THE DEVELOPMENT OF A NEW STRATEGY FOR THE SUSTAINABLE CONTROL OF BOVINE TRYPANOSOMOSIS IN SOUTHERN AFRICA

BY

PETER VAN DEN BOSSCHE

A thesis submitted to the University of Pretoria in the Faculty of Veterinary Science in fulfilment of the requirements for the degree

PHILOSOPHIAE DOCTOR

PRETORIA 2000

I declare that the thesis, which I submit hereby for the degree Philosophiae Doctor at the University of Pretoria is my own work and has not been submitted previously for a degree at any other university.

.....

Peter Van den Bossche

16 May 2000

I certify that the deponent acknowledged that he knew and understood the contents of the above declaration, did not have any objection to taking the prescribed oath and considered it to be binding on his conscience. I thereafter administered the oath by causing him to utter the following words: "I swear that the contents of this declaration are true, so help me God" — and thereafter the deponent in my presence signed the declaration at PRETORIA on which date and which place I also signed this certificate:

.....

16/5/2000

PROF H M TERBLANCHÉ
DEPUTY DEAN
FACULTY OF VETERINARY SCIENCE
PRIVATE BAG X 04
ONDERSTEPOORT 0110

ACKNOWLEDGEMENTS

Many people have assisted me during the nearly ten years it took to conduct the work presented in this thesis. The results are part of work carried out during my official duties while working in Zambia and Zimbabwe. The work was partly funded by the Belgian Development Co-operation and the European Development Fund of the European Commission.

First, I would like to express my sincere gratitude to Prof. J. Mortelmans who introduced me to the field of tsetse and trypanosomosis. His enthusiasm and sincerity have inspired me throughout my stay in Africa.

In Zambia, I would like to thank the staff of the Department of Animal Health and Production for their expert field assistance. Special thanks go to Mr. L. Mukanta and Mr. L. Chanda for their assistance in the day-to-day running of the Chipopela Research Station. My colleagues at the Belgian Animal Disease Control Project (BADCP, Chipata) are acknowledged for the administrative and logistic support. I am also indebted to Drs. R. De Deken, J. Brandt and Prof. S. Geerts of the Institute of Tropical Medicine (Antwerp, Belgium) for their friendship, continuous support and useful suggestions while working in Zambia. Dr. C. Staak of the Federal Institute for Consumers Health Protection and Veterinary Medicine (Berlin, Germany) is acknowledged for the analysis of bloodmeals for host determination.

Thanks are due to my colleagues at the Regional Tsetse and Trypanosomiasis Control Programme (RTTCP, Harare). I am grateful for the valued friendship and guidance given to me by Drs. R. J. Connor, G. A. Vale and Mr. M. Doran. They all have provided comments and suggestions on various sections presented in this thesis. Special thanks go to Dr. W. Shumba and staff of the Zambian Veterinary Research Laboratory for the assistance with the analysis of the thousands of ELISA samples. Special thanks go to my co-workers at the Departments of Veterinary Services of Malawi, Namibia, Zambia and Zimbabwe. They are thanked for their co-operation and permission to use the field data.

Drs. L. Munsimbwe, D. Chigoma and C. Njagu are acknowledged for their assistance with the implementation of various field trials in Zambia and Zimbabwe.

I thank Drs. M. Billiouw, G. J. Rowlands and L. Duchateau for help with some of the statistical analyses. Furthermore, I thank Drs. E. Authié, J. Hargrove, J. A. Lawrence, F.T. Potgieter, M. Warnes, A. Wilson and Mr. D. F. Lovemore for their constructive comments on various aspects of the work.

Deep gratitude is owed to Prof. J. A. W. Coetzer and to my promoters Profs. R. J. Connor, R. J. Phelps and B. L. Penzhorn for their interest, guidance, criticism and encouragement throughout the writing up of the thesis.

Finally, I thank my wife Thérèse and children Julie, Christophe and Vincent, for allowing me to spend so many evenings, weekends and holidays undisturbed at my desk. This is for them.

SUMMARY

THE DEVELOPMENT OF A NEW STRATEGY FOR THE SUSTAINABLE CONTROL OF BOVINE TRYPANOSOMOSIS IN SOUTHERN AFRICA

BY

PETER VAN DEN BOSSCHE

Promoter : Prof. B. L. Penzhorn
Department of Veterinary Tropical Diseases
University of Pretoria
South Africa

Associate Promoters : Prof. R. J. Connor
OAU/IBAR
Nairobi
Kenya

: Prof. R. J. Phelps
9 Drayton Avenue
Harare
Zimbabwe

Previously, strategy formulation for large-scale eradication of tsetse in southern Africa was dominated by straightforward technical considerations. The current shift to localised control of tsetse-transmitted bovine trypanosomosis has changed the emphasis from the vector to the disease. Nagana remains the main reason for intervening but control methods will differ according to the local situation and interventions will be restricted to those areas where the disease is present. As a result,

the technical criteria to be considered will differ substantially from those considered in the planning for large-scale eradication. First, a clear picture of the extent and magnitude of the bovine trypanosomosis problem is required. Second, the selection of the most efficient intervention methods will vary according to the local epidemiological situation. Hence, the different epidemiological situations need to be identified and the effectiveness of available control methods needs to be evaluated in each of these situations. Finally, the long-term sustainability of an intervention will depend, to a large extent, upon the socio-economic impact of the disease and perceived benefits accruing from its control.

Tsetse-transmitted bovine trypanosomosis occurs in large areas of Malawi, Zambia, Zimbabwe and Namibia. The epidemiology of the disease differs substantially between areas. On the plateau of eastern Zambia, for example, cattle are kept in a tsetse-infested area. Because of the encroachment of people and cattle into the tsetse-infested area and the concomitant reduction in the number of game animals, tsetse have become highly dependent on cattle as their source of food. As a result, the distribution and density of tsetse is determined largely by the distribution and changes in the distribution or grazing pattern of cattle. *Trypanosoma congolense* is the main trypanosome species in tsetse and cattle. The prevalence of *congolense*-type trypanosomal infections in tsetse undergoes little variations between months and is affected mainly by the average age of the tsetse population. The incidence of bovine trypanosomosis is significantly correlated with the density of the tsetse population.

Bovine trypanosomosis is also prevalent in areas where cattle are kept adjacent to a tsetse-infested zone or where tsetse occasionally invade a tsetse-free area. In Malawi, for example, the main foci of bovine trypanosomosis are located adjacent to tsetse-infested national parks, game reserves or forest reserves. Bovine trypanosomosis also occurs far outside the known tsetse foci because of the seasonal movement of tsetse along rivers or because of, often small, undetected tsetse foci. Such foci have been detected in Malawi and in Zimbabwe. In most of the areas, bovine trypanosomosis is caused by *T. congolense*. However, the prevalence of *T. vivax* infections is high in areas where tsetse take a large proportion of feeds on game animals. This is the case in the Mamili area of the Eastern Caprivi. At the tsetse/cattle interface, the incidence of

bovine trypanosomosis is determined by the level of interaction between tsetse and cattle and is not necessarily correlated with the density of the tsetse population in the tsetse-infested area.

Determining the prevalence of bovine trypanosomosis accurately is fraught with difficulties. The parasitological diagnostic methods, that are commonly used, have low diagnostic sensitivity. Hence, a substantial proportion of the parasitologically positive animals will not be detected. This will result in an underestimate of the prevalence of infection. Therefore, the distribution of bovine trypanosomosis is determined best by combining parasitological diagnostic methods with methods that have higher sensitivity. The anti-trypanosmal antibody detection ELISA is a diagnostic test with high sensitivity and specificity. Furthermore, non-specific cross reactions with antibodies against common tick-borne parasites, such as *Anaplasma marginale* and *Babesia* spp., do not occur. Anti-trypanosomal antibodies are an indirect indication of a trypanosomal infection and persist up to 13 months after a trypanosomal infection has been treated successfully or has self-cured. Consequently, by using data on the prevalence of anti-trypanosomal antibodies, areas where trypanosomosis challenge is low, irregular or where trypanocidal drugs are used frequently can be identified.

An important determinant in the selection of priority areas for the control of bovine trypanosomosis is the effect of the disease on agricultural development. A usual consequence of a trypanosomal infection in susceptible cattle breeds is the development of anaemia. The level of anaemia is a good representation of the severity of the disease or the disease status of an infected animal. It is strongly correlated with the infected animal's performance. At the herd level, the herd average PCV decreases with increasing prevalence of trypanosomal infections. The shape of the relationship between herd average PCV and prevalence of trypanosomal infections, expressed by the slope of the regression line between average PCV and prevalence, is a useful indicator of (i) the impact of various levels of disease prevalence on herd health and (ii) the likely impact of control interventions on herd health. By establishing the slopes of the regression lines spatial and temporal comparisons can be made of the impact of trypanosomosis. On the plateau of eastern Zambia, for example, the impact of trypanosomal infections on herd average PCV is lowest in areas where challenge is

continuous and where tsetse feed mainly on cattle. Such conditions are conducive to the development of non-sterile immunity. In areas where challenge is irregular or where tsetse take a large proportion of their meals on game animals, the herd average PCV decreases significantly faster with increasing prevalence of trypanosomal infections. Season also plays an important role in determining the impact of trypanosomal infections on herd average PCV. During the dry season, when nutritional stress is highest, the decline in herd average PCV with increasing prevalence of trypanosomal infections is faster compared to the rainy season. The level and the effectiveness of trypanocidal drug treatments affect the direct socio-economic impacts of bovine trypanosomosis on animal production. In southern Africa, where trypanocidal drugs are readily available and where trypanocidal drug resistance is not widespread, mortality due to trypanosomosis is low and bovine trypanosomosis mainly reduces calving rates. The prevalence of the disease and the level of disease tolerance affect the reduction in calving rate due to the presence of the disease. The socio-economic impact of bovine trypanosomosis is generally highest in areas where cattle are kept adjacent to tsetse-infested zones such as the Vwaza area in the Northern Region of Malawi. All other, mainly indirect, impacts of bovine trypanosomosis are affected by non-trypanosomosis related factors such as the cattle owners' disease management practices, the potential for herd and arable land expansion and cash requirements. All these variables and their linkages have to be considered when planning for the localised control of bovine trypanosomosis. Failure to do so may result in an overestimate of the benefits accruing from control and is likely to affect the sustainability of an intervention.

Trypanocidal drugs are used widely in the southern African region. An analysis of drug-use practices has indicated that the majority of cattle owners prefer curative over chemoprophylactic drugs. Furthermore, cattle owners prefer to treat productive animals in the herd (oxen and cows) and appear to apply a production-oriented treatment strategy. This treatment strategy reduces the trypanosomosis-related mortality but has little effect on the calving rate. The sustainability of a drug-based trypanosomosis control strategy depends to a large extent on the drug-use practices. Survey results indicate that, even though farmers administer most trypanocides themselves, there is no evidence of frequent under-dosing and other factors enhancing the development of trypanocidal drug resistance were not present.

Odour-baited targets have proven to be an effective tsetse control method in large areas of homogenous vegetation. In relatively small, cultivated, areas also the presence of odour-baited, insecticide-treated targets at a density of approximately 4/km^2 results in a rapid decline in the tsetse population density and a reduction in the incidence of bovine trypanosomosis. Seasonal changes in the distribution of tsetse can be exploited by the deployment of targets in selected vegetation types. Targets are also effective barriers against the re-invasion of tsetse into cleared areas. Insecticide-treated cattle are a very effective means to control nagana in areas where tsetse take a large proportion of their blood meals from cattle. However, insecticide-treated cattle will only be effective when they sufficiently reduce the density of tsetse to reduce the incidence of bovine trypanosomosis. Insecticide-treated cattle do not constitute effective barriers against the re-invasion by tsetse. Even in the absence of an effect on the incidence of bovine trypanosomosis, insecticide treatments result in an immediate improvement of animal condition. This is best represented by the increase in the herd average PCV and is attributed to the effect of the insecticide-treatments on tick burden. Whereas the acaricidal effect of the insecticides to control tsetse may be beneficial in preventing tick-borne disease outbreaks, it may affect the development of enzootic stability. This is the case in eastern Zimbabwe where deltamethrin treatments at short intervals have resulted in a decline in the density of *Boophilus* spp. with a concomitant reduction in tick and *Babesia*-challenge and the development of an enzootic unstable situation.

Results of this thesis have shown that planning for the sustainable localised control of bovine trypanosomosis is a multidisciplinary exercise that requires a good understanding of the distribution and epidemiology of the disease. The choice of a particular control method will depend largely on the local epidemiological situation. By distinguishing the different epidmiological situation in southern Africa and by analysing their characteristics, appropriate methods to control bovine trypanosomosis have been identified.

OPSOMMING

ONTWIKKELING VAN 'N NUWE STRATEGIE VIR DIE VOLHOUBARE BEHEER VAN TRIPANOSOMOSE VAN BEESTE IN SUIDER-AFRIKA

DEUR

PETER VAN DEN BOSSCHE

Promotor: Prof. B.L. Penzhorn
Departement Veterinêre Tropiese Siektes
Fakulteit Veeartsenykunde
Universiteit van Pretoria
Suid-Afrika

Mede-promotors: Prof. R.J. Connor
OAU/IBAR
Nairobi
Kenya

Prof R.J. Phelps
Draytonweg 9
Harare
Zimbabwe

Formulering van strategieë vir die grootskaalse uitroeiing van tsetsevlieë in Suider-Afrika is vroeër deur eenvoudige tegniese oorwegings oorheers. Met die huidige oorgang na gelokaliseerde beheer van tsetse-oorgedraagde tripanosomose van beeste verskuif die klem van die vektor na die siekte. Nagana is steeds die hoofrede vir ingryping, maar

beheermetodes verskil na gelang van die plaaslike situasie en beheer word slegs toegepas waar die siekte voorkom. Die tegniese kriteria wat oorweeg word verskil dus aansienlik van dié wanneer grootskaalse uitroeiing beplan word. Eerstens moet die omvang van die probleem vasgestel word. Tweedens hang die keuse van die mees doeltreffende beheermetode van die plaaslike epidemiologiese toestande af. Die onderskeie epidemiologiese toestande en die effektiwiteit van beheermetodes moet dus bepaal word. Laastens hang die langtermyn volhoubaarheid van ingryping grootliks af van die sosio-ekonomiese uitwerking van die siekte en die gewaande voordele van beheer.

Tsetse-oorgedraagde tripanosomose van beeste kom in groot gedeeltes van Malawi, Zambië, Zimbabwe en Namibië voor. Die epidemiologie van die siekte verskil aansienlik van plek tot plek. Op die plato van Oos-Zambië word beeste bv. in 'n tsetsebesmette gebied aangehou. Deurdat mense en hul beeste tsetsebesmette gebiede binnedring, daal die wildgetalle en word tsetse afhanklik van beeste as voedingsbron. Die verspreiding en bevolkingsdigtheid van tsetse word dus grootliks deur die verspreiding van beeste en veranderings in hul weipatroon bepaal. Die voorkoms van *Trypanosoma congolense*, die belangrikste spesie van beide tsetsevlieë en beeste, ondergaan min verandering van maand tot maand en word veral deur die ouderdom van die tsetsebevolking beïnvloed. Die voorkoms van nuwe tripanosomosegevalle by beeste is betekenisvol gekorreleer met die digtheid van die tsetsebevolking.

Tripanosomose van beeste kom ook algemeen voor waar beeste langs 'n tsetsebesmette gebied aangehou word of waar tsetsevlieë soms 'n tsetsevrye gebied binnedring. In Malawi grens die hoof besmettingshaarde van tripanosomose aan tsetsebesmette nasionale parke, wildtuine of bosreservate. Weens die seisoenale beweging van tsetse langs riviere of die voorkoms van klein kolle tsetse wat oor die hoof gesien word, kom tripanosomose van beeste ook ver buite bekende tsetsekolle voor. Voorheen onbekende tsetsekolle is later in Malawi en Zimbabwe opgespoor. In die meeste gebiede word tripanosomose deur *T. congolense* veroorsaak. Die voorkoms van *T. vivax* is egter hoog waar tsetse dikwels op wild voed, soos in die Mamiligebied van Oos-Caprivi. Die voorkoms van tripanosomose hang af van die vlak van interaksie tussen tsetse en beeste en korreleer nie noodwendig met die digtheid van die tsetsebevolking nie.

Dis moeilik om die voorkoms van tripanosomose van beeste akkuraat te peil. Die parasitologiese metodes wat algemeen gebruik word het 'n lae diagnostiese sensitiwiteit. Dit lei tot onderskatting van die voorkoms van besmetting. Die verspreiding van tripanosomose word beter deur 'n kombinasie van parasitologiese en meer sensitiewe metodes bepaal. Die anti-tripanosoom-teenliggaambepalende ELISA is uiters sensitief en spesifiek; nie-spesifieke kruisreaksies met teenliggame teen algemene bosluisoorgedraagde antigene soos *Anaplasma marginale* en *Babesia* spp. kom nie voor nie. Anti-tripanosoom-teenliggaampies, 'n indirekte aanduiding van besmetting, bly behoue tot 13 maande nadat 'n besmetting suksesvol behandel is of selfgenesing ingetree het. Deur die voorkoms van anti-tripanosoom-teenliggaampies vas te stel kan gebiede waar daging met tripanosome laag of ongereeld is of waar tripanosoomdodende middels dikwels gebruik word, bepaal word.

Die uitwerking van die siekte op landbou-ontwikkeling is 'n belangrike bepaler by die keuse van voorkeurgebiede om tripanosomose te beheer. Besmetting van vatbare beesrasse lei gewoonlik tot bloedarmoede; die vlak daarvan is 'n goeie aanduider van die felheid van die siekte of die siektetoestand van 'n besmette dier en is sterk gekorreleer met produksie. Op kuddevlak daal die gemiddelde gepakte selvolume (GSV) met stygende voorkoms van tripanosoombesmetting. Die verwantskap tussen gemiddelde GSV van 'n kudde en die voorkoms van tripanosoombesmetting, as regressielyn uitgedruk, is 'n handige aanduider van (i) die uitwerking van verskeie voorkomsvlakke op kuddegesondheid en (ii) die waarskynlike uitwerking van beheeringrepe op kuddegesondheid. Deur die hellings van regressielyne te bepaal kan ruimtelike en tydgebonde vergelykings van die uitwerking van tripanosomose gemaak word. Op die Oos-Zambiese plato is die uitwerking van tripanosoombesmetting op gemiddelde GSV van kuddes op sy laagste by ononderbroke daging en waar tsetse veral op beeste voed. Sulke omstandighede bevorder die ontstaan van nie-steriele immuniteit. Waar daging ongereeld is en tsetse veral op wild voed daal die gemiddelde GSV van kuddes betekenisvol vinniger met toenemende tripanosoombesmetting. Seisoen speel ook 'n belangrike rol by die bepaling van die uitwerking van tripanosoombesmetting op gemiddelde GSV van kuddes. Tydens die droë seisoen, met voedingstres op sy hoogste, daal gemiddelde GSV van kuddes vinniger as tydens die reëntyd. Die vlak en doeltreffendheid van behandeling met tripanosoomdodende middels beïnvloed die direkte sosio-ekonomiese effek van tripanosomose op produksie van beeste. In Suider-Afrika, waar dié middels geredelik beskikbaar is, is mortaliteit weens

tripanosomose laag en lei besmetting veral tot verlaagde kalfpersentasies. Die voorkoms van die siekte en die vlak van weerstandigheid beïnvloed die daling in kalfpersentasies. Die sosio-ekonomiese uitwerking van tripanosomose is die hoogste waar beeste langs tsetsebesmette gebiede aangehou word, bv. die Vwazagebied in die noordelike streek van Malawi. Al die ander, veral indirekte, uitwerkings van tripanosomose word deur nie-verwante faktore beïnvloed, bv. siektebestuur, die moontlikheid om kuddes en bewerkte grond uit te brei en die behoefte aan kontant. Al hierdie veranderlikes en hul onderlinge verwantskap moet in ag geneem word wanneer plaaslike beheer van tripanosomose van beeste beplan word. Versuim mag lei tot die oorberaming van die voordele van beheer en kan dus die volhoubaarheid van die ingreep beïnvloed.

Tripanosoomdodende middels word algemeen in Suider-Afrika toegedien. 'n Ontleding dui daarop dat die meerderheid beeseienaars genesende middels bo voorkomende middels verkies. Eienaars verkies ook om produktiewe diere (osse en koeie) te behandel en pas veral 'n produksegerigte behandelingstrategie toe. Dié behandeling verlaag vrektes weens tripanosomose maar het weining invloed op die kalfpersentasie. Die volhoubaarheid van 'n middelgebaseerde beheerstrategie hang grootlik van die gebruikswyse van die middels af. Alhoewel boere die meeste middels self toedien, toon ontleding van opnames dat onderdosering nie juis voorkom nie en dat ander faktore wat die ontwikkeling van middelweerstand bevorder, ontbreek.

Teikens met geur as lokmiddel is 'n doeltreffende tsetsebeheermetode in groot gebiede met 'n eenvormige plantegroei. In relatief klein, bewerkte gebiede het sulke teikens, met insekdoders behandel, teen 'n digtheid van sowat 4/km^2 gelei tot 'n vinnige afname van die tsetsebevolking en 'n daling in die voorkoms van tripanosomose. Seisoenale veranderings in die verspreiding van tsetsevlieë kan uitgebuit word deur teikens in geselekteerde plantegroeitipes uit te plaas. Teikens is ook 'n doeltreffende versperring teen die herbesmetting van skoongemaakte gebiede deur tsetsevlieë. Beeste wat met insekdoders behandel is, is 'n belangrike metode om nagana te beheer in gebiede waar tsetse veral op beeste voed. Sulke beeste is egter slegs doeltreffend indien hulle die tsetsedigtheid genoegsaam verlaag om die voorkoms van tripanosomose te laat daal. Beeste wat met insekdoders behandel is, is onvoldoende om herbesmetting van 'n gebied deur tsetsevlieë te verhoed. Selfs waar daar geen effek op die voorkoms van tripanosomose is nie, lei

behandeling van beeste met insekdoders dadelik tot 'n verbetering in hul kondisie. Dit word weerspieël deur 'n styging in die kudde se gemiddelde GSV en word toegeskryf aan die middel se uitwerking op die bosluislading. Alhoewel die bosluisdodende effek van insekdoders voordelig mag wees om uitbreke van bosluisoorgedraagde siektes te voorkom, kan dit die ontstaan van ensoötiese stabiliteit beïnvloed. Dit het in Oos-Zimbabwe gebeur, waar deltametrienbehandeling met kort tussenposes tot 'n verlaging in die bevolkingsdigtheid van *Boophilus* spp. en dus in bosluis- en *Babesia*-daging gelei het; 'n ensoöties onstabiele toestand was die gevolg.

Die resultate van hierdie proefskrif toon dat beplanning vir die volhoubare beheer van tripanosomose van beeste op plaaslike vlak 'n multidissiplinêre oefening is wat 'n grondige begrip van die verspreiding en epidemiologie van die siekte verg. Die keuse van 'n bepaalde beheermetode hang grootliks af van die plaaslike epidemiologie van die siekte. Deur die verskeie epidemiologiese toestande in Suider-Afrika te onderskei en hul eienskappe te ontleed, is toepaslike metodes om tripanosomose van beeste te beheer geïdentifiseer.

CONTENTS

Page

Page

Page

Page

LIST OF TABLES

Page

Page

Page

LIST OF FIGURES

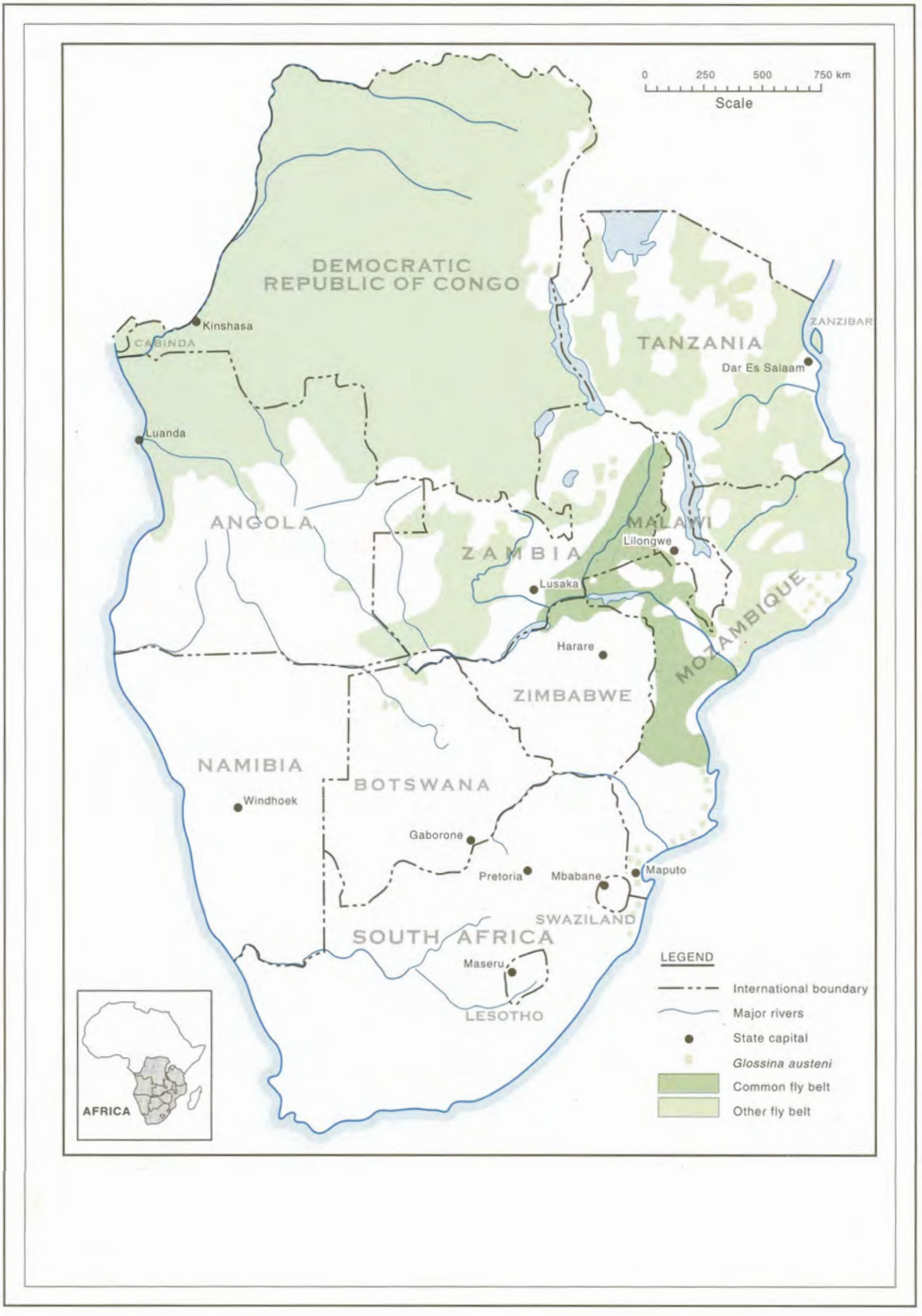
Page

INTRODUCTION

AND

AIMS OF THE STUDY

Figure 1: Approximate distribution of tsetse in the SADC Region



Tsetse-transmitted trypanosomosis is recognized widely as a major animal disease particularly, but not exclusively, in sub-Saharan Africa (Jordan, 1986). In the majority of 37 sub-Saharan countries affected by tsetse-transmitted trypanosomosis, the problem is classified as severe and ranks among the three top priority livestock diseases. Approximately seven million km^2 of the tsetse-infested areas would probably be suitable for livestock and agricultural development if trypanosomosis were controlled (Finelle, 1974).

In Malawi, Eastern Caprivi region of Namibia, eastern Zambia and Zimbabwe (the countries dealt with in the thesis and henceforth referred to as southern Africa), trypanosomosis poses a serious threat to cattle. Bovine trypanosomosis or "nagana" depresses all aspects of production: fertility is impaired; milk yields, growth and work output are reduced; and the mortality rate may reduce herd size (Connor, 1994a). Therefore, bovine trypanosomosis is a significant factor responsible for retarding rural development in much of southern Africa.

There are various ways of dealing with the bovine trypanosomosis problem (Jordan, 1986). They range from eradication of the vector, the tsetse fly, to treating trypanosomal infections with therapeutic drugs. In much of southern Africa, however, trypanosomosis control has focused on the large-scale control of the vector with the ultimate aim of its eradication (Jordan, 1985).

Throughout the long history of tsetse control in southern Africa impressive progress has been made in the development of effective means of controlling the fly (Vale, 1993b; Green, 1994). Tsetse control has evolved from indirect methods, such as altering the tsetse's environment, to direct methods using toxic substances (Allsopp, 1984). Applying a variety of methods, governments (sometimes assisted by donors) have been able to clear large areas of tsetse (Lovemore, 1986; Shereni, 1990). Unfortunately, very few countries have been able to sustain those reclamations. This is attributed to re-invasion by tsetse of cleared areas in the absence of permanent barriers (such as natural barriers) to tsetse invasion and the ineffectiveness of most tsetse control methods in preventing tsetse from re-invading cleared areas. In recent

years, new methods to control tsetse have been developed and these greatly improve the prospects for effective barriers against tsetse re-invasion (Muzari and Hargrove, 1996).

Despite the availability of effective tsetse control methods, however, the prospects for large-scale control of the vector are bleak. Due to the unfavourable economic situation in most African countries, costs involved in the large-scale control of tsetse and subsequently maintaining artificial barriers against re-invasion have become prohibitive. Nevertheless, bovine trypanosomosis is and will remain a serious constraint to rural development. This will be more so when tsetse-infested land is required to settle a continuously expanding human population (Hursey, 1998). There is thus a need to re-orient planning for the control of bovine trypanosomosis in line with the changing environment.

The current unfavourable economic environment has prompted a shift in emphasis from government/donor-funded, large-scale, tsetse control to small-scale, sustainable, trypanosomosis control. This shift reflects, in part, the reduction in government capacity and change in government's policy towards the control of endemic diseases such as trypanosomosis but also the changing attitude of donors towards more participatory and sustainable approaches (Umali *et al.*, 1994). This change from eradication of the vector, towards small-scale sustainable control has important implications for strategy formulation, which is a dynamic process that identifies, ranks and constantly adjusts priority areas for such sustainable trypanosomosis control. It addresses the following questions:

- *Why*?: The need for control should be established at the outset. Objectives should always be directed towards removing or alleviating the problems and constraints associated with trypanosomal infections. At the same time, the potential to create new problems should be recognised.
- *How*? : The effectiveness, transferability and sustainability of different control methods should be properly assessed. When communities are involved in control

operations, it is particularly important to evaluate the transferability and sustainability of the methods proposed.

- *When*?: Implementation schedules should be based on realistic assumptions about the availability of labour, management and financial resources. During planning, it may be necessary to quantify resource constraints at different levels (e.g. at the government, community and/or small holder levels).
- *Where*?: Priority areas for control operations must be identified and then ranked.
- *By whom*?: Responsibilities for the implementation and maintenance of control operations should be stated. Government, private contractor and/or community responsibilities must be clearly defined and understood by those involved.
- *For what benefits and costs*?: The direct and indirect benefits and costs of any proposed operation should, where possible, be identified and quantified. The hidden effects and potential conflicts associated with the implementation of control operations should be carefully considered as well.
- *Paid for by Whom*?: Financial responsibilities for control operations should be defined for the short, medium and long term.

To address these questions properly, potential control options should be screened by considering carefully socio-economic, institutional, technical and environmental criteria. A failure to consider adequately these different criteria can result in problems, which will undermine the sustainability of an intervention.

It is beyond the scope of this thesis to consider all of the above mentioned criteria. However, two of them, the technical and socio-economic ones, will be looked at in more detail.

Previously, strategy formulation for large-scale eradication of tsetse in southern Africa was dominated by straightforward technical considerations. The most cost effective and technically efficient means of controlling tsetse in an area was emphasised. The technical efficiency of an eradication campaign did not require a thorough knowledge or understanding of, for example, the distribution and epidemiology of tsetse-

transmitted bovine trypanosomosis. Notwithstanding the fact that nagana was the main reason for intervening, the disease itself would be dealt with indirectly by eradicating the vector from all areas including those where nagana was not present. The current shift to localised control of tsetse-transmitted bovine trypanosomosis has changed the emphasis from the vector to the disease. Nagana remains the main reason for intervening but control methods will differ according to the local situation and interventions will be restricted to those areas where the disease is present. As a result, the technical criteria to be considered will differ substantially from those considered in the planning for large-scale eradication. First, a clear picture of the extent and magnitude of the bovine trypanosomosis problem is required. Second, the selection of the most efficient intervention methods will vary according to the local epidemiological situation. Hence, the different epidemiological situations need to be identified and the effectiveness of available control methods needs to be evaluated in each of these situations. This will require an understanding of the numerous variables involved in the epidemiology of nagana in southern Africa. Finally, the long-term sustainability of an intervention will depend, to a large extent, upon the socio-economic impact of the disease and perceived benefits accruing from its control. Hence, the socio-economic impact of the disease and the determining factors need to be assessed and identified.

The epidemiology of tsetse-transmitted bovine trypanosomosis is complex (Rogers, 1988). Detailed information is available on the behaviour of most southern African tsetse species of economic importance (Phelps and Lovemore, 1994). Nevertheless, there is insufficient knowledge of the fly-related variables that determine the interaction between *Glossina morsitans morsitans*, the major vector of bovine trypanosomosis in southern Africa, and cattle. Data available on, for example, the seasonal distribution and abundance of this tsetse species and factors affecting those variables were collected in wildlife areas that cannot be compared with areas where the cattle/tsetse interface occurs (Pilson and Pilson, 1967). Moreover, *G. m. morsitans* populations have been investigated using sampling methods of unknown sensitivity and different sampling biases (Bursell, 1961). Although the epidemiology of trypanosomal infections in *G. pallidipes* has been investigated thoroughly

(Woolhouse *et al.*, 1993; Woolhouse *et al.*, 1994), too little information is available on the prevalence of trypanosomal infections in *G. m. morsitans*. More studies are thus required on the epidemiology of bovine trypanosomosis in southern Africa.

Accurate and up-to-date information on the distribution and prevalence of tsetse-transmitted bovine trypanosomosis in southern Africa is sparse. Moreover, the little information that is available has been collected using methods of low sensitivity (Paris *et al.*, 1982). This leaves many areas where trypanosomosis is present but unidentified. In areas where the disease has been detected, the magnitude of the problem is likely to be quantified poorly and underestimated. Hence, available information on the distribution is an unreliable source for the development of a strategy for sustainable localised control. The recent development of a more sensitive and practical indirect method (anti-trypanosomal antibody detection enzyme-linked immunosorbent assay (antibody ELISA)) to detect the presence of bovine trypanosomosis (Hopkins *et al.*, 1998), offers the possibility of determining more accurately the distribution of the disease. Unfortunately, the usefulness of this indirect method has not yet been fully assessed and interpretation of results needs to be improved.

Entomological and veterinary data rather than socio-economic principles formed the backbone for planning large-scale tsetse control or eradication campaigns. Socio-economics were only considered when improving the cost-effectiveness of control methods (Vale, 1993b; Barrett, 1994). Similarly, the impact of control interventions has been measured using entomological and veterinary indicators rather than socio-economic ones. As a result, little is known of the socio-economic impact of bovine trypanosomosis and its control on cattle productivity. Socio-economic aspects of the impact of the disease and expected impact of control interventions, on the other hand, form an essential component of planning for cost-effective control. From a socio-economic point of view, sustainable control can only be achieved when the benefits accruing from the control intervention are larger than its cost (Salmon and Barrett, 1994; Swallow and Woudyalew, 1994). There is thus a need to assess carefully the impact of bovine trypanosomosis on the productivity of cattle. Moreover, the

relationship between trypanosomosis, at various levels of disease prevalence, and productivity needs to be established.

Today, two tsetse control methods, based on bait technology, are used increasingly and more widely in southern Africa. Stationary baits (odour-baited, insecticide-treated, targets (Vale *et al.*, 1986)) have proven to be very effective in controlling tsetse in large, homogenous areas (Vale *et al.*, 1988a; Knols *et al.*, 1993). The effectiveness of this method in controlling tsetse in small, cultivated areas still needs to be assessed. The effectiveness of mobile baits (insecticide-treated cattle (Thomson, 1987)) in controlling tsetse or reducing tsetse challenge, under conditions prevailing in southern Africa, still needs to be tested. The effects of applying insecticide to cattle on tick challenge and the development of enzootic stability against some tick-borne diseases need to be determined. Moreover, the role that insecticide-treated cattle could play in preventing tsetse from re-invading previously cleared areas still has to be assessed. Despite the rather superficial knowledge of the effectiveness of these methods in the small-scale control of tsetse or in preventing the spread of tsetse, both methods are promoted widely. There is a need to investigate more thoroughly the effectiveness and socio-economic impact of these control method under different circumstances.

An alternative to the control of tsetse is the control of trypanosomosis with drugs (Peregrine, 1994). In most countries of southern Africa, farmers are able to buy trypanocides and can thus implement their own disease management strategies using therapeutic or prophylactic drugs. The long-term sustainability of such an approach is a function of the probability of trypanosomes developing resistance against those drugs (Geerts and Holmes, 1997). Trypanocidal drug resistance has been recorded in many countries in West and East Africa (Pinder and Authié, 1984; Dolan *et al.*, 1992). In southern Africa, little is known of the susceptibility of trypanosome strains to trypanocidal drugs. Sensitive methods to determine the susceptibility of trypanosomes to isometamidium chloride (the principal prophylactic trypanocide) have been developed and are being used on a trial basis (Eisler *et al.*, 1996). Unfortunately, those techniques are expensive and cannot be used to assess the susceptibility of

trypanosome strains to the most commonly used, therapeutic trypanocide, diminazene aceturate. A first step in determining the probability of drug resistance could be the establishment of the frequency with which trypanocides are used, the dose and the mode of application. This simple information is not available but could be collected during surveys. Results of such "drug use" surveys should form an integral part of the decision-making process on how to control trypanosomosis in a particular area.

Finally, the sustainability of a disease control intervention will to a large extent be determined by the cattle owner's attitude towards his animals. Although this information is difficult to quantify, data on trypanocide use could be used as an indirect and quantifiable indicator of this attitude.

By collecting the required information and improving the interpretation of the results, a significant contribution will be made to the development of a framework for the formulation of appropriate strategies for the effective control of tsetse-transmitted bovine trypanosomosis in the southern African region.

The specific aims of the investigations outlined in this thesis were:

1. *to obtain a better understanding of the interaction between tsetse and cattle in an area in southern Africa where tsetse-transmitted bovine trypanosomosis is endemic (Chapter 2);*

2. *to use improved methods to update distribution maps and improve the understanding of the epidemiology of bovine trypanosomosis in southern Africa (Chapter 3);*

3. *to assess the impact of bovine trypanosomosis on production and agricultural development in southern Africa (Chapter 4);*

4. *to investigate trypanocidal drug use and the impact of various tsetse control methods, under different epidemiological situations, in the southern Africa (Chapter 5).*

The findings obtained from these studies indicate that planning for the effective control of tsetse-transmitted bovine trypanosomosis in southern Africa requires an understanding of the dynamics of the tsetse population and accurate knowledge of the distribution of the disease, its impact on production and productivity, and the impact of control interventions under various epidemiological situations.

CHAPTER ONE

LITERATURE REVIEW

1.1 Introduction

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

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

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

1.2.1 The host-tsetse contact

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

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

1.2.1.1 Host preference

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

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

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

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

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

1.2.1.2 Probing response

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

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

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

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

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

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

1.2.1.3 Feeding frequency

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

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

1.2.2 The prevalence of trypanosomal infections in tsetse

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

1.2.2.1 Mechanical transmission of trypanosomes

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

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

1.2.2.2 Cyclical development of trypanosomes in the tsetse fly

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

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

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

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

1.2.2.3 Methods to detect trypanosomal infections in tsetse

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

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

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

1.2.2.4 Factors affecting the prevalence of trypanosomal infections in tsetse

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

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

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

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

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

1.2.2.5 The prevalence of trypanosomal infections in southern African tsetse species

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

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

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

b. 14442620
i. 14746050

1.2.3 The density of the tsetse population

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

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

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

1.2.4 The coefficient of transmission of a trypanosomal infection

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

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

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

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

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

1.2.5 The interaction between tsetse and cattle in southern Africa

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

1.3 The prevalence of tsetse-transmitted trypanosomosis in the host

1.3.1 Trypanosome development in the host

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

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

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

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

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

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

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

1.3.2 Methods to detect trypanosomal infections in the host

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

1.3.2.1 Parasitological diagnosis

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

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

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

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

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

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

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

1.3.2.2 Anti-trypanosomal antibody detection tests

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

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

1.3.2.3 Trypanosome antigen or DNA detection tests

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

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

1.3.3 The distribution of bovine trypanosomosis in southern Africa

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

1.4 Impact of tsetse-transmitted bovine trypanosomosis

1.4.1 Pathogenesis of bovine trypanosomosis

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

1.4.1.1 Anaemia

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

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

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

1.4.1.2 Myocarditis and myositis

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

1.4.1.3 Immunosuppression

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

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

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

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

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

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

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

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

1.4.2 Immunity to bovine trypanosomosis

1.4.2.1 Trypanotolerance

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

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

1.4.2.2 Acquired tolerance to trypanosomal infections

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

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

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

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

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

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

1.4.3 Effect of bovine trypanosomosis on productivity

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

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

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

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

1.4.4 The impact of bovine trypanosomosis in southern Africa

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

1.5 The control of tsetse-transmitted bovine trypanosomosis

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

1.5.1 The control of the parasite

1.5.1.1 The history of trypanocidal drug development

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

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

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

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

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

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

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

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

1.5.1.2 Resistance of trypanosomes to trypanocides

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

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

1.5.1.3.1Eastern Zambia

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

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

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

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

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

1.5.1.3.2 Zimbabwe

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

1.5.1.3.3 Malawi

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

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

1.5.2 The control of the vector

1.5.2.1 Indirect control methods

1.5.2.1.1 Vegetation clearance

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

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

1.5.2.1.2 Game elimination and game fences

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

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

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

1.5.2.2 Direct control methods

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

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

1.5.2.2.1 Application of insecticides to vegetation

Ground spraying

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

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

Aerial spraying

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

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

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

1.5.2.2.2 Application of insecticides to bait systems

Stationary baits

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

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

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

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

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

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

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

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

Mobile baits

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

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

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

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

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

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

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

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

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

CHAPTER TWO

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

2.1 Introduction

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

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

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

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

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

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

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

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

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

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

2.2.1 Introduction

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

2.2.2 Materials and methods

2.2.2.1 Study area

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

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

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

2.2.2.2 Tsetse sampling

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

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

2.2.2.3 Abundance and distribution of cattle

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

2.2.3 Results

2.2.3.1 Tsetse abundance and distribution

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

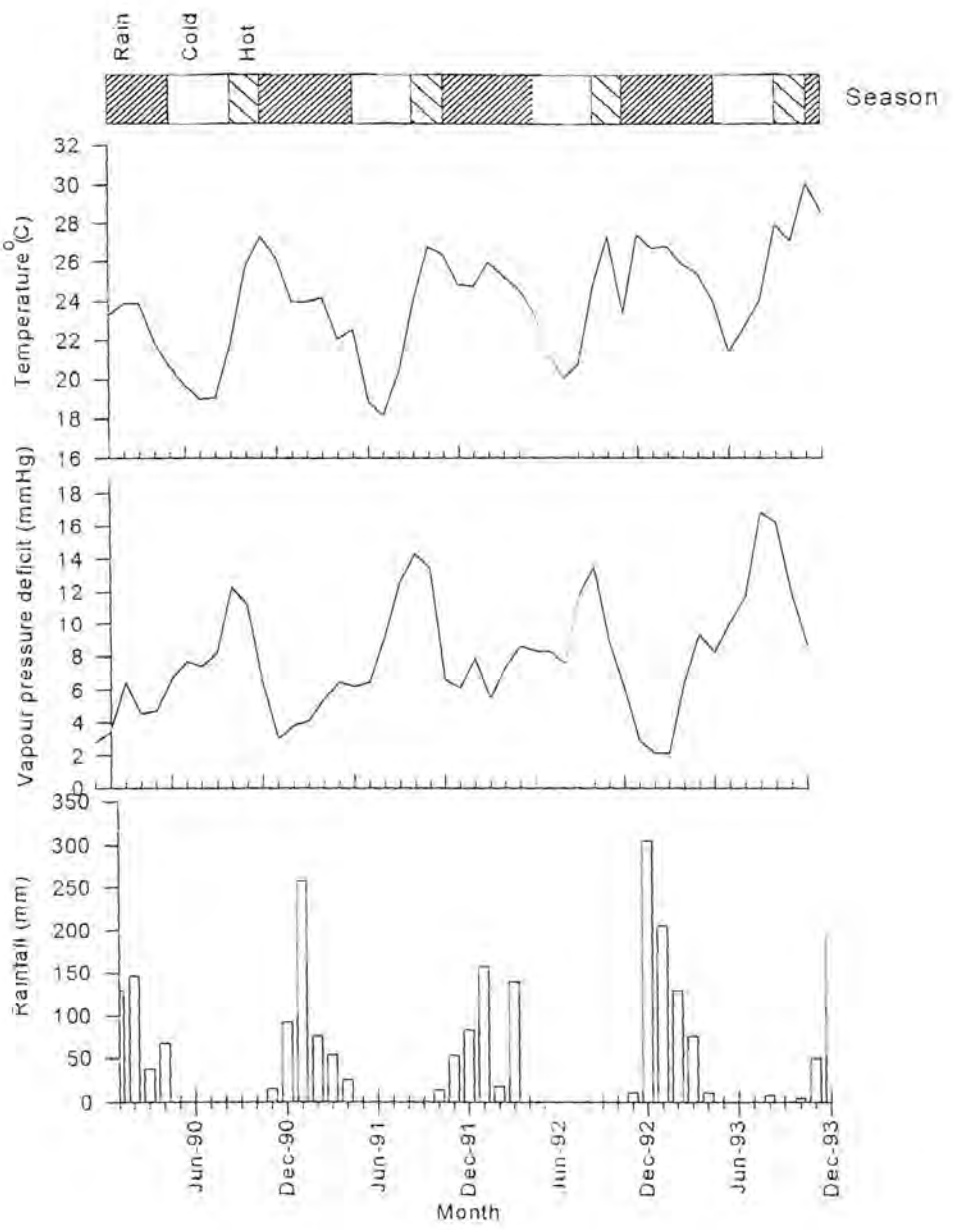


Figure 2.2.1: Monthly average ambient temperature, vapour pressure deficit and rainfall, Katete District, Eastern Province, Zambia.

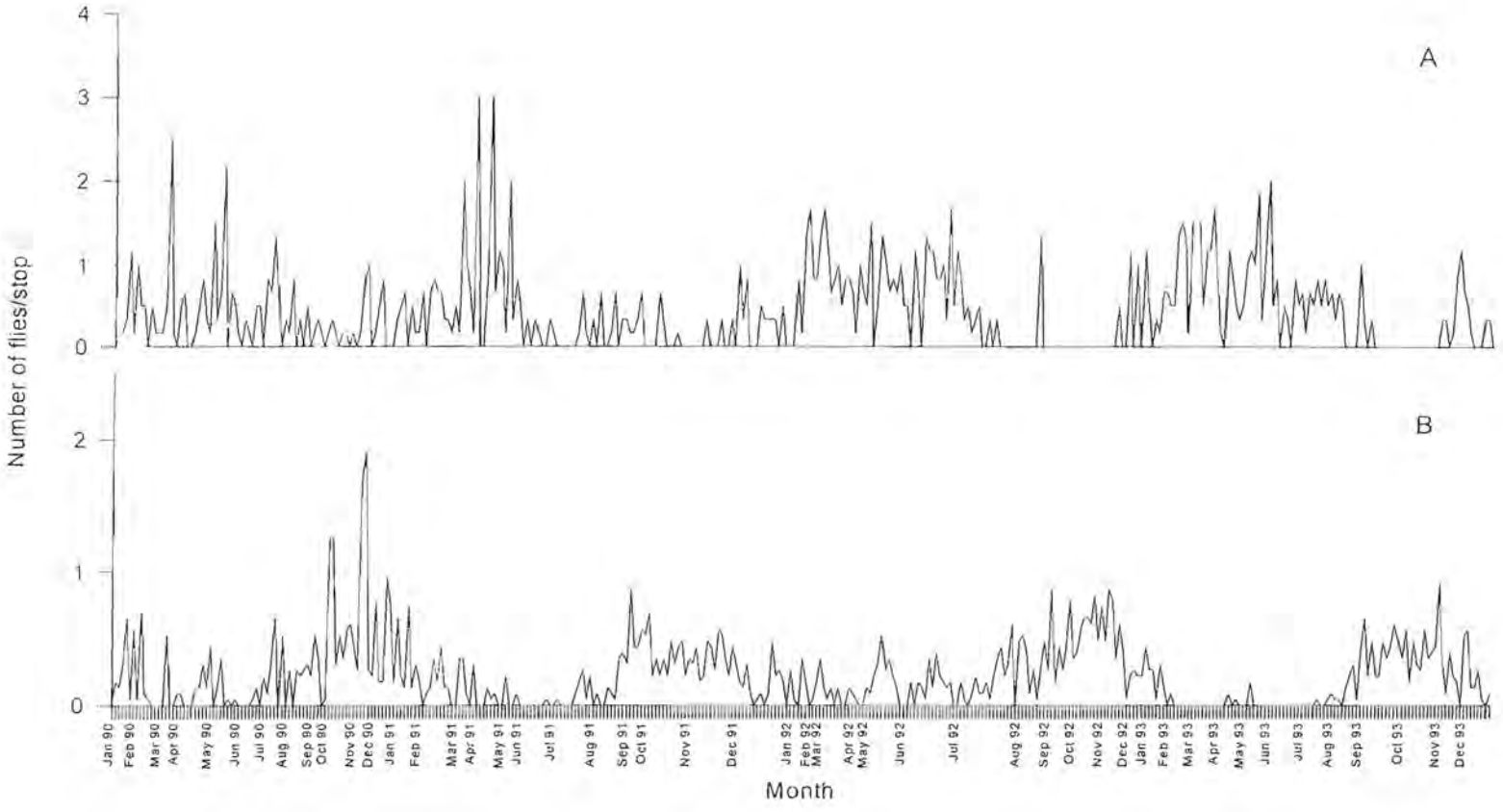


Figure 2.2.2: Number of *G. m. morsitans* captured per stop during each fly-round in miombo (A) and munga (B).

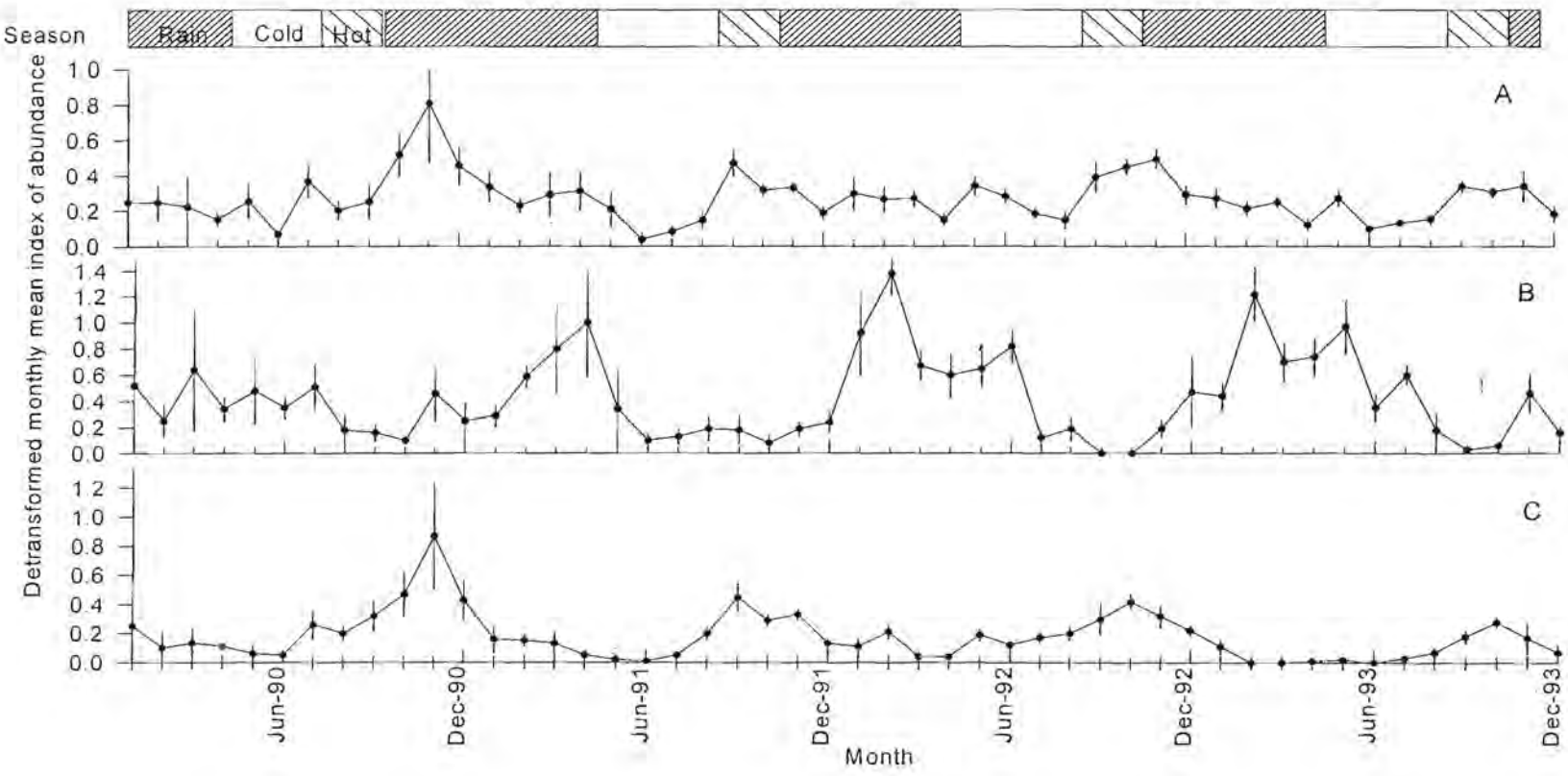


Figure 2.2.3: Detransformed monthly average index of abundance (± 1 s.e.) of *G. m. morsitans* in all vegetation types (A), in miombo (B) and in munga (C).

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

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

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

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

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

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

2.2.3.2 Cattle abundance and distribution

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

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

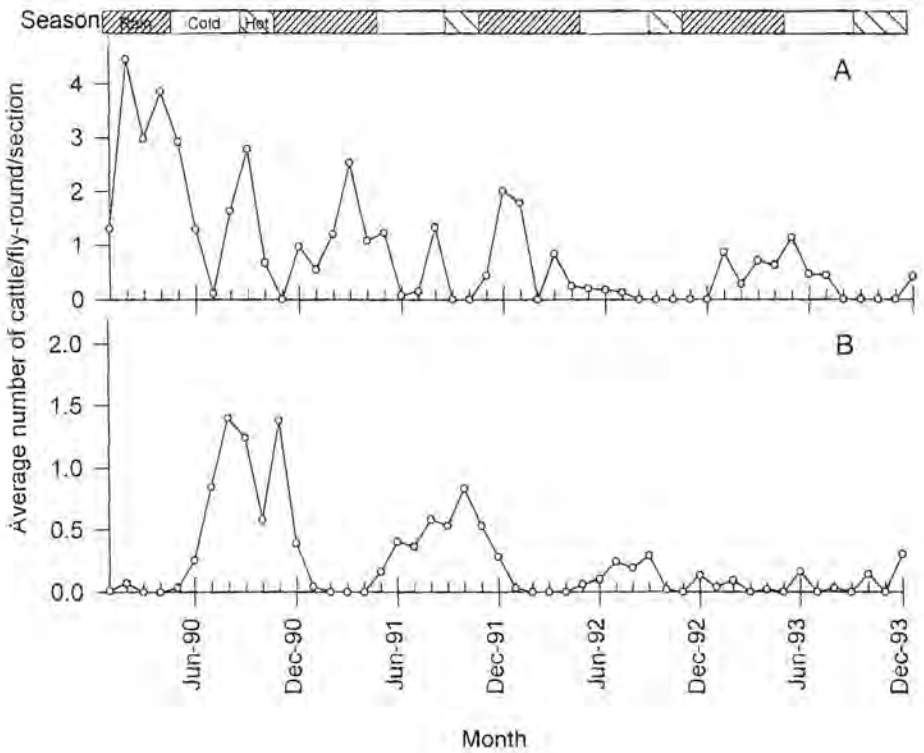


Figure 2.2.4: Monthly average number of cattle seen in each section per fly-round in miombo (A) and munga (B).

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

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

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

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

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

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

2.2.4 Discussion

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

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

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

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

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

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

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

2.3.1 Introduction

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

2.3.2 Materials and methods

2.3.2.1 Study area

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

2.3.2.2 Tsetse sampling

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

2.3.2.3 Bloodmeal collection

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

2.3.3 Results

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

2.3.4 Discussion

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

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

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

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

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

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

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

2.4.1 Introduction

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

2.4.2 Materials and methods

2.4.2.1 Study area

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

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

2.4.2.2 Capture/Mark/Release/Recapture experiment

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

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

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

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

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

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

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

2.4.3 Results

2.4.3.1 Tsetse abundance and distribution

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

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

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

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

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

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

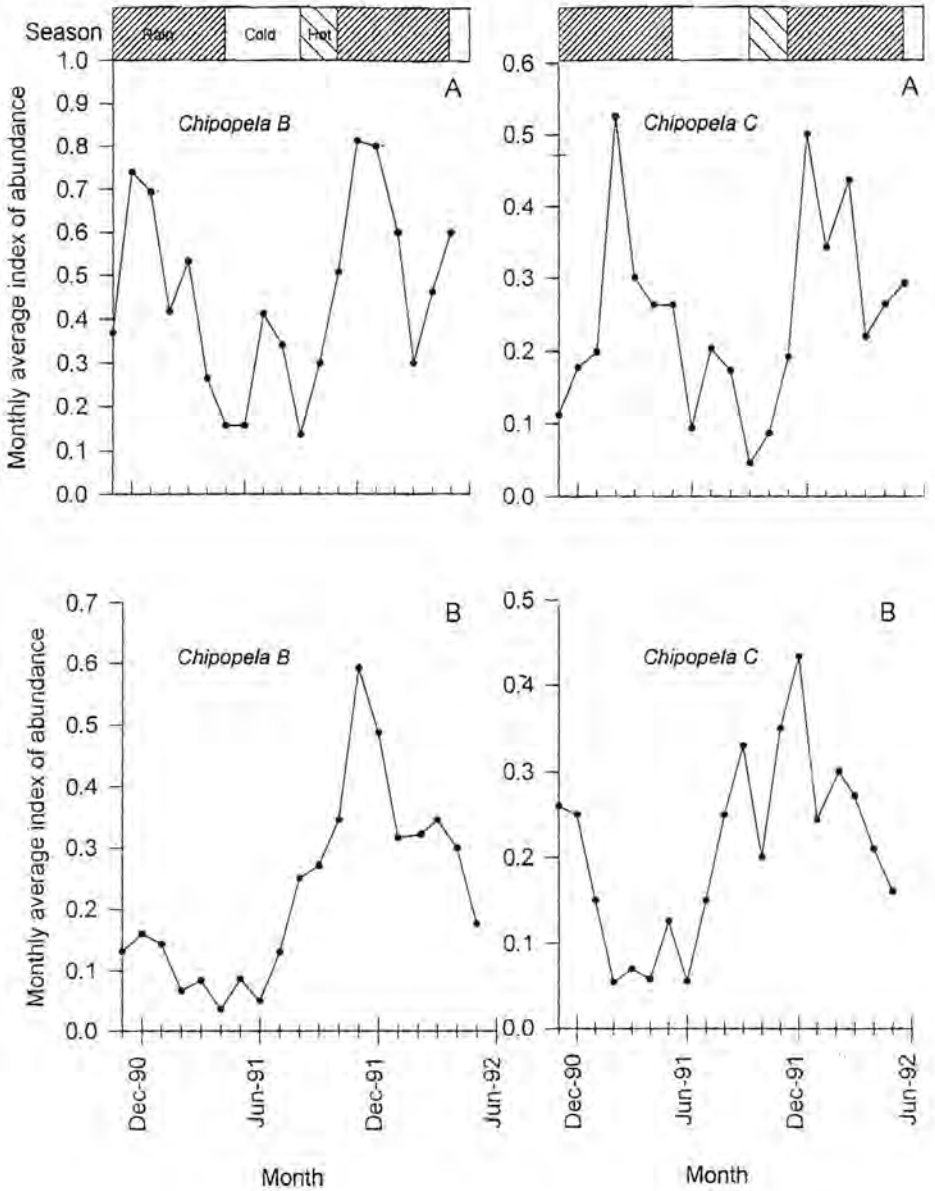


Figure 2.4.1: Monthly average index of abundance of *G. m. morsitans* in miombo (A) and munga (B) along Chipopela-B and Chipopela-C transects.

2.4.3.2 Recapture rates of tsetse

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

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

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

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

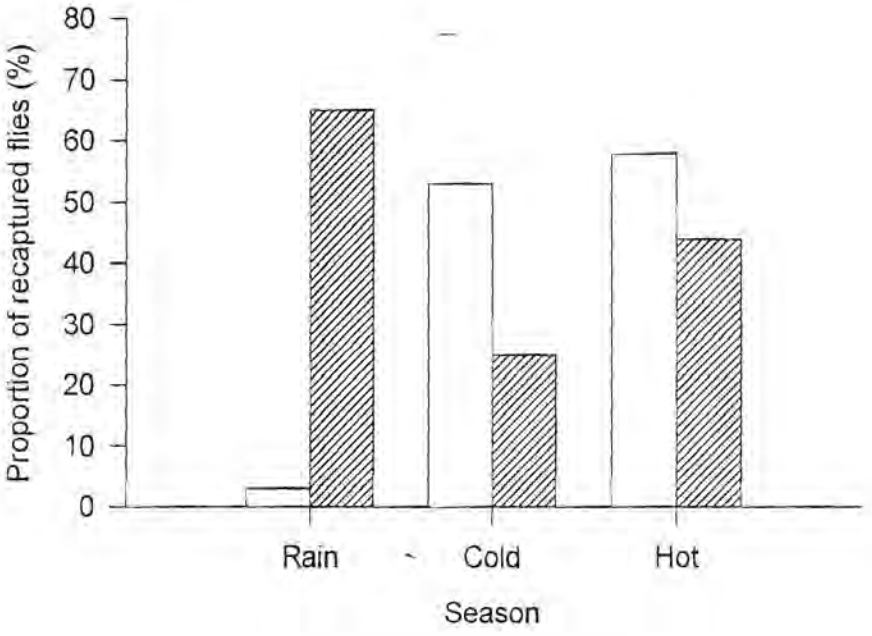


Figure 2.4.2: Proportion of *G. m. morsitans* (males plus females) marked in miombo and recaptured in munga (□) and marked in munga and recaptured in miombo (=) during the rainy, cold and hot dry season.

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

2.4.4 Discussion

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

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

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

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

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

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

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

2.5.1 Introduction

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

2.5.2 *Materials and methods*

2.5.2.1 Study area

The study area is described in Section 2.2.2.1.

2.5.2.2 Tsetse sampling

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

2.5.2.3 Tsetse dissection

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

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

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

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

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

2.5.2.4 Incidence of bovine trypanosomosis

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

2.5.2.5 Statistical data analysis

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

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

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

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

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

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

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

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

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

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

2.5.3 Results

2.5.3.1 Temporal variations in the prevalence of trypanosomal infections in tsetse

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

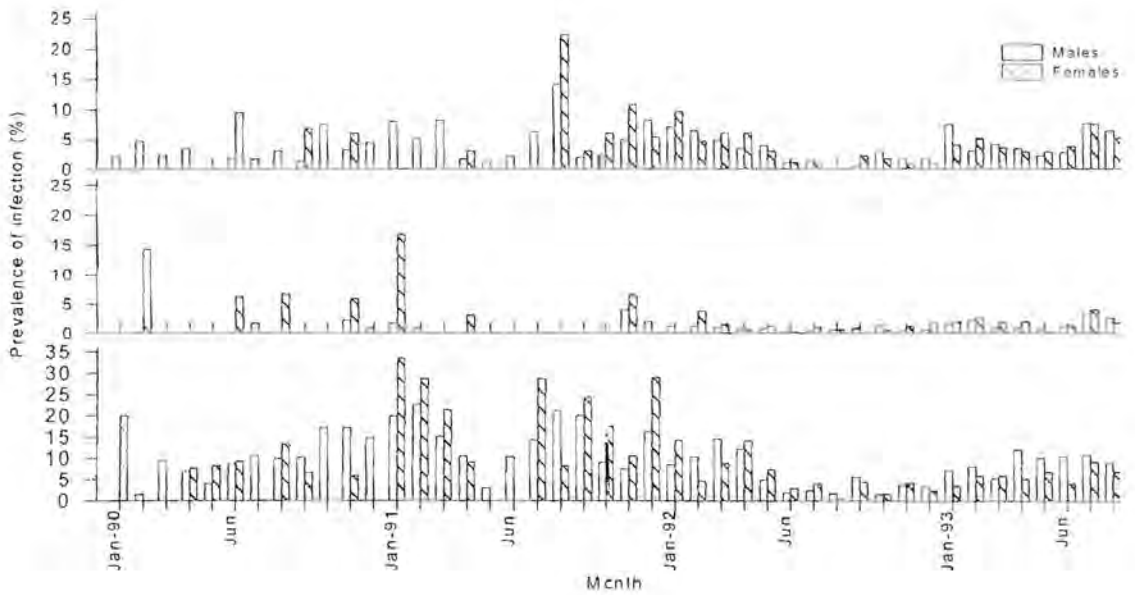


Figure 2.5.1: Monthly proportion of male and female *G. m. morsitans* with (top) *congolense*-type, (middle) *vivax*-type, and (bottom) immature trypanosomal infections.

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

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

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

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

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

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

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

2.5.3.3 Age-prevalence relationship

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

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

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

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

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

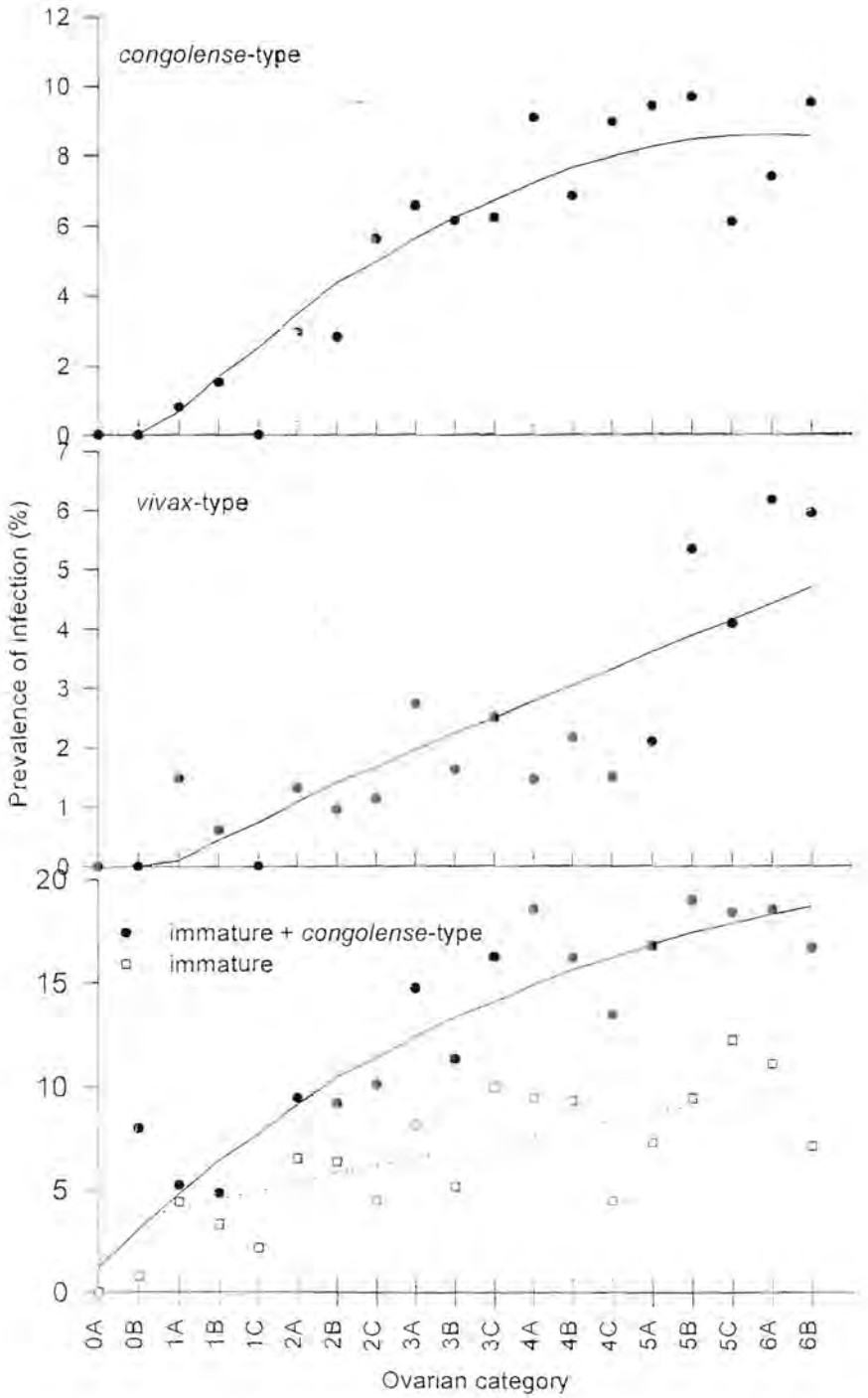


Figure 2.5.2: Variations in prevalence of *congolense*-type, *vivax*-type and *congolense*-type + immature (•) and immature (□) infections of female *G. m. morsitans* with fly age (by ovarian category). The best fit model (line) is ln(1-y) = -0.02107 - 0.002672age + 2.292e-07age^3 for *congolense*-type,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

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

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

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

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

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

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

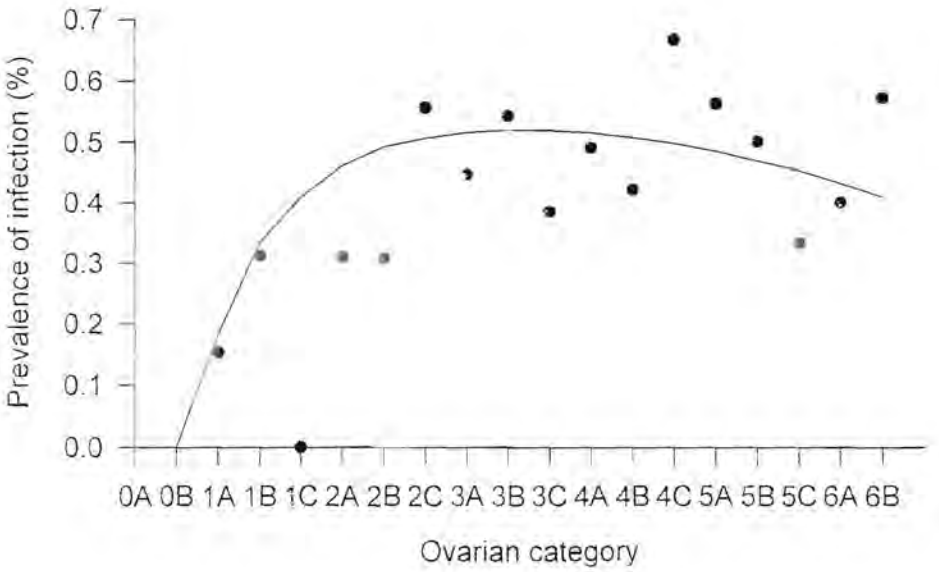


Figure 2.5.3: Variations in the ratio of *congolense*-type infections to (*congolense*-type + immature) infections of female *G. m. morsitans* with fly age (by ovarian category).

2.5.3.4 Incidence of bovine trypanosomosis

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

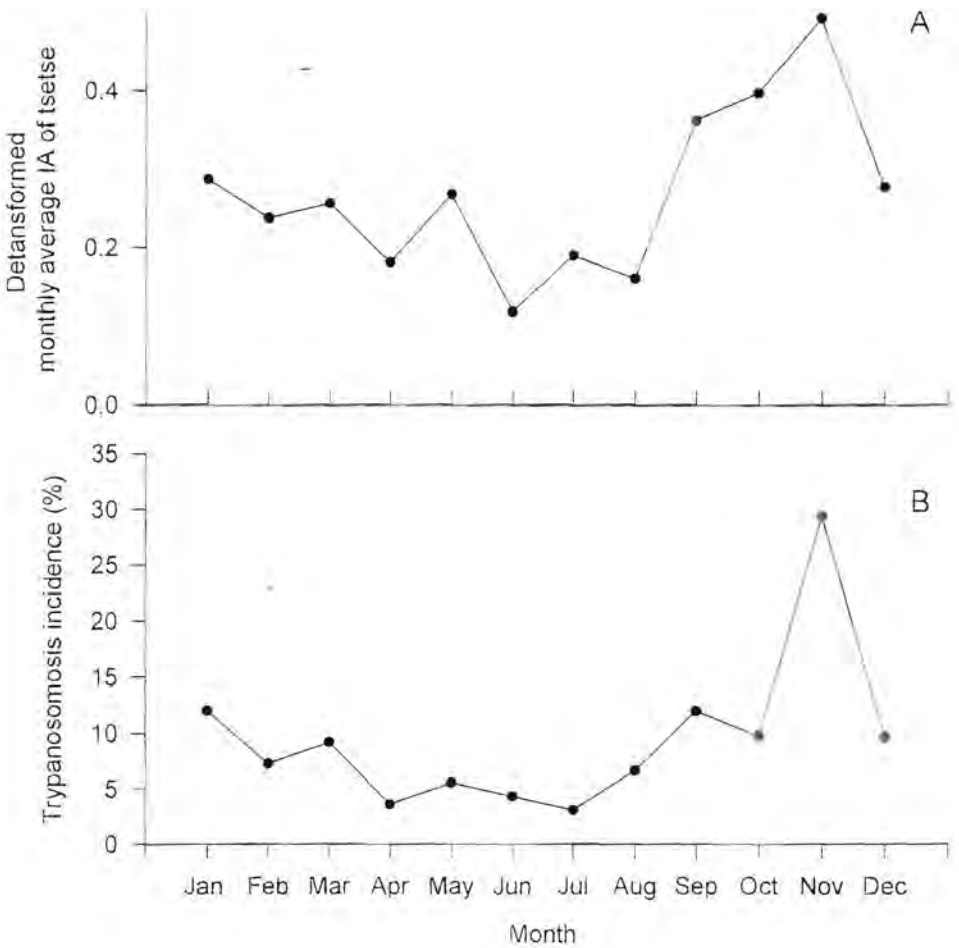


Figure 2.5.4: Detransformed monthly average index of abundance of *G. m. morsitans* (A) and monthly average incidence of trypanosomal infections in sentinel cattle (B).

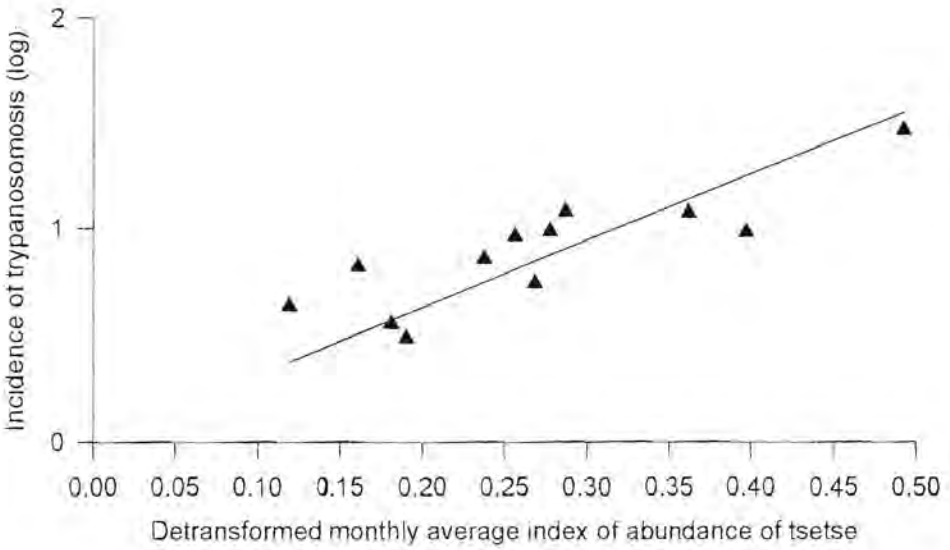


Figure 2.5.5: Relationship between the monthly average incidence of trypanosomal infections in cattle (log transformed) and the detransformed average monthly index of abundance of *G. m. morsitans* in the study area.

2.5.4 Discussion and conclusions

2.5.4.1 Prevalence of trypanosome species in tsetse

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

2.5.4.2 Temporal variation in the prevalence of trypanosomal infections in tsetse

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

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

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

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

2.5.4.3 Age-prevalence relationship for trypanosomal infections in female tsetse

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

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

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

2.5.4.4 The epidemiology of bovine trypanosomosis

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

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

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

CHAPTER THREE

BOVINE TRYPANOSOMOSIS IN MALAWI, NAMIBIA AND ZIMBABWE

3.1 Introduction

An important requirement for developing a strategy for the localised control of tsetse-transmitted bovine trypanosomosis is to have a clear picture of the extent and magnitude of the nagana problem and a basic understanding of its local epidemiology. For many decades, emphasis was placed on determining the distribution and density of the vector rather than the prevalence of the disease in cattle. This is not surprising in view of the past tsetse eradication policy and the absence of cattle in extensive areas of potential tsetse habitat. However, information on the distribution and density of tsetse does not suffice when developing a strategy for the localised control of tsetse-transmitted bovine trypanosomosis. There are several reasons for supplementing tsetse data with bovine trypanosomosis prevalence figures. First and foremost, using bovine trypanosomosis prevalence data areas where nagana is present or areas at risk can be identified. Moreover, areas can be classified according to the proportion of animals infected or levels of challenge and different epidemiological situations can be identified. This information can be used to identify priority areas for particular types of control. Second, disease prevalence data are essential in evaluating the effectiveness of control measures.

Surprisingly, accurate data of the distribution and prevalence of nagana in southern Africa are not available. This is partly due to the present entomological bias in the management of tsetse-transmitted trypanosomosis. Moreover, determining the prevalence of bovine trypanosomosis accurately is fraught with difficulties. The parasitological diagnostic methods in common use have low diagnostic sensitivity. Hence, a substantial proportion of the parasitologically positive animals will not be detected. This will result in an underestimate of the prevalence of infection. Consequently, an area cannot be declared disease-free on the basis of parasitological diagnostic tests alone. Moreover, trypanocidal drugs are used widely in most countries of the region. Parasitological diagnostic tests cannot distinguish between areas where the disease is absent and areas where the prevalence is low or where trypanocidal drugs are used effectively. Finally, in many countries of the southern African region, tsetse are confined to a particular habitat. The interaction between cattle and tsetse often occurs seasonally. This will not be detected easily by a one off

survey using parasitological diagnostic methods. The obvious shortcomings of the parasitological diagnostic method in determining the distribution of bovine trypanosomosis can be compensated for partly by surveillance. However, surveillance is time consuming, expensive and is, therefore, limited to areas of particular interest. Clearly, the methods currently used to determine the distribution and prevalence of bovine trypanosomosis need to be supplemented with more sensitive diagnostic tests.

The recently improved anti-trypanosomal antibody detection ELISA may be such a tool. The test was developed about 20 years ago (Luckins, 1977) but has hardly been used for extensive surveys. Recently, the assay was further developed for detection of anti-trypanosomal antibodies in eluted blood spots collected on filter papers. Anti-trypanosomal antibodies are an indirect indication of a trypanosomal infection and persist even after a trypanosomal infection has been cured. Whereas the persistence of antibodies is often considered a disadvantage, it may be advantageous under certain circumstances. Indeed, determining the prevalence of animals with anti-trypanosomal antibodies may identify areas where challenge is low or irregular. When assessing the effectiveness of control operations, on the other hand, an understanding of the dynamics of anti-trypanosomal antibodies after an infection has been cured is essential. A trial was conducted in eastern Zimbabwe to determine this decline in antibody levels after treatment (Section 3.2). Another factor, which may interfere with the interpretation of anti-trypanosomal antibody prevalence data, is non-specific cross-reactions with antibodies against other diseases. Such cross-reactions will lead to an overestimate of the antibody prevalence. Of particular importance, in this respect, are cross-reactions with antibodies against non-pathogenic trypanosomes (*T. theileri*) and/or antibodies against tick-borne parasites. The ELISA's species sensitivity with regard to *T. theileri* has already been assessed (Hopkins *et al.*, 1998). Tick-borne diseases, especially babesiosis and anaplasmosis, occur over large areas where tsetse are present. Non-specific cross-reactions with antibodies against these diseases, in their acute and/or latent phases, would seriously affect the ELISA's usefulness. Hence, a study was undertaken to determine if non-specific cross-reactions with antibodies against *Anaplasma marginale* and bovine *Babesia* spp. do occur (Section 3.3).

Promising preliminary results were obtained in eastern Zambia where a large-scale bovine trypanosomosis survey was conducted as part of the antibody ELISA's development and validation (Hopkins, 1997). Nevertheless, there is a need to use the test more widely in the Region. Therefore, both parasitological and anti-trypanosomal antibody detection diagnostic methods were used to determine the distribution of bovine trypanosomosis in Malawi (Section 3.4), Namibia (Section 3.5) and Zimbabwe (Section 3.6). Information on the parasitological prevalence of trypanosomosis and the prevalence of anti-trypanosomal antibodies in cattle was used to clarify the epidemiology of bovine trypanosomosis in each of these countries. Moreover, the usefulness of anti-trypanosomal antibody prevalence data in evaluating the effectiveness of tsetse control operations is assessed (Section 3.6).

3.2 The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge

3.2.1 Introduction

When determining the distribution, or studying the epidemiology, of bovine trypanosomosis use is usually made of parasitological diagnostic tests. These tests are simple but lack the diagnostic sensitivity required for an accurate assessment of the distribution of infected animals (Paris *et al.*, 1982). Recently, an anti-trypanosomal antibody-detection enzyme-linked immunosorbent assay (ELISA) was adapted for use with dried blood spots on filter paper (Hopkins *et al.*, 1998). The test has high diagnostic sensitivity and specificity. It has been used in large-scale bovine trypanosomosis surveys and for monitoring the effectiveness of tsetse control interventions in southern Africa. Although knowledge of the prevalence of anti-trypanosomal antibodies in cattle is very useful, interpretation of the results is often difficult. This is due to the persistence of anti-trypanosomal antibodies even after an animal has been cured (Bocquentin *et al.*, 1990). To facilitate the interpretation of data on anti-trypanosomal antibody prevalence in cattle, a study was undertaken to determine the changes in the antibody levels after treatment with trypanocidal drugs.

3.2.2 Materials and methods

3.2.2.1 Experimental animals

Anti-trypanosomal antibody levels were studied in a herd of adult Mashona breed cattle. They were kept under natural tsetse challenge (*G. m. morsitans* and *G. pallidipes*) in an area along the Zimbabwe/Mozambique border in Mudzi District (Mashonaland East Province), Zimbabwe. The monthly incidence of trypanosomosis in this area varied between months and was on average about 20%. Each month, blood taken from an ear vein of each animal was examined for trypanosomes using the haematocrit centrifuge, phase contrast technique (Murray *et al.*, 1977). Ear vein blood, contained in one heparinized microhaematocrit centrifuge capillary tube, was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect anti-trypanosomal antibody detection ELISA (Hopkins *et al.*, 1998). Use was made of a *T.*

congolense (IL 3000) invariable antigen batch prepared by the Parasitology Laboratory of the Department of Paraclinical Studies of the School of Veterinary Medicine, University of Zambia. Each blood spot was analysed three times, on different plates. A rigorous system of quality assurance was adopted. The Optical Density (OD) of each ELISA sample tested was expressed as a percentage (percentage positivity) of the strong positive reference standard (Wright *et al.*, 1993). A cut-off of 28% positivity was used. Animals with a percentage positivity equal to or larger than 50% were treated with diminazene aceturate (Berenil®, Hoechst) at 7.0 mg/kg body weight and transferred immediately to a tsetse-free zone. Once an animal was transferred, blood spots continued to be collected at monthly intervals and were screened for anti-trypanosmal antibodies using the ELISA.

Two animals, one in the tsetse-infested and one in the tsetse-free area, were not transferred and served as controls. Blood spots were collected at monthly intervals and anti-trypanosomal antibody levels were determined as described above.

3.2.2.2 Data analysis

The decline in anti-trypanosmal antibody levels over time in each experimental animal was examined by regression analysis. The significance of the difference between the slope of the regression lines was tested by an analysis of variance (Sokal and Rohlf, 1998). The probability of an animal having anti-trypanosomal antibodies in consecutive months after treatment was calculated using a survival analysis (Bland, 1987). All analyses were performed using the statistical package SPSS (SPSS Inc.).

3.2.3 Results

The standard deviation of the repeated measurement of the percentage positivity of each sample was very small. Therefore, monthly averages were calculated of the repeated measures of each sample. The averages were used in the analysis.

3.2.3.1 Decline in percentage positivity in individual animals

(i) Control animals

During the 12 months observation period, the average percentage positivity of bloodspots collected from the control animal, in the tsetse-free zone, varied between months but never exceeded the cut-off value (Fig. 3.2.1). The control animal, kept in the tsetse-infested zone, developed anti-trypanosomal antibodies. It remained positive from month 5 onwards (Fig. 3.2.1).

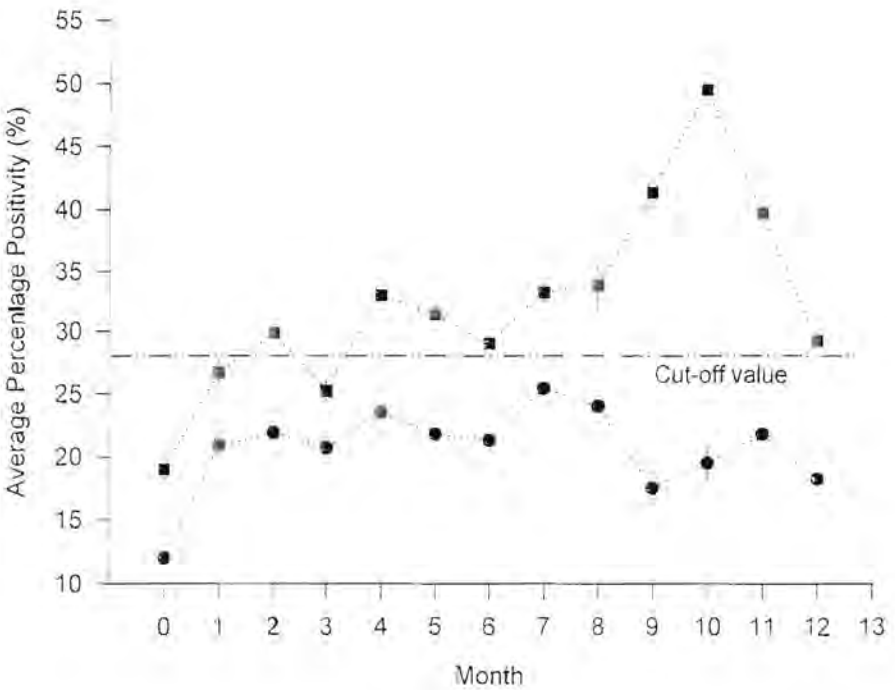


Figure 3.2.1: Average percentage positivity (%) (± 1 s.e) of the positive (■) and negative (●) control animal in consecutive months.

(ii) Animals transferred to tsetse-free zone

A total of 7 animals (henceforth referred to as animals 1 to 7) were treated and transferred from the tsetse-infested to the tsetse-free zone. The average percentage positivity of bloodspots at the moment of transfer was 70.6 ± 1.9%. The average percentage positivity declined rapidly in the absence of challenge and reached 37.1 ± 2.3% five months after transfer (Fig. 3.2.2). From month six onwards, the average

percentage positivity continued to decline but at a lower rate. It reached a level lower than the cut-off value (28%), 13 months after treatment (Fig. 3.2.2). The decline of anti-trypanosomal antibodies was linear with a change in slope six months after treatment (Fig. 3.2.2). Therefore, the decline in average percentage positivity was considered over two periods, i.e. months 0 to 5 and months 6 to 14.

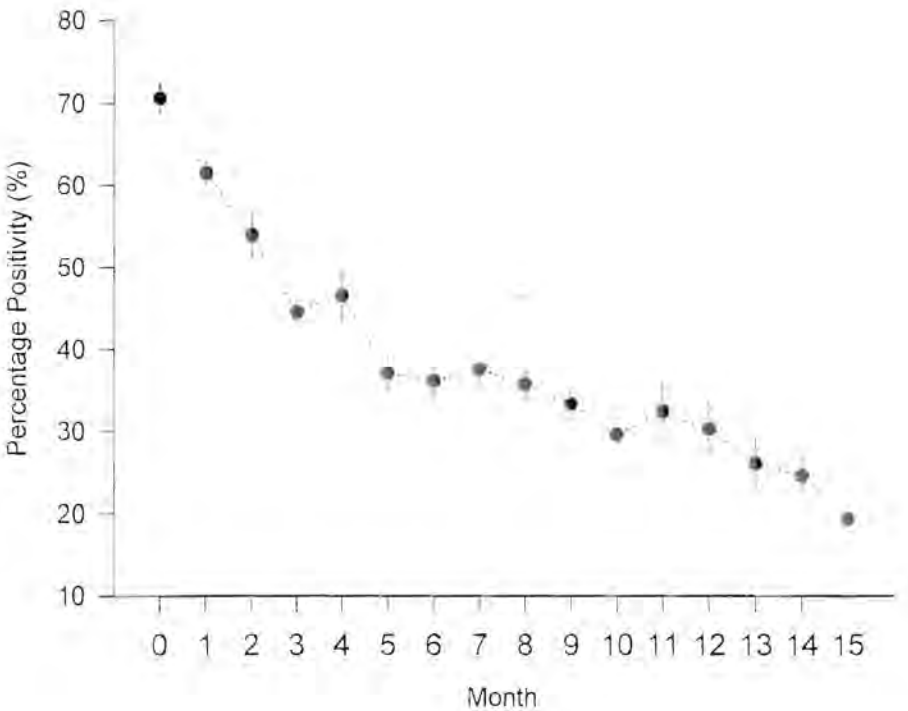


Figure 3.2.2: Average percentage positivity (%) (± 1 s.e.) in consecutive months of all experimental animals transferred to the tsetse-free zone.

3.2.3.2 Decline in percentage positivity during the first five months after transfer (Fig. 3.2.3)

The decline in the anti-trypanosomal antibody level, the first five months after treatment, was almost linear (Table 3.2.1). With the exception of animal 3, which had a temporary increase in anti-trypanosomal antibody level four months after treatment

(Fig. 3.2.3), the "time after treatment" explained between 91.4 and 98% of the variation in the anti-trypanosomal antibody level.

Table 3.2.1: Linear regression of anti-trypanosomal antibody level on months after treatment (months 0-5) for each of the experimental animals.

Animal	a	b	r	Significance
1	78.9	-9.1	0.98	P<0.001
2	76.8	-7.4	0.97	P<0.001
3	71.9	-5.2	0.65	P<0.004
4	70.8	-6.7	0.99	P<0.001
5	62.7	-8.1	0.96	P<0.001
6	60.4	-7.5	0.99	P<0.001
7	51.0	-4.7	0.96	P<0.001

a = intercept
b = regression coefficient
r = correlation coefficient

The decline in anti-trypanosomal antibody levels after treatment (quantified by the slope of the regression lines (b)) differed significantly between animals (Table 3.2.2) but was not affected by the antibody level at the moment of treatment (r = -0.51, P>0.05). The monthly decline in percentage positivity was, on average, 10% of the percentage positivity at the moment of treatment.

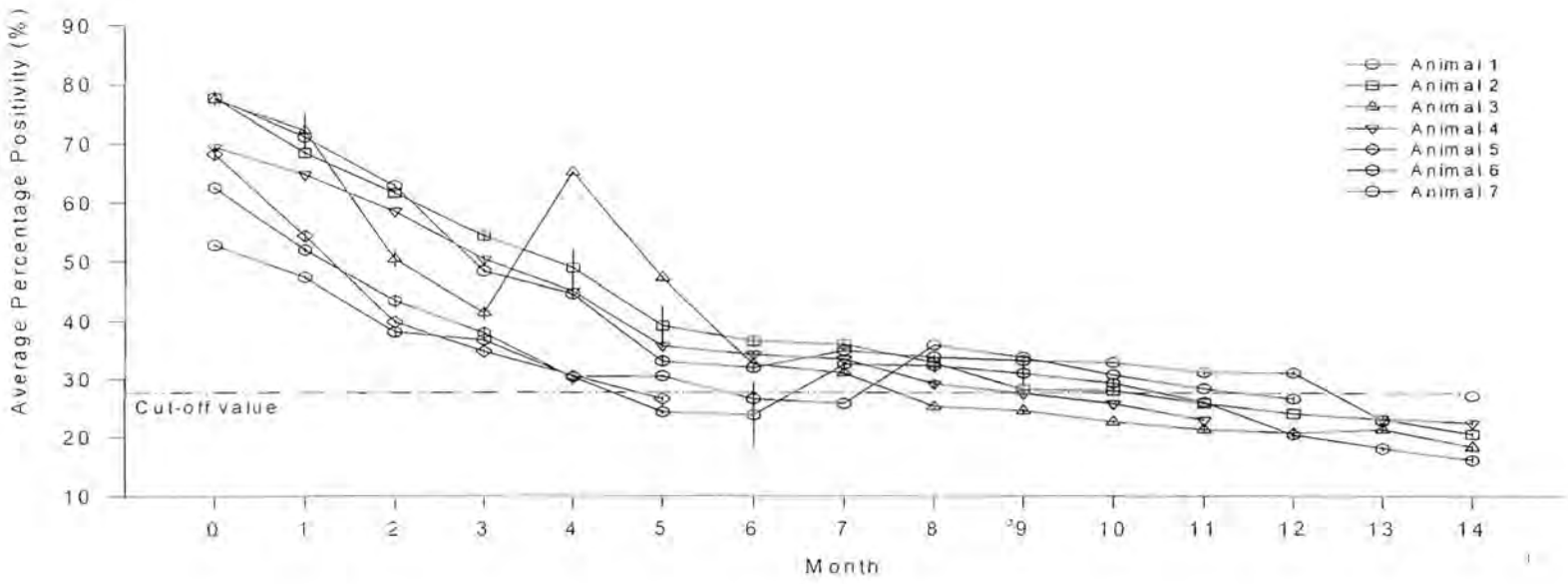


Figure 3.2.3: Average anti-trypanosomal antibody level (expressed as the average percentage positivity (%)) (± 1 s.e.) of animals 1-7 in consecutive months after treatment (month 0 being the month of treatment).

Table 3.2.2: Comparison between animals of the rate of antibody decline (slope of the regression lines) during the first five months after treatment.

	Animal						
	1	2	3	4	5	6	7
1	-	*	*	*	ns	*	*
2	*	-	ns	ns	ns	ns	*
3	*	ns	-	ns	ns	ns	ns
4	*	ns	ns	-	*	*	*
5	ns	ns	ns	*	-	ns	*
6	*	ns	ns	*	ns	-	*
7	*	*	ns	*	*	*	-

* = significant at the 0.05 level of P
ns = not significant

3.2.3.3 Decline in percentage positivity between months 6 and 14 after transfer (Fig. 3.2.3)

With the exception of animal 7, the anti-trypanosomal antibody level continued to decline between months 6 and 14 after treatment (Table 3.2.3). However, the rate of decline was substantially lower compared to the one observed during first five months.

Table 3.2.3: Table 2: Linear regression of anti-trypanosomal antibody level on months after treatment (months 6-14) for each of the experimental animals.

Animal	a	b	r	Significance
1	40.9	-1.0	0.67	P<0.01
2	48.9	-2.1	0.97	P<0.001
3	41.1	-1.7	0.93	P<0.001
4	43.1	-1.6	0.93	P<0.001
6	41.8	-1.6	0.68	P<0.001
7	34.8	-0.6	0.39	P>0.05

a = intercept
b = regression coefficient
r = correlation coefficient

The slope of the regression lines differed significantly between animals (Table 3.2.4) but was not affected by the antibody level at the moment of treatment (r = -0.76, P>0.05). For the majority of the animals the "time after treatment" explained most of the variation in the anti-trypanosomal antibody level (Table 3.2.3).

Table 3.2.4: Comparison between animals of the rate of antibody decline (slope of the regression lines) during months 6 and 14 after treatment.

	Animal					
	1	2	3	4	6	8
1	-	*	*	*	ns	ns
2	*	-	ns	*	ns	ns
3	*	ns	-	ns	ns	ns
4	*	*	ns	-	ns	ns
6	ns	ns	ns	ns	-	ns
8	ns	ns	ns	ns	ns	ns

* = significant at the 0.05 level of P
ns = not significant

Between month 6 and month 14, the monthly average decline in percentage positivity was 3.6% of the average percentage positivity in month 6 after treatment.

3.2.3.4 Survival analysis

Approximately 50% of the seropositive animals became seronegative 7.5 months after treatment (Fig. 3.2.4). All animals had have become seronegative 13 months after treatment (Fig. 3.2.4).

3.2.4 Discussion

The level of anti-trypanosomal antibodies, in specimens collected from all experimental cattle, declined rapidly after treatment with trypanocides. A similar rapid decline in the level of anti-trypanosomal antibodies after treatment has been observed by other workers (Wilson and Cunningham, 1971; Luckins, 1977; Dwinger *et al.*, 1988b; Bocquentin *et al.*, 1990). Luckins (1977), using a microplate ELISA with various antigens, found that antibodies persisted up to 83 days after treatment with diminazene aceturate. Bocquentin *et al.* (1990), using an ELISA with *T. congolense* antigen, detected anti-trypanosomal antibodies up to 116 days after treatment but persistence differed significantly between experimental animals. All these results concur with those obtained using the indirect fluorescent antibody test (IFAT) to detect anti-trypanosomal antibody (Wilson and Cunningham, 1971; Zwart *et al.*, 1973; Bocquentin *et al.*, 1990). However, contrary to the above-mentioned work, the majority of the experimental animals were still seropositive after this initial phase of rapid decline in antibody level. It took another 8 months before the anti-trypanosomal antibodies had disappeared in all experimental animals. During this period (months 6 to 13), the antibody level continued to decline significantly though at a much lower rate.

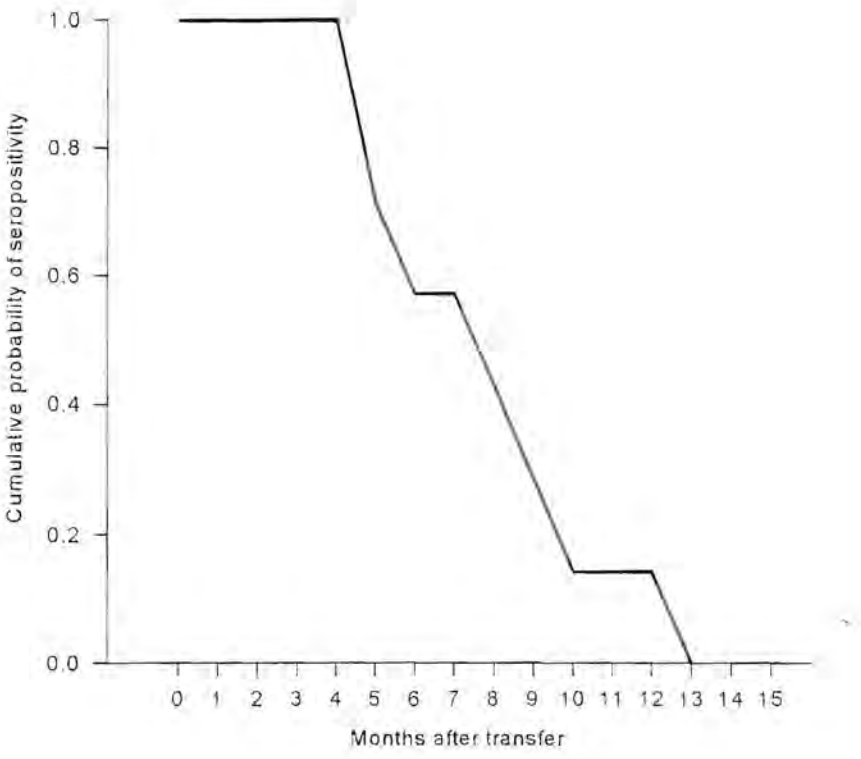


Figure 3.2.4: Cumulative probability of animals having anti-trypanosomal antibodies in consecutive months after treatment.

These data suggest that the persistence of anti-trypanosomal antibodies after treatment with trypanocidal drugs is much longer than has been suggested previously. This is in accordance with observations made by Authié *et al.* (1993) and Hopkins (1997). In both cases, anti-trypanosomal antibodies were present up to 10 months after treatment of a *T. congolense* infection with diminazene aceturate. The decline in the levels of anti-trypanosomal antibodies observed by Authié *et al.* (1993) was similar to the one in the present experiments. The antibody levels decreased progressively until Day 50 and remained higher than the pre-challenge levels for several months (Authié *et al.*, 1993). The question remains as to why the persistence of anti-trypanosomal antibodies is so much higher in the present study compared to those of many others. The most likely explanation is the difference in the sensitivity of the various tests. The high sensitivity of the test used in this experiment, compared to those used by other workers, can be explained as follows. First, the ELISA used in the present experiment made use of a *T. congolense* antigen for coating (Hopkins *et al.*, 1998). *Trypanosoma congolense* is the dominant trypanosome species in the area. Second, the cut-off value used in the present test was determined by making use of highly representative reference samples (Greiner *et al.*, 1997a). The cut-off value used for the antibody-ELISA used in this experiment was determined using blood spots collected from Mashona breed cattle kept in a tsetse-free area of Zimbabwe. The positive blood spots were collected from parasitologically positive animals in Zimbabwe and eastern Zambia. Hence, samples used to determine the cut-off value were obtained from representative reference populations. The accuracy of the cut-off can still be questioned. However, notwithstanding the fluctuations in the average percentage positivity of the negative control animal, the average percentage positivity never exceeded the cut-off value (28%). Moreover, during a five-month serosurveillance exercise conducted in the tsetse-free area, described above only five (2.2%) of a total of 222 animals had trypanosomal antibody values higher than the cut-off value. Both observations suggest that an appropriate cut-off value was used. Furthermore, if the experimental animals had been seronegative five months after treatment, the average percentage positivity would not be expected to decline over time but fluctuate around the same value as was the case in the negative control animal.

The observed dynamics of the anti-trypanosomal antibody levels in cattle after treatment with trypanocides have important practical implications. Sentinel herds of cattle are often used when monitoring the effectiveness of vector control operations. These herds are followed up at regular intervals and the parasitological incidence of trypanosomosis is determined. The value of this type of monitoring depends largely on the sensitivity of the diagnostic tests. Because of the low diagnostic sensitivity of tests for the parasitological diagnosis of trypanosomosis, results from such surveillance exercises should be interpreted with caution. The apparent absence of an infection does not necessarily mean the complete absence of challenge. More sensitive diagnostic methods are required to establish unequivocally the effect of the control intervention. The anti-trypanosomal antibody detection ELISA may offer this possibility. The present results have shown that, in the absence of challenge, the levels of anti-trypanosomal antibodies decline steeply in animals treated with trypanocidal drugs. Hence, the establishment of sentinel herds consisting of seropositive cattle that have been treated with trypanocides and the follow-up of the decline in the anti-trypanosomal antibody level over time may be a useful adjunct to evaluating the effectiveness of a tsetse control intervention.

3.3 An investigation of non-specific cross reactions in an anti-trypanosomal antibody detection ELISA for the diagnosis of bovine trypanosomosis

3.3.1 Introduction

The anti-trypanosomal antibody detection enzyme-linked immunosorbent assay (antibody-ELISA) has high diagnostic sensitivity and specificity (Luckins, 1977) which makes it a useful tool to supplement parasitological diagnostic methods that have variable, but generally low, sensitivity (Paris *et al.*, 1982; Desquesnes and Tresse, 1996). The diagnostic sensitivity may be affected by the occurrence of non-specific reactions. For example, the capillary tube agglutination test for measuring anti-trypanosomal antibodies in bovine and human serum gave positive reactions in cattle infected with *Theileria* spp. (Robson, 1972). When developing and evaluating the anti-trypanosomal antibody detection ELISA for bovines, the possibility of non-specific cross reactions was investigated using sera infected with various parasites (Luckins, 1977). Recently, the antibody ELISA was further developed for the analysis of blood samples collected on filter paper (Hopkins *et al.*, 1998). Although this new version of the antibody ELISA distinguishes between pathogenic and non-pathogenic (*T. theileri*) trypanosomes (Hopkins *et al.*, 1998), further research in non-specific cross reactions is required. Tick-borne diseases of cattle, especially anaplasmosis and babesiosis, are very common in many areas of southern Africa where tsetse-transmitted trypanosomosis is present. Non-specific cross reactions between the antibodies against those tick-borne parasites and the antibodies developed against trypanosomes would obviously result in misinterpretation of trypanosomosis survey and surveillance data. Therefore, the occurrence of such cross reactions in the acute and chronic phase of anaplasmosis (*Anaplasma marginale*) and babesiosis (*Babesia bigemina*) was investigated.

3.3.2 Materials and methods

3.3.2.1 Experimental animals

Blood samples were collected from Mashona breed adult cattle in Mudzi District (Mashonaland East Province) of Zimbabwe. Trypanosomosis-positive samples were obtained from cattle in areas immediately adjacent to the Mozambique

border where tsetse (*G. m. morsitans* and *G. pallidipes*) are present. Samples from the parasitologically negative and tick-borne parasite infected animals were obtained from cattle kept in areas cleared of tsetse immediately west of a target barrier that prevents tsetse from re-invading (Section 5.6). In these areas, trypanosomosis was still present but the incidence of trypanosomal infections was very low (Section 5.6). Tick control was irregular. The main tick-borne diseases in the sampling area are those caused by *Anaplasma marginale* or *Babesia bigemina* (Norval *et al.*, 1983). Heartwater and theileriosis were virtually absent (Norval *et al.*, 1985; Peter *et al.*, 1998).

In the first phase of the experiment, a survey was conducted in the two areas. Since no distinction could be made between acute or chronic tick-borne infections, a second phase was initiated in March 1999. During this phase (March -August 1999), two sentinel herds each of 62 adult Mashona breed cattle were established. One herd was based in the tsetse-infested area along the Mozambique border and the other was herded in the area protected by the target barrier. Sentinel animals were sampled at monthly intervals to detect new (acute) tick-borne infections. Parasitologically positive (trypanosomosis or tick-borne diseases) sentinel animals were treated when the packed cell volume (PCV) was lower than 20%.

3.3.2.2 Diagnostic methods

Parasitological and serological methods were used for diagnosis. Blood was collected from an ear vein into heparinized microhaematocrit centrifuge capillary tubes and onto glass slides, as thick and thin blood smears. The capillary tubes were sealed with "Cristaseal" (Hawksley) and centrifuged immediately in a microhaematocrit centrifuge for 5 min. at 9 000 rpm. After centrifugation, the PCV was determined. Animals with a PCV $\leq$ 24% were considered to be anaemic. The buffy coat and the uppermost layer of red blood cells of each specimen were extruded onto a microscope slide and examined for the presence of motile trypanosomes. Samples were examined with a phase-contrast microscope with a x 40 objective lens. Giemsa-stained thick and thin blood smears were examined under x 100 oil immersion objective lens for the presence of *Trypanosoma* spp., *A. marginale* or *B. bigemina*.

From most of the animals, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect ELISA (Hopkins *et al.*, 1998). A rigorous system of quality assurance was adopted. The Optical Density (OD) of each ELISA sample tested was expressed as a percentage (percentage positivity, PP) of the strong positive reference standard (Wright *et al.*, 1993). A cut-off of 28% positivity was used. At this cut-off the assay had a sensitivity of 88.5% and a specificity of 99.0%.

3.3.2.3 Statistical analysis

Samples were divided into four groups, i.e. parasitologically negative, *Trypanosma*-infected, *Anaplasma*-infected and *Babesia*-infected. A distinction was made between samples collected during the survey and those collected during surveillance. The average PCV and the percentage positivity of the four groups were compared using parametric or non-parametric statistical tests (Sokal and Rohlf, 1998). All statistical analyses were performed using the statistical package SPSS (SPSS Inc.).

3.3.3 Results

A total of 1 369 blood samples was collected. The average PCV differed significantly between the four groups ($P<0.01$ for samples collected during the survey and during surveillance) (Table 3.3.1 and Fig. 3.3.1). The PCV was highest in the parasitologically negative group and lowest in the animals infected with tick-borne parasites.

Table 3.3.1: Average PCV (± 1 s.e.) of parasitologically negative, trypanosome-infected and tick-borne parasite-infected samples collected during the survey and the surveillance.

Disease status	Survey		Surveillance	
	n	Average PCV (± 1 s.e.)	n	Average PCV (± 1 s.e.)
Negative	665	28.5 ± 0.3	513	30.4 ± 0.1
Trypanosoma spp.	36	26.4 ± 0.4[a]	23	26.7 ± 1.2[a]
A. marginale	60	23.4 ± 0.6[b]	51	25.1 ± 0.6[a]
B. bigemina	12	25.0 ± 1.1[ab]	9	24.3 ± 1.3[a]

Averages followed by the same letter are not significantly different at P<0.05.

A total of 1 251 blood spots was analysed for the presence of anti-trypanosomal antibodies. The average percentage positivity was significantly higher in the trypanosome-infected (92% *T. congolense*) group. It did not differ between parasitologically negative animals and animals infected with tick-borne parasites (Table 3.3.2 and Fig. 3.3.2). The differences in average percentage positivity of samples collected from cattle with tick-borne parasites, during the survey or during the surveillance, were not significant.

Table 3.3.2: Average percentage positivity (± 1 s.e.) of samples collected from parasitologically negative, trypanosome-infected and tick-borne parasite-infected cattle during the survey and the surveillance.

Disease status	Survey		Surveillance	
	n	Average PP (± 1 s.e.)	n	Average PP (± 1 s.e.)
Negative	665	19.2 ± 0.3[a]	401	19.3 ± 0.3[a]
Trypanosma spp.	36	39.8 ± 1.8	23	34.8 ± 1.9
A. marginale	60	18.8 ± 0.6[a]	46	19.8 ± 0.9[a]
B. bigemina	12	20.3 ± 2.1[a]	8	19.2 ± 2.9[a]

Averages followed by the same letter are not significantly different at P<0.05.

The majority (91.5%) of the samples collected from trypanosome-infected cattle had anti-trypanosomal antibodies (Table 3.3.3). The proportion of cattle, infected with *A.*

marginale or *B. bigemina*, with anti-trypanosomal antibodies was low (9.5%) and differed little from the proportion of parasitologically negative animals with anti-trypanosomal antibodies (10.1%) (Table 3.3.3).

Table 3.3.3: Number of samples with anti-trypanosomal antibodies collected from parasitologically negative, trypanosome-infected and tick-borne parasite-infected cattle during the survey and the surveillance.

Disease status	Survey		Surveillance	
	Number analysed	Number positive (%)	Number analysed	Number positive (%)
Negative	665	59 (8.9)	401	49 (12.2)
Trypanosoma spp.	36	34 (94.4)	23	20 (86.9)
A. marginale	60	2 (3.3)	46	7 (15.2)
B. bigemina	12	2 (16.7)	8	1 (12.5)

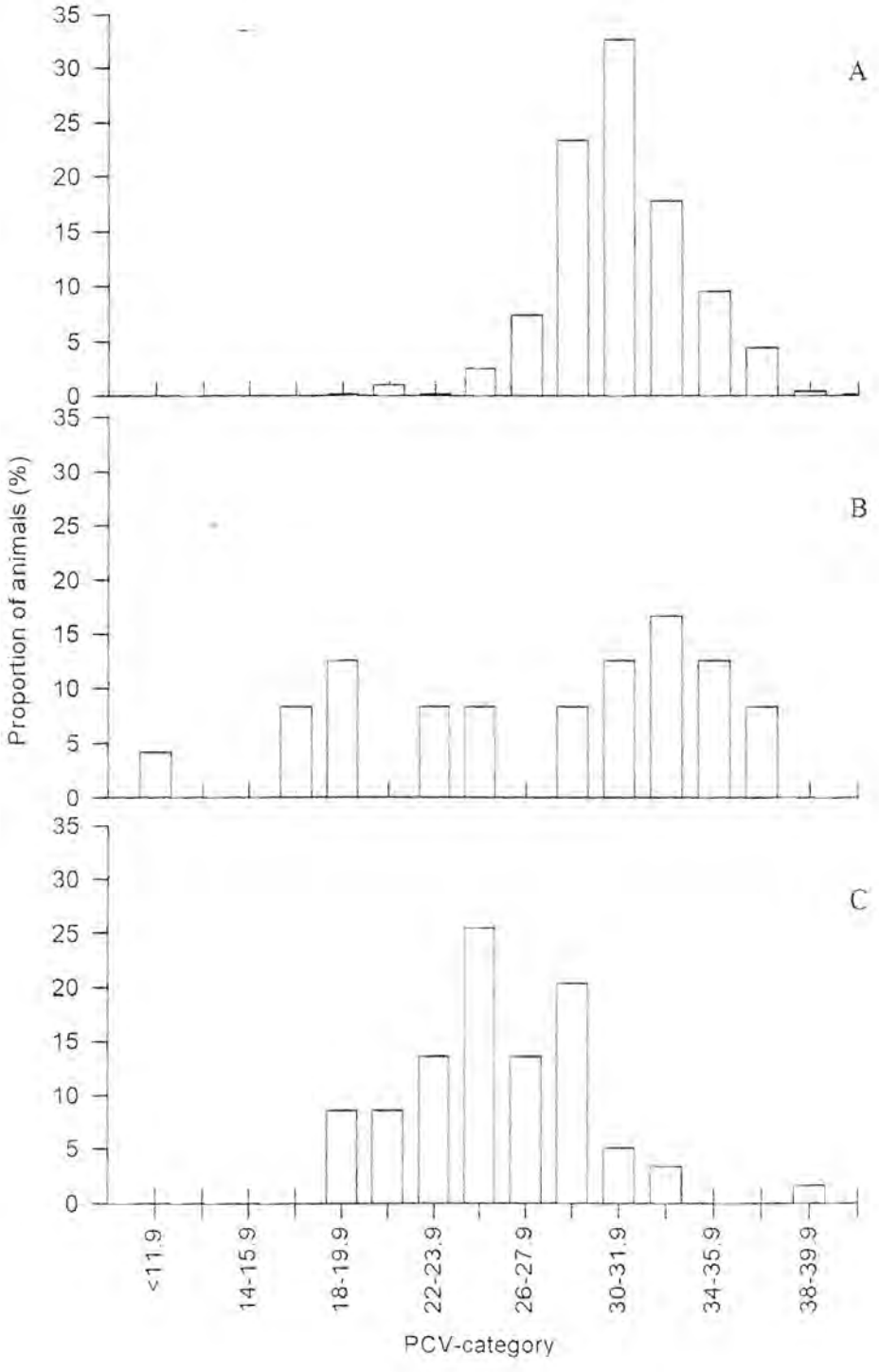


Figure 3.3.1: PCV-distribution of parasitologically negative (A), trypanosome infected (B) and tick-borne parasite-infected (C) sentinel cattle.

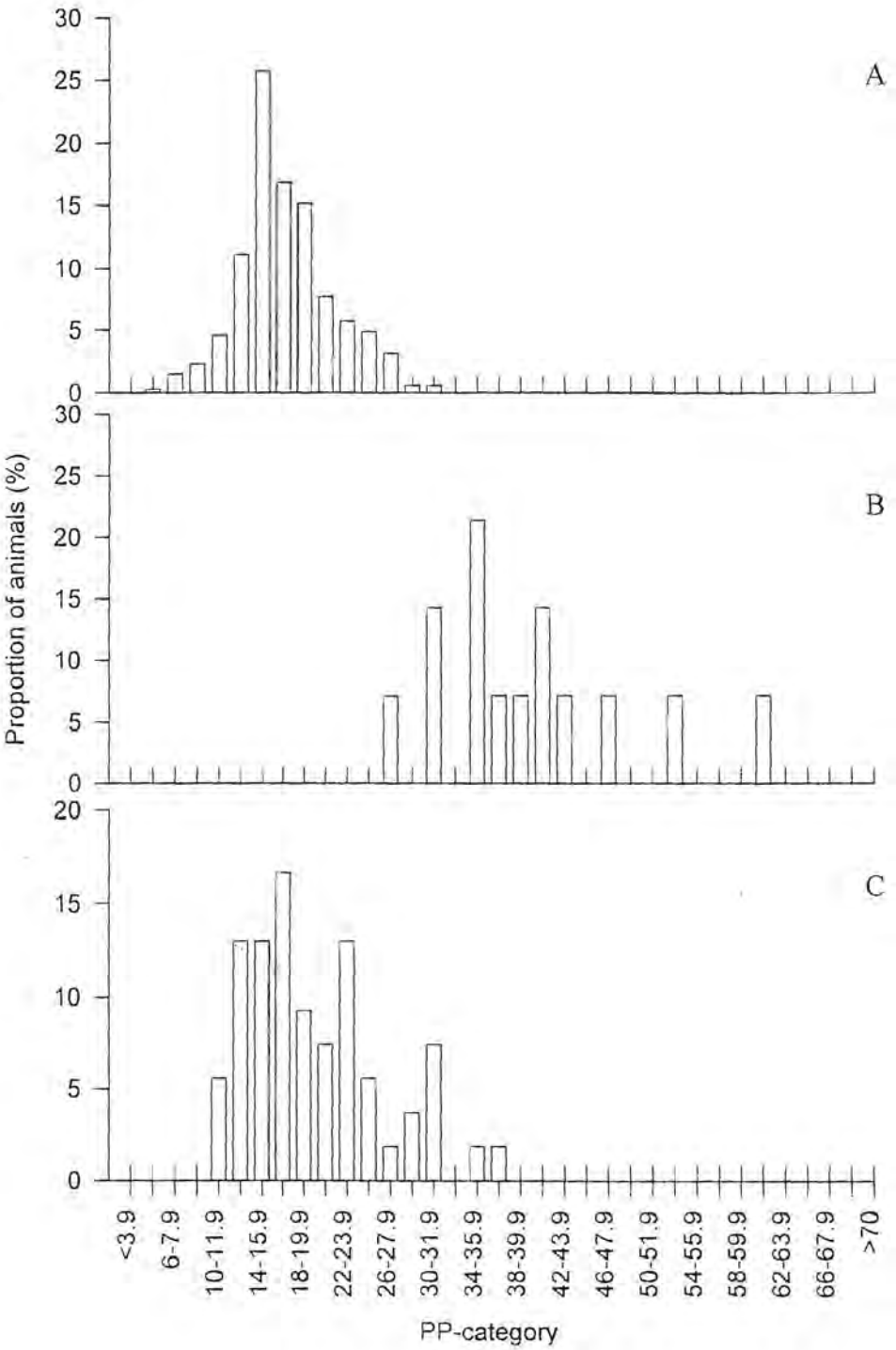


Figure 3.3.2: Distribution of the percentage positivity of samples collected from parasitologically negative (A), trypanosome-infected (B) and tick-borne parasite-infected (C) sentinel cattle.

3.3.4 Discussion

The results of this study show that the presence of a *B. bigemina* and *A. marginale* infection did not result in non-specific cross reactions. Although some animals infected with tick-borne parasites did react positively on the ELISA, the proportion of seropositives in the tick-borne parasite infected groups differed little from the proportion of seropositives in the parasitologically negative group. This observation suggests that the reasons for the occurrence of anti-trypanosomal antibodies in the cattle infected with tick-borne parasites do not differ from the reasons for the occurrence of anti-trypanosomal antibodies in the parasitologically negative animals. Such reasons could be false positive reactions and, since trypanosomosis is not completely absent, trypanosomal infections that were not detected or persistent anti-trypanosmal antibodies. It was difficult to establish if the cattle found to be infected with *Babesia* or *Anaplasma* during the survey were in the acute of chronic phase of infection. Progressive anaemia is a typical sign of patent *Babesia* and *Anaplasma* infections in cattle (de Vos and Potgieter, 1994; Potgieter and Stoltsz, 1994). The low average PCV of both the *Anaplasma*-and *Babesia*-infected group, therefore, suggests that a substantial proportion of the infected cattle must have been in the acute phase of the infection. Since sentinel cattle were sampled at monthly intervals, they were in the acute phase of the *Babesia* or *Anaplasma* infection upon the detection of the parasites. Despite the presence of this acute tick-borne infection, the proportion of sentinel cattle with anti-trypanosomal antibodies differed little from the proportion of parasitologically negative sentinel animals that had anti-trypanosomal antibodies. Hence, results show that non-specific cross reactions with antibodies against *A. marginale* and *B. bigemina* were absent in both the acute or latent stages of both tick-borne diseases.

This study showed that the ELISA was highly sensitive for trypanosomal infections. The majority of the animals infected with trypanosomes were identified by the anti-trypanosomal antibody ELISA. These results confirm the value of the test in trypanosomosis surveys.

3.4 The distribution and epidemiology of bovine trypanosomosis in Malawi

3.4.1 Introduction

Tsetse have been reported in Malawi since the end of the 19th century (Austen, 1903). Nevertheless, the distribution of bovine trypanosomosis in Malawi was only mapped in the late 1980s (Davison, 1990). This national survey used parasitological diagnostic methods and revealed a disease distribution pattern that in most areas was correlated with the distribution of the vector. Unfortunately, the parasitological diagnostic methods for trypanosomosis have relatively low sensitivity (Paris *et al.*, 1982). Hence, many areas where the disease is present at low prevalence or where trypanocidal drugs are used frequently may not be detected and may thus be considered disease-free. Similarly, the sampling methods for tsetse are heavily biased and lack sensitivity especially for *G. m. morsitans* and *G. brevipalpis* two species present in Malawi (Hargrove, 1980b).

To improve the accuracy of bovine trypanosomosis distribution maps, an anti-trypanosomal antibody detection Enzyme-Linked Immunosorbent Assay (ELISA) (Luckins, 1977) was recently further developed for use in large-scale surveys (Hopkins *et al.*, 1998). The test has high sensitivity and specificity. It has the advantage that it detects antibodies against current and past trypanosomal infections. This makes it possible to identify areas where tsetse challenge is seasonal, where tsetse are present but cannot be detected and/or where trypanocidal drugs are used frequently.

To support the development of a strategy for the control of tsetse-transmitted trypanosomosis in Malawi a survey was conducted to update the distribution of bovine trypanosomosis. Use was made of parasitological and serological diagnostic methods. This section summarizes the findings of this survey. The usefulness of anti-trypanosomal antibody prevalence data is discussed and the findings of the survey are used to clarify the epidemiology of bovine trypanosomosis in Malawi.

3.4.2 Material and methods

3.4.2.1 Sampling sites and sample selection

Between August 1995 and June 1997 a total of 9 309 adult cattle were examined at 159 sampling sites (henceforth referred to as herds). Sampling was restricted to areas where cattle were present permanently and attempts were made to distribute the sampling sites evenly over 23 districts in the Northern, Central and Southern Regions of Malawi. A cross-sectional sampling method was applied. Sample sizes were calculated according to Cannon and Roe (1982) and depended on the total cattle population at a particular sampling site but never exceeded 60 head of cattle at a single sampling site. Sample sizes were calculated to provide 95% certainty of detecting at least one positive case at a prevalence of 5%.

3.4.2.2 Diagnostic methods

The buffy coat, stained thick and stained thin smear were used as parasitological diagnostic tests (Section 3.3.2.2).

From most of the animals from 150 herds, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect ELISA (Section 3.3.2.2). All statistical analyses were performed using the statistical package SPSS (SPSS Inc.).

3.4.3 Results

3.4.3.1 Parasitological prevalence of bovine trypanosomosis

A total of 186 trypanosomal infections (1.9%) was diagnosed in cattle from 27 herds. The majority of infections were *T. congolense* (Table 3.4.1).

Table 3.4.1: Species prevalence of trypanosomal infections in cattle sampled in Malawi.

Trypanosome species	Number of infections	Trypanosome species prevalence (%)
T. congolense	176	94.6
T. vivax	9	4.8
T. brucei	1	0.6

The parasitological herd prevalence of trypanosomosis varied between 1.7% and 42.8% (on average 12.2 ± 2.1%).

3.4.3.2 Prevalence of anti-trypanosomal antibodies

A total of 966 blood spots out of a total of 6 810 samples (14.2%) had anti-trypanosomal antibodies (Table 3.4.2). The majority of parasitologically positive animals were seropositive (80.8%) and 12.9% of the parasitologically negative animals had anti-trypanosomal antibodies (Table 3.4.2).

Table 3.4.2: Number of parasitologically negative and positive animals with and without anti-trypanosomal antibodies.

	Serologically		Total
Parasitologically	Positive (%)	Negative (%)	
Positive	105 (80.8)	25 (19.2)	130
Negative	861 (12.9)	5819 (87.1)	6680

Only 6 out of 9 parasitologically positive samples (66.7%) of the animals infected with *T. vivax* had anti-trypanosomal antibodies whereas 99 out of 120 parasitological positive samples (82.5%) of the animals with a *T. congolense* infection were seropositive. The average percentage positivity was much higher in serologically

positive and parasitologically positive animals (53.7 ± 3.3%) compared to serologically positive but parasitologically negative animals (41.1 ± 0.7%).

The majority of the parasitologically positive herds (92.4%) were seropositive whereas 71.8% of the parasitologically negative herds were seropositive (Table 3.4.3). The prevalence of trypanosomal infections in the parasitologically positive herds was significantly correlated with the proportion of animals with anti-trypanosomal antibodies (r = 0.28, P<0.01) in those herds and their average percentage positivity (r = 0.29, P<0.01). Only 5.4% of all serologically negative herds contained animals with trypanosomal infections (Table 3.4.3).

Table 3.4.3: Number of parasitologically negative and positive herds with animals with anti-trypanosomal antibodies.

	Serologically		Total
Parasitologically	Positive (%)	Negative (%)	
Positive	24 (92.4)	2 (7.7)	26
Negative	89 (71.8)	35 (28.2)	124

3.4.3.3 Packed cell volume

The average PCV of parasitologically negative animals (31.4 ± 0.05%) was significantly higher (P<0.001) than the average PCV of parasitologically positive animals (22.5 ± 0.5%) (Fig. 3.4.1). It increased with decreasing percentage positivity (r = -0.12, P<0.001). Similarly, the average PCV was significantly lower in seropositive (30.1 ± 0.2%) compared to seronegative animals (31.9 ± 0.07%) (P<0.001). The average PCV of parasitologically negative animals that were also seronegative was significantly higher than the average PCV of parasitologically negative cattle in which anti-trypanosomal antibodies were detected (P<0.001) (Table 3.4.4).

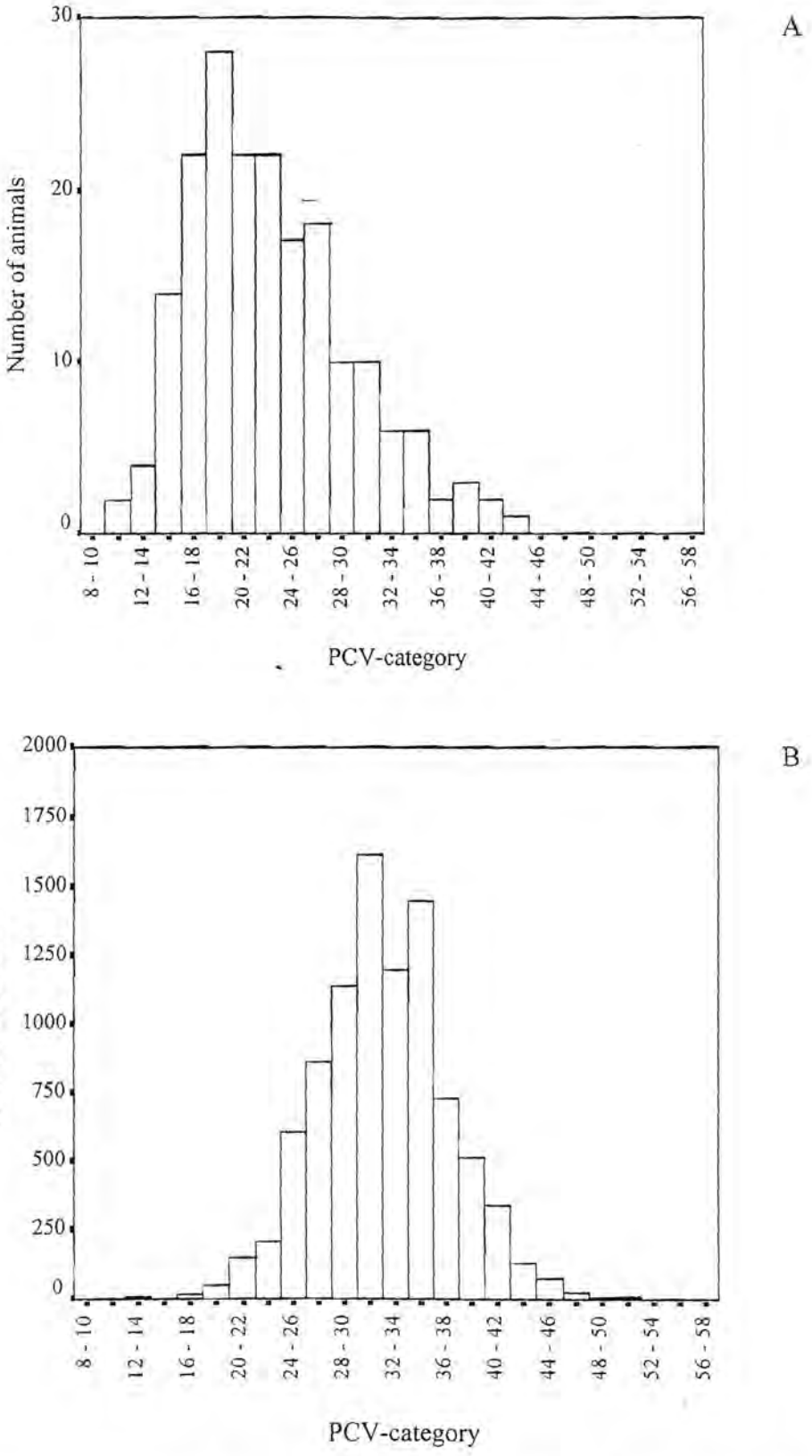


Figure 3.4.1: PCV-profile of parasitologically positive (A) and parasitologically negative (B) animals.

Table 3.4.4: Average PCV (% ± 1 s.e.) of serologically and parasitologically positive and negative animals.

	Serologically	
Parasitologically	Positive	Negative
Positive	21.7 ± 0.8[a]	22.8 ± 0.6[a]
Negative	30.6 ± 0.2	31.7 ± 0.1

Averages followed by the same letter are not significantly different at P<0.05 (Tukey-Kramer test)

The herd average PCV was significantly lower in parasitologically positive herds compared to parasitologically negative herds (29.9 ± 0.44% compared to 31.7 ± 0.18, P<0.001) and was significantly, negatively correlated with the prevalence of infection (r = -0.66, P<0.001,) (Fig. 3.4.2). It was highest in parasitologically and serologically negative herds (Table 3.4.5).

Table 3.4.5: Average PCV (% ± 1 s.e.) of serologically and parasitologically positive and negative herds.

	Serologically	
Parasitologically	Positive	Negative
Positive	29.8 ± 0.5[b]	31.6 ± 0.3[ab]
Negative	31.7 ± 0.2[ab]	31.9 ± 0.3[a]

Averages followed by the same letter are not significantly different at P<0.05 (Tukey-Kramer test)

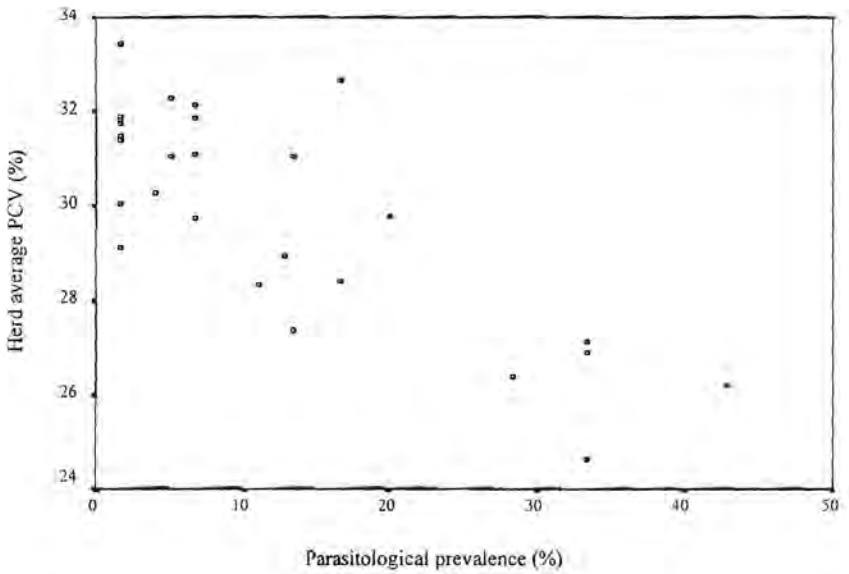


Figure 3.4.2: Scatterplot of the relationship between parasitological prevalence of trypanosomosis and herd average packed cell volume.

The herd average PCV of parasitologically negative but seropositive herds decreased with increasing average percentage positivity (r = -0.24, $P<0.05$) but was not correlated with the proportion of seropositive animals in the herd.

3.4.3.4 Distribution of bovine trypanosomosis in Malawi

(i) Northern Region

Several bovine trypanosomosis foci were identified in the Northern Region (Table 3.4.6 and Fig. 3.4.3).

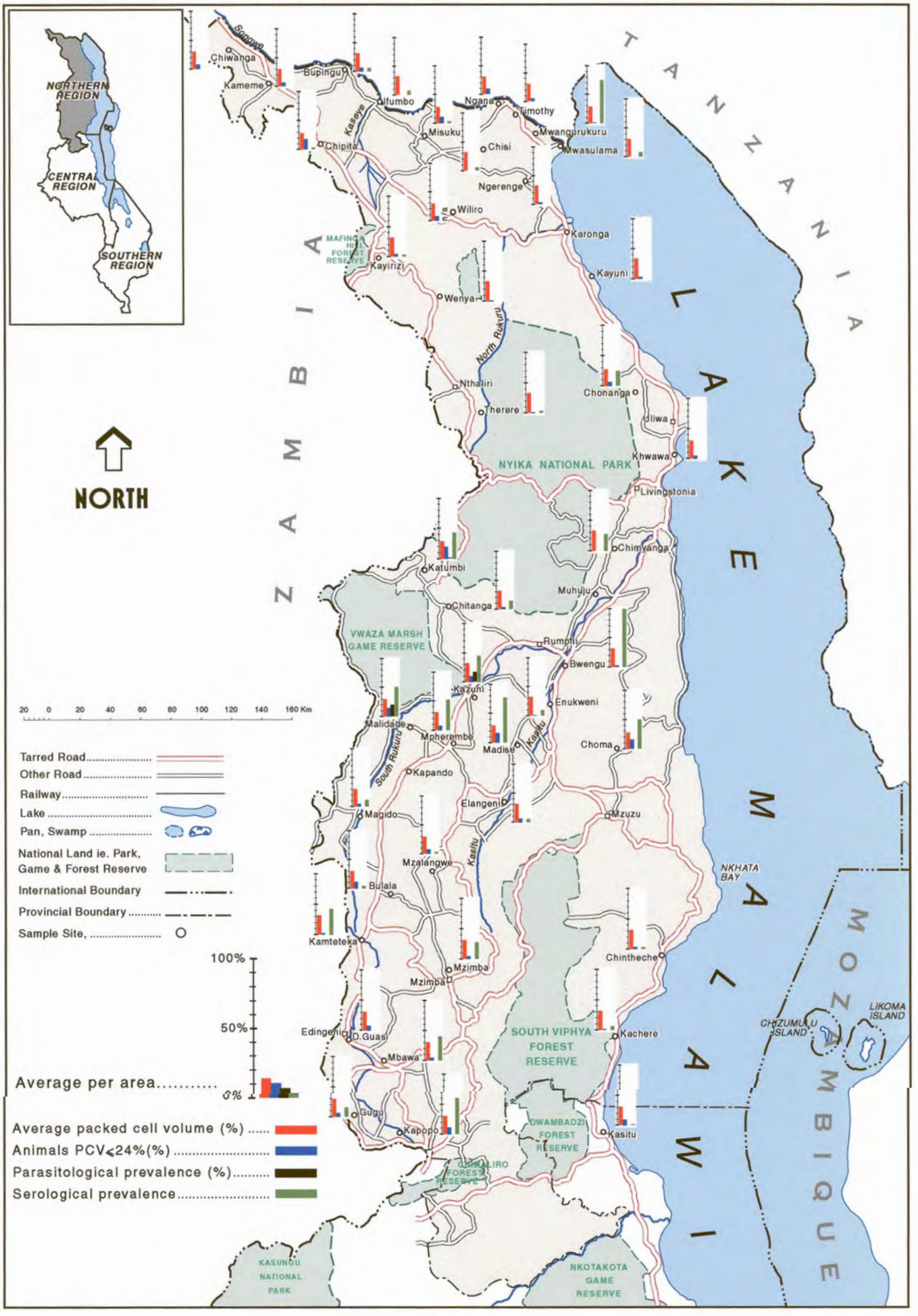
Trypanosomal infections were detected in animals sampled in Rumphi (*T. congolense* (9), *T. vivax* (2), *T. brucei* (1) and mixed (1)) and Mzimba (*T. congolense* (9) and *T. vivax* (1)) Districts. In Rhumpi District, parasitologically positive herds were located within 10-15 km from the edge of the Vwaza Game Reserve (Fig. 3.4.3). Anti-trypanosomal antibodies, on the other hand, were found in animals sampled up to 40 km east of the Game Reserve. The proportion of animals with anti-trypanosomal antibodies was high (on average 32.1 ± 10.7%) in herds sampled along the Zambian

border south west of Mzimba along the South Rukuru river (Fig. 3.4.3). However, no trypanosomal infections were detected using parasitological diagnostic tests. In Karonga and Chitipa Districts, no trypanosomal infections were detected and the prevalence of anti-trypanosomal antibodies was generally low (on average 5.0 ± 1.7%). One exception, however, was Mwangurukuru crushpen, situated close to Lake Malawi along the border with Tanzania (Fig. 3.4.3), where the prevalence of animals with anti-trypanosomal antibodies was high (75.1%). No trypanosomal infections were detected in cattle sampled along the shores of Lake Malawi. The prevalence of anti-trypanosomal antibodies was relatively high in cattle sampled at Chonanga and Chimyanga crushpens (28.2% and 26.5%, respectively), immediately east of the Nyika National Park (Fig 3.4.3).

Table 3.4.6: Average PCV (%), parasitological and anti-trypanosomal antibody prevalence (%) in each of the districts surveyed in the Northern Region of Malawi.

District	Number of herds	Average PCV (% ± 1 s.e.)	Parasitology		Serology	
			Number positive (%)	Number sampled	Number positive (%)	Number sampled
Chitipa	10	31.1 ± 0.2	0 (0)	600	11 (3.0)	364
Karonga	10	31.1 ± 0.2	0 (0)	600	45 (11.1)	404
Mzimba	13	30.7 ± 0.2	10 (1.1)	899	183 (26.3)	695
Nkhata Bay	2	32.1 ± 4.5	0 (0)	120	4 (4.6)	86
Rumphi	9	31.3 ± 0.2	13 (2.7)	481	134 (34.2)	392

Figure 3.4.3: Herd average PCV, prop[illegible]ic animals, parasitological and serological prevalence of bovine trypanosomosis in the Northern Region of Malawi.



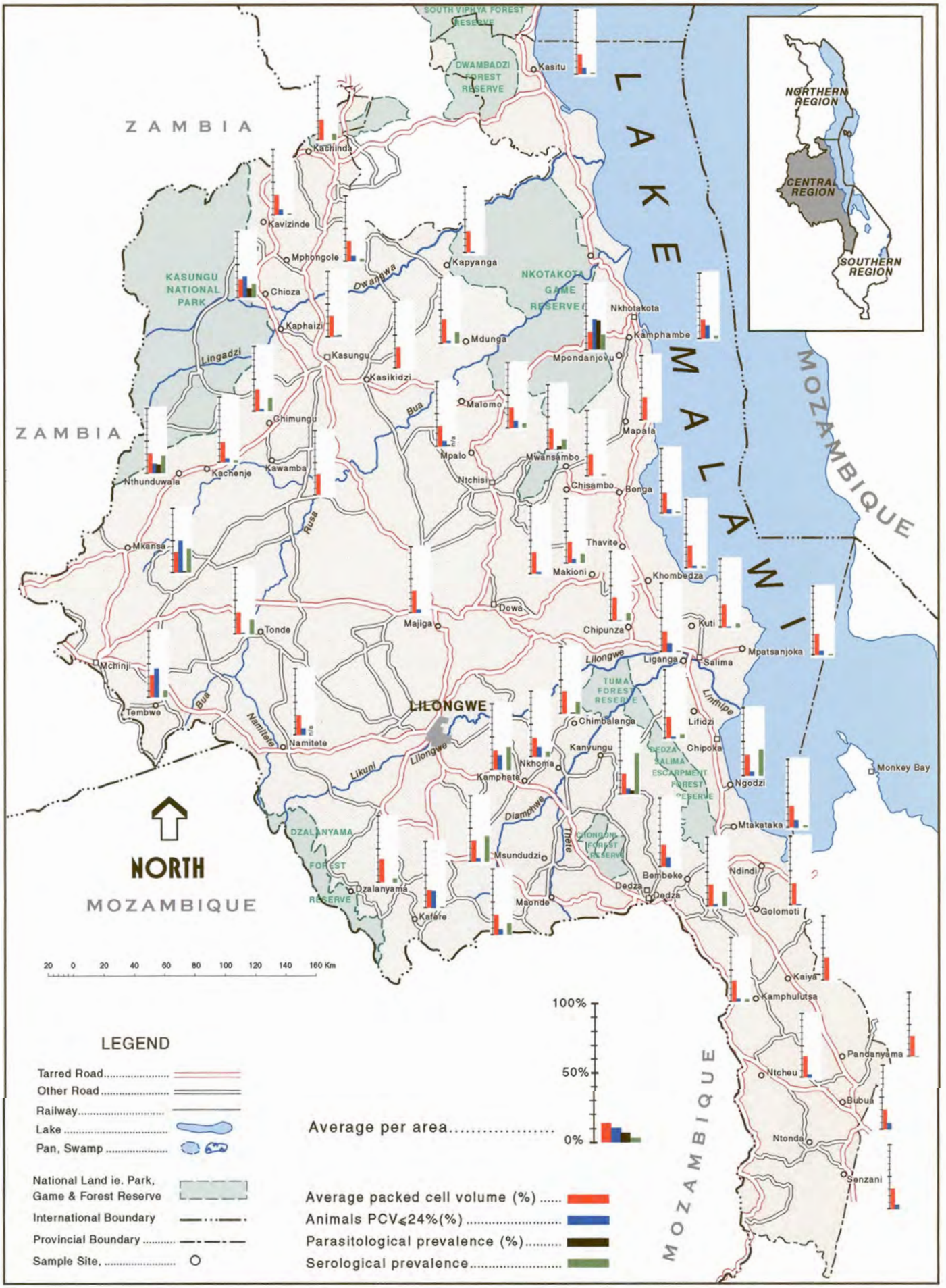
(ii) Central Region

In the Central Region, trypanosomal infections were detected in cattle sampled in the vicinity the Kasungu National Park and the Nkhotakota Game Reserve (Table 3.4.7 and Fig. 3.4.4). However, the prevalence of infection and the prevalence of cattle with anti-trypanosomal antibodies were generally low. The parasitological prevalence of trypanosomosis (42.8%, all *T. congolense*) and the proportion of anaemic animals (45.2%) was highest in cattle sampled at Mpondanjovu crushpen situated between the Nkhotakota Game Reserve and the shore of Lake Malawi (Fig. 3.4.4). Along the boundary of Kasungu National Park, trypanosomal infections were diagnosed in animals sampled at Chioza (8 *T. congolense* (13.7%)), Kaphaizi (1 *T. vivax* (1.7%)) and Nthunduwala crushpens (8 *T. congolense* (13.3%)) all situated within a 10-15 km wide band along the edge of the National Park (Fig. 3.4.4). The prevalence of anti-trypanosomal antibodies in cattle sampled in this band, was 11.3 ± 4.2%. In Lilongwe and Dedza Districts, trypanosomal infections (*T. congolense* (3) and *T. vivax* (1)) were found in cattle sampled along the Tuma Forest Reserve and the Dedza-Salima Escarpment Forest Reserve (Fig. 3.4.4). The prevalence of animals with anti-trypanosomal antibodies was generally high along the Lilongwe-Dedza road (Kanyungu (62.0%), Msundudzi (47.6%) and Kamphata (34.6%)). With the exception of animals sampled at Ngodzi crushpen (antibody prevalence of 38.5%), trypanosomosis was virtually absent in cattle sampled along the lakeshore in the Central Region (Salima District). Trypanosomosis was also absent in Ntcheu and Dowa Districts.

Table 3.4.7: Average PCV (%), parasitological and anti-trypanosomal antibody prevalence (%) in each of the districts surveyed in the Central Region of Malawi.

District	Number of herds	Average PCV (% ± 1 s.e.)	Parasitology		Serology	
			Number positive (%)	Number sampled	Number positive (%)	Number sampled
Dedza	4	31.2 ± 0.3	3 (1.3)	240	55 (28.6)	192
Dowa	1	32.9 ± 0.6	0 (0)	60	0 (0)	55
Mchinji	2	28.6 ± 0.5	1 (0.8)	120	20 (20.8)	96
Kasungu	13	31.6 ± 0.2	17 (2.6)	644	43 (8.5)	503
Lilongwe	7	31.0 ± 0.3	1 (0.2)	420	31 (11.1)	280
Nkhotakota	8	31.0 ± 0.3	25 (6.0)	414	29 (7.9)	368
Ntcheu	5	31.9 ± 0.3	0 (0)	300	4 (1.5)	257
Ntchisi	2	31.1 ± 0.5	1 (0.8)	120	3 (3.9)	76
Salima	16	32.3 ± 0.2	0 (0)	960	49 (6.2)	788

Figure 3.4.4: Herd average PCV, proportion of animals, parasitological and serological prevalence of bovine trypanosomosis in the Central Region of Malawi



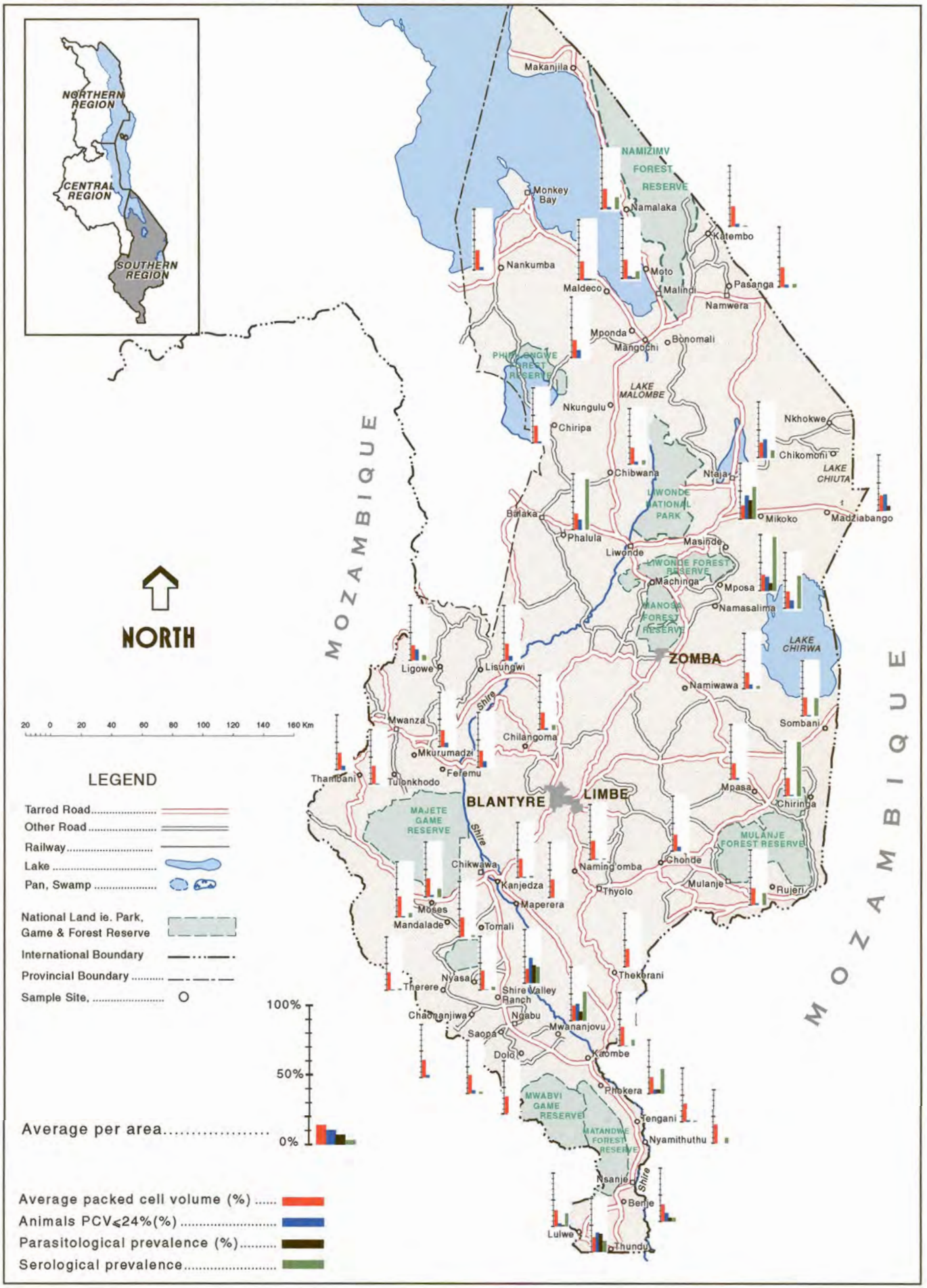
(iii) Southern Region

A major trypanosomosis focus in the Southern Region is the area east of Liwonde National Park and Liwonde Forest Reserve (Table 3.4.8 and Fig. 3.4.5). The prevalence of anti-trypanosomal antibodies was high in cattle sampled at Mposa (97.4%) and Namasalima (58.8%) crushpens (Machinga and Zomba Districts). Trypanosomal infections (7 *T. congolense* (12.7%)) were detected only in samples collected from animals at Mposa crushpen (Zomba District). The prevalence of cattle with trypanosomal infections and anti-trypanosomal antibodies was also high closer to the Mozambican border to the east (Fig. 3.4.5). Thirty-three percent of the animals sampled at Mikoko crushpen, north of Lake Chilwa were infected with trypanosomes (all *T. congolense*). The anti-trypanosomal antibody prevalence remained high (51.3 ± 24.5%) in animals sampled south of Lake Chilwa in the stretch along the Mozambican border (Mulanje District) (Fig. 3.4.5). However, trypanosomal infections were not detected. Phalula was the only crushpen west of the Shire River in Zomba and Machinga Districts that had a high proportion of cattle with anti-trypanosomal antibodies (91.1%). In Chikwawa District the parasitological prevalence of trypanosomosis was high in the herd sampled at Shire Valley Ranch (20 *T. congolense* (33.3%)) and Mwananjovu crushpen (10 *T. congolense* (16.7%)) (Fig. 3.4.5). Except for cattle sampled at these two sites, the prevalence of anti-trypanosomal antibodies was low (on average 3.7 ± 0.9%) in Chikwawa District. Trypanosomal infections (4 *T. congolense* (6.7%)) were also detected in cattle sampled at Phokera crushpen at the edge of Mwabvi Game Reserve (Nsanje District). In the southern part of Nsanje District, trypanosomosis was prevalent. A total of 25 *T. congolense* infections (13.9%) were detected in cattle sampled at Benje, Lulwe and Thundu crushpens (Fig. 3.4.5). Trypanosomosis was absent in Mwanza, Blantyre and Thyolo Districts.

Table 3.4.8: Average PCV (%), parasitological and anti-trypanosomal antibody prevalence (%) in each of the districts surveyed in the Southern Region of Malawi.

District	Number of herds	Average PCV (% ± 1 s.e.)	Parasitology		Serology	
			Number positive (%)	Number sampled	Number positive (%)	Number sampled
Blantyre	2	31.6 ± 0.4	0 (0)	110	3 (7.7)	39
Chikwawa	13	32.3 ± 5.7	30 (3.8)	780	38 (8.5)	449
Machinga	8	28.2 ± 0.3	44 (9.4)	470	50 (21.8)	229
Mangochi	10	31.9 ± 0.2	6 (1.0)	587	40 (8.6)	466
Mulanje	5	31.4 ± 0.3	0 (0)	281	62 (26.6)	233
Mwanza	6	30.6 ± 0.2	0 (0)	360	4 (2.7)	149
Nsanje	7	32.0 ± 0.3	29 (6.9)	420	55 (15.6)	352
Thyolo	2	33.8 ± 0.6	0 (0)	88	1 (1.1)	88
Zomba	4	30.7 ± 0.3	7 (3.0)	235	33 (22.4)	147

Figure 3.4.5: Herd average PCV, prop[illegible]c animals, parasitological and serological prevalence of bovine trypanosomosis in the Southern Region of Malawi.



3.4.4 Discussion and conclusions

3.4.4.1 Parasitological and serological prevalence of bovine trypanosomosis

In all areas surveyed, most trypanosomal infections were *T. congolense*. This is in accordance with observations made in most countries of southern Africa. The majority of the animals in which trypanosomal infections was diagnosed parasitologically had anti-trypanosomal antibodies. Hence, the diagnostic sensitivity of the antibody-detection ELISA was high but differed between trypanosome species. The sensitivity was highest for *T. congolense* infections and was similar to the original sensitivity, at a cut-off of 28%, when known positive samples collected in Zambia were used (Hopkins *et al.*, 1998). The sensitivity of the antibody ELISA to detect *T. vivax* infections, on the other hand, appeared to be much lower. Although *T. vivax* infections only constituted a small proportion of all trypanosomal infections, this observation requires further investigation. The prevalence of anti-trypanosomal antibodies was much higher than the parasitological prevalence of infection. Only a small proportion of the seropositive animals (10.9%) was infected with trypanosomes. This is not surprising considering the low sensitivity of parasitological diagnostic methods for trypanosomosis (Paris *et al.*, 1982) and the persistence of anti-trypanosomal antibodies in the absence of infection (section 3.2.3). Hence, some of the seropositive but parasitologically negative animals were likely to be infected with trypanosomes (false negatives) whereas others may have been infected with trypanosomes but the infection had been cured. It is difficult to allocate a seropositive animal to one of those two categories. However, on the basis of the PCV of a seropositive animal, assumptions can be made on its infection status. One of the most typical signs of bovine trypanosomosis is the development of anaemia which is best measured by determining the PCV (Stephen, 1986). Hence, the significantly lower PCV of cattle infected with trypanosomes and the significant correlation between parasitological prevalence of trypanosomosis and herd average PCV. The significantly lower PCV of parasitologically negative and seropositive animals compared to parasitologically negative and seronegative animals does indeed suggest that a proportion of the seropositive animals was infected at the time of sampling or had recently been infected with trypanosomes, which caused a reduction in their PCVs. Moreover, the correlation between the PCV and the percentage positivity of

these parasitologically negative but seropositive animals suggests that the false negative animals are most likely those with the highest antibody titre. Although, on the basis of these results, no statements can be made on the specificity of the antibody ELISA, the relationships described above do indicate that the antibody detection ELISA does detect false negative animals and can be used as a useful supplementary diagnostic test to improve the accuracy of trypanosomosis surveys.

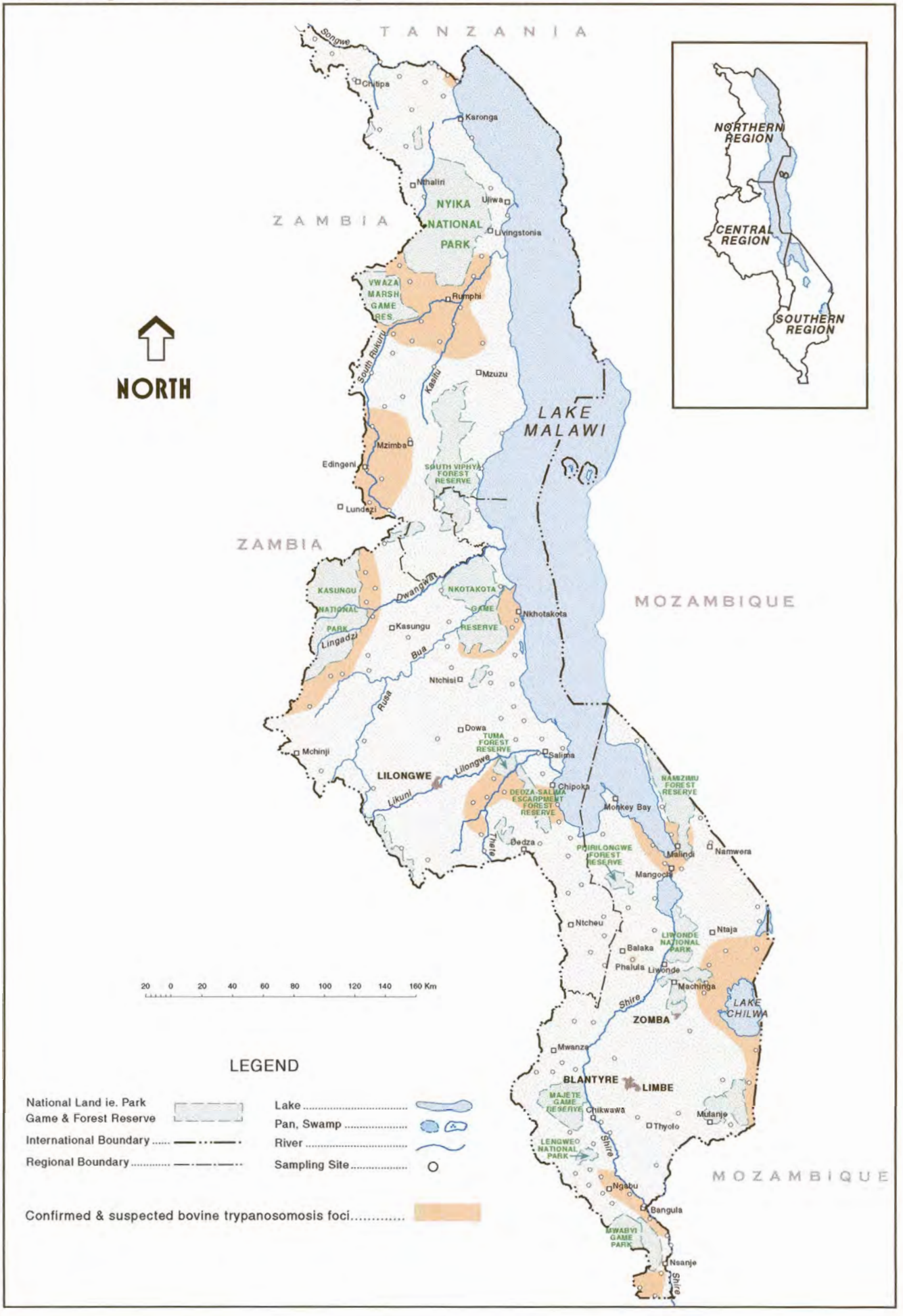
3.4.4.2 Distribution and epidemiology of bovine trypanosomosis in Malawi

The main factor affecting the distribution of tsetse and, hence, bovine trypanosomosis in Malawi is the expansion of the human population and concomitant destruction of the vegetation. As a result, *G. m. morsitans* and *G. pallidipes* primarily occupy national parks, game reserves and forest reserves where habitat is suitable and game animals which constitute the major food source occur. *Glossina brevipalpis* is mainly found along the shore of Lake Malawi or along rivers, often in small patches of dense vegetation (Sanderson, 1910; Mitchell and Steele, 1956). Parasitologically positive herds were located in the vicinity of known tsetse foci within Malawi or adjacent to tsetse-infested areas in neighbouring countries. Bovine trypanosomosis was diagnosed in areas surrounding the Kasungu National Park, the Nkhotakota Game Reserve, the Vwaza Game Reserve, the Liwonde National Park, the Lengwe National Park and the Tuma Forest Reserve (Fig. 3.4.6). Despite the abundance of tsetse in most of these foci, the parasitological prevalence of bovine trypanosomosis was generally low. This is not surprising considering the abundance of suitable wild hosts in the game areas and the restricted tsetse/cattle interface along the edges of the tsetse-infested areas. Moreover, the odour-baited, insecticide-treated, target barriers (Hargrove, 1993) along the edge of Kasungu National Park and the Nkhotakota Game Reserve have reduced substantially the prevalence of bovine trypanosomosis in herds surrounding both game areas.

The distribution of parasitologically positive herds correlated well with the picture obtained from the 1987-89 National Trypanosomosis Survey (Davison, 1990). The most northern trypanosomosis focus (Mwangurukuru crushpen) is still attributed to the presence of *G. brevipalpis*. This tsetse pocket was first identified in 1909

(Sanderson, 1910; Lamborn, 1915) and has reduced substantially in size since the last surveys (Mitchell and Steele, 1956; Davison, 1990). Hitherto, little was known of the distribution and the epidemiological importance of *G. brevipalpis* in Malawi. Because of ecological separation of its habitat and the grazing areas of cattle and the high proportion of feeds on hippopotamus (*Hippopotamus amphibius*), *G. brevipalpis* has rarely been implicated as an important vector of bovine trypanosomosis (Weitz, 1963). Nevertheless, *G. brevipalpis* has been observed feeding on cattle (Sanderson, 1910) and tsetse species of the *fusca*-group are good vectors of cattle trypanosomosis (Leak *et al.*, 1991). Moreover, it occurs outside the protected areas (game parks and forest reserves) and the gradual clearing of land for agriculture may result in an increased contact between cattle and this tsetse species. In some areas of the KwaZulu-Natal Province of South Africa, for example, *G. brevipalpis* is considered to be the main source of infection for cattle (Kappmeier *et al.*, 1998). The importance of *G. brevipalpis* in the epidemiology of bovine trypanosomosis in Malawi may, therefore, be underrated and could increase in the future. Since the distribution of *G. brevipalpis* is not well defined, insecticide treatments of cattle may be an effective means of controlling this tsetse species. The Vwaza Game Reserve was the main tsetse-infested area (*G. m. morsitans* and *G. pallidipes*) in the Northern Region of Malawi and determined the distribution of bovine trypanosomosis in the Vwaza area. The distribution of *G. m. morsitans* and *G. pallidipes* was, however, not restricted to the boundaries of the Game Reserve (Mitchell and Steele, 1956; Davison, 1990). This is indicated by the distribution pattern of cattle with anti-trypanosomal antibodies. The movement of tsetse away from their prime focus is common in Malawi and was first described by Shircore (1914). He found that, in the dry season, tsetse were confined to the most favourable habitat in their prime foci. However, during the rainy season when climatic conditions are favourable tsetse may move into surrounding areas. The extent of dispersion during the rainy season will depend largely on availability of suitable habitat and host density. The South Rukuru River provides an ideal conduit for such seasonal movements of tsetse in the Vwaza area, which probably explains the widespread distribution of cattle with anti-trypanosomal antibodies in the Rumphi District. Similar seasonal changes in the distribution of tsetse were observed by Davison (1990) in the Chief Chulu area along the fringe of the Kasungu National

Figure 3.4.6: Confirmed (trypanosomal) and suspected (anti-trypanosomal antibodies present) foci of bovine trypanosomosis in Malawi.



Park. These changes cause seasonal variations in the level of tsetse challenge to cattle that are often highly susceptible. The reasons for and the extent of the seasonal fly movements are not fully known but they appear to be an important part of the epidemiology of bovine trypanosomosis in Malawi as they were in the original situation in Zululand (South Africa) and very likely in parts of Zimbabwe, especially where *G. pallidipes* is present. They explain the presence of cattle with anti-trypanosomal antibodies in areas far removed from the original tsetse focus and the occurrence of bovine trypanosomosis epidemics such as those observed between 1982-85.

The presence of cattle with anti-trypanosomal antibodies south west of Mzimba is attributed to the Lundazi tsetse-belt (*G. m. morsitans*) in eastern Zambia which extends into Malawi.

In the Central Region, the Kasungu National Park and the Nkhotakota Game Reserve were the main tsetse foci (mainly *G. m. morsitans*). Tsetse density in both wildlife areas was high (Davison, 1990) and the prevalence of human sleeping sickness and bovine trypanosomosis reached epidemic proportions a decade ago (unpublished reports. Department of Animal Health and Industry). This problem was effectively alleviated by the deployment of a 6 km-wide odour-baited, insecticide-treated, target barrier (Hargrove, 1993) along the eastern edge of Kasungu National Park and the southern part (south of Bua River) of the Nkhotakota Game Reserve (RTTCP, 1996). Both target barriers have been very effective in reducing challenge. This is clearly reflected in the low parasitological prevalence of bovine trypanosomosis and, especially, the low prevalence of anti-trypanosomal antibodies in the areas surrounding these two tsetse foci. Moreover, whereas bovine trypanosomosis used to be prevalent throughout the area between the two game parks (Davison, 1990) it is now confined to the immediate vicinity (approximately 10-15 km) of both tsetse-infested zones. The effect of the target barriers is twofold. First, they effectively reduce contact between tsetse and cattle at the edge of the game parks and second, they almost entirely prevent the seasonal movement of tsetse. The absence of a target

barrier in the southern section of Kasungu National Park explains the southward spread of the distribution of bovine trypanosomosis.

Glossina m. morsitans has been recorded in the Dedza-Salima Escarpment Forest Reserve and the Tuma Forest Reserve (Mitchell and Steele, 1956). However, during the 1987-89 survey, flies were only found in the Tuma Forest Reserve. According to these survey results challenge occured in both areas. A distinct reduction in the distribution of tsetse and, hence, trypanosomosis was observed in the Salima area. Whereas *G. m. morsitans* used to infest most of the valley floor north of Salima (Shircore, 1914), bovine trypanosomosis was virtually absent at the time of the present survey. Trypanosomosis was also completely absent in cattle sampled near the Phirilongwe Forest Reserve focus. Although this forest reserve was infested with *G. m. morsitans* during the 1987-89 survey, progressive infiltration of settlements may have resulted in the eradication of the fly from this focus during the past decade. The presence of *G. m. morsitans* in the Mamizumu and the Mangochi Forest Reserves (Mitchell and Steele, 1956; Davison, 1990) explains the prevalence of bovine trypanosomosis along the southern part of Lake Malawi. The Shire River formed the boundary between a trypanosomosis-free and trypanosomosis-infested area in the northern part of the Southern Region. With the exception of the high prevalence of anti-trypanosomal antibodies in cattle sampled at Phalula, bovine trypanosomosis was absent in the area west of the river. Since Phalula is located close to the bridge across the Shire River, the high prevalence of cattle with antibodies can be attributed to the movement of cattle with anti-trypanosomal antibodies from the trypanosomosis-infested, eastern area into the trypanosomosis-free, western area.

The main tsetse focus (*G. m. morsitans*) east of the Shire River was the Liwonde National Park. The bovine trypanosomosis cases east of the National Park could have been due to challenge by tsetse from the Liwonde National Park although few cattle graze in the immediate vicinity of this tsetse focus. It is more plausible that the trypanosomal infections east of Liwonde National Park and north, west and south of Lake Chilwa were a result of fly-belts from Mozambique's Mecanhelas and Milange Districts extending into Malawi.

Despite the presence of *G. pallidipes* in Majete Game Reserve (Davison, 1990), the parasitological and serological prevalence of bovine trypanosomosis in herds sampled in areas surrounding the Game Reserve was low. This is in contrast with the 1987-89 trypanosomosis survey results (Davison, 1990). Nevertheless, the present results are in accordance with the limited distribution of tsetse in the Game Reserve as observed by Davison (1990). Similarly, cattle seem to have been challenged very little by the tsetse (*G. pallidipes* and *G. m. morsitans*) present in the Lengwe National Park. Moreover, the tsetse pockets south of the National Park and the bovine trypanosomosis focus in this area (Davison, 1990) seem to have disappeared. In view of significant reduction in the distribution of bovine trypanosomosis in areas surrounding the Lengwe National Park, the trypanosomal infections in cattle north and east of Ngabu (Shire Valley Ranch an Mwananjovu crushpen) cannot be attributed to challenge by tsetse from the Lengwe National Park. However, both sampling sites are located close to the Elephant Marsh. Little is known of the current tsetse situation in the Elephant Marsh but *G. brevipalpis* has been reported (Austen, 1903; Lamborn, 1915; Potts, 1954). Further investigations are required. The trypanosomosis cases detected in the most southern part of the country can be attributed to the presence of tsetse (*G. pallidipes* and *G. m. morsitans*) in the Mwabvi Game Reserve, the Matandwe Forest Reserve and challenge by tsetse from neighbouring Mozambique (Morrumbala and Mutarara Districts).

3.5 The parasitological and serological prevalence of bovine trypanosomosis in the Eastern Caprivi (Caprivi District, Namibia)

3.5.1 Introduction

In Namibia, tsetse-transmitted trypanosomosis or "nagana" is restricted to the Caprivi District. The distribution of tsetse (*G. m. centralis*) is confined for the greater part to the Linyanti-Mashi-Kwando drainage. The main foci are located along the Kwando River between Kongola and the Angolan and Zambian borders in the north and around Lupala and Nkasa "islands" in the south (Bingham *et al.*, 1995).

Between 1964 and 1994, human and animal trypanosomosis were controlled by ground-spraying operations along the eastern and western banks of the Kwando River (Bingham *et al.*, 1995). In 1994, a 5 km-wide, odour-baited, insecticide-treated, target barrier (Hargrove, 1993) was constructed along the western side of the Kwando River starting near the Botswana border and extending to the Angola border. In the same year, a similar target barrier was constructed along the northern edge of the Mamili National Park.

In 1984, an outbreak of trypanosomosis in cattle was reported in the Katima Mulilo area. The presence of nagana in this area is of great concern especially because of the possible spread of tsetse southwards across the Caprivi Strip to the Chobe River and thence into Botswana.

To establish the current distribution of tsetse-transmitted trypanosomosis in the Eastern Caprivi and to determine its spread in the Katima Mulilo area, a survey was conducted. Use was made of both parasitological and serological methods (antibody-detection). The value of these survey methods in establishing the distribution of tsetse-transmitted trypanosomosis is discussed in the light of the results obtained from the survey.

3.5.2 Materials and methods

3.5.2.1 Sampling area

The Eastern Caprivi lies to the east of the Kwando River (Fig. 3.5.1) in the Caprivi District (Namibia). It is over 11 600 km^2 in extent and has a population of over 122 000 head of cattle. The only tsetse species occurring is *G. m. centralis*.

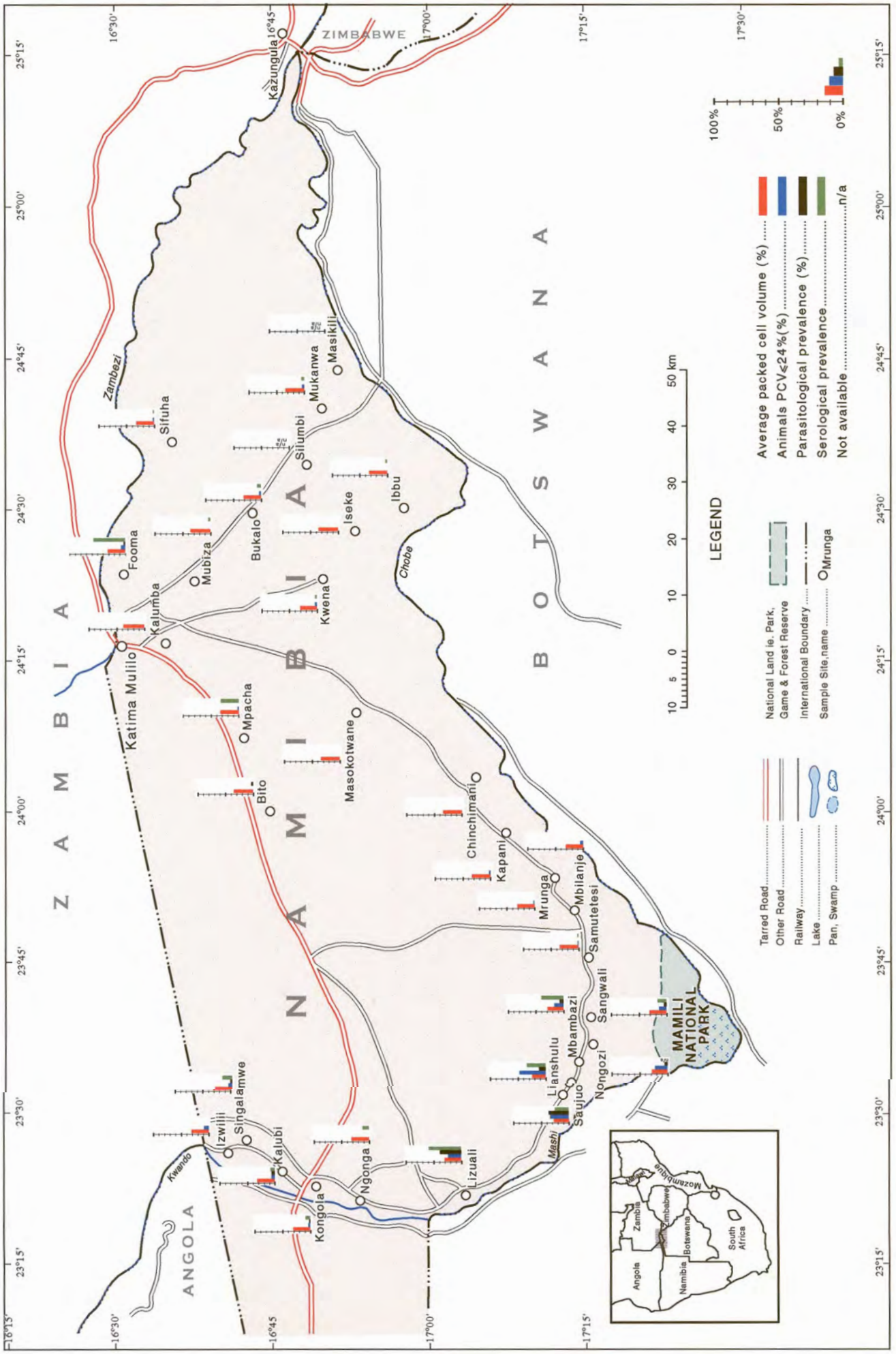
Between August 1995 and June 1997, a survey of bovine trypanosomosis was conducted at 33 sampling sites (Fig. 3.5.1).

To facilitate the interpretation of the survey results, the sampling area was subdivided into survey areas. Sampling sites were categorised according to the grazing areas of the cattle and allocated to one of the survey areas. Four survey areas were identified (Table 3.5.1):

Table 3.5.1: Survey areas and sampling sites in Eastern Caprivi, Namibia.

Survey area	Sampling site	Survey area	Sampling site
Katima Mulilo	Fooma	Linyanti/Chobe	Sangwali
	Kalumba		Malinda
	Mpacha		Samutetesi
	Bito		Mbilanje
	Mubiza		Mrunga
	Sifuha		Kapani
	Bukalo		Chinchimani
	Kwena		Ibbu
	Masokotwani		Mukanwa
	Iseke		Masikili
	Silumbi	Mamili	Lianshulu
Kwando	Izwilii		Saujuo
	Kalubi		Nongozi
	Kongola		Mbambazi
	Ngonga		Lizauli
	Singalamwe		Samudondo
			Malengalenga

Figure 3.5.1: Map of the survey area and location of the sampling sites in the Eastern Caprivi, Namibia.



- the "Katima Mulilo area" (cattle grazing along the Zambezi and immediately south of Katima Mulilo),
- the "Kwando area" (cattle grazing along the Kwando River, north of Ngonga),
- the "Mamili area" (cattle grazing along the Kwando/Mashi River north of the Mamili National Park) and
- the "Linyanti/Chobe area" (cattle grazing along the Linyanti and Chobe Rivers).

Odour-baited target barriers were in place west of the Kwando survey area and south of the Mamili survey area (Fig. 3.5.1). Trypanocides, mainly diminazene aceturate (Berenil®, Hoechst), were used frequently in cattle herds in the Mamili survey area.

3.5.2.2 Sampling size

A total of 1 481 adult cattle were examined. A cross-sectional sampling method was applied (Section 3.4.2.1).

3.5.2.3 Sampling method

Direct parasitological serological (anti-trypanosomal antibody detection ELISA) diagnostic tests were used (Section 3.3.2.2).

3.5.3 Results

3.5.3.1 Parasitological prevalence of bovine trypanosomosis

A total of 1 481 samples were examined. Tsetse-transmitted trypanosomes were detected in 66 animals (4.5%) sampled at 14 of the 33 sampling sites (Table 3.5.2).

All infections were detected on buffy coat and confirmed on thick and thin smears. The parasitological prevalence of *T. vivax*, *T. congolense* and mixed (*T. congolense* and *T. vivax*) infections was 81.8%, 16.7% and 1.5%, respectively. Overall parasitological prevalence was highest in the Mamili survey area. The proportion of *T. vivax* infections varied significantly between the survey areas. *T. vivax* infections were dominant in the Mamili area (95.9%) whereas all trypanosomal infections detected in cattle grazing in the Kwando area were *T. congolense*.

Table 3.5.2: Sample size, number of animals with trypanosomal infections and average PCV (% ± 1 s.e.) of herds sampled at various sampling sites in the different survey areas, Eastern Caprivi, Namibia.

Survey area	Sample site	Sample size	Number of trypanosomal infections				Average PCV (%) (± 1 s.e.)
			T. vivax	*T. congolense*	*T. brucei*	mixed (Tc/Tv)	
Katima	Mpacha	55	0	0	0	0	33.4 ± 0.5
Mulilo	Bito	28	1	0	0	0	35.6 ± 0.4
	Kalumba	10	0	0	0	0	37.2 ± 1.1
	Kwena	30	0	0	0	0	30.5 ± 0.8
	Masokotwani	37	0	0	0	0	33.9 ± 0.5
	Mubiza	40	0	0	0	0	36.1 ± 0.7
	Fooma	42	0	1	0	0	31.2 ± 0.8
	Bukalo	34	0	0	0	0	31.7 ± 0.7
	Iseke	30	0	0	0	0	35.2 ± 0.7
	Sifuha	50	0	0	0	0	32.8 ± 0.6
	Silumbi	10	0	0	0	0	-
Kwando	Kalubi	50	0	3	0	0	32.3 ± 0.5
	Kongola	60	0	1	0	0	30.7 ± 0.5
	Izwilii	60	0	0	0	0	30.8 ± 0.7
	Singalamwe	60	0	2	0	0	31.6 ± 0.5
	Ngonga	60	0	0	0	0	32.5 ± 0.5
Mamili	Mbambazi	60	4	0	0	0	29.9 ± 0.7
	Samudondo	60	3	0	0	0	31.6 ± 0.7
	Lianshulu	60	7	0	0	0	24.4 ± 0.8
	Saujuo	33	12	0	0	0	27.0 ± 1.0
	Nongozi	60	3	0	0	0	28.3 ± 0.6
	Lizauli	60	21	2	0	0	30.0 ± 0.9
Linyanti/	Sangwali	60	3	0	0	0	31.1 ± 0.7
Chobe	Malinda	60	0	0	0	0	33.8 ± 0.6
	Malengalenga	60	0	0	0	1	29.8 ± 0.6
	Samutetesi	60	0	0	0	0	34.5 ± 0.6
	Mbilanje	60	0	0	0	0	31.4 ± 0.7
	Kapani	40	0	0	0	0	33.4 ± 0.4
	Mrunga	60	0	2	0	0	31.3 ± 0.4
	Chinchimani	20	0	0	0	0	34.9 ± 0.8
	Mukanwa	25	0	0	0	0	34.5 ± 0.9
	Ibbu	33	0	0	0	0	33.7 ± 0.7
	Masikili	14	0	0	0	0	-

The mean parasitological prevalence was low in the Katima Mulilo, Kwando and Linyanti/Chobe survey area (Table 3.5.3). A significantly higher mean parasitological prevalence was found in the Mamili survey area (Table 3.5.3).

3.5.3.2 Packed cell volume

Table 3.5.2 summarizes the average PCV (± 1 s.e.) of herds sampled in each of the survey areas. Packed cell volume profiles for each of the four survey areas are presented in Figure 3.5.2.

The mean PCVs were significantly different between all survey areas ($P<0.001$). The percentage of anaemic animals at a sampling site and the parasitological prevalence of trypanosomal infections at the same sampling site were significantly correlated ($r = 0.71$, $P<0.001$).

3.5.3.3 Prevalence of anti-trypanosomal antibodies

A total of 1 196 blood spots were screened for anti-trypanosomal antibodies (Table 3.5.4). Only 115 samples (9.6%) were serologically positive.

Table 3.5.3: Number of samples, average serological prevalence, parasitological prevalence of trypanosomosis and average packed cell volume of herds sampled in each of the survey areas, Eastern Caprivi, Namibia.

Survey area	Number of samples	Average parasitological prevalence (%) (± 1 s.e.)	Number of samples	Average serological prevalence (%) (± 1 s.e.)	Number of samples	Average PCV (%) (± 1 s.e.)
Katima Mulilo	397	1.2 ± 0.1	236	13.2 ± 1.3	422	33.6 ± 0.2
Kwando	290	2.2 ± 0.1	277	17.3 ± 1.4	288	31.2 ± 0.2
Mamili	360	11.4 ± 0.1	138	32.9 ± 0.6	272	28.0 ± 0.4
Linyanti/Chobe	432	0.8 ± 0.1	338	3.4 ± 0.3	398	32.2 ± 0.2

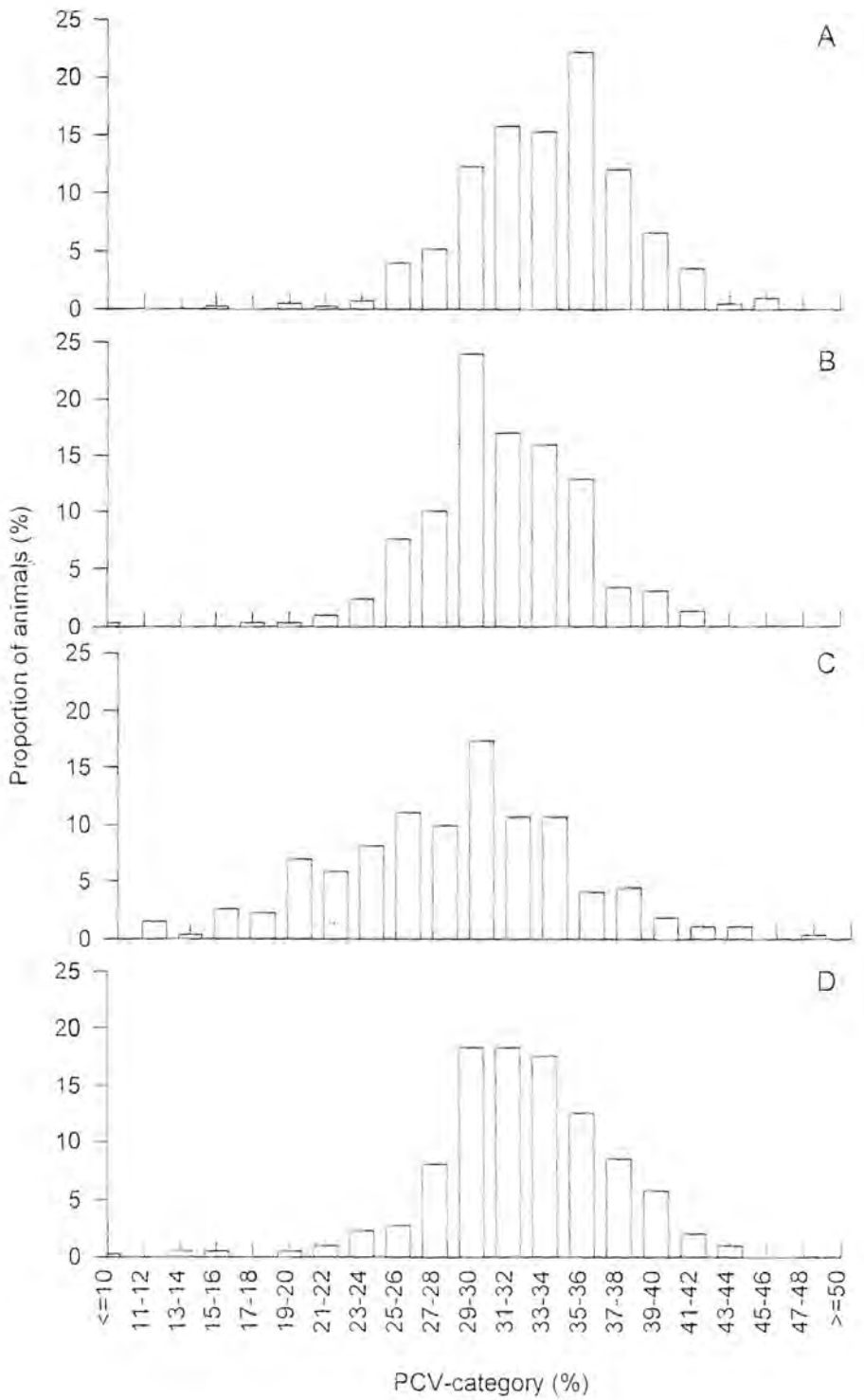


Figure 3.5.2: Comparison of PCV profiles of herds sampled in the Katima Mulilo (A), Kwando (B), Mamili (C) and Linyanti/Chobe (D) survey areas. Eastern Caprivi. Namibia.

Table 3.5.4: Sample size, number of positives, average percentage positivity (% ± 1 s.e.) and serological prevalence of samples collected at various sampling sites in the different survey areas, Eastern Caprivi, Namibia.

Survey area	Sample site	Sample size	Number serological positive	Average Percentage Positivity (± 1 s.e.)	Serological Prevalence (%)
Katima	Mpacha	49	14	21.3 ± 1.3	32.3
Mulilo	Bito	27	0	16.5 ± 0.7	0
	Kalumba	10	0	16.6 ± 1.5	0
	Kwena	30	1	18.4 ± 0.8	3.7
	Masokotwani	47	0	16.6 ± 0.5	0
	Mubiza	39	2	16.4 ± 0.9	3.7
	Fooma	40	2	28.6 ± 1.9	56.5
	Bukalo	31	2	18.6 ± 0.7	7.3
	Iseke	32	0	18.7 ± 0.7	0
	Sifuha	46	1	17.9 ± 0.0	2.5
	Silumbi	9	0	15.2 ± 1.8	0
Kwando	Kalubi	59	0	15.5 ± 2.0	7.8
	Kongola	57	4	21.5 ± 1.1	8.3
	Izwilii	59	0	15.5 ± 0.5	0
	Singalamwe	47	7	20.7 ± 1.2	17.5
	Ngonga	50	5	15.4 ± 1.1	11.8
Mamili	Mbambazi	53	18	22.2 ± 1.7	40.0
	Samudono	59	0	13.3 ± 0.7	0
	Lizauli	52	26	27.7 ± 1.7	58.8
	Lianshulu	52	15	24.5 ± 1.9	33.9
	Saujuo	33	7	21.2 ± 2.6	25.0
Linyanti/	Sangwali	57	8	15.7 ± 1.5	16.5
Chobe	Samutetesi	54	1	12.1 ± 0.5	2.2
	Mbilanje	57	0	13.5 ± 0.5	0
	Kapani	37	0	8.4 ± 0.8	0
	Mrunga	57	0	14.6 ± 0.5	0
	Chinchimani	20	0	15.6 ± 0.7	0
	Mukanwa	23	1	17.9 ± 0.8	4.8
	Ibbu	33	1	20.2 ± 0.6	3.3
	Masikili	13	0	19.2 ± 0.9	0

Anti-trypanosomal antibodies were detected in herds sampled at 18 out of the total of 30 sample sites where blood spots were collected (Table 3.5.4). The serological prevalence varied considerably between locations (Table 3.5.4). Cattle at three sampling sites (10%), where trypanosomes were detected using parasitological methods, had no anti-trypanosomal antibodies. A significant correlation (r = 0.58, $P<0.01$) was found between the parasitological and serological prevalence of trypanosomosis at the various sampling sites.

Anti-trypanosomal antibody titres were found in cattle from nine sampling sites where animals were parasitologically negative. The average serological prevalence in the parasitologically negative herds was 7.9% compared to 29.4% in herds that were parasitologically positive. The average serological prevalence in each of the survey areas is summarised in Table 3.5.4.

The percentage of anaemic animals at a sampling site was significantly correlated (r = 0.59, $P<0.01$) with the serological prevalence at that site.

3.5.4 Discussion and conclusions

3.5.4.1 Parasitological and serological prevalence of bovine trypanosomosis

According to the parasitological and serological prevalences, tsetse-transmitted trypanosomal infections in the Eastern Caprivi are confined to the Kwando River drainage and the vicinity of Katima Mulilo. The Kwando River infestation complies with the scanty information on the historical distribution of tsetse in the Eastern Caprivi (Bingham *et al.*, 1995). However, the Kwando River target barrier seems to have significantly reduced the spread of tsetse. The effectiveness of the target barrier in reducing tsetse challenge is clearly reflected in the low parasitological prevalence of trypanosomosis and the low prevalence of anti-trypanosomal antibodies in cattle sampled in the Kwando survey area.

South of the barrier, the parasitological prevalence of trypanosomosis was unexpectedly high. This is explained by the recent capture of tsetse at Lianshulu (R. Mkandawire, personal communication, 1996). Parasitological and serological

prevalence figures from the Mamili survey area indicate that trypanosomal infections are probably acquired when cattle graze and water along the Kwando River. The possibility that tsetse challenge may occur when cattle or tsetse cross the 5 km-wide Mamili target barrier cannot be excluded. Considering these results, regular follow-up surveys are needed to monitor the possible spread of tsetse and trypanosomosis into the Mamili area of the Eastern Caprivi.

The parasitological and serological prevalence rates confirm the reports of recent trypanosomosis outbreaks in the Katima Mulilo area. According to the serological data, cattle from two sampling sites (Fooma and Mpacha) face regular tsetse challenge. During an extensive tsetse survey conducted in the vicinity of Katima Mulilo, no tsetse were trapped (P. Van den Bossche, unpublished, 1995) although tsetse flies are present in the adjacent Sesheke area of Zambia. It is, therefore, assumed that trypanosomal infections are being acquired when cattle graze along the Zambezi River. Unfortunately, the low sensitivity of trapping methods for *G. m. centralis* makes it impossible to draw conclusions from tsetse survey results alone. Information obtained from the serological survey, however, clearly indicates that tsetse have not been able to establish themselves in Katima Mulilo and areas south of Katima Mulilo. Nevertheless, there will be a need for close vigilance in these areas until the threat of invasion from the north has been removed.

None of the animals sampled south of Katima Mulilo and in the Linyanti/Chobe survey area were infected with trypanosomes. These data are, however, not sufficient to conclude that the disease is absent. The seroprevalence of anti-trypanosomal antibodies was also low. Since the predictive value of a serological test declines as the prevalence of the disease declines, the low seroprevalence of anti-trypanosomal antibodies in cattle sampled south of Katima Mulilo and in the Linyanti/Chobe survey area could even be an overestimation of the true prevalence (Thrusfield, 1986). From the serological prevalence data and with the sample sizes used in this survey it can be assumed, with a high degree of confidence, that trypanosomosis is absent in those areas.

Of particular epidemiological interest is the difference in the prevalence of *T. vivax* infections in cattle from two areas despite their relatively close proximity. There may be several explanations. First, this could be explained by the occurrence of mechanical transmission although the role of other biting flies in transmitting *T. vivax* to cattle in tsetse-infested areas remains an unresolved issue (D'Amico *et al.*, 1996). Second, there may be differences in host availability or host preference between the two areas. Antelopes, which are abundant in the Mamili area, are generally accepted to be reservoir hosts of *T. vivax* from which the infection is transmissible to domestic ruminants (Hoare, 1970). An increased feeding frequency by tsetse on antelopes could, therefore, result in a high *T. vivax* prevalence in cattle. Finally, for a tsetse fly to become infective, it must live longer than the developmental period of the trypanosome. Since *T. vivax* has the shortest developmental cycle, a high proportion of tsetse infected with *T. vivax* is expected in areas where large numbers of young tsetse flies are present. Proportionately larger numbers of younger flies than older flies may be recorded either when mortality is high in a relatively stable tsetse population, or when the mortality is low in an expanding tsetse population. The Mamili survey area is situated at the edge of the fly-belt. Ecological conditions for tsetse, at the edge of a fly-belt, are normally less favourable resulting in a high mortality rate of tsetse. This high mortality rate would, nevertheless, permit the development of *T. vivax* infections in tsetse and could explain the high *T. vivax* prevalence rate in cattle sampled in the Mamili survey area.

3.5.4.2 Packed cell volume and trypanosomosis prevalence

Although anaemia can be caused by factors other than trypanosomosis, it remains one of the most important indicators of tsetse-transmitted trypanosomosis in cattle (Stephen, 1986). The PCV profile and average PCV of a herd is affected by the number of trypanosome-infected animals or the parasitological prevalence of trypanosomosis. This is clearly seen in the shift of the PCV distribution to the lower PCV values in survey areas where trypanosomosis was detected (Fig. 3.5.2). This observation suggests that PCV profiles can be used as an additional indicator of trypanosomosis even when trypanosomes could not be detected by parasitological diagnostic tests.

3.5.4.3 Interpretation of serological data

In contrast with the parasitological methods, the serological test used in this survey had high sensitivity and specificity. Nevertheless, interpreting anti-trypanosomal antibody prevalence rates remains difficult. This is mainly because such antibodies can persist for several months even after successful trypanocidal drug therapy or self-cure (Bocquentin *et al.*, 1990). The effect of tsetse control measures on the transmission of bovine trypanosomosis is often assessed by determining the parasitological incidence of trypanosomosis in sentinel cattle. This type of surveillance is expensive and lacks adequate sensitivity (Paris *et al.*, 1982). Although antibody detection tests cannot form the basis of identifying infected animals (Nantulya, 1990), a decline in antibody prevalence can be used to assess the impact of tsetse control operations on the trypanosomosis challenge. Effectiveness of tsetse control measures can, therefore, be monitored by regular surveys to establish the prevalence of anti-trypanosomal antibodies. These types of surveys are easy to conduct, less time consuming than the normal surveillance and have high sensitivity and specificity. Once anti-trypanosomal antibodies have disappeared, seroprevalence surveys can continue to be used as a sensitive monitoring system. Such a monitoring system is extremely useful in countries where tsetse-cleared areas are protected by, for example, target barriers to prevent re-invasion of tsetse from infested areas (Van den Bossche and Mudenge, 1997).

An important reason for conducting this serological survey was to determine whether the population or herd had been exposed to trypanosomosis. In this respect, areas of particular interest were those where disease prevalence was too low to detect parasites by current parasitological diagnostic methods or where tsetse could not be captured. In such cases, the determination of the anti-trypanosomal antibody prevalence of a herd made it possible to distinguish with a high degree of confidence between low challenge and no challenge. One such example was the Katima Mulilo survey area where the trypanosomosis situation could only be explained by combining parasitological and serological data.

Although some animals with recent trypanosomal infections may not have developed antibody response at the time of sampling, 90 % of the parasitologically positive herds were also serologically positive. Moreover, herd seroprevalence was positively correlated to parasitological prevalence and the percentage of anaemic animals. These findings indicate that, on a herd basis, the prevalence of anti-trypanosomal antibodies may be used to assess the infection status or extent of disease.

3.6 An evaluation of the usefulness of the anti-trypanosomal antibody detection ELISA as a tool for monitoring the effectiveness of tsetse control operations in Zimbabwe

3.6.1 Introduction

In Zimbabwe, tsetse control has a long history. During the past 65 years, large portions of land have been cleared from tsetse through concerted effort of Zimbabwe's Tsetse and Trypanosomiasis Control Branch (T&TCB). Between 1986 and 1998, for example, approximately 20 400 km^2 of area was cleared of tsetse using a variety of control methods (Lovemore, 1999). In most areas, odour-baited target barriers (in some areas supported by insecticide-treated cattle) have been put in place to prevent tsetse from re-invading cleared areas. In other areas, where the risk of reinvasion by tsetse is low, artificial barriers are absent. The effectiveness of these barriers or the absence of tsetse in areas not protected by barriers is monitored through continuous tsetse and irregular trypanosomosis surveillance. Such surveillance exercises are time consuming and expensive. Moreover, due to the low sensitivity of the currently available tsetse and trypanosomosis surveillance methods (Paris *et al.*, 1982; Hargrove, 1980a), low-density tsetse populations and areas where the prevalence of trypanosomosis is low may be missed and, hence, regarded erroneously as tsetse or disease-free. The recently improved anti-trypanosomal antibody detection enzyme-linked immunosorbent assay (antibody ELISA) (Hopkins *et al.*, 1998) could be used as an additional tool for monitoring the effectiveness of tsetse control operations. The assay has high diagnostic sensitivity and specificity in detecting anti-trypanosomal antibodies in cattle. Moreover, the assay detects antibodies against current and past trypanosomal infections. Finally, because of the use of filter papers to collect blood samples, sample collection and storage is simplified.

To determine the usefulness of the anti-trypanosomal antibody detection ELISA as an additional tool to monitor the effectiveness of tsetse control operations, a trypanosomosis survey was conducted along Zimbabwe's tsetse front. The prevalences of anti-trypanosomal antibodies in cattle were determined at each of the sampling sites and were compared with the parasitological prevalence of

trypanosomal infections and current and historical data on the distribution and density of tsetse. Conclusions were drawn on the current trypanosomosis situation in the country and the usefulness of the antibody ELISA as a monitoring tool is discussed.

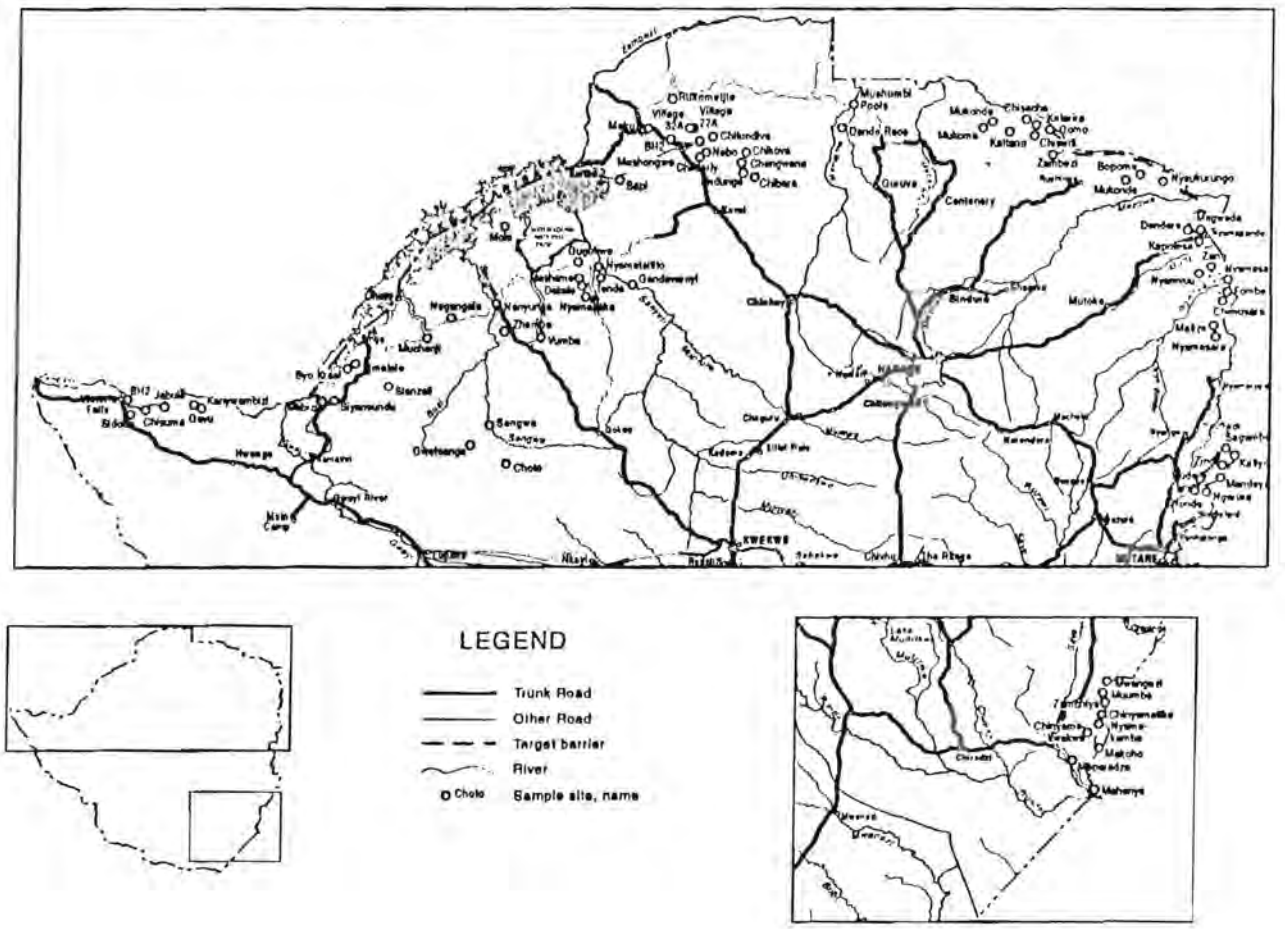
3.6.2 Materials and methods

3.6.2.1 Sampling sites and sample selection

Between January 1998 and September 1999, a total of 3 988 adult cattle were examined at 62 sampling sites in the southeastern, eastern/northeastern, northern and western regions of Zimbabwe (Fig. 3.6.1). Since the aim of the survey was to monitor the current bovine trypanosomosis situation, sampling was restricted to areas, supposedly cleared of tsetse, adjacent to the tsetse invasion front or along barriers to prevent re-invasion.

A cross-sectional sampling method was applied (Section 3.4.2.1).

Figure 3.6.1: Location of sampling sites in Zimbabwe.



3.6.2.2 Survey areas

(i) Chipinge area

Tsetse were eradicated from the south-east lowveld as the result of a large-scale joint ground spraying operation between the governments of Mozambique, South Africa and Zimbabwe (then Rhodesia) (Robertson and Kluge, 1968). Between 1962 and 1981, about 5 700 km^2 of the southeastern lowveld was cleared of *G. m. morsitans* and *G. pallidipes* (Robertson and Kluge, 1968). Furthermore, because of the spraying campaigns in Mozambique the tsetse front was pushed up to Massangena about 60 km east of the Zimbabwe border. Despite the absence of measures to contain the westerly advance of the fly front, no cases of bovine trypanosomosis have been diagnosed in the southeastern lowveld since the spraying operation. In 1997, however, two tsetse flies were captured at Mavué along the Zimbabwe/Mozambique border immediately south of the Save River (RTTCP, 1999a). Moreover, the prevalence of cattle with anti-trypanosomal antibodies sampled at Mavué was high (59%). Because of the potential threat of reinvasion of tsetse into Zimbabwe, intensive tsetse surveillance was initiated in 1998 in the Chipinge area. No tsetse were captured. Nevertheless, the threat of tsetse reinvading the southeastern lowveld is present.

(ii) Honde Valley

In the Honde Valley bovine trypanosomosis was recorded in 1959 (Thakersi, 1992). In 1992 a major outbreak of the disease occurred. This outbreak was attributed to the spread of *G. pallidipes*, *G. m. morsitans* and *G. austeni* from neighbouring Mozambique (Thakersi, 1992) and was controlled by dipping of cattle in 0.00375% deltamethrin (Decatix®, Coopers) at two-weekly intervals. Because of the significant improvement of the trypanosomosis situation insecticide treatments of cattle ceased at the end of 1996. Since that period little information is available on the tsetse and trypanosomosis situation in the Honde Valley.

(iii) Eastern/northeastern region

In Centenary, Mount Darwin, Rushinga, Mudzi and Nyanga Districts, tsetse are restricted to the international border with Mozambique. An odour-baited, insecticide-treated, targets barrier is present in most of the border areas. It was

removed between Musengezi River and Chigango. Because of intense invasion pressure of tsetse (*G. m. morsitans* and *G. pallidipes*) between Nyamapanda and the Ruenya River in Mudzi District, the target barrier was supplemented by an additional target operation, on average 10 km wide, with a target density of 4 targets/km^2. In Guruve District, target operations supplemented by deltamethrin-treated cattle are active in the Dande Communal Land and the Dande Safari Area. Tsetse surveillance and survey results are used to evaluate the effectiveness of the eastern/north eastern border tsetse control operation. Trypanosomosis surveys are conducted at irregular intervals by the Department of Veterinary Services.

(iv) Northern region

Between the eastern edge of Lake Kariba and the Manyame River, tsetse-cleared areas are protected from re-invasion by active odour-baited, insecticide-treated, target operations of varying width (Lovemore, 1999). The effectiveness of this target barrier is evaluated as in the eastern/north eastern border region.

(v) Western region

With the exception of the area covered by the Matusadona National Park, large-scale aerial and ground spraying operations were conducted between 1982 and 1987 with the aim to clear the western region of tsetse (Hursey and Allsopp, 1984; Allsopp and Hursey, 1986; Lovemore, 1990). Despite all these efforts, tsetse (*G. pallidipes*) were still being captured along the drainage of the Busi and Sengwa Rivers and in the communal land south of the Chirisa Safari Area (Sengwa Gorge) (Lovemore, 1990). As a result of these catches and the detection of trypanosomal infections in cattle sampled in areas surrounding the Chirisa Safari Area, a target operation and dipping of cattle in deltamethrin was initiated at the end of 1988. At the same time, a 10 months' trypanosomosis surveillance exercise was initiated at six diptanks (including Choto and Gwetsanga) until July 1989 (RTTCP, 1989). Following these measures no tsetse have been caught since December 1989, and the last trypanosomal infection in the sentinel cattle was detected in March 1989 (RTTCP, 1989).

Tsetse disappeared from the banks of the Zambezi between Victory Falls and the Gwayi River after the rinderpest outbreak at the end of the 19th century (Jack, 1914). However, in 1972 an isolated outbreak of bovine trypanosomosis was reported at Katchetechete (Lovemore and Napier Bax, 1972). Since that period, no bovine trypanosomosis outbreaks have been reported from the area. In 1990, a trypanosomosis survey conducted along the Zambezi River east of Victoria Falls could not detect trypanosomal infections (RTTCP, 1990). However, trypanosomosis surveys in Zambia revealed a southerly advance from the tsetse (*G. m. centralis*) infestation centred on the Kafue National Park (RTTCP, 1991). The threat to Zimbabwe of this southerly advance of tsetse is present.

3.6.2.3 Diagnostic methods

Direct parasitological and serological (anti-trypanosomal antibody detection ELISA) diagnostic tests were used (Section 3.3.2.2).

3.6.3 Results

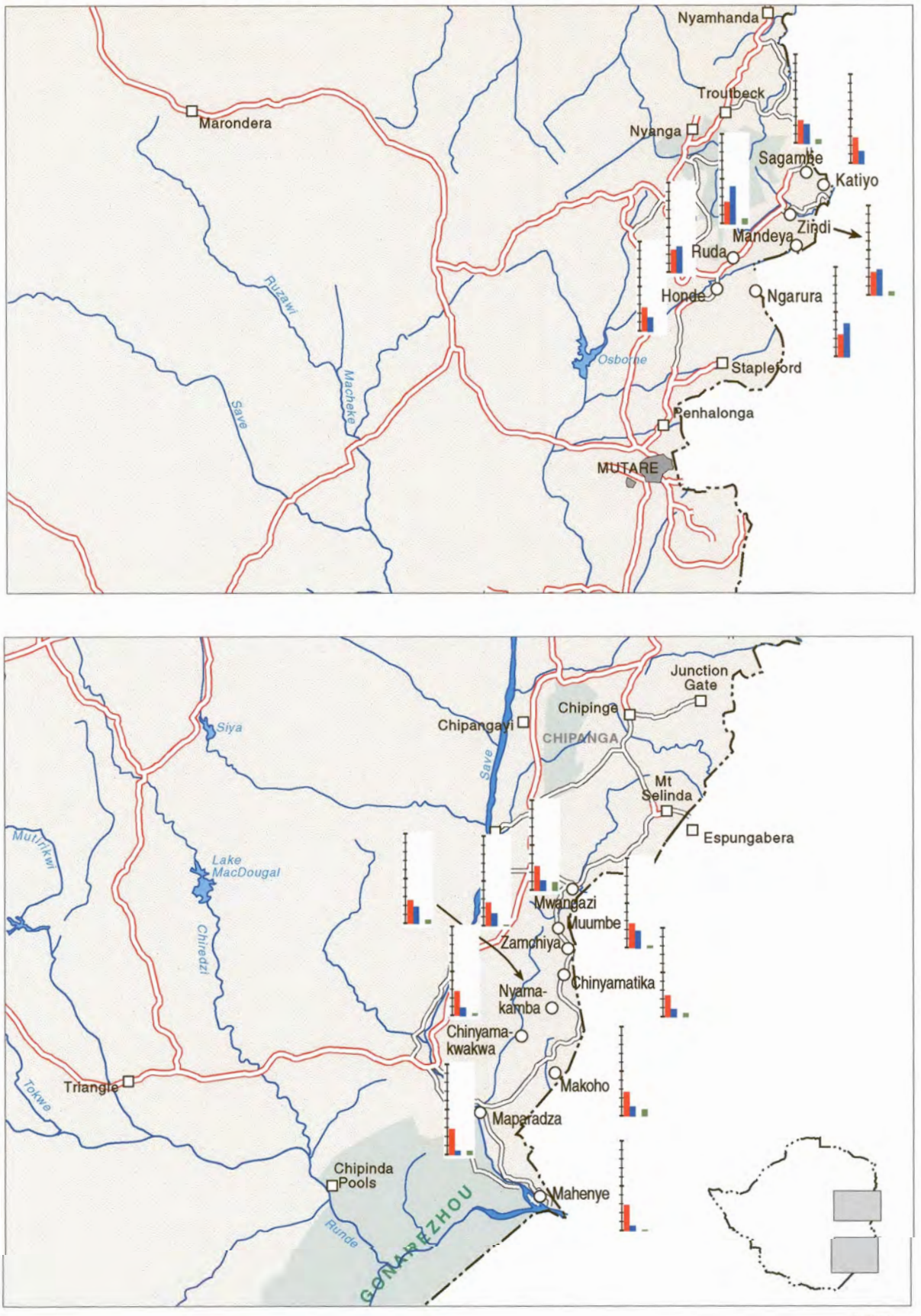
3.6.3.1 Chipinge area

A total of 540 head of cattle, from nine sampling sites, was sampled in the Chipinge area (Table 3.6.1 and Fig.3.6.1). No trypanosomal infections were detected. Anti-trypanosomal antibody levels were present in cattle sampled at each site. However, the average proportion of cattle with anti-trypanosomal antibodies at each sampling site was low (4.8 ± 0.9%). The average percentage positivity of the seropositive samples was 34.0 ± 1.1%. The average PCV of the seropositive animals animals (28.1 ± 0.9%) did not differ significantly from that of the seronegative animals (28.6 ± 0.9%) ($P>0.05$).

Table 3.6.1: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the Chipinge area.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV≤24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Mahenye	60	30.6	6.7	0	1.7
Maparadza	60	29.9	5.0	0	5.0
Makoho	60	28.4	11.7	0	8.3
Chinyamukwaka	60	28.9	10.0	0	3.3
Nyamakamba	60	27.3	20.0	0	5.0
Chinyamatika	60	25.5	10.0	0	5.0
Zamuchiya	60	27.5	15.0	0	1.7
Muumbe	60	28.4	20.0	0	3.3
Mwangazi	60	27.9	11.7	0	10.0

Figure 3.6.2: Herd average PCV, aemic animals, parasitological and serological prevalence of bovine trypanosomosis in the Chipinge area and the Honde Valley in Zimbabwe



3.6.3.2 Honde Valley

In the Honde Valley 363 head of cattle were sampled at seven sampling sites. No trypanosomal infections were detected. Cattle with anti-trypanosomal antibodies were present at three of the seven sites (Table 3.6.2 and Fig. 3.6.2). The average PCV of the seropositive animals (26.0 ± 1.2%) did not differ significantly from that of the seronegative animals (26.8 ± 0.3%) (P>0.05).

Table 3.6.2: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the Honde Valley, Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Ngarura	50	25.9	38.3	0	0
Honde	60	27.6	16.7	0	0
Ruda	60	26.1	30.0	0	0
Mandeya	60	25.5	43.3	0	7.1
Zindi	60	27.0	30.0	0	5.0
Katiyo	20	30.0	15.0	0	0
Sagambe	53	27.3	22.6	0	5.6

3.6.3.3 Eastern/northeastern region

A total of 1 594 head of cattle was sampled at 23 sampling sites. Trypanosomal infections (25 *T. congolense* and 13 *T. vivax*) were detected in cattle from four sampling sites most of them located in Mudzi District or adjacent to the target barrier (Table 3.6.3 and Fig. 3.6.3).

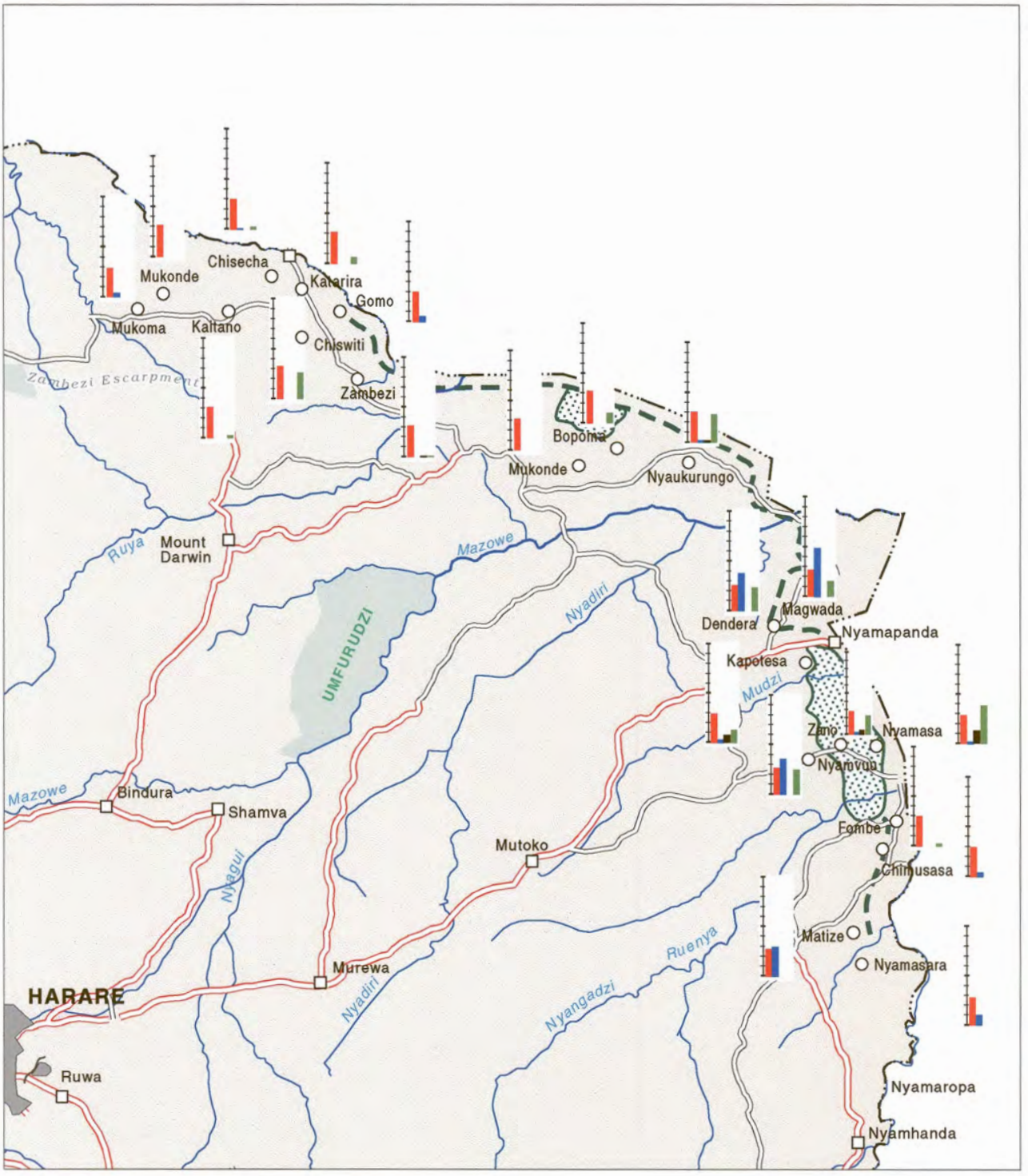
Anti-trypanosmal antibodies were most prevalent in cattle sampled in Mudzi and Guruve Districts. With the exception of cattle sampled at Nyaukurungo and Chiswiti.

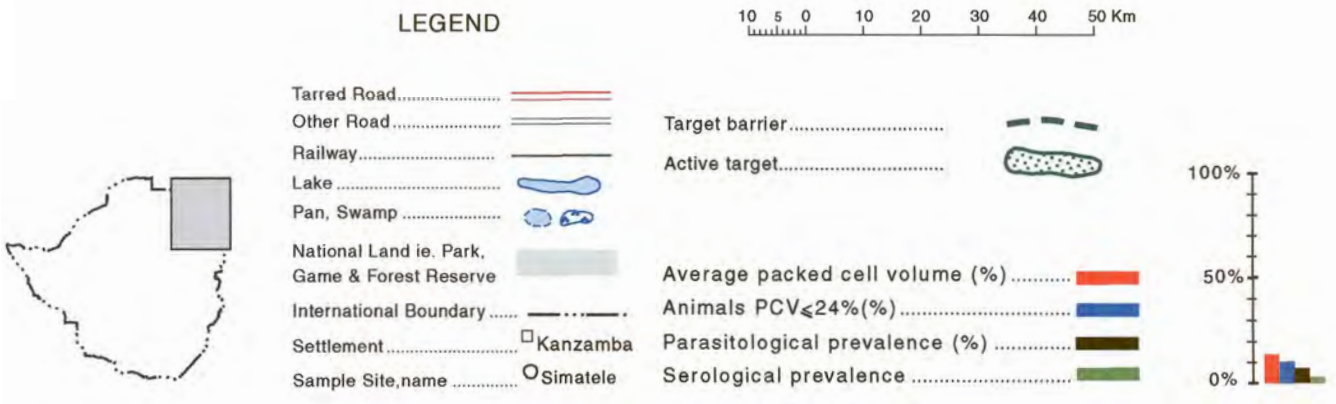
anti-trypanosomal antibodies were almost absent in cattle sampled in the remaining parts of the east/northeastern region (Table 3.6.3). The average PCV of all seropositive animals (27.2 ± 0.2%) differed significantly from that of the seronegative animals (29.8 ± 0.2%) ($P<0.001$).

Table 3.6.3: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites along the eastern/northeastern region. Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Nyamasara	60	29.0	11.2	0	0
Matize	60	28.1	30.5	0	0
Chimusasa	60	30.5	5.5	0	0
Fombe	60	31.0	0	0	3.6
Nyamvuu	60	27.7	36.2	0	25.9
Nyamasa	208	28.9	2.4	12.9	38.6
Zano	60	28.2	3.6	5.9	23.4
Kapotesa	112	29.4	1.8	8.3	13.4
Magwada	60	27.5	50.0	0	16.3
Dendera	60	26.8	38.8	0	24.6
Nyaukurongo	64	32.1	3.2	3.1	29.2
Bopoma	60	33.7	0	0	10.9
Mukonde	60	32.7	0	0	0
Zambezi	60	32.7	0	0	0
Gomo	65	29.7	0	0	1.6
Chiswiti	60	33.9	0	0	27.7
Katarira	60	32.5	0	0	7.3
Chisecha	60	31.0	1.7	0	3.6
Kaitano	60	32.3	0	0	3.4
Mukonde	60	32.7	0	0	0
Mukoma	65	29.9	4.6	0	0
Dande	60	31.1	10.0	0	15.7
Mashumbi	60	31.6	1.7	0	27.6

Figure 3.6.3: Herd average PCV, prop animals, parasitological and serological prevalence of bovine trypanosomosis in the eastern/northeastern region in Zimbabwe.





3.6.3.4 Northern region

A total of 762 head of cattle was sampled at 16 sampling sites in the northern region. Trypanosomosis was diagnosed in 93 animals from 11 sampling sites (Tables 3.6.4 and 3.6.5) most of them located within or north of the active target operations (Fig. 3.6.4). Trypanosomal infections were mainly due to *T. congolense* (50.3%) and *T. vivax* (40.8%) (Table 3.6.4).

The average prevalence of anti-trypanosomal antibodies was high in all areas (Table 3.6.5). It was, however, substantially higher in cattle sampled at sites north of the target operations (34.5 ± 7.2%) compared to cattle sampled within (13.5 ± 7.6%) or south (12.4 ± 1.5%) of the target operations.

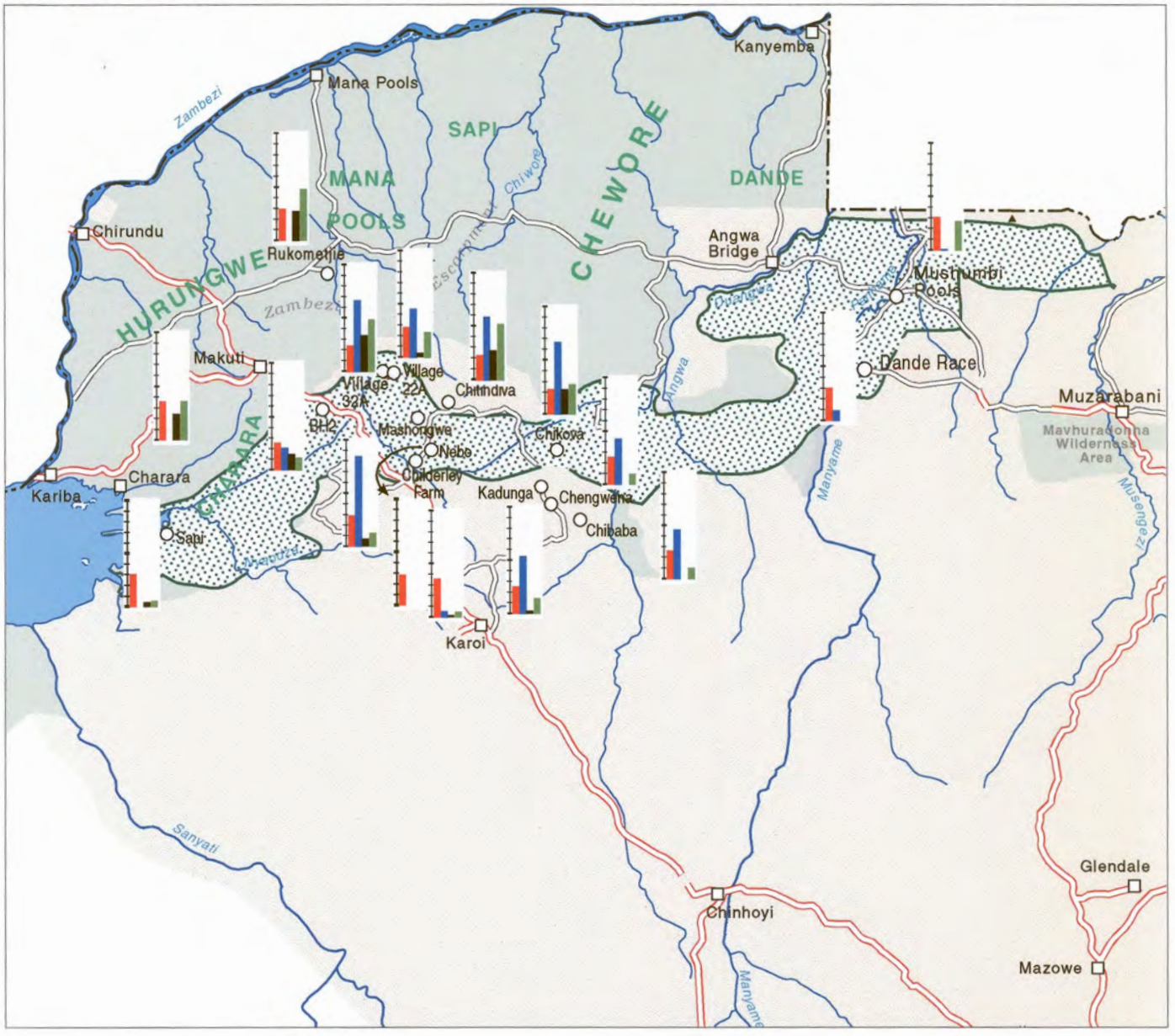
Table 3.6.4: Number of trypanosomal infections detected and trypanosome species involved at various sampling sites in the northern region, Zimbabwe.

Sampling site	Number sampled	Trypanosome species		
		T. congolense	*T. vivax*	*T. brucei*
Chikova	67	16	1	0
Kadunga	66	1	1	0
Chitindiva	73	10	9	1
Childerly Farm	41	1	0	0
Village 22A	44	1	0	1
Mashongwe	25	1	1	0
Village 32A	76	6	20	0
Rukometjie	32	5	1	3
BH2	46	3	4	0
Makuti	23	2	1	3
Sapi	22	1	0	0

Table 3.6.5: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the northern region, Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Chibara	60	27.1	47.2	0	11.1
Chengweha	61	26.5	43.8	0	10.7
Chikova	67	24.1	67.5	25.4	28.6
Kadunga	66	26.1	54.4	3.0	15.3
Chitindiva	73	23.3	59.5	27.4	52.7
Nebo Farm	6	29.2	0	0	0
Childerly Farm	41	36.7	6.1	2.4	5.6
Village 22A	44	28.8	46.6	4.5	24.4
Mashongwe	25	29.6	85.4	8.0	13.6
Village 32A	76	24.6	67.5	34.2	49.3
Rukometjie	32	27.8	28.1	28.1	49.3
BH2	46	26.2	21.7	15.2	12.5
Makuti	23	30.7	26.1	26.1	37.1
Sapi	22	31.4	0	4.5	6.2

Figure 3.6.4: Herd average PCV, proportion of anaemic animals, parasitological and serological prevalence of bovine trypanosomosis in the northern region in Zimbabwe.



3.6.3.5 Western region

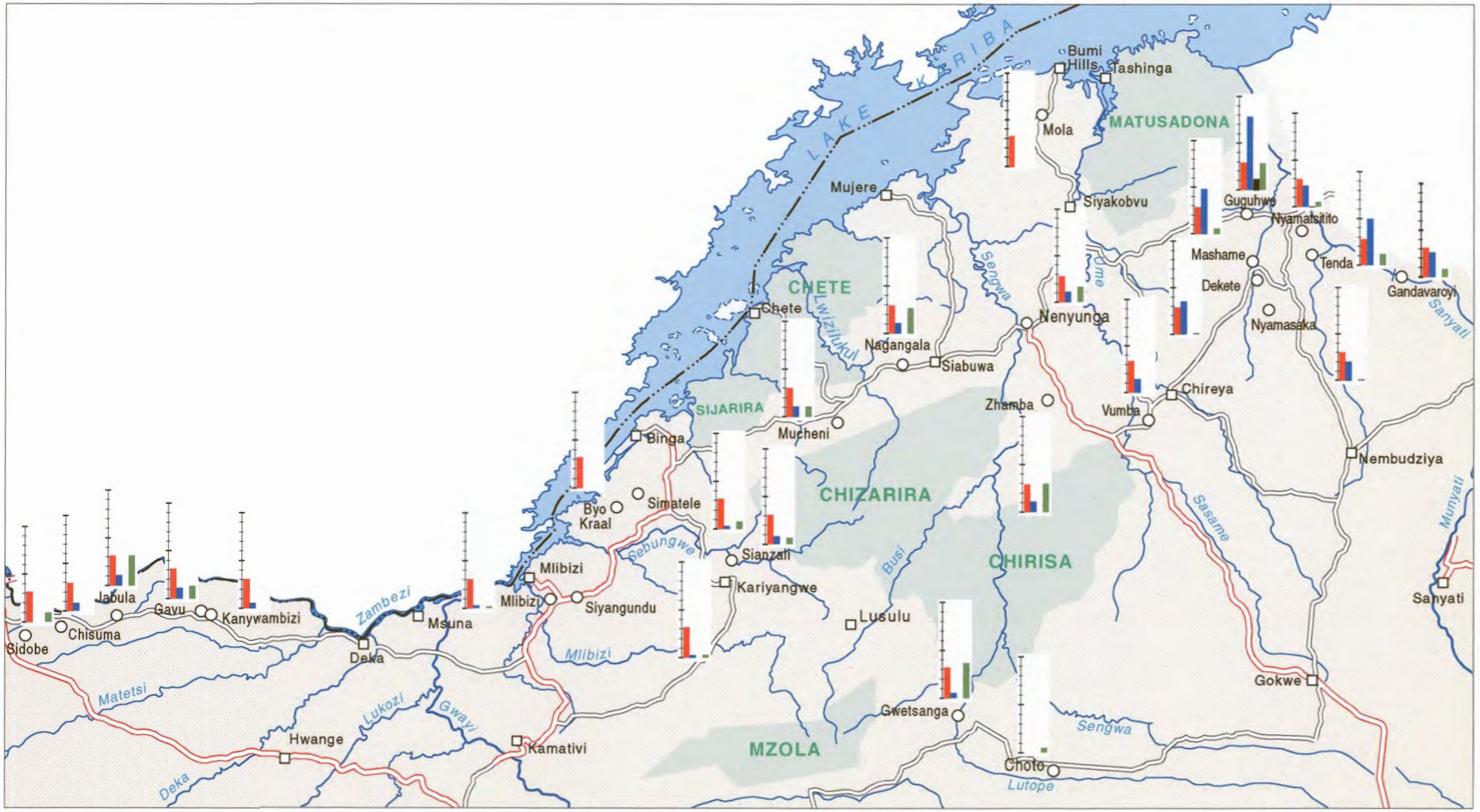
A total of 1 441 head of cattle was sampled at 25 sampling sites in the western region. Trypanosomal infections (4 *T. congolense*) were detected in cattle sampled at two sampling sites (Table 3.6.6). They were both located south of the tsetse-infested Matusadona National Park (Fig. 3.6.5).

Table 3.6.6: Number of animals sampled, average PCV, proportion of anaemic animals and parasitological and serological prevalence of bovine trypanosomosis at various sampling sites in the western region, Zimbabwe.

Sampling site	Number sampled	Average PCV (in %)	Proportion PCV ≤ 24% (in %)	Prevalence (in %)	
				Parasitological	Serological
Gandavaroyi	60	29.9	25.0	0	8.3
Tenda	60	26.8	47.2	0	11.7
Nyamatsito	61	28.2	21.8	1.6	5
Nyamasaka	60	28.9	19.5	0	1.7
Guguhwe	26	27.6	74.0	11.5	26.9
Dekete	60	27.4	33.3	0	1.7
Mashama	62	27.5	45.6	0	5.8
Vumba	61	32.2	13.8	0	0
Mola	30	33.4	0	0	0
Zhamba	60	29.3	11.2	0	30.0
Nenyunga	60	28.5	11.2	0	16.7
Nagangala	60	29.7	11.2	0	26.7
Mucheni	60	30.3	11.2	0	11.7
Choto	60	n/a	n/a	n/a	5.0
Gwetsanga	60	32.3	5.5	0	36.7
Sianzali	60	30.8	8.3	0	7.1
Byo Kraal	60	32.5	0	0	1.7
Simatele	60	32.1	2.8	0	8.3
Siyangundu	60	32.3	2.8	0	3.3
Mlibizi	60	31.0	2.8	0	1.7
Kanywambizi	61	30.5	5.5	0	0
Gavu	60	31.5	11.2	0	13.3
Jabula	60	31.5	11.2	0	31.5
Chisuma	60	29.7	8.3	0	0
Sidobe	60	32.1	0	0	10.0

n/a = not available

Figure 3.6.5: Herd average PCV, proportion of anaemic animals, parasitological and serological prevalence of bovine trypanosomosis in the western region in Zimbabwe.



The prevalence of cattle with anti-trypanosomal antibodies was relatively high at samplings sites located along the Sanyati River, in the vicinity of the Chizarira National Park and the Chirisa Safari Area and along the border with Zambia in the vicinity of Victoria Falls.

3.6.4 Discussion and conclusions

3.6.4.1 Distribution and prevalence of bovine trypanosomosis

(i) Chipinge area

Whereas no trypanosomal infections were diagnosed using parasitological diagnostic methods, anti-trypanosomal antibodies were detected in some of the animals at all sampling sites. However, the average prevalence of cattle with antibodies was low. Moreover, since the anti-trypanosomal antibody detection ELISA is not 100% specific and sensitive, a proportion of these positive reactions are likely to be false positives. This proportion of false positives can even increase with decreasing prevalence of infection (Thrusfield, 1986). Such false positive reactions may partly explain the very low prevalence of cattle with anti-trypanosomal antibodies at most of the sampling sites. However, the prevalence of anti-trypanosomal antibodies in cattle sampled at Makoho and Mwangazi does suggest a degree of tsetse challenge. Since anti-trypanosomal antibodies persist, these antibody levels could be the due to a current trypanosomal infection that was not detected or an infection that was cured within a period of a maximum of 13 months previous to sampling (section 3.2.3). Because of the low sensitivity of parasitological methods for diagnosing trypanosomosis, a substantial proportion of the trypanosomal infections were not diagnosed (Paris *et al.*, 1982). Hence, some animals with anti-trypanosomal antibodies may, in fact, have be infected with trypanosomes. If this was the case in this survey, the average PCV of seropositive animals could be expected to be lower than that of seronegative animals. This was not the case. The results, therefore, suggest that trypanosomosis challenge in the Chipinge area is low and probably irregular. This was confirmed by the absence of tsetse catches during a 12-month intensive tsetse surveillance exercise in the Chipinge area (Chihiya, personal communication, 2000). Nevertheless, more focused surveillance is required to

elucidate the origins of the challenge. Moreover, surveillance units need to be established to monitor the possible spread of tsetse in the Gonarezou National Park.

(ii) Honde Valley

In the Honde Valley, only three sampling sites had cattle with anti-trypanosomal antibodies. At each of these sites, the prevalence of cattle with anti-trypanosomal antibodies was low. Moreover, the average PCV of seropositive animals did not indicate the presence of active trypanosomal infections. As in the Chipinge area, the Honde Valley survey results suggest that a low degree of tsetse challenge was present. This is not surprising because of the absence of tsetse control measures and the possible presence of tsetse species such as *G. austeni* that would be difficult to detect. Regular monitoring of the situation is required.

(iii) East/northeastern region

Along the east/northeastern border with Mozambique, tsetse challenge was highest in Mudzi, in the southern part of Rushinga and in Gururve Districts. Despite the presence of a 10 km-wide target barrier in Mudzi District, several trypanosomal infections were diagnosed and high proportions of animals had anti-trypanosomal antibodies. This challenge is attributed to the high invasion pressure of tsetse (*G. m. morsitans* and *G. pallidipes*) along this part of the Zimbabwe/ Mozambique border (Section 5.6.3.2). Whereas entomological and disease prevalence data indicate that targets are able to cope with this invasion pressure, cattle are likely to be challenged when grazing close to the tsetse invasion front.

In areas south and north of the Mudzi District, the parasitological prevalence of trypanosomosis and the prevalence of anti-trypanosomal antibodies was low. These results suggest that the areas have been cleared effectively of tsetse and that the control measures left in place cope effectively with the invasion pressure from neighbouring Mozambique. Exceptions were the relatively high prevalence of anti-trypanosomal antibodies in cattle sampled at Magwada and Dendera (Rushinga District), at Katarira (Centenary District), at Chiswiti (Centenary District) and Dande and Mashumbi (Gururve District) and the presence of a trypanosomal infection and anti-trypanosomal antibodies in cattle sampled at Nyaukurongo (Mount Darwin

District). The anti-trypanosomal antibody levels detected in cattle sampled at Katarira and Chiswiti could be explained by the presence of a residual tsetse focus. In 1996, tsetse flies (*G. m. morsitans*) were captured southeast of Mukumbura (Davison, 1996). However, a more detailed assessment of the situation is required. The origin of the trypanosomal infection detected in Nyaukurongo is not clear. It could be attributed to the movement of cattle into tsetse-infested habitat in Mozambique or the reinvasion by tsetse. Again, more detailed investigations are required. Since Mashumbi is located close to the tsetse invasion front (Fig. 3.6.3), the high prevalence of cattle with antibodies (27.7%) is not surprising. Challenge still occurs at Dande but at a much lower rate compared to Mashumbi. According to these results, the effectiveness of the target barrier in Rushinga District needs to be reviewed.

(iv) Northern region

Not surprisingly, the prevalence of trypanosomal infections and anti-trypanosomal antibodies was high in animals sampled north of the target-protected area. *Trypanosoma congolense* and *T. vivax* were the dominant trypanosome species diagnosed. In southern Africa, *T. congolense* is the most prevalent trypanosome species in cattle (Section 3.4). The high proportion of *T. vivax* infections detected in the northern region was, therefore, unusual. Since *T. vivax* has a short developmental cycle in the tsetse fly (Davies, 1977), a high proportion of *T. vivax* is expected in younger age-groups of tsetse. Hence, *T. vivax* infections are likely to dominate in areas where the proportion of young tsetse flies is high because of high mortality rates. Such high mortality rates certainly occur within or along a target barrier.

South of the target barrier, only one trypanosomal infection was detected in an animal sampled at Kadunga. However, the proportion of cattle with anti-trypanosomal antibodies suggests challenge at all other sampling sites located south of the target barrier. These findings are not surprising since cattle immediately south of the target barrier can move into the barrier zone and become infected. Furthermore, the presence of tsetse south of the barrier cannot be excluded.

(v) Western region

In the Western region, trypanosomal infections were detected in cattle sampled along the Sanyati River and south of the tsetse-infested Matusadona National Park. The infections south of the National Park indicate that tsetse were present south of the target barrier surrounding the National Park. Hence, before commencing with the clearing of tsetse from the Matusadona National Park, detailed information on the spread of the fly outside the National Park's boundaries should be obtained. The trypanosomal infections detected in cattle sampled along the Sanyati River could be due to movement of tsetse from the Matusadona National Park. However, tsetse (*G. pallidipes*) have been captured in large numbers in this area suggesting that residual foci of tsetse may still be present (TTCB, 1991).

According to the survey results, bovine trypanosomosis was absent from most areas sampled along the southern shoreline of Lake Kariba. However, trypanosomal antibodies were prevalent in cattle sampled at sites surrounding the Chizarira National Park and the Chirisa Safari Area. This suggests that, despite the efforts made to clear the area from tsetse (Lovemore, 1990), challenge still occurs. Intensive tsetse surveys are required to elucidate the tsetse situation.

A high proportion of cattle with anti-trypanosomal antibodies was detected along the Zambezi River in the western part of the country. The most likely explanation for this occurrence is the southerly advance of the *G. m. centralis* fly belt in Zambia or movement to the west of tsetse (*G. m. morsitans* or *G. pallidipes*) from the Lusitu area (Southern Province, Zambia). Indeed, bovine trypanosomosis was detected in cattle sampled on the other side of the Zambezi River immediately north of Jabula (Lovemore, 1989; RTTCP, 1991). A high level of vigilance is urgently required.

3.6.4.2 Monitoring of the effectiveness of tsetse control interventions

The effectiveness of tsetse control operations is assessed by establishing their impact on the density of the tsetse population. Once tsetse have been cleared and the threat of reinvasion remains, continuous monitoring is required to confirm the tsetse-free status. This was in progress in large areas of Zimbabwe. Monitoring may either be direct or indirect. Direct monitoring involves determining the presence or absence of tsetse. This approach has the obvious advantage that, if flies are captured,

conclusions are unequivocal. Moreover, this type of monitoring can be conducted in all areas since it does not rely on the presence of cattle. However, interpretation of results is difficult when no tsetse are caught. The absence of tsetse catches in traps or along fly-round transects does not necessarily mean the total absence of the fly (Hargrove, 1980a). Therefore, information on the abundance of tsetse is supplemented usually by indirect indicators of the presence of tsetse. Useful indirect indicators are data on the parasitological prevalence or incidence of bovine trypanosomosis in sentinel herds or locally owned cattle. Information on the prevalence or incidence of nagana is particularly helpful when trypanosomal infections are detected in areas where, according to the outcome of tsetse samplings, no flies are present. However, interpreting results is difficult when no trypanosomal infections are detected. Indeed, the absence of detectable trypanosomal infections does not necessarily mean the absence of the disease. The currently available methods for the parasitological diagnosis of trypanosomosis have variable but generally low sensitivity (Paris *et al.*, 1982) and many infections are not detected. Although this may not be such a problem in areas where nagana is highly prevalent, the low sensitivity has important consequences in areas where the disease is present at low prevalence. The latter areas are usually those where tsetse, because of their low population density, cannot be captured with the conventional sampling methods. The low sensitivity of the tsetse sampling methods and methods for the parasitological diagnosis of trypanosomosis can be compensated for by surveillance. However, surveillance is time consuming and labour intensive. Therefore, it is frequently beyond the financial means of a tsetse control department. Moreover, whereas surveillance may be used to declare an area vector-free it is practically impossible to continue such intensive surveillance to monitor the vector-free status of the whole tsetse-cleared area in, for example, Zimbabwe. Clearly there is a need for a simple and relatively inexpensive method to assess quickly and accurately the effectiveness of control measures. The anti-trypanosomal antibody detection ELISA may meet this requirement. The sampling procedure has been simplified. Whole blood samples can be collected on filter papers, which makes the technique user-friendly and less expensive (Hopkins *et al.*, 1998). It also dispenses with the need for a cold chain. More importantly, the test is robust and has high sensitivity and specificity. The test detects antibodies against current and past infections. Although this is often considered as a disadvantage, it may be an

advantage when the antibody-ELISA is used to monitor the tsetse-free status of an area. Indeed, the anti-trypanosomal antibody levels in a herd will give an indication of the presence or absence of challenge during the previous 13 months (Section 3.2.3). Like most diagnostic methods with specificity of less than 100%, false positive reactions may make interpretation of results difficult. This is especially the case when challenge is low or absent (Thrusfield, 1986). Indeed, even in the absence of challenge, false positive reactions are likely to occur. Ideally, when prevalence is very low, a more sensitive and specific test is required. Such a test is not available for routine use. In practice, however, repeated testing of the same specimen can solve the problem of false positive reactions.

From the results of the survey conducted in Zimbabwe, the antibody-ELISA is a useful monitoring tool in areas where;

1) tsetse have been absent for many years but where the threat of tsetse reinvasion is present (Chipinge area, Honde Valley and western region);
2) the effectiveness of barriers to reinvasion of tsetse needs to be assessed (east/northeast and northern regions);
3) residual tsetse foci may be present (western region)

Although monitoring should not rely solely on the antibody-ELISA, it is certainly a useful additional tool in situations where a high degree of sensitivity and specificity is required. The test can only be applied to those areas where cattle are present but its results do not indicate where and when challenge occurred. However, in some cases it may be useful to introduce seronegative sentinel cattle. By monitoring the serological status of the sentinel animals over time and accurately establishing their movement patterns, useful information can be obtained on the time and place of challenge.

Despite routine tsetse surveillance in Zimbabwe, the results obtained from the survey described in this paper have improved greatly the knowledge of the bovine trypanosomosis situation in Zimbabwe. Similar follow-up surveys could be conducted at, for example, six-monthly intervals or when required.

CHAPTER FOUR

BOVINE TRYPANOSOMOSIS AS A HERD DISEASE AND AN ASSESSMENT OF ITS SOCIO-ECONOMIC IMPACTS

4.1 Introduction

An important determinant in the selection of priority areas for the control of bovine trypanosomosis is the effect of the disease on agricultural development. These effects can be either direct or indirect (Section 1.4.3). The indirect socio-economic impacts of nagana are often difficult to qualify and quantify. They are a result of the effects of the disease on agricultural production and farming practices. They are likely to be affected by factors other than trypanosomosis and are, therefore, subject to temporal and spatial variability. Hence, the indirect effects of trypanosomosis can only be quantified and qualified after a thorough analysis of the farming system and the factors constraining it. Most of the direct effects, on the other hand, can be quantified relatively easily.

The direct effects of bovine trypanosomosis and its control on herd productivity are a result of changes in the health status and performance of cattle in areas under challenge. The proportion of infected animals and disease tolerance, to a large extent, determines the health status of a herd. A wide range of factors can increase an animal's susceptibility to trypanosomosis (Section 1.4.2.2). Hence, susceptibility is also subject to temporal and spatial variations. With bovine trypanosomosis, tolerance to the disease is associated with an animal's ability to control the intensity, prevalence and duration of parasitaemia and to limit the pathological effect of parasites, the most prominent of which is anaemia. Consequently, the relationship between prevalence of trypanosomal infections in a herd and the herd's average packed cell volume could be a useful indicator of disease tolerance. A study was undertaken to investigate this relationship and the usefulness of this relationship as an adjunct to the rapid assessment of the impact of bovine trypanosomosis (Section 4.2).

Bovine trypanosomosis is considered to be one of the major constraints to livestock development in large parts of southern Africa. Despite the perceived importance of the disease, the real impacts of nagana on productivity are unknown. This is surprising since the sustainability of a control intervention will depend largely on the benefits accruing from such an intervention. To quantify the impact of bovine trypanosomosis on production and predict the potential impacts of controlling the disease under the

different epidemiological situations occurring in the southern African region socio-economic surveys were conducted. Information was collected about the proportion of households directly affected by the disease, cattle marketing practices and cattle productivity under different epidemiological situations and current disease management practices (Section 4.3). Conclusions were then drawn about the socio-economic impact of trypanosomosis control.

4.2 The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume

4.2.1 Introduction

The distribution of tsetse-transmitted bovine trypanosomosis is usually established by demonstrating trypanosomal infections in cattle. By comparing the proportion of infected animals between herds, areas of low, medium or high disease prevalence can be distinguished from disease-free areas. Several factors including trypanosome strain, age of the infected animal, breed and nutritional status affect the tolerance of trypanosomal infections in susceptible breeds (Connor, 1994a). Therefore, decisions to intervene and control trypanosomosis cannot be based solely on the presence of trypanosome-infected animals but should be supported by an assessment of the impact of the infection on animal condition and, hence, animal production. In cattle and other domestic animals, anaemia is a well-recognized and inevitable consequence of an infection with pathogenic trypanosomes (Murray and Dexter, 1988). It is best measured by determining the PCV. In the absence of other factors causing anaemia, the PCV gives a reliable indication of the disease status of a trypanosome-infected animal and is strongly correlated with its performance (Trail *et al.*, 1991; 1993). Consequently, the herd average PCV should give a good indication of the health status of a herd. Furthermore, by establishing the relationship between herd average PCV and prevalence of infection in an area, useful information can be obtained on (i) the impact of various levels of disease prevalence on herd health and (ii) the likely impact of control interventions on herd health. Although the herd average PCV is expected to decrease with increasing prevalence of trypanosomosis, this relationship has not been quantified sufficiently. Data obtained from trypanosomosis surveys conducted in eastern Zambia were used to study this relationship in more detail. The usefulness of this relationship as an adjunct to the rapid assessment of the impact of bovine trypanosomosis is discussed.

4.2.2 Materials and methods

The herd mean prevalence of trypanosomal infections and herd mean PCV were obtained from cattle sampled at 141 sampling sites (crushpens) in Katete, Petauke, Chipata and Lundazi Districts (Chipangali area) of eastern Zambia. All districts are ecologically similar, have similar farming systems (RTTCP, 1998; 1999) and are situated on the eastern plateau. Bovine trypanosomosis, transmitted by *G. m. morsitans*, is endemic. Bovine trypanosomosis is managed using trypanocidal drugs mainly diminazene aceturate (Berenil ®, Hoechst). The proportion of animals treated and the treatment frequency is low (Section 5.2.3). Two separate disease surveys were conducted. The first survey covered all four districts. It was conducted during the rainy season (November-March 1996). Sampling sites were selected depending on their location and were evenly spread over each district. During a second survey, sampling was repeated at the sampling sites in Petauke District only but different animals were sampled. The second survey was conducted during the dry season (August-September 1996).

A "random" sample of communally managed adult cattle (Angoni breed) was selected at each sampling site (Section 3.4.2.1). The buffy coat, stained thick and stained thin smears were used as parasitological diagnostic methods (Section 3.3.2.2). The PCV of each blood sample was determined. The level of bovine trypanosomosis at a sampling site was calculated as the proportion of cattle with a trypanosomal infection; it is henceforth referred to as "herd prevalence". The PCV of all animals sampled at each site was averaged; this is referred to as the "herd average PCV". From all animals, blood contained in one heparinized microhaematocrit centrifuge capillary tube was extruded onto a filter paper (Whatman n° 4, Whatman®). Eluted blood spots were screened for the presence of trypanosomal antibodies using an indirect ELISA (Section 3.3.2.2).

The relationship between the parasitological prevalence of trypanosomal infections and herd average PCV was examined by regression analysis using the estimates of herd average PCV as the dependent variable and the prevalence of trypanosomal infections in a herd as the independent variable. The effects of area of sampling

(district) and season of sampling on the herd average PCV was also investigated by analysis of variance using data obtained from the four districts in Zambia during the rainy and one district during the dry season. In addition use was made of a multiple linear regression model with herd average PCV as the dependent variable and combinations of season, area of sampling (district) and herd prevalence of trypanosmal infections as independent variables.

The models fitted were:

$y_{ij} = a + d_i + b_i x_{ij} + r_{ij}$ for all four districts ($i = 1, \ldots, 4$) during the rainy season and

$y_{ij} = a + s_i + b_i x_{ij} + r_{ij}$ for rainy and dry season ($i = 1, 2$) for herds in Petauke District

where y_{ij} = herd PCV for herd j within respectively district or season i

a = average intercept

d_i ($i = 1,\ldots,4$) = effect of district on mean herd PCV

s_i ($i = 1, 2$) = effect of season on mean herd PCV

b_i = regression coefficient for district in season i

x_{ij} = prevalence of trypanosomal infections in a herd

r_{ij} = residual

All analyses were performed using the statistical package SPSS (SPSS Inc.).

4.2.3 Results

A total of 8 640 head of cattle were sampled in four districts (Chipata, Lundazi, Petauke and Katete) of the Eastern Province of Zambia. Trypanosomal infections, mainly due to *congolense* (86.4%), were detected in 1 252 animals (14.5%) from 97 herds out of a total of 141 herds sampled.

The herd average prevalence of trypanosmal infections in the rainy season varied between areas. It was highest in the Petauke and Lundazi Districts and lowest in Chipata District (Table 4.2.1).

Table 4.2.1: Number of herds sampled, number of trypanosome-positive herds, average parasitological prevalence of trypanosomosis and average PCV of parasitological positive and negative herds in four districts of the Eastern Province of Zambia during the rainy season and in Petauke District during the dry season.

Area	Season	Number of herds sampled	Number of positive herds	Average prevalence (% ± 1 s.e.)	Average PCV (% ± 1 s.e.)	
					positive herds	negative herds
Chipata	rain	21	10	8.4 ± 2.3	29.9 ± 0.9	31.8 ± 0.8
Katete	rain	16	13	11.4 ± 1.6	27.7 ± 0.7	29.7 ± 0.8
Lundazi	rain	22	22	17.8 ± 1.8	27.1 ± 0.5	-
Petauke	rain	43	38	24.7 ± 2.8	26.5 ± 0.3	29.6 ± 0.9
Petauke	dry	39	39	26.1 ± 4.2	26.1 ± 0.5	-

The average PCV of parasitologically positive herds decreased with increasing average prevalence of infection. The majority of the parasitologically negative herds (68.4%) contained animals with anti-trypanosomal antibodies. However, the herd average PCV of parasitologically negative and seronegative herds (31.8 ± 1.2%, n = 6) did not differ from the average herd PCV of parasitologically negative but seropositive herds (30.5 ± 0.5%, n = 13) (Fig. 4.2.1)

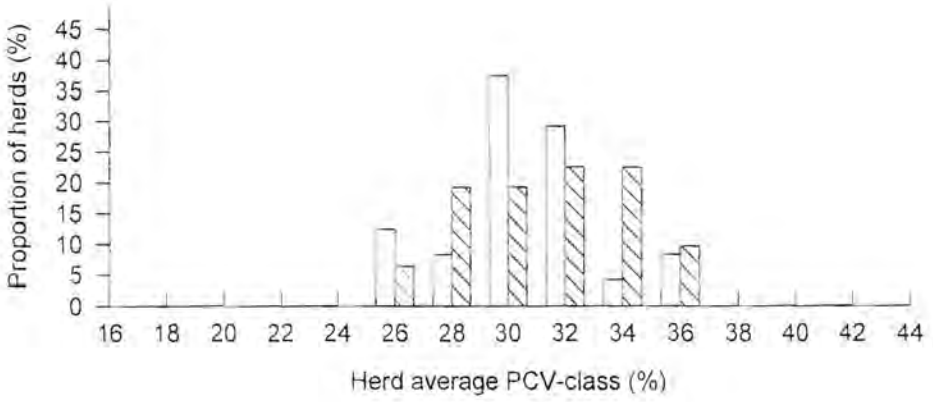


Figure 4.2.1: Frequency distribution of average packed cell volume (PCV) of parasitologically negative herds that are serologically negative (-) and serologically positive (=).

In all areas, regression analyses showed that the herd average PCV of parasitologically positive herds decreased with increasing prevalence of trypanosomal infections (Table 4.2.2).

Table 4.2.2: Linear regression of herd average PCV (%) on parasitological prevalence of trypanosomosis (%) in four districts of the Eastern Province of Zambia during the rainy season.

Area	Season	Number of herds	a*	b*	r*	Significance
Chipata	rainy	10	32.8 ± 0.8	-0.35 ± 0.08	0.84	P<0.001
Katete	rainy	13	31.3 ± 1.3	-0.31 ± 0.11	0.65	P<0.05
Lundazi	rainy	22	30.6 ± 0.9	-0.20 ± 0.04	0.71	P<0.001
Petauke	rainy	38	28.4 ± 0.4	-0.08 ± 0.02	0.65	P<0.001

* a = intercept (± 1 s.e.)
b = regression coefficient (± 1 s.e.)
r = correlation coefficient

Removal of district from the multiple linear regression model did not result in a significant change in its fit ($P > 0.05$). Hence, the intercepts for area of sampling shown in Table 4.2.2 were not significantly different. The slope of the equation between mean PCV and trypanosome prevalence (parameter b in Table 4.2.2), however, decreased with increasing prevalence of trypanosomal infections suggesting a curvilinear relationship with PCV (Fig. 4.2.2). Season of sampling also affected the slope of the equation between mean PCV and trypanosome prevalence (parameter b in Table 4.2.3).

Table 4.2.3: Linear regression of herd average PCV (%) on parasitological prevalence of trypanosomosis (%) in Petauke District of the Eastern Province of Zambia during the rainy and dry season.

Area	Season	Number of herds	a*	b*	r*	Significance
Petauke	rainy	38	28.4 ± 0.4	-0.08 ± 0.02	0.65	P<0.001
Petauke	dry	39	28.3 ± 0.6	-0.20 ± 0.03	0.71	P<0.001

* a = intercept (± 1 s.e.)
b = regression coefficient (± 1 s.e.)
r = correlation coefficient

For the same increase in prevalence of infection, the decrease in herd average PCV was higher in the dry compared to the rainy season ($P<0.01$) (Table 4.2.3 and Fig. 4.2.3).

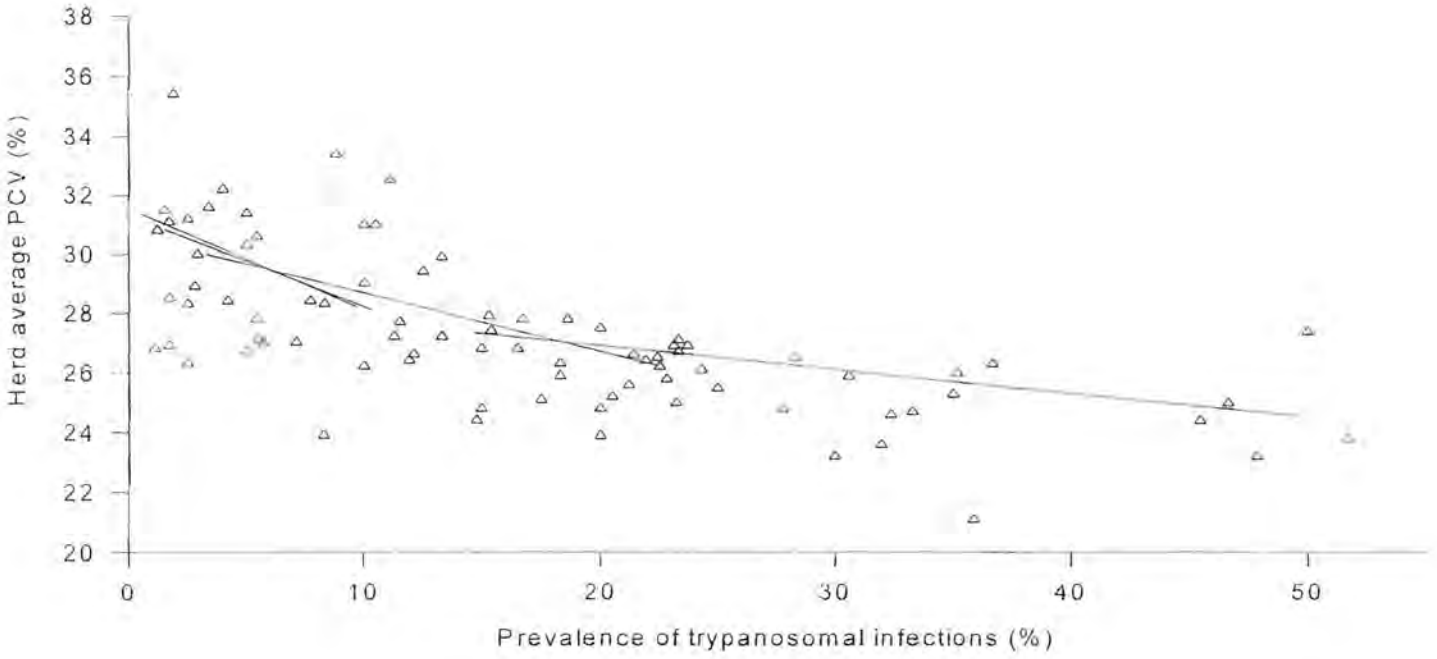


Figure 4.2.2: Relationship between herd average PCV and prevalence of trypanosomal infections in herds sampled during the rainy season in eastern Zambia. Lines are fitted by linear regression; see Table 4.2.1 for parameter estimates and significance levels.

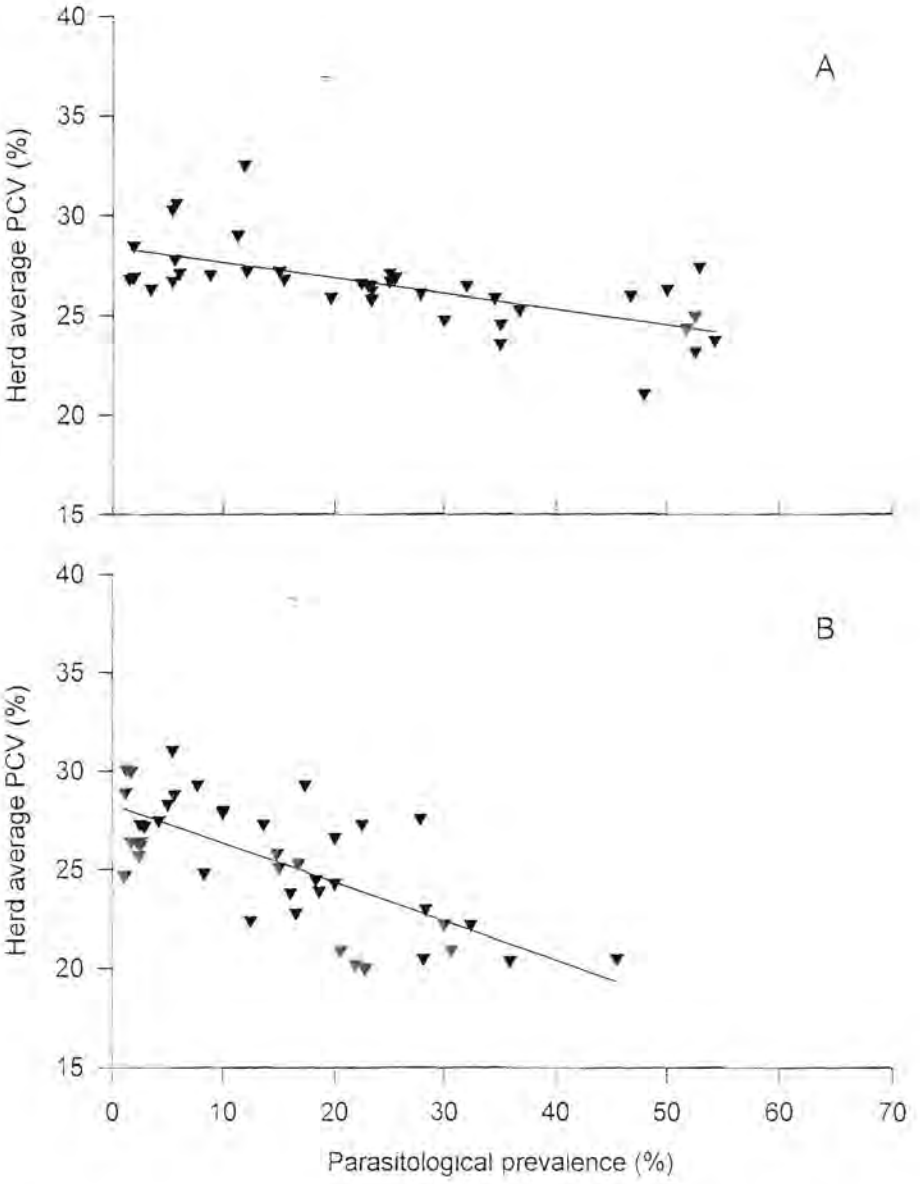


Figure 4.2.3: Relationship between herd average PCV and parasitological prevalence of trypanosomal infections in herds sampled during the rainy (A) and dry (B) season in Petauke District.

4.2.4 Discussion

The development of anaemia is one of the most typical signs of trypanosomosis caused by *T. congolense* in susceptible cattle breeds (Murray and Dexter, 1988). The level of anaemia, as indicated by the PCV, usually gives a reliable indication of the disease status and productive performance of infected animals (Trail *et al.* 1991, 1993). Bovine trypanosomosis control aims at reducing the prevalence of infection with a concomitant increase in the herd average PCV (Bauer *et al.*, 1999). Nevertheless, reduction in trypanosomosis prevalence does not necessarily result in an increase in the herd average PCV (Rowlands *et al.*, 1996). Therefore, establishing the relationship between the prevalence of trypanosomal infections and herd average PCV is a useful tool in the preliminary assessment of the expected impact of a control intervention. Conversely, the slope of the regression line can be used as an indicator of the impact of trypanosomosis on herd average PCV and, hence, herd health. By comparing the slopes, temporal and spatial comparisons could be made of the impact of trypanosomosis. However, the herd average PCV is affected by factors other than trypanosomosis (Connor, 1994). These confounding factors are not always easily identifiable but they are likely to affect both trypanosomosis positive and negative animals. Hence, the average PCVs of trypanosomosis-free herds are a good indicator of the levels of anaemia in the absence of trypanosomosis and could form the baseline for comparison between areas. Determining the PCV-distribution of parasitologically negative herds may be difficult with parasitological diagnostic methods of low sensitivity at the individual animal level (Paris *et al.*, 1982). However, according to the above results, the herd-level sensitivity of the buffy coat method for the detection of at least one positive sample in a positive herd is high at the sample sizes used in the survey (Martin *et al.*, 1992). Indeed, the herd average PCV of parasitologically negative herds does not differ from the herd average PCV of serologically negative or true negative herds. The PCV-distribution of the parasitologically negative herds can thus be used as the baseline herd average PCV distribution in the absence of trypanosomosis.

The slope of the regression equation between PCV and trypanosome prevalence decreased with increasing prevalence of trypanosomal infections. Hence, for the same

increase in prevalence, the decrease in herd average PCV is lower in herds under high challenge compared to herds under low challenge. This could be explained by differences in trypanocidal drug use. The effect of increased prevalence of infection on PCV would, for example, be minimal if the majority of drug treatments were given in the early stages of infection before or during the early phase of anaemia development. Such a trypanocidal drug treatment regimen requires early diagnosis of the trypanosomal infection. Diagnostic facilities are, however, not readily available in the study area. Hence cattle owners mainly treat clinically sick animals in the later stage of infection when, often severe, anaemia has developed (Section 5.2.3).

For the same increase in the prevalence of infection, the decrease in herd PCV is higher in the areas with low to medium prevalence (Chipata and Katete Districts). In the absence of an effect of trypanocidal drug use, the main factor determining the slope of the equation between PCV and trypanosome prevalence is the infected animal's ability to control the development of anaemia. This will differ between cattle breeds and will be affected by factors such as intercurrent disease, nutrition and the level of acquired immunity. The effect of cattle breeds is well-known. The development of anaemia tends to be more severe in exotic breeds whereas it is well-controlled in trypanotolerant breeds (d'Ieteren *et al.*, 1998). All cattle sampled during the survey were of the trypanosusceptible Angoni breed. The effect of the other factors on the relationship between prevalence and PCV is difficult to quantify. Differences in the prevalence of intercurrent diseases, aggravating the effect of trypanosomal infections on PCV, could explain the difference between areas. However, the prevalence of such diseases is unlikely to differ much between adjacent areas such as the Petauke and the Katete Districts and the Chipata and Lundazi Districts and can thus not be the main reason for the observed differences in the effect of prevalence of trypanosomal infections on herd average PCV. Nutritional stress also may exacerbate the severity of trypanosomosis. However, livestock management practices do not differ between the different survey areas and none of the areas are, as yet, subject to overgrazing (RTTCP, 1999c). Furthermore, surveys were conducted during the rainy season when grazing is at its best. Hence, differences in nutritional stress are unlikely to be the reason for the observed differences in the relationship

between prevalence of infection and herd average PCV. Field trials have shown that infection with one serodeme of trypanosome can induce immunity to reinfection with that particular serodeme but not to heterologous challenge. The immunity conferred, however, is short lived and is no longer apparent six months after the initial challenge (Murray and Urquhart, 1977). The development of such a "nonsterile immunity" to bovine trypanosomosis is difficult to assess but has been observed in areas of high challenge by a resident tsetse population that feeds mainly on cattle and where curative drugs are used to treat the acutely sick animals (Hornby, 1941; Boyt, 1967; Wilson *et al.*, 1976; Bourn and Scott, 1978). Such conditions do prevail in the Petauke District of eastern Zambia (Chapter 2 and Section 5.2.3) and may explain the observed difference in the relationship between the prevalence of infection and the herd average PCV in the Petauke District and in the other three districts. Indeed, herds in the Katete and Chipata Districts are subject to low and irregular challenge. This level of challenge may be insufficient for the development of protective immunity in cattle resulting in higher decrease in PCV for the same increase in prevalence compared to cattle in the Petauke area. Herds in the Lundazi District are subject to similar challenge as herds in Petauke District. However, because of the invasion of tsetse from the adjacent Lukusuzi and Kasungu National Park challenge is likely to be more heterologous and will not result in the same level of "nonsterile immunity" in cattle.

A major factor affecting the PCV is the plane of nutrition. Poor nutrition is known to result in a lower PCV (Sawadogo *et al.*, 1991; Katunguka-Rwakishaya *et al.*, 1995). A study, investigating the effect of season on the food intake of Angoni cattle in eastern Zambia, showed that poor pasture conditions and high temperatures cause nutritional stress during the dry but especially the hot dry season (De Clercq, 1997). Thus, the observed effect of season on the association between prevalence and herd average PCV is very likely to have been due to poor nutrition in the dry season. Whereas the season of sampling has no effect on the average PCV of the parasitologically negative herds, trypanosomosis seems to be less well tolerated during the dry season. This may explain the high proportion of trypanocidal drug treatments administered during the dry season (Section 5.2.3).

The above results indicate that the relationship between the prevalence of trypanosomal infections and the average PCV could be a useful tool in the management of trypanosomosis and planning of its control. Further research should be conducted in the temporal and spatial variations of this relationship and the factors affecting it.

4.3 An assessment of the impacts of bovine trypanosomosis on herd performance and offtake rates

4.3.1 Introduction

Bovine trypanosomosis occurs in vast areas of southern Africa (Connor, 1994). The distribution and prevalence of the disease is well established (Chapter 3). Its impact on cattle production, on the other hand, is less well known. Assessments of the socio-economic impact of the disease are, therefore, frequently based on assumptions. Nevertheless, the socio-economic impact of bovine trypanosomosis and the expected impact of its control are important criteria in determining the appropriateness of and need for a particular intervention.

The impact of bovine trypanosomosis on cattle production and marketing is best assessed by comparing various variables before and after the implementation of a control intervention in a particular area. This longitudinal approach is often difficult and time consuming. Alternatively, animal production and marketing can be compared between ecologically similar areas where cattle are either kept under tsetse challenge or where trypanosomosis is absent or has been controlled. This approach was adopted in collecting the results described in this section. To minimise the differences in cattle production variables and cattle marketing for reasons other than trypanosomosis, data were collected in a tsetse-infested and adjacent, ecologically similar, tsetse-free areas. Results were compared and the impact of bovine trypanosomosis was deduced.

4.3.2 Materials and methods

4.3.2.1 Survey areas

Surveys were conducted in six areas. In three areas (Petauke, Chipangali and Vwaza (adjacent to the game reserve,)), bovine trypanosomosis was prevalent. In the three other areas (Katete, Kasungu and Vwaza (>20 km from the edge of the game reserve) bovine trypanosomosis is virtually absent.

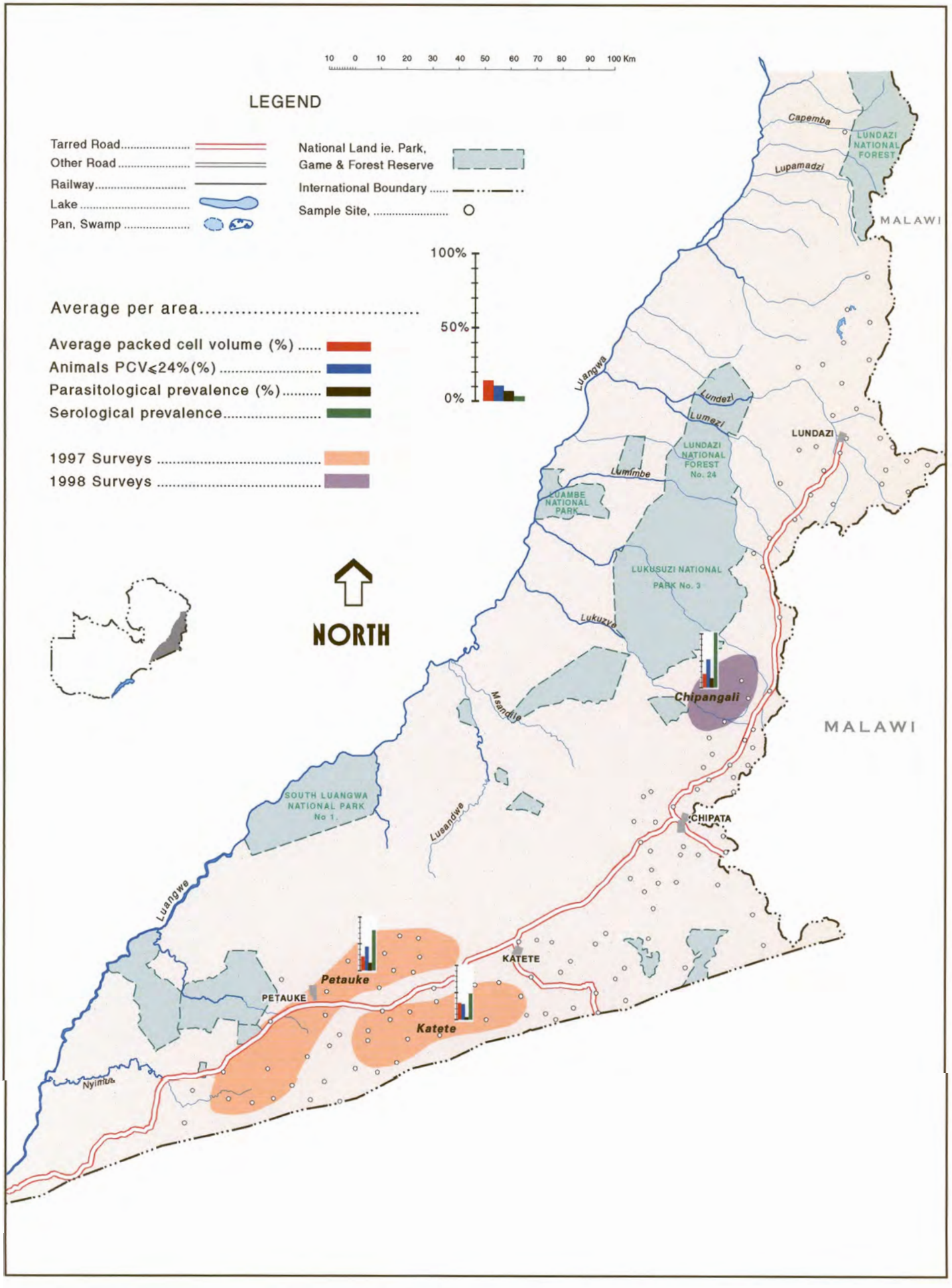
Petauke area

Baseline socio-economic surveys were conducted in an area of medium to high tsetse challenge (Petauke/Nyimba) of Petauke District in the Eastern Province of Zambia (Fig. 4.3.1) (Section 2.5.3.4). The area is situated on the eastern plateau and is part of the eastern tsetse-belt (Chapter 2). *Glossina m. morsitans* is the only tsetse species present and takes about 75% of its blood meals from cattle (Section 2.3.3). The monthly average incidence of bovine trypanosomosis is *ca.* 9% (Section 2.5.3.4). Trypanocidal drugs (diminazene aceturate and isometamidium chloride) are readily available. Trypanocides are used by the majority of the cattle owners (85%) (Section 5.2.3.1). In the use of trypanocides, preference is given to curative rather than prophylactic drugs. The majority of treatments are given to oxen and cows with each treated animal receiving *ca.* 1.5 treatments/year. The density of the trypanosusceptible Angoni breed cattle is on average 10 animals/km^2 but varies significantly between areas. Human density varies between 60-75 people/ km^2.

Katete area

A second survey was conducted in the tsetse-free Mvuvye/Katete South area of Katete District in eastern Zambia (Fig. 4.3.1). The area lies adjacent to the Petauke survey area. It is is ecologically similar and crops and crops combinations grown are virtually identical. Tsetse were controlled effectively using odour-baited, insecticide-treated, targets in the Mvuvye area and tsetse are absent in the Katete South area (Section 5.3.3). The area carries *ca.* 24 head of cattle/km^2. The human density is *ca.* 90 people/km^2.

Figure 4.3.1: Location of socio-economic survey areas in eastern Zambia



Chipangali area

The Chipangali area is located in the north-eastern corner of Chipata and the southern part of Lundazi Districts in the Eastern Province of Zambia (Fig. 4.3.1). To the north the area is bounded by the Lukusuzi National Park and to the east by the Kasungu National Park (Malawi). Both national parks are heavily infested with tsetse (*G. m. morsitans*). The Chipangali area is subject to tsetse invasion from both national parks (Wilson, 1975). The parasitological prevalence of bovine trypanosomosis is comparable to the prevalence in the Petauke area. Trypanocidal drug use practices are similar to the ones observed in the Petauke area (Section 5.2). Human and livestock densities are relatively low.

Vwaza area

The Vwaza survey area is located along the edge of the Vwaza Marsh Game Reserve in the Northern Region of Malawi (Fig. 3.4.3). The Vwaza Game Reserve is infested with tsetse (*G. m. morsitans* and *G. pallidipes*) (Davison, 1990). The survey was conducted within a band of *ca.* 40 km along the perimeter of the game reserve. Bovine trypanosomosis is present in herds located within 0-20 km from the edge of the game reserve (Vwaza < 20 km) (Section 3.4.3.4). The average prevalence of trypanosomal infections is about 7%. Bovine trypanosomosis is controlled with trypanocidal drugs by only 40% of the cattle owners (RTTCP, 1999b). Bovine trypanosomosis is absent between 20 to 40 km from the perimeter of the game reserve. Human population density in the Vwaza area is on average 85 people/km^2.

Kasungu area

The Kasungu survey area is situated along the edge of the Kasungu National Park in the Central Region of Malawi (Fig. 3.4.4). The Kasungu National Park is infested with tsetse (*G. m. morsitans*). A 6 km-wide barrier of odour-baited, insecticide-treated, targets (Section 3.4.4.2) prevents the spread of tsetse into the areas surrounding the eastern edge of the national park. Hence, the prevalence of bovine trypanosomosis is very low in the survey area (Section 3.4.4.2). The area is heavily settled, with a human population density of 104/km^2.

Because of spatial and temporal variations in the performance variables of cattle, comparisons between the trypanosomosis-infected areas could not be made. Therefore, the performance variables of each area where bovine trypanosomosis was prevalent were compared with those recorded in an adjacent ecologically similar trypanosomosis-free area (control area) surveyed during the same year (Table 4.3.1).

Table 4.3.1: Trypanosomosis-infected and their adjacent trypanosomosis-free (control) survey areas.

Survey areas		
Trypanosomosis-infected		Control
Petauke	⇔	Katete South
Chipangali	⇔	Kasungu
Vwaza (0-20 km)	⇔	Vwaza (>20 km)

4.3.2.2 Sampling methods

All surveys were conducted in the dry season between July/August and September/October. A rapid rural appraisal method was used (ILCA, 1990). Households interviewed were selected by using a two-stage cluster sampling method (ILCA, 1990). Detailed household counts were made for all the villages in each of the survey areas. Villages were then clustered, each cluster consisting of *ca.* 200 households. Twenty-five percent of the clusters were selected by random sampling and full household listings were made of all selected clusters. Households chosen for interview were selected by systematic sampling. Approximately 500 households were interviewed in each area.

A standard questionnaire was used (Annex 1). The questionnaire was pre-tested and revised to clarify specific questions and ensure that the average interview time did not exceed 45 minutes. Enumerators were trained for several days before the interviews were conducted. The questionnaire contained *ca.* 200 questions covering issues such as household characteristics, off-farm employment and income generation, crop and

livestock practices and asset ownership. Replies were coded, transferred to a coding sheet and entered into a database. The Statistical Package for Social Sciences (SPSS, SPSS Inc.) was used to analyse the data.

4.3.2.3 Variables measured

The following variables were measured for the 12-month period prior to interview:

Mortality rates of different sex and age categories of cattle

- (Number of deaths/Total herd opening number for a sex or age category) x 100

Cattle performance

- Calving rate (%) = [(Calves born)/(Cow closing number + cow deaths + cow offtake – cow purchases)] x 100
  = (Calves born/Cow opening number) x 100
- Weaning rate (%) = [(Calves born - Calf deaths)/Cow opening x 100
- Offtake rate (%) = Total cattle offtake/total herd opening number x 100
- Sales rate (%) = Total cattle sales/total herd opening number x 100
- Commercial offtake rate (%) = Cattle sales + Exchanges/ total herd opening x 100

4.3.3 Results

The surveys were conducted between June 1997 and October 1998. A total of 2622 households were interviewed (Table 4.3.2).

Table 4.3.2: Number of households interviewed in each survey area.

	Survey area					
	Petauke	Chipangali	Vwaza <20km	Vwaza >20km	Katete	Kasungu
Households	546	424	277	255	541	579

4.3.3.1 Cattle ownership

The majority of the households in all survey areas did not own cattle (Table 4.3.3). The average proportion of households owning cattle in all survey areas was only 33.9%. Furthermore, average herd sizes per owner were small (Table 4.3.3). Cattle ownership was highly skewed with 5% and 10% of the households owning half of the cattle in the Malawian and Zambian survey areas, respectively. In all areas surveyed, the proportion of cattle owners lending cattle to relatives and other farmer was < 10%. Bovine trypanosomosis was reported as the main reason for cattle loss in the Petauke, Chipangali and Vwaza survey areas.

4.3.3.2 Herd structure

Herd structures differed substantially between survey areas. Herd structure in the Kasungu survey area was fairly typical for African sedentary farming systems in southern Africa where oxen constituting *ca.* 20% and cows between 30-35% of the average herd. In both tsetse-infested areas of Zambia the oxen/cow ratio was high (1.30 and 0.96 for the Petauke and Chipangali area, respectively). In the Vwaza area of Malawi, on the other hand, a very low ox/cow ratio was recorded (on average 0.38) (Table 4.3.4).

4.3.3.3 Cattle performance variables

Mortality rates

Except for the relatively high average mortality rates in the Petauke survey area, cattle mortality rates in all age and sex categories in the tsetse-infested areas were low (Table 4.3.5). Differences between the average mortality rates in the

Table 4.3.3: Frequency distribution of cattle ownership in each survey area.

Herd size	Survey area					
	Petauke[*]	Katete[o]	Chipangali[+]	Kasungu[o]	Vwaza[*]	Vwaza[o]
No cattle	69.3	51.4	66.7	71.2	83.0	78.8
1-5	12.0	24.7	15.6	14.3	8.3	10.9
6-10	11.6	11.1	9.0	7.4	4.5	7.3
11-15	3.6	6.2	4.0	3.8	1.8	2.4
>15	3.5	6.6	4.8	3.3	2.4	0.6
Average per owner	8.1	8.5	8.9	8.0	8.3	9.1

[*] trypanosomosis-infected areas
[o] control areas

Table 4.3.4: Proportion of cattle (%) in each age and sex category in each survey area.

Age and sex category	Survey area					
	Petauke[+]	Katete[o]	Chipangali[+]	Kasungu[o]	Vwaza[+]	Vwaza[o]
Male calves	6.6	9.7	5.7	8.3	9.2	10.1
Female calves	6.6	9.6	6.1	8.7	8.7	12.2
1-4 yr old males	6.8	11.0	9.8	8.3	11.4	7.1
1-4 yr old females	8.1	10.8	13.9	10.4	12.8	9.4
Cows	30.3	30.1	30.7	34.3	35.4	41.4
Oxen	39.3	25.6	29.4	24.8	14.9	14.3
Bulls	2.1	3.1	4.4	5.2	7.6	5.5

[+] trypanosomosis-infected areas
[o] control areas

Table 4.3.5: Average mortality rates (% ± 1 s.e.) per age and sex category of cattle in each survey area.

Age category	Average mortality rate per survey area (%)					
	Petauke[+]	Katete[°]	Chipangali[+]	Kasungu[°]	Vwaza[+]	Vwaza[°]
Cow	10.2 ± 2.2	10.3 ± 1.5	6.6 ± 1.7	4.7 ± 1.4	3.1 ± 1.9	3.7 ± 1.5
Oxen/bulls	8.4 ± 1.6	11.8 ± 1.8	4.6 ± 1.4	3.3 ± 1.2	2.3 ± 2.2	2.3 ± 1.5
Young stock	5.4 ± 1.7	3.6 ± 1.1	1.6 ± 1.2	8.1 ± 2.2	3.4 ± 3.4	17.5 ± 6.0
Calves	13.2 ± 2.1	8.7 ± 1.6	7.1 ± 2.8	8.4 ± 2.5	7.1 ± 2.6	8.1 ± 3.7

Table 4.3.6: Average calving and weaning rates (% ± 1 s.e.) per survey area.

	Survey area					
	Petauke[+]	Katete[°]	Chipangali[+]	Kasungu[°]	Vwaza[+]	Vwaza[°]
Calving rate	46.9 ± 4.6	47.8 ± 4.0	37.1 ± 4.0	49.2 ± 3.4	36.0 ± 3.2	53.7 ± 4.1
Weaning rate	40.7 ± 3.8	43.6 ± 3.5	34.4 ± 4.0	45.5 ± 3.5	33.5 ± 3.8	49.3 ± 3.7

[+] trypanosomosis-infected areas
[°] control areas

Petauke area and the adjacent Katete area were, however, not significant (P>0.05). were comparable to those recorded in their respective control areas (Table 4.3.5). Except for young stock, average mortality rates in the Chipangali and Vwaza (0-20 km) areas

Calving and weaning rates

With the exception of the Petauke survey area, average calving rates were significantly lower (P<0.01) in all trypanosomosis-infected areas compared to their control areas (Table 4.3.6).

4.3.3.4 Cattle sales, offtake rates and purchases

Mean sales rates and offtake rates were low in all areas (Table 4.3.7) and did not differ between cattle owners with small (less than the median number of animals) and large (above the median number of animals) herds (P>0.05). This suggests that the majority of cattle owners in tsetse-infested and tsetse-free areas meet cash needs by alternative means. In all areas, commercial reasons for disposal (sales and exchange) accounted for more than 50% of total offtake (Table 4.3.8). Emergency slaughter was particularly high in the Katete area (Table 4.3.8). This is attributed to an East Coast Fever (ECF, *Theileria parva*) outbreak a few months before the survey was conducted. Market-related reasons for not disposing of cattle (market prices too low, unable to sell cattle or not enough buyers) were relatively unimportant (Tables 4.3.9 and 4.3.10). They constituted less than 5% of all the reasons for not selling more animals.

In the year preceding the survey, a substantial proportion of households and cattle owners bought cattle in the Zambian survey areas (Table 4.3.11). The differences between the Zambian survey areas were not significant (P>0.05). In the Malawian survey areas, on the other hand, cattle purchases were substantially lower (Table 4.3.11). The cattle owners' strategies for stock purchase differed markedly between areas (Table 4.3.12). In the tsetse-infested areas in Zambia and most areas in Malawi, the majority of all purchases were draught animals. Most of the cattle (99%) were purchased from farmers living within the same area or a nearby village.

Table 4.3.7: Average gross offtake rate, sales rate and commercial offtake rate per cattle owner in each survey area.

Offtake measure	Survey area					
	Petauke[+]	Katete[o]	Chipangali[+]	Kasungu[o]	Vwaza[+]	Vwaza[o]
Gross offtake rate	7.0	7.9	9.0	13.7	19.4	17.9
Sales rate	3.7	3.5	2.6	5.3	8.1	6.5
Commercial offtake rate	3.9	3.7	3.9	5.8	8.6	9.5

Table 4.3.8: Frequency distribution of reasons for cattle disposal in each survey area.

Reason for disposal	Survey area					
	Petauke[+]	Katete[o]	Chipangali[+]	Kasungu[o]	Vwaza[+]	Vwaza[o]
Cash-sale	55.9	49.8	45.4	42.1	42.5	45.2
Home consumption	7.8	3.7	7.5	4.9	8.5	5.4
Lobola/bride price	5.0	2.3	20.1	26.2	8.5	15.0
Ceremonies	0	4.6	3.4	7.9	10.6	6.5
Emergency slaughter	15.6	25.6	9.2	11.0	23.6	19.3
Exchange	11.3	11.9	11.0	6.7	4.2	6.5
Other reasons	4.4	2.1	3.4	1.2	2.1	2.1

[+] trypanosomosis-infected areas
[o] control areas

Table 4.3.9: Frequency distribution of reasons for not selling more cattle in each survey area.

Reason for not selling more cattle	Survey area					
	Petauke[+]	Katete[°]	Chipangali[+]	Kasungu[°]	Vwaza[+]	Vwaza[°]
No more cash required	22.4	30.8	26.5	36.0	43.5	51.9
Needed to keep oxen	43.3	33.7	25.8	13.4	8.7	1.9
Market prices too low	1.5	1.5	0	1.2	0	0
Not enough buyers	0	0	0	0	4.3	0
Herd too small	32.3	31.5	45.3	39.1	42.3	44.2
Other reasons	0.5	0.5	1.1	10.3	1.2	2.0

Table 4.3.10: Frequency distributions of reasons for cattle sale.

Reason for sale	Survey area					
	Petauke[+]	Katete[°]	Chipangali[+]	Kasungu[°]	Vwaza[+]	Vwaza[°]
Cash for specific purpose	85.6	82.6	100.0	91.6	87.5	88.6
Animals ready for market	4.8	2.7	0	2.8	3.1	0
Animals were sick	4.8	12.4	0	2.8	6.3	9.1
Other reasons	4.8	2.7	0	2.8	3.1	2.3

[+] trypanosomosis-infected areas
[°] control areas

Table 4.3.11: Proportion of households and owners buying cattle in each survey area.

Proportion buying (%)	Survey area					
	Petauke[+]	Katete[o]	Chipangali[+]	Kasungu[o]	Vwaza[+]	Vwaza[o]
Households	7.5	10.4	9.0	4.3	3.2	1.6
Owners	23.2	21.1	27.0	15.0	19.1	7.4

Table 4.3.12: Cattle purchases (%) within different stock categories per cattle owner in each survey area.

Stock purchase category	Survey area					
	Petauke[+]	Katete[o]	Chipangali[+]	Kasungu[o]	Vwaza[+]	Vwaza[o]
Males ≤ 4 yrs old	25.4	29.1	20.9	20.6	28.6	25.0
Males ≥ 4 yrs old	44.8	18.5	43.3	41.2	71.4	25.0
Females ≤ 4 yrs old	10.4	33.0	16.4	14.7	0	50.0
Females ≥ 4 yrs old	19.4	19.4	19.4	23.5	0	0

[+] trypanosomosis-infected areas
[o] control areas

4.3.4 Discussion

4.3.4.1 Cattle ownership

In all survey areas the proportion of households owning cattle was low and ownership was highly skewed. Several studies in East and West Africa (Swallow, 1998) have indicated that the control of bovine trypanosomosis will lead to an increased proportion of households owning cattle with a concomitant increase in the average herd size per owner. Such an increase in cattle numbers after trypanosomosis control could be one reason for the higher proportion of households owning cattle and the larger average herd size in the Katete area compared to the adjacent, tsetse-infested, Petauke area of eastern Zambia. Increased cattle ownership and increased herd size should, however, not be considered as a normal consequence of trypanosomosis control. Indeed, notwithstanding the absence of trypanosomosis in the area 20-40 km from the edge of the Vwaza Game Reserve, the proportion of households owning cattle and the average herd size per owner does not differ significantly from the cattle ownership and average herd size in the area immediately adjacent to the game reserve where trypanosomosis is prevalent. In the Vwaza area, it is likely that the land pressure has placed severe limitations on the potential to increase herd size and cattle ownership.

Since wealth in terms of livestock ownership tends to be skewed towards the cattle owner (RTTCP, 1999b), trypanosomosis control interventions will tend to benefit the wealthiest part of the population most. Although difficult to quantify, trypanosomosis control will also have indirect effects on the non-cattle owners through, for example, increased availability of oxen for hire for land preparation. Therefore, an important indirect impact of trypanosomosis control is likely to be an expansion in the area ploughed per household with a concomitant reduction in the proportion of deficit food producing households (Swallow, 1998). However, the capacity to expand the cultivated areas depends on the amount of land available. The amount of land available is extremely limited in both Malawi survey areas with human population densities in excess of 100 people/km^2 around both game areas. In such circumstances the indirect impact on crop production will be limited.

4.3.4.2 Herd structure

Data on herd structures can be instructive. The observed variations between the different survey areas may reflect differences in management strategies or investment priorities that may result from particular constraints affecting the cattle owners. It is possible that some of the observed differences in herd structure are a result of the presence or absence of bovine trypanosomosis. The relatively high proportion of cows in herds in the tsetse-free area compared to both tsetse-infested areas of eastern Zambia, for example, could be the result of the farmers' emphasis on herd growth and the accumulation of breeding animals after trypanosomosis has been removed. Indeed, in areas where bovine trypanosomosis is endemic, the reproductive performance of cows is low with, consequently, low returns from investments in breeding stock (Section 1.4.1). This emphasis on oxen in tsetse-infested areas can clearly not be generalised suggesting that other factors, not related to the presence of tsetse, affect the herd structure. The low proportion of oxen in the Vwaza area of Malawi, for example, could again be a result of the low availability of arable land and small plot sizes, which reduces the need for draught power. Hence, the survey results indicate that it is dangerous to draw conclusions on the impact of bovine trypanosomosis, and its control, on herd structure.

Although the effect of nagana and its control on herd structure may be difficult to predict, knowledge of the existing herd structure is perhaps more important in the design of a strategy for the control of bovine trypanosomosis. The structure of the herd can, to a significant extent, determine the impact of trypanosomosis control on herd growth and, where land is available, the rate of arable land expansion. For example, in the Katete area, where herd structures are normal, herd size is likely to increase by *ca.* 3% per annum after trypanosomosis has been controlled (Doran, pers. comm.). In the Petauke area, on the other hand, where oxen constitute a large proportion of the herd (39.3%) annual herd growth will be substantially lower (0.9%) after trypanosomosis has been controlled.

4.3.4.3 Performance variables

The estimation of cattle performance parameters was an important objective of the survey. Parameter estimation is, however, fraught with problems since performance will be influenced by intra-and inter-seasonal climatic changes, disease outbreaks and levels of drug use, etc.

Mortality rate

Despite the presence of trypanosomosis, average mortality rates recorded in all Zambian survey areas fell within the ranges expected for communally kept cattle in Zambia (Perry *et al.*, 1984). In the Malawian survey areas also, average mortality rates were low (Soldan and Norman, 1994). The relatively high average mortality rates in most age and sex categories of stock in the Petauke and Katete areas were attributed to an outbreak of ECF during the February-June period preceding the survey. Swallow (1998), reviewing studies on the impact of trypanosomosis in various parts of Africa, stated that trypanosomosis increases calf mortalities by between 10-20% and reduces the density of cattle between 37-70%. Survey results collected in the trypanosomosis-infected areas in Zambia and Malawi cannot confirm those findings. The ranges of calf and adult mortality rates in the tsetse-infested areas of Zambia and Malawi were not atypical for traditional African livestock-keeping systems without trypanosomosis challenge and fell within the ranges recorded by Perry *et al.* (1984) (4-32% range and 4-10% range for calves and adults, respectively). A likely reason for the low mortality rate in all age categories of stock could be the effective treatment with trypanocides. However, trypanocidal drug use figures, collected in the survey area, indicate that the proportion of calves treated with trypanocides is low (Section 5.2.3.2). A more likely explanation for the low mortality in calves is, therefore, the low levels of tsetse challenge calves undergo. Research has shown the low attractiveness of calves to tsetse compared to adult animals (Torr, personal communication, 1999). This low level of attractiveness must result in a lower level of challenge and, hence, trypanosomosis-related mortality.

Calving and weaning rates

In two of the tsetse-infested areas, calving rates were significantly lower compared to their tsetse-free control areas. These results confirm observations made by other authors that trypanosomosis does affect the calving rate (reviewed by Swallow, 1998). Reproductive disorders are frequently seen in animals infected with trypanosomes (Section 1.4.1). They are attributed mainly to the effects of trypanosomosis on the endocrine system of cows resulting in irregular oestrus, foetal death and abortion (Ikede *et al.*, 1988; Gombe, 1989). The effect of trypanosomal infections on reproduction are reversible after diminazene aceturate treatment but it may take several months before normal cyclical activity resumes (Llewelyn *et al.*, 1988). It is, therefore, not surprising that the curative diminazene aceturate treatments administered by the cattle owners contribute little to improved reproductive performance in the tsetse-infested area.

The relatively high average calving rate in the Petauke survey area is more difficult to explain. Disease prevalence is comparable to the one in the Chipangali area and trypanocidal drug use practices do not differ (RTTCP, 1999c). However, a study investigating the effect of trypanosomal infections on herd average PCV showed that the effect was more severe in the Chipangali (Lundazi District) than in the Petauke survey area (Section 4.2.3). These results indicate differences in trypanosomosis tolerance between the two areas. In the Petauke area, the increased tolerance was attributed to the high level of homologous challenge by a resident tsetse population almost entirely feeding on cattle which induces a level of "nonsterile immunity" in the cattle population. Cattle in the Chipangali area, on the other hand, are subject to challenge by tsetse invading from the adjacent game areas (Lukusuzi National Park and the Kasungu Game Reserve). Since a substantial proportion of the invading flies obtained trypanosomal infections from game animals, cattle will undergo heterologous challenge and "nonsterile immunity" is unlikely develop. This may explain the lower tolerance of cattle to trypanosomosis in the Chipangali area (Section 4.2.3) and, hence, the low calving rate. Reasons for the low calving rate observed along the vicinity of the Vwaza Game Reserve are likely to be similar.

4.3.4.4 Offtake, sales rates and cattle purchases

In the context of planning for trypanosomosis control, it is important to obtain information about the sales and offtake rates and the reasons for cattle disposal. In all survey areas, sales rates were low and were influenced predominantly by the need to obtain cash to pay for food, school fees, clothes and inputs (*e.g.* drugs and/or fertiliser). Market-related factors (*e.g.* prices or animal condition) did not seem to constrain the offtake of cattle.

Sales and offtake rates are often assumed to increase after control operations become effective and benefits of this kind are sometimes incorporated in economic and financial appraisals of trypanosomosis control interventions. Swallow (1998), for example, stated that trypanosomosis control may result in a 30% increase in the commercial offtake of cattle. However, the direct relationship between cattle sales rates and cash needs observed in all survey areas has important implications. When, for example, alternative sources of cash are available these are likely to be used to meet cash needs in preference to the sale of cattle (Doran *et al.*, 1979; Low *et al.*, 1980). Significant improvements in, for example, crop revenue earning capacity thus tend to be associated with reduced sales rates and more rapid herd accumulation. Animal disease interventions, such as trypanosomosis control, which increase the availability of draught power and hence the capacity to expand the areas under cultivation are, therefore, likely to have similar effects on the rate of herd accumulation over time. Other things being equal, the greater the agricultural potential of an area and the greater the capacity for arable land expansion, the greater will be the capacity for herd accumulation and the lower the aggregate offtake and sales rates.

Survey results indicate that trypanosomosis control did not affect the proportion of households or cattle owners purchasing cattle. In the Zambian survey areas, however, the cattle purchase data suggest that, under tsetse challenge, emphasis is given to the purchase of especially adult males for draught purposes. Removal of the trypanosomosis constraint, appears to shift the emphasis to a longer term herd accumulation strategy in which the purchase of breeding females is given priority. As

suggested above, the high proportion of male cattle purchased in tsetse-infested areas can be explained by the low reproductive performance of females. Other factors may also affect the purchase strategy.

The outcomes of the socio-economic surveys conducted in Zambia and Malawi indicate that is it is dangerous to make generalizations on the impact of bovine trypanosomosis and its control on cattle production and offtake. With the exception of the impact of the disease on calving rates, all other direct and indirect impacts are affected by non-trypanosomosis related factors such as the cattle owners' disease management practices, the potential for herd and arable land expansion and cash requirements. All these factors and their linkages and the local decision making process have to be considered when planning for the localised control of bovine trypanosomosis. Failure to do so may result in an overestimate of the benefits accruing from control and is likely to affect the sustainability of an intervention.

CHAPTER FIVE

OPTIONS FOR THE CONTROL OF BOVINE TRYPANOSOMOSIS

5.1 Introduction

Control of tsetse-transmitted trypanosomosis can be based on the control of the causal agent, the trypanosome, the control of the vector, the tsetse fly, the genetic selection of resistant cattle or a combination of all.

Despite the progress made in the development of effective tsetse control methods, trypanocidal drugs are, and will continue to be, an important means of controlling bovine trypanosomosis. In several countries of southern Africa (Malawi, Zambia and Mozambique), trypanocidal drugs (diminazene aceturate and isometamidium chloride) are readily available. The current and future effectiveness of a trypanosomosis control strategy based on the use of trypanocidal drugs will be determined largely by the prevalence of trypanosome strains resistant to those drugs. In areas where drug resistance is absent or present at low prevalence, the potential for widespread resistance in trypanosomes to these compounds is an important component determining the sustainability of a control strategy based on trypanocides. Detailed information on the distribution and prevalence of trypanocidal drug resistance in trypanosomes is not available. Moreover, tests to detect resistance are labour intensive, expensive and are unlikely to provide information on time or on a scale required for planning. The factors contributing to the development of resistance to trypanocidal drugs, on the other hand, are well-known (Section 1.5). Hence, detailed area-specific data on, for example, levels of trypanocidal drug use, trypanocidal drug preference, dose and frequency of application could be used as a practical, indirect indicator of the likelihood of the development of resistance to trypanocidal drugs in trypanosomes.

To determine current trypanocidal drug use in a bovine trypanosomosis endemic area and assess the potential for the development resistance a survey on trypanocidal drug use was conducted in eastern Zambia (Section 5.2). The survey results were used to determine the appropriateness of a bovine trypanosomosis control strategy based on trypanocides in the area. Moreover, general principles of cattle owners' attitudes towards trypanosomosis and its control were deduced and conclusions, relating to the implementation of other control methods were drawn (Section 5.2).

During the past 20 years much effort has gone into the development of low-technology and cost-effective tsetse control methods (Section 1.5). At the moment, most tsetse control operations rely on the use of stationary (odour-baited, insecticide-treated targets) or mobile (insecticide-treated cattle) baits. Odour-baited targets have been used to clear tsetse from large areas of Zimbabwe (Lovemore, 1999). They have proved to be highly effective when used in large areas where human and cattle population densities are often low. Moreover, odour-baited, insecticide-treated target barriers are highly effective in preventing the re-invasion of tsetse (Section 3.6.3) or significantly reducing the contaqct between tsetse and cattle at the edge of tsetse-infested areas (Section 3.4.3). Despite the successful implementation of the target technology under these conditions, the effectiveness of the method in controlling tsetse in small cultivated areas with high cattle and human population densities, where trypanosomosis is endemic and tsetse habitat patchy, still needs to be assessed. This situation occurs on the plateau area of eastern Zambia (Chapter 2). To evaluate the effectiveness of the target technology under the conditions prevailing on the plateau area of eastern Zambia, a tsetse control trial using odour-baited, insecticide-treated targets was conducted (Section 5.3).

Much of the development of insecticide-treated cattle as mobile baits to control tsetse has been conducted in southern Africa (Section 1.5). Nevertheless, with the exception of a few small trials, the method has never been used on a large-scale. Furthermore, the effectiveness of insecticide-treated cattle under the various epidemiological situations prevailing in southern Africa (Chapter 3) still needs to be assessed. It is, for example, important to clarify the effect of insecticide treatments on the tsetse's feeding responses and trypanosomosis transmission. Any repellent or irritant effect that the insecticide has on tsetse could be of particular importance in situations where the interaction between tsetse and cattle occurs along the edge of a tsetse-infested area (Section 3.4.3.4). Therefore, a trial was conducted to evaluate the effect of deltamethrin (the most commonly used pyrethroid to control tsetse) applied to cattle on the transmission of bovine trypanosomosis (Section 5.4). In view of the localised control of tsetse, effective barriers are required to prevent re-invasion. Targets

effectively prevent tsetse re-invasion (Hargrove, 1993). However, the maintenance of a target barrier is costly and constant vigilance is required in order to prevent the barrier breaking down. Recent work has indicated that the efficacy of insecticide treatment of cattle against tsetse might be greater than was originally supposed (Baylis and Stevenson, 1998) and it has been suggested that cattle treatments alone might be sufficient to stem the re-invasion of tsetse. To assess the potential use of insecticide-treated cattle as a barrier to re-invasion of tsetse, a trial was conducted in northeastern Zimbabwe (Section 5.6).

Insecticide-treated cattle have lead to the clearance of mainly riverine species of tsetse from large areas in West Africa (Section 1.5.2.2.2). The effectiveness of the method in cultivated areas, where tsetse (savannah species) distribution is patchy still needs to be assessed. A trial was, therefore, initiated in eastern Zambia (Section 5.7).

Finally, the pyrethroid insecticides used to control tsetse have good acaricidal activity. Hence, regular treatment of cattle with pyrethroids is likely to have a significant effect on the tick population. This may affect the development and maintenance of enzootic stability to certain tick-borne diseases. To determine the effect of regular deltamethrin treatments on the epidemiology of babesiosis, a survey was undertaken in northeastern Zimbabwe (Section 5.5).

5.2 An analysis of trypanocidal drug use in the Eastern Province of Zambia

5.2.1 Introduction

In many parts of Africa where bovine trypanosomosis is a serious constraint to development, trypanocidal drug treatments constitute the principal method of controlling the disease. Despite the availability of effective vector control methods, it is very likely that in the foreseeable future, chemotherapy and chemoprophylaxis will continue to contribute significantly to the control of bovine trypanosomosis.
Only a small group of chemoprophylactic and chemotherapeutic compounds are currently in use and new compounds are unlikely to become available in the near future (Peregrine, 1994). Moreover, there is growing concern that the effectiveness of this control method will be severely reduced by the widespread development of resistance in trypanosomes to these compounds (Geerts and Holmes, 1998).

Notwithstanding the importance of this trypanosomosis control method and the threat of drug resistance, little is known of how African communal farmers use trypanocides. This is partly because of the decline in services offered by veterinary departments in many African countries, which has resulted in the unsupervised use of many veterinary drugs, including trypanocides.

Information on the use of trypanocides is required when determining options for the control of trypanosomosis in an area. Depending on the drug-use strategy, areas can be selected where chemoprophylaxis or chemotherapy may be an effective and sustainable way of controlling trypanosomosis. In other areas, information on trypanocides may indicate that they are not a viable option and other methods, such as vector control, should be adopted.

In the course of developing strategy options for the control of bovine trypanosomosis in Zambia, a survey was conducted to quantify and qualify the current use of trypanocidal drugs in the Eastern Province. Use was made of a rapid rural appraisal method. The survey aimed at determining the way that communal cattle owners use trypanocides. The value of this rapid rural appraisal approach is discussed.

5.2.2 *Materials and methods*

5.2.2.1 Survey area

A survey was conducted in Petauke District of the Eastern Province of Zambia in December 1997. Two areas were surveyed; Mvuvye (referred to as tsetse-controlled) and Petauke/Nyimba (referred to as tsetse-infested). They are situated on the eastern plateau and are ecologically similar with medium to high agricultural potential and mixed farming systems. *Glossina m. morsitans* was the only tsetse species present. The annual climatic cycle comprises three seasons; the rainy season (from November to April), the cold dry season (from May to August) and the hot dry season (from September to October). In the Mvuvye area (*ca.* 900 km^2), an odour-baited, insecticide-treated target, tsetse control operation was initiated in 1989 (Section 5.3). Despite the resulting decline in trypanosomosis prevalence, animals that graze outside the area may still contract trypanosomosis. The monthly average incidence of trypanosomal infections was 3.3% (mainly due to *T. congolense*) (Department of Animal Health and Production, unpublished data).

In the Petauke/Nyimba area (*ca.* 2000 km^2), tsetse are not controlled. The monthly average incidence of trypanosomal infections at the time of the survey, also mainly due to *T. congolense*, was *ca.* 10% (Section 2.5).

In both survey areas, farmers buy the trypanocidal drugs, diminazene aceturate (Berenil®, Hoechst, in sachet of 2.36 g) and isometamidium chloride (Samorin®, Rhône Mérieux, in sachet of 1 g), from the offices of the Department of Veterinary Services or from a Government Veterinary Assistant at cost. Widespread resistance of trypanosomes to either of the trypanocidal compounds used in the survey areas had not been reported at the time of the survey.

5.2.2.2 Sample selection and questionnaire

Cattle owners using trypanocides were identified during a socio-economic survey conducted in the same areas from June to September 1997 (Section 4.2). A total of 262 cattle owners indicated that they had purchased trypanocidal drugs during the year preceding the survey. Two hundred and seven of these trypanocidal drug users (101 in the tsetse-controlled and 106 in the tsetse-infested area) were interviewed in a more detailed follow-up survey to determine trypanocidal drug use practices. A uniform questionnaire was used (Annex 2). The questionnaire was pre-tested on a pilot basis. It was revised to clarify specific questions and ensure that the average time taken to interrogate each respondent was not more than 45 minutes. Enumerators were trained for several days before interviewing farmers. Questions were posed on herd structure, trypanocidal drug preference, treatment rationale, reason for treatment, method of treatment and treatment frequency. The information obtained was coded and entered into a database. Statistical analyses (chi-square tests) were conducted using the Statistical Package for Social Sciences (SPSS, SPSS Inc.) software.

5.2.3 Results

5.2.3.1 Drug purchase and drug administration

The majority of the cattle owners, in both survey areas, had used trypanocides during the year before interviews (Table 5.2.1). In each area, farmers preferred to use diminazene aceturate rather than isometamidium chloride (Table 5.2.1). Nevertheless, about 30% of trypanocide users used a combination of both drugs. The choice of drug did not differ between areas ($P>0.05$).

Table 5.2.1: Proportion of farmers owning cattle, proportion of cattle owners using trypanocidal drugs and drug preference in the two survey areas.

Survey area	Cattle - owners (%)	Cattle owners using trypanocides (%)	% trypanocide users using		
			diminazene aceturate	isometamidium chloride	both drugs
Tsetse-controlled	49.7	73.7	63	4	33
Tsetse-infested	30.1	85.1	68	5	27

Most of the trypanocidal drugs purchased (98.9%) were obtained from representatives of the Department of Veterinary Services. In the majority of cases (66.7%), cattle owners themselves administered the drugs. Only a small proportion of users (12.1%) indicated that veterinary personnel administered treatments. There was no difference between areas ($P>0.05$).

During the year preceding the survey, *ca.* 26% of the cattle owners in the tsetse-controlled and 15% of cattle owners in the tsetse-infested area used no trypanocidal drugs at all. The main reasons given (more than 90 % of the answers in both areas) were that "the animals were not sick and did not require treatment" or "the drugs were too expensive". Few cattle owners (less than 5 %) reported that they could not obtain the drugs.

5.2.3.2 Frequency of treatments and category of cattle treated

Application rates and the proportion of the herd treated with each trypanocide did not differ significantly between areas ($P>0.05$) and are thus not correlated with the disease challenge. Diminazene aceturate users treated, on average, 4.9 animals per herd (approximately 50% of the average total number of animals in the herd). Isometamidium chloride users treated, on average, 7.6 animals per herd (about two

thirds of the average total number of animals in the herd). The proportion of oxen and cows receiving treatment with either drug was substantially higher compared to other classes of stock (Table 5.2.2). Irrespective of the type of drug and the age and sex category of stock, an average of *ca.* 1.5 treatments was given annually to each animal (Table 5.2.2).

5.2.3.3 Reason for trypanocide use

In both areas, the reasons for trypanocidal drug use were very similar (Table 5.2.3). More than 75% of the diminazene aceturate treatments were given to clinically sick animals (Table 5.2.3). However, only 15% and 13% of the diminazene aceturate treatments in the tsetse-controlled and tsetse-infested area, respectively, were given to animals with trypanosomal infections or suspected of having trypanosomal infections (Table 5.2.3). Only about 30% of all isometamidium chloride treatments, in both survey areas, were given to prevent trypanosomosis (Table 5.2.3). On the other hand, almost half of the isometamidium chloride treatments were for the reason that "animals were sick but the reason was unknown" (Table 5.2.3).

Table 5.2.2: Proportion of cattle treated with each trypanocide and average number of treatments by age and sex category (since proportion of cattle treatment and number treated did not differ between survey areas, data for both areas were pooled).

Category	Diminazene aceturate		Isometamidium chloride	
	% treated in each category	Treatments/animal/ year for those treated (± 1 s.e.)	% treated in each category	Treatments/animal/ year for those treated (± 1 s.e.)
Calves (0-1 year old)	2.5	2.00 ± 0.77	16.4	1.82 ± 0.48
Young stock (1-4years old)	22.2	1.22 ± 0.09	32.8	1.54 ± 0.26
Cows	55.1	1.40 ± 0.07	61.2	1.49 ± 0.17
Bulls	8.6	1.41 ± 0.27	23.9	1.69 ± 0.35
Oxen	83.8	1.52 ± 0.07	82.1	1.63 ± 0.23

Table 5.2.3: Reasons for diminazene aceturate and isometamidium chloride use in each of the survey areas.

Reason for use	Survey area			
	Tsetse-controlled (%)		Tsetse-infested (%)	
	Diminazene	Isometamidium	Diminazene	Isometamidium
Trypanosomosis diagnosed	5.2	0	4.0	0
To prevent trypanosomosis	-	33.3	-	29.0
Trypanosomosis suspected	11.3	-	8.9	-
Animals sick (reason unknown)	63.9	47.2	66.3	48.4
Combination	17.5	5.6	9.9	9.7
Other reasons	2.1	13.9	10.9	12.9

5.2.3.4 Season of treatment

The majority of treatments were given during the dry season (Table 5.2.4). The seasonal pattern of treatment was almost identical for both areas.

Table 5.2.4: Proportion of treated animals receiving diminazene aceturate and isometamidium chloride during different seasons of the year.

Season of Treatment	% of drug users using	
	Diminazene aceturate	Isometamidium chloride
Wet Season	20.2	26.7
Dry Season	60.4	53.4
Both Seasons	19.4	19.9

5.2.3.5 Dosage rate and drug application

Evidence from the survey suggests that most of the farmers who used trypanocides did not under-dose with either diminazene aceturate or isometamidium chloride (Tables 5.2.5 and 5.2.6). On the contrary, at a normal dose rate of 3.5 mg diminazene aceturate/kg body weight and 0.5 mg isometamidium chloride/kg body weight, most calves and young stock in the 1-4 year age category were being overdosed (Table 5.2.5).

Only 17.4% of the trypanocide users indicated that they used boiled water to dilute the drug. Most of them (75%) diluted the trypanocides in water from potentially contaminated sources (dam, river, well or a combination of these). Sixty-seven percent

Table 5.2.5: Frequency distribution of the number of animals treated with 1.05 g of diminazene aceturate.

Number of animals treated	Category (in %)		
	Adult stock	Young stock (1-4 years old)	Calves (0-1 year old)
1	97.9	84.5	2.0
2	2.1	14.9	87.6
>2	0.0	0.6	10.4

Table 5.2.6: Frequency distribution of the number of animals treated with 1g of isometamidium chloride.

Number of animals treated	Category (in %)		
	Adult stock	Young stock (1-4 years old)	Calves (0-1 year old)
8	42.4	1.6	0.0
10	56.1	71.2	4.5
12	1.5	10.6	4.5
14	0.0	3.0	0.0
16	0.0	9.1	22.7
20	0.0	4.5	62.2
>20	0.0	0.0	6.1

of the users kept residual isometamidium chloride powder for later use. The remainder of the farmers (33%) sold the residual powder to other cattle owners.

5.2.4 Discussion

5.2.4.1 Drug purchase and drug administration

In all areas, preference was given to the use of a curative (diminazene aceturate) rather than a prophylactic drug (isometamidium chloride). Most trypanocides were obtained from representatives of the Department of Veterinary Services. This arrangement reduces the risk of administering generic products which may have unknown efficacy and offers a useful mechanism to monitor drug sales and drug application rates. Given the recent decline in the level of services offered by the veterinary department, cattle owners administer most trypanocides themselves. This may increase the risk of underdosing. However, survey results indicate that this does not seem to be the case. On the contrary, most of the calves and young stock receive an overdose of both drugs. The use of non-sterile water may induce the formation of abscesses that can reduce the availability of the drug. Advice is required to improve the mode of application

5.2.4.2 Trypanocidal drug-use strategy

In both areas, the majority of trypanocidal treatments are given to clinically sick animals that are not necessarily infected with trypanosomes. Moreover, irrespective of the type of trypanocidal drug used, oxen and cows received the majority of treatments. This suggests that farmers prefer to treat the productive animals in the herd and appear to apply a production-oriented curative treatment strategy. This may also be the reason why most treatments were given during the dry season when body condition and tolerance of infection are lowest (Doran, unpublished data).

Despite the differences in the monthly incidence of trypanosomosis between the two areas, the trypanocide-use tactics adopted are similar. This suggests that after the implementation of tsetse-control measures and the concomitant reduction of the

challenge, cattle owners are unlikely to automatically change their pattern of trypanocide use. This is not surprising given the history of tsetse challenge before tsetse control and the treatment strategy, which focuses on the treatment of clinically sick animals rather than only on animals infected with trypanosomes. At the observed treatment frequency in the tsetse-controlled area, the number of trypanocide treatments given is higher than the monthly average incidence of trypanosomal infections (3.3%). Indeed, on the assumption that all animals infected with trypanosomes in the tsetse-controlled area were clinically sick and were treated, 68% of the diminazene aceturate treatments given to oxen (26% of treated herds) and 48% to cows (30.1% of treated herds) were inappropriate or administered to animals that were not infected with trypanosomes. In young stock (21.8% of treated herds), 46% of the diminazene treatments were given inappropriately. Obviously, the proportion of inappropriate treatments increases when isometamidium use is taken into consideration. Moreover, many animals infected with trypanosomes may not be clinically sick which will increase the number of inappropriate treatments even more. Since diagnostic facilities are not readily available, it will be difficult for the cattle owner to distinguish between clinically sick animals infected with trypanosomes and clinically sick animals infected with other disease agents. Cattle owners in the tsetse-controlled areas would, therefore, benefit from improved diagnosis and improved veterinary extension advisory services.

At the observed treatment frequency in the tsetse-infested area, on the other hand, 95% of the trypanosomal infections in oxen (39.3% of treated herds), 64% of the trypanosomal infections in cows (30.9% of treated herds) and 22% of the trypanosomal infections in young stock (14.9% of treated herds) could have been treated with diminazene aceturate. These figures will also increase when isometamidium treatments are taken into consideration. In the tsetse-infested area, a large proportion of the clinically sick animals is likely to be infected with trypanosomes often concealed by the presence of more readily detectable secondary infections (Connor, 1994b). Hence treatment of clinically sick animals in the trypanosomosis endemic area may, in the absence of microscopic diagnosis, be an effective way of combating trypanosomosis-related mortality. This seems to be the

case since the cattle mortality rate in the tsetse-infested area did not differ from that in an adjacent tsetse-free area (Section 4.2). Moreover, the strategy of administering curative treatment to clinically sick animals in the tsetse-infested area is likely to keep oxen in reasonable condition during the ploughing season. Despite the substantial proportion of treatments given to cows, compared to other categories of cattle, many trypanosomal infections in cows were not treated. The treatment regime adopted would have boosted the condition of clinically sick cows but would not have improved their reproductive performance. Hence, the significantly lower calving rate recorded in the tsetse-infested area compared to that in the adjacent tsetse-free area (44.1% compared to 60.4%) (Doran, personal commication, 1999). Trypanosomosis is known to reduce reproductive performance of cattle (Losos and Ikede, 1972). There are, however, numerous examples of susceptible cattle being kept successfully under tsetse challenge. This may be achieved by applying strict treatment regimes with chemotherapeutic or, especially, chemoprophylactic drugs (Boyt, 1979; ILCA, 1988). A curative treatment strategy, as adapted in the tsetse-infested survey area, is not sufficient to maintain normal reproductive performance of cows.

5.2.4.3 Risk of development of trypanosome resistance

A major drawback, which affects the sustainability of chemotherapy in the control of bovine trypanosomosis, is the development of resistance by trypanosomes to trypanocides. Resistance to mainly chemoprophylactic trypanocides used in cattle has been reported at sites in West, Central, East and southern Africa (Peregrine, 1994). It is widely accepted that the best way to delay the development of drug resistance is to reduce selection pressure on parasite populations. This is best achieved by using the correct dose, decreasing the treatment frequency and reducing the number of animals treated (Geerts and Holmes, 1998). The survey results presented in this section show that, even though farmers administer most of the trypanocides themselves, there is no evidence of frequent under-dosing. On the assumption that all diminazene aceturate treatments in the tsetse-infested area were given appropriately, only about half (51%) of all trypanosomal infections in a herd were treated. The risk of trypanosomes developing resistance associated with the frequent and large-scale use of chemoprophylactic drugs in the tsetse-infested area is,

therefore, minimal. Additionally, only a small proportion of owners use isometamidium chloride and treatment frequency is low (Tables 5.2.1 and 5.2.2). Hence, the risk of resistance developing in trypanosomes associated with the frequent and large-scale use of chemoprophylactic drugs is also minimal. Since the incidence of trypanosomosis is low in the tsetse-controlled areas, the risk of resistance to trypanocides developing in this area appears to be extremely low. Thus, information obtained from the survey indicates that the factors enhancing the development of resistance to trypanocides are not present in any of the areas surveyed.

There is a lack of reliable information on the prevalence of trypanocidal drug resistance in Africa. Despite the availability of highly sensitive drug resistance monitoring tools (Geerts and Holmes, 1998), systematic surveys are too expensive for countries where trypanosomosis is a problem. It is, therefore, very unlikely that in the foreseeable future reliable data on the true prevalence of drug resistance will become available from systematic surveys. In this respect, the results of this and similar farmer-based surveys could provide a useful baseline to indicate the likelihood of the development of resistance to trypanocides in an area. Such information could be used to focus more technically based approaches to map both the temporal and spatial distribution of trypanocidal drug resistance.

Information obtained from this survey clarified the pattern of trypanocidal drug use by communal cattle owners in a trypanosomosis endemic area of Zambia. The outcome of similar surveys, conducted in Malawi and Mozambique, shows a similar production-oriented, curative strategy (Doran, unpublished data). According to these survey results the use of trypanocides is an appropriate option for the control of bovine trypanosomosis in these areas. However, a more rational strategic use of chemoprophylactic drugs could improve the reproductive performance of cows.

Valuable data have been obtained on the frequency of treatment, method of application and disease management strategies that could form the baseline for monitoring drug resistance. Improving veterinary extension could reduce the inappropriate use of trypanocides in areas where tsetse have been controlled.

However, in the absence of practical, affordable and easy tests for the diagnosis of trypanosomosis a certain level of inappropriate usage appears to be unavoidable.

The type of information obtained from this survey is essential when developing strategy options for the control of bovine trypanosomosis and similar approaches could be adopted for planning of animal disease control in general. Communal farmers' attitudes towards the control of cattle diseases and the manner in which they spend money on veterinary medicines should form the baseline from which an animal disease control strategy is developed. This approach is likely to improve the acceptability and, hence, sustainability of animal disease control in communal areas.

5.3 The control of *G. m. morsitans* (Diptera: Glossinidae) in a settled area in Petauke District (Eastern Province, Zambia) using odour-baited targets

5.3.1 Introduction

In much of Zambia, bovine trypanosomosis retards agricultural development. The disease transmitted by tsetse, *Glossina* spp., depresses every aspect of livestock production making it impractical to keep domestic animals in areas heavily infested by the flies. During the past decades several attempts, using both aerial and ground spraying, have been made to control tsetse in the Eastern Province (Evison and Kathuria, 1984). Most of these operations caused considerable reduction in fly population density, or removed flies completely from some areas. Unfortunately, due to financial constraints on the Department of Veterinary and Tsetse Control Services and the lack of effective means of preventing re-invasion, most of the former controlled areas have been reinfested with the flies.

The development of odour-baited targets as a low-technology method of controlling *G. m. morsitans* and *G. pallidipes* in Zimbabwe (Vale *et al.*, 1986), and the successful application of this method to control *G. m. centralis* in the Western Province of Zambia (Willemse, 1991), led to the trial described in this section. The objective of the trial was to investigate the efficacy of odour-baited targets to control *G. m. morsitans* in highly cultivated areas with patchy tsetse distribution and high cattle densities. At the same time, a methodology for use of odour-baited targets in such cultivated areas was developed.

5.3.2 *Materials and methods*

5.3.2.1 Trial area

The trial was conducted in 300 km^2 of the Chimpundu area in Petauke District. It is situated between the Great East Road in the north, the Mozambican border in the south, the Chikalawa Road in the west and the Sinda Road in the east (Fig. 5.3.1). The trial area lies southwest of the area described in Section 2.2.2.1. The main vegetation type was miombo (Section 2.2.2.1). Hills in the area carry extensive woodlands of *Brachystegia* spp. whereas *ca.* 70 % of the lowland (munga, Section 2.2.2.1) is cleared for cultivation (subsistence farming). The area carries about 8-10 head of cattle/km^2 together with goats, pigs and a few game animals, mainly small antelopes. The tsetse species present was *G. m. morsitans* which takes 75% of its blood meals from cattle at this locality (Section 2.3). There are three main seasons : the rainy (November to April), cold dry (May to August) and the hot dry (September to October) (Section 2.2).

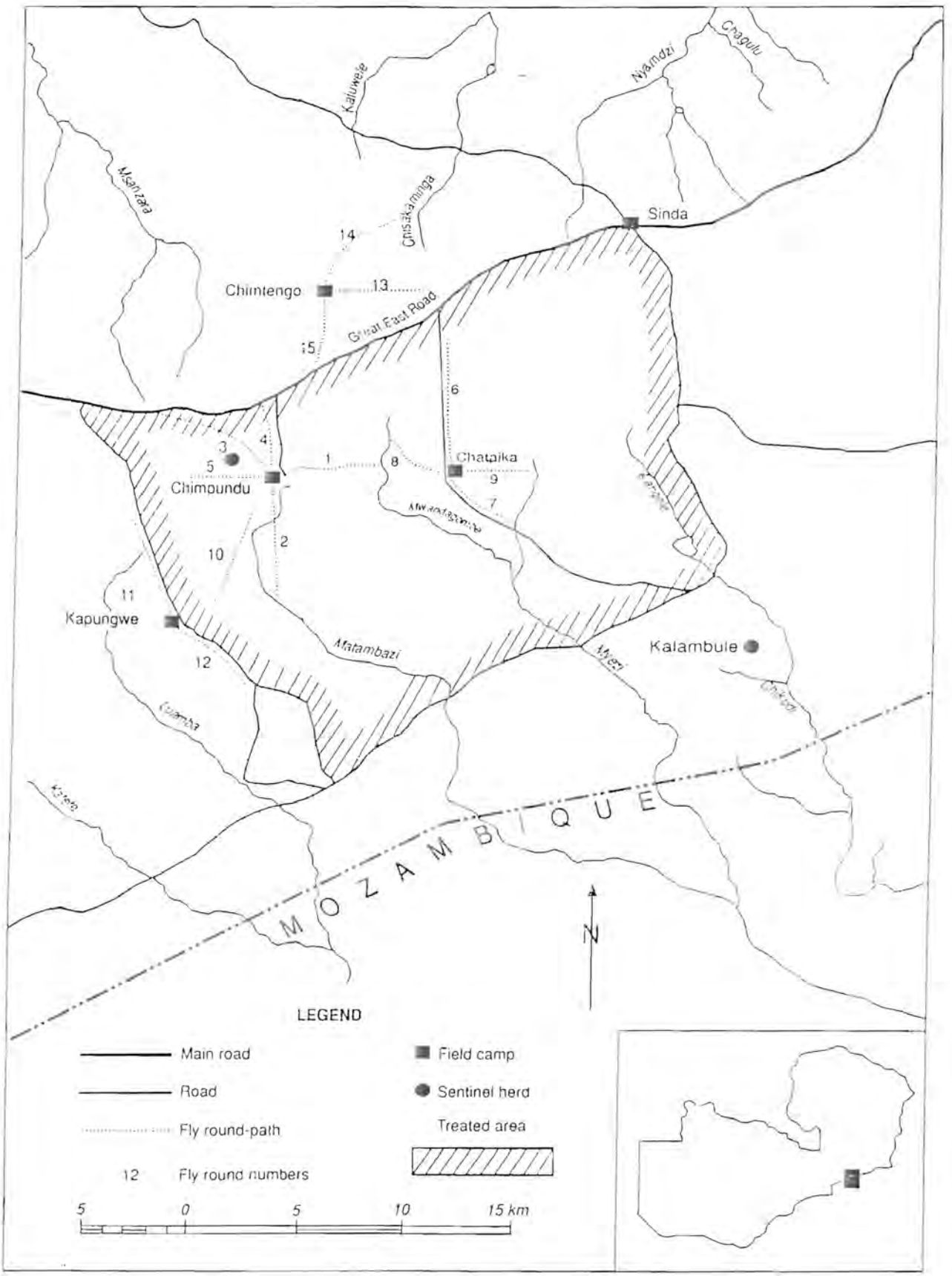
5.3.2.2 Targets

The S-type target (Vale *et al.*, 1988a), developed in Zimbabwe, was used in the trial. It consists of a central piece of black cotton cloth, 0.7 x 1.0 m, flanked on each side by fine black terylene netting, 0.5 x 1.0 m. Targets were fixed on a metal frame rotating in the wind about a central post.

5.3.2.3 Odour attractants

Acetone was dispensed from 500 ml brown glass bottles. Its vapour diffused through a 4.5 mm aperture in the lid resulting in an average dose of 250 mg/h. Bottles were placed in front of the target or attached to the top of its horizontal support; they were replenished at three-monthly intervals. For the first three months of the trial, 3-*n*-propylphenol/1-octen-3-ol/4-methylphenol (ratio of mixture: 1/4/8) in polyethylene sachet dispensers (150 µm thick, surface area 30 cm^2) were used as an additional odour attractant (Vale and Hall, 1985b). The sachet was placed in a pocket at the top of the central panel of the cloth.

Figure 5.3.1: Map of the trial area, fly round transects, location of field camps and sentinel herds.



5.3.2.4 Insecticide

All targets were treated with 0.1% deltamethrin (Glossinex 200 S.C.®, Coopers) applied by knapsack sprayers to both sides of the cloth and netting until run-off. Spraying intervals varied from two months during the rainy season to three months during the dry season. Eighteen months after the initial deployment, all targets were resprayed with 0.6% deltamethrin, and the spraying interval was increased to nine months. This significantly reduced the amount of maintenance work.

5.3.2.5 Target deployment

The targets were deployed mainly in miombo woodland. Patches of woodland, suitable for target deployment, were identified using 1/50 000 scale maps and 1/30 000 scale aerial photographs. Target deployment was facilitated by erecting most of the targets at 250 m intervals along roads. All equipment was transported using 4 WD vehicles and was hand-carried from the road to the selected deployment site. Each target site was identified by blazing trees along the roads. Teams, of 12 people each, deployed an average of 25 targets/day. The risk of targets being burned by bush fires was reduced by clearing all vegetation within 3 m of the target. All targets were numbered and mapped. A total of 980 targets were deployed during the cold dry season (July) giving an overall target density of 3.3 targets/km^2.

5.3.2.6 Target maintenance

Three permanent field camps within the trial area were used for maintenance operations (Fig. 5.3.1). Every working day, maintenance teams were sent to inspect and, if necessary, maintain targets. Torn, stolen, or faded cloths were replaced, odours were replenished and regenerating vegetation around the target was cleared. Extra casual labourers were employed for target resprays. To improve mobility, all labourers were issued with bicycles.

5.3.2.7 Monitoring of the tsetse population

The tsetse population was monitored along fly-round transects of *ca.* 6 km long with stops at 200 m intervals (Section 2.2.2.2). A total of 15 transects were traversed inside (transect number 1, 2, 3, 4, 5, 8 and 10), adjacent to (transect number 6, 7, 11, 12, 13 and 15), and outside the trial area (transect number 9 and 14) (Fig. 5.3.1). To produce sufficient pre-control data, tsetse monitoring started 18 months before the onset of the trial. The monthly average index of abundance (IA) of tsetse was calculated as the average number of flies (males and females) captured per stop and per fly-round. The monthly average indices of abundance in the trial area and adjacent area were expressed as a percentage of the monthly mean indices of abundance in the untreated area.

The corrected percentage was calculated using the following formula (Küpper *et al.*, 1982):

$$[\,[\,E_t \cdot (C_m/C_t) - E_m\,] / [\,E_t \cdot (C_m/C_t)\,]\,] \cdot 100$$

where: E_t = Initial IA in the trial area

E_m = IA in the control area per month

C_t = Initial IA in the untreated area

C_m = IA in the untreated area per month

5.3.2.8 Trypanosomosis monitoring

To monitor the effect of tsetse control on the transmission of tsetse-transmitted trypanosomosis, the trypanosomosis incidence in cattle was assessed using two sentinel herds, one inside the trial area (Chimpundu) and one 5 km outside (Kalambule) (Fig. 5.3.1). Each herd consisted of 20 adult Ngoni breed cattle belonging to local farmers. The cattle were kept under traditional village management. Each month blood collected from each sentinel animal was examined using parasitological diagnostic methods (Section 3.3.2.2). Animals infected with trypanosomes received a curative treatment of diminazene aceturate (Berenil®, Hoechst), at a dose of 7mg/kg body weight for *T. brucei* or 3.5 mg/kg body weight for

T.congolense or *T.vivax*, by intramuscular injection. To evaluate the effect of the tsetse control measures on the prevalence of anti-trypanosomal antibodies, blood samples were taken from 20 adult and 20 young (6 -12 months old) head of cattle inside and outside the trial area 15 months after the start of the trial. Since the antibody-ELISA was not available at the time the trial was conducted, the anti-trypanosomal antibody levels were determined using the Immunofluorescent Antibody Test (IFAT) (Katende *et al.*, 1987).

5.3.3 Results

5.3.3.1 Index of abundance of tsetse

The monthly average IA of tsetse outside the trial area (Fig. 5.3.2) tended to be lowest in May/June (cold dry season) and highest a few months later (hot dry season). Allowing for this seasonal effect (Section 2.2.3.1), there was a fairly steady increase in the monthly average IA of tsetse over the four years of this study. The monthly average IA inside the trial area were, except for the 6 months before the onset of the trial, about the same as outside the trial area. After the targets were deployed the catches inside the trial area declined rapidly. One month after target deployment, tsetse catches in the trial area were 81.8% lower compared to catches outside the trial area (Fig. 5.3.3). A 94.8% reduction was reached three months later.

Except for four flies caught during the 1991 rainy season, no further fly catches were recorded.

A less drastic reduction in catches was observed on the area adjacent to the trial area. Here the monthly average IA declined by 38.4%, compared to catches outside the trial area, in the first month after target deployment. The monthly mean IA gradually decreased during the following months, reaching a maximum reduction of 97.6% eight months after target deployment (Fig. 5.3.3).

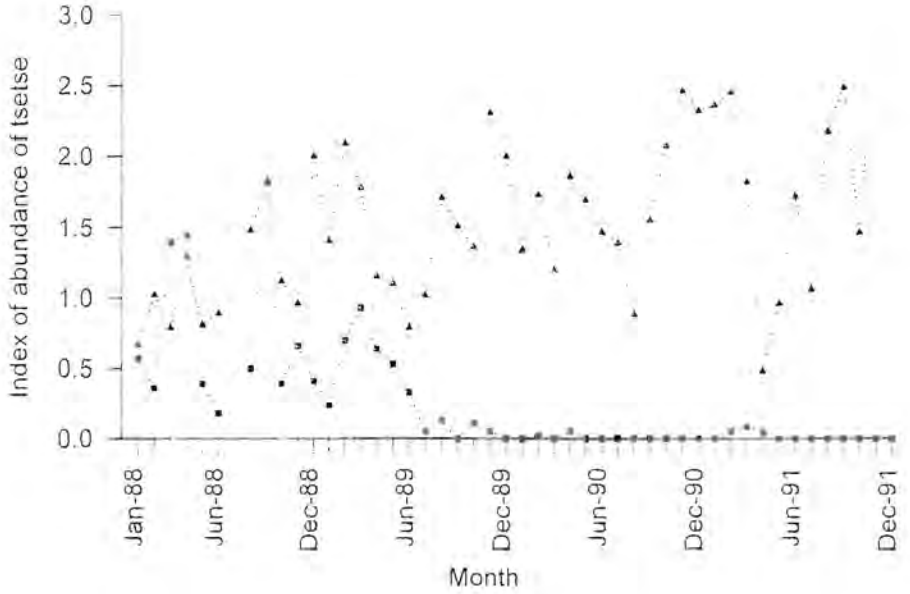


Figure 5.3.2: The monthly average index of abundance of *G. m. morsitans* inside (■) and outside (▲) the trial area.

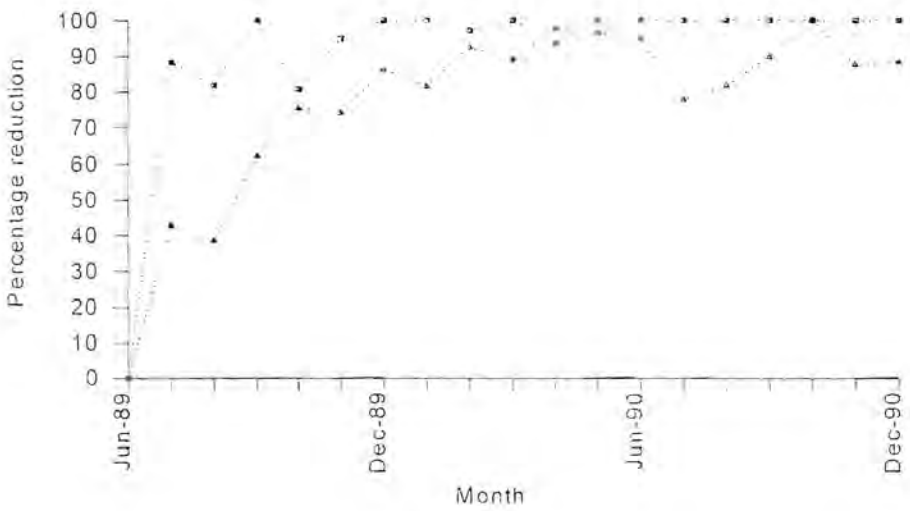


Figure 5.3.3: Reduction in the monthly average index of abundance of *G. m. morsitans* (%) inside (■) the trial area and adjacent (▲) to the trial area compared to catches outside the trial area.

5.3.3.2 Incidence of bovine trypanosomosis

Except for September 1990, trypanosomosis incidence was higher in the sentinel herd outside the trial area than in the herd inside the trial area (Fig. 5.3.4). None of the sentinel cattle were parasitologically positive in February 1990, June 1991, October1991 and December 1991.

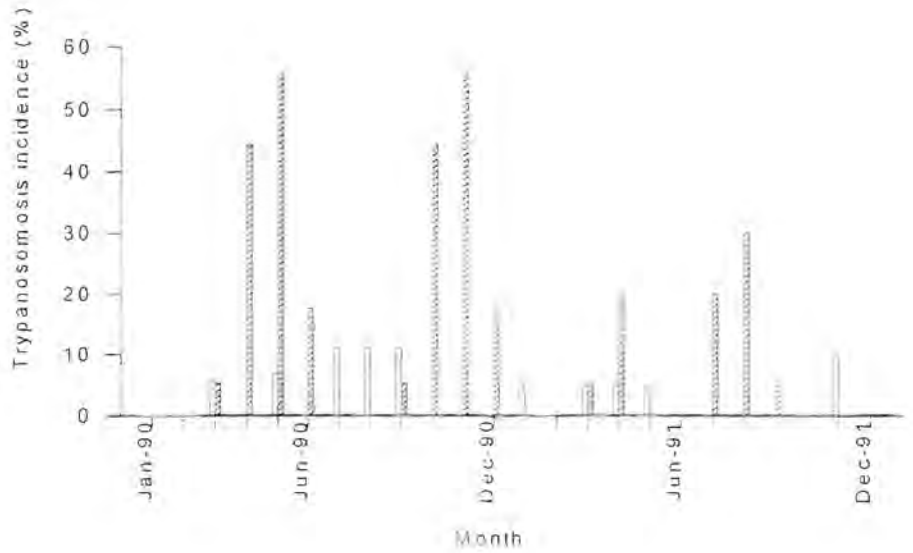


Figure 5.3.4: Monthly incidence of trypanosomal infections (%) in sentinel cattle inside (□) and outside (=) the trial area.

Inside the trial area, the incidence of trypanosomosis in sentinel cattle decreased significantly from an annual mean of 35.7% prior to target deployment to means of 5.4% and 2.3% in 1990 and 1991, respectively. However, outside the trial area trypanosomosis incidence remained high (Table 5.3.1). *Trypanosoma congolense* accounted for 96.1% of all infections.

Table 5.3.1: Annual average parasitological incidence of trypanosomal infections and average PCV in sentinel cattle inside and outside the target trial area and anti-trypanosomal antibody prevalence in adult and young cattle, 15 months after the start of the trial.

Location	Year	Average parasitological incidence (%)	Average PCV (%) (± 1 s.e.)	Antibody Prevalence (%) 15 months after trial start	
		Sentinel herds		Adults	Young
Inside	1990	5.4 ± 4.9	29.3 ± 3.5	88.9	20
	1991	2.3 ± 1.4	27.9 ± 4.0	-	-
Outside	1990	30.7 ± 21.5	25.6 ± 4.7	100	47.9
	1991	13.3 ± 11.7	25.2 ± [illegible]	-	-

Differences between mean annual PCVs of sentinel herds kept inside or outside the tsetse-controlled area were statistically significant ($P<0.01$). Fifteen months after the start of the trial, 88.9% of the adult cattle sampled in the trial area had anti-trypanosomal antibodies. However, the prevalence of anti-trypanosomal antibodies in young animals kept in the tsetse-controlled area was substantially lower that the prevalence of anti-trypanosomal antibodies in animals of the same age-category but grazing outside the trial area (Table 5.3.1).

5.3.4 Discussion

The results of the trial indicate that odour-baited targets are very effective in controlling *G. m. morsitans*, under the conditions prevailing in the Eastern Province of Zambia. Compared to other areas where odour-baited targets have been used to control *G. morsitans* (Vale *et al.*, 1988; Willemse, 1991), the distribution of tsetse in the trial area is patchy and the contact between tsetse and cattle is high (Chapter 2).

The methodology used in this trial differs from that applied in other tsetse control campaigns using odour-baited targets (Vale *et al.*, 1988; Willemse, 1991). Due to the

high level of cultivation and the subsequent patchy distribution of tsetse habitat, targets were not deployed along gridlines but deployment was restricted to suspected tsetse habitat. This resulted in an irregular distribution with concentration of targets in miombo and an overall target density lower than the recommended four targets/km^2 (Vale *et al.*, 1988). The deployment of targets along roads greatly facilitated their deployment and maintenance. Though it was beyond the scope of this trial, it is expected that access to targets could be an important parameter when the responsibility for target maintenance is ultimately handed over to the local community. As was observed by Vale *et al.* (1988) population density of *G. m. morsitans* was reduced for several kilometres outside the trial area. This effect is attributed to the movement of tsetse into the target-treated zone.

The decline of the tsetse population density was associated with a significant reduction in the incidence of bovine trypanosomosis and a significant increase in the average PCVs of cattle grazing in the trial area. This is not surprising in view of the highly significant regression between the index of abundance of tsetse and the incidence of bovine trypanosomosis (Fig 2.5.5). The PCV is a reliable indicator of anaemia (Saror, 1979), which is a major characteristic of bovine trypanosomosis (Murray and Dexter, 1988). Significant differences between herd PCVs could, therefore, be used as an additional indicator of trypanosomal infections and tsetse challenge. It is not surprising that the serological prevalence of trypanosomosis in adult cattle was high for 15 months after targets were deployed. The decline of anti-trypanosomal antibody levels is slow evan after challenge is reduced (Section 3.2.3), hence the high proportion of adult cattle with anti-trypanosomal antibodies inside the trial area. The prevalence of anti-trypanosomal antibodies in calves born in the trial area after the onset of the trial, on the other hand, was about 50% lower than in the control area. This clearly indicates a significant decrease in trypanosome challenge in the trial area. The sero-monitoring of young animals, born after tsetse have supposedly been cleared, could be a useful additional monitoring tool of a tsetse control campaign (Section 3.6.4).

From six months after the start of the trial, almost no tsetse were captured in the trial area. Trypanosomes were, however, still detected, though at a much lower incidence rate. There may be many reasons for this. The difficulty in detecting low density populations of *G. m. morsitans* and the limited area covered by fly-rounds hinders objective interpretation of entomological results. Moreover, tsetse eradication in the whole trial area could not be guaranteed because of the invasion pressure from surrounding areas. Hargrove (1993) suggested that an 8 km-wide barrier, with four targets/km^2, is needed to prevent re-invasion. This means that, in the case of this trial, only the most central part of the target area could be considered to have been re-invasion pressure free. Moreover, movements of sentinel cattle into tsetse-infested areas outside the trial block complicates interpretation of the incidence of trypanosomosis.

Expanding the treated area, creating a central area where tsetse are likely to be eradicated, could solve the problem of tsetse re-invasion. However, even this might not solve the problem of cattle moving to tsetse-infested areas, which can occur during the dry season when they search for grazing. Trypanosomosis incidence is determined by various host and vector-related parameters (Rogers, 1988; see Chapter 1). Theoretical disease transmission thresholds and basic rates of reproduction emphasise the difficulty of controlling trypanosomosis caused by *T. vivax* or *T. congolense* by any strategy other than total elimination of the vector (Rogers, 1988). Results of this trial and the epidemiological considerations indicate the importance of the scale at which such vector-control operations should be conducted.

Due to the rapidly growing human population, increasing number of people will have to settle in or near tsetse-infested habitats. Tsetse-transmitted trypanosomosis is expected to be a serious constraint to rural development for those communities. Results of this trial show that odour-baited targets can be used to control tsetse in such settled areas. It should, however, be realised that trypanosomosis control will only be achieved through large-scale vector control resulting in almost complete absence of challenge.

5.4 The effect of deltamethrin pour-on (Spoton®, Coopers) applied to cattle on the transmission of bovine trypanosomosis

5.4.1 Introduction

Tsetse flies (*Glossina* spp.), and the disease that they transmit, have been controlled successfully by applying insecticide to cattle or to artificial baits, termed targets (Bauer *et al.*, 1995; Green, 1994; Mérot *et al.*, 1984) (Section 5.3). With both types of application the disease transmission is reduced due to a slow decline of tsetse population densities in the surrounding areas, and hence a gradual reduction in challenge. However, there is some evidence that disease transmission can also be reduced more directly and immediately by the inhibition of the flies' feeding responses on insecticide-treated animals (Van den Bossche *et al.*, 1987; Bauer *et al.*, 1992a). Other evidence contradicts this (Thomson, 1987; Baylis *et al.*, 1994; Gouteux *et al.*, 1996).

It is necessary to clarify the importance of this direct effect of insecticide treatments on the tsetse's feeding responses or trypanosome transmission because it is the one which could offer an immediate benefit to the farmer who treats his cattle, irrespective of whether the cattle are treated in adjacent areas or irrespective of its effect on the tsetse population density (Echessah *et al.*, 1996). In contrast, the effect that depends on the decline of tsetse population density cannot be achieved by one farmer alone. If cattle in nearby areas are untreated, or treated only sporadically, the flies will persist there, allowing a steady stream of flies to invade the areas where cattle may be treated properly. In this case, deltamethrin treatments on cattle will have less effect on the incidence of trypanosomosis in treated cattle. A related problem occurs where cattle are kept immediately adjacent to a game reserve from which tsetse can continuously invade (Section 3.4.3).

The present work elucidated the importance of the direct effect by studying the incidence of trypanosomosis in groups of deltamethrin-treated and untreated cattle herded in the same area and subject to a similar and constant tsetse challenge.

5.4.2 *Materials and methods*

5.4.2.1 *Trial area*

The trial was conducted between August 1992 and December 1992 in the Katete District, Eastern Province, Zambia (31°50' E -13°05' S) (Section 2.2.2.1).

5.4.2.2 *Experimental animals and treatments*

Twenty-seven randomly selected adult oxen (Ngoni breed), aged between 1.5 and 3 years, were divided into two herds: a control herd (n = 15) and one treated with deltamethrin pour-on (Spoton®, Coopers) (n = 12). At the start of the trial (Week 0), all animals were eartagged and treated with diminazene aceturate (Berenil®, Hoechst) at 7.0 mg/kg body weight. Deltamethrin pour-on (Spoton®, 1% deltamethrin a.i.) was applied to all animals of the treated herd in a line along each side of the animal at a dose of 10 ml/100 kg body weight, using a T-shaped hand applicator. Pour-on treatment was repeated at 4-week intervals (Weeks 0, 8, 12 and 16).

To avoid the risk of contamination, oxen treated with deltamethrin pour-on were kept as one group and kraaled together. All animals were exposed to the same natural field challenge of tsetse by herding them in the same area (*ca.* 10 km^2). Different herdsmen looked after the treated and the untreated groups and kept the two herds separate.

To allow for a prophylactic effect of a double dose of diminazene aceturate (7mg/kg), all animals were considered to be protected during the first four weeks after the initial treatment. Trypanosomosis incidence in both herds was calculated at two-weekly intervals from Week 5 onwards. On each occasion, ear vein blood of all animals was examined for trypanosomes using the haematocrit centrifugation technique and the PCV was measured (Section 3.3.2.2). Since resistance to diminazene aceturate has not been reported in the trial area, trypanosomal infections were treated with diminazene aceturate at a dose of 7mg/kg body weight for *T. brucei* or 3.5 mg/kg body weight for *T.congolense* or *T.vivax*. Animals given diminazene were considered to be protected during the subsequent two weeks and were therefore excluded from the next calculation of incidence.

5.4.2.3 Statistical analysis

Packed cell volumes of treated and untreated cattle were compared using a t-test (Sokal and Rohlf, 1998). A one-sided Fisher's exact test (Sokal and Rohlf, 1998) was used to test whether the trypanosomosis incidence in the deltamethrin-treated herd was significantly lower compared to the incidence in the untreated herd (SPSS, SPSS Inc.).

5.4.2.4 Monitoring of tsetse population

The index of abundance of tsetse in each herd's grazing area was monitored using five epsilon traps baited with acetone (at a release rate of 200 mg/h) (Hargrove and Langley, 1990). Traps were sited in munga and miombo (Section 2.2.2.1). In addition, five epsilon traps (control traps) were deployed 10 km south of the grazing area. Trap cages were emptied daily. Live flies were dissected to determine trypanosome infection rate (Lloyd and Johnson, 1924). The monthly mean index of abundance (IA) of tsetse was calculated as the average number of flies (males and females) captured per day and per trap.

5.4.3 Results

5.4.3.1 Index of abundance of tsetse

The IA in the grazing area was similar to the IA outside the grazing area (Fig. 5.4.1). This is not surprising considering the low number of deltamethrin-treated cattle in the trial area.

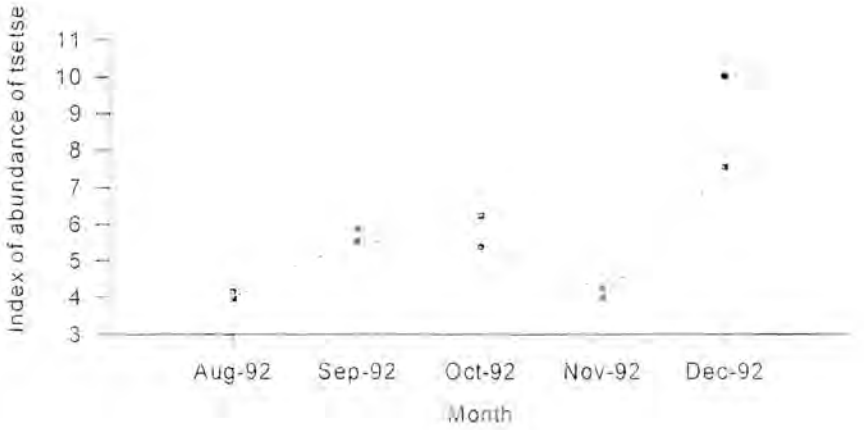


Figure 5.4.1: Monthly average index of abundance of *G. m. morsitans* inside the grazing area (■) and in the control area (◆).

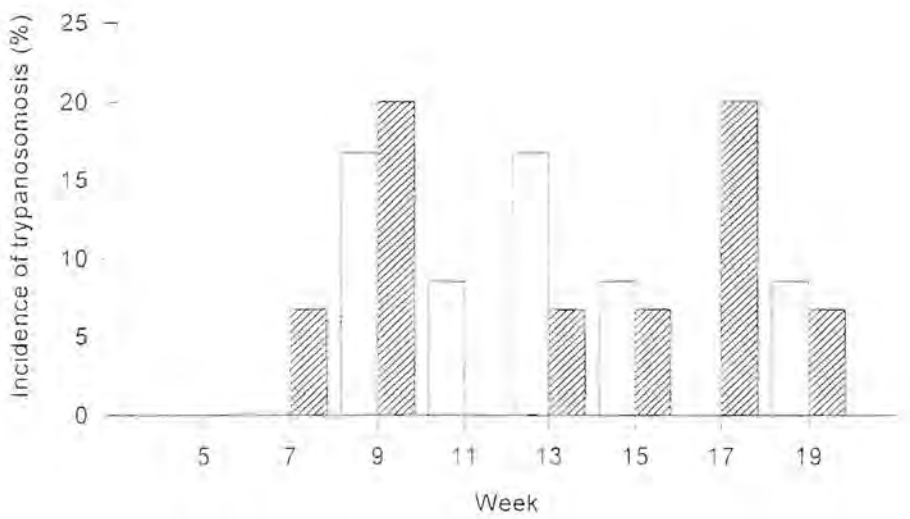


Figure 5.4.2: Two-weekly incidence of trypanosomosis in control (▨) and deltamethrin-treated (▤) herd.

During the trial period, the monthly proportion of infected tsetse increased gradually from 0.63% to 2.5%. A total of 62.5% of the trypanosomal infections in tsetse were *congolense*-type, the remaining being *vivax*-type.

5.4.3.2 Incidence of bovine trypanosomosis

The first trypanosmal infection was detected seven weeks after the onset of the trial (Fig. 5.4.2). No trypanosomal infections were detected in Week 17 in the control herd and Weeks 7 and 17 in the deltamethrin-treated herd. Trypanosomosis incidence varied considerably between herds and between weeks. The average two-weekly trypanosomosis incidence, however, was 8.1% and 7.8% for the control and the deltamethrin-treated herd, respectively. A total of 16 trypanosomal infections were detected. *Trypanosoma congolense* accounted for the majority (87.5%) of the infections. The remaining 12.5% was attributed to *T. vivax*. The probabilities for the null hypothesis of no difference between the trypanosomosis incidence in the deltamethrin-treated and the untreated herds area is shown in Table 5.4.1.

Table 5.4.1: Two-weekly incidence of trypanosomal infections in deltamethrin-treated and untreated, control, herd and significance of Fisher' exact test.

Week	Control herd		Deltamethrin-treated herd		P-value
	Infected	Not infected	Infected	Not infected	
7	1	14	0	12	1.00
9	2	12	2	10	0.64
11	[illegible]	13	[illegible]	9	0.44
13	1	14	2	9	0.38
15	1	13	1	10	0.70
17	3	11	0	11	1.00
19	1	11	1	11	0.76

For none of the weeks was the difference between the incidence of trypanosomal infections significant.

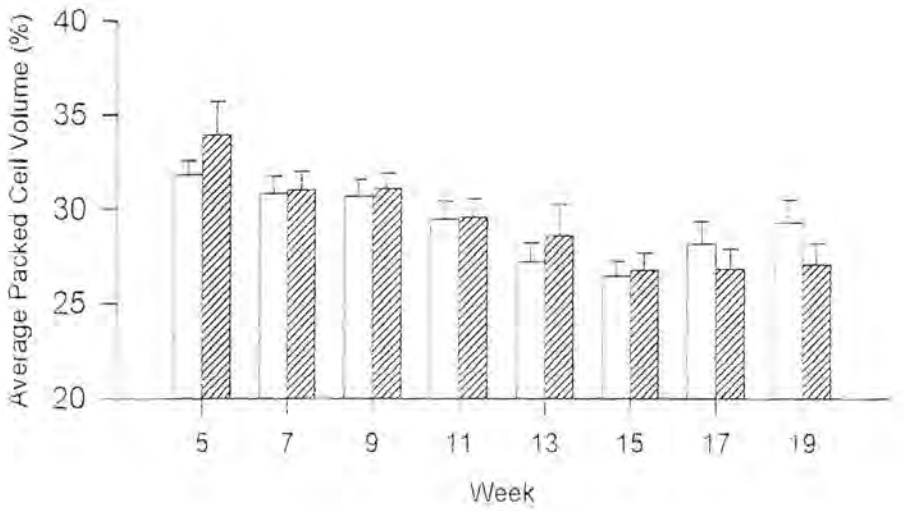


Figure 5.4.3: Two-weekly average packed cell volume (PCV) (± 1 s.e.) of the control (□) and deltamethrin-treated (▨) animals.

The two-weekly average PCVs of both herds decreased gradually, from 32.8% in Week 5, to 26.6% in Week 15. From Week 17 onwards the average PCV increased, reaching 28.2% in Week 19. None of the differences between average PCV of untreated and deltamethrin-treated herds was statistically significant ($P>0.05$) (Fig. 5.4.3).

5.4.4 Discussion

During the three-month observation period, the incidence of tsetse-transmitted trypanosomosis was never statistically lower in the deltamethrin-treated herd compared to the untreated herd. This lack of association between deltamethrin treatments and disease incidence and the variations in the incidence of trypanosomosis between herds and between samplings could have been due to the low sensitivity of the parasitological diagnostic methods to detect trypanosomal infections (Paris *et al.*,

1982). Such low sensitivity could lead to the misclassification of non-diseased animals and consequently affect parasitological incidence. This low diagnostic sensitivity is, however, non-discriminatory and would have affected both the deltamethrin-treated and untreated herd. Therefore, it cannot affect the degree of association (Thrusfield, 1986). It can, nevertheless, cause substantial variations in the parasitological incidence of trypanosomosis between herds and between consecutive samplings.

Measuring indirect effects of trypanosomosis in both herds could partly compensate for the low diagnostic sensitivity. A major characteristic of bovine trypanosomosis is anaemia (Murray and Dexter, 1988) and the PCV is a reliable indicators of anaemia (Saror, 1979). Significant differences between herd PCVs could, therefore, be used as an additional indicator of trypanosomal infections and tsetse challenge. No significant differences were observed between the average two-weekly PCVs of the deltamethrin-treated and untreated herds. The gradual decrease in the PCV during the first 15 weeks of the trial followed by an increase during the last four weeks is attributed to seasonal changes in the pasture condition (Sawadogo *et al.*, 1991).

According to the parasitological incidence and PCVs, there was no difference between the incidence of tsetse-transmitted trypanosomosis in deltamethrin-treated and untreated herds.

A repellent or irritant effect of the deltamethrin pour-on, applied at the dose rate and treatment interval used in this trial, cannot be excluded from the current experimental design. This could affect the preference of tsetse for either treated or untreated animals. Nonetheless, results indicate that even if such effects do occur, they are too small to reduce the trypanosomosis incidence to a level that would be a direct benefit accruing to the owners of treated animals.

Consequently, the effect of deltamethrin-treatment of cattle on the incidence of tsetse-transmitted trypanosomosis observed in other experiments or control campaigns seems to be a result of its effect on the population density of tsetse or tsetse challenge

rather than its direct effect on the tsetse's feeding response. Successful control of tsetse-transmitted trypanosomosis using deltamethrin-treated cattle (at a dose rate of 10 ml Spoton®/100 kg body weight and at monthly treatment intervals) will, therefore, depend on the level of induced tsetse mortality and tsetse invasion pressure. The use of this tsetse control method in areas where, for whatever reason, the tsetse population density cannot be sufficiently reduced to reduce disease challenge will not result in a decline in trypanosomosis incidence.

5.5 The effect of short-interval deltamethrin applications to control tsetse, on the seroprevalence of babesiosis in cattle

5.5.1 Introduction

Regular treatments with acaricides have long been regarded as the most effective means of controlling ticks and tick borne-diseases. However, considerable evidence from several epidemiological studies has demonstrated that such intensive dipping has often been the main cause of tick-borne disease problems (Norval, 1983). As a result, most tick-borne diseases are nowadays managed by integrating the strategic use of acaricides, the application of vaccines if available and the exploitation of endemic stability if present.

The concept of endemic stability is well established. An endemically stable situation is one in which the majority of the host population acquires protective immunity to a particular tick-borne disease, through infection when young while still protected by passively-acquired and non-specific factors (Norval, 1983). For endemic stability to develop, infected vectors must be present in sufficient numbers to ensure regular challenge of young animals. Effective tick control, in areas where endemic stability is present, may cause endemic instability due to infrequent disease transmission. Consequently, animals will become susceptible and when challenged would develop clinical disease.

Regular treatments of cattle with pyrethroids, to control tsetse, might have a significant effect on the density of the tick population. The degree of tick control will, however, depend upon the interval between treatments, the proportion of animals treated, the acaricidal activity of the compound and the dose at which the pyrethroid is applied.

For the past decade, deltamethrin treatment of cattle along Zimbabwe's eastern/north-eastern border has been part of an integrated approach to counteract continuous invasion of tsetse from the Mozambique fly-belt (Shereni, 1990). To determine the effect of these deltamethrin treatments on the epidemiology of babesiosis, a survey

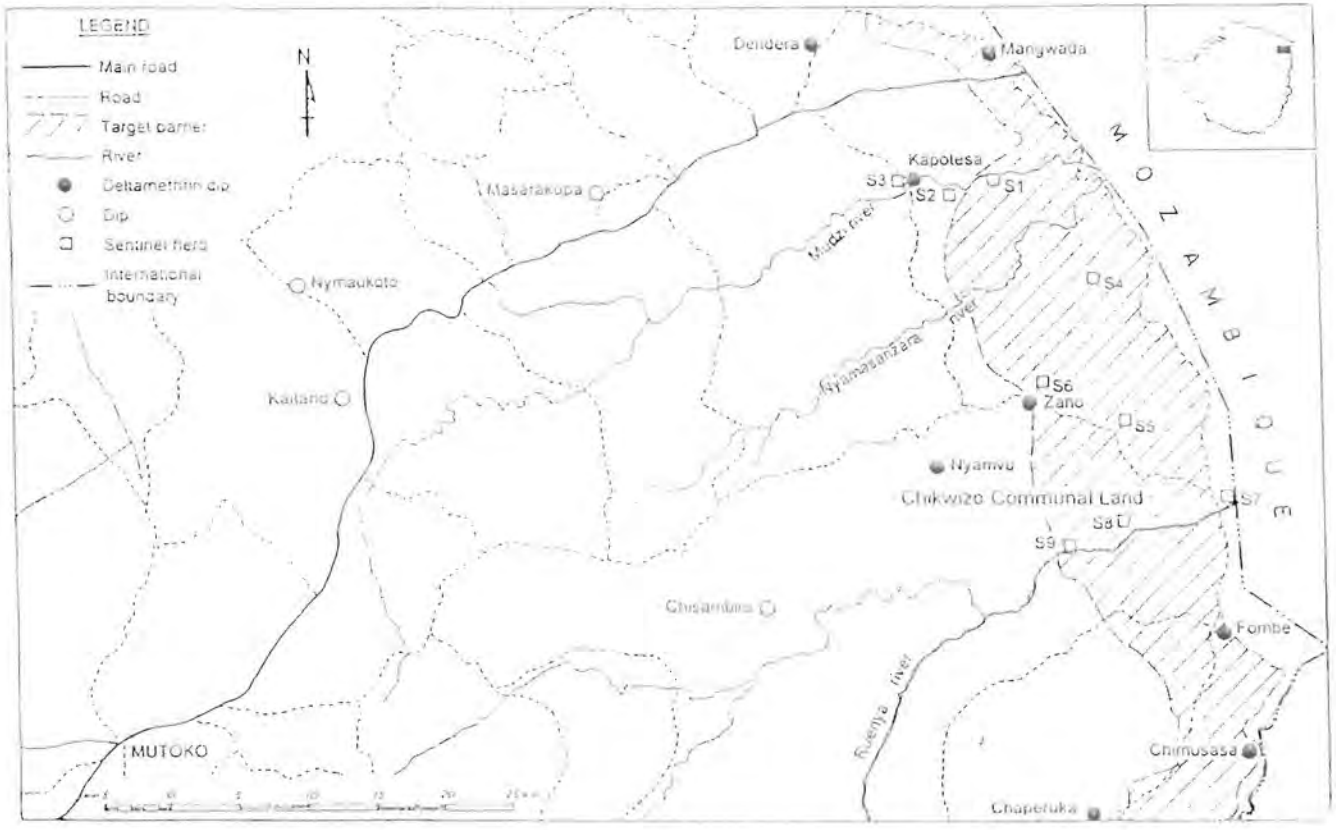
was conducted to estimate the prevalence of antibodies against *Babesia bigemina* in adult cattle. The seroprevalence figures were compared with those from a survey conducted before the implementation of the tsetse control measures (Norval *et al.*,1983). The seroprevalence of *B. bigemina* in adjacent areas, where cattle are not treated with deltamethrin, was also determined for comparison.

5.5.2 *Materials and methods*

5.5.2.1 Trial area

The survey was conducted between November 1995 and February 1997 in the Chikwizo Communal Land (Mudzi District, Mashonaland East Province) (Fig. 5.5.1). The survey area had been cleared of tsetse for many years but is subject to continuous invasion of tsetse flies (*G. pallidipes* and *G. m. morsitans*) from the Mozambique fly-belt (Shereni, 1990; Van den Bossche and Mudenge, 1997; see Section 5.6). To protect tsetse-free areas, a barrier of odour-baited targets (Hargrove, 1993) was erected along the Mozambique border. From 1986 onwards, the effect of the target barrier was supplemented by compulsory treatments of cattle in a zone of 10-15 km wide (deltamethrin treatment zone, DTZ) (Fig. 5.5.1) by dipping them in 0.00375% deltamethrin (Decatix®, Coopers) at two-weekly intervals (Thomson and Wilson, 1992). In areas adjacent to the DTZ (Fig. 5.5.1), cattle were treated with short residual acaricides (Amitraz, Triatix®, Coopers). *Babesia bigemina* is widespread in cattle in communal areas of Zimbabwe (Norval *et al.*,1983); the survey was, therefore, restricted to establishing its prevalence. The effect of regular deltamethrin treatments on the prevalence of *B. bigemina* was determined by comparing the serological prevalence of *B. bigemina* in the DTZ with its serological prevalence in cattle outside but adjacent to this zone.

Figure 5.5.1: Map of the trial area indicating location of the sentinel herds and the areas covered by the survey.



5.5.2.2 Sample collection and analyses

Jugular blood was collected from at least 30, randomly selected, adult communal cattle at 8 localities inside and 4 localities outside the DTZ (Fig.5.5.1). Serum was separated from the clotted blood and stored at –20°C prior to serological testing. The indirect fluorescent antibody test was used to detect anti-*B. bigemina* antibodies (Norval *et al.*, 1983).

To determine the level of *B. bigemina* transmission, 90 head of adult sentinel cattle were introduced into the DTZ in January 1996. Before introduction, all the animals were tested for antibodies to *B. bigemina*. Only serologically negative animals were retained and assigned to nine sentinel herds (S1-S9) (Fig. 5.5.1). Sentinel animals were not treated with deltamethrin. The prevalence of anti-*B. bigemina* antibodies in the sentinel cattle was determined at 3-monthly intervals.

Estimates of the monthly coverage with deltamethrin-treatments were obtained by comparing the number of animals dipped in a particular month with the total number of animals registered at that dip in the same month. The monthly average dipping coverage in the DTZ, expressed as a percentage of the total cattle population registered, was calculated for 1996.

5.5.3 *Results*

5.5.3.1 Proportion of cattle dipped

The proportion of animals dipped each month in the DTZ varied between 50 and 80% of the total population registered (Fig. 5.5.2). Attendance for dipping was lowest during the hot dry season and at the beginning of the rainy season.

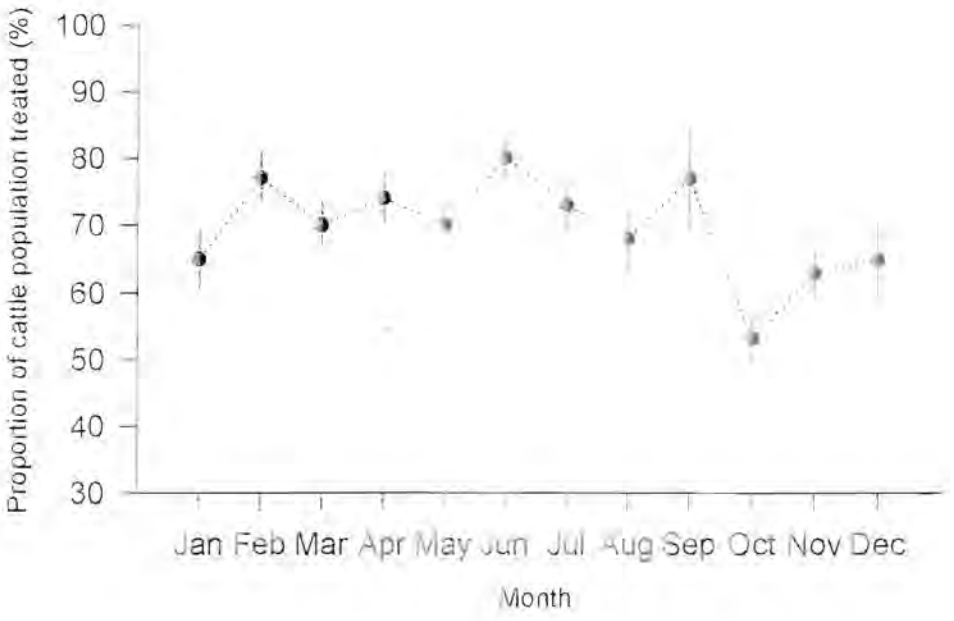


Figure 5.5.2: Monthly average proportion (± 1 s.e.) of the total cattle population treated in the "deltamethrin treatment zone" during 1996.

*5.5.3.2 Prevalence and incidence of cattle with anti-*B. bigemina *antibodies*

Anti-*B. bigemina* antibodies were detected in sera from cattle sampled at only two locations (25%) in the deltamethrin-treated zone. At those locations, the prevalence of serologically positive cattle was low, indicating low levels of disease transmission (Table 5.5.1).

Table 5.5.1: Prevalence of antibodies against *B. bigemina* in cattle sampled at various locations in the deltamethrin-treatment zone.

Location	Sample size	Number positive	Prevalence (%)
Zano	34	0	0
Nyamvu	35	0	0
Kapotesa	35	4	11.4
Mangwada	35	0	0
Dendera	35	0	0
Fombe	35	0	0
Chimusasa	33	0	0
Chaperuka	35	2	5.7

Serologically positive animals were present at all locations outside the DTZ (Table 5.5.2). The mean prevalence of antibodies against *B. bigemina* in cattle sampled at locations inside and outside the DTZ was 2.1 ± 1.5% and 43.2 ± 3.6%, respectively.

Table 5.5.2: Prevalence of cattle with antibodies against *B. bigemina* sampled at various locations outside the deltamethrin-treatment zone.

Location	Sample size	Number positive	Prevalence (%)
Masarakupa	30	13	43.3
Kaitano	43	20	46.0
Nyamukoto	63	32	50.0
Chisambiro	30	10	33.3

The proportion of sentinel cattle exhibiting antibody titres against *B. bigemina* varied between months (Table 5.5.3). However, incidence was generally low and did not increase in time.

Table 5.5.3: Variations in the proportion of sentinel cattle with antibodies against *B. bigemina* in the deltamethrin-treatment zone.

Month	Sample size	Number positive	Prevalence (%)
February '96	80	4	5.0
May '96	74	13	17.6
August '96	90	4	4.4
October '96	76	4	5.3
December '96	95	10	10.5
February '97	69	3	4.3
April '97	82	6	7.3

5.5.4 Discussion

A survey, conducted in 1980-81, on the prevalence of antibodies to *B. bigemina* and the distribution of ticks of the genus *Boophilus* in Zimbabwe revealed that *B. bigemina*, together with its main vector *Boophilus decoloratus*, occurred throughout the country (Norval *et al.*, 1983; Mason and Norval, 1980). In most areas where dipping was non-existent or irregular, the prevalence of antibodies against *B. bigemina* was high, suggesting that endemic stability was present. Unfortunately, the 1980-81 survey did not cover the area in this study. The above results showed that the prevalence of antibodies to *B. bigemina* was much higher in areas where dipping with a non-pyrethroid acaricide was conducted than in the DTZ. Seroprevalence is, however, insufficient to assume endemic stability for *B. bigemina* (Norval *et al.*, 1983).

The compulsory dipping of cattle in deltamethrin to control tsetse appears to have been very successful in also controlling *Boophilus* spp. The relatively low prevalence of *B. bigemina* antibodies in the 90 susceptible adult animals at risk of natural infection in the DTZ (Table 5.5.3) confirmed that the challenge of *Boophilus* spp. in that area was low. This low population density of *Boophilus* spp. reduced the chance of cattle receiving an immunizing infection as young animals when they would have

been relatively resistant and may have acquired protective immunity. The spread of infected ticks to such susceptible populations of cattle or the introduction of susceptible cattle to endemic areas could lead to serious disease outbreaks (Lawrence *et al.*,1980).

Both deltamethrin and amitraz have good acaricidal activity when applied at two-weekly intervals (Norval *et al.*,1992; Fox *et al.*,1993; Chizyuka and Luguru,1986). This is certainly the case for one-host ticks such as *Boophilus* spp. The better control of *Boophilus* spp. in the DTZ is attributed to the regular use of deltamethrin to control tsetse and the stringent supervision of dipping practices by Government services. This resulted in a high coverage of the animals and, consequently, good tick control (Fig. 5.5.2). In the adjacent zone in which amitraz was used, on the other hand, dipping was often disrupted due to problems with water or acaricide supply. As a result, tick control was less rigorous and permited the development of endemic stability

The dose of deltamethrin required to control tsetse is far below that required to control ticks. Therefore, the most obvious solution to avoid potential adverse effects of severely reducing the population of ticks would be to extend the interval between deltamethrin treatments. Although a certain degree of tick control is unavoidable, pyrethroids used to control tsetse should be applied at intervals or doses that give optimal tsetse control without affecting the transmission of tick-borne disease agents and, hence, permit the development of endemic stability. The intervals, varying from 1 to 3 months depending on the insecticide and its formulation, at which pyrethroid insecticides should be applied to control tsetse have been derived from laboratory studies (Bauer *et al.*,1992a; 1989; 1988) and have been adopted in the field. At such extended treatment intervals no immediate effect on tick-borne disease transmission is expected. Nevertheless, the long-term effect of such treatments on tick populations is still unknown. If, for whatever reason, the intervals between treatments used to control tsetse need to be shortened, adverse effects on the tick population and transmission of tick-borne diseases are to be expected.

The results of this survey clearly demonstrate the importance of an integrated approach towards disease control. Potential adverse effects of pyrethroid treatments used to control tsetse on the transmission of tick-borne disease should be taken into consideration at the onset of tsetse control operations.

5.6 Evaluation of insecticide-treated cattle as a barrier to re-invasion of tsetse to cleared areas in northeastern Zimbabwe

5.6.1 Introduction

In Zimbabwe over recent years, a combination of tsetse control methods has successfully eradicated the fly from large parts of the country's interior, leaving infestations in the northeastern Zambezi Valley and along the eastern border with Mozambique (Shereni, 1990; Lovemore, 1999). As a result, a large proportion of the tsetse control budget in Zimbabwe (20%) is now spent on maintaining the barriers to tsetse re-invasion from neighbouring countries (Shereni, 1990). These barriers consist of odour-baited, insecticide-treated, targets in a band, 8 km wide, at an operational density of 4/km² (Hargrove, 1993). Such barriers are supported by the compulsory treatment of all cattle adjacent to the barrier with the synthetic pyrethroid deltamethrin, either as a dipwash (Decatix®, Coopers) at two-weekly intervals, or as a pour-on (Spoton®, Coopers) at monthly intervals.

The maintenance of a target barrier is costly. Target service intervals are usually shorter in target barriers due to the increased theft problems associated with the semipermanent layout of the targets, and constant vigilance is required in order to prevent the barrier breaking down. The treatment of cattle with deltamethrin pour-on or dip is also more costly than the acaricide that is routinely used for tick control in Zimbabwe, further increasing the cost of maintaining the barrier.

Recent work has suggested that the efficacy of insecticide treatment of cattle against tsetse might be greater than was originally supposed (see Bauer *et al.*, 1992b, 1995; Fox *et al.*, 1993) and it has been suggested that cattle treatments alone might be sufficient to stem the re-invasion of tsetse into cleared areas of Zimbabwe. If this were case, a considerable cost saving could be made.

This section reports the results of a field trial which was undertaken to see if insecticide-treatments of local cattle alone could act as a barrier to the re-invasion of tsetse into cleared areas of Zimbabwe.

5.6.2 *Materials and methods*

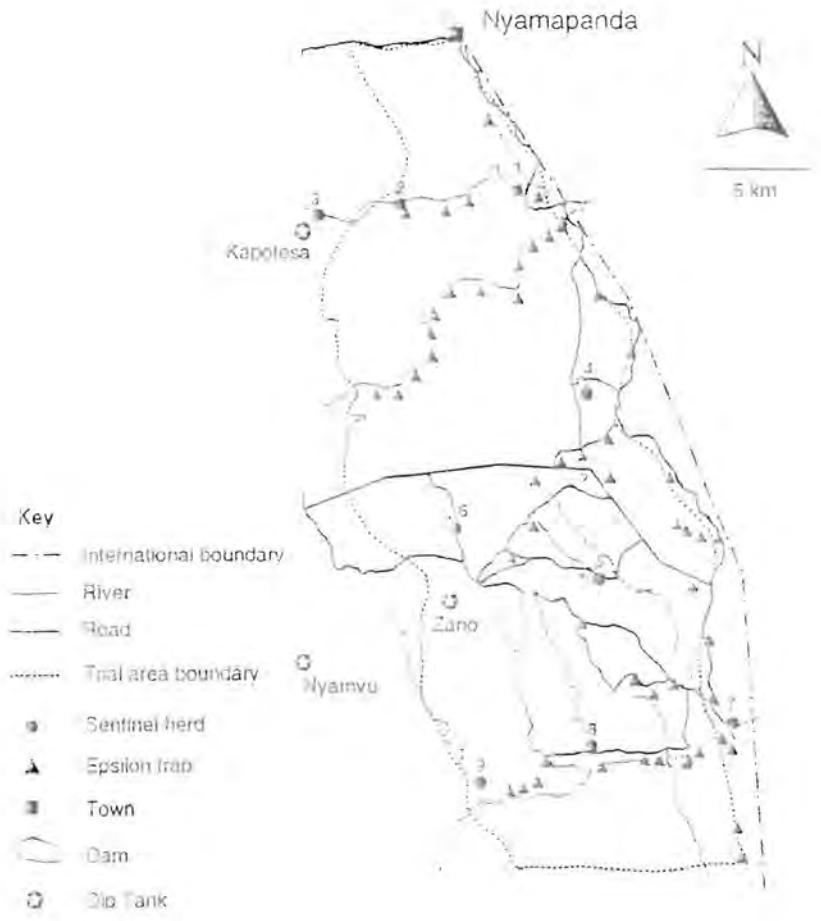
5.6.2.1 *The trial area*

An area of 428km² (≈40 km long and 5-15 km wide) adjacent to the Mozambique border and to the south of the Tete road in northeast Zimbabwe, was chosen for the trial (Fig. 5.6.1). Archive data showed that this area suffered a high invasion pressure from populations of both *G. m. morsitans* and *G. pallidipes* in neighbouring Mozambique (TTCB, 1992). Much of the area was heavily settled although the distribution of settlement was patchy. The remaining land consisted of a mosaic of alluvial woodland and dry forest, with patches of thicket adjacent to the Ruenya, Nyamusandzara and Mudzi Rivers, which feed in a northeasterly direction through the trial area towards the Zambezi River. A cattle census revealed a population of between eight and twelve cattle per km² in the 428 km² of the trial area, which should be sufficient for an effective control of tsetse fly by insecticide treatment (Bauer *et al.*, 1992b). However, the cattle were not evenly distributed, reflecting the patchiness of the settlements, and the grazing areas could not be controlled.

The target barrier consisted of blue/black/blue 'S-type' targets (Vale *et al.*, 1988a) with the central black portion of the target treated with deltamethrin 0.54% (Glossinex®, Coopers) and baited with butanone and a mixture of 4-methyl phenol, 1-octen-3-ol and 3-*n*-propyl (Torr *et al.*, 1997). These were arranged in transects 0.5 km apart running in an east-west direction, with targets placed at 0.5 km intervals. The layout was strengthened by additional target lines along the rivers and roads to give an operational density of 5.4 targets per km².

The target barrier was supported by the insecticide treatment of cattle in, and adjacent to, the barrier in an area some 20 km wide, west of the tsetse re-invasion front. Some 5 400 head of cattle at three inspection sites in the area (Zano, Kapotesa and Nyamvu, Fig. 5.6.1) were dipped in 0.00375% deltamethrin (Decatix®, Coopers) at two-weekly intervals. After each dipping, the deltamethrin concentration in the dips was checked and, if necessary, adjusted. Whenever dipping could not be conducted (due to water

Figure 5.6.1: Trial area in northeastern Zimbabwe along the border with Mozambique. Targets were placed in transects from left to right every 0.5 km. Additional targets were placed in transects along all the rivers and roads shown on the map, giving a density of 5.4 targets per km^2.



shortage), the cattle were treated with pour-on 1% deltamethrin (Spoton®, Coopers) at monthly intervals. The pour-on was applied in a line along each side of the animal, close to the dorsal mid-line, at a dose of 10ml/100kg body weight. Records were kept of the number of animals treated every month.

5.6.2.2 Tsetse monitoring

Tsetse population monitoring began in January1996 using 54 permanent Epsilon traps (Hargrove and Langley, 1990), baited with mixture of butanone, 4-methyl phenol, 1-octen-3-ol and 3-*n*-propyl phenol (Torr *et al.*, 1997). The traps were spaced at 4 km intervals along the border road, and ≈1 km apart through the trial area, along rivers and roads (Fig. 5.6.1). In addition, from March 1996, five ox-fly-round teams operated between 450 and 500 km of fly-round transect, either each month or every other month. These followed the same defined paths and covered the whole trial area each month. Tsetse catch sites were plotted by geographical co-ordinates, and the distance of each catch from the re-invasion front (easterly side of Fig. 5.6.1) was calculated to facilitate a clear visual presentation of the results. The fly-round teams and the traps were operated until the end of the trial in August 1997.

5.6.2.3 Trypanosomosis monitoring

Monthly trypanosomosis incidence was monitored using nine sentinel herds, each consisting of nine or ten adult cattle (Mashona breed), located at various distances from the tsetse re-invasion front. Three herds grazed along the tsetse invasion front (1, 4 and 7, Fig. 5.6.1), three herds ≈5km into the trial area (2, 5 and 8, Fig. 5.6.1), and three herds ≈10 km into the trial area (3, 6 and 9, Fig. 5.6.1). The sentinel herds followed a strict grazing rota within their allotted grazing areas. Sentinel cattle were not treated with insecticide.

At the start of the trial, all the sentinel animals were ear-tagged and received a curative treatment of diminazene aceturate (Berenil®, Hoechst) by intramuscular injection at a dose of 7.0 mg/kg. Each month, blood taken from the jugular vein of each sentinel animal was examined for trypanosomes (Section 3.3.2.2). Infected animals were cured by intramuscular injection of diminazene aceturate at a dose of 7mg/kg body weight

for *T. brucei* or 3.5 mg/kg body weight for *T. congolense* or *T. vivax* infections. The incidence of trypanosomosis was calculated and presented as average incidence at the various distances (0, 5 and 10 km) from the tsetse re-invasion front.

Grazing areas for the local cattle attending the three inspection sites were *ca.* 0-15, 10-18 km and 20-25 km west of the tsetse re-invasion front for Zano, Kapotesa and Nyamvu, respectively. The prevalence of trypanosomosis in these cattle was determined at regular intervals by taking cross-sectional samples of the adult cattle population at each inspection site (Section 3.4.2.1).

5.6.2.4 Experimental design

The target barrier was maintained for eight months until September 1996 when the targets were removed, leaving only the insecticide treatment of the local cattle to stem the tsetse re-invasion. It was planned to have no targets deployed for 12 months, in order to allow for any seasonal changes in tsetse numbers, but, in March 1997 the prevalence of trypanosomal infections in local cattle became unacceptably high and the target barrier was re-deployed in the following month. The tsetse population and trypanosomosis in the sentinel herds continued to be monitored for a further five months.

5.6.3 Results

5.6.3.1 The abundance of tsetse

Glossina pallidipes accounted for 73% of the catch at the Epsilon traps, but only 3.5% of the catch on ox-fly-rounds throughout the trial period. For the analysis, the results from both species were grouped, but the Epsilon trap catches (Table 5.6.1) were predominately *G. pallidipes* whereas the ox-fly-round catches (Table 5.6.2) were predominantly *G. m. morsitans*, reflecting the known sampling biases of these two sampling systems (Hargrove, 1980a).

Table 5.6.1: Catch per trap per day in the trial area, with distance from the re-invasion front. The number of trap days was variable due to trap theft, vandalism, or weather damage.

Distance from re-invasion front:	0-1km	1-2km	2-3km	3-4km	4-5km	5-6km	6-7km
No.traps	19	6	3	7	3	3	13
Traps days (range)	773-380	201-89	116-51	222-124	111-57	112-54	572-287
Target barrier plus cattle treatments							
Jan. 96	0.0194	0.01					
Feb. 96	0.0162	0.012					
Mar. 96	0.0552	0.0152	0.0132	0.0127			
Apr. 96	0.0225	0.0121					
May. 96	0.031	0.0058		0.0126			
Jun. 96	0.0446	0.0051					
Jul. 96	0.013						
Aug. 96	0.0304	0.0076		0.0161			
Cattle treatments only							
Sep. 96	0.0211	0.0047					
Oct. 96	0.0466	0.0075		0.0128			
Nov. 96	0.0579	0.0674			0.0108		
Dec. 96	0.0881	0.0326	0.0196				
Jan. 97	0.0305	0.0052		0.0056	0.0109		
Feb. 97	0.0104	0.0094		0.0106		0.0127	
Mar. 97	0.0064	0.0345		0.0057			
Target barrier plus cattle treatments							
Apr. 97	0.0085	0.0057	0.012				
May. 97	0.0021						
Jun. 97							
Jul. 97							
Aug. 97	0.0056						

Table 5.6.2: Total catch of tsetse from 450 to 500km of ox-fly round covering the trial areas each month, with distance from the re-invasion front.

Distance from re-invasion front:	0-1km	1-2km	2-3km	3-4km	4-5km	5-6km	6-7km	7-8km	8+	Total catch
Target barrier plus cattle treatments										
Mar. 96										0
May 96	1	1	1							3
Jul. 96	7	1								8
Cattle treatments only										
Sep. 96	0	2	11	10	2					25
Oct. 96	12	5	3	9			2		1	32
Nov. 96	5	13	11	6	1	1			1	38
Dec. 96	7	5	5	2	1	3	1			24
Jan. 97	11	5	4	1	3			1	2	27
Feb. 97	6		2	3	2					13
Mar. 97	11	4	3	2					2	22
Target barrier plus cattle treatments										
Apr. 97	7		1							8
May 97										0
Jun. 97		1								1
Jul. 97										0
Aug. 97										0

In both cases the removal of the target barrier in September 1996 caused an increase in the catch along the re-invasion front, and a change in the distribution of the catch as the flies moved into the trial area. The positions of all ox-fly-round catches throughout the experiment are plotted in Figure 5.6.2. Prior to the removal of the target barrier, catches were confined to an area 10-15 km long and stretching 2-3 km into the target barrier (Fig. 5.6.2a). Once the target barrier was removed, the flies quickly moved into the trial block (Fig 5.6.2b, Tables 5.6.1 and 5.6.2) and the fly front, or the area where re-invasion occurred, expanded. When the targets were re-deployed in April 1997, the catch dropped and the position of capture rapidly reverted to the distribution that was seen before the removal of the targets (Fig.5.6.2c). It was not possible to control for seasonal changes in tsetse numbers and their availability at capturing devices, due to the high prevalence of trypanosomosis in the local stock in March 1997 (Table 5.6.3) which caused an early termination of the trial.

Figure 5.6.2: Position of ox-fly-round catches in the trial area; (a) before removal of the targets (total distance covered 1335 km); (b) after removal of the targets (total distance covered 3584 km); and (c) after the targets have been re-deployed (total distance covered 2296 km)

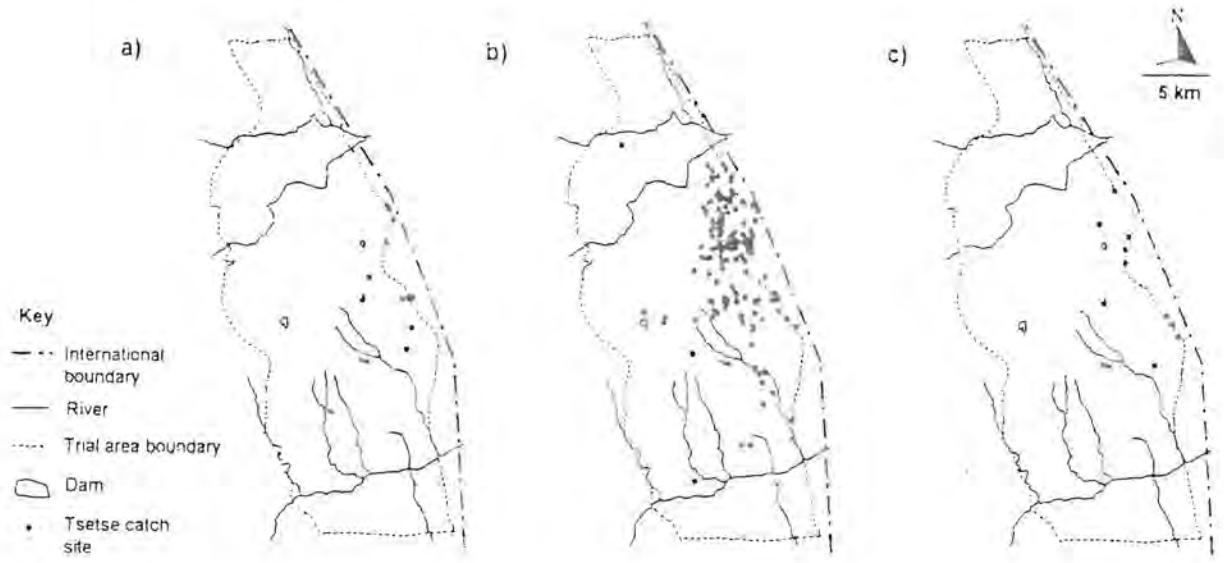


Table 5.6.3: Monthly prevalence of trypanosomosis (%) and average PCV (%) in local stock.

Month		Sampling site		
		Zano	Kapotesa	Nyamvu
Apr. 96	Prevalence	3.3	0	0
	PCV	29.7	32.3	30.5
Jun. 96	Prevalence	0	0	0
	PCV	29.5	30.6	30.3
Oct. 96	Prevalence	0	0	0
	PCV	30.6	32.8	29.9
Dec. 96	Prevalence	0	0	0
	PCV	29.9	31.6	28.9
Mar. 97	Prevalence	19.6	3.3	3.3
	PCV	23.9	32.4	30.4
Apr. 97	Prevalence	3.3	0	0
	PCV	26.3	32.4	31.7
Jun. 97	Prevalence	3.3	0	0
	PCV	28.7	30.3	30.1

However, the pattern of tsetse capture did not follow that which is usually observed due to seasonal changes in Zimbabwe (Phelps and Vale, 1978), suggesting that the expanding population from September through to March was a direct result of the removal of the target barrier. By implication also, the crash in tsetse catch and the immediate restriction in tsetse distribution after the targets were replaced in April 1997, suggest that this was a direct result of the increased mortality imposed on tsetse populations by the targets.

5.6.3.2 Incidence and prevalence of bovine trypanosomosis

Prior to the removal of the target barrier, trypanosomal infections were only diagnosed in sentinel cattle grazing along the tsetse re-invasion front (Fig.5.6.3a). During this period (February 1996-August 1996) the monthly average incidence for

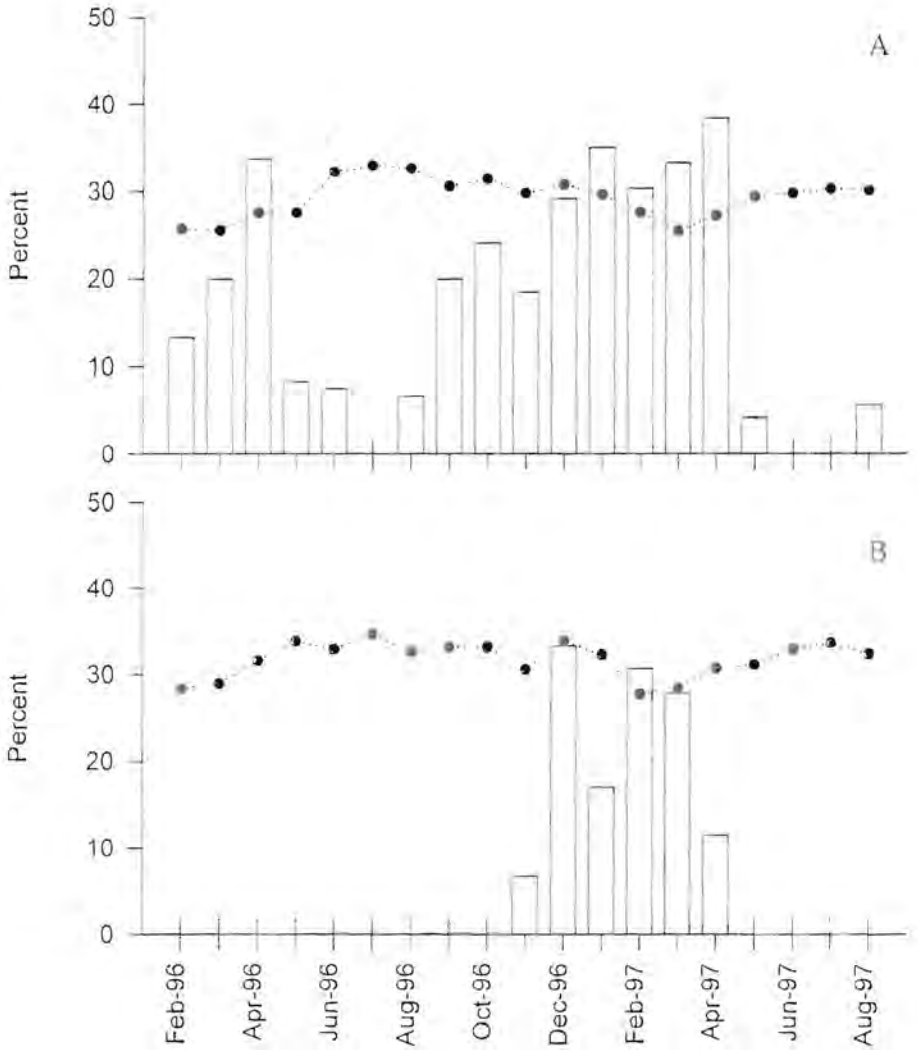


Figure 5.6.3: Incidence of trypanosomosis in sentinel cattle grazed (A) on or very close to the tsetse re-invasion front (herds 1, 4 and 7 of Fig. 5.6.1) and (B) 5 km west of the tsetse re-invasion front (herds 2, 5 and 8 of Fig. 5.6.1). Bars show monthly incidence and dots show average monthly PCV.

the three sentinel herds (1, 4 and 7; Fig 5.6.1) varied from 33.7% in April 1996 to 0% in July 1996 (Fig 5.6.3a). After the removal of the target barrier, the monthly average incidence rose steadily in these herds, reaching a peak of 38.4% the following April. The herds grazing ≈5km from the re-invasion front (2, 5 and 8; Fig 5.6.1), first showed positive to a trypanosomal infection in November 1996, two months after the removal of the target barrier. The trypanosomosis incidence reached 33% in December 1996, and remained high until after the targets were replaced the following April (Fig.5.6.3b).

After re-deploying the target barrier (April 1997), the incidence of trypanosomal infections in all the sentinel cattle returned to a level that was similar to that before the removal of the target barrier.

For seven of the 10 months of the trial period during which targets were present, the monthly average PCVs of sentinel herds grazing along the tsetse re-invasion front were significantly lower ($P<0.05$) than the monthly average PCVs of sentinel cattle grazing either 5 or 10 km from the re-invasion front. The monthly average PCVs of sentinel herds at the tsetse invasion front were highly correlated ($r = -0.90$, $P<0.01$) with the monthly incidence of trypanosomal infections in those animals and reflect the challenge that animals undergo even in the presence of an odour-baited target barrier.

Removal of the target barrier resulted in a decline in the average PCV of herds grazing 5 km from the tsetse re-invasion front (Fig. 5.6.3b) but it did not affect the PCVs of cattle grazing 10 km away. Between January 1997 and April 1997, the average PCVs of sentinel herds grazing 5 km west of the invasion front were not significantly different from those of sentinel herds at the re-invasion front. The re-deployment of targets resulted in a rapid increase in the average PCVs of sentinel herds 5 km west of the invasion front (Fig. 5.6.3b) The prevalence of trypanosomal infections in the local cattle population at each of the three inspection sites was greatly increased by removal of the target barrier (Table 5.6.3).

cases the effect of treatment of cattle on tsetse populations and trypanosomosis *control* was investigated. This is different from our investigation, which was designed to see if treated cattle can prevent tsetse *invasion*. Clearly the answer to this last question, under the circumstances that prevailed, is "no".

Even if tsetse have a high feeding preference for the insecticide-treated animals and a high proportion of the cattle are treated, re-invasion will only be prevented if the treated cattle are evenly distributed over the whole area and if the probability of tsetse contacting treated animals is high. In this trial we had no control over where the cattle grazed at any particular time, and it is probable that for large portions of the trial there were very few cattle, treated or untreated, close to the re-invasion front. Studies in Zimbabwe (Scoones, 1995) have shown that communal cattle grazing patterns can be split according to season. In the cropping season (November-March) cattle are kraaled and herded away from cropped areas, usually under supervision, to protect the crops. In the early dry season (April-July), after the crops have been gathered, the cattle are allowed to roam free and feed unsupervised, mainly on crop residues. As the dry season progresses - late dry season (August - October) - the cattle are forced to move further afield and to graze or browse on diverse food sources. Therefore, one would expect, and observations confirm, a more even distribution of cattle in our trial area during the late dry season and a more patchy distribution at other times of year. This seasonality in the grazing pattern of cattle is common in most communal areas in southern Africa. Consequently, it is almost impossible to assure an even distribution of insecticide-treated cattle throughout the year. This implies that, if insecticide-treated cattle are used to prevent re-invasion of tsetse, the probability of tsetse encountering a treated host will vary according to the season and therefore efficacy of the insecticide-treated cattle barrier will vary accordingly.

It was not possible to test the efficacy of the target barrier in the absence of insecticide-treated cattle. However, the level of management of the target barrier was high and resources were not a limiting factor. Due to the logistical difficulties involved in maintaining a target barrier, and variable resource inputs, it is probably wise to continue insecticidal dipping of cattle in the barrier, to cover for possible breakdowns in barrier efficacy. The additional cost of using a deltamethrin-based dip rather than

5.6.4 Discussion

Under the conditions of this trial, the regular insecticide treatment of cattle did not prevent the tsetse from re-invading the trial area. After the second month without the target barrier in place, tsetse were caught up to 8 km west of the re-invasion front. At the same time, the trypanosomosis incidence in sentinel cattle increased, with a concomitant decrease in the PCV. Furthermore, the high prevalence of trypanosomosis in the local cattle suggested that the insecticide treatments afforded little protection from tsetse challenge and subsequent trypanosomal infection. This is in agreement with the results of Baylis *et al.* (1994) and the results presented in Section 5.4.3. After 7 months, the prevalence of bovine trypanosomosis in the local cattle was unacceptably high in the trial area and the trial was stopped prematurely.

Although it was not possible to investigate the effect of the target barrier in the absence of cattle treatments, it appears that the target barrier performed roughly as has been predicted by a mathematical analysis of tsetse movement (Hargrove, 1993) and earlier experimental investigations (Muzari & Hargrove, 1996). As expected, the targets did not afford complete protection for the cattle herded at the edge of the tsetse re-invasion front, but they gave almost full protection to cattle herded ≈5 km inside the barrier, and complete protection to cattle herded more than 5 km into the area. When the targets were removed, the trypanosomosis incidence in cattle on the re-invasion front and the tsetse catch there increased, indicating that the target barrier was having an effect on the adjacent tsetse populations, as was suggested by Vale *et al.* (1988a).

Previous studies on the effects of insecticide-treated cattle for tsetse control have given mixed but promising results. Bauer *et al.* (1995), working in Burkina Faso, reported good control of tsetse and trypanosomosis in stock using deltamethrin pour-on, and Fox *et al.* (1993) in Tanzania reported reduced tsetse population and increased herd health after the deltamethrin treatment of cattle on a large commercial ranch. Baylis & Stevenson (1998), reporting on a trial on the Galana Ranch in south-east Kenya, concluded that the effect on herd health was greater than could be expected from the minimal effects on tsetse density caused by cattle treatments. In all of these

the routinely used acaricide is low compared to the cost of mopping up tsetse populations that become established after penetrating a poorly maintained target barrier.

Monthly deltamethrin treatment coverage of adult cattle in the trial area varied between 76 and 87% of the total cattle population. This variability is explained by the poor turn-out at dips on wet days, a failure of stock owners in outlying homesteads to trek to the inspection sites every time, and the free roaming of cattle during the dry season.

5.7 The large-scale use of a 1% cyfluthrin pour-on (Cylence®, Bayer) to control bovine trypanosomosis in eastern Zambia

5.7.1 Introduction

In the mid-1900s, the attractiveness of hosts to tsetse was first exploited as a tsetse control method (Whiteside, 1949; Vanderplank, 1947; Du Toit, 1954). Despite initial successes, this promising tsetse control method was abandoned because of the low persistence of the insecticides used.

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

The promising results of the initial controlled trials were followed by several field trials in the southern African Region. A small-scale trial, conducted in the Eastern Province of Zambia, involving the weekly dipping in deltamethrin of 400 head of cattle, resulted in a reduction of the trypanosomosis incidence from 40% at the beginning of the trial to 5% eight months later (Chizyuka and Luguru, 1986). Similar effects were observed in other parts of Zambia (Wiersma and Schoonman, 1992) and in Zimbabwe (Thompson *et al.*, 1991). Despite the successful application of this method in other parts of Africa (Bauer *et al.*, 1988; Bauer *et al.*, 1992b; Fox *et al.*, 1993; Leak *et al.*, 1995; Bauer *et al.*, 1995), it has not been used widely in southern Africa. This is largely a result of the strategy of tsetse eradication from large areas where cattle are absent. However, the shift from a strategy of tsetse eradication to localised trypanosomosis control makes the insecticide treatment of cattle an attractive method of control. To evaluate the effectiveness of the method in an intensively cultivated area of southern Africa where bovine trypanosomosis was endemic, a trial

was initiated in eastern Zambia. Use was made of a 1% cyfluthrin pour-on (Cylence®, Bayer) formulation.

5.7.2 Materials and methods

5.7.2.1 Trial area

The trial was carried out in an area of about 2 000 km^2 situated between 30°44'and 31°08' E and between 14°07' and 14°42' S in Petauke and Nyimba Districts of the Eastern Province of Zambia (Fig. 5.7.1). The area is intensively cultivated and carries a cattle population (Angoni breed) of *ca.* 11 animals/km^2 (based on an aerial survey conducted in August 1997). The total number of adult cattle in the area was 20 130 (based on census figures of the Department of Veterinary Services). Vegetation and climate are described in Section 2.2.2.1. *Glossina m. morsitans*, the only tsetse species present, takes most of its blood meals from cattle (Section 2.3.3). Tsetse-transmitted bovine trypanosomosis was endemic (Fig. 5.7.1) with a monthly average incidence of 9.7% (Section 2.5.3.4). The index of abundance of tsetse explains 74% of the variance in the incidence (log transformed) of nagana (Section 2.5.3.4).

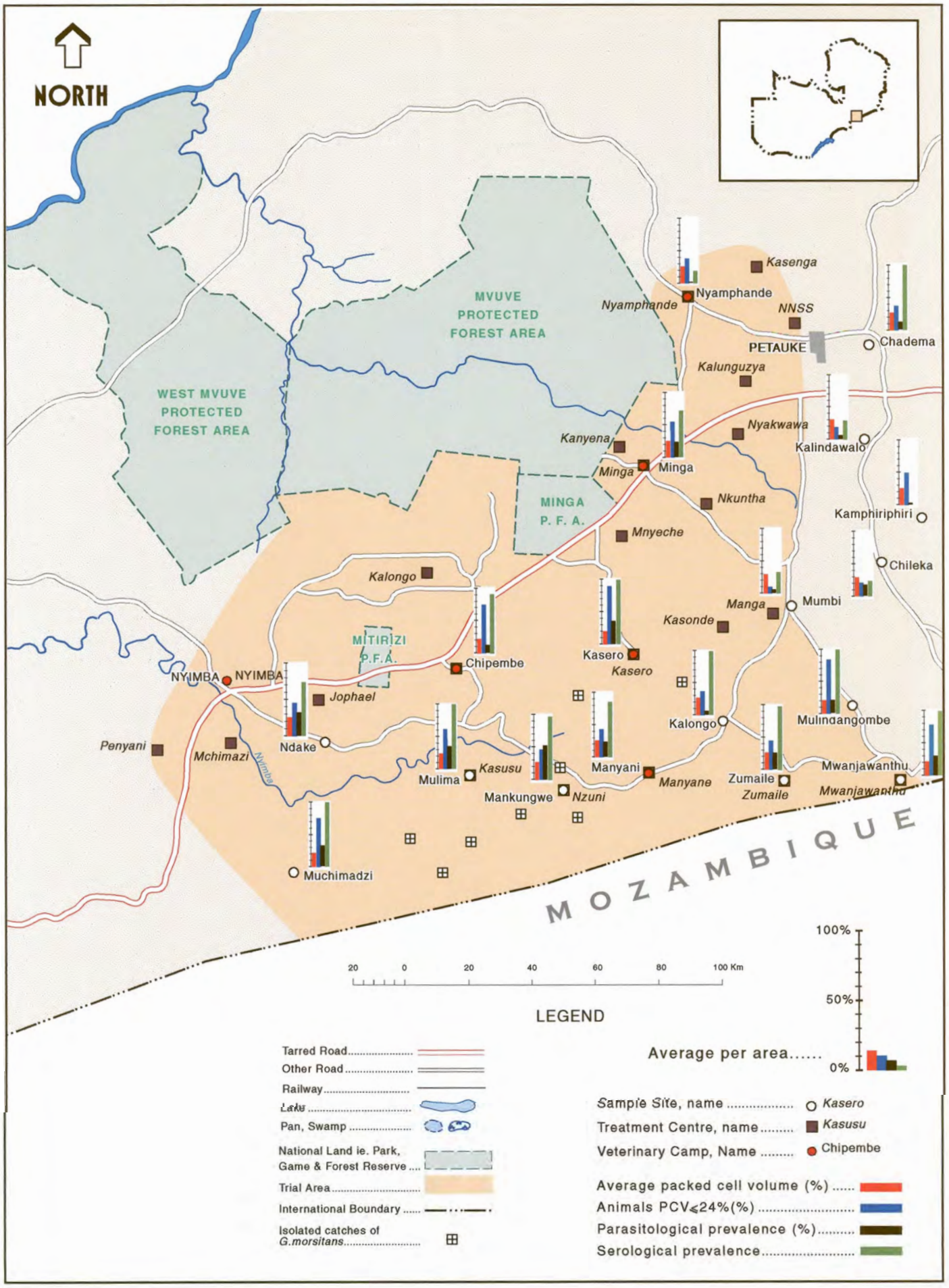
5.7.2.2 Insecticide treatments

A 1% cyfluthrin pour-on (Cylence®, Bayer) was applied in one line along the spine of the animal, from shoulder to tail base, at a dose of 15 ml/100 kg body weight using an automatic pour-on applicator. Treatments started in November 1998 and were repeated at *ca.* 7-week intervals. They were applied free of charge. To facilitate the treatment of all adult cattle in the trial area, 22 treatment centres were established. They were supervised by 7 veterinary camps (Fig. 5.7.1). Records were kept of the number of animals treated at each centre during each treatment. The total number of animals treated was expressed as a proportion of the total number of animals in the trial area.

5.7.2.3 Trypanosomosis monitoring

The effect of the application of the pour-on on the tsetse population was monitored indirectly by determining the incidence of trypanosomosis in eight sentinel

Figure 5.7.1: Map of the trial area indicating centres, location of sentinel herds, bovine trypanosomosis and tsetse survey results.



herds situated throughout the trial area (Fig. 5.7.1). Each herd consisted of 20, eartagged, adult Angoni cattle. They were kept under traditional village management but not treated with cyfluthrin pour-on. Each month blood collected from each sentinel animal was examined using parasitological diagnostic methods (Section 3.3.2.2). Animals infected with trypanosomes received a curative treatment of diminazene aceturate (Berenil®, Hoechst) by intramuscular injection, at a dose of 7mg/kg body weight for *T. brucei* or 3.5 mg/kg body weight for *T. congolense* or *T. vivax*,.

To evaluate objectively the cattle owner's perception of the effect of the pour-on on animal condition, records were obtained from the Veterinary Offices of the Districts on the sales of diminazene aceturate (Berenil®, Hoechst). Diminazene aceturate sales to cattle owners between January 1999 and June 1999 were compared with the sales during the same period in 1998.

5.7.3 Results

5.7.3.1 Proportion of animals treated

The number of animals treated with the cyfluthrin pour-on, expressed as a proportion of the total number of animals in the trial area, increased throughout the trial. It was low (47%) during the first treatment but increased gradually (Fig. 5.7.2). It was again low (about 30%) during the last two treatments (Fig. 5.7.2).

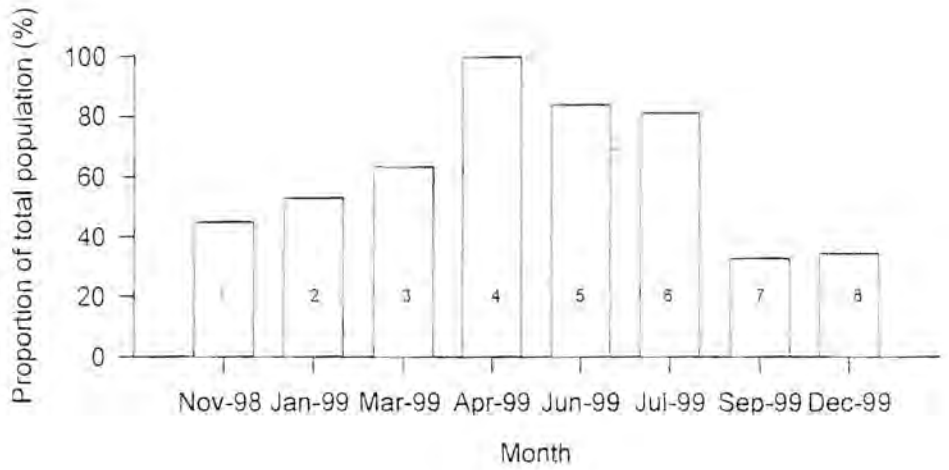


Figure 5.7.2: Proportion of the total cattle population treated with cyfluthrin pour-on during consecutive treatments.

5.7.3.2 Incidence of bovine trypanosomosis

During the first eight months of the trial (November - June), the monthly mean incidence of trypanosomosis in the sentinel herds differed little from the the monthly mean incidence before treatment was initiated (Fig. 5.7.3).

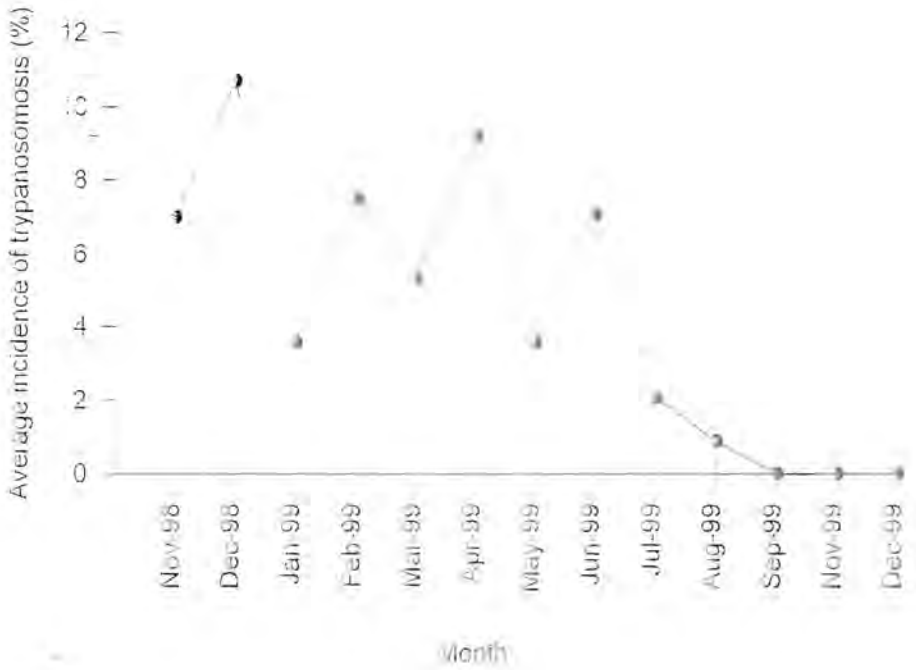


Figure 5.7.3: Monthly average incidence of trypanosomal infections in sentinel herds.

From July onwards, however, the monthly mean incidence started to decline steeply. It reached 0.8% in August. From September onwards no trypanosomal infections were detected.

Between January 1999 and June 1999 a total of 3 738 doses of diminazene aceturate were sold compared to 13 134 doses during the same period in 1998.

5.7.3.3 Packed cell volume

The application of the pour-on resulted in an immediate increase in the average PCV of the sentinel cattle. The average PCV remained relatively high (compared to the PCV values during the months preceding the start of the pour-on application) during treatment period (Fig. 5.7.4). It reached peak values of on average 32.4% between September and December 1999.

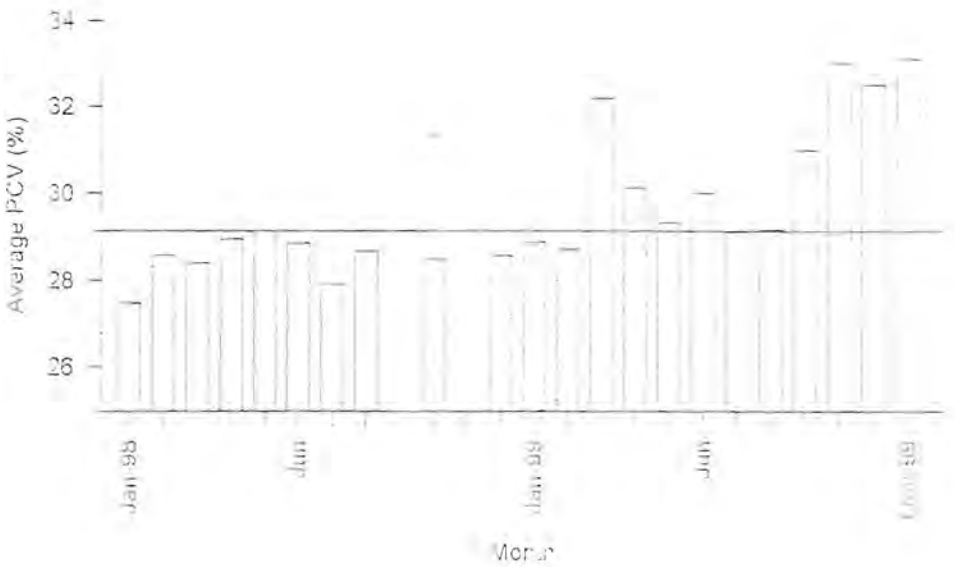


Figure 5.7.4: Monthly average PCV of sentinel cattle before and after the start of the pour-on application.

5.7.4 Discussion

5.7.4.1 Effect of cyfluthrin pour-on on the incidence of nagana

Several studies have shown that regular treatment of cattle with pyrethroid insecticides such as deltamethrin, flumethrin or cypermethrin can significantly reduce the incidence of nagana (e.g. Chizyuka and Luguru, 1986; Bauer *et al.*, 1992; Leak *et al.*, 1995). Results from this trial show that a 1% cyfluthrin pour-on (Cylence® Bayer) applied at 15 ml/100 kg body weight at 7-week intervals also results in a significant reduction in the incidence of bovine trypanosomosis. However, a significant effect on the incidence of bovine trypanosomosis was only observed eight months after the application started (July 1999). Several reasons explain this delayed

effect. First, the proportion of animals treated between November 1998 and March 1999 was substantially lower than the total coverage between March 1999 and July 1999. Despite an intensive extension exercise, the low turn-up is explained by the presence of crops in the fields during the rainy season. This made it difficult for a large proportion of the cattle owners to reach the nearest treatment centre. Second, mud on the skin of treated animals and heavy rain showers may have reduced the availability of the insecticide to tsetse during the rainy season. A second possible reason for the sudden increase in the effectiveness of the pour-on in July is the ecology of tsetse in the trial area. Throughout the trial area, tsetse are highly dependent on cattle as their source of food (Section 2.3.3). Because of this dependence, seasonal changes in the grazing patterns of cattle affect the distribution and abundance of tsetse (Sections 2.2.3 and 2.4.3). Such a sudden change in the grazing pattern of cattle occurs in June/July, when cattle are allowed to roam freely (Section 2.2.4). The abrupt reduction in host availability together with the high proportion of cattle treated with cyfluthrin in June probably contributed significantly to the reduction in the density of the tsetse population and concomitant reduction in the incidence of bovine trypanosomosis.

The high proportion of animals treated during this period seems to have had a severe impact on the tsetse population density. Indeed, despite the low treatment frequency from September onwards no trypanosomal infections were detected in the sentinel cattle.

5.7.4.2 Effect of cyfluthrin pour-on on packed cell volume

Whereas the effect of the application of the pour-on on the incidence of nagana was only seen clearly eight months after the start of the application, the effect on the herd average PCV was observed from four months (March 1999) onwards. Between March 1999 and December 1999, the monthly herd average PCV was either equal to or higher than the maximum monthly average PCV recorded during the period preceding the pour-on treatments. Since the first months of treatment had little effect on the incidence of nagana, the increase in average PCV may be attributed to the effect of the cyfluthrin treatment on ticks and, hence, tick-borne disease challenge.

Babesiosis and anaplasmosis occur in the trial area. Both tick-borne diseases cause anaemia and significantly reduce the PCV (Section 3.3.3, Table 3.3.1). Cyfluthrin applied at the dose rate and treatment interval used in this trial is an effective acaricide. It is, therefore, not surprising that the average PCV of the sentinel cattle increased substantially even during periods of highest tick challenge. This effective control of ticks (including *Rhipicephalus appendiculatus*) contained an East Coast Fever (*Theileria parva parva*) outbreak that occurred during the trial period in Petauke (Lubinga, pers. comm.).

The increase in the herd average PCV is probably the best reflection of the improved condition of cattle in the trial area after cyfluthrin treatments were initiated. An indirect reflection of this improved herd health is the substantial reduction in the sales of diminazene aceturate (Berenil®, Hoechst). The majority of the cattle owners (85.1%) in the trial area use trypanocides (Section 5.2.3.1, Table 5.2.1). In the absence of microscopic diagnosis, treatment was usually given to clinically sick animals or animals in poor condition (Section 5.2.4.2). An improvement in animal condition resulting from a reduction in tsetse and, especially during the first months of the trial, tick challenge may be the most likely explanation for the reduction in diminazene aceturate sales. Similar indirect results have been observed elsewhere with other pyrethroid insecticides (Bauer *et al.*, 1992; Baylis and Stevenson, 1998; Bauer *et al.*, 1999).

CHAPTER SIX

CONCLUSIONS

The formulation of a strategy for the sustainable control of tsetse-transmitted bovine trypanosomosis is a dynamic process in which areas suitable for control are identified, ranked and adjusted over time. The control strategy addresses questions such as *where, how, why, when, by whom,* and *for what benefits and costs* nagana should be controlled. To answer these questions accurately, potential control options are screened by carefully considering socio-economic, technical, environmental and institutional criteria.

The work presented in this thesis aimed at contributing to a framework for the formulation of appropriate strategies for the sustainable localised control of tsetse-transmitted bovine trypanosomosis in southern Africa. Some of the questions mentioned above were addressed, after considering carefully the technical and socio-economic criteria.

A prerequisite for the development of a strategy for the sustainable control of tsetse-transmitted bovine trypanosomosis is accurate knowledge of the distribution of the disease. Usually, the distribution of nagana is established by determining the distribution of cattle with trypanosomal infections. The diagnosis of bovine trypanosomosis is, however, fraught with difficulties. Because of the low diagnostic sensitivity of the commonly used parasitological diagnostic tests, areas where bovine trypanosomosis is present at low prevalence or where it occurs seasonally are often missed. Hence, maps of the parasitological distribution of bovine trypanosomosis often provide an inaccurate basis for the formulation of a control strategy. In Malawi and Zimbabwe, for example, large areas where bovine trypanosomosis occurs would not have been identified if reliance had been placed only on parasitological diagnostic methods (Sections 3.4.3 and 3.6.3). This sensitivity problem could be solved largely by using the recently developed PCR method (Section 1.3.2.3). The test is promising but not yet available for use in large-scale surveys. However, by sampling a sufficiently large part of the population, and by combining parasitological diagnostic methods with indirect tests, a more accurate picture of the distribution and the dynamics of the disease can be obtained that could form a sound basis for strategy formulation (Sections 3.4, 3.5 and 3.6). Such indirect tests detect the animal's immune response to trypanosomal infection, and include the anti-trypanosomal antibody-detection ELISA (antibody-ELISA). The high sensitivity and

specificity of the antibody-ELISA improves accuracy and also, and of equal importance, this ELISA detects anti-trypanosomal antibodies that persist in cattle long after infections have been cured (Section 3.2.3). As a result, areas of low or irregular tsetse challenge can be identified in the absence of the causal agent.

The value of a diagnostic test such as the antibody-ELISA depends on its characteristics. These characteristics can be defined in terms of repeatability, sensitivity and specificity. The antibody-ELISA used in the work presented in this thesis had high repeatability (Section 3.2.3). Furthermore, the test's sensitivity and specificity for anti-*T. congolense* antibodies was high (Sections 3.3.2 and 3.3.3) and non-specific cross reactions with antibodies against *B. bigemina* and *A. marginale* were not found to occur (Section 3.3.5). The test's sensitivity and specificity for antibodies against pathogenic trypanosome species other than *T. congolense* requires further investigation. However, since use has been made of antigens derived from whole trypanosomes the test was probably not species-specific (Section 3.5.3.3). The standardization of technical parameters and continuous quality control are essential steps towards the sustained use of the antibody-ELISA. Standardization could be improved by introducing defined recombinant antigens. The use of representative reference standards as routine quality controls would ensure accurate test results. Failure to standardise and validate the antibody-ELISA may produce incorrect results that will constitute a false basis for the formulation of a strategy for the control of bovine trypanosomosis.

A variety of methods are available to control nagana (Section 1.5). Most of them have been developed with large-scale vector control in mind (Section 1.5.2). On a smaller scale, the suitability of a control method or combination of methods is likely to depend on the local circumstances. In southern Africa, bovine trypanosomosis occurs in areas where cattle are kept inside or adjacent to a tsetse-infested area (Chapter 3). The areas where cattle are kept inside a tsetse-infested zone are generally well-known. The eastern plateau of Zambia is such an example (Chapter 2). Usually, however, tsetse and cattle do not occur in the same area and the distribution of bovine trypanosomosis is restricted to the zone where the tsetse-belt and the cattle grazing

areas overlap (the "cattle/tsetse interface"). In such areas, nagana is an "ecotonal or edge" problem. Three types of cattle/tsetse interface have been identified in southern Africa, *i.e.* (i) areas where cattle are kept immediately adjacent to a tsetse-infested zone, (ii) areas where bovine trypanosomosis is prevalent because of the occasional or seasonal invasion by tsetse into the tsetse-free area, and (iii) areas where bovine trypanosomosis is present because of small, often unidentified, tsetse pockets (Chapter 3).

(i) *Areas where cattle are kept immediately adjacent to a tsetse-infested zone*

This type of cattle/tsetse interface is common in Malawi where the distribution of tsetse is restricted to national parks, game reserves and forest reserves. Bovine trypanosomosis is prevalent in the adjacent, tsetse-free, areas (Section 3.4.3.4). A similar cattle/tsetse interface occurs in areas where the movement of tsetse is restricted because of natural or artificial barriers. In the Eastern Caprivi, for example, the Zambezi River restricts the spread of tsetse from the Sesheke area of Zambia into the Katima Mulilo area. Nevertheless, cattle are still challenged when moving into or close to tsetse-infested areas in Zambia (Section 3.5.3). A similar situation occurs along Zimbabwe's eastern/ northeastern border where an artificial barrier (odour-baited, insecticide-treated, targets) separates the tsetse-belt in Mozambique from the tsetse-cleared areas in Zimbabwe (Section 3.6.2.2).

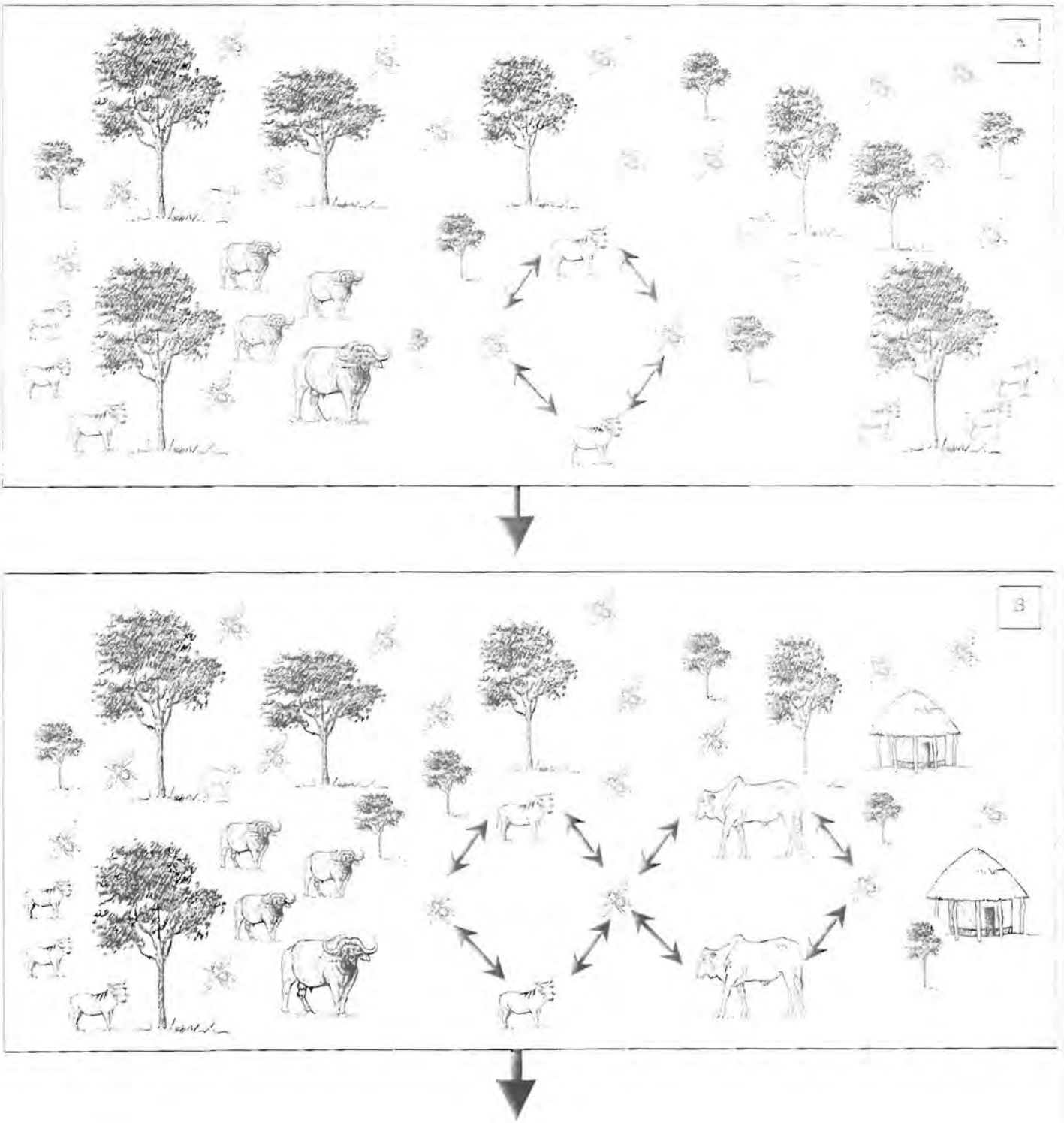
(ii) Areas where bovine trypanosomosis occurs because of occasional or seasonal invasion by tsetse.

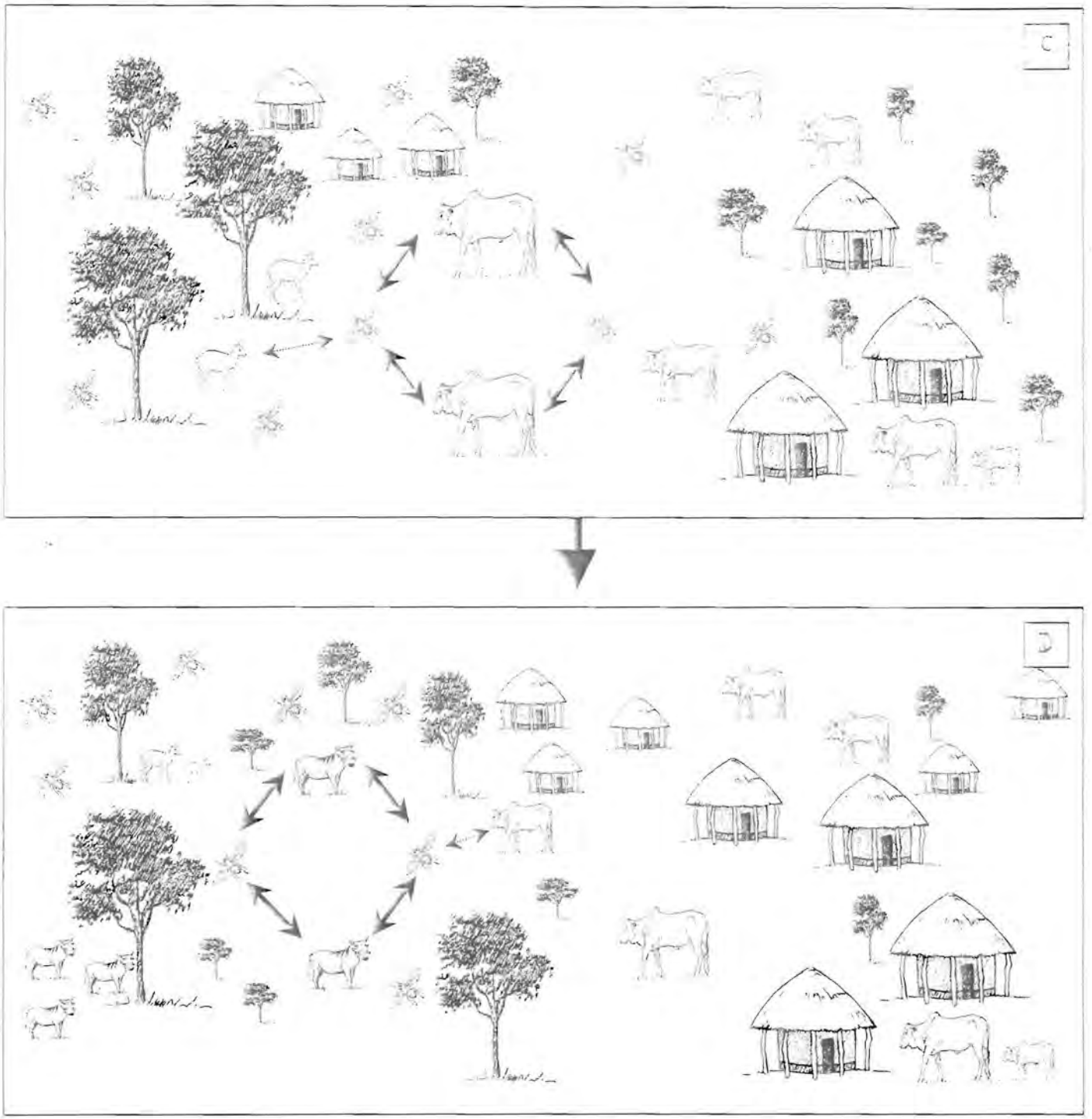
This type of interface is again common in Malawi where bovine trypanosomosis occurs far outside know tsetse foci (Section 3.4.3.4). It is attributed mainly to the seasonal movement of tsetse along rivers such as the South Rukuru River in the Northern Region of the country (Section 3.4.4.2).

(iii) Areas where bovine trypanosomosis is present because of small, often unidentified, tsetse pockets.

This type of cattle/tsetse interface occurs in Malawi where *G. brevipalpis* is present in several small unidentified foci and in western Zimbabwe where small foci of tsetse (probably *G. pallidipes*) have gone undetected for the past 20 years (Sections 3.4.3 and 3.6.3).

Figure 6.1: The consequences of gradual encroachment of people and cattle into a tsetse-infested area on the epidemiology of bovine trypanosomosis.





Cattle kept in a tsetse-infested area

A usual consequence of the introduction of people and cattle into tsetse-infested areas is a reduction of the habitat suitable for tsetse because of the clearing of vegetation for cultivation and a reduction in the density of game animals (Figs. 6.1a-c). Tsetse adapt rapidly to the reduced availability of wild hosts by increasing the proportion of feeds that they take from cattle. Ultimately, the flies become highly dependent on cattle for their survival. In some areas of eastern Zambia, for example, tsetse take 75% of their blood meals from cattle (Section 2.3.3). The high dependence of tsetse on cattle as source of food and the gradual reduction in suitable tsetse habitat have important repercussions (Sections 2.2 and 2.4), which should be taken into consideration when a strategy for the localised control of bovine trypanosomosis is formulated.

Because of the close relationship between cattle and tsetse, bovine trypanosomosis has an endemic character. Its incidence is correlated with the density of the tsetse population (Section 2.5.3.4), which in turn, can be exploited in the timing of prophylactic trypanocidal drug campaigns. Increasing the mortality rate in the tsetse population has two effects on the transmission of nagana. First, increased tsetse mortality will have an immediate and significant effect on the incidence of bovine trypanosomosis (Sections 5.3.3 and 5.7.3). Second, because of the age-dependent prevalence of trypanosomal infections in tsetse, it will reduce the infection rate in the fly population (Section 2.5.3.3).

Probably the most appropriate method of controlling a tsetse population that is highly dependent on cattle as its food source is the treatment of cattle with insecticides. This method was highly effective in eastern Zambia but only after a high proportion of the cattle population was treated, over a large area for 6 consecutive months (Sections 5.4 and 5.7). Stationary baits also were highly effective in controlling tsetse in cultivated areas and seasonal variations in the distribution of cattle and tsetse have been exploited successfully by deploying stationary baits in selected habitats (Section 5.3). Furthermore, the effectiveness of a control operation can be increased if it starts just

before the time that the tsetse population is subject to additional mortality, caused, for example, by the sudden changes in the grazing pattern of cattle.

Cattle kept at the cattle/tsetse interface

In areas where cattle are kept at the edge of tsetse-infested areas (usually game reserves) (Chapter 3) or where cattle are subject to seasonal challenge by tsetse, the importance of cattle as hosts is likely to be minimal. Consequently, cattle are challenged at irregular intervals and the level of challenge is not necessary correlated with the density of the tsetse population. Bovine trypanosomosis, therefore, has an epidemic character at the cattle/tsetse interface. Such nagana epidemics occurred along the Kasungu National Park in Malawi in the mid-1980s, and along Umfolozi Game Reserve in Zululand (South Africa) in 1990. In such situations, control methods should aim at either controlling the tsetse population in the tsetse-infested area or reducing the interaction between tsetse and cattle along the interface. Odour-baited, insecticide-treated targets are effective in controlling tsetse in game areas but are also very effective in reducing the contact between tsetse and cattle at the edge of such tsetse-infested areas. Moreover, they prevent the spread of tsetse from an infested area into a tsetse-free area (Section 3.4.3, 3.5.3 and 3.6.3). The treatment of cattle with insecticides at the edge of a tsetse-infested area is unlikely to have a dramatic effect on the density of tsetse inside the tsetse-infested zone. Furthermore, the insecticide treatments will not protect the animal from tsetse challenge (Section 5.4.3). It is, therefore, an inappropriate method in such situations. The simplest and probably most effective method would be to prevent cattle from grazing in the vicinity of the tsetse-infested area. However, severe pressure for land often makes it impossible to change grazing patterns and to enforce restrictions on livestock movement.

The direct and indirect socio-economic impacts of bovine trypanosomosis on agricultural development are important determinants in the selection of priority areas for the localised control of bovine trypanosomosis. The indirect impacts on agricultural production and farming practices are often difficult to quantify. Furthermore, they vary substantially between areas (Section 4.3.3). The direct impacts, on the other hand, are easier to quantify. In southern Africa, nagana has a

direct impact mainly on adult mortality and calving rates (Section 4.3.3). The degree to which both production variables are affected by the disease is, however, determined by factors such as the innate and acquired immunity to the disease (see below) and stress factors (Section 4.2.3). Trypanocidal drug treatments also may significantly affect the direct impact of nagana on especially mortality rates (Section 4.3.3). Consequently, the socio-economic impacts of bovine trypanosomosis on agricultural development are subject to significant temporal and spatial variations. Since the sustainability of a control intervention will depend largely on the benefits accruing from it, decisions to intervene or not should be based on an objective assessment of the local impacts of the disease.

The high dependence of tsetse on cattle as a source of food also has a repercussion on the impact of bovine trypanosomosis. Several field studies in tsetse-infested areas of Africa (Section 1.4.2.2) have demonstrated that susceptible cattle breeds which survive trypanosomosis because of treatment with trypanocides or because of self-cure are subsequently more resistant to rechallenge with the same serodeme(s). Such immunity is likely to develop in areas where trypanocides are used and where a resident tsetse population feeds almost entirely on cattle. This is the case in some parts of eastern Zambia (Sections 2.3.3 and 5.2.3). Furthermore, the close interaction between tsetse and cattle must have played a role in the natural selection of the indigenous cattle population and the gradual disappearance of highly pathogenic trypanosome strains and trypanosome species such as *T. vivax*. It is, therefore, not surprising that results from the socio-economic surveys conducted in the Petauke District of the Eastern Plateau in Zambia suggest some degree of resistance to trypanosomosis in the resident cattle population (Sections 4.2.3 and 4.3.3).

Because of the irregular challenge and the high proportion of new trypanosome strains in tsetse acquired from game animals, protective immunity to trypanosomal infections is unlikely to develop in cattle along the cattle/tsetse interface. Furthermore, no selection has occurred against highly pathogenic trypanosome strains. The impact of bovine trypanosomosis along such interfaces is, therefore, expected to be higher (Chapter 4). In areas where curative trypanocidal drugs are used, this higher impact

directly affects reproduction in cattle, hence the lower calving rates in cattle kept along the Vwaza Game Reserve in Malawi but also in the Chipangali area of eastern Zambia where cattle are challenged by tsetse from the Kasungu National Park and Lukusuzi Game Reserve (Section 4.3.3.3). In areas where trypanocides are not routinely used, high mortality rates are expected. Such high mortality rates were observed during the nagana epidemics along the edge of the Kasungu National Park and adjacent to the Umfolozi Game Reserve. The significant effects of trypanosomosis on cattle production and the potential for devastating nagana epidemics along the cattle/tsetse interface should be considered when formulating a strategy for the localised control of bovine trypanosomosis.

The level of resistance to trypanosomosis in cattle is an important determinant in strategy formulation. A useful indicator of resistance to trypanosomosis can be obtained by establishing the relationship between herd average PCV and prevalence of trypanosomal infections in an area (Section 4.3).

In many parts of Africa, trypanocidal drug treatments constitute the principal method of controlling bovine trypanosomosis. Despite the availability of various effective vector control methods it is likely that, in the foreseeable future, chemotherapy and chemoprophylaxis will continue to contribute significantly to the control of the disease. Furthermore, trypanocidal drug treatments play a crucial role in the acquisition of immunity in cattle kept in tsetse-infested areas (Sections 4.2.3 and 4.3.3). In areas where resistance in trypanosomes to trypanocidal drugs is absent or present at low prevalence, the sustainability of a drug-based control strategy will depend on the risk of large-scale resistance development. Surveys to investigate the trypanocidal drug-use practices by cattle owners provide a useful baseline to indicate the likelihood of the development and spread of such resistance (Section 5.2.3) and, therefore, should form part of the control strategy formulation process. Furthermore, the results of drug-use surveys provides a good picture of the farmer's attitudes towards control of cattle diseases and the manner in which they spend money on veterinary medicines. This information is important when determining the

appropriateness of a strategy for the control of bovine trypanosomosis, in particular, and animal health in general.

The appropriateness of a tsetse control method will depend on its suitability, transferability and sustainability. The suitability of a control method is a technical question, which involves an assessment of the technical efficiency of a method under a particular situation. The results presented in this thesis indicate that odour-baited targets are suitable in all epidemiological situations. They are effective in clearing tsetse from relatively small areas and are effective barriers against tsetse re-invasion (Sections 5.3.3, 5.6.3, 3.4.3, 3.5.3 and 3.6.3). The use of insecticide-treated cattle, on the other hand, is most effective when it is restricted to areas where the tsetse population is isolated or where reinvasion of flies is low. Furthermore, insecticide treatments of cattle do not provide an effective barrier against the invasion of tsetse in the areas studied here (Section 5.6.3).

The transferability of a tsetse control method is influenced by the willingness and ability of a farmer to adopt a particular technique or combination of techniques. It was beyond the scope of this thesis to study in depth the sociological aspects of trypanosomosis control. However, the results of the drug-use survey clearly showed that cattle owners in southern Africa have a curative approach towards the control of nagana (Section 1.2.4.2). In the absence of widespread trypanocidal drug resistance, transferability of tsetse control methods that are essentially prophylactic measures may be low. The sustainability of a control method involves an assessment of the ability of a farmer or community to sustain the use of a technique or combination of techniques. Of particular importance are the risks associated with unwanted side effects such as the development of resistance in trypanosomes to trypanocidal drugs (Section 5.2.3) or the effect of pour-on treatments on enzootic stability in cattle against some tick-borne diseases (Section 5.5.3). Furthermore, the implementation of tsetse control operations in areas where cattle have developed immunity to the disease is likely to result in the loss of such immunity. Whereas this may not be a problem as long as tsetse control measures are effective, the loss of immunity may have important consequences after the tsetse control operation collapses and a highly susceptible cattle population becomes subject to tsetse challenge.

The formulation of a strategy for the sustainable localised control of bovine trypanosomosis is a dynamic process that has to take into account a wide range of variables that determine the epidemiology of the disease and, hence, its appropriateness in a particular area. Several of the variables have been presented and discussed in this thesis. It is not difficult to imagine that the various epidemiological situations that were identified are part of a logical sequence triggered by a single phenomenon, *i.e.* the encroachment of people and their livestock into tsetse-infested areas. Human encroachment directly affects the population densities of cattle and game animals, the availability of suitable habitat for tsetse. These factors and their consequences for the impact of bovine trypanosomosis determine, to a large extent, the appropriateness of strategies for the sustainable localised control of tsetse-transmitted bovine trypanosomosis in southern Africa (Table 6.1 and Figs. 6.1a-e).

Previously, strategy formulation for *large-scale eradication* of tsetse in southern Africa was dominated by straightforward technical considerations. The most cost effective and technically efficient means of controlling tsetse in an area was emphasised. The success of a control intervention was largely dependent on the availability of manpower, equipment and the organizational capacity of the implementer. Results of this thesis have shown that the planning for the *sustainable localised control* of bovine trypanosomosis is a multidisciplinary exercise that requires a good understanding of the distribution and epidemiology of the disease. This will require the necessary expertise to analyse the local situation and draw conclusions relevant to the formulation of a control strategy. However, it may be facilitated greatly by the use of geographical information systems. The choice of a particular control method will depend largely on the local epidemiological situation. By distinguishing the different epidemiological situations in southern Africa and by analysing their characteristics, appropriate methods to control bovine trypanosomosis have been identified. This thesis has, therefore, contributed to a better understanding of the epidemiology and control of bovine trypanosomosis in southern Africa and made a significant contribution to the development of a framework for the formulation of appropriate strategies for the effective control of tsetse-transmitted bovine trypanosomosis in the southern African region.

Table 6.1: Expected effects of the introduction of cattle and vegetation clearing in a tsetse-infested area on the epidemiology, impact and control of bovine trypanosomosis (- = no impact, + = slight, ++ = intermediate, +++ = great).

Events	Figure	Population density			Host preference		Disease	Suitability of control method[2]		
		Tsetse	Cattle	Game	Game	Cattle	impact	Trypanocides[1]	Targets	Insecticide-treaments
Cattle-free, tsetse-infested area	6.1a	+++	-	+++	+++		-	-	+++	-
Introduction of cattle and people	6.1b	+++	+	++	++	+	+++	++	+++	-
Progressive clearing of vegetation	6.1b	++	++	+	+	++	++	+++	+++	+++
Significant reduction in tsetse habitat	6.1c	+	+++	+	+	+++	+	+++	+++	+++
No habitat left, challenge only at interface	6.1d	+	+++	+	+++	+	+++	+++	+++	-

[1] Trypanocidal drug resistance is not present
[2] Not taking into account transferability and sustainability

CHAPTER SEVEN

REFERENCES

Adlington, D., Randolph, S.E. & Rogers, D.J. (1996) Flying to feed or flying to mate: gender differences in the flight activity of tsetse (*Glossina palpalis*). *Physiological Entomology* **21**. 85-92.

Agyemang, K., Dwinger, R.H., Little, D.A., Laperre, P. & Grieve, A.S. (1992) Interaction between physiological status in N'Dama cows and trypanosome infections and its effect on health and productivity of cattle in The Gambia. *Acta Tropica* **50**. 91-99.

Agyemang, K., Dwinger, R.H., Little, D.A., & Rowlands, G.J. (1997) *Village N'Dama cattle production in West Africa. Six years of research in The Gambia.* International Livestock Research Institute (ILRI)/International Trypanotolerance Centre (ITC), Nairobi. 131 pp.

Akol, G.W. & Murray, M. (1982) Early events following challenge of cattle with tsetse infected with *Trypanosoma congolense*: development of local skin reaction. *Veterinary Record* **110**. 295-302.

Akol, G.W.O. & Murray, M. (1985) Induction of protective immunity in cattle by tsetse-transmitted cloned isolates of *Trypanosoma congolense*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**. 617-627.

Akol, G.W.O., Authié, E., Pinder, M., Moloo, S.K., Roelants, G.E. & Murray, M. (1986) Susceptibility and immune responses of Zebu and Taurine cattle of West Africa to infection with *Trypanosoma congolense* transmitted by *Glossina morsitans centralis*. *Veterinary Immunology and Immunopathology* **11**. 361-373.

Allsopp, R. (1984) Control of tsetse flies (Diptera: Glossinidae) using insecticides: a review and future prospects. *Bulletin of Entomological Research* **74**. 1-23.

Allsopp, R. and Hursey, B.S. (1986) Integrated chemical control of tsetse flies in Western Zimbabwe. *Tsetse and Trypanosomiasis Control Branch*, Harare, 39 pp.

Austen, E.E. (1903) *A monograph of the tsetse-flies (Genus Glossina, Westwood).* Johnson Reprint Corporation, New York. 319 pp.

Authié, E. (1994) Trypanosomiasis and trypanotolerance in cattle: A role for Congopain ? *Parasitology Today* **10**. 360-264.

Authié, E. & Pober, T. (1990) Serum haemolytic and C3 levels in bovine trypanosomiasis under natural conditions of challenge-early indication of individual susceptibility to disease. *Veterinary Parasitology* **35**. 43-59.

Authié, E., Muteti, D.K. & Williams, D.J.L. (1993) Antibody responses to invariant antigens of *Trypanosoma congolense* in cattle of differing susceptibility to trypanosomiasis. *Parasite Immunology* **15**. 101-111.

Bailey, J.W. & Smith, D.H. (1992) The use of the acridine orange QBC technique in the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 630

Baldry, D.A.T. (1980) Local distribution and ecology of *Glossina palpalis* ~~*tachinoides*~~ and *G. tachinoides* in forest foci of west African human trypanosomiasis, with special reference to associations between peri-domestic tsetse and their hosts. *Insect Science and its Application* **1**, 85-93.

Baldry, D.A.T., Taze, Y. & Bushrod, F.M. (1987) Preliminary observations on the feeding habits of *Glossina morsitans centralis* Machado in Mubwa district, Zambia. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 17th meeting, Arusha, pp 419-422.

Barrett, J.C. (1994) *Economic issues in trypanosomiasis control: case studies from Southern Africa.* PhD-thesis, University of Reading, Reading, 485 pp.

Bauer, B., Petrich - Bauer, J. & Pohlit, H. (1988) Effects of flumethrin pour-on against *Glossina palpalis gambiensis*. *Tropical Medicine and Parasitology* **39**, 151-152.

Bauer, B., Meyer, F. & Kabore, I. (1989) Effects of flumethrin pour-on against *Glossina palpalis gambiensis* (Diptera : Glossinidae) during releases in a fly proof stable. *Tropical Medicine and Parasitology* **40**, 478-479.

Bauer, B., Kabore, I. & Petrich-Bauer, J. (1992a) The residual effect of deltamethrin Spoton tested against *Glossina palpalis gambiensis* under fly chamber conditions. *Tropical Medicine and Parasitology* **43**, 38-40.

Bauer, B., Kabore, I., Liebisch, A., Meyer, F., & Petrich-Bauer, J. (1992b) Simultaneous control of ticks and tsetse flies in Satiri, Burkina Faso, by the use of flumethrin pour on for cattle. *Tropical Animal Health and Production* **43**, 41-46.

Bauer, B., Amsler-Delafosse, S., Clausen, P.H., Kabore, I. & Petrich-Bauer, J. (1995) Successful application of deltamethrin pour on to cattle in a campaign against tsetse flies (Glossina spp.) in the pastoral zone of Samarogouan, Burkina Faso. *Tropical Medicine and Parasitology* **46**, 183-188.

Bauer, B., Amsler-Delafosse, S., Kaboré, I., & Kamuanga, M. (1999) Improvement of cattle productivity through rapid alleviation of African Animal Trypanosomosis by integrated disease management practices in the agropastoral zone of Yalé, Burkina Faso. *Tropical Animal Health and Production* **31**, 89-102, 1999.

Bauer, F. (1955) Trypanosomen- und Babesiernerkrankungen in Afrika und ihre Behandlung mit dem neuen Präparat "Berenil". *Zeitschrift für Tropenmedizin und Parasitologie* **6**, 129-140.

Baylis, M. (1996) Effect of defensive behaviour by cattle on the feeding success and nutritional state of the tsetse fly, *Glossina pallidipes* (Diptera: Glossinidae). *Bulletin of Entomological Research* **86**, 329-336.

Baylis, M. (1997) The daily feeding rate of tsetse (Diptera: Glossinidae) on cattle at Galana Ranch, Kenya and comparison with trypanosomiasis incidence. *Acta Tropica* **65**, 81-96.

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

Baylis, M. & Nambiro, C.O. (1993b) The nutritional state of male tsetse flies, *Glossina pallidipes*, at the time of feeding. *Medical and Veterinary Entomology* **7**, 316-322.

Baylis, M., Mbwabi, A.L. & Stevenson, P. (1994) The feeding success of tsetse flies, *Glossina pallidipes* (Diptera: Glossindae), on oxen treated with pyrethroid pour-ons at Galana Ranch, Kenya. *Bulletin of Entomological Research* **84**, 447-452.

Baylis, M. & Mbwabi, A.L. (1995) Feeding behaviour of tsetse flies (*Glossina pallidipes* Austen) on *Trypanosoma*-infected oxen in Kenya. *Parasitology* **110**, 297-305.

Baylis, M. & Stevenson, P. (1998) Trypanosomiasis and tsetse control with insecticidal pour-ons - fact and fiction ? *Parasitology Today* **14**, 77-82.

Bealby, K.A., Connor, R.J., & Rowlands, G.J. (1996) *Trypanosomosis in goats in Zambia*. International Livestock Research Institute (ILRI), Nairobi, 88 pp.

Bevan, E.W. (1928) A method of inoculating cattle against trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **22**, 147-156.

Bingham, M.G., de Rooij, R.C., Flanagan, F.O., Vale Henriques, J.O., James, A.D., Lovemore, D.F., Maclaurin, A.R., Thakersi, H.C.D. & Timberlake, J.R. (1995) *Study of the tsetse fly problem common to Angola, Botswana, Namibia and Zambia*. Report prepared by Zambezi Livestock and Lands Ltd. And RDP Livestock Services for the Regional Tsetse and Trypanosomosis Control Programme, Harare, 174 pp.

Bland, M. (1987) *An introduction to medical statistics*. Oxford University Press, Oxford, 365 pp.

Bocquentin, R., Very, P. & Duvallet, G. (1990) Cinétique des anticorps aprés traitement trypanocide chez des bovins infectés expérimantalement ou naturellement. Intérêt épidémiologique. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **43**, 479-483.

Bosompem, K.M., Masake, R.A., Assoku, R.K.G., Opiyo, E.A. & Nantulya, V.M. (1996) Field evaluation of a dot-ELISA for the detection and differentiation of trypanosome species in infected tsetse flies (*Glossina* spp.). *Parasitology* **112**, 205-211.

Bourn, D. & Scott, M. (1978) The successful use of work oxen in agricultural development of tsetse infested land in Ethiopia. *Tropical Animal Health and Production* **10**, 191-203.

Boyt, W.P. (1967) The veterinary contribution to the control of trypanosomiasis. *Proceedings and Transactions of the Rhodesia Scientific Association* **52**, 16-20.

Boyt, W.P. (1971) Trypanosomiasis control in Rhodesia. *Bulletin Office Internationale Epizooties* **76**, 301-306.

Boyt, W.P. (1978) Food hosts of tsetse. *The Rhodesia Science News* **12**, 141-143.

Boyt, W.P. (1979) Trypanosomiasis in Zimbabwe Rhodesia. *Rhodesia Veterinary Journal* **10**, 54-63.

Boyt, W.P. (1986) *Guide pratique pour le diagnostic, le traitement et la prévention de la trypanosomiase animale Africaine*. Food and Agricultural Organization (FAO), Rome, 281pp.

Boyt W.P., Lovemore D.F., Pilson R.D., & Smith I.D. (1963) A preliminary report on the maintenance of cattle by various drugs in a mixed *G. morsitans* and *G. pallidipes* fly belt. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 9th meeting, Conacry, pp. 71 -79.

Boyt, W.P., Mackenzie, P.K.I. & Ross, C. (1970) An attempt to demonstrate the natural transmission of bovine trypanosomiasis by agents other than *Glossina* in the Sabi Valley of Rhodesia. *Rhodesia Veterinary Journal* **1**, 7-20.

Boyt, W.P., Mackenzie, P.K.I. & Pilson, R.D. (1972) The importance of the donkey (*Equinus asinus*) as a source of food and a reservoir of trypanosomes for *Glossina morsitans* Westw. *The Rhodesia Science News* **6**, 18-20.

Boyt, W.P., Mackenzie, P.K.I. & Pilson, R.D. (1978) The relative attractiveness of donkeys, cattle, sheep and goats to *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen in middle-veld area of Rhodesia. *Bulletin of Entomological Research* **68**, 497-500.

Burnett, G.F. (1954) The effect of poison bait cattle on populations of *Glossina morsitans* Westw. and *G. swynnertoni* Aust. *Bulletin of Entomological Research* **45**, 411-421.

Bursell, E. (1961) The behaviour of tsetse flies (*Glossina swynnertoni* Austen) in relation of sampling. *Proceedings of the Royal Entomological Society London (A)* **36**. 9-20.

Bursell, E. (1966) The nutritional state of tsetse flies from different vegetation types in Rhodesia. *Bulletin of Entomological Research* **57**. 71-180.

Bursell, E. (1970) Dispersal and concentration of *Glossina*. In: H.W. Mulligan (Ed.), *The African Trypanosomiasis*. George Allen and Unwin/Ministry of Overseas Development. London. pp. 382-394.

Bursell, E. (1981) Energetics of haematophagous arthropods: influence of parasites. *Parasitology* **82**. 107-108.

Bursell, E., Billing, K.C., Hargrove, J.W., McCabe, C.T. & Slack, E. (1974) Metabolism of the bloodmeal in tsetse flies. *Acta Tropica* **31**. 297-320.

Bursell, E. & Taylor, P. (1980) An energy budget for Glossina. *Bulletin of Entomological Research* **70**. 187-196.

Burtt, E.D. (1946a) Incubation of tsetse pupae: increased transmission rate of *Trypanosoma rhodesiense* in *Glossina morsitans*. *Annals of Tropical Medicine and Parasitology* **40**. 18-28.

Burtt, E. (1946b) The sex ratio of infected flies found in transmission-experiments with *Glossina morsitans* and *Trypanosoma rhodesiense*. *Annals of Tropical Medicine and Parasitology* **40**. 74-79.

Burtt, E. (1946c) Salivation of *Glossina morsitans morsitans* on to glass slides: a technique for isolating infected flies. *Annals of Tropical Medicine and Parasitology* **40**. 141-143.

Buxton, P.A. (1955) *The natural history of tsetse flies*. H.K. Lewis & Co. Ltd., London. 816 pp.

Camus, E. (1991) Epidemiologie et incidence clinique de la trypanosomose dans le nord de la Côte d'Ivoire. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **34**. 289-295.

Cannon, R.M. & Roe, R.T. (1982) *Livestock disease surveys. A field manual for Veterinarians*. Australian Government Publishing Service, Canberra. 26 pp.

Carmichael, J. & Bell, F.R. (1944) The use of a new phenanthridinium compound 1553 in the treatment of *Trypanosoma congolense* infection in cattle. *Veterinary Record* **56**. 495-496.

Challier, A. (1965) Amélioration de la méthode de détermination de l'âge physiologique des Glossines. Etudes faites sur *Glossina palpalis gambiensis* Vanderplank, 1949. *Bulletin de la Societe de Pathologie Exotique* **58**. 250-259.

Chapman, N.G. (1976) Aerial spraying of tsetse flies (*Glossina* spp.) in Rhodesia with ultra low volumes of endosulfan. *Transactions of the Rhodesian Scientific Association* **57**, 12-21.

Chitambo, H. & Arakawa, A. (1991) Therapeutic effect of Berenil and Samorin in mice infected with four trypanosome populations isolated from Zambian cattle. *Veterinary Parasitology* **39**, 42-52.

Chitambo, H. & Arakawa, A. (1992) *Trypanosoma congolense*: manifestation of resistance to Berenil and Samorin in cloned trypanosomes isolated from Zambian cattle. *Zentralblat für Bakteriologie* **277**, 371-381.

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

Chorley, J.K. (1929) Experiments in grass fires against *Glossina morsitans* in Southern Rhodesia. *Bulletin of Entomological Research* **20**, 377-390.

Clarke, J.E. (1964). Game elimination as a means of tsetse control with special reference to host preference. *The Puku* **2**, 67-75.

Clarke, J.E. (1969) Trypanosome infection rates in the mouthparts of Zambian tsetse flies. *Annals of Tropical Medicine and Parasitology* **63**, 15-34.

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

Claxton, J.R., Leperre, P., Rawlings, P., Snow, W.F. & Dwinger, R.H. (1993) Trypanosomiasis in cattle in the Gambia: incidence, prevalence and tsetse challenge. *Acta Tropica* **50**, 219-225.

Cockbill, G.F. (1967) The history and significance of trypanosomiasis problems in Rhodesia. *Proceedings and Transactions of the Rhodesian Scientific Association* **52**, 7-15.

Cockbill, G.F., Lovemore, D.F. & Phelps, R.J. (1963) The control of tsetse flies (*Glossina*: Diptera, Muscidae) in a heavily infested area of Southern Rhodesia by means of insecticide discharged from aircraft, followed by settlement of indigenous people. *Bulletin of Entomological Research* **54**, 93-106.

Cockbill, G.F., Pilson, R.D. & Vale, G.A. (1969) Observations on populations of *Glossina morsitans* and of game animals on island 173/174 Lake Kariba, Rhodesia. *Bulletin of Entomological Research* **85**, 495-500.

Connor, R.J. (1989) *Final report of the Regional Trypanosomiasis Expert*. Regional Tsetse and Trypanosomiasis Control Programme (RTTCP), Harare, 123 pp.

Connor, R.J. (1994a) The impact of nagana. *Onderstepoort Journal of Veterinary Research* **61**, 379-383.

Connor, R.J. (1994b) African animal trypanosomiases. In: J.A.W. Coetzer, G.R. Thomson, and R.C. Tustin (Eds.), *Infectious diseases of livestock with special reference to southern Africa*, Oxford University Press, Cape Town, pp. 166-203.

Connor, R.J. & Halliwell, R.W. (1987) Bovine trypanosomiasis in southern Tanzania: parasitological and serological survey of prevalence. *Tropical Animal Health and Production* **19**, 165-172.

Croft, S.L., East, J.S. & Molyneux, D.H. (1982) Anti-trypanosomal factor in the haemolymph of *Glossina*. *Acta Tropica* **39**, 293-302.

Crump, A.J. & Brady, J. (1979) Circadian activity patterns in three species of tsetse fly: *Glossina palpalis jaunsteni* and *morsitans*. *Physiological Entomology* **4**, 311-318.

Cuisance, D. & Itard, J. (1973) Comportement de mâles stériles de *Glossina tachinoides* Westw. lâchés dans les conditions naturelles - environs de Fort-Lamy (Tchad). I. Transport, lâchers, rythme d'activité, action sur la population sauvage. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **26**, 55-76.

Dagnogo, M., Lohuirignon, K. & Gouteux, J.P. (1985) Comportement alimentaire des populations péridomestiques de *Glossina palpalis* (Robineau-Desvoidy) et de *Glossina tachinoides* Westwood du domaine guinéen de Côte d'Ivoire. *Cahier ORSTOM Série Entomologie Médicale et Parasitologie* **23**, 3-8.

Dale, C., Welburn, S.C., Maudlin, I. & Milligan, P.J.M. (1995) The kinetics of maturation of trypanosome infections in tsetse. *Parasitology* **111**, 187-191.

D'Amico, F., Gouteux, J.P., Le Gall, F. & Cuisance, D. (1996) Are stable flies (Diptera: Stomoxynae) vectors of *Trypanosoma vivax* in the Central African Republic? *Veterinary Research* **27**, 161-170.

Dargie, J.D. (1978) *Erythropoietic response in bovine trypanosomiasis*. International Development Research Centre, Ottawa, pp. 128 -134.

Dargie, J.D., Murray, P.K., Max Murray, Grimshaw, W.R.I. & McIntyre, W.I.M. (1979) Bovine trypanosomiasis: the red cell kinetics of N'Dama and Zebu cattle infected with *Trypanosoma congolense*. *Parasitology* **78**, 271-286.

Davey, D.G. (1950) Experiments with "Antrycide" in the Sudan and East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **43**, 583-616.

Davies, J.E. (1980) *The history of tsetse fly control in Botswana*. Department of Veterinary Services, Maun.

Davies, C.E. (1982) Thrombocytopenia: a uniform complication of African trypanosomiasis. *Acta Tropica* **39**, 123-133.

Davies, H. (1977) *Tsetse flies in Nigeria*. Oxford University Press, Ibadan, 186 pp.

Davison, G. (1990) *National tsetse and trypanosomiasis survey 1987-1989*. Ministry of Agriculture, Lilongwe, 60 pp.

Davison, G. (1996) *Terminal report of the technical assistance officer, RTTCP, Zimbabwe*. Regional Tsetse and Trypanosomiasis Control Programme (RTTCP), Harare, 20 pp.

Dean, G.J.W., Paget, J. & Wilson, F. (1969) Observations on the behaviour of tsetse flies (*Glossina morsitans orientalis* Vanderplank and *Glossina pallidipes* Austen) during an attempt to concentrate breeding around cattle. *Journal of Applied Ecology* **6**, 13-26.

De Clercq, K. (1997) *Feeding evaluation of the Angoni cattle during the late dry season in Chipata (Zambia)*. MSc thesis, University Ghent, Ghent, 143 pp.

Desquesnes, M. (1997) Evaluation of a simple PCR technique for the diagnosis of *Trypanosoma vivax* infection in the serum of cattle in comparison to parasitological techniques and antigen-enzyme-linked-immunosorbent assay. *Acta Tropica* **65**, 139-148.

Desquesnes, M. & Tresse, L. (1996) Evaluation de la sensibilité du test de Woo pour la détection de *Trypanosoma vivax*. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **49**, 315-321.

de Vos, A.J., & Potgieter, F.T. (1994) Bovine babesiosis. In: J.A.W. Coetzer, G.R. Thomson, and R.C. Tustin (Eds.), *Infectious diseases of livestock with special reference to southern Africa*, Oxford University Press, Cape Town, pp. 278-294.

Diarra, B., Diall, O., Geerts, S., Kageruka, P., Lemmouchi, Y., Schacht, E., Eisler, M.C. & Holmes, P. (1998) Field evaluation of the prophylactic effect of an isometamidium sustained-release device against trypanosomiasis in cattle. *Antimicrobial Agents and Chemotherapy* **42**, 1012-1014.

d'Ieteren, G.M.D., Authié, E., Wissocq, N., & Murray, M. (1998) Trypanotolerance, an option for sustainable livestock production in areas at risk of trypanosomosis. *Revue Scientifique technique de l'office International des Epizooties* **17**, 154-175.

Distelmans, W., D'haeseleer, F., Kaufman, L. & Rousseeuw, P. (1982) The susceptibility of *Glossina palpalis palpalis* at different ages to infection with *Trypanosoma congolense*. *Annales de la Société Belge de Médicine Tropicale* **62**, 41-47.

Dolan, R.B. (1998) *The Orma Boran: a trypanotolerant East African breed. Fifteen years of research on Galana Ranch in Kenya.* Kenya Trypanosomiasis Research Institute (KETRI), Nairobi. 88 pp.

Dolan, R.B., Okech, G., Alashula, H., Mutugi, M., Stevenson, P., Sayer, P.D. & Njogu, A.R. (1990) Homidium bromide as a chemoprophylactic for cattle trypanosomiasis in Kenya. *Acta Tropica* **47**, 137-144.

Dolan, R.B., Stevenson, P.G.W., Alushula, H. & Okech, G. (1992) Failure of chemoprophylaxis against bovine trypanosmiasis on Galana Ranch in Kenya. *Acta Tropica* **51**, 113-121.

Doran, M.H., Low, A.R.C. & Kemp, R.L. (1979) Cattle as a store of wealth in Swaziland: implications for livestock development and overgrazing in eastern and southern Africa. *Amercian Journal of Agricultural Economics* **61**, 41-47.

Du Toit, R. (1954) Trypanosomiasis in Zululand and the control of tsetse flies by chemical means. *Onderstepoort Journal of Veterinary Research* **26**, 317-387.

Du Toit, R., Kluge, E.B., & Fiedler, D.G.H. (1954) The eradication of *Glossina pallidipes* from Zululand by chemical means. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 5th meeting, Leopoldville, p. 141.

Dwinger, R.H., Grieve, A.S., Jeannin, P, Agyemang, K. & Faye, J. (1988a) Anti-trypanosomal antibodies in sequentially collected sera of N'Dama cattle under natural trypanosomiasis risk in The Gambia. In: *Livestock production in tsetse affected areas of Africa*, International Livestock Centre for Africa (ILCA), Addis Ababa, pp. 100-108.

Dwinger, R.H., Rudin, W., Moloo, S.K. & Murray, M. (1988b) Development of *Trypanosoma congolense*, *T. vivax* and *T. brucei* in the skin reaction induced in goats by infected *Glossina morsitans centralis*. *Research in Veterinary Science* **44**, 154-163.

Dwinger, R.H., Grieve, A.S., Snow, F.W., Rawlings, P., Jabang, B. & Williams, D.J.L. (1992) Maternal antibodies in N'Dama cattle kept under trypanosomiasis risk in the Gambia. *Parasite Immunology* **14**, 351-354.

Echessah, P.N., Swallow, B.M., Kamara, D.W. & Curry, J.J. (1996) Willingness to contribute labor and money to tsetse control: application of contingent valuation in Busia District, Kenya. *World Development* **25**, 1-15.

Edwards, E.E., Judd, J.M. & Squire, F.A. (1956) Observation on trypanosomes in domestic animals in West Africa. I. The daily index of infection and the weekly haematological values in goats and sheep infected with *Trypanosoma vivax*, *T. congolense* and *T. brucei*. *Annals of Tropical Medicine and Parasitology* **50**, 223-241.

Eisler, M.C., Elliott, C.T. & Holmes, P.H. (1996) A simple competitive enzyme immunoassay for the detection of the trypanocidal drug isometamidium. *Therapeutic Drug Monitoring* **18**, 73-79.

Elce, B.J. (1971) The transmission of *Trypanosoma congolense* through *Glossina morsitans* and the white mouse. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 239.

Evison, C. (1980) *Development of tsetse control in Zambia, 1942 to 1979 and its relation to animal trypanosomiasis control within the Third National Development Plan. I. Review of developments and current status of tsetse control.* United Nations Development Programme (UNDP)/Food and Agriculture Organization (FAO) Project RAF 75/001, Lusaka, 11 pp.

Evison, C. & Kathuria, K.D.S. (1984) *A review of fixed wing aerial spraying against* Glossina morsitans *in Zambia during the period 1968-1978.* Ministry of Agriculture and Water Development, Lusaka, 15 pp.

Fiennes, R.N.T.W. (1953) The therapeutic and prophylactic properties of antryeide in trypanosomiasis in cattle. *British Veterinary Journal* **109**, 280-295.

Fiennes, R.N.T.W. (1970) Pathogenesis and pathology of animal trypanosomiasis. In: H.W. Mulligan (Ed.), *The African Trypanosomiases*. George Allen and Unwin/Ministry of Overseas Development, London, pp. 729-750.

Finelle, P. (1974) African animal trypanosomiasis. Part IV. Economic problems. *World Animal Review* **10**, 15-18.

Flint, S. (1985) A comparison of various traps for *Glossina* spp. (Glossinidae) and other Diptera. *Bulletin of Entomological Research* **75**, 529-534.

Foil, L.D. (1989) Tabanids as vectors of disease agents. *Parasitology Today* **5**, 88-96.

Ford, J. (1960) Feeding and other responses of tsetse flies to man and ox and their epidemiological significance. *Acta Tropica* **26**, 249-294.

Ford, J., Glasgow, J.P., Johns, D.L., & Welch, J.R. (1959) Transect flyrounds in the field studies of *Glossina*. *Bulletin of Entomological Research* **50**, 275-285.

Ford, J. & Leggate, B.M. (1961) The geographical and climatic distribution of trypanosome infection rates in *Glossina morsitans* group of tsetse-flies. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **55**, 383-397.

Ford, J. & Blaser, E. (1971) Some aspects of cattle raising under prophylactic treatment against trypanosomiasis on Mkwaja ranch, Tanzania. *Acta Tropica* **28**, 69-80.

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

Frame, I.A., Ross, C.A. & Luckins, A.G. (1990) Characterization of *Trypanosoma congolense* Serodemes in Stocks Isolated from Chipata District, Zambia. *Parasitology* **101**, 235-241.

Fuller, C. & Mossop, M.C. (1929) Entomological notes on *Glossina pallidipes*. *Union of South Africa, Department of Agriculture, Science Bulletin No. 67*.

Gardiner, P.R. (1989) Recent studies in the biology of *Trypanosoma vivax*. *Advances in Parasitology* 229-316.

Geerts, S. & Holmes, P.H. (1998) *Drug management and parasite resistance in bovine trypanosomiasis in Africa*. Food and Agriculture Organization (FAO), Rome, 19 pp.

Gibson, W. (1994) Identification of trypanosomes in animals, humans and *Glossina*. *Bulletin de la Societe de Pathologie Exotique* **87**, 315-318.

Gidudu, A.M., Cuisance, D., Reifenberg, J.M. & Frézil, J.L. (1995) Amélioration de la technique de salivation des glossines pour la détection des métatrypanosomes infectants: étude de quelques facteurs biologique et non biologique sur le comportement de sondage des glossines. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **48**, 153-160.

Gingrich, J.B., Macken, L.M., Jackson, P.R. & Roberts, D.R. (1985) *Trypanosoma brucei*: enhancement of infection rates in the tsetse fly, *Glossina morsitans* by feeding artificial bloodmeal mixtures. *American Journal of Tropical Medicine and Hygiene* **34**, 73-77.

Glasgow, J.P. (1961) The feeding habits of *Glossina swynnertoni* Austen. *Journal of Animal Ecology* **30**, 77-85.

Gledhill JA & Caughey W. (1963) Report on a field trial in the use of Dieldrin for the control of *Glossina morsitans* in the Zambesi Valley - 1961. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 9th meeting, Conacry, pp. 239.

Glover, P.E. (1961) *The tsetse problem in northern Nigeria*. Patwa News Agency Ltd., Nairobi. 383 pp.

Glover, P.E., Jackson, C.H.N., Robertson, A.G., & Thomson, W.E.F. (1955) The extermination of the tsetse fly *Glossina morsitans* Westw. at Abercorn, Northern Rhodesia. *Bulletin of Entomological Research* **46**, 57-67.

Godfrey, D.G. (1961) Types of *Trypanosoma congolense*. II Differences in the courses of infection. *Annals of Tropical Medicine and Parasitology* **55**, 154-166.

Gombe, S. (1989) Endocrine effects of trypanosomiasis: recent studies. *Discovery and Innovation* **1**, 30-33.

Gouteux, J.P., D'-Amico, F., Cuisance, D., Blanc, F., Demba, D., Staak, C., Clausen, P.H., Kota-Guinza, A., Gall, F., le, Le-Gall, F. & Guinza, A., Kota-. (1994) Feeding behaviour of *Glossina fuscipes fuscipes* Newstead, 1910 (Diptera: Glossinidae) in 2 cattle breeding areas of the Central African Republic. *Veterinary Research* **25**, 16-28.

Gouteux, J.P., Le Gall, F., Guillerme, J.M. & Demba, D. (1996) Traitement épicutané (Pour on et Spot on) du bétail contre *Glossina fuscipes* en République Centrafricaine. *Veterinary Research* **27**, 273-284.

Gray, A.R. & Luckins, A.G. (1980) The initial stage of infection with cyclically transmitted *Trypanosoma congolense* infection in rabbits, calves and sheep. *Journal of Comparative Pathology* **90**, 499-512.

Green, C.H. (1994) Bait methods for tsetse fly control. *Advances in Parasitology* **34**, 229-291.

Greiner, M., Bhat, T.S., Patzelt, R.J., Kakaire, D., Schares, G., Dietz, E., Böhning, D., Zessin, K.H. & Mehlitz, D. (1997a) Impact of biological factors on the interpretation of bovine trypanosomosis serology. *Preventive Veterinary Medicine* **30**, 61-73.

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

Griffin, L., Allonby, E.W. & Preston, J.W. (1981) The interaction of *Trypanosoma congolense* and *Haemonchus contortus* infections in two breeds of goats. I. Parasitology. *Journal of Comparative Pathology* **91**, 85-95.

Habtemariam, T., Ruppanner, R., Riemann, H.P. & Theis, J.H. (1983a) An epidemiologic systems analysis model for African trypanosomiasis. *Preventive Veterinary Medicine* **1**, 125-136.

Habtemariam, T., Ruppanner, R., Riemann, P. & Theis, J.H. (1983b) Epidemic and endemic characteristics of trypanosomiasis in cattle : a simulation model. *Preventive Veterinary Medicine* **1**, 137-145.

Hall, P.E. (1910) Notes on the movements of *Glossina morsitans* in the Lundazi district, North Eastern Rhodesia. *Bulletin of Entomological Research* **1**, 183-184.

Hall, M.J.R., Kheir, S.M., Rahman, A.H.A. & Noga, S. (1983) Tsetse and trypanosomiasis survey of Southern Darfur Province, Sudan. I. Bovine trypanosomiasis. *Tropical Animal Health and Production* **15**, 191-206.

Hall, D.R., Beevor, P.S., Cork, A., Nesbitt, B.F. & Vale, G.A. (1984) 1-Octen-3-ol: a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Science and its Application* **5**, 335-339.

Hargrove, J.W. (1976) The effect of human presence on the behaviour of tsetse near a stationary ox. *Bulletin of Entomological Research* **66**, 173-178.

Hargrove, J.W. (1980a) The effect of model size and ox odour on the alighting response of *Glossina morsitans* Westwood and *G. pallidipes* Austen (Diptera: Glossinidae). *Bulletin of Entomological Research* **70**, 229-234.

Hargrove, J.W. (1980b) Improved estimates of the efficiency of traps for *Glossina morsitans morsitans* and *Glossina pallidipes*, with a note on the effect of concentration of accompanying host odour on efficiency. *Bulletin of Entomological Research* **70**, 579-587.

Hargrove, J.W. (1991) Ovarian ages of tsetse flies (Diptera: Glossinidae) caught of mobile and stationary baits in the presence and absence of humans. *Bulletin of Entomological Research* **81**, 43-50.

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

Hargrove, J.W., & Vale, G.A. (1978) The effect of host odour concentration on catches of tsetse flies (Glossinidae) and other Diptera in the field. *Bulletin of Entomological Research* **68**, 607-612.

Hargrove, J.W. & Vale, G.A. (1980) Catches of *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen (Diptera: Glossinidae) in odour baited traps in riverine and deciduous woodlands in the Zambesi valley of Zimbabwe. *Bulletin of Entomological Research* **70**, 571-578.

Hargrove, J.W. & Langley, P.A. (1990) Sterilizing Tsetse (Diptera, Glossinidae) in the Field - A Successful Trial. *Bulletin of Entomological Research* **80**, 397-403.

Hargrove, J.W. & Packer, M.J. (1993) Nutritional status of male tsetse flies (*Glossina* spp.) (Diptera: Glossinidae) caught in odour-baited traps and artificial refuges: models for feeding and digestion. *Bulletin of Entomological Research* **83**, 29-46.

Hargrove, J.W., Holloway, M.T.P., Vale, G.A., Gough, A.J.E. & Hall, D.R. (1995) Catches of tsetse (*Glossina* spp.) (Diptera: Glossinidae) from traps and targets baited with large doses of natural and synthetic host odour. *Bulletin of Entomological Research* **85**, 215-227.

Harley, J.M.B. (1965) Activity of *Glossina pallidipes* Aust., *G. palpalis fuscipes* Newst. and *G. brevipalpis* Newst. *Bulletin of Entomological Research* **56**, 141 – 160.

Harley, J.M.B. (1966) Studies on age and infection rate of *Glossina pallidipes* Austen, *G. palpalis fuscipes* Newst. and *G. brevipalpis* Newst. in Uganda. *Bulletin of Entomological Research* **57**, 23-37.

Harley, J.M.B. (1971a) Comparison of the susceptibility to infection with *Trypanosoma rhodesiense* of *Glossina pallidipes*, *G. morsitans*, *G. fuscipes* and *G. brevipalpis*. *Annals of Tropical Medicine and Parasitology* **65**, 185-189.

Harley, J.M.B. (1971b) The influence of the age of the fly at the time of the infecting feed on infecting *Glossina fuscipes* with *Trypanosoma rhodesiense*. *Annals of Tropical Medicine and Parasitology* **65**, 191-196.

Harley, J.M.B. & Wilson, A.J. (1968) Comparison between *Glossina morsitans*, *G. pallidipes*, and *G. fuscipes*, as vectors of trypanosomes of the *Trypanosoma congolense* group: the proportions infected experimentally and the number of infective organisms extruded during feeding. *Annals of Tropical Medicine and Parasitology* **62**, 178-187.

Hoare, C.A. (1970) Systematic description of the mammalian trypanosomes of Africa. In: H.W. Mulligan (Ed.), *The African Trypanosomiasis*, George Allen and Unwin/Ministry of Overseas Development, London, pp. 24-59.

Holloway, M.T.P. (1989) Alternatives to DDT for use in ground spraying control operations against tsetse flies (Diptera: Glossinidae). *Transactions of the Zimbabwe Scientific Association* **64**, 33-40.

Holmes, P.H., Mammo, E., Thomson, A., Knight, P.A., Lucken, R., Murray, P.K., Murray, M., Jennings, F.W. & Urquhart, G.M. (1974) Immunosuppression in bovine trypanosomiasis. *The Veterinary Record* **95**, 86-87.

Hopkins, J.S. (1997) *Epidemiological investigations of bovine trypanosomosis in the common fly belt of Zambia*. Thesis presented for the degree of Doctor of Veterinary Medicine and Surgery, University of Edinburgh, Edinburgh, 224 pp.

Hopkins, J.S., Chitambo, H., Machila, N., Luckins, A.G., Rae, P.F., Van den Bossche, P. & Eisler, M.C. (1998) Adaptation and validation of the antibody trapping ELISA using dried blood spots on filter paper, for epidemiological surveys of tsetse transmitted trypanosomosis in cattle. *Preventive Veterinary Medicine* **37**, 91-99.

Hornby, H.E. (1941) Immunisation against bovine trypanosomosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **35**, 165-176.

Hursey, B.S. (1998) Livestock and human needs: conflict or compatibility ? *World Animal Review* **90**, 1.

Hursey, B.S., & Allsopp, R. (1984) *The eradication of tsetse flies in Western Zimbabwe by integrated aerial and ground spraying*. Tsetse and Trypanosomiasis Control Branch (TTCB), Harare, 19 pp.

Ibrahim, E.A.R., Ingram, G.A. & Molyneux, D.H. (1984) Haemagglutinins and parasite agglutinins in haemolymph and gut of *Glossina*. *Tropical Medicine and Parasitology* **35**, 151-156.

Ikede, B.O., Elhassan, E. & Akpavie, S.O. (1988) Reproductive disorders in African trypanosomiasis: a review. *Acta Tropica* **45**, 5-10.

Ilemobade, A.A., Adegboye, D.S., Onoviran, O. & Chima, J.C. (1982) Immunodepressive effects of trypanosomal infection in cattle immunized against contagious bovine pleuropneumonia. *Parasite Immunology* **4**, 273-282.

Imbuga, M.O., Osir, E.O., Labongo, V.L., Darji, N. & Otieno, L.H. (1992a) Studies on tsetse midgut factors that induce differentiation of bloodstream *Trypanosoma brucei brucei in vitro*. *Parasitology Research* **78**, 10-15.

Imbuga, M.O., Osir, E.O. & Labongo, V.L. (1992b) Inhibitory effect of *Trypanosoma brucei brucei* on *Glossina morsitans* midgut trypsin *in vitro*. *Parasitology Research* **78**, 273-276.

International Livestock Centre for Africa (ILCA) (1988) *Livestock production in tsetse affected areas of Africa*. English Press, Nairobi, 473 pp.

International Livestock Centre for Africa (ILCA) (1990) *Livestock systems research manual. Volume 1. ILCA working paper no 1.* ILCA, Addis Ababa, 25 pp.

Jack, R.W. (1914) Tsetse fly and big game in Southern Rhodesia. *Bulletin of Entomological Research* **5**, 97-110.

Jackson, C.H.N. (1933) The causes and implications of hunger in tsetse-flies. *Bulletin of Entomological Research* **24**, 443-482.

Jackson, C.H.N. (1946) An artificially isolated generation of tsetse flies. *Bulletin of Entomological Research* **37**, 291-299.

Jackson, C.H.N. (1954) The hunger cycle of *Glossina morsitans* Westwood and *Glossina swynnertoni* Austen. *Journal of Animal Ecology* **23**, 368-372.

Jackson, P.R., Honigberg, B.M. & Holt, S.C. (1978) Lectin analysis of *Trypanosoma congolense* bloodstream trypomastigote and culture procyclic surface saccarides by agglutination and electron microscopic technics. *Journal of Protozoology* **25**, 471-481.

Jackson, P.R. & Diggs, C.L. (1983) *Trypanosoma rhodesiense* bloodstream trypomastigotes and culture procyclic cell surface carbohydrates. *Journal of Protozoology* **30**, 662-668.

Jefferies, D., Helfrich, M.P. & Molyneux, D.H. (1987) Cibarial infection of *Trypanosoma vivax* and *T. congolense* in *Glossina*. *Parasitology Research* **73**, 289-292.

Jemal, A. & Hugh-Jones, M.E. (1995) Association of tsetse control with health and productivity of cattle in the Didessa Valley, western Ethiopia. *Preventive Veterinary Medicine* **22**, 29-40.

Jenni, L., Marti, S., Schweizer, J., Betschart, B., Le Page, R.W.F., Wells, J.M., Tait, A., Paindavoine, P., Pays, E. & Steinert, M. (1986) Hybrid formation between African trypanosomes during cyclical transmission. *Nature* **322**, 173-175.

Jordan, A.M. (1963) The host of *Glossina* as the main factor affecting trypanosome infection rates of tsetse flies in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **59**, 423-431.

Jordan, A.M. (1965) Bovine trypanosomiasis in Nigeria. V. The tsetse-fly challenge to a herd of cattle trekked along a trade-cattle route. *Annals of Tropical Medicine and Parasitology* **59**, 270-276.

Jordan, A.M. (1974) Recent developments in the ecology and methods of control of tsetse flies. A review. *Bulletin of Entomological Research* **63**, 361-399.

Jordan, A.M. (1976) Tsetse flies as vectors of trypanosomes. *Veterinary Parasitology* **2**, 143-152.

Jordan, A.M. (1985) Tsetse eradication plans for Southern Africa. *Parasitology Today* **1**, 121-123.

Jordan, A.M. (1986) *Trypanosomiasis control and African rural development.* Longman. London. 357 pp.

Joshua, R.A., Obwolo, M.J., Bwangamoi, O. & Mandebvu, E. (1995) Resistance to diminazene aceturate by *Trypanosoma congolense* from cattle in the Zambezi Valley of Zimbabwe. *Veterinary Parasitology* **60**, 1-6.

Kappmeier, K., Nevill, E.M. & Bagnall, R.J. (1998) Review of tsetse and trypanosomosis in South Africa. *Onderstepoort Journal of Veterinary Research* **65**, 195-203.

Katakura, K., Lubinga, C., Chitambo, H. & Trada, Y. (1997) Detection of *T. congolense* and *T. brucei* subspecies in cattle in Zambia by polymerase chain reaction from blood collected on a filter paper. *Parasitology Research* **83**, 241-245.

Katende, J.M., Musoke, A.J., Nantulya, V.M. & Goddeeris, B.M. (1987) A new method for fixation and preservation of trypanosomal antigens for use in the indirect immunoflourescence antibody test for diagnosis of bovine trypanosomiasis. *Tropical Medicine and Parasitology* **38**, 41-44.

Katunguka-Rwakishaya, E., Parkins, J.J., Fishwick, G., Murray, M. & Holmes, P.H. (1995) The influence of energy intake on the pathophysiology of *Trypanosoma congolense* infection in Scottish Blackface sheep. *Veterinary Parasitology* **59**, 207-218.

Kazadi, J.M., Van Hees, J., Jochems, M. & Kageruka, P. (1991) Etude de la capacité vectorielle de *Glossina palpalis gambiensis* (Bobo-Dioulasso) vis-á-vis de *Trypanosoma brucei brucei* EATRO 1125. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **44**, 437-442.

Kazadi, J.M., Jochems, M., Kabore, H., Mbeng, C., Van Hees, J. & Kageruka, P. (1995) Standardisation et évaluation de la technique de salivation manuelle pour le dépistage des infections par trypanosomes chez la glossine (Diptera: Glossinidae). *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **48**, 171-175.

Kazadi, J.M., Losson, B., and Kageruka, P. (1998) Développement biologique de *Trypanosoma (Nannomonas) congolense* IL 1180 chez *Glossina morsitans morsitans* Westwood 1851 (Diptera: Glossinidae). *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **51**, 219-224.

Kelley, S. & Schillinger, D. (1983) Improved field diagnostic technique for trypanosomiasis by use of a mini-centrifuge. *The Veterinary Record* **113**, 219

Kettle, D.S. (1984) *Medical and veterinary entomology*. Croom Helm, London & Sydney, 658 pp.

Killick-Kendrick, R. (1968) The diagnosis of trypanosomiasis in livestock: a review of current techniques. *The Veterinary Bulletin* **38**, 191-197.

Knols, B.G.J., Willemse, L., Flint, S. & Mate, A. (1993) A trial to control the tsetse fly, *Glossina morsitans centralis*, with low densities of odour-baited targets in west Zambia. *Medical and Veterinary Entomology* 7, 161-169.

Komoin-Oka, C., Truc, P., Bengaly, Z., Formenty, P., Duvallet, G., Lauginie, F., Raath, J.P., N'Depo, A.E. & Leforban, Y. (1994) Etude de la prévalence des infections à trypanosomes chez différent espèces d'animaux sauvages du parc national de la Comoé en Côte d'Ivoire: résultats préliminaires sur la comparison de trois méthodes de diagnostic. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **47**, 189-194.

Kratzer, R.D. & Ondiek F.O. (1989) The buffy coat double centrifugation technique, an improved method for the diagnosis of African trypanosomiasis. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 20th meeting, Mombasa.

Küpper, W., Eibl, F., Van Elsen, A.C. & Clair, M. (1982) The use of the biconical Challier - Laveissière trap impregnated with deltamethrin against Glossina. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **35**, 157-163.

Küpper, W., Staak, C., Kröber, T. & Späth, J. (1990) Natural hosts of *Glossina tachinoides* in northern Côte d'Ivoire. *Tropical Medicine and Parasitology* **41**, 217-218.

Kyorku, C. & Brady, J. (1994) A free-running bimodal circadian rhythm in the tsetse fly *Glossina longipennis*. *Journal of Insect Physiology* **40**, 63-67.

Lamborn, W.A. (1915) Second report on *Glossina* investigations in Nyasaland. *Bulletin of Entomological Research* **6**, 249-265.

Lambrecht, F.L. (1980) Ecological and physiological factors in the cyclic transmission of African trypanosomiasis. *Insect Science and its Application* **1**, 47-54.

Langley, P.A. & Wall, R. (1990) The implications of hunger in the tsetse fly, *Glossina pallidipes*, in relation to its availability to trapping techniques. *Journal of Insect Physiology* **36**, 903-908.

Lanham, S.M. & Godfrey, D.G. (1970) The isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Experimental Parasitology* **28**, 521-534.

Laveissière, C., Hervouët, J.P., Couret, D., Eouzan, J.-P. & Merouze, F. (1985) La campagne pilote de lutte contre la trypanosmiase humaine dans le foyer de Vavoua (Côte d'Ivoire) 2.La mobilisation des communautés rurales et l'application du piégeage. *Cahier ORSTOM Série Entomologie Médicale et Parasitologie* **23**,167-185. 1985.

Lawrence, J.A., Foggin, C.M. & Norval, R.A.I. (1980) The effect of war on the control of diseases of livestock in Rhodesia (Zimbabwe). *The Veterinary Record* **107**, 82-85.

Leach, T.M. & Roberts, C.J. (1981) Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the eastern hemisphere. *Pharmacology and Therapeutics* **13**, 91-147.

Leak, S.G.A. (1980) *A review of the tsetse situation in Petauke and- Katete Districts of Eastern Province over the ten year period 1970-1979*. Department of Veterinary and Tsetse Control Services, Chipata, 14 pp.

Leak, S.G.A. (1998) *Epidemiology of trypanosomosis in domestic livestock*. CABI Publishing/International Livestock Research Institute (ILRI), Wallingford, 592 pp.

Leak, S.G.A., Awoume, K., Colardelle, C., Duffera, W., Feron, A., Mahamat, B., Mawuena, K., Minengu, M., Mulungo, M., Nankodaba, G., Ordner, G., Pelo, M., Sheria, M., Tikubet, G., Touré, M., & Yangari, G. (1988) Determination of tsetse challenge and its relationship with trypanosome prevalence in trypanotolerant livestock at sites of the African Livestock Network. In: *Livestock production in tsetse affected areas of Africa*, International Livestock Centre for Africa (ILCA), Nairobi, pp. 43-54.

Leak, S.G.A., Colardelle, C., d'Ieteren, G., Dumont, P., Feron, A., Jeannin, P., Minengu, M., Mulungu, M., Ngamuna, S., Ordner, G., Sauveroche, B., Trail, J.C.M. & Yangari, G. (1991) *Glossina fusca* group tsetse as vector of cattle trypanosomiasis in Gabon and Zaire. *Medical and Veterinary Entomology* 5, 111-120.

Leak, S.G.A., Mulatu, W., Authie, E., D'-Ieteren, G.D.M., Peregrine, A.S., Rowlands, G.J. & Trail, J.C.M. (1993) Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropica* **53**, 121-134.

Leak, S.G.A., Woudyalew Mulatu, Rowlands, G.J., & d'Ieteren, G.D.M. (1995) A trial of a cypermethrin 'pour-on' insecticide to control *Glossina pallidipes*, *G. fuscipes* fuscipes and *G. morsitans submorsitans* (Diptera: Glossinidae) in south-west Ethiopia. *Bulletin of Entomological Research* **85**, 241-251.

Leak, S.G.A. & Rowlands, G.J. (1997) The dynamics of trypanosome infections in natural population of tsetse (Diptera: Glossinidae) studied using wing-fray and ovarian ageing techniques. *Bulletin of Entomological Research* **87**, 273-282.

Leeflang, P., Buys, J. & Blotkamp, C. (1976) Studies on *Trypanosoma vivax* infectivity and serial maintenance of natural bovine isolates in mice. *International Journal for Parasitology* **6**, 413-417.

Lefrançois, T., Solano, P., Rocque, S., de-la, Bengaly, Z., Reifenberg, J.M., Kabore, I., Cuisance, D. & De-la-Rocque, S. (1998) New epidemiological features on animal trypanosomiasis by molecular analysis in the pastoral zone of Sideradougou, Burkina Faso. *Molecular Ecology* 7, 897-904.

Leggate, B.M. (1963) Trypanosome infections in *Glossina morsitans* Westw. and *G. pallidipes* under natural conditions. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 9th meeting, Conacry, pp. 213-227.

Leggate, B.M. & Pilson, R.D. (1961) The diurnal feeding activity of *Glossina pallidipes* Aust. in relation to trypanosome challenge. *Bulletin of Entomological Research* **51**, 697-704.

Lewis, A.R. & Thomson, J.R. (1974) Observations on an isometamidium resistant strain of *Trypanosoma congolense* in Rhodesia. *Rhodesia Veterinary Journal* **4**, 62-67.

Llewelyn, C.A., Munro, C.D., Luckins, A.G., Jordt, T., Murray, M. & Lorenzini, E. (1988) The effects of *Trypanosoma congolense* infection on the oestrus cycle of the Boran cow. *British Veterinary Journal* **144**, 379-387.

Lloyd, L.L. (1912) Notes on *Glossina morsitans* Westw. in the Luangwa valley, Northern Rhodesia. *Bulletin of Entomological Research* **3**, 233-239.

Lloyd, L.L. (1916) Report on the investigation into the bionomics of *Glossina morsitans* in Northern Rhodesia, 1915. *Bulletin of Entomological Research* **7**, 67-79.

Lloyd, H.M. (1935) Notes on the bionomics of *Glossina swynnertoni* Austen. *Bulletin of Entomological Research* **26**, 439-468.

Lloyd, L.L. & Johnson, W.B. (1924) The trypanosome infections of tsetse flies in Northern Nigeria and a method of estimation. *Bulletin of Entomological Research* **14**, 225-227.

Losos, C.J., & Ikede, B.O. (1972). Review of pathology of diseases in domestic animals caused by *Trypanosoma congolense, T. vivax, T. brucei, T. rhodesiense*, and *T. gambiense*. *Veterinary Pathology* **9**, 1-71.

Lovemore D.F. (1986) Tsetse control by chemical means in Malawi, Mozambique, Zambia and Zimbabwe. In: Cavalloro R. (Ed.), *Integrated tse-tse fly control: methods and strategies*, A. A. Balkema, Rotterdan/Brookfield, pp. 27-41.

Lovemore, D.F. (1989) *Assessment of the reported southerly advance of tsetse flies towards Livingstone, Zambia.* Regional Tsetse and Trypanosomiasis Control Programme, Harare, 5 pp.

Lovemore, D.F. (1990) *A history of tsetse and trypanosomiasis control in Zimbabwe's western region during the period, 1970-1990.* Harare, 17 pp.

Lovemore, D.F. (1999) *Final report for the Zimbabwe component of the first and second phases of the Regional Tsetse and Trypanosomiasis Control Programme Malawi, Mozambique, Zambia and Zimbabwe 1986-1998.* Harare, RTTCP, 163 pp.

Lovemore, D.F. & Napier Bax, P. (1972) An isolated outbreak of cattle trypanosomiasis near Victoria Falls, Rhodesia. *The Rhodesia Science News* **6**, 14-17.

Low, A.R.C., Kemp, R.L. & Doran, M.H. (1980) Cattle wealth and cash needs in Swaziland: price response and rural development implications. *Journal of Agricultural Economics* **31**, 225-236.

Luckins, A.G. (1977) Detection of antibodies in trypanosoma-infected cattle by means of a microplate enzyme-linked immunosorbant assay. *Tropical Animal Health and Production* **9**, 53-62.

Luckins, A.G. & Mehlitz, D. (1978) Evaluation of an indirect fluorescent antibody test, enyme-linked immunosorbent assay and quantification of immunoglobulins in the diagnosis of bovine trypanosomiasis. *Tropical Animal Health and Production* **10**, 149-159.

Luckins, A.G. & Gray,A.R. (1979) Trypanosomes in the lymph nodes of cattle and sheep infected with *Trypanosoma congolense*. *Research in Veterinary Science* **27**, 129-131.

Luckins, A.G., Sutherland, D., Mwangi, D. & Hopkins, J. (1994) Early stages of infection with *Trypanosoma congolense*: parasite kinetics and expression of metacyclic variable antigen types. *Acta Tropica* **58**, 199-206.

Luguru, S.M., Bennett, S.R. & Chizyuka, H.G.B. (1993) Observations on the incidence of bovine trypanosomiasis in cattle dipped in deltamethrin in tsetse infested area in Zambia. *Tropical Animal Health and Production* **25**, 129-130.

Lumsden, W.H.R., Kimber, C.D., Evans, D.A. & Doig, S.J. (1979) *Trypanosoma brucei*: miniature anion-exchange centrifugation technique for detection of low parasitaemias: adaptation for field use. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**, 312-317.

Majiwa P.O.A. & Otieno L.H. (1990) Recombinant DNA probes reveal simultaneous infection of tsetse flies with different trypanosoma species. *Molecular and Biochemical Parasitology* **40**, 245-254.

Makumi, J.N., Green, C.H. & Baylis, M. (1996) The role of cattle as hosts of *Glossina longipennis* at Galana Ranch, south-eastern Kenya. *Medical and Veterinary Entomology* **10**, 331-336.

Makumyaviri, A.M., Demey, F., Claes, Y., Verhulst, A. & Le Ray, D. (1984) Characterization of the vectorial capacity of *Glossina morsitans morsitans* towards *Trypanosoma brucei brucei*, EATRO 1125, (Antar 1). *Annales de la Société Belge de Médicine Tropicale* **64**, 365-372.

Martin, S.W., Shourki, M. & Thorburn, M.A. (1992) Evaluating the health status of herds based on tests applied to individuals. *Preventive Veterinary Medicine* **14**, 33-43.

Masake, R.A., Nantulya, V.M., Musoke, A.J., Moloo, S.K. & Nguli, K. (1987) Characterization of *Trypanosoma congolense* serodemes in stocks isolated from cattle intoduced onto a ranch in Kilifi, Kenya. *Parasitology* **94**, 349-357.

Masiga, D.K., Smyth, A.J., Hayes, P., Bromidge, T.J. & Gibson, W.C. (1992) Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International Journal for Parasitology* **22**, 909-918.

Masiga, D.K., Mcnamara, J.J., Laveissière, C., Truc, P. & Gibson, W.C. (1996) A high prevalence of mixed trypanosome infections in tsetse flies in Sinfra, Côte d'Ivoire, detected by DNA amplification. *Parasitology* **112**, 75-80.

Mason, C.A. & Norval, R.A.I. (1980) The ticks of Zimbabwe. I. The genus *Boophilus*. *Zimbabwe Veterinary Journal* **11**, 36-43.

Matson, B.A. (1959) *An investigation into the livestock problems of the Lower Shire Valley of Nyasaland with particular reference to the disease trypanosomiasis.* Blantyre, Department of Veterinary Services and Animal Industry, Nyasaland, 13 p.

Maudlin, I., Kabayo, J.P., Flood, M.E.T. & Evans, D.A. (1984) Serum factors and the maturation of *Trypanosoma congolense* infections in *Glossina morsitans*. *Zeitschrift für Parasitenkunde* **70**, 11-19.

Maudlin, I. & Welburn, S.C. (1987) Lectin mediated establishment of midgut infections of *Trypanosma congolense* and *Trypanosoma brucei* in *Glossina morsitans*. *Tropical Medicine and Parasitology* **38**, 167-170.

Maudlin, I. & Welburn, S.C. (1988) The role of lectins and trypanosome genotype in the maturation of midgut infections in *Glossina morsitans*. *Tropical Medicine and Parasitology* **39**, 56-58.

McNamara, J.J. & Snow, W.F. (1990) Improved identification of Nannomonas infections in tsetse flies from The Gambia. *Acta Tropica* **48**, 127-136.

Mehlitz, D., Heidrich-Joswig, S., Fimmen, H.O., Freitas, E.K. & Karbe, E. (1983) Observations on the colostral transfer of anti-trypanosoma antibodies in N'Dama calves and the immune response to infection with *Trypanosoma* (Duttonella) *vivax* and *T.* (Nannomonas) *congolense*. *Annales de la Société Belge de Médicine Tropicale* **63**, 137

Mérot, P., Politzar, H., Tamboura, I. & Cuisance, D. (1984) Résultats d'une campagne de lutte contre les glossines riveraines en Burkina par l'emploi d'écrans imprégnés de Deltaméthrine. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **37**, 175-184.

Mihok, S., Otieno, L.H., Darji, N. & Munyinyi, D. (1992) Influence of D (+)-glucosamine on infection rates and parasite loads in tsetse flies (*Glossina* spp.) infected with *Trypanosoma brucei*. *Acta Tropica* **51**, 217-228.

Mihok, S., Olubayo, R.O., Darji, N. & Zweygarth, E. (1993) The influence of host blood on infection rates in *Glossina morsitans* spp. infected with *Trypanosoma congolense*, *T. brucei* and *T.simiae*. *Parasitology* **107**, 41-48.

Mihok, S., Maramba, O., Munyoki, E. & Kagoiya, J. (1995) Mechanical transmission of *Trypanosoma* spp. by African Stomoxyinae (Diptera: Muscidae). *Tropical Medicine and Parasitology* **46**, 103-105.

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

Mitchell, B.L. & Steele, B. (1956) *A report on the distribution of tsetse flies in Nyasaland.* Government Printer. Zomba, 67 pp.

Moloo, S.K. (1981) Effects of maintaining *Glossina morsitans morsitans* on different hosts upon the vector's subsequent infection rates with pathogenic trypanosomes. *Acta Tropica* **38**, 125-136.

Moloo, S.K. (1993) The distribution of *Glossina* species in Africa and their natural hosts. *Insect Science and its Application* **14**, 511-527.

Molyneux, D.H. (1976) Vector relationships in Trypanosomatidae. *Advances in Parasitology* **15**, 1-82.

Morlais, I., Grebaut, P., Bodo, J.M., Djoha, S., Cuny, G. & Herder, S. (1998) Detection and identification of trypanosomes by polymerase chain reaction in wild tsetse flies in Cameroon. *Acta Tropica* **70**, 109-117.

Moser, D.R., Cook, G.A., Ochs, D.E. & Bailey, C.P. (1989) Detection of *T. congolense* and *T. brucei* subspecies by DNA amplification using the polymerase chain reaction. *Parasitology* **99**, 57-66.

Msangi, A.R., Whitaker, C.J. & Lehane, M.J. (1998) Factors influencing the prevalence of trypanosome infection of *Glossina pallidipes* on the Ruvu flood plain of eastern Tanzania. *Acta Tropica* **70**, 143-155.

Mulla, A.F. & Rickman, L.R. (1988) How do African game animals control Trypanosome infections? *Parasitology Today* **4**, 352-354.

Mulligan, H. W. (1970) *The African Trypanosomiases*, Gearge Allen and Uwin/Ministry of Overseas Development, London. 950 pp.

Münstermann, S. (1984) *Identifizierung der Wirtstierart von Tsetse-Fliegen (Diptera, Glossinidae) -Blutmahlzeiten unter Einsatz von KBR und ELISA.* Freie Universität Berlin, Berlin, 125 pp..

Murray, M. (1974) The pathology of African trypanosomiases. In: L. Brent and J. Holborow (Eds.), *Progress in immunology*, North-Holland Publishing Company, pp. 181-192.

Murray, M. & Urquhart, G.H. (1977) Immunoprophylaxis against African trypanosomiasis. In: L.H. Miller, J.A. Pino and J.J. McKelvey (Eds.) *Immunity to blood parasites of animals and man*. Plenum Press. London and New York, pp 209-241.

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

Murray, M., & Morrison, W.I. (1979a). Pathogenesis and pathology of African trypanosomiasis in domestic livestock. Food and Agriculture Organization (FAO), Rome, 14pp.

Murray, M., Nguyen, H.C., Lambert, P.H. & Gerber, H. (1979b) The anaemia of African trypanosomiasis. Demonstration of a haemolytic factor. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 15th meeting, pp. 460 -469.

Murray PK, Murray M, Wallace M, Morrison WI, and McIntyre WIM. (1979c) Trypanosomiasis in N'Dama and Zebu cattle. II. The influence of weight infection on the severity of the disease. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 15th meeting, pp. 482 -487.

Murray, M., Clifford, D.J., Snow, W.F., Gettinby, G. & McIntyre, W.I.M. (1981) Susceptibility to African trypanosomiasis of N'Dama and Zebu cattle in an area of *Glossina morsitans submorsitans* challenge. *The Veterinary Record* **109**, 503-509.

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

Murray, M. & Dexter, T.M. (1988) Anaemia in bovine African Trypanosomiasis: a review. *Acta Tropica* **45**, 389-432.

Mutayoba, B.M., O'hara-Ireri, H.B. & Gombe, S. (1988a) Trypanosome-induced depression of plasma thyroxine levels in prepubertal and adult female goats. *Acta Endocinologica* **119**, 21-29.

Mutayoba, B.M., Gombe, S., Kaaya, G.P. & Waindi, E.N. (1988b) Effect of chronic experimental *Trypanosoma congolense* infection on the ovaries, pituitary, thyroid and adrenal glands in female goats. *Research in Veterinary Science* **44**, 140-146.

Mutharia, L.M. & Pearson, T.W. (1987) Surface carbohydrates of procyclic forms of African trypanosomes studied using fluorescence activated cell sorter analysis and agglutination of lectins. *Molecular and Biochemical Parasitology* **23**, 165-172.

Muzari, M.O. & Hargrove, J.W. (1996) The design of target barriers for tsetse flies, *Glossina* spp. (Diptera: Glossinidae). *Bulletin of Entomological Research* **86**, 579-583.

Mwangelwa, M.I., Otieno, L.H. & Reid, G.D.F. (1987) Some barriers to *Trypanosoma congolense* development in *Glossina morsitans morsitans*. *Insect Science and its Application* **8**, 33-37.

Mwangelwa, M.I., Dransfield, R.D., Otieno L.H., & Mbata, K.J. (1990) Distribution and diel activity patterns of *Glossina fuscipes fuscipes* Newstead on Rusinga island and mainland Mbita, Kenya. *Insect Science and its Application* **11**, 315-321.

Nagel, P. (1995) *Environmental monitoring handbook for tsetse control operations*. Magraf Verlag, Weikersheim, 323 pp.

Nankodaba, G., Coulibaly, L., Hecker, P., Leak, S.G.A., & Scheutterle, A. (1988) Trypanosome prevalence in cattle herds exposed to a range of tsetse challenge levels in northern Côte d'Ivoire. In: *Livestock production in tsetse affected areas of Africa*, International Livestock Centre for Africa (ILCA), Nairobi, pp. 55-62.

Nantulya, V.M. (1986) Immunological approaches to the control of animal trypanosomiasis. *Parasitology Today* **2**, 168-173.

Nantulya, V.M. (1990) Trypanosomiasis in domestic animals: the problems of diagnosis. *Revue Scientifique technique de l'office International des Epizooties* **9**, 357-367.

Nantulya, V.M., Doyle, J.J. & Jenni, L. (1978) Studies on *Trypanosoma* (Nannomonas) *congolense* 2.Observations on the cyclical transmission of three field isolates by *Glossina morsitans morsitans*. *Acta Tropica* **35**, 339-344.

Nantulya, V.M., Musoke, A.J., Barbet, A.F. & Roelants, G.E. (1979) Evidence for reappearance of *Trypanosoma brucei* antigen types in relapse populations. *Journal of Parasitology* **65**, 673-679.

Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R., Saigar, N. & Minja, S.H. (1987) Monoclonal antibodies that distinguish *Trypanosoma congolense*, *T. vivax* and *T. brucei*. *Parasite Immunology* **9**, 421-431.

Naylor, D.C. (1971) The haematology and histopathology of *Trypanosoma congolense* infection in cattle Part II: Haematology (including symptoms). *Tropical Animal Health and Production* **3**, 159-168.

Ndao, M., Pandey, V.S., Zinsstag, J., Pfister, K. & van Meirvenne, N. (1995) Evaluation of sodium dodecyl sulfate (SDS) as a haemolytic agent for the detection of microfilaria and trypanosomes in the blood of cattle. *Annales de la Société Belge de Médicine Tropicale* **75**, 145-148.

Ndegwa, P.N., Irungu, L.W. & Moloo, S.K. (1992) Effect of puparia incubation temperature: increased infection rate of *Trypanosoma congolense* in *Glossina morsitans centralis*, *G. fuscipes fuscipes* and *G. brevipalpis*. *Medical and Veterinary Entomology* **6**, 127-130.

Ndoutamia, G., Moloo, S.K., Murphy, N.B. & Peregrine, A.S. (1993) Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. *Antimicrobial Agents and Chemotherapy* **37**, 1163-1166.

Neave, S.A. (1911) Report on a journey to the Luangwa Valley, north-eastern Rhodesia, from July to September 1910. *Bulletin of Entomological Research* **1**, 303-317.

Nguu, E.K., Osir, E.O., Imbuga, M.O. & Olembo, N.K. (1996) The effect of host blood in the *in vitro* transformation of bloodstream trypanosomes by tsetse midgut homogenates. *Medical and Veterinary Entomology* **10**, 317-322.

Nitcheman, S. (1988) Comparaison des longivités des glossines (*Glossina morsitans morsitans*) infectées par les trypanosomes (*Trypanosoma congolense* Broden, 1904) et des glossines saines. *Annales de Parasitologie Humaine Comparée* **63**, 163-164.

Nitcheman, S. & Jacquiet, P. (1990) Utilisation de sourcieaux pour la mise en évidence de l'infectivité des glossines. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **43**, 219-223.

Norval, R.A.I. (1983) Arguments against intensive dipping. *Zimbabwe Veterinary Journal* **14**, 19-25.

Norval, R.A.I., Fivaz, B.H., Lawrence, J.A. & Daillecourt, T. (1983) Epidemiology of tick-borne diseases of cattle in Zimbabwe. I. Babesiosis. *Tropical Animal Health and Production* **15**, 87-94.

Norval, R.A.I., Fivaz, B.H., Lawrence, J.A. & Brown, A.F. (1985) Epidemiology of tick-borne diseases of cattle in Zimbabwe. III. *Theileria parva* group. *Tropical Animal Health and Production* **17**, 19-28.

Norval, R.A.I., Perry, B.D. & Hargreaves, S. (1992) Tick and tick-borne disease control in Zimbabwe: what might the future hold ? *Zimbabwe Veterinary Journal* **23**, 1-15.

Ogwu, D. & Njoku, C.O. (1987) Effect of pregnancy on clinical manifestation of bovine trypanosomiasis. *Veterinary Parasitology* **24**, 25-33.

Okiwelu, S.N. (1976) Resting sites of *Glossina morsitans morsitans* Westwood (Diptera, Glossinidae) during the dry season in the Republic of Zambia. *Bulletin of Entomological Research* **66**, 413-419.

Okiwelu, S.N. (1977a) Host preference and trypanosome infection rates of *Glossina morsitans morsitans* Westwood in the Republic of Zambia. *Annals of Tropical Medicine and Parasitology* **71**, 101-107.

Okiwelu, S.N. (1977b) Observations of resting sites of *Glossina morsitans morsitans* (Diptera: Muscidae) during the wet season in the republic of Zambia, Africa. *Journal of Medical Entomolology* **13**, 595-599.

Okiwelu, S.N. & Maiga, S. (1981) Natural host of *Glossina morsitans submorsitans* and *Glossina palpalis gambiensis* Vanderplank in the Republic of Mali. *Cahier ORSTOM Série Entomologie Médicale et Parasitologie* **19**, 179-186.

Okoth, J.O. & Kapaata, R. (1988) The host of *Glossina fuscipes fuscipes* in Busoga, Uganda, and epidemiological implications for trypanosomiasis. *Annals of Tropical Medicine and Parasitology* **82**, 517-518.

Ole-MoiYoi, O.K. (1987) Trypanosome species-specific DNA probes to detect infection in tsetse flies. *Parasitology Today* **3**, 371-374.

Ooijen, C.J. (1986). *Bovine trypanosomiasis study Gwembe District (July '84 - Nov '85) Zambia.* Unpublished report, 63 pp.

Ormerod, W.E. (1986) A critical study of the policy of tsetse eradication. *Land Use Policy* **3**, 85-99.

Otieno, L.H. (1983) Inadequacy of the dissection method of estimating trypanosome infection rates. *Annals of Tropical Medicine and Parasitology* **77**, 329-330.

Otieno L.H. & Darji, N. (1979) The abundance of pathogenic African trypanosomes in the salivary secretions of wild *Glossina pallidipes*. *Annals of Tropical Medicine and Parasitology* **73**, 583-588.

Otte, M.J. & Abuabara, J.Y. (1991) Transmission of South American *Trypanosoma vivax* by the neotropical horsefly *Tabanus nebulosus*. *Acta Tropica* **49**, 73-76.

Owaga, M.L., Hassanali, A., & McDowell, P.G. (1988) The role of 4-cresol and 3-n-propylphenol in the attraction of tsetse flies to buffalo urine. *Insect Science and its Application* **9**, 95-100.

Owaga, M.L.A., Okelo, R.O. & Chaudhury, M.F.B. (1993) Diel activity patterns of the tsetse fly *Glossina austeni* Newstead (Diptera: Glossinidae) in the field and in the laboratory. *Insect Science and its Application* **14**, 701-705.

Paling, R.W., Moloo, S.K., Scott, J.R., McOdimba, F.A., Logan-Henfrey, L.L., Murray, M. & Williams, D.J.L. (1991) Susceptibility of N'Dama and Boran cattle to tsetse-transmitted primary and rechallenge infections with a homologous serodeme of *Trypanosoma congolense*. *Parasite Immunology* **13**, 413-425.

Paris, J., Murray, M. & McOdimba, F. (1982) A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta Tropica* **39**, 307-316.

Pays, E. (1989) Pseudogenes, chimaeric genes and the timing of antigen variation in African trypanosomes. *Trends in Genetics* **5**, 389-391.

Peregrine, A.S. (1994) Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology* **54**, 223-248.

Perry, B.D., Mwanaumo, B., Schels, H.F., Eicher, E. & Zaman, M.R. (1984) A study of health and productivity of traditionally managed cattle in Zambia. *Preventive Veterinary Medicine* **2**, 633-653.

Peter, T.F., Perry, B.D., O'Callagham, C.J., Medley, G.F., Shumba, W., Madzima, W., Burridge, M.J. & Mahan, S.M. (1998) Distribution of vectors of heartwater, *Amblyomma hebraeum* and *Amblyomma variegatum* (Acari: Ixodae), in Zimbabwe. *Experimental & Applied Acarology* **22**, 725-740.

Phelps, R.J. & Burrows, P.M. (1969) Prediction of pupal duration of *Glossina morsitans orientalis* Vanderplank under field conditions. *Journal of Applied Ecology* **6**, 323-337.

Phelps, R.J. & Vale, G.A. (1978) Studies on populations of *Glossina morsitans morsitans* and *G. pallidipes* in Rhodesia. *Journal of Applied Ecology* **15**, 743-760.

Phelps, R.J., & Lovemore, D.F. (1994) Tsetse flies. In: J.A.W. Coetzer, G.R. Thomson, and R.C. Tustin (Eds.), *Infectious diseases of livestock with special reference to southern Africa*. Oxford University Press, Cape Town, pp. 25-52.

Pilson, R.D. & Harley, J.M.B. (1959) The importance of cattle as a host of *Glossina morsitans* in Ankole. *East African Trypanosomiasis Research Organization report* **58**, 45-46.

Pilson, R.D. & Pilson, B.M. (1967) Behaviour studies of *Glossina morsitans* Westw. in the field. *Bulletin of Entomological Research* **57**. 227-257.

Pilson, R.D., Boyt, W.P. & Mackenzie, P.K.I. (1978) The relative attractiveness of donkeys, cattle, sheep and goats to *Glossina morsitans morsitans* Westwood and G. pallidipes Austen (Diptera : Glossinidae) in the Zambezi Valley of Rhodesia. *Bulletin of Entomological Research* **68**. 489-495.

Pinder, M. & Authié, E. (1984) The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. *Acta Tropica* **41**, 247-252.

Pollock, J.N. (1986) *Training manual for tsetse control personnel Volume 1. Biology, morphology and distribution of tsetse.* Food and Agriculture Organization (FAO), Rome, 307 pp.

Potgieter, F.T., & Stoltsz, W.H. (1994) Bovine anaplasmosis. In: J.A.W. Coetzer, G.R. Thomson, and R.C. Tustin (Eds.), *Infectious diseases of livestock with special reference to southern Africa*. Oxford University Press, Cape Town, pp. 408-430.

Potts, W.H. (1930) A contribution to the study of numbers of tsetse-fly (*Glossina morsitans* Westw.) by quantitative methods. *South African Journal of Sciences* **27**, 491-497.

Potts, W.H. (1954) *Maps of the distribution of tsetse species in Africa*. Directorate of Colonial Surveys, London.

Power, R.J.B. (1964) The activity pattern of *Glossina longipennis* Corti (Diptera : Muscidae). *Proceedings of the Royal Entomological Society London (A)* **39**. 5-14.

Randolph, S.E. & Rogers, D.J. (1978) Feeding cycles and flight activity in field populations of tsetse (Diptera : Glossinidae). *Bulletin of Entomological Research* **68**, 655-671.

Randolph, S.E. & Rogers, D.J. (1984) Movement patterns of the tsetse fly *Glossina palpalis palpalis* around villages in the pre-forest zone of Ivory Coast. *Bulletin of Entomological Research* **74**, 689-705.

Randolph, S.E., Rogers, D.J. & Kiilu, J. (1991a) The feeding behaviour, activity and trappability of wild female *Glossina pallidipes* in relation to the pregnancy cycle. *Medical and Veterinary Entomology* **5**, 335-350.

Randolph, S.E., Rogers, D.J., Dransfield, R.D. & Brightwell, R. (1991b) Trap-catches, nutritional condition and the timing of activity of the tsetse fly *Glossina longipennis* (Diptera: Glossinidae). *Bulletin of Entomological Research* **81**, 455-464.

Rawlings, P., Wacher, T. & Snow, W.F. (1994) Cattle-tsetse contact in relation to daily activity patterns of *Glossina morsitans submorsitans* in The Gambia. *Medical and Veterinary Entomology* **8**, 57-62.

Raymond, H.L. (1990) *Tabanus importunus*, vecteur mécanique expérimental de *Trypanosoma vivax* en Guyane Francaise. *Ann Parasitol Hum Comp* **65**, 44-46.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1989) *Mission report of the Regional Trypanosomiasis Expert (46/RTE/89)*. 4pp.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1990) *Mission report of the Regional Trypanosomiasis Expert (55/RTE/89)*. 3pp.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1991) *Mission report of the Regional Co-ordinator. Report for Zambia Mission 10-22/11/91*. 11 pp.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1996) *Annual report for 1998. Office of the Regional Co-ordinator*. RTTCP, Harare, 93 pp.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1999a) *Annual report of the Office of the Regional Co-ordinator*. RTTCP, Harare, 92 pp.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1999b) *Socio-economic surveys of the Kasungu, Nkhotakota and Vwaza areas of Malawi*. RTTCP, Harare, 48 pp.

Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) (1999c) *Socio-economic surveys of tsetse-free and tsetse-infested areas of Eastern Province, Zambia, 1998*. RTTCP, Harare, 40 pp.

Reifenberg, J.M., Solano, P., Bauer, B., Kabore, I., Cuny, G., Duvallet, G. & Cuisance, D. (1997) Apport de la technique PCR pour une meilleure compréhension de l'épizootiologie des trypanosomoses bovines: exemple de la zone d'aménagement pastoral de Yalé au Burkina Faso. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **50**, 14-22.

Rickman, L.R. & Kolala, F. (1982) Effects of some African game animal sera on *Trypanosoma brucei rhodesiense* and *T. b. brucei* clones. *Tropenmedizin und Parasitologie* **33**, 129-135.

Roberts, C.J., Gray, M.A. & Gray, A.R. (1969) Local skin reactions in cattle at the site of infection with *Trypanosoma congolense* by *Glossina morsitans* and *G. tachinoides*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63**, 620-624.

Roberts, L.W., Welide, B.T. & Reardon, M.J. (1989) Mechanical transmission of *Trypanosoma brucei rhodesiense* by *Glossina morsitans morsitans*. *Annals of Tropical Medicine and Parasitology* **83**, 127-131.

Robertson, A.G. (1983) The feeding habits of tsetse flies in Zimbabwe (formerly Rhodesia) and their relevance to some tsetse control measures. *Smithersia* **1**, 1-72.

Robertson, A.G. & Kluge, E.B. (1968) The use of insecticide in arresting an advance of *Glossina morsitans* Westwood in the south east lowveld of Rhodesia. *Proceedings and Transactions of the Rhodesia Scientific Association* **53**, 17-33.

Robson, J. (1972) The results of field trials of the capillary agglutination test for the detection of bovine trypanosomal antibodies, carried out in the Lambwe Valley. *Bulletin of the World Health Organization* **47**, 779-780.

Roditi, I. & Pearson, T.W. (1990) The procyclic coat of African trypanosomes (or the not-so-naked trypanosome). *Parasitology Today* **6**, 79-81.

Roeder, P.L., Scott, J.M. & Pegram, R.G. (1984) Acute *Trypanosoma vivax* infection of Ethiopian cattle in the apparent absence of tsetse. *Tropical Animal Health and Production* **16**, 141-147.

Rogers, D. (1977) Study of a natural population of *Glossina fuscipes fuscipes* Newstead and a model of fly movement. *Journal of Animal Ecology* **46**, 309-330.

Rogers, D.J. (1979) Tsetse population dynamics and distribution : a new analytical approach. *Journal Animal Ecology* **48**, 825-849.

Rogers, D.J. (1988) A general model for the African trypanosomiasis. *Parasitology* **97**, 193-212.

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

Rogers, D.J. & Randolph, S.E. (1978) Metabolic strategies of male and female tsetse (Diptera: Glossinidae) in the field. *Bulletin of Entomological Research* **68**, 639-654.

Rogers, D.J. & Randolph, S.E. (1986) Distribution and abundance of tsetse flies. *Journal Animal Ecology* **55**, 1007-1025.

Rosenberg, N.J., Blad, B.L., & Verma, S.B. (1983) *Microclimate. The biological environment*. John Welley & Sons. London, 495 pp.

Rottcher, D. (1975). *Summary of Dr. J.S. Dillman's work as a veterinary wildlife officer in Zambia*. Department of Veterinary Services, Lusaka, 18 pp.

Rowcliffe, C., & Finlayson, L.H. (1982) Active and resting behaviour of virgin and pregnant females of *Glossina morsitans morsitans* in the laboratory. *Bulletin of Entomological Research* **72**, 271-288.

Rowlands, G.J., Woudyalew Mulatu, Authié, E., d'Ieteren, G.D.M., Leak, S.G.A. & Nagda, S.M. (1994) Effects of trypanosomiasis on reproduction of East African Zebu cows exposed to drug-resistant trypanosomes. *Preventive Veterinary Medicine* **21**, 237-249.

Rowlands, G.J., d'Ieteren, G.D.M., Coulibaly, L., Hecker, P.A., Leak, S.G.A. & Nagda, S.M. (1996) Assessment of the effect of tsetse control on livestock productivity - a case study in northern Côte d'Ivoire. *Preventive Veterinary Medicine* **28**, 17-32.

Rurangirwa, F.R., Tabel, H., Losos, G., Masiga, W.N. & Mwambu, P. (1978) Immunosuppressive effect of *Trypanosoma congolense* and *Trypanosoma vivax* on the secondary immune response of cattle to *Mycoplasma mycoides* subsp *mycoides*. *Research in Veterinary Science* **25**, 395-397.

Rurangirwa, F.R., Tabel, H., Losos, G.J. & Tizard, I.R. (1979) Suppression of antibody response to *Leptospira biflexa* and *Brucella abortus* and recovery from immunosuppression after Berenil treatment. *Infection and Immunity* **26**, 822-826.

Rurangirwa, F.R., Mushi, Tabel, H., Tizard, I.R. & Losos, G.J. (1980) The effect of *Trypanosoma congolense* and *T. vivax* infections on the antibody response of cattle to live rinderpest virus vaccine. *Research in Veterinary Science* **28**, 264-266.

Salmon, J. & Barrett, J.C. (1994) Social issues in animal trypanosomiasis control. *Tropical Science* **34**, 191-202.

Sanderson, M. (1910) Notes on *Glossina fusca*, Walk., in north Nyasa. *Bulletin of Entomological Research* **1**, 299-302.

Saror, D.I. (1979) Classification of the anaemia of bovine trypanosomiasis. *Veterinary Record* **105**, 96-98.

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

Saunders, D.S. (1960) The ovulation cycle in *Glossina morsitans* Westwood (Diptera: Muscidae) and a possible method of age determination for female tsetse flies by examination of their ovaries. *Transactions of the Royal Entomological Society London* **112**, 221-238.

Sawadogo, G.J., Oumarou, A.A., Sene, M. & Diop, M. (1991) Effect of poor pasture conditions and type of feeding on some biochemical values of Gobra Zebu in Senegal. *British Veterinary Journal* **147**, 538-544.

Scoones, I. (1995) Exploiting heterogeneity: habitat use by cattle in dryland Zimbabwe. *Journal of Arid Environments* **29**, 221-237.

Scott, J.M., Pegram, R.G., Holmes, P.H., Pay, T.W.F., Knight, P.A., Jennings, F.W. & Urquhart, G.M. (1977) Immunosuppression in bovine trypanosomiasis: field studies using foot-and-mouth disease vaccine and clostridial vaccine. *Tropical Animal Health and Production* **9**, 159-165.

Sehof, C.F.H. (1975) *Tsetse bloodmeal collections from the Kakumbi area of the Luangwa Valley (1973 - 1974)*. Department of Veterinary Services, Lusaka. 5 pp.

Sekoni, V.O. (1990) Effect of Novidium (Homidium chloride) chemotherapy on genital lesions induced by *Trypanosoma vivax* and *Trypanosoma congolense* infections in Zebu bulls. *British Veterinary Journal* **146**, 181-185.

Sekoni, V.O., Njoku, C.O., Kumi-Diaka, J. & Saror, D.I. (1990) Pathological changes in male genitalia of cattle infected with *Trypanosoma vivax* and *Trypanosoma congolense*. *British Veterinary Journal* **146**, 175-180.

Sharpe, R.T., Langley, A.M., Mowat, G.N., Macaskill, J.A. & Holmes, P.H. (1982) Immunosuppression in bovine trypanosomiasis: response of cattle infected with *Trypanosoma congolense* to foot-and-mouth disease vaccination and subsequent live virus challenge. *Research in Veterinary Science* **32**, 289-293.

Shereni, W. (1984) The use of cloth screens and acetone vapour as alternatives to a bait-ox for sampling populations of tsetse flies (Diptera: Glossinidae). *Transactions of the Zimbabwe Scientific Association* **62**, 22-27.

Shereni, W. (1990) Strategic and tactical developments in tsetse control in Zimbabwe (1981-1989). *Insect Science and its Application* **11**, 399-409.

Shereni, W., & Pope, A.R.J. (1992). An evaluation of deltamethrin as an alternative to DDT for ground spraying against tsetse flies, Glossina spp. (Diptera: Glossinidae) in Zimbabwe. Unpublished report, Tsetse and Trypanosomiasis Control Branch, Harare.

Shircore, J.O. (1914) Suggestions for the limitation and destruction of *Glossina morsitans*. *Bulletin of Entomological Research* **5**, 87-90.

Slingenbergh, J. (1992) Tsetse control and agricultural development in Ethiopia. *World Animal Review* **70-71**, 30-36.

Snow, W.F. (1980) Host location and feeding patterns in tsetse. *Insect Science and its Application* **1**, 23-30.

Snow, W.F. & Boreham, P.F.L. (1979) The feeding habits and ecology of the tsetse fly *Glossina morsitans morsitans* Newstead in relation to nagana transmission in The Gambia. *Acta Tropica* **36**, 47-51.

Snow, W.F., Tarimo, S.A., Staak, C. & Butler, L. (1988) The feeding habits of tsetse, *Glossina pallidipes* Austen on the South Kenya coast. in the context of its host range and trypanosome infection. *Acta Tropica* **45**, 339-349.

Sokal, R.R. & Rohlf, F.J. (1998) *Biometry*. W.H. Freeman & Company, New York, 887 pp.

Solano, P. & Amsler-Delafosse, S. (1995) *Trypanosoma congolense* chez différents espèces de taons (Diptera: Tabanidae) au Burkina Faso. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **48**, 145-146.

Solano, P., Agiro, L., Reifenberg, J.M., Yao Yao & Duvallet, G. (1995) Field application of the polymerase chain reaction (PCR) to the detection and characterization of trypanosomes in *Glossina longipalpis* (Diptera: Glossinidae) in Côte d'Ivoire. *Molecular Ecology* **4**, 781-785.

Soldan, A.W. & Norman, T.L. (1994) *Livestock disease evaluation project, dipping trial, 1993 report*. Livestock Disease Evaluation Unit, Lilongwe, 48pp.

Spielberger, U., Na'isa, B.K., Koch, K., Manno, A., Skidmore, P.R., & Coutts, H.H. (1979) Field trials with the synthetic pyrethroids permethrin, cypermethrin and decamethrin against *Glossina* (Diptera: Glossinidae) in Nigeria. *Bulletin of Entomological Research* **69**, 667-689.

Staak, C., Kämpel, U. & Korkowski, G. (1986). Species identification of blood-meals from tsetse flies: results 1979-1985. *Tropical Medicine and Parasitology* **37**, 59-60.

Steel W.S. (1958) The Broken Hill - Mulungushi dam tsetse eradication scheme: 1941 - 1956. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 7th meeting, Brussels, pp. 247 -267.

Steel, W.S. & Gledhill, J.A. (1955) *A survey of the distribution of Glossina species and factors influencing their control in the territory of Northern Rhodesia (Zambia).* Department of Veterinary and Tsetse Control Services, Lusaka, 82pp.

Stephen, L.E. (1986) *Trypanosomiasis. A veterinary perspective.* Pergamon Press, Oxford, 551 pp.

Sternberg, J. & Tait, A. (1990) Genetic exchange in African trypanosomes. *Trends in Genetics* **6**, 317-322.

Swallow, B.M. (1998) *Impacts of African Animal Trypanosomosis on migration, livestock and crop production.* Food and Agriculture Organization (FAO), Rome, 19pp.

Swallow, B.M. & Woudyalew, M. (1994) Evaluating willingness to contribute to a local public good: application of contingent valuation to tsetse control in Ethiopia. *Ecological Economics* **11**, 153-163.

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

Symes, C.B., Hadaway, A.B., Barlow, F., & Galley, W. (1948) Field experiments with DDT and Benzene Hexachloride against tsetse (*G. palpalis*). *Bulletin of Entomological Research* **38**, 591

Tarimo, S.A., Gates, D.B. & Williamson, D.L. (1981) Feeding preference of Glossina in Northeast Tanzania. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 17th meeting, Arusha, pp. 415-418.

Tarimo, S.A., Snow, W.F. & Butler, L. (1984) Trypanosome infections in wild tsetse *Glossina pallidipes* Austen on the Kenya coast. *Insect Science and its Application* **5**, 415-418.

Tarimo, S.A., Otieno, L.H. & Onyango, P. (1987) Mixed trypanosome infection in wild *Glossina pallidipes* Aust. *Insect Science and its Application* **8**, 25-27.

Tarimo Nesbitt, S.A., Njau, B.C. & Otieno, L.H. (1991) Epizootiology of trypanosomiasis in Lambwe Valley, Kenya, East Africa. *Insect Science and its Application* **12**, 379-384.

Taylor, K.A. (1998) Immune responses of cattle to African trypanosomes: protective or pathogenic? *International Journal for Parasitology* **28**, 219-240.

Thakersi, H. (1992) New records of tsetse flies in Eastern Zimbabwe. *Transactions of the Zimbabwe Scientific Association* **66**, 30-34.

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

Thompson, J.W. & Wilson, A. (1992) A review of developments in tsetse fly (*Glossina* spp.) control by application of insecticide to cattle. *Bulletin of Animal Health and Production in Africa* **40**, 1-4.

Thompson, M.C. (1987) The effect on tsetse flies (*Glossina* spp.) of deltamethrin applied to cattle either as a spray or incorporated into ear-tags. *Tropical Pest Management* **33**, 329-335.

Thrusfield, M. (1986) *Veterinary epidemiology*. Butterworths, Bodmin, 280 pp.

Torr, S.J. (1994) Response of tsetse flies (Diptera: Glossinidae) to warthog (*Phacochoerus aethiopicus* Pallas). *Bulletin of Entomological Research* **84**, 411-419.

Torr, S.J., Holloway, M.T.P., & Vale, G.A. (1992) Improved persistence of insecticide deposits on targets for controlling *Glossina pallidipes* (Diptera: Glossinidae). *Bulletin of Entomological Research* **82**, 525-533.

Torr, S.J., Hall, D.R. & Smith, J.L. (1995) Responses of tsetse flies (Diptera: Glossinidae) to natural and synthetic ox odour. *Bulletin of Entomological Research* **85**, 157-166.

Torr, S.J. & Mangwiro, T.N.C. (1996) Upwind flight of tsetse (*Glossina* spp.) in response to natural and synthetic host odour in the field. *Physiological Entomology* **21**, 143-150.

Torr, S.J., Hall, D.R., Phelps, R.J., & Vale, G.A. (1997) Methods for dispensing odour attractants for tsetse flies (Diptera: Glossinidae). *Bulletin of Entomological Research* **87**, 299-311.

Trail, J.C.M., Sones, K., Jibbos, J.M., Durikin, J., Light, D. & Max Murray (1985) *Productivity of Boran cattle maintained by chemoprophylaxis under trypanosomiasis risk*. ILCA, Addis Ababa, 76 pp.

Trail, J., d'Ieteren, G.D.M., Feron, A., Kakiese, O., Mulungo, M. & Pelo, M. (1991) Effect of trypanosome infection, control of parasitaemia and control of anaemia development on productivity of N'Dama cattle. *Acta Tropica* **48**, 37-45.

Trail, J.C.M., d'Ieteren, G.D.M., Murray, M., Ordner, G., Yangari, G., Maille, J.C., Viviani, P., Colardelle, C., & Sauveroche, B. (1993) Measurements of trypanotolerance criteria and their effect on reproductive performance of N'Dama cattle. *Veterinary Parasitology* **45**, 241-255.

Truc, P., Aerts, D., Mcnamara, J.J., Claes, Y., Allingham, R., Le Ray, D. & Godfrey, D.G. (1992) Direct isolation *in vitro* of *Trypanosoma brucei* from man and other animals, and its potention value for the diagnosis of gambien trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 627-629.

Tsetse and Trypanosomiasis Control Branch (TTCB) (1991) *Senior Tsetse Field Offier (STFO) - monthly report. April 1991.* Department of Veterinary Services, Harare, 4 pp.

Tsetse and Trypanosomiasis Control Branch (TTCB) (1992) *Annual report of the Tsetse and Trypanosomiasis Control Branch.* Department of Veterinary Services, Harare, 46 pp.

Turner, M.J. (1985) The biochemistry of the surface antigens of the African trypanosomes. *British Medical Bulletin* **41**, 137-143.

Turner, C.M.R., Barry, J.D. & Vickerman, K. (1988) Loss of variable antigen during transformation of *Trypanosoma brucei rhodesiense* from bloodstream to procyclic forms in the tsetse fly. *Parasitology Research* **74**, 507-511.

Uilenberg, G. & Giret, M. (1972) Etudes immunologiques sur les trypanosomes. I. Existence d'un type antigénique de base chez une souche de *Trypanosoma congolense* Broden, 1904. Variations aprés transmission cyclique. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **25**, 37-52.

Umali, D.L., Feder, G. & De Haan, C. (1994) Animal health services: finding the balance between public and private delivery. *World Bank Research Observer* **9**, 71-96.

Urquhart, G.M. (1980) The pathogenesis and immunology of African trypanosomiasis in domestic animals. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 726-729.

Vail, L. (1977) Ecology and history: the example of eastern Zambia. *Journal of Southern African Studies* **3**, 129-155.

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

Vale, G.A. (1977) Feeding responses of tsetse flies (Diptera: Glossinidae) to stationary hosts. *Bulletin of Entomological Research* **67**, 635-649.

Vale, G.A. (1980) Field studies of the response of tsetse flies (*Glossina*) and other diptera to carbon dioxide, acetone and other chemicals. *Bulletin of Entomological Research* **70**, 563-570.

Vale, G.A. (1982a) The trap orientated behaviour of tsetse flies and other diptera. *Bulletin of Entomological Research* **72**, 71-93.

Vale, G.A. (1982b) The improvement of traps for tsetse flies. *Bulletin of Entomological Research* **72**, 95-106.

Vale, G.A. (1993a) Visual responses of tsetse flies (Diptera: Glossinidae) to odour-baited targets. *Bulletin of Entomological Research* **83**, 277-289.

Vale, G.A. (1993b) Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. *Journal of Medical Entomology* **30**. 831-842.

Vale, G.A. (1998) Responses of tsetse flies (Diptera: Glossinidae) to vegetation in Zimbabwe: implications for population distribution and bait siting. *Bulletin of Entomological Research* **80 Supplement 1**, 1-59.

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

Vale, G.A. & Phelps, R.J. (1978) Sampling problems with tsetse flies. *Journal of Applied Ecology* **15**, 715-726.

Vale, G.A. & Hall, D.R. (1985a) The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp., to ox odour. *Bulletin of Entomological Research* **75**, 209-217.

Vale, G.A. & Hall, D.R. (1985b) The role of 1-octen-3-ol, acetone and carbon dioxide to improve baits for tsetse flies. *Bulletin of Entomological Research* **75**, 219-231.

Vale, G.A., Hargrove, J.W., Cockbill, G.E., & Phelps, R.J. (1986) Field trials of baits to control populations of *Glossina morsitans morsitans* Westwood and *Glossina pallidipes* Austeni. *Bulletin of Entomological Research* **76**, 179-194.

Vale, G.A., Lovemore, D.F., Flint, S. & Cockbill, G.F. (1988a) Odour-baited targets to control tsetse flies, *Glossina* spp., in Zimbabwe. *Bulletin of Entomological Research* **78**, 31-49.

Vale, G.A., Hall, D.R. & Gough, A.J.E. (1988b) The olfactory responses of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to phenols and urine in the field. *Bulletin of Entomological Research* **78**, 293-300.

Vale, G.A., Mutika, G. & Lovemore, D.F. (1999) Insecticide-treated cattle for controlling tsetse flies (Diptera: Glossinidae): some questions answered, many posed. *Bulletin of Entomological Research*, In press.

Van den Bossche, P., Van Hees, J. & Mortelmans, J. (1987) Observations on the persistence of deltamethrin acaricide liquid on tsetse flies under laboratory conditions. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 19th meeting, Lome, pp. 422-424.

Van den Bossche, P. & Mudenge, D. (1997) Prevalence of tsetse-transmitted trypanosomosis along the eastern/north eastern border of Zimbabwe. *Zimbabwe Veterinary Journal* **28**, 49-59.

Van den Bossche, P. & Hargrove, J.W. (1999) Seasonal variation in nutritional levels of male tsetse flies *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) caught using fly-rounds and electric screens. *Bulletin of Entomological Research* **89**, 381-387.

Vanderplank, F.L. (1947) Experiments with DDT on various species of tsetse flies in the field and laboratory. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **40**, 603-620.

Vaughan-Jones, T.G.C. (1948) A short survey of the aims and functions of the game and tsetse control department of Northern Rhodesia. *Rhodes-Livingstone Institute Journal* **6**, 37-47.

Vickerman, K. (1985) Developmental cycles and biology of pathogenic trypanosomes. *British Medical Bulletin* **2**, 105-114.

Vickerman, K., Tetley, L., Hendry, K.A.K. & Turner, M.R. (1988) Biology of African trypanosomes in the tsetse fly. *Biology of the Cell* **64**, 109-119.

Voller, A., Bidwelll, D.E. & Bartlett, A. (1975) A serological study on human *Trypanosoma rhodesiense* infections using microscale ELISA. *Tropenmedizin und Parasitologie* **26**, 247-251.

Wacher, T.J., Rawlings, P. & Snow, W.F. (1993) Cattle migration and stocking densities in relation to tsetse-trypanosomiasis challenge in The Gambia. *Annals of Tropical Medicine and Parasitology* **87**, 517-524.

Wacher, T.J., Milligan, P.J.M., Rawlings, P. & Snow, W.F. (1994) Tsetse-trypanosomiasis challenge to village N'Dama cattle in The Gambia: field assessments of spatial and temporal patterns of tsetse-cattle contact and the risk of trypanosomiasis infection. *Parasitology* **109**, 149-162.

Watkins, T.I. & Woolfe, G. (1952) Effect of changing the quaternizing group on the trypanocidal activity of dimidium bromide. *Nature* **169**, 506.

Watkins, T.I. & Woolfe, G. (1956) Prophylaxis of trypanosome infections in cattle. *Nature* **178**, 368

Weitz, B. (1963). The feeding habits of *Glossina*. *Bulletin of the World Health Organization* **28**, 711-729.

Weitz, B. & Glasgow, J.P. (1956) The natural hosts of some species of *Glossina* in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **50**, 593-612.

Welburn, S.C., Maudlin, I. & Ellis, D.S. (1989) Rate of trypanosome killing by lectins in midguts of different species and strains of *Glossina*. *Medical and Veterinary Entomology* **3**, 77-82.

Welburn, S.C. & Maudlin, I. (1992) The nature of the teneral state in *Glossina* and its role in the acquisition of trypanosome infection in tsetse. *Annals of Tropical Medicine and Parasitology* **86**, 529-536.

Wellde, B.T., Kovatch, R. & Chumo, D. (1978) *Trypanosoma congolense*: thrombocytopaenia in experimentally infected cattle. *Experimental Parasitology* **36**, 6-19.

Wellde, B.T., Reardon, M.J., Onyango, F., Chumo, D.A., Muriithi, R.M. & Roberts, L.M. (1989) Natural and acquired resistance to *Trypanosoma vivax* in cattle. *Annals of Tropical Medicine and Parasitology* **83 Supplement 1**, 185-194.

Wells, E.A. (1972) The importance of mechanical transmission in the epidemiology of Nagana: a review. *Tropical Animal Health and Production* **4**, 74-89.

Whellan, J.A. (1950) Tsetse fly in S. Rhodesia; 1949. *Rhodesian Agricultural Journal* **47**, 416-427.

Whitelaw, D.D., Scott, J.M., Reid, H.W., Holmes, P.H., Jennings, F.W. & Urquhart, G.M. (1979) Immunosuppression in bovine trypanosomiasis: studies with louping-ill vaccine. *Research in Veterinary Science* **26**, 102-107.

Whitelaw, D.D. & Jordt, T. (1985) Colostral transfer of antibodies to *Trypanosoma brucei* in goats. *Annales de la Société Belge de Médicine Tropicale* **65**, 199

Whiteside, E.F. (1949) An experiment in control of tsetse with DDT-treated oxen. *Bulletin of Entomological Research* **40**, 123-134.

Whiteside, E.F. (1960) Recent work in Kenya on the control of drug-resistant cattle trypanosomiasis. *International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*, 8th meeting, Jos, pp. 141 -154.

Wiersma, E., & Schoonman, L. (1992). *Lusu spoton trial Sesheke.* Department of Veterinary Services Zambia, Mongu, 14 pp.

Wijers, D.J.B. (1958) Factors that may influence the infection rate of *Glossina pallidipes* with *Trypanosoma gambiense* I. The age of the fly a the time of the infective feed. *Annals of Tropical Medicine and Parasitology* **52**, 385-390.

Wijers, D.J.B. (1959) Polymorphism in *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, and the significance of intermediate forms. *Annals of Tropical Medicine and Parasitology* **53**, 59-68.

Willemse, L. (1991) A trial of odour baited targets to control the tsetse fly, *Glossina morsitans centralis* (Diptera: Glossinidae) in west Zambia. *Bulletin of Entomological Research* **81**, 351-357.

Willemse, L., Mwangelwa, M.I. & Mwanza, L.M. (1983) *A short term study on the ecology and trypanosome infection rate of a* Glossina *population in an endemic sleeping sickness focus of Luangwa valley, Zambia.* State University of Leiden, Leiden, 93 pp.

Willett, K.C. (1955) A special method for the dissection of *Glossina*. *Annals of Tropical Medicine and Parasitology* **49**, 376-383.

Williams, D.J.L., Naessens, J. & Scott, J.T. (1991) Analysis of peripheral leucocyte population in N'Dama and Boran cattle following a rechallenge infection with *Trypanosoma congolense*. *Parasite Immunology* **13**, 171-185.

Williams, D.J.L., Taylor, K.A., Newson, J. & Gichuki, B. (1996) The role of anti-variable surface glycoprotein antibody responses in bovine trypanotolerance. *Parasite Immunology* **18**, 209-218.

Wilson, A.J. (1969) Value of the indirect fluorescent antibody detection test as a serological aid to diagnosis of *Glossina*-transmitted bovine trypanosomiasis. *Tropical Animal Health and Production* **1**, 89-95.

Wilson, A.J. & Cunningham, M.P. (1971) Immunological aspects of bovine trypanosomiasis IV. Patterns in the production of common antibodies. *Tropical Animal Health and Production* **3**, 133-139.

Wilson, A.J., Dar, F.K. & Paris, J. (1972) A study on the transmission of salivarian trypanosomes isolated from wild tsetse. *Tropical Animal Health and Production* **4**, 14-22.

Wilson, A.J., Paris, J., Luckins, A.G., Dar, F.K. & Gray, A.R. (1976) Observations on a herd of beef cattle maintained in a tsetse area II. Assessment of the development of immunity in association with trypanocidal drug treatment. *Tropical Animal Health and Production* **8**, 1-12.

Wilson, V.J. (1975) Game and tsetse fly in Eastern Zambia. *Occasional papers of the National Museums and Monuments of Rhodesia, Series B, Natural Sciences* **5**, 339-404.

Woo, P.T.K. (1970) The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica* **27**, 384-386.

Woolhouse, M.E.J. (1989) On the interpretation of age-prevalence curves for schistosome infections of host snails. *Parasitology* **99**, 47-56.

Woolhouse, M.E.J., Hargrove, J.W. & Mcnamara, J.J. (1993) Epidemiology of trypanosome infections of the tsetse fly *Glossina pallidipes* in the Zambezi Valley. *Parasitology* **106**, 479-485.

Woolhouse, M.E.J., Bealby, K., Mcnamara, J.J. & Silutongwe, J. (1994) Trypanosome infections of the tsetse fly *Glossina pallidipes* in the Luangwa Valley, Zambia. *International Journal for Parasitology* **24**, 987-993

Woolhouse, M.E.J. & Hargrove, J.W. (1998) On the interpretation of age-prevalence curves for trypanosome infections of tsetse flies. *Parasitology* **116**, 149-156.

Wragg, W.R., Washbourne, K., Brown, K.N. & Hill, J. (1958) Metamidium: a new trypanocidal drug. *Nature* **182**, 1005-1006.

Wright, P.F., Nilsson, E., Van Rooij, E.M.A., Lelenta, M. & Jeggo, M.H. (1993) Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. *Revue Scientifique technique de l'office International des Epizooties* **12**, 435-450.

Zwart, D., Perie, N.M., Keppler, A. & Goedbloed, E. (1973) A comparison of methods for the diagnosis of trypanosomiasis in East African domestic ruminants. *Tropical Animal Health and Production* **5**, 79-87.

PARTS OF THE RESULTS PRESENTED IN THIS THESIS HAVE BEEN PUBLISHED OR ACCEPTED FOR PUBLICATION:

Van den Bossche, P. (1997) The control of *Glossina morsitans morsitans* (Diptera: Glossinidae) in a settled area in Petauke District (Eastern Province, Zambia) using odour-baited targets. *Onderstepoort Journal of Veterinary Research* **64**, 251-257.

Van den Bossche, P. & Staak, C. (1997) The importance of cattle as a food source for *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae) in Katete District, Eastern Province, Zambia. *Acta Tropica* **65**, 105-109.

Van den Bossche, P. & Duchateau, L. (1998) The effect of deltamethrin pour-on applied to cattle on the transmission of bovine trypanosomosis. *Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux* **51**, 123-126.

Van den Bossche, P. & Mudenge, D. (1999) The effect of short-interval deltamethrin applications, to control tsetse, on the prevalence of babesiosis. *Tropical Animal Health and Production* **31**, 215-222.

Van den Bossche, P., Mudenge, D., Mubanga, J. & Norval, A. (1999) The parasitological and serological prevalence of tsetse-transmitted bovine trypanosomosis in the Eastern Caprivi (Caprivi District, Namibia). *Onderstepoort Journal of Veterinary Research* **66**, 103-110.

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

Van den Bossche, P., Shumba, W. & Makhambera, P. (2000) The distribution and epidemiology of bovine trypanosomosis in Malawi. *Veterinary Parasitology* **88**, 163-176.

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

Van den Bossche, P., Chigoma, D., & Shumba, W. (In press) The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs in the absence of tsetse challenge. *Acta Tropica*.

Van den Bossche, P., Shumba, W., Njagu, C., & Shereni, W. (In press) The distribution of bovine trypanosomosis in Zimbabwe and an evaluation of the usefulness of the anti-trypanosomal antibody detection ELISA as a tool for monitoring the effectiveness of tsetse control operations. *Tropical Animal Health and Production*.

A PART OF THE RESULTS PRESENTED THIS THESIS HAS BEEN PRESENTED AS A PAPER AT A SCIENTIFIC MEETING:

Doran, M. & Van den Bossche, P. (1999) An assessment of the socio-economic impact of bovine trypanosomosis and its control in the southern African region. *25th meeting of the International Scientific Council for Trypanosomiasis Research and Control*, Mombasa, Kenya.

A PART OF THE RESULTS PRESENTED IN THIS THESIS HAS BEEN PRESENTED AS A POSTER AT A SCIENTIFIC MEETING:

Munsimbwe, L., Van den Bossche, P., Jooste, R., Mubanga, J., & Lumamaba, D. (1999) Preliminary results of a large-scale field trial to assess the effect of 1% cyfluthrin pour-on (Cylence®, Bayer) treatment of cattle on the incidence of bovine trypanosomosis in a tsetse-infested area of eastern Zambia. *25th meeting of the International Scientific Council for Trypanosomiasis Research and Control*, Mombasa, Kenya.

ANNEX 1