BOVINE TRYPANOSOME PREVALENCE AT GAME/LIVESTOCK INTERFACE OF HLUHLUWE-UMFOLOZI GAME RESERVE IN KWAZULU-NATAL PROVINCE, SOUTH AFRICA

By

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Declaration

I (Lundi Ntantiso) declare that the dissertation which I hereby submit for the degree Magister Scientiae (Veterinary Science) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

Signature…….Date……

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Acronyms and Abbreviations

%	Percentage
CAAT	Card agglutination test
DDT	Dichloro-diphenyl-trichloroethane
DNA	Deoxyribonucleic acid
EDTA	Ethylene-diamine-tetra-acetic acid
ELISA	Enzyme-linked immuno-sorbent assay
HAA	Herd average anaemia
HAP	Herd average prevalence
HA-PCV	Herd average packed cell volume
HCT	Haematocrit centrifugation technique
LAMP	Loop-mediated isothermal amplification
NKZN	Northern Kwa-Zulu Natal
PCR	Polymerase Chain Reaction
PCV	Packed cell volume

ABSTRACT

In South Africa, trypanosomosis also known as Nagana, transmitted by *Glossina brevipalpis* and *G. austeni*, is the major cause of anaemia and chronic debilitating condition in cattle. There is a wealth of entomological information on the ecology of the two tsetse species generated following the devastating outbreak in cattle due to Nagana in 1990. However, it is unfortunate that these entomological data has not been supported by parallel studies on the epidemiology of the disease. Therefore, the present study presents the first intensive epidemiological investigations since 1990 to address the problem of animal trypanosomosis in South Africa.

The relationship between trypanosomes herd average prevalence (HAP), herd average anaemia (HAA) and herd average packed cell volume (HA-PVC) were investigated in cattle in three communal diptanks located by the Hluhluwe-uMfolozi Game Reserve by regular monthly sampling for 15 months. The tsetse challenge with *G. brevipalpis* in two of the diptanks, Mvutshini and Ekuphindisweni, was high but low in the third (Ocilwane). In addition, *G. brevipaplis* and *G. austeni* coexist in Mvutshini diptank. This high and low tsetse challenge presented different disease scenarios. Cattle at Mvutshini and Ekuphindisweni diptanks had the highest HAP of 12.3% and 8.9%, respectively, which is significantly different ($p = 0.001$) from the HAP obtained from cattle at Ocilane (2.9%). Both cattle herds at Mvutshini and Ekuphindisweni diptanks also had the highest HAA, 27.7 and 33.4%, respectively, while cattle at Ocilwane had the lowest, 11.1% (statistically different; $p = 0.001$). Conversely, cattle at Ocilwane diptank had the highest HA-PCV, ranging between 29-32% while cattle at Mvutshini and Ekuphindisweni diptanks had the lowest HA-PCV (24-29%). The interaction between HAP and HAA is significant ($p = 0.021$). The overall effect of HAP on the animal health condition is clearly demonstrated when comparing the anaemia in trypanosomes infected and uninfected cattle at the 3 diptanks. Fifty percent, 63% and 100% of trypanosomes infected cattle were anaemic at Mvutshini, Ekuphindisweni and Ocilwane diptanks, respectively. In comparison, the prevalence of anaemia in uninfected cattle in the 3 diptanks was 20, 30 and 10% at

Mvutshini, Ekuphindisweni and Ocilwane diptanks, respectively. By combining the data from the 3 diptanks (1,800 observations), the overall HAA in infected and uninfected cattle was 62 and 20%, respectively.

The results of trypanosomes seasonal surveys conducted at 7 communal diptanks in tsetse infested areas, showed that all cattle at the diptanks were infected with trypanosomes with mean HAP and HAA of 10.3 and 35.3%, respectively. The highest HAP (range 15-31%, n=4) was recorded in Ngwenyambili diptank. This high infection in the cattle herds produced high values of HAA (50%; range 40-60).

The infection rate with trypanosomes in *G. brevipalpis* caught from the field showed immature infections in the midgut of 3.5% (16/458) while only one fly was found with mature infection in the proboscis (1/458, 0.22%). Very few *G. austeni* were collected (total of 9) during the same period and dissected. The infection rate with trypanosomes immature and mature infections was found to be very high; 5/9 (55.5%).

Blood samples were collected from a total of 132 buffaloes randomly immobilized for tuberculosis testing by the Hluhluwe-uMfolozi Game Reserve Authority. Two buffaloes were found to have *T. congolense* infection by the buffy coat technique. The presence of trypanosomes infected buffaloes in this study confirms the occurrence of sylvatic cycle at the tsetse/livestock/Hluhluwe-uMfolozi Game Reserve, thus, presenting a high risk of serious disease to cattle.

The objective of the study on the strategic treatment of trypanosomosis conducted on one farm in endemic area was to treat adult cows and calves at an arbitrary HAP threshold before the disease produces any clinical symptoms or production losses. The strategic use of ethidium bromide and novidium chloride produced attractive results whereby cattle were protected for an extended period of 3 to 6 months with no development of anaemia during this period. Therefore, two to four treatments per year may be sufficient to keep cattle productivity on the farm under the tsetse challenge.

CHAPTER 1
GENERAL INTRODUCTION

Bovine trypanosomosis, also known as Nagana, is caused by an infection with different species of the genus *Trypanosoma*. The protozoan parasites are transmitted by blood sucking flies of the genus *Glossina*, commonly known as tsetse. This disease reduces the overall fitness of livestock, resulting in anaemia, loss of weight and condition and subsequent loss of meat production and decrease in milk yield. Other production losses include abortion, stunted growth in calves rendering them unsuitable for sales and loss of draught power (Connor, Van den Bossche, 2004). Bovine trypanosomosis occurs in 36 sub-Saharan African countries; more than 50 million cattle and more than 60 million people are affected by the disease (Swallow, 2000). In South Africa the disease is restricted in northern Kwa-Zulu Natal (NKZN) extending from North of uMfolozi River up to the Mozambique border (Sigauque, Van den Bossche, Moisana, Jamal, Neves, 2000) coinciding with the presence of two species of tsetse; *G. austeni* and *G. brevipalpis* (Kappmeier, 2000). The disease affecting cattle was discovered in 1895 by a Scottish pathologist and microbiologist David Bruce in areas around the mountains of Ubombo (Bruce, 1895). He conducted his classical experiments and proved that a species of trypanosome caused animal trypanosomosis and that the disease was transmitted by the tsetse fly. The disease in cattle known as Nagana was already a well-known disease among the local people as “Nakane” meaning worrying disease. Local people knew the fly-infested areas, they organised game drives to try and reduce the number of game in the area so that the contact between cattle and game is prevented (Bruce, 1895).

South Africa historically, had four species of tsetse flies, namely *Glossina morsitans, G. pallidipes, G. brevipalpis* and *G. austeni*. *Glossina morsitans* was restricted to the lowveld of the old Transvaal Province and in and around Kruger National Park (Fuller, 1923, Henning, 1956). It disappeared shortly after the devastating rindepest epidemic of 1896 which resulted in the removal of most bovid animals. *Glossina pallidipes* was restricted to the Savannah areas of low-lying parts of northern Natal, which was known as Zululand.

This species was eradicated by 1953, primarily using aerial application of DDT, leaving the two remaining, so called minor vectors species, *G. brevipalpis* and *G. austeni* in the thickest coastal bush forest areas that were not suitable for *G. pallidipes* (Du Toit, 1954). Thereafter, these two fly species remained in the thick riverine and coastal bush areas which were considered unsuitable for cattle grazing (Du Toit, 1954).

The current tsetse infested areas support about 200, 000 head of cattle, 57, 585 small stock and 15 719 stock owners who are affected by Nagana. The human population in the Nagana affected area are estimated at 572, 340 and household of 89, 820 with high levels of unemployment and poverty. Livestock give source of income and social prestige, used in ploughing and other cultural activities to local people. Because of pressures for more grazing during dry season, cattle tend to graze adjacent to tsetse infested game reserves. The introduction of eucalyptus plantations for commercial purposes has been associated with artificial changes in land cover and the plantations with their surroundings became protected areas. Thicket expanded considerably over the years after cattle farmers shifted to game ranching, thus, increasing the protected tsetse areas. This man-made habitat resulted in the extension of the distribution and multiplication of both tsetse species when compared to the 1954 reported distribution maps (Esterhuizen, Kappmeier, Nevill, Van den Bossche, 2006).

In 1952 isolated cases of Nagana were reported at irregular intervals from Zululand and most cases occurred around St. Lucia Lake, well known with high infestations with *G. brevipalpis* and *G. austeni* (Du Toit, 1954). Kappmeier, Nevill, Bagnall (1998) reviewed Nagana situation up to 1990. In the review the authors referred to a survey carried by the Veterinary Services in 1980 which showed a rise in infections with trypanosomes on a number of farms in the lower Mkuze area. In 1987, an outbreak in Nibela area was associated with clinical disease and mortalities. In 1990, during a severe drought, about 10 000 cattle died of Nagana and 116, 000 were treated during this outbreak using ethidium bromide in the low lying areas of Zululand (Kappmeier et al., 1998). Further control measurements included the use of pyrethroid chemical in dip tanks to treat cattle against

ticks and since it is also insecticide, to reduce the challenge by the vector tsetse fly. These were indications that Nagana was not eliminated and the two remaining tsetse species *G. brevipalpis* and *G. austeni* were playing the major role since the eradication of *G. pallidipes* in 1954.

Sixteen years following the 1990 Nagana outbreak, a once-of sampling of a total of 76 cattle limited to one diptank by the edge of Hluhluwe-uMfolozi Reserve was carried by Van Den Bossche, Esterheizen, Nkuna, Matjila, Penzhorn, Geerts, Marcotty (2006). The result obtained from this survey, showed that the incidence of Nagana was even higher than in 1990. They concluded that the disease contribute significantly to the overall disease situation in KZN and recommended that further research was needed to develop appropriate control methods.

There is a wealth of entomological and ecological data on the two tsetse species since 1990 including the development of efficient tsetse traps, dispersal and re-invasion and limited control trials, (Kappmeier, Nevill, 1999a,b; Kappmeier, 2000, 2003; Hendrickx, Nevill, Biesemans, Kappmeier Green, Van Camp, Williams, 2003; Esterhuizen et al., 2006). Several basic studies on the feasibility of tsetse eradication were carried out for the KZN Department of Agriculture, Environmental Affairs (DAEA), by the assistance of the International Atomic Energy Agency. The Geographical tsetse presence/absence modeling and prediction models were developed for the two tsetse species (Hendrickx, 2002, 2007). The situation analysis of the feasibility and desirability for tsetse eradication in NKZN was prepared by Parker (2003). This study ascertained that the NKZN terrain was suitable for the Sequential Aerosol Technique (SAT), and an aerial spraying-based suppression programme similar to that used successfully in the Okavango Delta of Botswana can be simulated (Parker, 2003). An environmental impact analysis indicated that environmental impacts of SAT are likely to be minimal and short-term in nature (Grant, 2003). Lastly, an economic study and a cost-benefit analysis showed that there will be substantial financial benefits following the creation of a tsetse free zone in KZN and southern Mozambique (Knight, 2006). On the other hand, the Conservation and Wildlife Department of the KZN-

DAEA has objected to these studies and of the opinion that tsetse flies "must not be eradicated" as it is environmentally unacceptable (Armstrong, 2003). However, it is unfortunate that these entomological data and the accompanying studies or the objection to eradication have not been supported or refuted by parallel studies on the epidemiology of the disease which form the basis of further studies on the impact of the disease and its socio-economics on the livelihood of the resource-poor farming communities in NKZN. Therefore, the present study presents the first intensive epidemiological investigations to address the problem of animal trypanosomosis in South Africa.

The general aim of the study:

The current incidence of trypanosomosis in NKZN is unknown; it is thought to be relatively high and is a cause of concern to the Department of Agriculture and Environmental affairs Kwa-Zulu Natal and livestock farmers. This provides an ideal opportunity to study the epidemiology of the disease "Nagana" in cattle where tsetse flies are known to occur and to obtain baseline data against which the success of future control operations could be measured. The ultimate beneficiaries of tsetse control will be livestock owners, mainly resource-poor farmers, of Kwa-Zulu Natal Province.

CHAPTER 2
LITERATURE REVIEW

2.1 Background

Bovine trypanosomosis is caused by several species of the genus *Trypanosoma*, a parasitic protozoan infecting blood and tissues of the host animal. Trypanosomes are transmitted cyclically by several species of blood sucking flies of the genus *Glossina*, commonly known as tsetse flies. In South Africa the disease occurs in the northern Kwa-Zulu Natal Mkhanyakude District, covering an estimated area of 16,000 square kilometres. The disease which is locally known as Nagana, was first diagnosed in 1894 by Sir David Bruce (Bruce, 1895) in Ubombo District northern KwaZulu-Natal. Nagana was already a well-known disease among the local communities even before Bruce's time; it was called "Nakane" (worrying disease) or "Munca" meaning sucked out (Bruce, 1895).

2.2 Classification of trypanosome species

The genus *Trypanosoma* falls under the family Trypanosomatidae, order Kinetoplastida of the class Zoomastigophora (Levine, Corliss, Cox, Grain, Derouxg, Honigberg, Leedale, Loeblich, Lom, Lynn, Merinfeld, Page, Poljansky, Sprague, Wallace, 1980). *Trypanosoma* species are all parasitic and known to infect a wide variety of vertebrate species throughout the world. They feed by absorption of blood and fluids in solution in their environment (Lapage, 1968). Tsetse-transmitted trypanosomes in vertebrates are classified according to their developmental sites within the tsetse fly vector (Hoare, 1964). The salivaria group (Hoare, 1964) in which the trypanosome parasites development is completed in the mouth parts and subsequent transmission is by inoculation, are divided into three groups:

The *vivax* group subgenus Duttonella: the site of development is proboscis. The *vivax* group consists of one species, *T. vivax*.

The *congolense* group subgenus Nannomonas: the site of development is the midgut and proboscis (Hoare, 1964). This group consists of *T. congolense* and *T. simiae*.
The *brucei* group subgenus trypanozoon: the site of development is midgut and salivary glands. The brucei group consists of *T. brucei brucei*, *T. brucei rhodensiense* and *T. brucei gambiense, T. evansi, T. equiperdum*.

The second section is the stercoraria in which the development of trypanosomes is posterior (posterior station group). This section includes *T. theileri*, *T. lewisi* and *T. cruzi*. The subsequent transmission is by contamination.

2.3 Pathogenesis of the diseases

One of the major effects of infection with pathogenic trypanosome is anaemia (Leak, 1998). The disease varies from acute to chronic forms. The acute form occurs soon after the infection, characterise by high parasitaemia and rapid fall of packed cell volume (PCV) due to the destruction of red blood cells caused by foreign body proteins from the parasites in the host blood and tissues. This induces an autoimmunity or erythrophagocytosis, resulting in fever. Death normally occurs within 10 days in susceptible animals (Connor, Van Den Bossche, 2004). The extent of the acute or chronic forms of the disease is determined by a number of factors; complete tolerance (no-illness) in the case of game, virulence of the *Trypanosoma* species e.g. *T. congolense* and the level of parasitaemia (Connor, Van Den Bossche, 2004). The chronic stage which is typical in indigenous breeds can persist for an extended period (months) in which case the affected animals loose condition, become increasingly anaemic and lethargic (Itty, 1996). Any stressful situation on the animal such as shortage of food may result in recurrence of the acute form of the disease. Occasionally, the infected animals may recover spontaneously (Nantulya, Musoke, Rurangirwa, Minja, Moloo, 1984).

Murray and Gray (1984) in their review of pathogenicity of trypanosomes, discussed parasitaemia in relation to host susceptibility. They referred to susceptibility of an animal

as the "capacity to limit, reduce or control parasitaemia", a phenomenon confirmed in cattle and mice. Thus, the trypanotolerant Ndama cattle show a superior capacity to control parasitaemia than the susceptible Zebu cattle. As such, the severity of anaemia, and hence the disease, is directly related to the level of parasitaemia, particularly at the early phase of the infection. Recently, Marcotty, Simukoko, Berkvens, Verfuysse, Praet and Van den Bossche (2008), evaluated PCV values as "indicator of trypanosomosis infections in cattle" in tsetse infested areas. In this study, they compared three diagnostic tests, the buffy coat, PCR and PCV values.

The epidemiology of trypanosomosis is complex. In eastern and southern Africa, *T. congolense* is considered the most pathogenic species infecting cattle and produces serious disease (Connor, Van den Bossche, 2004). However, it has been established that *T congolense* is a species comprising three distinct genotypes; *T. congolense* Savannah-type is most virulent, the forest type is of low pathogenicity and Kilifi-type which is non-pathogenic (Majiwa, 1992, Bengaly, Sidibe, Ganaba, Desquesnes, Boly, Sawadogo, 2002). Furthermore, virulence testing of *T. congolense* Savannah-type revealed three virulence categories in mice; extremely virulent strains, moderately virulent and low virulence strains (Masumu, Marcotty, Geysen, Geerts, Vercruysse, Dorny, Van den Bossche, 2006). Recently, two *T. congolense* genotypes, Savannah-type and Kilifi-type have been shown to infect cattle in areas around Hluhluwe-uMfolozi Game Park (Mamabolo, Ntantiso, Latif, Majiwa, 2009).

2.4 Methods of diagnosis

Confirmatory diagnosis of Nagana is reached by demonstration and identification of the trypanosome parasites in the blood of an infected animal. Techniques for diagnosis of trypanosome parasites can either be direct by parasitological methods (Cunningham and Van Hoeve, 1965) or indirect by serological methods (Nantulya, 1990). Direct methods involve identification of the trypanosomes in thick and thin blood smears or by the buffy coat preparation by using the haematocrit centrifugation technique (HCT). Blood smears

stained with conventional stains e.g. Giemsa's stain; identify the parasites by the aid of compound microscope. The HCT, a wet preparation, is quick and suitable for screening large number of animals in the field. Experience is required to identify the parasites to species level by noting the type of movement under the field of microscope. It is widely used in epidemiological surveys and at the same time the status of anaemia in the animal can be assessed (Woo, 1970). However, the method is insensitive as some infected animals showing low parasitaemia may pass un-detected (Nantulya, 1990). On the other hand, thin smears allow better identification of the trypanosomes to species level.

Inoculation of infected blood into mice proved to be the most sensitive technique for detection of *T. brucei*. However, results will vary according to the ratio of infective to non-infective forms (Paris, Murray, McOdimba, 1982). The value of inoculation of *T. congolense* or *T. vivax* suspected blood into rodents is limited as not all field isolates become established (Paris et al, 1982). Leeflang and Janny, Coby, (1978) found mouse inoculation to be significantly less efficient for the diagnosis of *T. vivax* in comparison with other diagnostic tests including HCT, thick and thin blood smears.

Indirect method involve serological techniques like ELISA and IFA (Williams, Duxbury, Anderson, Sadun, 1963, Wilson, 1969; Nantulya, Musoke, Rurangirwa, Saigar, N., Minja, 1987) to identify antibodies against the parasites. Molecular tools using polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) were used as diagnostic tests and for characterization to species and subspecies level (Gibson, 1994; Thekiose, Inoue, Kuboki, Tuntasuvan, Bunnoy,Borisutsuwan, Igarashi, Sugimoto, 2005; Thekiose, Bazie, Coronel-Servian, Sugimoto, Kawazu, Inoue, 2009). Van den Bossche et al. (2006) conducted trypanosomosis survey in cattle at Mvutshini dip tank, South Africa, and found the prevalence of 34% by using the buffy coat method while the PCR method was found to be more sensitive giving a prevalence of 61%. Other tests include the indirect haemagglutination test (Gills, 1964). The test is difficult to standardize regarding sensitivity and specificity. The commercially available "card agglutination test for

trypanosomosis CATT" is simple, fast and mostly used under field conditions (Luong-To, Le-Ngoc, 1995).

2.5 Disease dynamics

Results obtained from trypanosomosis surveys conducted in Malawi, Mozambique, Zambia, Zimbabwe and Namibia have indicated that the epidemiology follows a sequence which can be divided into four distinct epidemiological situations (Van den Bossche, 2001). Those epidemiological circumstances are a consequence of human encroachment into tsetse-infested wild areas and the subsequent gradual alterations to the environment because of cleaning of vegetation and the introduction of cattle. As a result, the cycle of trypanosomes transmission changes from a sylvatic cycle (tsetse and game), to a combined sylvatic/domestic cycle (tsetse/game/livestock interface) and sylvatic cycle with occasional challenge of tsetse at the game or cattle interface (Van den Bossche, 2001).

2.5.1 Sylvatic cycle

This epidemiological situation occurs in tsetse-infested game areas of Southern Africa, such as the valley of the Luangwa and Zambezi rivers, or Hlulhuwe-uMfolozi Game Reserve in Kwa-Zulu Natal, where tsetse can thrive in absence of livestock. Obviously in the absence of cattle, bovine trypanosomosis does not pose a problem (Van den Bossche, 2001).

2.5.2 Mixed sylvatic/domestic cycle

The change from a sylvatic to a mixed sylvatic/domestic cycle of trypanosomosis is usually as the consequence of the introduction of cattle into tsetse-infested (game) areas. Despite the presence of game, livestock will be readily fed upon and infected with trypanosomes. Since the game constitute an important reservoir of trypanosome infections, cattle are likely to be challenged with a plethora of trypanosome stocks including highly pathogenic ones.

In such circumstances, bovine trypanosomosis often has an epidemic character (Van den Bossche, 2001).

2.5.3 Domestic cycle

The increasing human density and the progressive cleaning of vegetation reduce the density of the game. Large game animals usually disappear from such highly cultivated areas. For their survival, tsetse will have to rely mainly on feeding on cattle. Hence, the trypanosome transmission cycle changes from a mixed sylvatic/domestic cycle into a domestic cycle. This change in the epidemiological situation has important repercussions on the way in which trypanosomosis manifests itself in the cattle. Since cattle serve as reservoir of trypanosomes, highly pathogenic strains are likely to disappear and the diversity of the trypanosome strains will be limited (Van den Bossche, De deken, Brandt, Geerts, Geysen, Berkvens, 2004a). The disease becomes endemic with a high proportion of cattle population infected but with limited impact on cattle production. Endemic trypanosomosis occurs in a large part of the plateau area of eastern Zambia (Van den Bossche, Munsimbwe, Mubanga, Jooste, Lumamaba, 2004b).

2.5.4 Sylvatic cycle with challenge at the game/cattle interface

Finally, intensive cleaning of vegetation and the concomitant elimination of suitable tsetse habitat restricts the presence of the tsetse to protected areas such as game reserves, national parks and forests reserves. Game animals will again become the main host of tsetse and source of trypanosomes. Cattle will be subjected to irregular challenge along the edges of the protected areas (the interface). Because of the irregular challenge and trypanosome reservoir in game, bovine trypanosomosis again has an epidemic character. Currently, such interfaces are found along the Kasungu National Park and Nkhotakota Game Reserve in Malawi, along the Malawi National Park and the Kwando River in the Eastern Caprivi of Namibia (Van den Bossche, Shumba, Makhambera, 2000).

2.5.5 New threats

The epidemiology of bovine trypanosomosis in Southern Africa is clearly subject to changes. It is, thus, conceivable that in the years to come, those changes will have considerable repercussions on the impact of bovine trypanosomosis and its control strategies. It is expected that land reforms in South Africa, in presence of tsetse flies, will have one or another of these epidemiological scenarios.

2.5.6 Land utilization

Land use in Zululand in South Africa has in fact changed dramatically since *G. pallidipes* was eradicated and many of these changes and associated activities have possibly led to the re-appearance of Nagana as a threat to the health of livestock, especially of cattle. Some of the factors that may have contributed to the 1990 outbreak are mentioned below (Kappmeier et al., 1998):

- In that year there was serious drought and malnutrition, exacerbated by over-stocking in communal farming areas.
- Continuous uninterrupted use of a tick-detaching dipping agent (Amitraz) which does not have chemical properties to kill tsetse flies, thus leading to a build-up of all fly species associated with cattle.
- Increase in game numbers in existing game reserves following depletion in the early 1940's when game eradication was implemented as a control strategy.
- Change in commercial farming areas from cattle ranching to game farming which become in custody of conservation.
- Afforestation of large tracts of land previously unsuitable for tsetse flies.

2.6 Tsetse fly, the vector transmitting the disease

Tsetse fly species of the genus *Glossina*, act as the main vectors for several trypanosomes species. The distribution of the *Glossina* species is limited to Africa South of the Sahara,

covering an area of 10 million square km (Jordan, 1986). Tsetse flies are grouped according to their habitat preference, behaviour and morphometric features. There are three groups; the fusca which inhabits fringe forest areas, the palpallis, occupying rain forest around lake areas and morsitans group occupying Savannah areas (Phelps, Lovemore, 1994). There are a number of factors that affect tsetse fly infection by a trypanosome parasite. These are climatic factors related to the vector, host association and the parasite factors (Mattioli, 1997).

Habitats with increasing high light intensity, increased solar rays and an increase in environmental temperatures become less favourable biotopes and detrimental to tsetse survival. Consequently mean age tsetse population diminishes following these unfavourable local climatic conditions. This leads to a lower trypanomosal infection rate in vector population as generally the oldest fraction of *Glossina* are the most infected (Mattioli, 1997).

Field and laboratory investigations reported large variations in the proportion of trypanosome infected flies in different species of *Glossina* (Moloo, Kutuza, 1988). Innate differences amongst the various *Glossina* species seem responsible for this phenomenon, as sensitivity to trypanosome infections in tsetse flies is genetically modulated not only amongst a species, but also amongst individuals of the same species (Makumyaviri, Demey, 1985). Under laboratory conditions *G. m. morsitans* is a more effective vector of *T. congolense* than *G. tachinoides*. Once the trypanosome are ingested by a tsetse fly following an infective blood meal, the establishment and development of mature trypanosome infecting forms are moderated by the internal environment conditions contingent to the vector (Maudlin, Welburn, 1988).

Odours released by a host animal, such as cutaneous secretions and phenolic components of urine, are potent attractant for tsetse flies (Vale, Hall, Gough, 1988). The flies are also attracted by moving objects, such as man, animals, vehicle while their colour is a further stimulus in directing the insects towards a potential nourishment source (Vale, 1974). Thus,

these attractants indicative for each mammalian species lead the tsetse to feed selectively on a specific animal species, in particular wild mammals, despite a higher proportion of domestic ruminants co-existing in the same area (Gates, Williamson. 1984). These trophic preferences affect the probability of acquiring the infection and establishing mature parasites in tsetse flies (Moloo, 1981). The level of patent parasitaemia in the host, which is related to its susceptibility to trypanosomal infection (Paling, Moloo, Scott, Mcodimba, Logan-Henfrey, Murray, Williams, 1991), does not seem to have a central role on the acquisition of the parasite by tsetse fly (Moloo, 1982).

2.7 Tsetse distribution and control in South Africa

South Africa originally had four species of tsetse flies, namely *Glossina morsitans*, *G. pallidipes*, *G. brevipalpis* and *G. austeni*. *Glossina morsistans* was restricted to the lowveld of the old Transvaal Province (Fuller, 1923; Henning, 1956) and especially to the Kruger National Park. It disappeared shortly after the rinderpest epidemic of 1896 which cleared the national herd and most of the buffalo population on which the flies feed. *G. pallidipes* was restricted to the Savannah areas of low-lying parts of northern Natal, which was known as Zululand. This species was eradicated by 1953, primarily using aerial application of DDT, leaving the two remaining so called minor vectors species, *G. brevipalpis* and *G. austeni* in areas that were not suitable for *G. pallidipes*, thick coastal bush forest (Du Toit, 1954). The two species *G. brevipalpis* and *G. austeni* remained in the thick riverine and coastal bush areas and were not considered to be important vectors and because it was thought that cattle do not graze into these areas or places due to the thickness of the vegetation or trees (Du Toit, 1954).

The previous surveys of tsetse fly distribution in Zululand were conducted around 1946 and focused primarily on the determining the distribution of *G. pallidipes* (Du Toit, 1954). To determine the present distribution of the remaining two species (Figure 1.1) an intensive survey was conducted from the year 1993 to 2000 to cover the entire area of approximately 16 000km^2 where Nagana had been recorded. Sticky traps baited with artificial ox odour

were first used followed by the use of the more efficient H-traps (Kappmeier et al., 1998; Kappmeier, Nevill, 1999a,b; Esterhuizen, Kappmeier Green, Marcotty, Van den Bossche, 2005). The results of the surveys showed that both species required well shaded areas provided by evergreen bushes or riverine. *G. brevipalpis* is commonly associated with natural areas in game reserves while *G. austeni* occurs widely in communal farming areas. Distribution and prediction models based on collections data of presence/absence were developed for the two species (Hendrickx et al., 2002; Hendrickx, 2007). It is important to note that the data observed from this model suggests some avenues for future sampling or research; areas like Mkuze game reserve, communal areas north of Hlulhuwe game reserve and further south coastal belt, south of uMfolozi River e.g. Maphelane Nature Reserve and Mseleni Game Reserves.

Kwa-Zulu Natal Veterinary Services conducted a survey in 1990 after the outbreak to investigate the actual prevalence of trypanosomosis covering the area between uMfolozi River and Mozambique border (Kappmeier et al., 1998). The results showed that the prevalence was between 10-15% in the entire dip tanks that were sampled. Dipping of cattle in pyrethroid coupled with therapeutic treatment successfully contained the epidemic of 1990 (Kappmeier et al., 1998). However, controlling the tsetse flies around Hluhluwe-uMflozi Game Park using target technology did not produce satisfactory results and the control trial was discontinued (Kappmeier et al., 1998). There is no control policy in South Africa for the tsetse fly and the diseases they transmit other than reacting to the outbreak crises when it occurs. Strategy for an area-wide control with sterile insect technique to establish a tsetse-free zone was suggested as the only sustainable and profitable method (Kappmeier Green, Potgieter, Vreysen, 2007). The concept is based on an area-wide integrated pest management (AW-IPM) approach that includes tsetse suppression (sequential aerosol technique, impregnated targets and traps, pyrethroid-bait cattle) and followed by the release of sterile male insects. Tsetse eradication was considered successful in the Okavango Delta, Botswana, and the environmental concerns were found to be minimum (Perkins, Ramberg, 2004).

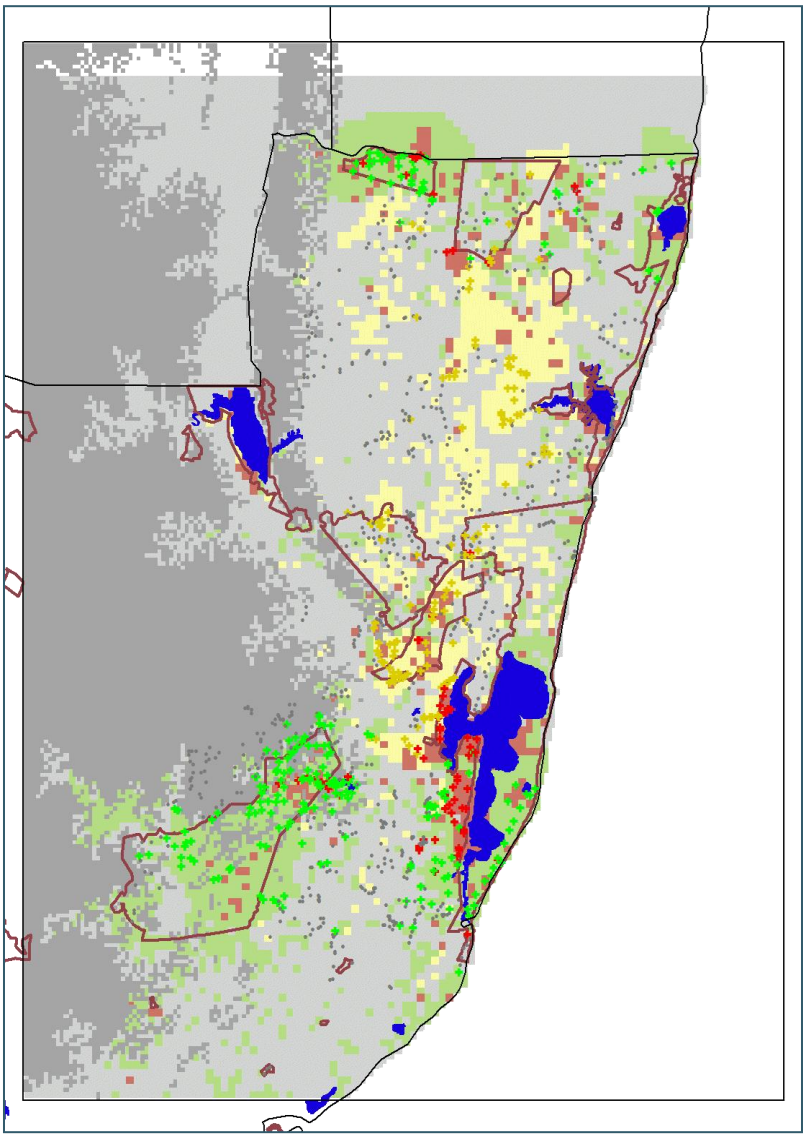


Fig 2.1: Map showing tsetse distribution in Northern KwaZulu-Natal, South Africa (Hendrickx, 2007)

2.8 Specific objectives of the study

- To assess the prevalence of trypanosomosis in cattle population in three communal diptanks in relation to cattle/game interface.
- To monitor the tsetse challenge in the three dip tanks at the game/livestock interface.
- To assess the infection rate with trypanosomes in the buffalo population in Hluhluwe-uMfolozi Game Park.
- To assess the infection rate with trypanosome parasites in the tsetse flies collected from the field.
- To survey the incidence of trypanosomosis in cattle population in 7 communal diptanks further away from Hluhluwe-uMfolozi Game Park.
- To assess a strategy of trypanocidal drug treatment in one commercial farm at Boomerang farm.

CHAPTER 3
MATERIALS AND METHODS

3.1 The study area

The study was conducted in Hlabisa Municipality, which is part of Umkhanyakude District (DC 27) around the edges of Hluhluwe-uMfolozi Game Reserve and along St. Lucia Wetlands Park which are the two major conservation and protected areas in the District. This area is part of the16, 000 km^2 tsetse belt in northern Kwazulu-Natal where both *G. brevipalpis* and *G. austeni* are present (Kappmeier Green, 2002). The vegetation consists of bush land, sand forest and forest plantation. Temperature ranges are between 22^0C– 28^0C annually, rainfall in summer is around 950 mm and winter 260 mm. This district has 200, 000 head of cattle, 57, 585 small stock, 15, 719 stock owner's and a population of 572, 340 and 89, 820 households (Emslie, 2005).

3.2 Boomerang Farm (commercial farm)

This farm is situated by Charter's Creek Nature Reserve which is part of Isimangaliso Wetland Park (Table 3.1). It has 180 herds of cattle with an output of about 30 cattle sold per year. Cattle were grazed into 300 hectares of indigenous forest during dry seasons. They were also supplemented by the cane sugar residues after the harvest. H-traps were deployed by the Entomology Division of the Onderstepoort Veterinary Institute for intensive tsetse population studies. Two tsetse species are present on the farm; *G. austeni* and *G. brevipalpis* (Esterhuizen et al., 2005). There was no history of any treatment for trypanosome before or the previous year. All cattle were generally in poor health condition though the herd received regular antihelminthics.

3.2.1 Strategic use of trypanocides

A treatment strategy using two drugs, Homidium bromide (Ethidium, CAMCO, Animal Health, UK) at a dose rate of 1.0 mg/kg and Homidium chloride (Novidiun, Rhone-Merieux, France) at a dose rate of 1.0 mg/kg , was attempted which would allow the herd of cattle to thrive in the high tsetse challenge area ((Esterhuizen et al., 2005). Both drugs have therapeutic and prophylactic actions. The drugs are not yet registered in South Africa but special import permit was granted by the then National Department of Agriculture (DOA), now known as the Department of Agriculture, Forestry and Fisheries (DAFF). The adult cattle and weaned calves (6 months old) borne in 2005 and 2006 were all monitored monthly for trypanosomes herd average prevalence (HAP, Section 3.4) and herd average anaemia (HAA, Section 3.4) over a period of 12 months. The threshold for treatment was decided arbitrary to be a herd average prevalence of 20 which was noticed to produce HAA of around 25%.

3.3 Trypanosomosis survey and surveillance

A total of 10 communal diptanks and one commercial farm were selected for trypanosomosis survey and surveillance which are located at various distances from the edge of the Hluhluwe-uMfolozi Game Reserve (Figure 3.1). The selected diptanks for the surveys were: Gwenyambili, Mahlambanyathi, Bukhipha, Mzineni, Nhlwathi, Qakwini, and Nibela (Table 2) and 3 for surveillance, Mvutshini, Ekuphindisweni and Ocilwane (Table 3.1). The cattle sampling depended on the total cattle population provided by the community at a particular sampling site but rarely exceeded 60 herds of cattle at a single sampling site. Cattle were surveyed three to four times per year to cover different seasons; mainly dry and wet seasons. A total number of cattle sampled during the study period was 2624.

Excellent working relation was made with communities at the 3 diptanks by the edge of Hluwhluwe-uMfolozi Game Reserve; Ekuphindisweni, Mvutshini and Ocilwane. To ensure the commitment by the communities, 10 sentinel cattle were purchased by the project from the herd owners of each diptank and at least 40 more animals were offered by the

community for sampling purposes on monthly bases for a period of 15 months. The herds were kept under normal village conditions and grazed in the normal grazing areas.

Treatment against livestock trypanosomosis owned by the local communities in NKZN has not been a regular practice and in most cases non-existent (Latif, pers. Communication, 2005). For ethical reasons, parasitological positive as well as negative animals with a PCV lower or equal to 24 % were treated with diminazene aceturate (Berenil®, Hoechst) at the rate of 3.5 mg/kg body weight. Animals given diminazene were considered to be protected during the subsequent two weeks and were therefore excluded from the next calculation of incidence.

3.4 Sampling

Blood was collected from the tail or jugular veins using 10 ml vaccutainer tubes coated with EDTA as anticoagulant. Here a generator was used as source of electric power for the haematocrit centrifuge and the microscope (Figure 3A, 3B). Further processing was done at the OVI Field Research Station, Kuleni or at the site of the diptank if it is further away from the station.. In the laboratory, blood from each sample was decanted into plain microhaematocrit centrifuge capillary tubes that were sealed with cristseal and centrifuged in microhaematocrit centrifuge for 5 minutes at 9,000 rpm. After centrifugation, the packed cell volume (PCV) was determined. Animals with a PCV of 24% or less were considered anaemic (Murray, Dexter, 1988; Van den Bossche, Shumba, Makhambera, 2000). The buffy coat of each sample was extruded onto a microscope slide, covered with a cover slip and examined for motile trypanosomes under a compound microscope using X40 magnification.

Trypanosoma congolense (Figure 3.2) is the dominant species infecting cattle and buffalo in the study area (Van den Bossche et al., 2006, Mamabolo et al, 2009). Few mixed infections of *T. congolense* and *T vivax* were reported in some diptanks and not a single *T. brucei* was reported from cattle in the area (Mamabolo et al., 2009). Furthermore, in

southern Africa *T. congolense* is more pathogenic to cattle than *T. vivax* and produces serious disease (Connor and Van den Bossche, 2004). Therefore, *Trypanosoma* infection in cattle i.e. the prevalence, in this study refers to infections with *T. congolense*.

The level of bovine trypanosomosis of a herd at specific site was calculated as the proportion of cattle with trypanosome infection and referred to as the herd average prevalence of trypanosome infection (HAP) (Van den Bossche and Rowlands, 2001). The percentage anaemia i.e. percentage of cattle in a herd with PCV of 24% or less was calculated. The PCV of cattle at each sampling was averaged and referred to as "herd average PCV" (HA-PCV) (Van den Bossche, Rowlands, 2001). Furthermore, the percentage of anaemic cattle in a herd is referred to as the herd average anaemia (HAA). The HAP, the HA-PCV and HAA which give good indication of the health status of the herd (Trail, d'Ieteren, Colardelle, Maille, Ordner, Sauveroche, Yangari, 1991; Trail, Murray, Sones, Jibbo, Durkin, Light, 1985), were obtained from herds at each dipank.

3.5 Tsetse population monitoring at the study sites

A total of four H-traps (Figure 3.3) were deployed at the diptanks of Ekuphindisweni (from 2006-2008), Mvutshini (2005-2008) and Ocilwane (2007-2008). Fly catches, males and females were collected once every two weeks, identified into species and counted. The total flies collected from the four traps per month were recorded for each site.

3.6 Infection rate with trypanosomes in tsetse flies

Tsetse flies were collected using the H-trap (Figure 3.3) from Hluhluwe-uMfolozi Game Park, Mvutshini, Charter's Creek and Boomerang farm. The traps were visited every morning and caught flies were collected and transported in a cool-box with ice to the laboratory at the Kuleni Field Station. They were kept in the cold condition, identified into species and immediately dissected. Dissection was performed, in phosphate buffered saline in 2% glucose, in chilled flies where the midgut and the proboscis of each fly were

separated, mounted on a separate microscopic slide and examined under a stereomicroscope. The infection with trypanosome parasites in each organ was recorded. As *T. bruce*i was never encountered during the surveys (Van den Bossche et al., 2006, Mamabolo et al., 2009), the salivary glands were not examined. *Trypanosoma congolense* immature infections were found in the midguts while mature infections were found in the proboscis (Jordan, 1976).

3.7 Infection rate with *Trypanosoma* species in buffalo at Hluhluwe-uMfolozi Game Park

Buffaloes were immobilized by the Park Authority to test for tuberculosis as a routine management of the disease. The opportunity arose in 2007 and a total of 132 buffaloes were tested using the buffy coat method as described above (Section 3.4).

3.8 Statistical analyses

Variation in HAP, HA-PCV and HAA between cattle herds at the 3 diptanks were analysed using the statistical program GenStat® (Payne, Murray, Harding, Baird, Soutar, 2007). The Chi-squared (χ^2) test was selected for the analysis because observations made were frequencies of occurrences categorized in distinct classes. In cases where the minimal allowed expected frequencies were less than 5, Fisher's Exact test was used instead of the Chi-squared (χ^2) test. Testing was done at the 5% level where *P*-values of <0.05 were used as the cut-off for statistical significance. Means and standard deviations were also obtained for HAP, HAA and HA-PCV for cattle herds in different locations.

Figure 3.A: generator, source of electric power for the haematocrit centrifuge and for the microscope.

Figure 3.B: Examination of blood smears in the field.

Table 3.1 Communal diptanks sampled monthly (surveillance)

DIP TANK NAME	DIP TANK NO.	CO-ORDINATES
Ekuphindisweni	328	26.57 S 32.46 E
Mvutshini	945	28.07 S 32.09 E
Ocilwane	831	28.26 S 32.00 E

Table 3.2: Communal diptanks surveys (seasonally)

DIP TANK NAME	DIP TANK NO.	CO-ORDINATES
Bukhipha	962	27.57 S 32.12 E
Nhlwathi	675	28.01 S 31.59 E
Mzineni	526	27.59 S 32.09 E
Nibela	817	27.51 S 32.27 E
Gwenyambili	788	28.06 S 32.21 E
Qakwini	692	28.08 S 32.20 E
Mahlambanyathi	963	28.11 S 32.19 E

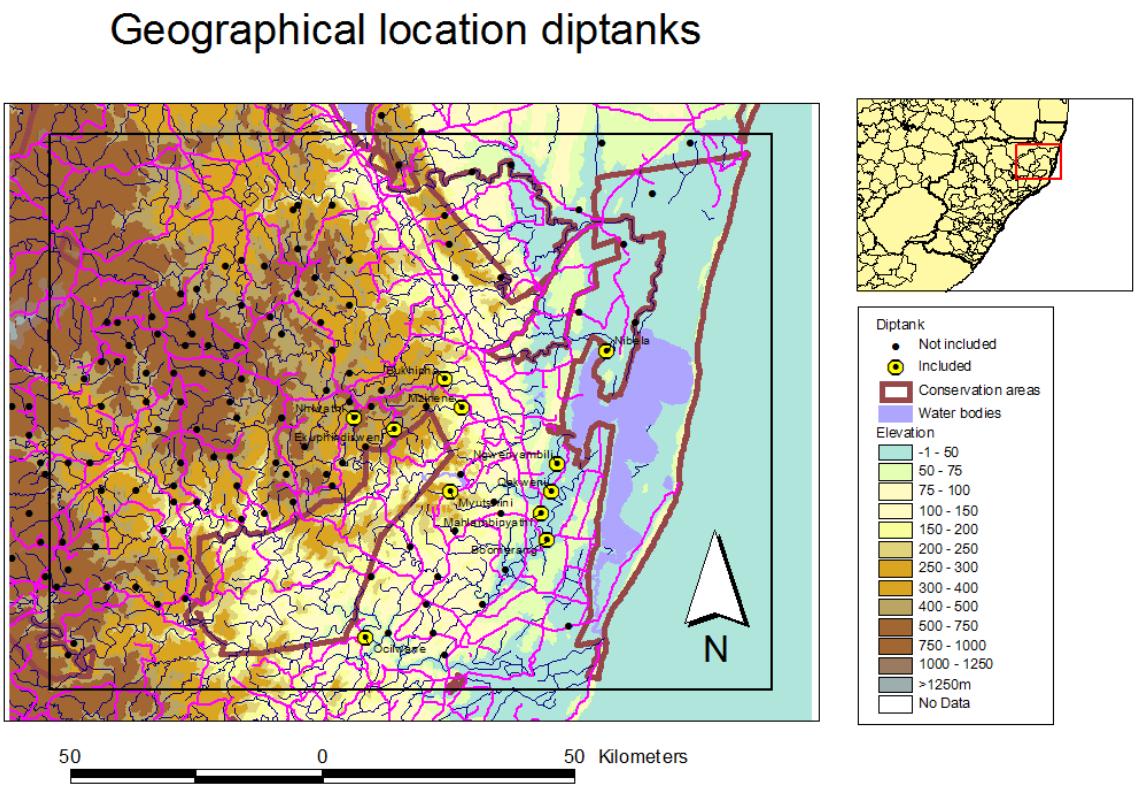


Figure 3.1: Hlabisa District (study area): showing the location of the selected diptanks and a commercial farm "circled" at the edges of Hluhluwe-uMfolozi Game Park and further from game parks.

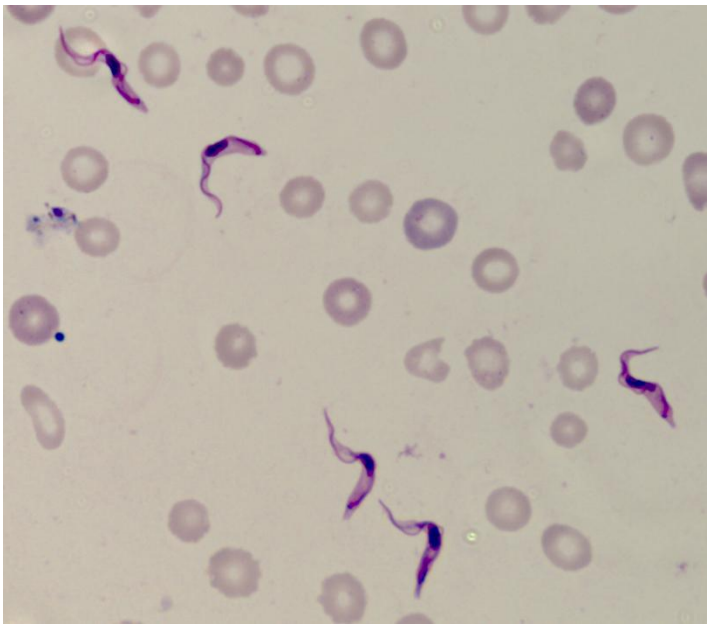


Figure 3.2: Blood smear showing *T. congolense*, a major species infecting cattle in the study sites. Identified by the absence of free flagellum and small size.

Figure 3.3: H-trap (Kappmeier, 2000) and containers showing fly catches

CHAPTER 4
RESULTS

4.1 The study sites and tsetse risk

Figure 4.1 shows the tsetse risk map drawn for the 10 diptanks within the study area. *Glossina brevipalpis* and *G. austeni* coexist in two of the diptanks, namely Mvutshini adjacent to Hluhluwe-uMfolozi Game Park and Boomerang farm near Charters Creek Nature Reserve. *G. brevipalpis*, on the other hand, is present on all of the study locations.

4.2 Tsetse abundance in the 3 surveillance diptanks

The two fly species were collected from Mvutshini diptank (Figure 4.2), though with varying abundance. *G. brevipalpis* abundance was high, ranging between 100-200 flies per month. It is noted that there was high peaks coinciding with the months of March-April in 2006 and 2007. However, the fly population density was almost stable in the year 2008. *G. austeni* apparent density population was very low of less than one fly catches per month from 2005-2008.

Figure 4.3 shows *G. brevipalpis* population density in Ekuphindisweni and Ocilwane diptanks as it was the only tsetse species recorded in these two sites. Ekuphindisweni diptank, lying at the most northern side of the Hluhluwe-uMfolozi Game Park, had high fly abundance of 50-250 fly catches per month. However, Ocilwane diptank, lying at the southern side of the park, had very low fly abundance demonstrated by 5-25 fly catches per month. It is noted that though the high peak of fly catches at Ocilwane diptank is similar to that of Mvutshini i.e. during the month of March-April, the peak of catches at Ekuphindisweni was coinciding with the months of August in 2007 and 2008.

Figure 4.4 shows the summary of the fly catches at the 3 diptanks. Essentially, it shows that Mvutshini and Ekuphindisweni diptanks, both situated at the most northern part of the

Hluhluwe-uMfolozi Park, had very high fly abundance of *G. brevipalpis* compared to the very low abundance at Ocilwane diptank, situated at the southern part of the park.

4.3 Trypanosomes surveillance: Herd average prevalence (HAP), herd average anaemia (HAA) and herd average PCV (HA-PCV) of cattle at the 3 diptanks

Cattle at Mvutshini and Ekuphindisweni diptanks had the highest HAP of 12.3%, 8.9%, respectively, which is significantly different ($p = 0.001$) from the HAP obtained from cattle at Ocilane (2.9%) (Table 4.1 and Figure 4.5). Both cattle herds at Mvutshini and Ekuphindisweni diptanks also had the highest HAA, 27.7, 33.4%, respectively while cattle at Ocilwane had the lowest, 11.1% (Table 4.2; $p = 0.001$). Conversely, cattle at Ocilwane diptank had the highest HA-PCV, ranging between 29-32%, average 30.5% while cattle at Mvutshini and Ekuphindisweni diptanks had the lowest HA-PCV (24-29%, average of 27.4 and 26.6, respectively) reaching 30% in only one observation (Figure 4.6; Table 4.2). The interaction between HAP and HAA is also significant ($p = 0.021$). This was also demonstrated by comparing the health condition of cattle during the rainy season at these diptanks. Figure 4.7 shows good body conditions of most cattle at Ocilwane compared to the poor conditions of cattle at Ekuphindisweni diptank; photographs were taken during the same week.

The overall effect of HAP on the animal health condition is clearly demonstrated in Figure 4.8. Comparing the anaemia in trypanosomes infected and uninfected cattle at the 3 diptanks showed that: 50, 63 and 100% of trypanosomes infected cattle were anaemic at Mvutshini, Ekuphindisweni and Ocilwane diptanks, respectively. It is noted that only 7 trypanosomes cases were reported in cattle at Ocilwane and all were found anaemic (100%). In comparison, the prevalence of anaemia in uninfected cattle in the 3 diptanks was 20, 30 and 10% at Mvutshini, Ekuphindisweni and Ocilwane diptanks, respectively. This is clearly related to high tsetse/trypanosomes challenge (Mvutshini, Ekuphindisweni) compared to low tsetse/trypanosomes challenge (Ocilwane diptank). By combining the

data from the 3 diptanks (total of 1,800 observations), the overall HAA in infected and uninfected cattle was 62 and 20%, respectively.

4.4 Trypanosomes survey: Herd average prevalence (HAP) and herd average anaemia (HAA) of cattle at the 7 diptanks

Table 4.3 shows the results of trypanosomes surveys conducted at 7 communal diptanks. Generally, all cattle at the diptanks were infected with trypanosomes with HAP of 10.3% and 35.3% of cattle were anaemic. It is noteworthy to mention that the standard deviation values were high for both parameters; 10.3 $\pm$ 8.5, 35.3 $\pm$ 19.0 for HAP and HAA, respectively, due to the different levels of trypanosomes challenge at each location. One exception is that trypanosome infections were not detected at Qakwini from one sampling in 2005. However, the HAP recorded in 2008 from the same diptank confirmed the presence of infected cattle with HAP of 16%. The highest HAP (range 15-31%, n = 4) was recorded in Gwenyambili diptank. This high infection in the cattle herds produced high records of HAA (range 40-60%; average 50%). It is worth mentioning that this diptank is located inside a dense indigenous forest (Figure 4.9) suitable as tsetse habitat next to Simangaliso Wetland Park. One cow with a PCV of 12% and in very poor body condition was presented for treatment (Figure 4.10). In contrast, the lowest trypanosomes infections, HAP of 4%, were recorded in cattle at Nhlwathi diptank. This is also reflected in the health of cattle at this diptank with only 12-18% of animals were anaemic. It is worth mentioning that this diptank is situated far from the game reserve. Again, the rainy seasons did not result in improvement of health conditions of trypanosomes infected cattle. This is demonstrated from results obtained from cattle at Gwenyambili diptank where the HAP was 22% and the HAA was 55% (28 February 2007, Table 4.3). Likewise, the HAP and HAA were 9 and 50% in cattle at Nibela diptank (27 January 2007; Figure 4.11).

4.5 The infection rate with trypanosomes in tsetse flies collected from the field

Table 4.4 shows the summary of results of fly dissection performed on a total of 458 *G. brevipalpis*. The infection with the immature infections in the midgut was 3.5% (16/458) while only one fly was found with mature infection in the proboscis (1/458, 0.22%). Very few *G. austeni* (total of 9) were collected during the same period and dissected. The infection rate with trypanosomes immature and mature infections was found to be very high; 5/9 (55.5%).

4.6 Prevalence of trypanosomes parasites in buffalo population at Hluhluwe-uMfolozi Game Park

Blood samples were collected from a total of 132 buffaloes randomly immobilized for tuberculosis testing by the Park Authority. Two buffaloes were found to have *T. congolense* infection by the buffy coat technique.

4.7 Strategic treatment of adult cattle and weaned calves at Boomerang farm using trypanocidal drugs

Table 4.5 and figure 4.12 show the results of HAP, HAA and strategic treatment with ethidium bromide and novidium chloride. The primary survey conducted in June 2006 on the farm revealed very high trypanosomes HAP (51%) and HAA (25%). Subsequently, all cattle in the herd received treatment with ethidium bromide. Thereafter, the regular monthly sampling for the next 5 months showed a decline in HAP of 2-4%. However, the HAP of 16% i.e. 5 times the previous month was recorded. This high level of infection was maintained for the following two months with a similar rise in the HAA (from zero to 20%) before the herd was treated using novidium chloride.

In December 2006, at a time when the HAP of the adult cows was very low (3%), the weaned calves (2005 group) experienced very high HAP of 72 while 37% of them were anaemic. This demonstrated the high trypanosomes challenge during this period. All of the 2005 calves received treatment with novidium chloride. The calves continued to be negative for trypanosomes infections for the following 3 months. This was followed by a second relapse or re-infection and a high HAP (17 and 26%) was recorded for 2 consecutive months. The herd was treated with novidium chloride. Two months later the HAP was found to be 3 and very few cases of anaemia were recorded (6%).

A second group of weaned calves of 2006 was also examined in May 2007. The HAP was found to be 26 and 20% was recorded in the following month. The calves were treated using Novdium chloride and remained uninfected for 2 months when the observations on the farm cattle were terminated. It is worth mentioning that the 2006 calf group, whether infected or uninfected, had no signs of anaemia.

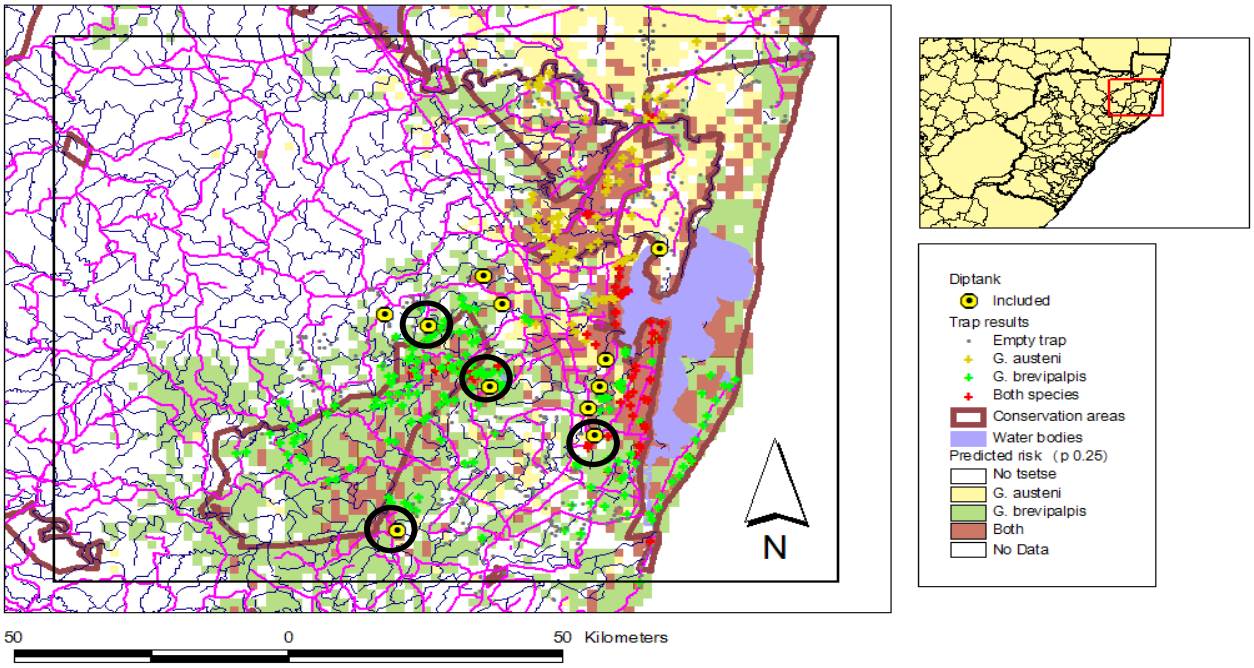
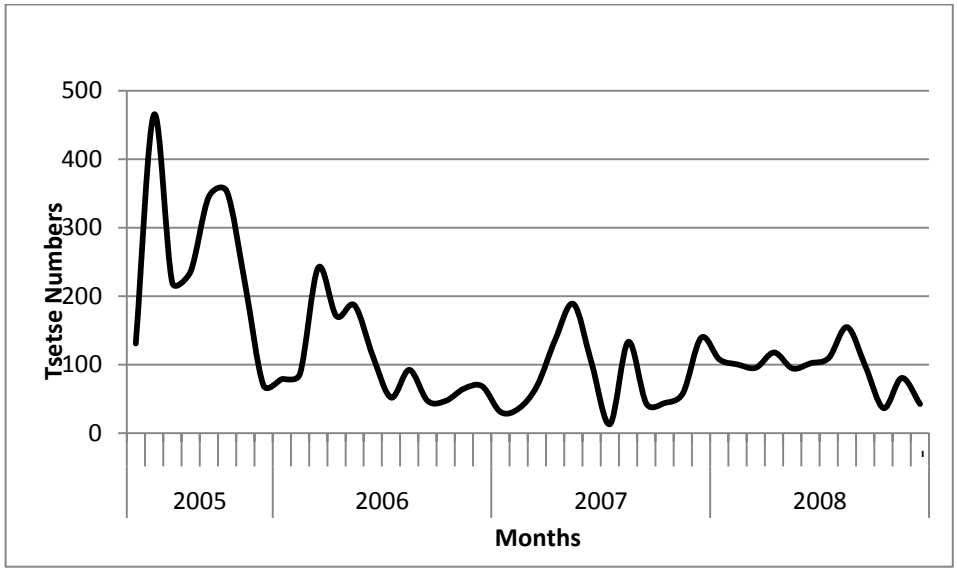


Figure 4.1: Tsetse distribution in the study sites of both *G. austeni* and *G. brevipalpis*; circled sites around the edges of Hluhluwe-uMfolozi Game Park are Mvutshini (eastern side of the Park), Ekuphindisweni (western side of the Park) and Ocilwane (southern side of the Park) and the further away is Boomerang Farm. (Prepared by Hendrickx, G. specifically for the this study, 2007)

A



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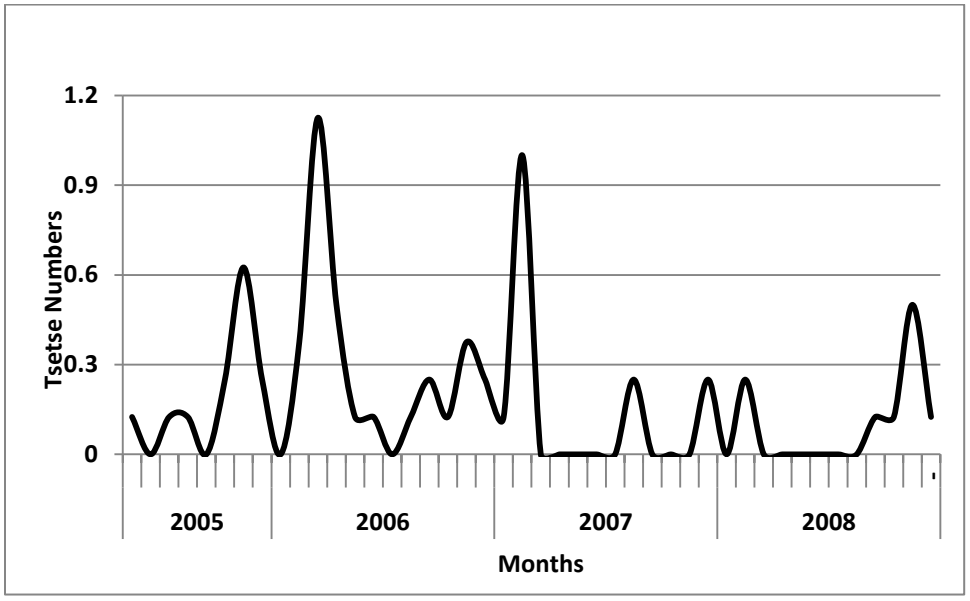

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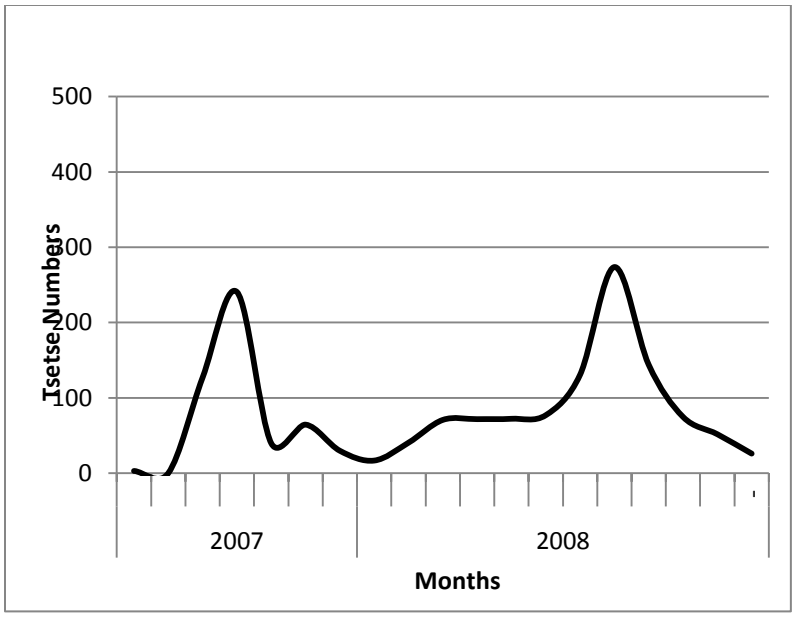
Fig 4.2: Tsetse population of A: *G. austeni* and B: *G. brevipalpis*; Mvutshini diptank. Tsetse numbers: the total of 4 traps per month.

A



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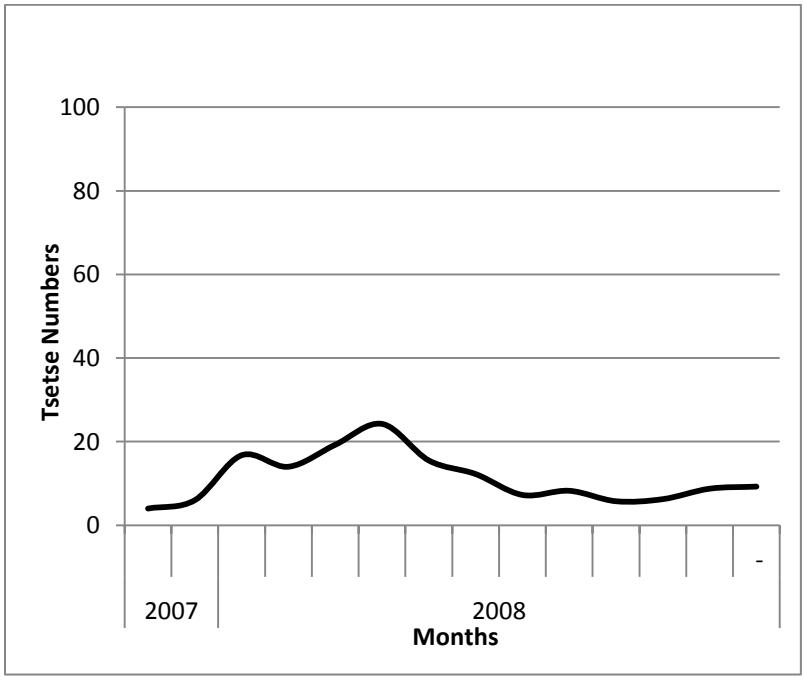


Fig 4.3: Tsetse populations of *G. brevipalpis* at Ekuphindiseni (A) and Ocilwane (B) diptanks. Tsetse numbers: the total of 4 traps per month.

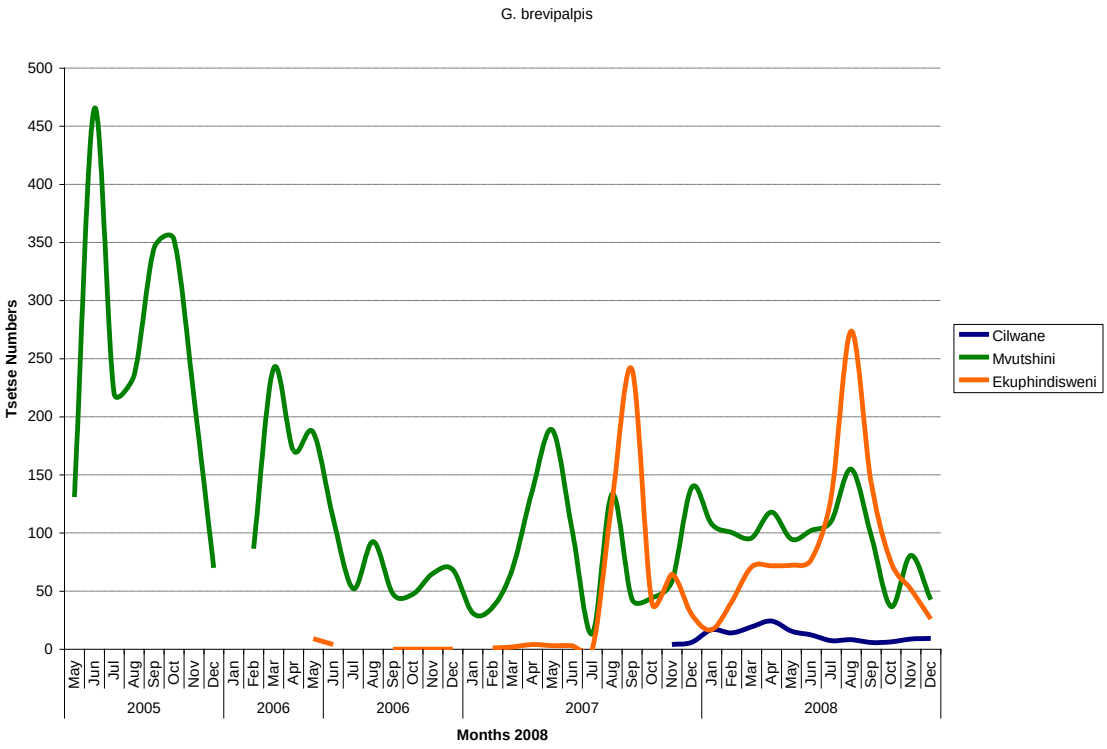
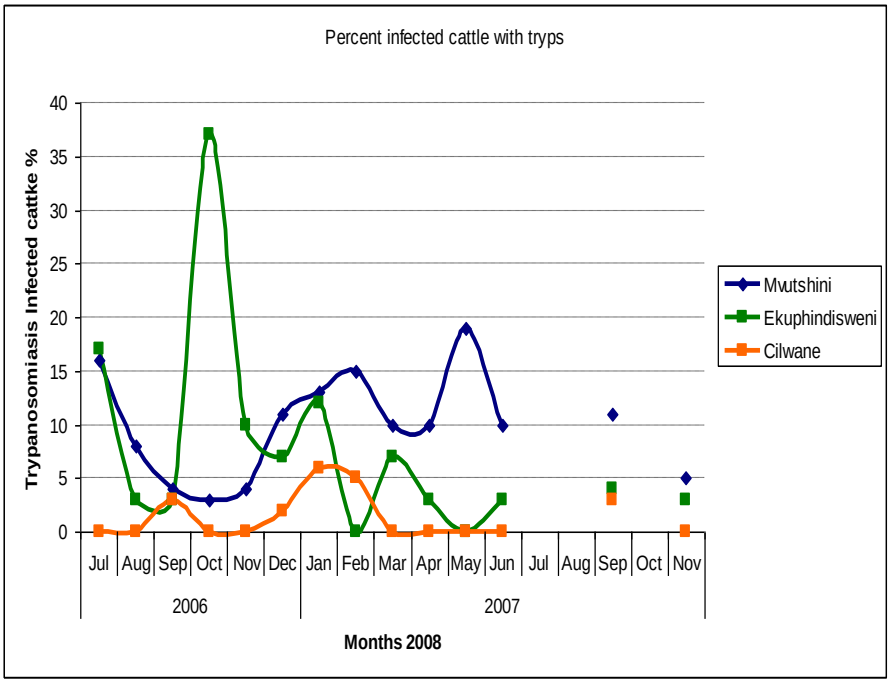


Figure 4.4: Comparison of *G. brevipalpis* populations in the 3 diptanks.

Table 4.1 Trypanosome infections in cattle kept in the 3 communal diptank areas; Herd average prevalence (HAP)

Date	**Mvutshini**	**Ekuphindisweni**	**Ocilwane**
26.01.2005	34 (76)	ND	ND
22.05.2006	18 (40)	18 (40)	8 (60)
11.07.2006	16 (38)	17 (46)	0 (25)
14.08.2006	8.3 (24)	3 (37)	0 (43)
18.09.2006	4.0 (26)	3 (34)	3.4 (29)
23.10.2006	3.2 (31)	37 (27)	0 (33)
22.11.2006	3.5 (28)	10 (42)	0 (16)
23.12.2006	11 (9)	7 (38)	19 (16)
22.01.2007	13 (15)	12 (26)	6 (18)
26.02.2007	15 (19)	0 (20)	5 (19)
26.03.2007	10 (21)	7 (30)	0 (19)
18.04.2007	10 (19)	3 (34)	0 (24)
21.05.2007	19 (37)	0 (24)	0 (10)
18.06.2007	10 (19)	ND	0 (21)
03.09.2007	11 (36)	4 (25)	3 (34)
20.11.2007	10 (21	3 (30)	0 (39)
Total examined	**459**	**453**	**406**
HAP±SD	**12.3±7.**	**8.9±9.9**	**2.9±5.1**

A



B

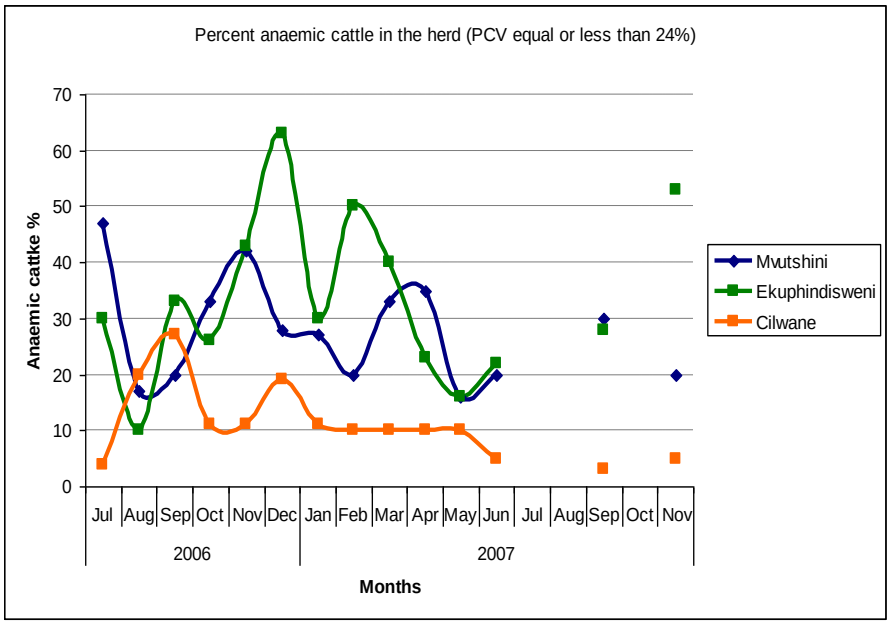


Fig 4.5: Comparing trypanosomes A: herd average prevalence (HAP) and B: herd average anaemia (HAA) in cattle in the three dip tanks.

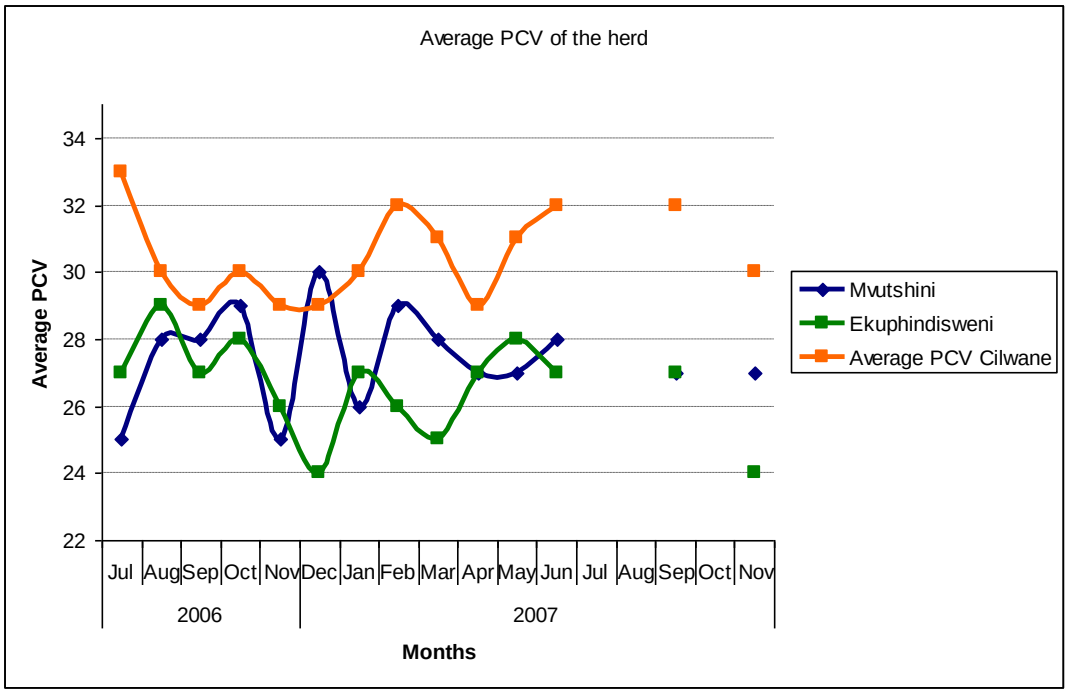
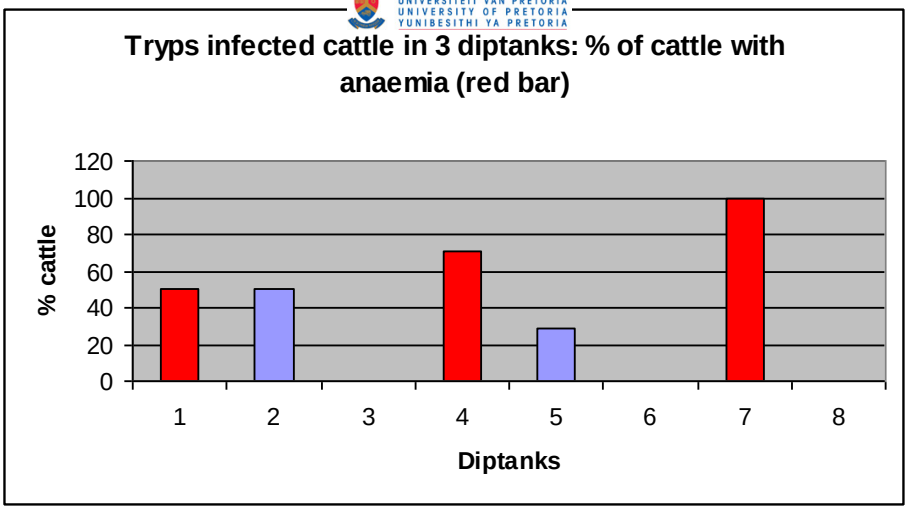


Figure 4.6: Herd average PCV (HA-PCV) of cattle at the three dip tanks.

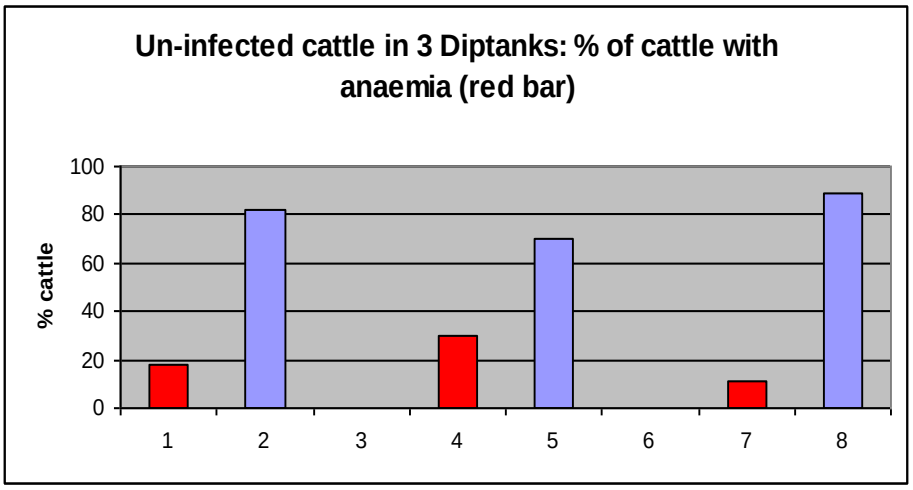
A

B

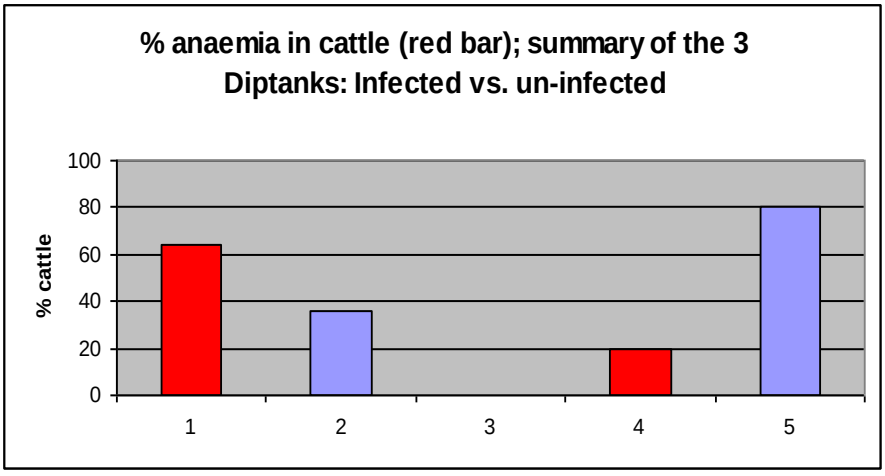
Figure 4.7: Cattle at Ocilwani diptank: **A** excellent body condition and **B** Nagana infected cattle at Ekuphindisweni diptank. Both photographs were taken in the same week during the rainy season of 2007.



Mvutshini (1 and 2); Ekuphindisweni (4 and 5); Ocilwane (7 and 8)



Mvutshini (1 and 2); Ekuphindisweni (4 and 5); Ocilwane (7 and 8)



Infected cattle (1 and 2); un-infected cattle (4 and 5)

Figure 4.8: Comparing herd average anaemia in infected versus un-infected cattle in the three surveillance diptanks. Observation over 15 consecutive months.

Table 4.2: Summary of the relationship between trypanosome herd average prevalence (HAP), herd average anaemia (HAA) and herd average PCV (HA-PCV) in cattle at the three communal diptank areas

Epidemiological factor	Mvutshini	Ekuphindisweni	Ocilwane
HAP	12.3[a]	8.9[a]	2.9[b]
HAA	27.7[a]	33.4[b]	11.1[c]
HA-PCV	27.4[a]	26.6[a]	30.5[b]

Figures followed by different letters are significantly different (analysis below)

Accumulated analysis

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ HAP	1	1303.28	1303.28	119.73	<.001
+ HAP.HAA	1	15949.86	15949.86	1465.31	<.001
+ HAP.HAA	1	58.42	58.42	5.37	0.021
Residual	1284	13976.34	10.89		
Total	1287	31287.91	24.31		

Table 4.3: Results of the trypanosome survey at the seven communal diptanks showing means ± SD (n = 710) of herd average prevalence (HAP) and herd average anaemia (HAA)

Dip tank	Date	HAP %	HAA %	No of cattle
Gwenyambili	08.10.2005	18	50	28
	14.07.2006	31	60	35
	24.10.2006	15	40	39
	28.02.2007	22	55	32
Mahlambanyathi	10.10.2005	2	20	43
	30.11.2005	6	30	50
	5.09.2006	7	15	40
	15.09.2006	15	40	45
	06.03.2007	3	20	30
Qakwini	11.10.2005	0	10	44
	15.08.2008	16	40	56
Nhlwathi	06.02.2006	7	12	50
	06.05.2006	2	15	43
	05.06.2006	3	18	40
Mzineni	20.09.2006	19	60	37
Nibela	25.11.2006	4	70	28
	27.01.2007	9	50	34
Bukhipha	23.11.2006	6	30	36
Mean ± SD		**10.3 ± 8.5**	**35.3 ± 19.0**	**39**

Figure 4.9 Gwenyambili diptank showing indigenous forest by a riverbed; suitable tsetse habitat

Figure 4.10: Trypanosome infected cow with a PCV of 12 presented for treatment; Gwenyambili diptank. Chronic disease is associated with progressive emaciation and eventually cachexia. Note classical signs: Condition "skin and bone", hair sparse, rough coat, dry scaly, lost hair at the tail switch, skin dull and staring and the animal is isolated from the herd (Connor, Van den Bossche, 2004). Furthermore, some individual animals in the herd, like the one just standing behind, are in good condition.

Figure 4.11: An infected cow and its calf showing poor health conditions.

Note the availability of green grass cover during the rainy season (Mvutshini diptank).

Table 4.4: Trypanosome infection rate in *G. brevipalpis*

Site of collection	Number flies dissected	Positive infection (%)	
		proboscis	midgut
Hluhluwe iMpholozi	209	0	3 (1.9)
Muvtshini	93	1	4 (4.3)
Charters Creek	156	0	9 (5.8)
Total	458	1 (0.22)	16 (3.5)

Only 9 *G. austeni* were collected from Charters Creek. Five out of 9 were infected in the midgut and proboscis

Table 4.5: Treatment strategy using Ethidium (Homidium bromide) and Novidium (Homidium chloride)

Date	Prevalence (n)	% cattle anaemia
	A. Breading Cows	
23.06.2006	51 (49)	25
	Herd Treatment Ethidium bromide (July) 2006	
18.08.2006	2 (46)	2
22.09.2006	4 (34)	0
27.10.2006	2 (50)	6
21.11.2006	0 (34)	6
13.12.2006	3 (36)	0
25.01.2007	16 (31)	13
28.02.2007	20 (35)	15
15.03.2007	20 (30)	20
	Herd Treatment (March 2007)	
19.04.2007	4 (45)	11
25.05.2007	0	
22.06.2007	0 (37)	0
04.09.2007	4 (24)	10
21.11.2007	21 (14)	
	B. Weaned Group (2005)	
12.12.2006	72 (39)	28
25.01.2007	76 (42)	37
	Herd Treated Novidium chloride (February 2007)	
28.02.2007	0 (38)	16
15.03.2007	0 (34)	12
19.04.2007	0 (39)	3
25.05.2007	17 (42)	10
21.06.2007	26 (42)	7
	Herd Treated Novidium chloride (18.07.2007)	
04.09.2007	3 (36)	6

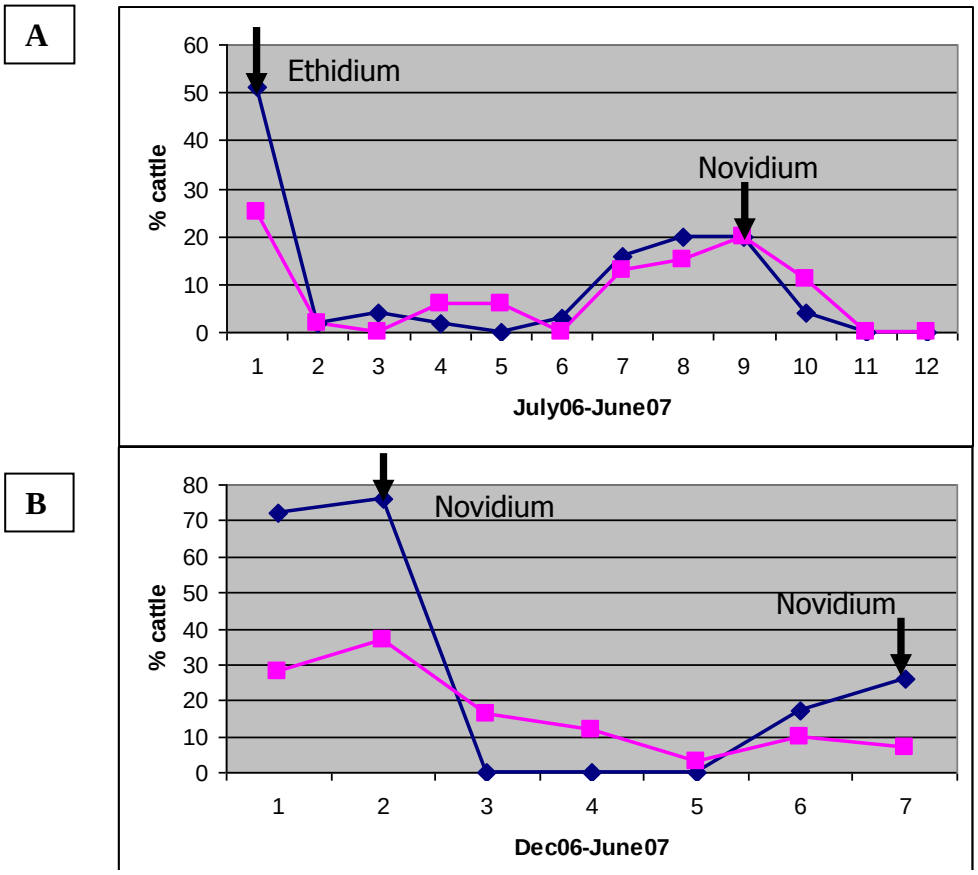


Figure 4.12: Responses to trypanocidal treatment (A: ethidium; B novidium) of adult cattle and weaned calves monitored over a period of 6 and 12 months at Boomerang Farm (Blue lines: herd average prevalence (HAP); red lines: herd average anaemia (HAA).

CHAPTER 5
DISCUSSION AND CONCLUSIONS

5.1 Nagana surveillance and surveys

In 1990, during a severe drought in the tsetse infested low lying areas of Zululand, about 10 000 cattle died of Nagana and 116, 000 were treated during this outbreak using Ethidium bromide (Kappmeier et al., 1998). Further control measures included the use of pyrethroid dip on a four year interval to reduce the challenge by the vector tsetse fly. Sixteen years following the 1990 outbreak, 76 cattle suspected to be infected with trypanosome were bled at one communal diptank by the edge of Hluhluwe-uMfolozi Park and the results were reported in a research communication by Van den Bossche et al. (2006). Thirty four percent of cattle were found to be infected with *T. congolense* and 83% were anaemic. This once off survey demonstrated that Nagana was still prevalent and recommended further research to develop appropriate control methods. There is a wealth of entomological information on the ecology of the two tsetse species since 1990 (Kappmeier, Nevill, 1999a,b; Kappmeier, 2000, 2003; Hendrickx et al., 2006). However, it is unfortunate that these entomological data has not been supported by parallel studies on the epidemiology of the disease, Nagana. Therefore, the present study presented the first intensive epidemiological investigations to address the problem of animal trypanosomosis in South Africa since 1990 outbreak.

The relationships between trypanosome herd average prevalence, herd average anaemia and herd average PCV was investigated in cattle in three communal diptanks for 15 months. The tsetse challenge with *G. brevipalpis* in two of the diptanks was high (Mvutshini and Ekuphindisweni) but is low in the third (Ocilwane). In addition, *G. brevipaplis* and *G. austeni* coexist in Mvutshini diptank. This high and low tsetse challenge also presented different disease scenarios. The HAP and the consequent HAA were significantly higher in the high fly challenge situation than in low challenge. The HA-PCV also correlated well with other two factors; Low HA-PCV in cattle in the high fly challenge

and significantly higher values in cattle in the low challenge. The three epidemiological parameters were previously shown to reflect the health conditions in cattle in relation to tsetse challenge (Van den Bossche, Rowlands, 2001). Though the percentage of HAA in infected cattle is worryingly high (62%), more un-infected animals were also anaemic, however, trypanosome parasites could not be detected by the conventional buffy coat examination. Other sensitive molecular tools when compared with conventional diagnostics revealed higher levels of trypanosome infections in field cattle (Geysen, Delespaux, Geerts, 2003).

Cattle herd at Ocilwane diptank are subjected to irregular low tsetse challenge along the most southern edges of the reserve. It is known that this the most southerly distribution limit of tsetse in NKZN (Hendrickx, 2002). Only few animals got infected (2.9%) but all of the infected ones became anaemic (100%). This is a unique situation where the interface with the irregular tsetse challenge and the presence of trypanosomes reservoir in game, as demonstrated here in the buffalo, produced an epidemic character. Such interfaces are found along the Kasungu National Park and Nkhotakota Game Reserve in Malawi, along the Malawi National Park and the Kwando River in the Eastern Caprivi of Namibia (Van den Bossche et al, 2000). In contrast to the other two diptanks with high tsetse challenge the disease situation becomes endemic; most of cattle were infected and anaemic but some did not show anaemia.

Anaemia due to trypanosome infections (Murray, Morrison, Murray, Clifford, Trail, 1979; Murray, Dexter, 1988; Suliman, Feldman, 1989; Nok, Balogun, (2003) is the result of the parasite damage red blood cells (RBC) by releasing biochemical molecules. The subsequent removal of the damaged RBCs from the circulation by phagocytosis, particularly following the initial infection and increased parasitaemia produce a fall in PCV. The chronic phase of the disease is of economic importance as animals lose weight, condition and as a result of the extended process of dyshaemopoiesis, remain anaemic (Connor, Van den Bossche, 2004). Despite the apparent disappearance of the parasites from circulation or becoming undetectable by conventional diagnostic tools, RBCs destruction

continues resulting in persistent anaemia. The PCV of individual animals and the HA-PCV are useful indicators of anaemia and in trypanosome endemic areas are the most typical signs of Nagana in domestic animals (Murray, Dexter, 1988; Trail et al., 1991, Marcotty et al., 2008). HAA supplements parasitological diagnosis. Parasitologically negative animals in endemic tsetse areas that have low PCV are regarded as having trypanosomes infections. In the present study only 62% of the anaemic animals were found to be infected using direct parasitological methods and therefore, all of the anaemic cattle should be considered infected.

The survey conducted in the seven diptanks under tsetse challenge highlighted the magnitude of Nagana as a major risk to animal health. The HAP was very high, up to 31%, and the consequent HAA was also high (up to 70%). Anaemia in cattle is clearly related to trypanosome infections as cattle continued to be anaemic during the rainy season. This is also demonstrated by comparing the health conditions in cattle under high and low tsetse challenge (Mvutshini and Ekuphindisweni vs. Ocilwane).

5.2 Trypanosome infection rate in tsetse fly

Several epidemiological factors identified in trypanosomosis and their interactions with the vector tsetse fly, livestock, presence of game and the climate. The infection rate is of prime importance (Connor, Van den Bossche, 2004). The degree of risk depends on “challenge” which is related to the number of infective bites an animal receives, host preference and host susceptibility and parasite virulence (Connor, Van den Bossche, 2004). These factors are of importance in determining the “impact of tsetse control” in a country. Motloang, Masumu, Mans and Latif (2011) investigated the vector competence of *G. brevipalpis* and *G. austeni* collected from the same communal diptaks and Hluhluwe-uMfolozi Park. They found the infection with mature parasites in *G. austeni* to be 8% while no mature infections were found in *G. brevipalpis*. Moreover, *G. austeni* and *G. brevipalpis* collected from the same sites were applied to feed onto susceptible cattle. *G. austeni* transmitted *T. congolense* and no transmission occurred with *G. brevipalpis*. They concluded that *G.*

austeni is the major vector in the transmission of trypanosomes in the area. These findings support the results from the present study in two ways; firstly, only *G. austeni* had trypanosome infective stages but not *G. brevipalpis* (only 1 out of 458 was positive) and, secondly only *T. congolese* was transmitted confirming the species to be the major parasite infecting cattle in the area. It was previously believed that *G. brevipalpis* was the potential vector due to its high abundance in areas where high HAP was recorded (Van den Bossche et al., 2006). The present study and Motloang et al. (2011) work both do not agree with results obtained using molecular tools to assess the infection rate in tsetse in KZN. Mamabolo et al. (2009) found 89% of flies from KZN (species not indicated) to have trypanosomes DNA but the inoculation of proboscis homogenates into 20 mice did not produce any patent parasitaemia. Though assessment by PCR produces higher infection rate in tsetse flies, a drawback is that it does not differentiate between recent infections, developing immature and mature infections as the processing involves disintegrating the whole fly body to extract DNA (Mamabolo et al., 2009; Mekata, Konnai, Simunza, Chembensofu, Kano, Witola, Tembo, Chitambo, Inoue, Onuma, Ohashi, 2008). Furthermore, it has also been shown that there are barriers in the tsetse fly to both initial parasites establishment and maturation of infection after blood meal. As such, the vast majority of trypanosomes fail to successfully develop to maturity and most failures occur in the midgut or during migration (Roditi, Lehane, 2008).

Other major effects of prevalence of infection in *G. pallidipes* are the month of sampling and age of the fly. an (Rowlands, Woudyalew, Authie, d'Ieteren, Leak, Nagda, Peregrine, 1993; Msangi, Whitaker, Lehane, 1998). Similar results were reported earlier for *G. brevipalpis* (Harley, 1966). The are some deficiencies in the present data on infection rates with trypanosomes in wild caught tsetse flies considering all factors determining the infectivity in flies. The flies were not sexed (Waiswa, Picozzi, Katunguka-Rwakishaya, Olaho, Musoke, Welburn, 2006) or aged, as mentioned above, and both factors had major effects on infection rate. In their review of tsetse-trypanosome interactions, Welburn and Maudlin (1999) discussed the hazards faced by both parasite and fly that affect vector competence of tsetse species.

The prevalence of *T. congolense* in cattle on Mafia Island, Tanzania, was surprisingly very low (0.8%) in spite of the wide spread distribution of *G. brevipalpis* and being the only species present (Goossens, Mbwambo, Msangi, Geysen, Vreysen, 2006). One of the three reasons advocated for the low prevalence of pathogenic trypanosomes is the low vectorial capacity of *G. brevipalpis*, which is in support of the very low infection rate reported in the present study. Other reasons were the possible low feeding frequency of the fly on cattle on the Island and usage of prophylactic treatment with trypanocidal drugs (Goossens et al., 2006).

5.3 *G. austeni* low population density but high vectorial capacity

Low fly population density can cause a serious disease problem (Jenni, Molyneux, Livesey, Galun, 1980; Molyneux, Jefferies, 1986; Roberts, 1981). This observation supports the HAP in cattle at all diptanks surveyed with low or no apparent *G. austeni* presence. The apparent abundance of *G. brevipalpis* was substantially higher than that of *G. austeni* in three main vegetation types in Zululand (Esterhuizen et al., 2005). It was not possible to attribute these findings to the real density of the two species or to sampling bias. Though the H-trap was developed to target the two species and performed better than any other tested traps for *G. austeni* (Kappmeier, 2000).

5.4 Trypanocides

The intention of the study was to treat adult cows and calves at an arbitrary HAP threshold of 20% before the disease produces any clinical symptoms or production losses. Ethidium and Novidium strategic use produced attractive results whereby cattle were protected for extended period of up to six months. Therefore, two to three treatments per year may be sufficient to keep cattle productivity on the farm. It is noted that the trypanosomes HAP and the consequent HAA reached very high level in 2005 born calves (76% and 37%, respectively). If this group had not been treated the weaned calves could have experienced

a state of "stunted growth" and become unfit for sale. On the other hand, calves, with reference to the group born in 2006, seemed to resist HAP of up to 20% without showing any signs of anaemia (none of the calves was found anaemic). This observation proved the "arbitrary threshold for treatment" adopted in this study. Age-related resistance to trypanosomes is recognized (Maclennan, 1974; Murray et al., 1982; Wellde, Hockmeyer, Kovatch, Bhogal, Diggs, 1981) as well as young animal are less attractive to tsetse flies. Ethidium and Novidium were reported to give protection for a period of up to 4 months (Brander, Pugh, 1979). There were some successes reported of farming in tsetse and trypanosomes challenge areas (Holmes, Scott, 1982; Logan, Goodwin, Tembely, Craig, 1984; Moloo et al., 1988; Trail et al., 1985) and in Zimbabwe and Mozambique (Boyt, 1979; Takken, Taylor-Lewis, Woodford, 1988). The strategic use of trypanocides requires close monitoring of the HAP while treatment of the herd becomes expensive and not affordable to many communal cattle owners. Moreover, treatment does not cure chronic infections as in many situations the condition becomes irreversible (Connor, Van den Bossche, 2004). The strategy also requires veterinary supervision, surveillance and strict administration of the drugs (Holmes, Scott, 1982) to avoid under/overdosing of a drug which may shorten the time period for the trypanosomes to build resistance against the drug. The problem of development of resistance in trypanosomes is the threat to the sustainability of the strategy. Recently, an investigation into drug-resistant strains in NKZN was carried out and the results did not reveal the presence of any resistant strains (Masumu, unpublished data, 2010). In absence of tsetse eradication policy, integrated approaches can be applied for the control of trypanosomosis (Murray, Black, 1985; Holmes, 1997).

5.5 Sylvatic cycle

The presence of trypanosomes infected buffaloes in this study confirms the occurrence of sylvatic cycle. Wildlife, in this case buffaloes, is reservoir of trypanosomes and shows no clinical signs of the disease (Mulia, Rickman, 1988). The sylvatic cycles of transmission presents a high risk of serious disease to introduced cattle. Fly dispersal for up to 5 km is the major factor in the epidemiology of Nagana at the game/livestock/tsetse interface. It is

not necessarily that cattle come in contact with buffalo for initiation of infection. Once more, the tsetse flies once infected become infected for the rest of its life and as such become another reservoir host (Connor, Van den Bossche, 2004).

5.6 Tsetse and trypanosomosis: A hanging veterinary and socioeconomic problem in South Africa

In NKZN, tsetse habitat of 16,000 sq km is conserved in nature reserves, national and private game parks and resorts. Man-made habitat such as exotic timber forests is extended to form extensions to indigenous forests. Patches of evergreen indigenous forests are found around river-beds. The habitat has been extended when the majority of cattle farming areas have moved into game farming and resorts, thus, allowing bushes, trees and vegetation to grow, thus, creating more suitable tsetse breeding sites. Estheruizen et al. (2005) reported more extension of *Glossina brevipalpis* than was previously known. Currently, two species of tsetse species coexist, one proven more efficient vector. *Glossina brevipalpis* and *G. austeni* have different behavioural activities. Dispersal and re-invasion of the flies is continuous between different habitats (Estheruizen et al., 2005). The present study has shown that buffaloes at Hluhluwe-uMfolozi Game Park are carrier of *T. congolense* and as such, are risk as reservoir hosts. Other animals have not been examined but it is well known that game/livestock interface presents risk (Connor, Van den Bossche, 2004). The limited grazing, small areas, and degraded communal land force cattle to move to such tsetse habitat and exposing them to Nagana challenge. The present study showed that Nagana is prevalent at all the 10 diptanks surveyed and affecting mainly resource poor farmers. In South Africa, there is no control policy for tsetse and Nagana. The only response is to “manage the crises” when the problem is out-of-hand. This policy is proved to be un-sustainable over the years and demonstrated as in this study, by the high level of trypanosome infections in cattle. What is required is a politically supported decision to eradicate tsetse flies to increase cattle productivity. A strategy for an area-wide control to establish tsetse-free zone in South Africa has been advocated (Kappmeier Green et al., 2007).

5.7 Outcome of the study

The present study generated a wealth of information of the epidemiological factors related to the wide spread trypanosomes infected cattle and reservoir game animals. It is also shown that Nagana is a disease of economic importance impacting on the livelihood of resource-poor farmers. The knowledge accumulated forms the bases to assist in better control strategies in the future.

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