kappmeier *et al.* 1998). Tsetse numbers increased with their wild game hosts and Du Toit (1954) described severe outbreaks between 1907 and 1921 where settlers lost large numbers of cattle.

In response to complaints from the settlers the Natal Provincial Administration embarked on a tsetse control programme. Initial game eradication campaigns and trapping of tsetse flies using the newly developed Harris trap resulted in localized reduction in tsetse numbers but failed to resolve the problem. Between 1942 and 1946 the most severe nagana outbreak ever experienced in Zululand killed in excess of 60000 cattle (Du Toit 1954; Kappmeier *et al.* 1998).

At the end of the Second World War synthetic insecticides were introduced into the tsetse control operations. These pesticides were applied from the ground, from the air and on

cattle with the result that by 1954 the most important vector of trypanosomosis in Zululand, *Glossina pallidipes*, had been eradicated, leaving much reduced populations of the 2 remaining tsetse species (*Glossina austeni* and *Glossina brevipalpis*) which were considered to be unimportant in the transmission of trypanosomosis (Du Toit 1954; Kappmeier *et al.* 1998).

Over the next 30 years sporadic cases of trypanosomosis in cattle, horses and dogs were diagnosed (Bagnall 1993) and the disease was thought to be under control. In 1990 cattle in several diptank areas adjacent to Hluhluwe Game Reserve were diagnosed with trypanosomosis. Further investigation revealed a widespread outbreak that extended from the Umfolozi River to the Mozambique border. Trypanosomosis prevalences of 10% to 15% were found in cattle in 61 out of 132 diptank areas (Carter 1993, 1994; Bagnall 1993). Although cattle mortalities were high it was impossible to differentiate between losses associated with trypanosomosis and those resulting from other causes such as starvation due to drought conditions (Bagnall 1994).

Emergency disease-control measures were implemented in the form of metaphylactic treatment of cattle with injectable trypanocides and dipping of cattle with synthetic-pyrethroid-based insecticides to reduce tsetse numbers. By 1994 cases of trypanosomosis had been dramatically reduced, trypanocide

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treatments had ceased and cattle dipping with synthetic pyrethroids had been discontinued, due to concerns about development of resistance in tick populations (Bagnall 1993, 1994).

The occurrence of such a large outbreak showed that the 2 tsetse species remaining in Zululand were both important vectors of trypanosomosis and a collaborative research venture, between the Directorate of Veterinary Services (Department of Agriculture) and the Onderstepoort Veterinary Institute was initiated in order to better understand the population dynamics and behaviour of the 2 tsetse species. A method of effectively trapping both species of tsetse in order to be able to monitor populations, and a target system able to effectively attract and destroy both species, were objectives of this research, which is ongoing (Kappmeier *et al.* 1998; Kappmeier *et al.* 1999a, 1999b, 1999c; Kappmeier 2000a).

While efforts were being made to understand the behaviour and population dynamics of the tsetse vector, less attention had been given to the effects which trypanosomosis exerted in the rural communal cattle farming community. Therefore in 2000 a research project, which forms the basis of this dissertation, was initiated at 4 diptanks at which cattle had been diagnosed with high trypanosomosis prevalences during the 1990-1994 outbreak.

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The objectives of the project included the quantification of the effects of trypanosomosis in communal stockowner cattle, and the evaluation of 3 possible trypanosomosis control strategies, in terms of disease incidence and financial impact.

CHAPTER 2

2   LITERATURE REVIEW

   2.1   Trypanosomosis

   2.1.1   Trypanosomes

Trypanosomosis is a disease resulting from infection with protozoa of the genus *Trypanosoma*. These protozoa can parasitize all classes of vertebrate and with the exception of *Trypanosoma equiperdum*, which is the cause of dourine and is venereally transmitted, are transmitted from host to host by haematophagous insects. Usually a cycle of development and maturation occurs in the vector, after which the parasites are transmitted to another vertebrate host as the vector feeds. Transmission is either by inoculation of trypanosomes with saliva (subgenus Salivaria) or by contamination of mucosa or broken skin with trypanosomes in the vector's faecal material (subgenus Stercoraria).

Mechanical transmission can also occur where a biting insect passes the infection from an infected to an uninfected animal in the course of interrupted feeding. The time elapsed between feeds is crucial for effective transmission because the trypanosomes die when the blood dries. Large biting insects such as tabanids, and even tsetse flies, are more likely to act as mechanical vectors as they carry relatively large volumes of blood. This mode of transmission has proved to be sufficiently effective to maintain

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Trypanosoma vivax and *Trypanosoma evansi* in South and Central America, and the latter species in North Africa and Asia as well, in the absence of tsetse flies. Mechanical transmission can also occur iatrogenically by means of contaminated needles or surgical instruments.

In Africa the pathogenic trypanosomes that cause sleeping sickness in humans and nagana in domestic animals are salivarian, and cyclical development takes place in the tsetse vectors. *Trypanosoma brucei*, *T.congolense* and *T.vivax* (Fig. 1 and Table 1) are the trypanosome species responsible for stock and production loss in southern Africa (Connor 1994; Stephen 1986; Uilenberg 1998).

The pathogenic trypanosomes are organisms that vary in size from 8 to over 50μm in length. They comprise an outer envelope or membrane filled with cytoplasm in which various structures, including a nucleus, basal body, kinetoplast and volutin granules are suspended. A flagellum arises from the basal body in the posterior end of each trypanosome and runs the length of the organism in a fold of cell membrane called the undulating membrane. The flagellum may continue past the anterior end of the trypanosome as a whip-like free flagellum (Fig. 2). The size, shape, extent of development of the undulating membrane and presence or absence of a free flagellum vary between trypanosome species, as well as

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between different stages in cyclical development of trypanosomes, and are important characteristics in the identification of trypanosomes during microscopy (Fig. 1) (Connor 1994; Uilenberg 1998).

Trypanosomes are motile by virtue of a flagellum and undulating membrane. The free flagellum, when present, acts as a propeller by which the organism is pulled forward through blood plasma or tissue fluids. Each species has its own locomotory characteristics, especially *T.vivax* which moves rapidly forward between blood cells, which can be useful in the identification of trypanosomes in wet preparations during microscopy (Murray 1977; Paris *et al.* 1982) (Table 1).
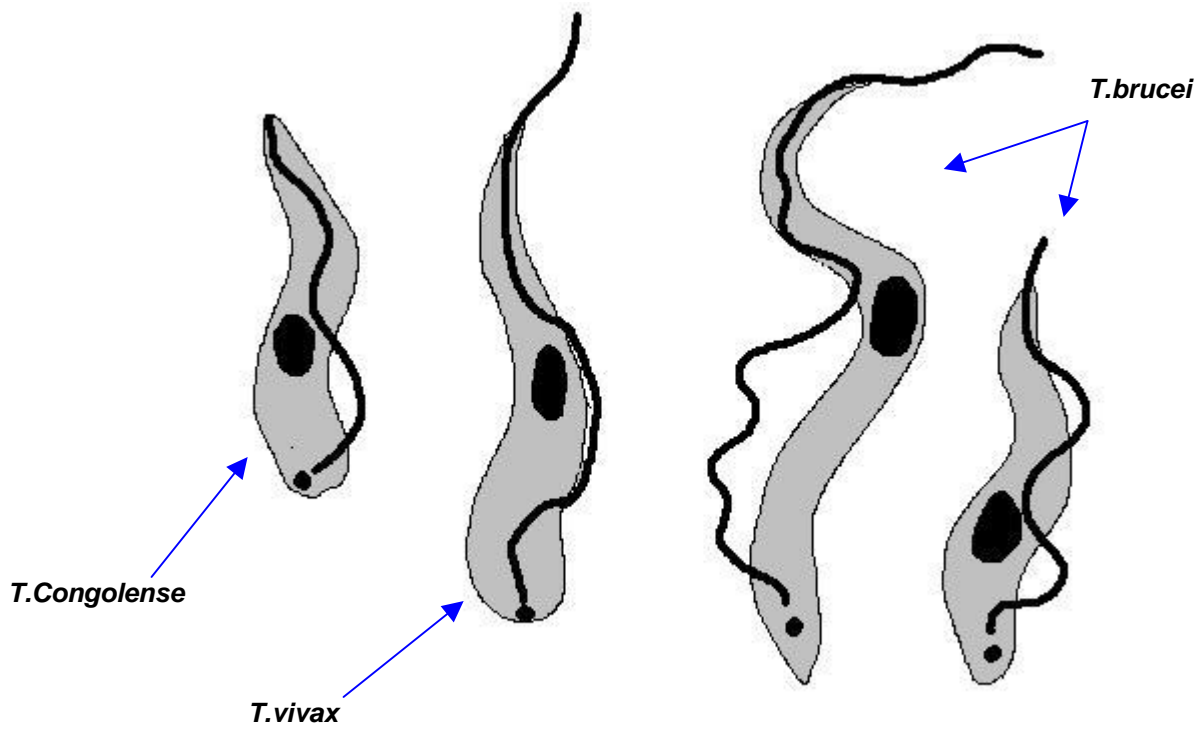
Figure 1   **Diagram showing comparison of the important pathogenic trypanosomes in South Africa**

Energy and respiration requirements are simply absorbed through the trypanosome's outer membrane from the surrounding body fluids. Metabolic waste is excreted into the extracellular fluids by a reverse process.

Reproduction is mainly by means of binary fission and can result in vast trypanosome populations within the host in a very short period of time. There is also evidence of some degree of exchange and recombination of genetic material in the tsetse fly.

The pathogenic trypanosomes usually undergo a cyclical development that requires a vertebrate and an invertebrate host. Blood stream trypanosome forms, called trypomastigotes, are ingested by the tsetse fly vector during feeding. These undergo considerable change in metabolism and morphology, developing first into long slender forms called epimastigotes that multiply before developing into the infective metatrypanosomes. Metatrypanosomes enter the mammalian host when the infected invertebrate vector feeds. Development and multiplication of trypanosomes occur at the site of infection, and may result in chancre (localized inflammatory reaction at inoculation site) formation in the skin, before mature blood trypomastigotes are released into blood circulation via lymph nodes and lymph vessels (Connor 1994; Leak 1998; Uilenberg 1998).

Trypanosomes are classified into three groups on the basis of their morphology and behaviour (Table 1.). *Trypanosoma vivax* is the most common pathogenic trypanosome from the subgenus *Duttonella*. This is a large trypanosome varying in length from 18 to 26μm, which has an inconspicuous undulating membrane, a free flagellum in the anterior and a rounded posterior end. In wet preparations the monomorphic (all parasites similar in appearance) trypanosomes can be seen making rapid progressive movement across the field, between the blood cells. *T.vivax* infections account for the majority of trypanosomosis cases in cattle which are in close proximity to areas of natural vegetation containing wild animals at the game: cattle interface.

In cattle herds kept further away from wild game hosts, where modification of natural vegetation has occurred, the bulk of trypanosomosis cases result from infections with *T. congolense*, subgenus *Nannomonas* (Van den Bossche 2001), which is the smallest of the pathogenic trypanosomes, varying in length from 9 to 22μm. In wet preparations infections are usually monomorphic with parasites that have no free flagellum, and poorly developed undulating membranes, making slow non-progressive movements between blood cells. This trypanosome arguably has the greatest impact on cattle production in Africa.

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The subgenus *Trypanozoon* comprises 5 members: *T. brucei rhodesiense* and *T. brucei gambiense*, which are the aetiological agents of East and West African human sleeping sickness, respectively; *T. equiperdum* the cause of the venereally transmitted equine disease dourine; *T. evansi,* the aetiological agent resulting in trypanosomosis in several mammal species in North Africa, Asia, Central and South America; and *T. brucei brucei* which is the third pathogenic trypanosome species diagnosed in trypanosomosis infections in cattle in Sub-Saharan Africa, but which results in disease of greater severity in dogs and horses. *Trypanosoma brucei brucei* infections are usually polymorphic with long slender (23 to 30μm) forms with a free flagellum, short stumpy (17 to 22μm) forms with no free flagellum that are adapted to life in the intermediate tsetse host, and intermediate parasite forms with a free flagellum. Parasite movement in wet preparations is usually slow but progressive.
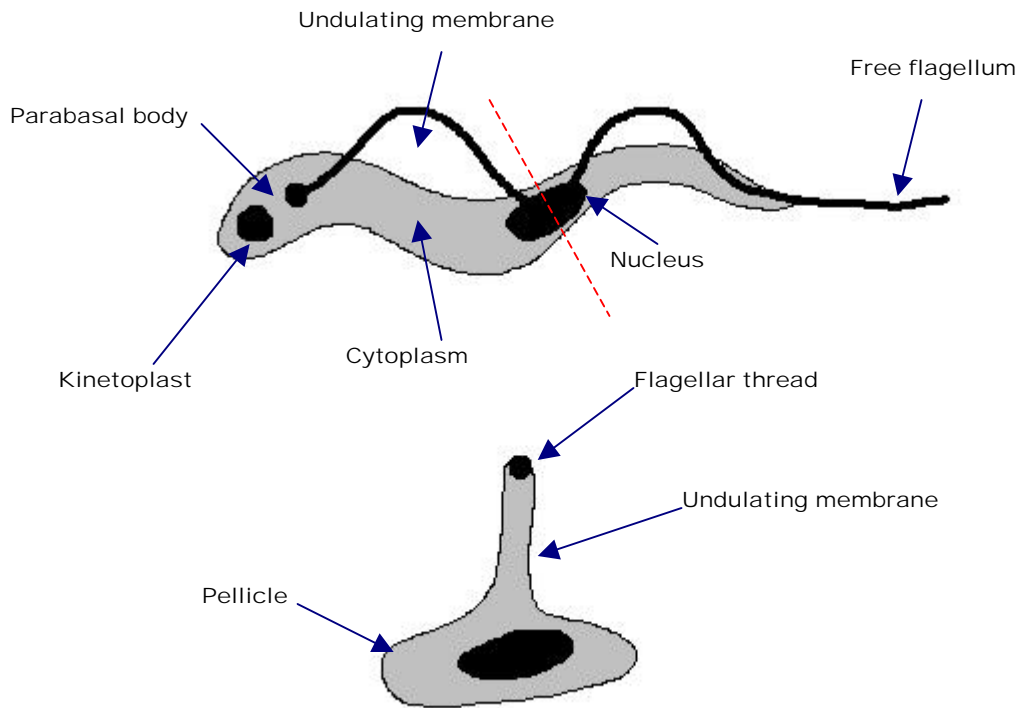
Figure 2   **Diagram of a trypanosome showing major anatomical features**

Table 1

Classification of the pathogenic African Trypanosomes

Subgenus	Species/ Group	Development/ Transmission
Duttonella	Vivax group: *T. vivax* *T. uniforme*	In tsetse: proboscis only Can persist by mechanical transmission
Nannomonas	Congolense group: *T. congolense* *T. simiae* *T. godfreyi*	In tsetse: midgut and proboscis Not known to maintain itself by mechanical transmission
Trypanozoon	Brucei group: *T. brucei brucei* *T. evansi* *(T. equiperdum)*	In tsetse: midgut and salivary glands (venereal transmission)

2.1.2   Tsetse flies

Tsetse flies are haematophagous (blood-sucking) dipterids belonging to the family Glossinidae and genus *Glossina*. They are distributed through Africa, mostly Sub-Saharan, and its islands. These flies have one pair of functional membranous wings with a characteristic 'hatchet'-shaped discal cell (Fig. 3), useful in identification, which are folded one on top of the other at rest (Fig. 4) in contrast to other biting flies such as horse flies (*Tabanus* spp.) and stable flies (*Stomoxys* spp.). The proboscis points forward at rest and the antennae have bristles on the aristae.

Proboscis points forward at rest

Wings extended to show hatchet-shaped discal cell
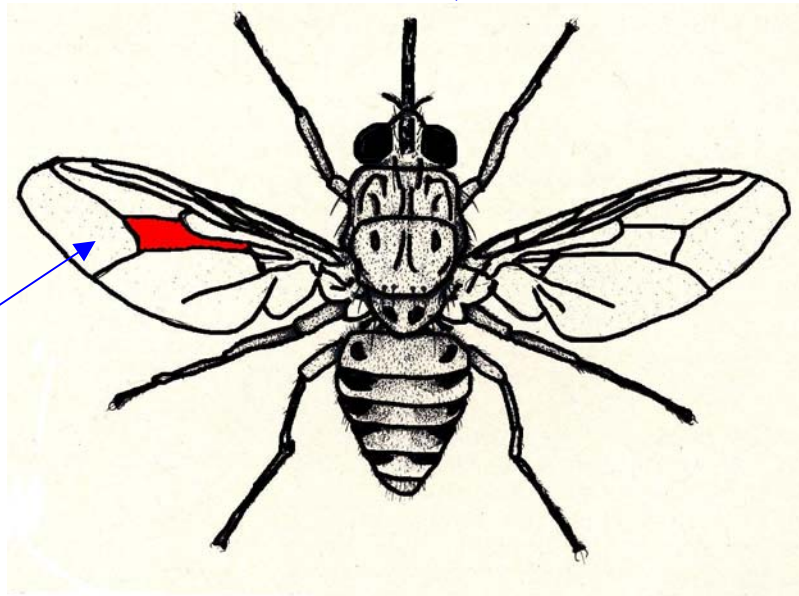
Figure 3   Dorsal view of tsetse fly with extended wings
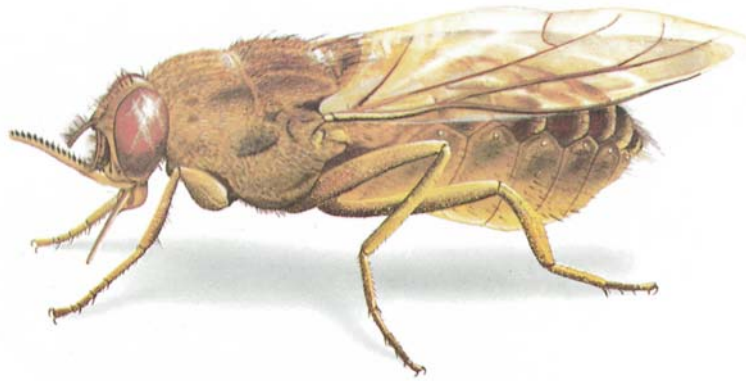
Figure 4   Diagram showing tsetse fly at rest

Both sexes are obligate blood feeders and seek their host through olfactory (volatile substances in urine and breath) and optical (movement and colour) stimuli (Phelps & Lovemore 1994; Kappmeier *et al.* 1999a, 1999b and 1999c). Blood is the source of hydration and energy, and does not allow sufficient energy for long sustained flight, which influences the behaviour patterns of the fly. After emerging from their puparia the unfed (teneral) tsetse flies immediately seek a blood meal. If this happens to be from an animal infected with a high trypanosomosis parasitaemia then flies may become infected with trypanosomes after which they act as biological vectors transmitting the infection to animals on which they subsequently feed at 3 to 5-day intervals. The trypanosomes become established in the mouthparts, mid-gut, salivary glands or a combination of these depending on the species of trypanosome involved. Tsetse flies mate only once, at or around the time of their first feed, and are viviparous, with the females depositing their first larvae in well-shaded soft substrates approximately 14 days later. The female flies continue to deposit larvae at 10-day intervals thereafter and live to an age of approximately 8 weeks, while males usually die after 4 weeks.

Freshly deposited larvae are in the third instar stage and immediately pupate. Adult flies emerge from the puparia after a period that is influenced greatly by environmental temperature but

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which is usually between 27 and 30 days at 25°C. Female flies emerge before males (Leak, 1998).

Tsetse behaviour is also influenced by environmental temperature. They are inactive below 15°C and above 35°C they seek out refuge sites where they rest. Thus temperatures have to remain between 16°C and 35°C for long enough during the day (diurnal) for them to remain active and successfully seek food (Kappmeier 2000b).

Tsetse distribution, and consequently trypanosomosis distribution in Africa, is determined largely by vegetation type. In turn vegetation type is influenced by temperature and humidity, with all forms of woodland, from savannah to rain forest and including agricultural plantations and modified vegetation types, providing suitable habitat for one or more tsetse species. However, no vegetation type is suitable for all species (Phelps & Lovemore 1994).

The abundance and distribution of tsetse flies is also closely correlated with the availability of hosts, with each tsetse species having its own preferred hosts. *Glossina morsitans* and *G. pallidipes* are usually associated with wild animals where warthog, bushpig, certain Bovidae, elephant, rhinoceros and African buffalo constitute suitable hosts, while *G. palpalis* feed extensively on reptiles. In the absence of preferred hosts tsetse flies can survive on the blood of

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other host species. Domestic animals such as cattle and donkeys, but not sheep and goats, can be good hosts for tsetse flies with evidence that *G. morsitans* can survive entirely on cattle. And, in West Africa, *G. palpalis* is known to adapt to feeding on peridomestic animals such as dogs and pigs (Phelps & Lovemore 1994).

There are 3 groups of species within the genus *Glossina*, which are distinguished on their habitat preferences, behaviour and anatomical features (Table 2):

The *fusca* group inhabits rain forest or heavy riparian forest. The *palpalis* group also inhabits rain forest but some species extend along the riparian fringes far out into savannah woodland. The *morsitans* group is restricted largely to savannah woodlands, where in the wet season they are spread throughout the woodland, while in the hot dry season they are associated with vegetation along drainage lines (Phelps & Lovemore 1994; Leak 1998).

Table 2

Classification of Southern African tsetse species

***fusca* group**	*Glossina schwetzi* Newstead & Evans
	Glossina tabaniformis Westwood
	Glossina nashi Potts
	Glossina brevipalpis Newstead
***palpalis* group**	*Glossina palpalis palpalis* (Robineau-Desvoidy)
	Glossina fuscipes fuscipes Newstead
	Glossina fuscipes quanzensis Pires
	Glossina fuscipes martini Zumpt
	Glossina pallicera newsteadi (Austen)
***morsitans* group**	*Glossina austeni austeni* Newstead
	Glossina austeni mossurizensis Travassos Dias
	Glossina morsitans morsitans Westwood
	Glossina morsitans centralis Machado
	Glossina pallidipes Austen

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2.1.3   Trypanosomosis in mammalian host

The ability of trypanosomes to successfully establish an infection in the invertebrate tsetse vector depends not only on the trypanosome species involved but also on the age and species of tsetse. The feeding behaviour and host preference will determine to what extent the tsetse population concerned is exposed to trypanosome infections, and to what trypanosome species they are exposed. *Trypanosoma vivax* infections are more common in tsetse flies which feed mostly on wild animals, while *T. congolense* infections are more common in tsetse feeding predominantly on cattle. Van den Bossche (2001) described in great detail the dynamics between trypanosomes, tsetse and mammalian host (sylvatic cycles), as well as the influence that habitat modification has on African animal trypanosomosis (AAT).

When the infected tsetse fly feeds, metacyclic trypanosomes are injected into the skin of the host. These trypanosomes divide and multiply, then invade the lymphatics and lymph nodes after which they enter the blood stream. Localized inflammation at the injection site gives rise to a swelling called a chancre.

Each trypanosome is clad in a dense surface glycoprotein coat; these proteins are antigenic and stimulate an immune response by the host's humoral and cell-mediated defence systems. This immune response is associated with a febrile reaction that can

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be detected clinically by an increase in body temperature. After a few days the host's antibodies succeed in destroying most of the trypanosomes, with a resultant drop in body temperature. Before the infection is sterilized, however, the few remaining parasites replace their layer of surface glycoproteins with new proteins which are no longer recognized by the host and a new immune response has to be mounted while the trypanosomes once again increase in number. The trypanosomes have a vast repertoire of different surface glycoproteins which results in the host repeatedly having to mount new immune responses while never managing to successfully eliminate the infection, and is the reason that no successful vaccine has been developed yet (Lubega *et al.* 2002; Roditi & Liniger 2002). This seesawing pattern of peaks in parasitaemia and body temperature, interspersed with periods during which the host's immune system seems to be gaining the upper hand is characteristic of acute trypanosomosis. Eventually cattle herds which are repeatedly exposed to a trypanosome population will have been exposed to all the glycoprotein variants and will develop a protective immunity. This can be seen in the trypanotolerant N'Dama cattle of West Africa (Connor 1994; Uilenberg 1998)

One of the main symptoms of trypanosome infection is anaemia. This is the result of lysis of erythrocytes as a result of toxins released by the trypanosomes, as well as increased

phagocytosis of red blood cells which have become coated in material from destroyed trypanosomes (auto-immunity). Not only do the trypanosomes increase the destruction of red blood cells, but they suppress the production of new ones by means of toxins which affect the haemopoietic system. These toxins also result in an immunodepression which compromises the host's ability to mount effective immune responses and leads to an increase in concurrent infections such as *Babesia*, *Theileria* and *Anaplasma*, which can confound the diagnosis.

The various trypanosome species differ in their pathogenicity and ability to penetrate and cause damage to various organs and tissues. *Trypanosoma congolense* is confined to the blood stream while *T. vivax* and *T. brucei* also invade various tissues causing direct damage. *T. vivax* is found in the lymph and even in the chamber of the eye while *T. brucei* is known to invade the central nervous system in man (human sleeping sickness), horses, goats and dogs. Myocarditis, especially in European cattle breeds, which is exacerbated by nutritional and exertional stresses often results in heart failure and death. Trypanosomosis can lead to increased vascular permeability which results in oedema, especially in dogs and horses.

Generally trypanosomosis is a chronic wasting disease associated with reduced fat reserves and degenerative changes in

muscle and other tissues. The progressive anaemia leads to a
decrease in tissue oxygenation which contributes to the tissue
damage and degeneration. In pigs, however, the disease caused by
T.simiae is a hyperacute, fulminating, condition which results in
death and a congested haemorrhagic carcase (Uilenberg 1998).

2.2   Trypanosomosis in South & Southern Africa

Tsetse-transmitted trypanosomosis has a severe negative impact
on both human and animal health in Africa. An area of approximately
10 million square kilometres is tsetse infested, which places 45-50
million people at risk of contracting human African trypanosomosis
(HAT) or sleeping sickness, and 260-300 million cattle at risk of
contracting AAT or nagana. Agricultural losses due to
trypanosomosis are estimated at between 1.3 and 5 billion US$ per
annum, whilst there may be as many as 300 000 new cases of
sleeping sickness annually (McDermott & Coleman 2001). HAT has
not been recorded in South Africa (Kappmeier *et al.* 1998). AAT has
had a dramatic impact on animal production and agriculture.

A major scientific breakthrough occurred in 1895 when Bruce
demonstrated the association between tsetse flies and the disease
known as nagana, in Zululand, South Africa. He identified
Trypanosoma as the causative organism, that wild animals served
as a reservoir of the disease and that tsetse flies transmitted the

infection to healthy animals (Bruce 1895). In addition to *T. brucei*, the trypanosome identified by Bruce, *T. vivax* and *T. congolense* were identified as causative agents of AAT in cattle (Henning 1956).

Four species of tsetse flies have been recorded in South Africa. *Glossina morsitans morsitans*, *G. pallidipes*, *G. austeni* and *G. brevipalpis*. *Glossina m. morsitans* was distributed in the northern parts of the country and reports of "fly-disease" affecting cattle belonging to pioneers date back to 1836. This tsetse-infested area was dramatically reduced between 1872 and 1888 through the shooting out of game animals and stocking of land with cattle. *Glossina m. morsitans* eventually disappeared from South Africa during the rinderpest epizootic from 1896-1897 and has not been recorded since (Fuller 1923; Phelps & Lovemore 1994; Kappmeier *et al*. 1998). In the northeastern area of the province of KwaZulu-Natal, known as "Zululand", three species were identified: *G. austeni*, *G. brevipalpis* and *G. pallidipes*, although only the latter was considered to be of epidemiological significance (Du Toit 1954).

A number of trypanosomosis epizootics, varying in extent and severity, occurred in Zululand from the late 1800's. These disease outbreaks increased in importance as settlement and the development of livestock production increased. Eventually angry stockowners forced provincial authorities to implement disease-control measures. Three game eradication campaigns, an initial

campaign on the 'Makatini Flats' north of the Mkuze River in 1917, followed by two larger more organized campaigns around the Umfolozi 1929-1930 and Hluhluwe Game Reserves 1942-1950, resulted in the slaughter of hundreds of thousands of wild animals. In addition to the destruction of wild animals large areas of vegetation, comprising suitable tsetse habitat, were cleared around the Hluhluwe and Umfolozi Game Reserves in 1942. These game destruction and vegetation clearing tactics resulted in varied levels of success (Du Toit 1954; Connor 1994).

In 1931 a tsetse fly trap, the "Harris trap", was introduced into Hluhluwe Game Reserve and resulted in massive tsetse catches. By 1940 over 26 000 Harris traps were deployed yet still the trypanosomosis problem persisted and during 1945-1946 Hluhluwe and Mkuze farmers lost over 60 000 cattle (Du Toit 1954).

At the end of the Second World War (1945) synthetic insecticides were introduced into tsetse-control operations and DDT and benzene hexachloride (BHC, now HCH) were applied to suitable tsetse habitat on a large scale by aerial and ground spraying. By 1954 *G. pallidipes* had disappeared entirely from Zululand leaving *G. austeni* and *G. brevipalpis* (Fig. 5) as the two remaining vectors which were not considered to be of significance (Du Toit 1954; Kappmeier *et al.* 1998).

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Sporadic outbreaks of trypanosomosis were reported in dogs, cattle and horses until 1990 (Bagnall 1993). Then in 1990 a widespread outbreak was diagnosed with communal cattle at 61 out of 132 diptank areas, between the Umfolozi River and Mozambique border, showing trypanosomosis prevalences of around 10 -15% (Carter 1993, 1994; Bagnall 1993, 1994; Kappmeier *et al.* 1998). Disease-control measures were implemented. State subsidized cattle dips were changed to a pyrethroid-based dip (cyhalothrin) until March 1993, after which they were changed back to an amitraz dip and animals received spot treatments of a deltamethrin pour-on in order to continue tsetse control. In addition to vector control measures, cattle in affected diptank areas were subject to chemotherapy and chemoprophylaxis using diminazene aceturate or homidium bromide (Bagnall 1993). The additional costs of pyrethroid dipping in order to control tsetse were approximately US$400 000 over the two year control period, whilst costs of homidium treatments amounted to US$65 000 (Bagnall 1994).

During 1992-1993 targets and traps were evaluated in Hluhluwe-Umfolozi Park as a means of tsetse control. Use was made of targets and traps that had been developed and used successfully in Zimbabwe against *G. pallidipes* and *G. m. morsitans*. However, these were found to be less effective against the resident populations of *G. austeni* and *G. brevipalpis* (Bagnall 1993;

Kappmeier *et al*. 1998). Kappmeier and co-workers developed traps and targets with increased efficacy against the two resident species (Kappmeier *et al.* 1999a; Kappmeier *et al.* 1999b; Kappmeier *et al*. 1999c; Kappmeier 2000a) that are currently being evaluated in field control trials in Zululand.
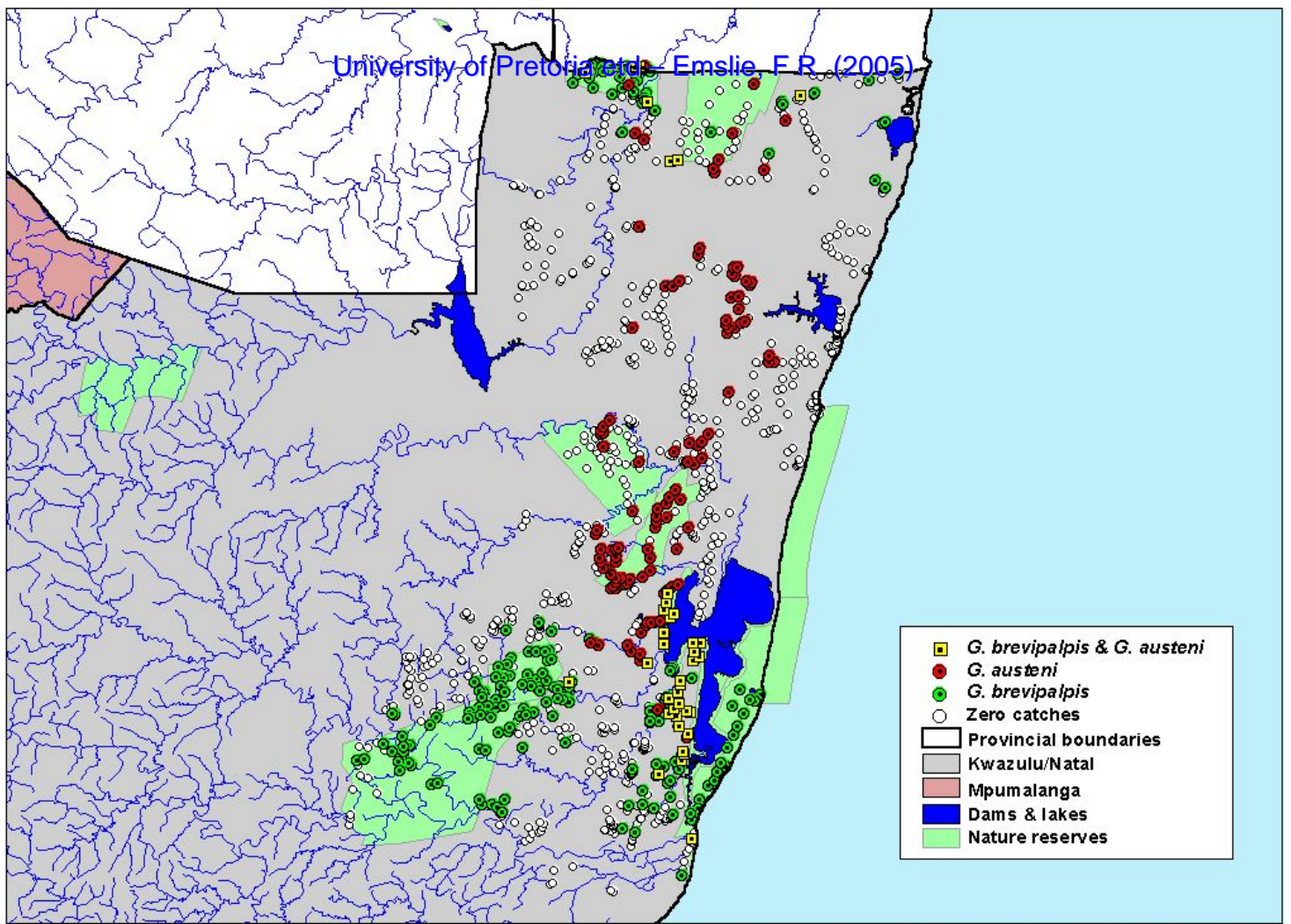
Figure 5   Map of north-eastern KwaZulu-Natal showing

tsetse distribution based on trap catches (1998)

Due to the necessity to implement rapid trypanosomosis control measures no attempt was made to quantify trypanosomosis incidence or the impact of the disease in the local stockowner community. Only the distribution and prevalence of trypanosomosis and tsetse distribution (Fig. 5) were established prior to implementation of disease-control measures. No quantification of trypanosomosis incidence was made after cessation of disease-control measures in 1994. Sporadic outbreaks are reported in Zululand, in cattle horses and dogs, to date.

Vector-control measures have relied mainly on the application of synthetic insecticides: to suitable tsetse habitat by aerial or ground spraying, fogging or smoking; on odour-baited traps or targets; or on live animals serving as bait. Bauer *et al.* (1988) evaluated the efficacy of flumethrin pour-on on animals against *G. palpalis gambiensis*, and for the integrated control of ticks and tsetse flies, in West Africa. Baylis and Stevenson (1998), Löhr *et al.* (1991) and Kamau *et al.* (2000) evaluated pour-on efficacy against *G. pallidipes* and *G. longipennis* populations in East Africa. McDermott and Coleman (2001) judged vector-control measures to be more effective than either a potential vaccine (Aksoy *et al.* 2001; Doyle *et al.* 1984; Lubega *et al.* 2002; Roditi & Liniger 2002) or trypanocidal drug. This is supported by Schofield and Maudlin (2001) who suggest that the

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application of insecticides to the tsetse's natural host as a live-bait strategy is the logical progression from developing odour-baited stationary targets impregnated with insecticide. Furthermore, insecticide application can be used for the integrated control of both tsetse and ticks, which makes it more sustainable, and cost-effective than other trypanosomosis control strategies (Bauer *et al.* 1992; Hargrove *et al.* 2000; Munsimbwe 1999; Holmes 1997).

Kappmeier (1999c) and Kappmeier *et al.* (1998, 1999a, 1999b) found that trap and target technology, developed primarily for Zimbabwean tsetse species (Mangwiro *et al.* 1999), lacked efficacy against the two tsetse species endemic in Zululand. They developed trap and target technology which would be more effective against *G. austeni* and *G. brevipalpis* (Kappmeier 2000a; Kappmeier *et al.* 1999c), but due to behavioural differences between the species this technology still lacks efficacy against the former species. The efficacy of insecticide-treated cattle in controlling *G. austeni* and *G. brevipalpis* has not been evaluated.

In many areas, especially West and Central Africa, no effort has been made to control tsetse populations and instead livestock farmers have relied solely on the prophylactic use of trypanocidal drugs (Allsopp 2001). Frequent use of these drugs has resulted in the widespread development of resistance, in trypanosome populations, against commonly use trypanocidal drugs (Anene *et al.*

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2001; Eisler *et al.* 2001; Geerts *et al.* 2001). Fortunately, use of trypanocidal drugs in South Africa was restricted to a period of approximately 2 years (Kappmeier *et al.* 1998), which should have precluded the development of resistance in Zululand trypanosome populations. This is contrary to findings in Mozambique, which shares a common tsetse belt with South Africa (Sigauque *et al.* 2000), where multiple resistances to trypanocides has been reported (Sigauque 2003, unpublished data).

In order to determine the current incidence of trypanosomosis, quantify the impact the disease has on the local stockowner community, and evaluate the efficacy of three trypanosomosis-control strategies, a longitudinal incidence study was carried out in an area of high trypanosomosis challenge in Zululand during 2000 and 2001. Two vector-control strategies and a chemoprophylaxis strategy were compared with an untreated control (UTC) in order to evaluate their efficacy in controlling trypanosomosis.

CHAPTER 3

3 MATERIALS & METHODS

3.1 Trial diptank and animal selection

In KwaZulu-Natal, cattle belonging to rural subsistence farmers are dipped in State-subsidized plunge dips, which serve the community within a radius of approximately 7km (De Waal *et al.* 1998). These diptanks were built from the late 1930's as part of a State-subsidized compulsory dipping campaign to eradicate East Coast Fever (ECF, caused by *Theileria parva*) from South Africa. This dipping continued after the eradication of ECF and serves to concentrate rural communal cattle farmers around their local diptank. As a result no differentiation can be made between herds belonging to separate stockowners and instead the cattle populations from each specific diptank area are considered as individual herds.

Four diptank herds were selected for trial purposes in an area of high trypanosomosis challenge (Bagnall 1993). The diptank areas selected were matched on the basis of biotype, climate, and cattle population. All four diptank areas were situated in sub-tropical sandy coastal palm veld and subject to similar temperature variation and rainfall. Meteorological data were recorded from the station (Mbazwane recording station) closest to the trial diptanks. Average daily rainfalls as well as daily maximum and minimum temperatures

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were recorded and these data were collated in four-week periods corresponding with those of the trial. Very little trading of cattle has occurred in the trial area due to the cultural values associated with livestock, and distances from suitable markets. Cattle are kept as symbol of status, wealth, and for payment for new wives (Lobola), and are only slaughtered on special occasions such as at weddings and funerals. Large distances and lack of adequate handling facilities have discouraged speculators from sourcing cattle in this area, and very few animals are brought in from more distant commercial farming operations. As a result a uniform and typical animal type is found across the Makhathini Flats area of Zululand. All of the trial herds were comprised of these local 'Nguni' animals or crosses thereof, with similar age and sex composition across the herds (Fig.7).
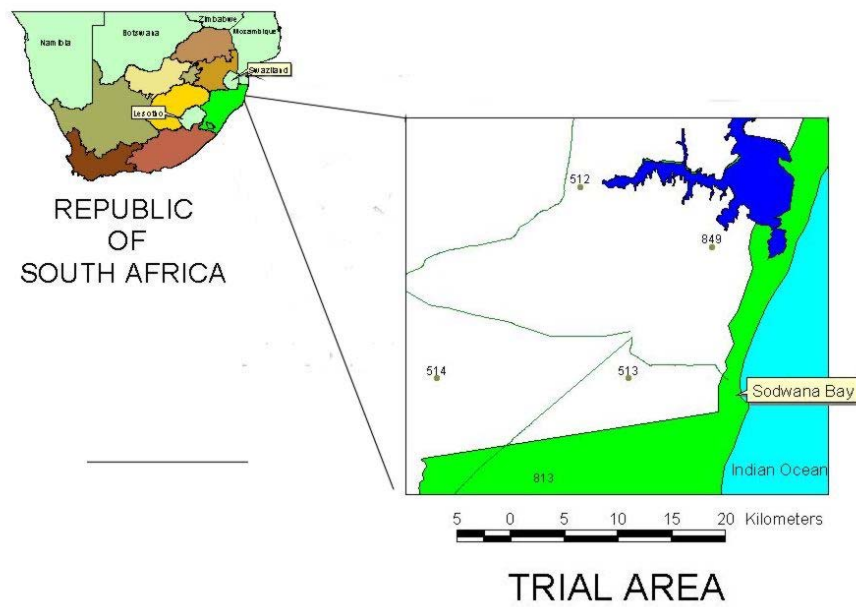
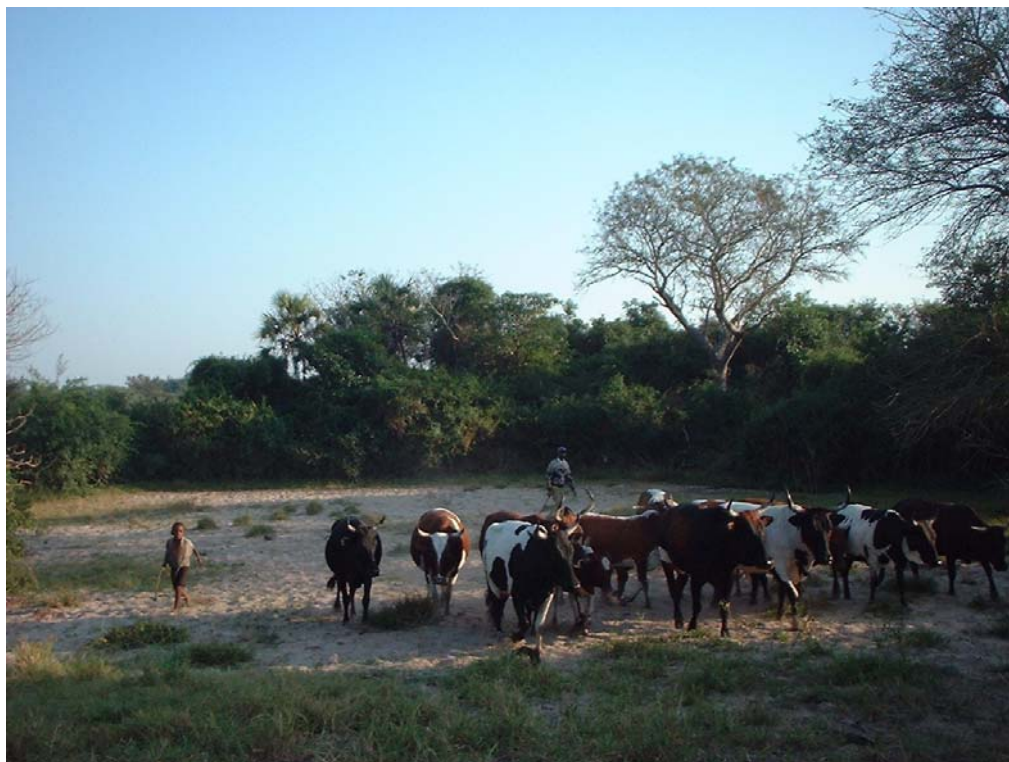**Figure 6   Map showing location of trial diptanks in SA**

Figure 7   Typical 'Nguni' cattle in the trial area

Two owners per diptank were purposively selected based on their willingness to participate, herd size and representivity of their animals to the local community's cattle. These animals were sampled every four weeks over the course of the study period. A sample size of 35 animals per diptank was calculated based on an assumed trypanosomosis prevalence of 10%, and an allowable error of 10% (Thrusfield 1995). This number was doubled (n=70) in order to compensate for loss of animals due to slaughter, death from other causes, or absenteeism on sampling day. A cohort of 35 cattle per selected owner was selected by systematic random sampling, and each animal was individually identified by means of numbered ear tags.

3.2   Trypanosomosis incidence and diagnosis

All sentinel animals were treated with diminazene aceturate (Berenil®, Intervet), at a dose rate of 3.5mg/kg body weight, by deep intramuscular injection, in order to sterilize existing trypanosome infections. Following diminazene treatment, a period of four weeks was allowed to elapse after which the trial cattle were screened in order to ensure that all animals were trypanosome free. Eight weeks after diminazene treatment the trial herds were screened in order to establish their baseline (pre-treatment) trypanosomosis incidence rate.

Diagnostic screening of trial animals was by means of dark-ground microscopy, using the buffy-coat technique (Murray 1977), which is the most sensitive direct parasitological diagnostic technique available for use under field conditions (Paris *et al*. 1982). Heparinized central-venous blood samples were collected from all trial animals, and were transported to the local State Veterinarian's office for processing. The packed cell volume (PCV) of each sample was established, followed by microscopic examination to determine the animal's trypanosomosis status. Animals diagnosed with trypanosomosis were treated within 24 hours using diminazene aceturate, which is rapidly excreted and has little prophylactic activity (Leak 1998; Uilenberg 1998).

Figure 8   Screening blood samples using the 'Buffy smear' technique
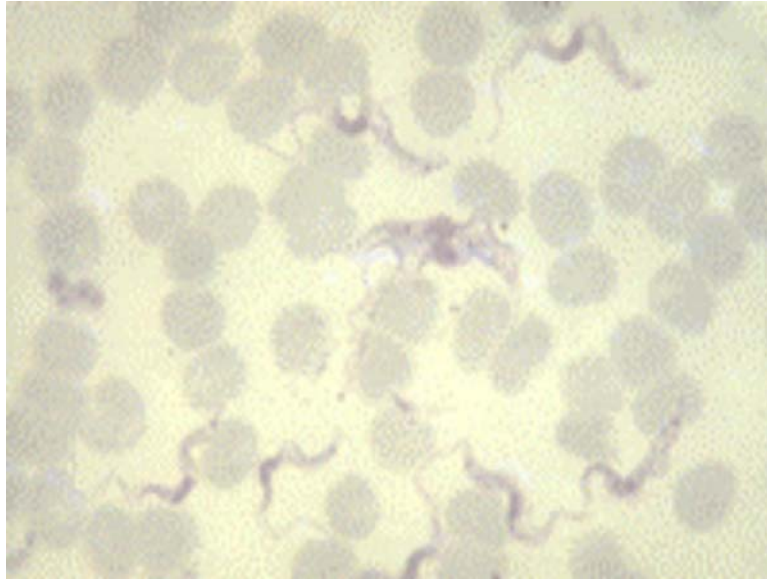
Figure 9   Trypanosomes in fixed thin blood smear

(1000x magnification)

3.3   <u>Trypanosomosis control strategies</u>

Each of three herds was then subjected to a different trypanosomosis control strategy, while the fourth herd served as an UTC: The first herd was subjected to 2-weekly dipping in a flumethrin plunge dip (Bayticol®, Bayer), and the second herd had a cyfluthrin pour-on (Cylence®, Bayer) applied 6-weekly at 15ml/100kg (Munsimbwe 1999). These two herds were therefore subject to vector-control measures.

In the third herd isometamidium chloride (Veridium®, Ceva) was administered 6-weekly, at a dose rate of 0.5mg/kg by deep intramuscular injection, as a trypanocidal chemotherapy and chemoprophylactic. The fourth herd, serving as an UTC, was subject

to 2-weekly dipping in an amitraz plunge dip (Triatix TR® Intervet) as per standard State dipping policy. Amitraz has no effect on tsetse populations (Kappmeier *et al.* 1998) yet is an effective acaricide and was used in the UTC out of animal welfare considerations. The entire diptank cattle population, including the sentinel animals, at each dip was subjected to the respective treatment strategy. All four trial herds were screened 4-weekly, using the buffy-coat technique, and new trypanosomosis cases were recorded.

3.4   Partial budget assessment

In an attempt to quantify the impact that the various trypanosomosis-control strategies would have on livestock and farmers, the benefits and costs of each strategy were presented in the form of a partial budget.

Additional returns:

It was assumed that an infected animal would die if it received no trypanocidal treatment, and that this would result in a loss of R2000.00 per animal based on sales figures (Stockowners Association, personal communication) during the time that the trial was conducted. Thus any animal, which survived as a result of a trypanosomosis control strategy, would result in an additional return equal to R2000.00 per animal.

Foregone returns:

The area of Zululand known as the 'Makhathini Flats' comprises approximately 7 500km² (Dr. R. Bagnall personal communication) of communal subsistence farming land and game reserves. Approximately 196 000 cattle (Carter 1994) are owned by communal cattle farmers in this area which has resulted in dramatic modification of the vegetation that used to occur in this area. This has been further exacerbated by frequent drought years. As a result large numbers of cattle are subject to nutritional stress and many animals die in dry periods.

The increased numbers of cattle surviving as a result of trypanosomosis-control strategies would therefore have a negative environmental impact. This negative impact was quantified in the partial budget as a loss of grazing where it was assumed that one cow would consume 8kg (personal communication, Dr.W.Schultheiss, 2002) of dry matter per day at a cost of R0.12 per kg (R0.96/cow/day).

Additional costs:

Consideration was given to the fact that crush-pen infrastructure would be required for all three trypanosomosis strategies at a cost of approximately R20 000.00 each. And, that if this cost were paid over a 10-year period, it would amount to R153.85 per 4-week period.

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In addition, a diptank would have to be constructed in order to implement Bayticol plunge dipping. It was assumed that this infrastructure would cost approximately R250 000.00, and if paid over a 20-year period, would result in a cost of R961.54 per 4-week period.

Since adequate crush-pen and diptank infrastructure exists through most of the tsetse distribution area it was deemed unnecessary to include these construction costs. However, if a community wished to institute one of the control strategies in a new area it would be prudent to give this requirement consideration. In order to facilitate comparisons between the trial groups in the partial budget, all data were transformed to a group size of 100 animals per 4-week period. The financial impact of each treatment strategy was then calculated as the difference between each treatment strategy and the UTC. Once the impact of each strategy, versus the UTC, had been calculated a between strategy comparison was made by comparing these results (Table 6).

3.5 Packed Cell Volume (PCV)

Several authors, including Uilenberg (1988) and Van den Bossche *et al.* (2004), place importance on anaemia as a clinical indicator and result of trypanosomosis. In this trial packed cell volumes (PCV) were determined for each trial animal at every

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screening. Mean 4-weekly PCVs were calculated for each trial group over the duration of the trial and the results were compared in a graphically (Fig. 12).

3.6   Reed-Frost model

The Reed-Frost model (Thrusfield, 1995) is a simple chain binomial model, which describes the major factors that play a role in herd immunity in the context of a hypothesized disease outbreak. While the model is simple, it is useful in demonstrating those factors that are of importance in herd immunity.

The trypanosomosis incidence curve observed in the UTC was compared with a projected Reed-Frost curve for a population of similar size (Fig. 13) in order to establish the model's suitability for describing the dynamics of trypanosomosis in cattle in KZN. Then the effects, which the trial control strategies had on trypanosomosis incidence, were explored using this model (see Fig. 14).

CHAPTER 4

4 <u>RESULTS</u>

4.1   <u>Incidence and Standardized Incidence</u>

Incidence rates recorded prior to initiation of control measures were considered to be the baseline or pre-treatment incidence rates for the respective trial groups. These pre-treatment (weeks -4 to 0) incidence rates varied slightly between trial groups. Trypanosomosis incidence rates for the four groups over the entire study period can be seen in Table 3.

Table 3

Trypanosomosis incidence in trial groups

Incidence rates (cases/ 100 animals)

Week	Control	Bayticol	Cylence	Veridium
0	4.29	6.35	10.00	6.15
4	1.19	8.00	0.00	0.00
8	4.78	3.72	0.00	0.00
12	11.11	3.57	1.75	0.00
16	4.76	1.82	1.85	0.00
20	5.00	11.54	9.62	0.00
24	3.39	3.92	0.00	0.00
28	5.08	4.08	5.66	0.00
32	3.64	0.00	0.00	1.72
36	4.92	1.89	0.00	0.00
40	4.21	1.54	1.74	0.00
44	4.44	4.55	2.32	0.00
48	5.17	4.65	0.00	

To compensate for the variation between trial group trypanosomosis challenge and facilitate between group

comparisons, the post-treatment incidence rates were subjected to a standardizing procedure similar to that used by Rowlands *et al.* (1996) in Côte d'Ivoire. For each group the pre-treatment incidence rate was subtracted from each post-treatment incidence rate resulting in the change-in-incidence or Standardized Incidence Rate (SIR). Trial group SIRs are shown in Figure 10.

Figure 10

Chart showing comparison of SIR between trial groups
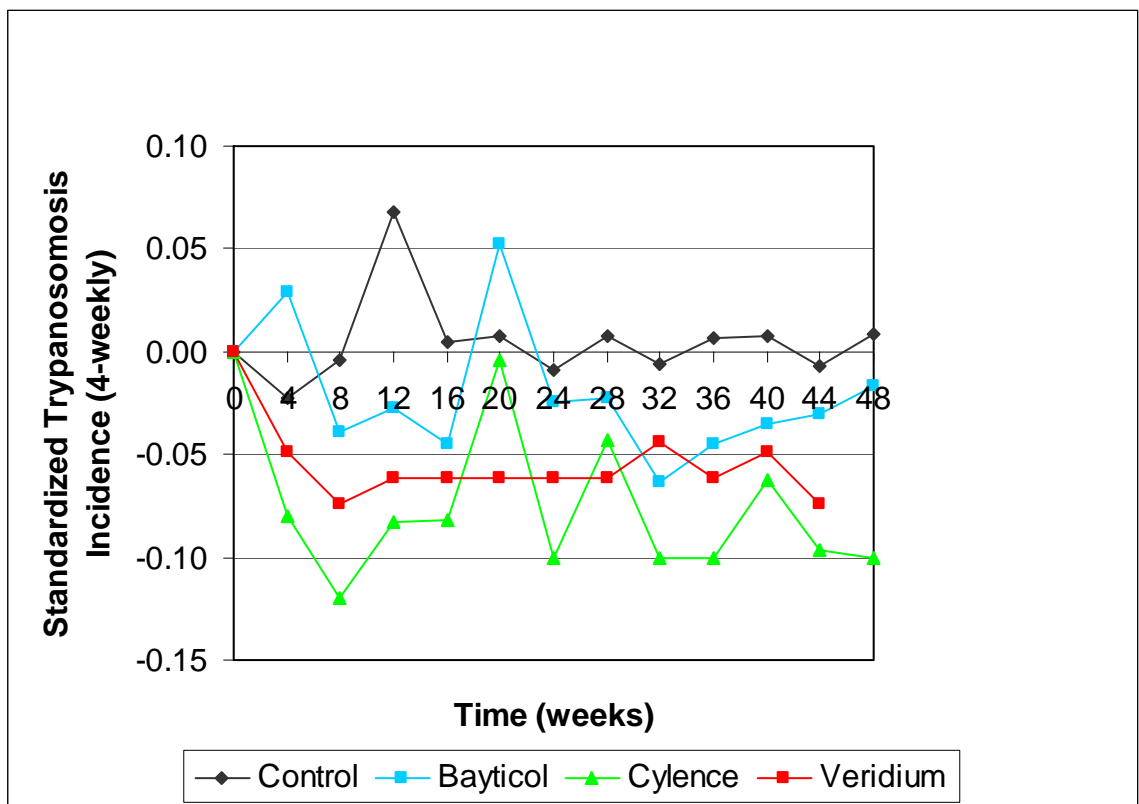
4.2   Statistical significance

The SIRs for the four trial groups were compared using Analysis Of Variance (ANOVA) and significant between-group variation was established (p<0.05) as shown in Table 4. The group SIRs were then compared, with the UTC and each other, using a two-tailed Student's T Test. All 3of the treatment groups showed SIRs significantly (p<0.05) different to the UTC; the Veridium and Cylence group SIRs were significantly (p<0.05) different to the Bayticol group SIR; whilst no significant difference (p>0.05) could be established between the Veridium and Cylence group SIRs.

Table 4   Analysis of variance (ANOVA single factor) between trial group SIRs.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.048457	3	0.016152	19.7599	1.98E-08	2.802352
Within Groups	0.038419	47	0.000817			
Total	0.086876	50				

4.3   Area Under Curve

To evaluate and compare the rate and extent of change in trypanosomosis incidence, in and between trial groups, over the course of the trial period the Area Under the Curve (AUC) of the trial group SIRs was calculated using the trapezoidal method (Hintz, 2003) (Table 5). By comparing each treatment group AUC with that of the UTC the effectiveness of each strategy can be quantified. A comparison of the AUC supported the comparison of SIRs, which

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showed all three strategies to be effective in reducing trypanosomosis incidence. If the magnitude of the impact of the strategies in reducing trypanosomosis is considered, however, then the Cylence group shows a much greater impact than the Veridium group, which in turn shows a greater impact than the Bayticol group.

Table 5   Total Area Under the Curve of trial groups

Control	Bayticol	Cylence	Veridium
0.23	-1.04	-3.68	-2.49

4.4   Weather

The mean rainfall, minimum and maximum temperatures by 4-week trial period are reflected in Figure 10. A period of high rainfall around Week +12 of the trial, combined with high temperatures, coincided with a peak in trypanosomosis incidence in the UTC group.

Figure 11

Chart showing Rainfall and Temperature data over trial period
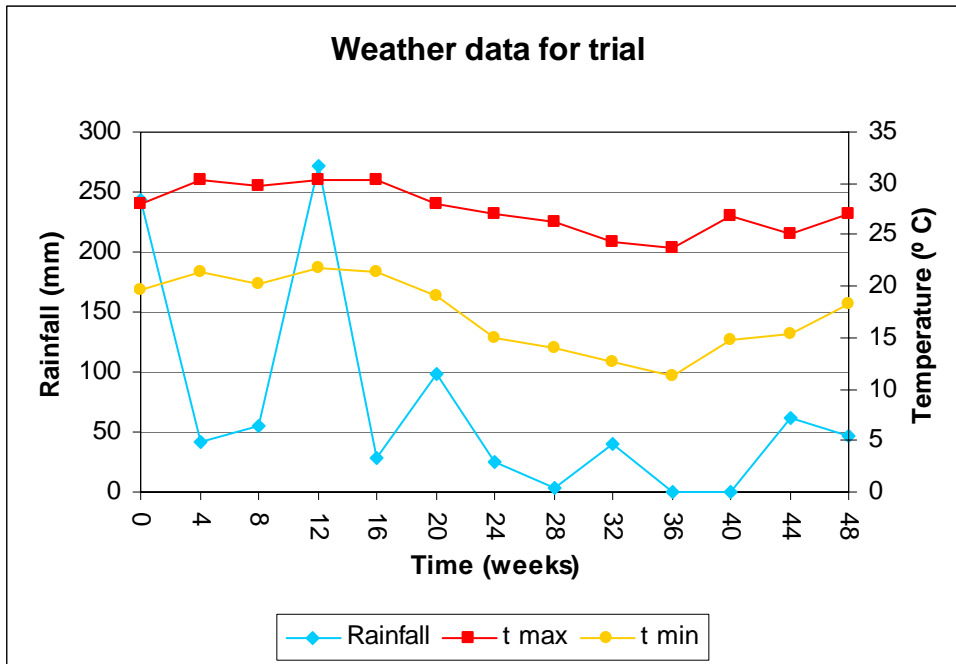
4.5   Partial Budget

All three strategies showed a net benefit when compared with the UTC group. The Cylence group resulted in the greatest returns of approximately R16 000, with the Veridium group delivering a slightly lower return of approximately R12 000. Although the Bayticol group resulted in a positive return of approximately R5 000, this was lower than the other two strategies. The partial budget, including the differences between group and UTC per 4-week period, is given in Table 6.

Table 6

PARTIAL FARM BUDGET ANALYSES TO DETERMINE THE ECONOMIC IMPACT OF THREE CONTROL STRATEGIES

Components	Control Group	Cylence Group	Bayticol Group	Veridium Group	Difference per Group per month - Cylence	Difference per Group per month - Bayticol	Difference per Group per month - Veridium
Additional returns							
Surviving Stock	(R1,044.83)	R16,176.22	R4,484.56	R11,994.21	R17,221.04	R5,529.39	R13,039.04
Foregone returns							
Loss in Grazing	(R14.04)	R217.41	R60.27	R161.20	R231.45	R74.31	R175.24
Additional Costs Incurred							
Cost of treatments	R0.00	R864.00	R298.00	R841.00	R864.00	R298.00	R841.00
Costs no longer incurred							
None					R0.00	R0.00	R0.00
					R 16,125.59	**R 5,157.07**	**R 12,022.80**

4.6   Packed Cell Volume

The mean 4-weekly PCVs for all of the trial groups were compared graphically (Fig. 12).

Figure 12
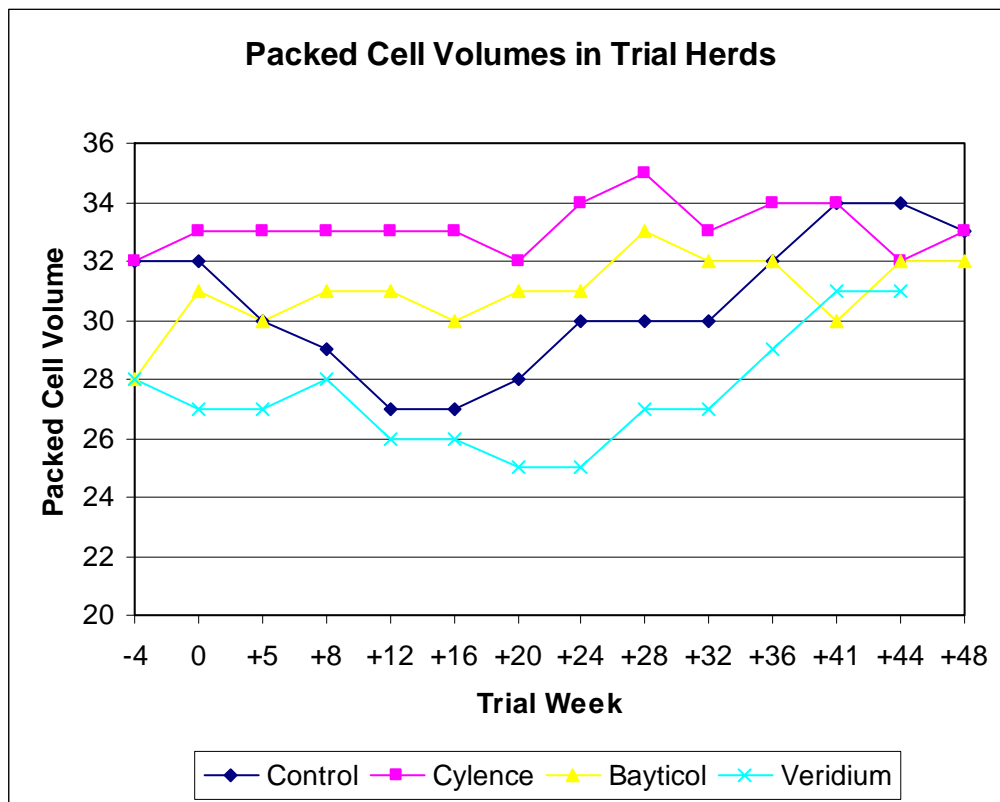
The mean PCVs for both the insecticide-treated groups of animals increased post treatment and remained elevated until the trial conclusion. The PCVs for animals in the control and trypanocide-treated groups dropped after trial initiation and only recovered toward the trial conclusion when they reached levels similar to those of the insecticide treated animals. When the trial

group mean PCVs were subjected to ANOVA significant (p<0.05)

between-group variation was found to exist (see Table 7).

Table 7   Analysis of variance (ANOVA single factor)

between trial group PCVs.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	220.8264	3	73.60879	25.64703	2.96E-10	2.78623
Within Groups	146.3736	51	2.870071			
Total	367.2	54				

4.7   Reed-Frost Model

The trypanosomosis incidence in the UTC group was compared

with a projected Reed-Frost curve for a population of similar size.

By obtaining a 'best fit' between the observed and projected curves

the probability of adequate contact (viz. the probability of

transmission occurring) was determined to be 0.04 (Q=0.96). The

UTC group showed an initial increase in incidence, which

approximated the incidence projected with the Reed-Frost model,

but failed to reach the same peak number of cases. After reaching

peak incidence the UTC curve had an elongated tail with a constant

low number of cases while the Reed-Frost curve followed a normal

distribution and showed a zero incidence after a short tail.

Figure 13

Reed-Frost Model for the transmission of trypanosomes in northern KwaZulu-Natal, South Africa
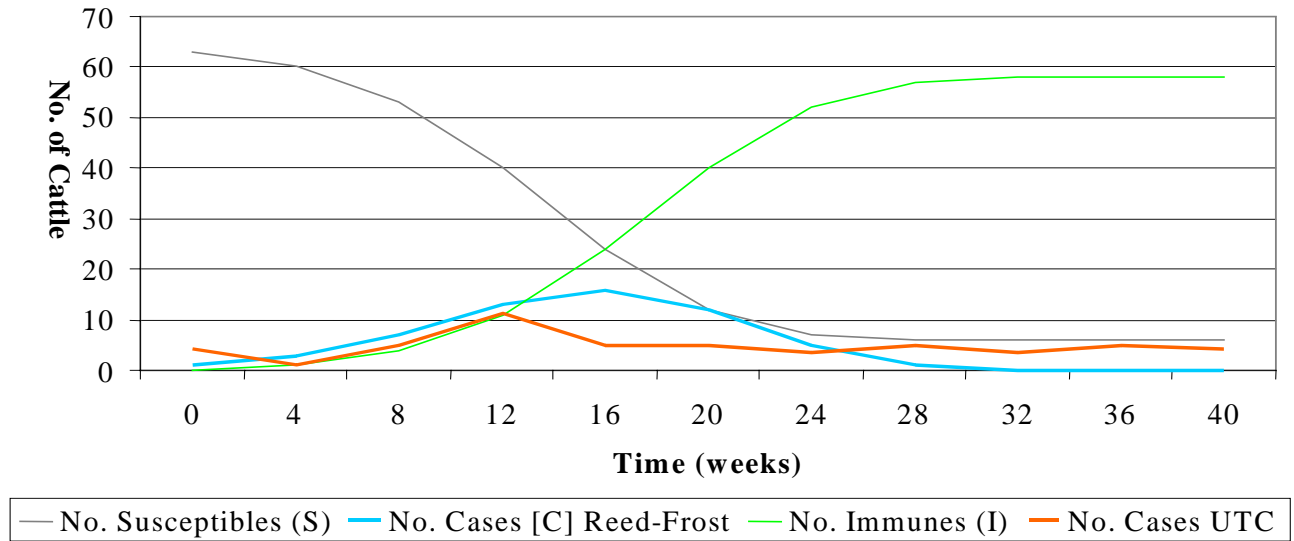
CHAPTER 5

5   <u>DISCUSSION</u>

5.1   <u>Standardized Incidence Rate</u>

5.1.1 Untreated Control group

The standardized incidence rate for the UTC group showed a peak in trypanosomosis incidence around Week +12 (February 2002) of the trial that corresponded closely with a peak in rainfall (Fig. 10), otherwise the incidence rate remained fairly constant with trypanosomosis incidence remaining similar to baseline pre-treatment levels. Increased humidity and rainfall promote conditions that are suitable for survival and dispersal of the tsetse vector, which would account for this increased transmission and peak in trypanosomosis incidence in the control herd.

The cattle in the control herd were dipped with an Amitraz-based acaricide in order to manage the large tick burdens in the trial area, and prevent the occurrence of tick-borne diseases. This active has no effect on nuisance flies and cattle owners in this area complained about the large numbers of house and nuisance flies around their houses and cattle kraals. Despite an increase in nuisance flies and a higher trypanosomosis incidence than in the treatment herds, the cattle owners were satisfied with the treatment strategy (UTC) as their cattle were relatively tick free. This emphasized the importance that cattle owners place on the control of tick vectors, which are

more visible, rather than on the control of other insect vectors and tick and vector-borne diseases which are less tangible to them. This perception can bias the evaluation of the efficacy of a disease-control strategy or product within these communities (Hargrove *et al.* 2000; Bauer *et.al.* 1992; Kamau 2000; Munsimbwe 1999).

5.1.2 Injectable trypanocide strategy

As would be expected with the prophylactic use of trypanocidal drugs, the SIR curve in the Veridium group showed a dramatic drop in trypanosomosis incidence up to Week +8 of the trial after which it stabilized and remained uniform for the duration of the trial. The AUC for the Veridium SIR showed a reduction in trypanosomosis incidence greater than the control, greater than the Bayticol group and equivalent to the Cylence group SIR. When compared with the UTC group incidence curve and the projected Reed-Frost curve (Fig. 12) it can be seen that the Veridium group curve does not approximate that of the Reed-Frost model and is instead level indicating that there is insufficient probability of adequate contact associated with this treatment strategy and an outbreak can not occur. These results show no evidence of the development of resistance against the commonly used trypanocidal drugs, which has been reported from numerous other African countries (Anene *et al.* 2001; Eisler *et al.* 2001; Geerts *et al.* 2001; Holmes 1997;

McDermott & Coleman 2001; Mugunieri & Murilla 2003 and Sigauque 2003, unpublished data).

Although resistance to trypanocidal drugs has not been recorded in South Africa, the significant efficacy observed in this trial is not justification for the reliance on trypanocidal drugs in the absence of any other control measures. Trypanocidal drugs remain an effective measure only when used as part of an integrated control programme (Anene *et al.* 2001; Holmes 1997; McDermott 2001).

5.1.3 Vector control strategies

Two pyrethroid insecticides were evaluated in the context of vector-control agents in order to control trypanosomosis. These insecticides not only serve to reduce the absolute number of tsetse flies in the environment thereby reducing the tsetse/ trypanosomosis challenge, but they also reduce the mechanical transmission of trypanosomosis within the herd. When compared with the projected incidence curve of the Reed-Frost model and that of the UTC (Fig. 12) it can be seen that in both the Cylence and Bayticol groups the peak in incidence was delayed, and the area under the tail of the curve was reduced. The curves of both groups were distorted, with one big peak and a number of smaller peaks, and the incidence of trypanosomosis was reduced showing that both products were successful in reducing the probability of adequate contact through

reduction of the vector population. However, both curves still approximated the projected Reed-Frost curve closely enough, especially on the descending slope, to show that the probability of transmission was still adequate for an outbreak to occur, albeit with reduced numbers of animals being infected.

Flumethrin, in a pour-on formulation, has been evaluated against tsetse populations in West Africa and East Africa, by Bauer *et al.* (1988, 1992) and Löhr *et al.* (1991), respectively. These authors established that flumethrin was an effective glossinicide. In this trial flumethrin was evaluated in the form of a dipwash (Bayticol ®, Bayer) administered fortnightly in a plunge dip. The Bayticol group SIR showed an initial increase in trypanosomosis incidence at Week +4, which then dropped to below the control SIR for the duration of the trial with the exception of a peak increase in incidence at Week +20. This peak was probably the result of an increase in tsetse vector activity associated with increased rainfall and temperature around Week +12.

Cyfluthrin in a pour-on formulation (Cylence ®, Bayer) was evaluated in Zambia by Munsimbwe (1999) and Van den Bossche *et al.* (2004) who observed a decrease in the use of trypanocidal drugs by stockowners when Cylence was applied to their animals during a large-scale tsetse control trial. The SIR curve of the Cylence group showed a dramatic drop in trypanosomosis incidence during the first

eight weeks post-treatment, after which it fluctuated but remained below the control SIR for the duration of the trial. A peak increase in incidence at Week +20 corresponded closely with the peak increase in incidence observed in the Bayticol group SIR.

Both vector-control measures proved to be effective trypanosomosis control strategies. However the cyfluthrin pour-on had a greater impact on trypanosomosis incidence than the flumethrin dipwash. Both vector control groups showed a peak in trypanosomosis incidence at Week +20 while the UTC group showed a trypanosomosis peak at Week +12. These peaks in trypanosomosis incidence were probably all associated with increased rainfall and temperature conditions during Week +12, which were conducive to increased tsetse vector activity and increased trypanosomosis transmission. The delay of 8 weeks, and the reduction in magnitude of the peak in trypanosomosis incidence, in these 2 groups is explained by the reduced probability of adequate contact (Reed-Frost model) resulting from the insecticide treatments, and decreased trypanosomosis transmission relative to the control group. Some degree of wash off of active ingredient by high rainfall may have resulted in a shorter duration of efficacy, which would have to be compensated for by increased frequency of application.

Tsetse flies are slow reproducing insects, which make them vulnerable to control measures. This, coupled with the high toxicity and long residual activity of pyrethroid insecticides allows for effective vector control using these products (Schofield & Maudlin 2001). McDermott & Coleman (2001) rated vector control to be the most effective form of trypanosomosis control, followed by a potential vaccine and trypanocidal drugs, respectively. Since the application of insecticides to cattle allows for simultaneous control of ticks and nuisance flies this strategy is usually the most cost effective (Holmes 1997; Hargrove *et al.* 2000; Bauer *et al.* 1992), the most acceptable to cattle owners, and therefore the most sustainable tsetse-control strategy. This strategy also has limited adverse effects on the environment (Grant 2001), which make it suitable for large-scale eradication (Kabayo 2002) and control campaigns.

Insecticides have also been applied on odour-baited targets, which attract tsetse flies leading to contact between the fly and the treated target cloth resulting in the death of the tsetse fly. This target technology has received much attention and has been employed with varying efficacy in tsetse-control campaigns in many countries (Mangwiro *et al.* 1999; Kappmeier 2000; Kappmeier *et al.* 1998; 1999a; 1999b and 1999c).

Since cattle are a natural host of the tsetse vector they should be more attractive than synthetic targets, and if treated with an effective insecticide would be the more logical control method than odour-baited targets (Allsopp 2001; Baylis & Stevenson 1998). Furthermore, cattle move through vegetation thereby covering a larger area, increasing contact opportunities between treated animals and tsetse flies as opposed to targets and traps, which are stationary (Hargrove *et al.* 2000).

5.2   Partial budget analysis

Cattle have always played an important role in Zulu tradition and culture and form the basis of agricultural activity. When the State introduced compulsory dipping of cattle, in a bid to eradicate East Coast Fever from SA, plunge diptanks were built across the whole of Zululand at regular intervals. These diptanks served as a central point around which cattle dipping and handling occurred and eventually each diptank evolved into a community centre. The cattle herds in these diptank areas roam freely, attended by a herd-boy (a young boy whose duties are similar to those of a shepherd), through the communal grazing area, however all herds are associated with a specific diptank. Thus all animals dipped in a specific diptank can be considered to come from a single diptank herd, and each diptank serves as a sampling unit. In this trial each trial group was

comprised animals, which represented the animals from their respective diptank cattle populations. Thus in each trial group the respective trypanosomosis control strategy was applied to every animal in the diptank population even though only a small representative group was monitored.

Host-parasite and vector-parasite interactions, lack of diagnostic method sensitivity, vector-environment dynamics, control strategy interactions with vector and environment, and difficulty with identifying and quantifying direct and indirect effects associated with trypanosomosis and trypanosomosis-control strategies make financial analysis extremely difficult (Rowlands *et al.* 1996).

In compiling the partial budget analysis for this trial an attempt was made to include only those variables that would be changed through the implementation of a control strategy.

The partial budget showed that the Cylence group yielded a return greater than the Veridium and Bayticol groups, whilst the Veridium group yielded returns greater than the Bayticol group. Thus overall the Cylence and Veridium groups showed the greatest financial benefits, whilst the Bayticol strategy still proved to be more beneficial than the zero-treatment strategy.

5.3 Packed Cell Volumes

Fasciolosis was diagnosed in a number of trial animals, especially in the trypanocide-treated group, during the course of the study. These helminth burdens necessitated anthelmintic treatment in order to prevent loss of animals. Since acute Fascioliasis can result in anaemia it is probable that variable helminth burdens affected the trial group PCVs and confounded any effect which trypanosomosis control measures had on PCV values during the study.

Trypanosomosis was diagnosed early in the course of the disease as a result of routine 4-weekly screening of blood smears, and positive animals were immediately treated with an effective trypanocide. Any effect that trypanosomosis or trypanosomosis-control measures would have exerted on the trial group PCVs would have been moderated by this early treatment.

Any conclusions of trypanosomosis control strategy efficacy, based on changes in trial group PCVs, would therefore have to be viewed with circumspection as a result of confounding by helminth burdens and trypanosomosis treatment protocol.

5.4 Reed-Frost model

When an infectious disease enters a host population the progression of the resulting outbreak is a function of a number of

factors including; the number and proportion of immune individuals in the population, and the probability of the infectious agent being transferred from infected to healthy individuals.

The Reed-Frost model (Thrusfield, 1995) is a simple chain binomial model, which describes the major factors that play a role in herd immunity in the context of a hypothesized disease outbreak. While the model is simple, it is useful in demonstrating those factors that are of importance in herd immunity. A number of assumptions are made in the model; infection is assumed to be spread from infected to healthy individuals by "adequate contact" only, once a susceptible individual has been in contact with an infected individual it will develop the disease and be infectious in the next time period after which it will be immune, and there is a fixed probability of adequate contact between two individuals.

The numbers of cases (clinically diseased or infected individuals), susceptible and immune individuals are recorded at each time period after the introduction of the first infected individual. The single factor that carries the epidemic from one time period to the next is the probability of adequate contact, which is defined as the likelihood in any time period that an infected individual will have sufficient contact with another susceptible individual to transmit the infection.

The mathematical formulation of the Reed-Frost model is $C_{t+1} = S_t(1-Q^c{}_t)$, where C is the number of cases, S is the number of susceptibles, and Q is the probability of no adequate contact. (The probability of no adequate contact is found by subtracting the probability of adequate contact [P] from 1.) The subscript t serves as a time counter, and the length of the time period usually is set equal to the incubation or latent period of the disease. The time at which the first case enters the population is time 0 and each unit of time thereafter is numbered sequentially.

Specifically, the model equates the number of cases at any time to the number of susceptibles in the immediately preceding time period and the probability of contact of each individual with a case. This and other models together with studies of actual epidemics demonstrate that epidemics die out because of a combination of a low rate of adequate contact and a reduced number of susceptible individuals. Specifically, if P x S is greater than 1, the epidemic can occur; whereas if P x S is less than 1, the epidemic will die out or not occur in the first instance.

In the case of trypanosomosis probability of adequate contact is a complex factor, which is the product of the interaction between host, vector and parasite variables. Trypanosomosis incidence in host animals was the variable that was measured in this study so it was possible to simplify transmission and quantify it as probability

of adequate contact. The probability of adequate contact in the UTC group (viz. the probability of transmission occurring) was determined to be 0.04 (Q=0.96). The trypanosomosis-control strategies evaluated in the study resulted in either a reduction in the population of tsetse vectors in the trial area, or a reduction in the number of animals with trypanosomosis with a subsequent reduction in the number of infected tsetse flies. Both strategies effectively reduced the probability of transmission and modified the nature of trypanosomosis occurrence in the trial groups (Fig. 14).

Figure 14

Reed-Frost model showing the modifying effect of control strategies on trypanosomosis incidence
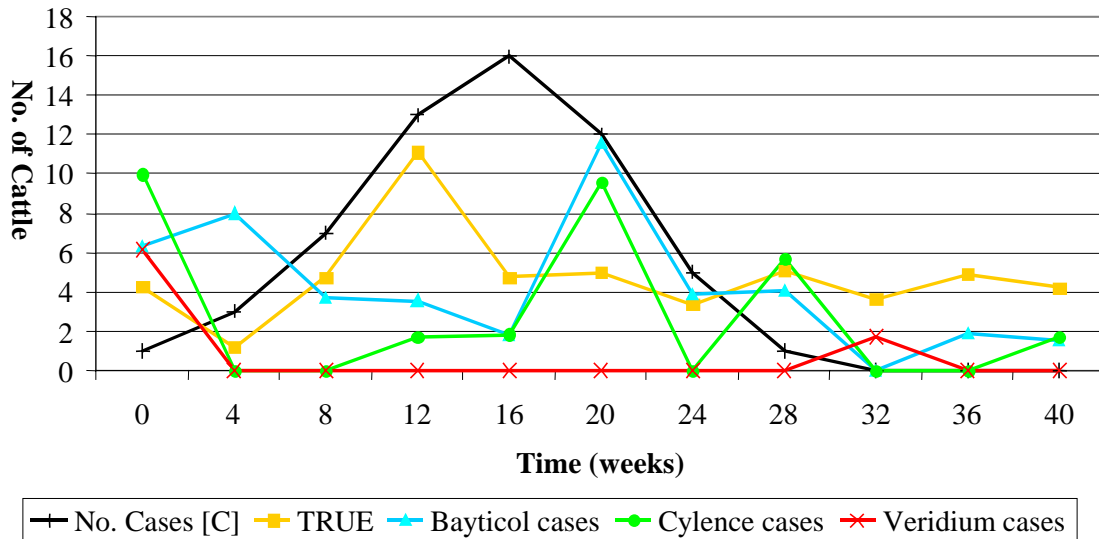
The UTC incidence curve closely approximated the ascending slope of the Reed-Frost curve but failed to reach the same peak and instead had an elongated tail area. This elongated tail area resulted from the treatment protocol used during the trial where animals diagnosed with trypanosomosis were immediately treated, for ethical and welfare reasons, with diminazene. Instead of dying or becoming immune following an active infection, the trypanosomosis positive animals were therefore treated and recovered without forming a protective immunity. In contrast to the assumptions made in the Reed-Frost model the animals were returned to a susceptible status after infection and, given a constant probability of adequate contact, this practice ensured the continued low trypanosomosis incidence

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rate reflected in the extended tail of the UTC group incidence curve. In addition to having an extended tail area, the UTC curve failed to reach the same peak in incidence as that seen in the Reed-Frost curve. This can also be explained by the treatment protocol; as animals were treated and cured of trypanosomosis the probability of new tsetse vectors becoming infected was reduced which in turn resulted in a reduction in the probability of adequate contact. The incidence rate in the UTC was moderated and failed to reach the peak projected using the Reed-Frost model.

Both of the groups where vector control measures, in the form of insecticides (Bayticol and Cylence), were used to control trypanosomosis showed incidence curves with a delayed peak approximating the descending slope of the Reed-Frost curve. The peaks were reduced and both curves showed a much reduced tail area when compared with the UTC group curve.

CHAPTER 6

6   <u>CONCLUSION</u>

Both vector-control and prophylactic trypanocide strategies were successful in reducing trypanosomosis incidence.

The cyfluthrin pour-on proved to be more effective than the isometamidium trypanocide or the flumethrin dip and resulted in the most favourable financial returns. Ease of application and long residual action made this strategy popular with the local stockowners.

The flumethrin dip was the least effective of the three strategies, yet still resulted in a significant reduction in trypanosomosis, and yielded favourable financial returns when compared with the no-treatment strategy. Both the cyfluthrin pour-on and the flumethrin dip strategies were adversely affected by heavy rains which resulted in peaks in trypanosomosis resulting from increased tsetse vector challenge.

The injectable isometamidium trypanocide prevented all but one trypanosomosis case resulting in no peaks in incidence during the trial. SIR and AUC comparison showed this strategy to be almost as effective as the cyfluthrin pour-on, and more effective than the flumethrin dip, while it yielded financial returns significantly better than the flumethrin dip. No evidence of trypanocide resistance was found during the trial.

Although all three strategies proved effective in reducing trypanosomosis, they should not be relied on solely in absence of an integrated control programme, where both vector control and trypanocide strategies are used simultaneously in order to benefit from the strengths of different approaches.

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REFERENCES

8   REFERENCES

1. Aksoy, S., O'Neill, S.L., Maudlin, I., Dale, C. and Robinson, A.S., 2001, Prospects for control of African trypanosomiasis by tsetse vector manipulation. Parasitol. Today. 17, 29-35

2. Allsopp, R., 2001, Options for vector control against trypanosomiasis in Africa. Parasitol. Today. 17, 15-19

3. Anene, B.M., Onah, D.N. and Nawa, N., 2001, Drug resistance in pathogenic trypanosomes: what hopes for the future? Vet. Parasitol. 96, 83-100

4. Bagnall, R.J., 1993, Trypanosomosis in Zululand, In: Proceedings of the twenty-fourth meeting of SARCCUS standing committee for animal health, Mbabane, Swaziland, 1993:41-49

5. Bagnall, R.J., 1994, Country Report – Republic of South Africa, In: Seminar on tsetse and trypanosomosis control, Nairobi, December 1994.

6. Bauer, B., Petrich-Bauer, J., Pohlit, H. and Kabore, I., 1988, Effects of Flumethrin Pour-on against *Glossina palpalis gambiensis* (Diptera, Glossinidae). Trop. Med. Parasitol. 39, 151-152

7. Bauer, B., Kaboré, I., Liebisch, A., Meyer, J. and Petrich-Bauer, J., 1992, Simultaneous control of ticks and tsetse flies

in Satiri, Burkina Faso, by use of flumethrin pour on for cattle.
Trop. Med. Parasitol. 43, 41-46

8. Baylis, M. and Stevenson, P., 1998, Trypanosomiasis and tsetse control with insecticidal pour-ons – fact and fiction? Parasitol. Today. 14, 77-82

9. Bruce, D., 1895, Preliminary report of the tsetse fly disease or nagana in Zululand. Ubombo, Zululand. Bennett & Davis, Durban.

10. Carter, R., 1993, Mabedlana region, Annual report of the Department of Agriculture and Forestry, Veterinary Services, KwaZulu Government Service, South Africa, for 1992/1993.

11. Carter, R., 1994, Mabedlana region, Annual report of the Department of Agriculture and Forestry, Veterinary Services, KwaZulu Government Service, South Africa, for 1993/1994.

12. Connor, R.J., 1994, African animal trypanosomiasis, In: Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (Eds.) Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press, 1:167-205.

13. De Waal, D.T., Carter, R.C., Matthee, O. and Bagnall, R.J., 1998. Importance of antigen-detection ELISA in monitoring and implementing control strategies for trypanosome infection in KwaZulu-Natal, in *Antigen ELISAs for Trypanosomes.*

Evaluation of the Performance. Proceedings of a workshop held at ILRI, Nairobi, Kenya, 9-11 December 1996, edited by S. Morzaria, R. Masake, J. Rowlands & T. Musoke. Nairobi, Kenya: ILRI (International Livestock Research Institute).

14. Doyle, J.J., Moloo, S.K. and Borowy, N.K., 1984, Development of improved control methods of animal trypanosomiasis: a review. Prev. Vet. Med. 2, 43-52

15. Du Toit, R., 1954, Trypanosomiasis in Zululand and the control of tsetse flies by chemical means. Onderstepoort J. Vet. Res. 26, 317-387

16. Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.-H., Delespaux, V., Holmes, P.H., Ilemobade, A., Machila, N., Mbwambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung'u, J.M., Peregrine, A.S., Sidibé, I., Sinyangwe, L. and Geerts, S., 2001, Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. Vet. Parasitol. 97, 171-183

17. Fuller, C., 1923, Tsetse in the Transvaal and surrounding territories: an historical review. Pretoria: Government Printer (Union of South Africa, Department of Agriculture, Entomology Memoir, No.1)

18.   Geerts, S., Holmes, P.H., Eisler, M.C. and Diall, O., 2001, African bovine trypanosomiasis: the problem of drug resistance. Parasitol.Today. 17, 25-28

19.   Grant, I.F., 2001, Insecticides for tsetse and trypanosomiasis control: is the environmental risk acceptable? Parasitol. Today. 17, 10-14

20.   Hargrove, J.W., Omolo, S., Msalilwa, J.S.I. and Fox, B., 2000, Insecticide-treated cattle for tsetse control: the power and the problems. Med. Vet. Entomol. 14, 123-130

21.   Henning, M.W., 1956, Animal diseases in South Africa, 3rd ed., South Africa: Central News Agency.

22.   Hintz, J., 2003, NCSS 2001.

23.   Holmes, P.H., 1997, New approaches to the integrated control of trypanosomosis. Vet. Parasitol. 71, 121-135

24.   Kabayo, J.P., 2002, Aiming to eliminate tsetse from Africa. Trends Parasitol. 18, 473-475

25.   Kamau, S.W., Omukuba, J., Kiragu, J., Masika, P., Ndung'u, J.M., Wachira, P. and Mehlitz, D., 2000, Financial analysis of animal trypanosomiosis control using cypermethrin pour-on in Kenya. Prev. Vet. Med. 44, 231-246

26.   Kappmeier, K., 2000a, A newly developed odour-baited "H trap" for the live collection of *Glossina brevipalpis* and

Glossina austeni (Diptera: Glossinidae) in South Africa. Onderstepoort J. Vet. Res. 67, 15-26

27.   Kappmeier, K., 2000b, Diurnal activity patterns of *Glossina brevipalpis* and *G. austeni* (Diptera: Glossinidae) in South Africa, with reference to season and meteorological factors. Onderstepoort J. Vet. Res. 67, 179-189

28.   Kappmeier, K. and Nevill, E.M., 1999a, Evaluation of coloured targets for the attraction of *Glossina brevipalpis* and *G.austeni* (Diptera: Glossinidae) in South Africa. Onderstepoort J. Vet. Res. 66, 291-305

29.   Kappmeier, K. and Nevill, E.M., 1999b, Evaluation of conventional odour attractants for *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa. Onderstepoort J. Vet. Res. 66, 307-316

30.   Kappmeier, K. and Nevill, E.M., 1999c, Evaluation of a proposed odour-baited target to control the tsetse flies *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa. Onderstepoort J. Vet. Res. 66, 327-332

31.   Kappmeier, K., Nevill, E.M. and Bagnall, R.J., 1998, Review of tsetse flies and trypanosomosis in South Africa. Onderstepoort J. Vet. Res. 65, 195-203

32. Leak, S.G.A. (Ed.) 1998, Tsetse biology and ecology: Their role in the epidemiology and control of trypanosomosis. CABI publishing in association with the International Livestock Research Institute, Nairobi, Kenya.

33. Löhr, K.-F., Omukuba, J.N., Njogu, A.R., Maloo, S.H., Gisemba, F., Okedi, T. and Mwongela, S., 1991, Investigation of the efficacy of flumethrin pour-on for the control of high tsetse and trypanosomiasis challenge in Kenya. Trop. Med. Parasitol. 42, 131-134

34. Lubega, G.W., Byarugaba, D.K. and Prichard, R.K., 2002, Immunization with a tubulin-rich preparation from *Trypanosoma brucei* confers broad protection against African trypanosomosis. Exp. Parasitol. 102, 9-22

35. Mangwiro, T.N.C, Torr, S.J., Cox, J.R. and Holloway, M.T.P., 1999, The efficacy of various pyrethroid insecticides for use on odour-baited targets to control tsetse. Med. Vet. Entomol. 13. 315-323

36. McDermott, J.J. and Coleman, P.G., 2001, Comparing apples and oranges – model-based assessment of different tsetse-transmitted trypanosomosis control strategies. Int. J. Parasitol., 31, 602-608

37. Mugunieri, G.L. and Murilla, G.A., 2003, Resistance to trypanocidal drugs – suggestions from field survey on drug use in Kwale district, Kenya. Onderstepoort J. Vet. Res. 70, 29-36

38. Munsimbwe, L., 1999, The use of a 1% cyfluthrin pour-on (Cylence®, Bayer) on cattle to manage trypanosomosis in Petauke-Nyimba area, Eastern Province, Zambia. MSc dissertation, University of Zimbabwe, Department of Paraclinical Veterinary Studies.

39. Murray, M., 1977, An improved parasitological technique for the diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg., 71: 325-326

40. Paris, J., Murray, M and McOdimba, F., 1982, A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. Acta Trop., 39, 307-316

41. Phelps, R.J. & Lovemore, D.F., 1994, Vectors: Tsetse flies, in: Coetzer, J.A.W., Thomson, G.R. and Tustin, R.C. (Eds.) Infectious diseases of livestock with special reference to Southern Africa, Cape Town: Oxford University Press

42. Roditi, I. and Liniger, M., 2002, Dressed for success: the surface coats of insect-borne protozoan parasites. Trends in Microbiol., 10, 128-134

43. Rowlands, G.J., d'Ieteren, G.D.M., Coulibaly, L., Hecker, P.A., Leak, S.G.A. and Nagda, S.M., 1996, Assessment of the effect of tsetse control on livestock productivity – a case study in northern Côte d'Ivoire. Prev. Vet. Med., 28, 17-32

44. Schofield, C.J. and Maudlin, I., 2001, Trypanosomiasis control. Int. J. Parasitol. 31, 614-619

45. Sigauque, I., Van den Bossche, P., Moiana, M., Jamal, S. and Neves, L., 2000, The distribution of tsetse (Diptera: Glossinidae) and bovine trypanosomosis in the Matutuine District, Maputo Province, Mozambique. Onderstepoort J. Vet. Res., 67: 167-172

46. Stephen, L.E., 1986, Trypanosomiasis: a veterinary perspective. Pergamon Press, UK

47. Thrusfield, M. (Ed.) 1995, Veterinary epidemiology, Blackwell Science Ltd.

48. Uilenberg, G. (Ed.) 1998, A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis, FAO, Rome.

49. Van den Bossche, P., 2001, Some general aspects of the distribution and epidemiology of bovine trypanosomosis in southern Africa. Int. J. Parasitol., 31, 592-598

50. Van den Bossche, P., Munsimbwe, L., Mubanga, J., Jooste, R. and 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. Trop. Anim. Health Prod., 36, 33-43