# THE EPIDEMIOLOGY OF LIVESTOCK TRYPANOSOMOSIS IN A TRYPANOSOMOSIS ENDEMIC AREA OF EASTERN ZAMBIA

**Humphrey Simukoko**

**Dissertation submitted in fulfilment of the requirement for the degree of Doctor (Ph.D.) in Veterinary Science.**

**Promotors:**

**Prof. Dr. P. Van den Bossche**
**Prof. Dr. J. Vercruysse**

**Laboratory of Parasitology,**
**Department of Virology, Parasitology and Immunology,**
**Faculty of Veterinary Medicine,**
**Gent University, Salisburylaan, 133, Merelbeke.**

**This thesis is dedicated to my late brother, Dominic Musatwe Simukoko-my inspiration in academia.**

**Acknowledgements**

Training of citizens to the PhD level has become an essential, inevitable and priority undertaking for most developing countries because highly educated individuals are an essential ingredient in economic development. A research undertaking of the magnitude of a PhD requires substantial financial investment. In sub-Saharan Africa, and particularly in Zambia, governments are unable to provide that financial assistance to their citizens. The void in providing financial assistance for higher education training to developing countries has been ably filled by co-operating partners such as the Flemish inter university Council (VLIR). My PhD programme was fully sponsored by the VLIR. I therefore sincerely thank VLIR for providing the financial assistance in all areas of my PhD programme.

I am grateful to my employer, The University of Zambia (UNZA), for giving me a four-year paid leave to enable me to undertake the PhD training.

My technical skills in molecular biology and other laboratory techniques were largely mastered at the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. I am grateful to the ITM directorate and management and specifically to the ITM staff in the Animal Health Department. In particular, I would like to thank the following who were directly involved: Prof. P. Dorny for accepting me as a student at ITM; Dr. Geysen for guiding me during the molecular biology training; Dr. T. Marcotty for statistical analysis and guidance and; all the technical staff in the molecular biology laboratory.

The intellectual guidance of the entire PhD thesis was undertaken by my promoter Prof. P. Van den Bossche of the ITM. I am very grateful for his mentorship and counsel. I also recognize the great contributions of Prof. J. Vercruysse of the Laboratory of parasitology of Ghent University for his intellectual guidance and as facilitator of my PhD programme.

I thank the two people in Zambia who were instrumental in ensuring that my PhD programme progressed smoothly. To Dr. I.K. Phiri and Dr. S. Gabriel I say many

thanks. I thank Mr. Rodgers Phiri, who has since passed on, for his assistance during the molecular analyses of my samples at UNZA School of veterinary medicine.
I also thank the veterinary Department of the Eastern Province of Zambia for facilitating the survey that was conducted in 2003.

To my family, I salute all of you for being patient and understanding during the whole PhD training programme.

Lastly, but definitely not the least, I thank all the livestock farmers in Eastern Province who willingly provided their animals for the various aspects of my research.

*TABLE OF CONTENTS*

**List of abbreviations**

| | |
|---|---|
| AAT: | African Animal Trypanosomosis |
| Bp: | Base pair |
| DDT: | Dichloro--diphenyl-trichlorethylene |
| DNA: | Deoxynucleic Acid |
| dNTP: | dinucleotide tri-phosphate |
| Eco: | E-coli |
| EDTA: | Ethylenediamine tetraacetic acid |
| ELISA: | Enzyme Linked Immunosorbent Assay |
| HCH: | Hexachlorocyclohexane |
| HCL: | Hydrochloric Acid |
| KCL: | Potassium Chloride |
| MgCl: | Magnesium Chloride |
| m | Millimolar |
| PATTEC: | Pan African Tsetse and Trypanosomosis Eradication Campaign |
| PCR: | Polymerase Chain Reaction |
| PCV: | Packed Cell Volume |
| Pmol: | Picomole |
| RFLP: | Restriction Fragment Polymorphism |
| SSA: | sub-Saharan Africa |
| Taq: | Thermus aqueous |
| TBE: | Tris/Borate/EDTA |
| µl: | Microlitre |
| U: | Unit |
| V: | Volts |

**Figures, tables and annexes**

# General introduction

African Animal Trypanosomosis (AAT) is one of the economically most important livestock diseases in sub-Saharan Africa (hereafter designated SSA). It is important because it causes extensive economic losses and retardation in livestock productivity and rural development. Substantial resources are directed towards the control and management of livestock trypanosomosis (Swallow, 1998; Holmes, 1997). If trypanosomosis was not present, the resources currently used to curb the scourge of this disease could be channelled towards improving the living standards of the impoverished people in the tsetse-infested areas of SSA.

Tsetse flies, the insects that transmit the parasites that cause livestock trypanosomosis, are mostly found in wildlife zones. Therefore, livestock trypanosomosis mostly occurs in areas that border wildlife zones or those areas that were previously occupied by wildlife. It can be argued that livestock trypanosomosis would not be such a problem if livestock were reared away from tsetse-infested zones. However, it is not possible to prevent people in SSA from settling in tsetse-infested areas. This is due to the fact that the population in SSA keeps increasing and the people, who are highly dependent on natural resources for their survival, are continuously in search of fertile farming land. Thus, there is no choice but to continue looking for solutions that will either completely eliminate the tsetse fly and trypanosomosis in SSA or at least drastically reduce, in a sustainable way, its impact on livestock production.

A large range of trypanosomosis control methods has been developed. They are based on tsetse control, parasite control using anti-trypanocidal agents and/or the use of trypanotolerant livestock. Various tsetse control methods have been implemented successfully. However, the long-term success of those campaigns is usually threatened by the danger of reinvasion by tsetse of previously cleared areas. Although tsetse fly eradication is the ultimate goal, the eradication of the fly from the African continent will not be achieved in the near future.

Few anti-trypanocidal agents have been developed and are currently in use in most SSA countries that are afflicted by trypanosomosis. The anti-trypanocidal agents, currently in use, continue to help considerably in alleviating the impact of trypanosomosis in those countries. However, the drawback of this trypanosomosis control approach is the development of resistance by trypanosomes to most anti-trypanocidal drugs (Peregrine *et al*., 1991; Peregrine, 1994; Sinyangwe *et al*., 2004). Despite the development of (multiple) drug

resistance, very little research is currently directed towards developing new, cheap and non-toxic anti-trypanocidal agents.

In some SSA countries, livestock breeds that possess inherent trypanotolerant traits have been selected as livestock that can be reared in tsetse-infested and trypanosomosis-affected areas (Roberts and Gray, 1973; Mwangi *et al*., 1993). However, those trypanotolerant livestock are found mainly in western Africa.

The most effective and cost-effective way of combating infectious diseases is arguably immunization. Immunization makes an animal to remain protected against specific infectious diseases for long periods. However, in as far as trypanosomosis is concerned, the concept of immunization is rather elusive. The trypanosomes have an inherent knack for evading the immune system of an animal by constantly switching the characteristics of their surface antigens in a phenomenon called "antigenic variation" (Vickerman, 1985). Thus, immunization against trypanosomosis remains a pipedream.

Strategic disease control entails that control efforts are applied in such a way that the impact of the control measures is effective in reducing the incidence of the disease. This requires understanding certain facets of the epidemiology of the disease. The current school of thought, in as far as the control and management of trypanosomosis is concerned, is that use has to be made of the most appropriate range of currently available trypanosomosis control and management practices based on an efficient utilization of available resources. Resources can only be efficiently used if they are applied strategically and focused. This, however, requires a thorough understanding of specific local epidemiological situations so that its peculiarities can be exploited leading to effective and sustainable trypanosomosis control.

Recent studies have shown that in southern Africa the trypanosomosis epidemiological setting can vary substantially (Van den Bossche, 2001). The epidemiological circumstance where cattle are kept in a tsetse-infested area and have become the main host of the tsetse flies is of considerable economic importance because such places are important agricultural areas. Such a situation occurs on the plateau of eastern Zambia. The purpose of the research described in this thesis was to investigate in more detail the epidemiological specificities of this epidemiological setting and exploit its particularities in the control of the disease in livestock.

**References**

Holmes, P.H., (1997) New approaches to the integrated control of trypanosomosis. *Veterinary Parasitology,* **110**, 117-122.

Mwangi, E., Stevenson, P., Gettinby, G. and Murray, M. (1993) Variation in susceptibility to tsetse borne trypanosomosis among *Bos indicus* cattle breeds in East Africa. In: Sones, K. (ed) *Proceedings of the 22nd meeting of the International Scientific Council for Trypanosomosis Research and Control*. Publication no. **117**, pp. 125-128.

Peregrine, A.S. (1994) Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology,* **54**, 223-248.

Peregrine, A.S., Knowles, G., Ibitayo, A.I., Scott, J.R., Moloo, S.K. and Murphy, N.B. (1991) Variation in resistance to Isometomedium chloride and dimenazene aceturate by clones derived from a stock of *Trypanosoma congolense*. *Parasitology*, **102**, 93-100.

Roberts, C.J. and Gray, A.R. (1973) Studies on trypanosome-resistant cattle. I. The breeding and growth performance of N'Dama, Muturu and Zebu cattle maintained under the same conditions of husbandry. *Tropical Animal Health and Production*, **5**, 211-219.

Sinyangwe, L., Delespaux, V., Brandt, J., Geerts,S., Mubanga,J., Machila, N., Holmes, P.H. and Eisler, M.C. (2004) Trypanocidal drug resistance in eastern province of Zambia. *Veterinary Parasitology*, **119**, 125-135.

Swallow, B.M. (1998) *Impacts of African Animal Trypanosomosis on migration, livestock and crop production.* Food and Agriculture Organization (FAO), Rome, 19pp.

Vickerman, K. (1985) Developmental cycles and biology of pathogenic trypanosomes. *British Medical Bulletin,* **2**, 105-114.

Van den Bossche, P., (2001) Some general aspects of the distribution and epidemiology of bovine trypanosomsosis in southern Africa. *International Journal for Parasitology*, **31**, 592-598.

# CHAPTER 1

# The epidemiology and control of tsetse-transmitted trypanosomosis in southern Africa: A review

## 1.1. Introduction

To address the problems posed by tsetse-transmitted trypanosomoses in Africa, the African Union, through the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), has embarked on a tsetse and trypanosomosis (T&T) eradication campaign as the only way to effectively deal with the scourge of the disease (Kabayo, 2002). According to PATTEC the implementation of the protocol for eradicating the tsetse flies will be based on the concept, strategy, viability and principles of the area-wide approach in pest control in the context of the transboundary nature of the T&T problem.

Although there has been political commitment of Africa's leaders to the objectives of rendering Africa tsetse-free in the shortest possible time, it is unlikely that tsetse and trypanosomosis will be eradicated in the "shortest possible time". This is because the areas to be covered are vast, the T&T problem is complex and it may take a long time to mobilize the resources required to accomplish this task. In the meantime, however, tsetse-transmitted trypanosomosis will continue to threaten livestock production in areas where susceptible livestock are kept in tsetse-infested zones and where the disease is endemic.

The current livestock health policies in many southern African countries require active involvement of the private sector and the community in the control of many animal diseases, including trypanosomosis. This means that the individual livestock keepers are responsible for the costs associated with the control of animal trypanosomosis. The rural livestock holders in sub-Saharan countries, who are adversely affected by trypanosomosis, are mostly resource poor people. This has implications for the entire approach to tsetse and trypanosomosis control. Indeed, the tsetse and trypanosomosis control methods of choice in endemic areas have to be adapted to the local conditions, i.e. prevailing agro and socio-economic conditions and user and environmentally friendly.

Two interesting options are available for localized, geographically restricted T&T control. They are the use of insecticide-treated livestock and/or the use of trypanocidal drugs. It is logical to assume that any livestock disease control strategy to be used by resource poor farmers must be affordable and effective. Hence, for trypanosomosis, intervention methods must be effective in killing tsetse flies and/or in reducing the incidence and/or prevalence of trypanosomosis in livestock. In addition,

for these methods to be sustained by local livestock owners they must be affordable, i.e. control should be achieved at the lowest possible cost or strategies should be developed to reduce animal drug treatment frequency without jeopardizing effectiveness. This can be realized by targeting treatment with curative trypanocides in infected animals only and by using prophylactic treatment in animals that are challenged most during periods of highest T&T pressure. In addition, insecticide-treatments can be selectively applied on that fraction of the herd that is challenged most during periods of the year when challenge is highest.

In order to determine whether it is possible to further reduce the cost and improve effectiveness of available tsetse and trypanosomosis control options based on the conditions mentioned above, we need to fully understand the epidemiology of endemic livestock trypanosomosis. The objective of the subsequent sections of this chapter is to explore the various aspects of the epidemiology of trypanosomosis that could possibly contribute to the improvement of the control of trypanosomosis in the endemic areas. Through a critical analysis of the available literature related to the subject dealt, a number of knowledge gaps were identified and further investigated. The outcome of the research will contribute to the improved control of endemic livestock trypanosomosis in resource poor livestock-agricultural settings by increasing the effectiveness and cost-efficiency of available tools.

## 1.2. General aspects of livestock trypanosomosis

Trypanosomosis, caused by trypanosomes (protozoan of the genus *Trypanosoma*), can be included in the list of several debilitating long-term diseases occurring in sub-Saharan Africa. In humans, the disease is called sleeping sickness and Nagana in domestic animals. In the southern African region, the human disease is relatively rare, occurring mainly in a few foci (Van den Bossche and Vale, 2000). Nagana, on the other hand, is more common and it can virtually occur in all areas where tsetse and domestic animals co-exist or enter into contact (Van den Bossche and Vale, 2000). The epidemiology of livestock trypanosomosis is complex, varies from one locality to another and depends on the interaction between the tsetse fly, the parasite and the host. Anthropogenic drivers and livestock movements influence the degree of contacts between the vector (tsetse fly) and the host (humans, livestock) and, thus, affecting the epidemiology of trypanosomosis.

The causative parasites are known by the generic name “trypanosomes”, single-celled protozoans, zoologically classified, from their morphology, as:

Kingdom: Protista
Subkingdom: Protozoa
Phylum: Sarcomastigophora
Subphylum: Mastigophora
Class: Zoomastigophora
Order: Kinetoplastida
Family: Trypanosomatidae
Section: Salivaria
Genus: *Trypanosoma*

Another classification is based on the mode of vectorial transmission. Salivarian trypanosomes are those that undergo cyclic development in their vector (the tsetse fly) before they are transmitted to the host. Stercorarian trypanosomes are those that are transmitted to the host without undergoing cyclic transformation. Stercorarian trypanosomes are of little or negligeable economic importance in sub-Saharan Africa. Salivarian trypanosomes, on the other hand, are economically important in tsetse infested areas of sub-Saharan Africa and will, therefore, be described in some detail.

Salivarian trypanosomes can be divided into three major sub-genera: *Duttonella, Nannomonas* and *Trypanozoon*. The most economically important and widely spread species affecting domestic ruminants are, within the *Duttonella* sub-genus, *Trypanosoma vivax* and within the *Nannomonas* and *Trypanozoon* genera *T. congolense* and *T. brucei s.l.*, respectively. In southern Africa and East Africa *T. congolense* is considered the most pathogenic species in domestic ruminants. *Trypanosoma congolense* belongs to a group of small size trypanosomes with medium sized marginal kinetoplasts, no flagellum and a poorly developed undulating membrane. *Trypanosoma vivax* is the most important causative species in West African cattle (Stephen, 1986). Morphologically, it has a large kinetoplast, a distinct free flagellum and an inconspicuous undulating membrane. Within the sub-genus *Trypanozoon*, *T. brucei s. l.* are two closely related sub-species, *T. b. rhodesiense* and *T. b. gambiense*. These two trypanosomes, together with *T. b. brucei*, are the causative agents of human trypanosomosis or sleeping sickness.

Trypanosomes are mainly and cyclically transmitted to hosts by tsetse flies. Tsetse flies are two-winged, male and female blood feeding insects of the Order *Diptera*, family *Glossinidae*, Genus *Glossina* (Buxton, 1955). The Genus *Glossina* is sub-divided into three sub-genera: *morsitans*, also know as *Glossina* group, *Nemorhina* or *palpalis* group and *Austenina* or *fusca* group. The *morsitans* group inhabits most of the grassy woodlands of Africa. Their distribution is limited to the South by low temperatures and to the North by hot dry conditions. Within their distribution range, they are highly dependent on the presence of game animals. The *morsitans* tsetse group is considered an efficient vector of pathogenic trypanosomes.

The *palpalis* group inhabits the humid parts of Africa. When penetrating drier areas, the flies of this group are confined to dense riverine or lake shore vegetation. In comparison with the *morsitans* group, they are less efficient vectors of trypanosomes (Madubunyi, 1978; Laveissiere *et al*., 1990). Most of the *fusca* group species inhabit the thickly forested humid areas of West and Central Africa. Some species of this tsetse group need the denser canopy of rain forests while others thrive at the forest edge.

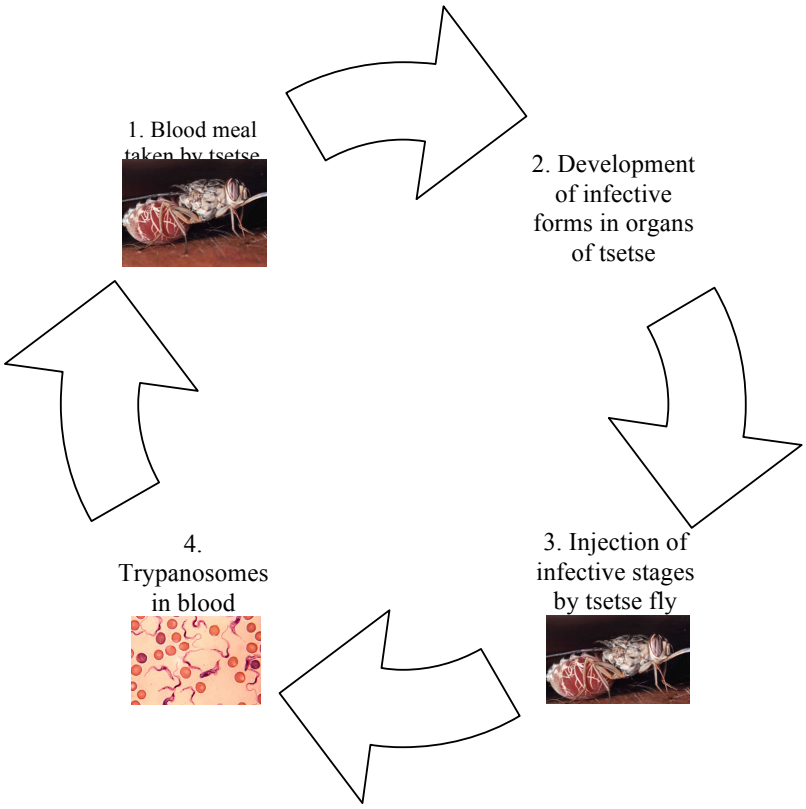
Trypanosomes can be transmitted cyclically only by tsetse flies. The trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on a susceptible host. Trypanosomes can also be mechanically transmitted by tsetse and other biting insects through the transfer of infected blood from one animal to another. This mode of transmission is negligeable in the epidemiology of Animal African Trypanosomosis (AAT).

All species of domesticated livestock can succumb to infections with pathogenic trypanosomes. Cattle, small ruminants, pigs, equines, and camelidae are all susceptible to AAT and, once infected, they show different clinical signs. The disease may have a sub-clinical, mild, chronic or acute course. If untreated, the disease is often fatal. Clinical manifestations of the disease vary according to the trypanosome species, the virulence of the strain and the susceptibility of the host species infected (Masumu *et al*., 2006). Acute disease is characterized by anaemia, weight loss, abortion and, if not treated, death. In some instances, infected animals show no overt signs of disease but can succumb if stressed, for example, by work, pregnancy, milking, adverse environmental and nutrition conditions and intercurrent pathological stress (Luckins, 1988).

### 1.3. The tsetse-transmitted trypanosomosis transmission cycle

Cyclic transmission of trypanosomes is the epidemiologically most important transmission cycle. When a tsetse fly feeds on the blood of a parasitized host it also ingests blood-stream forms of the trypanosome. Within the tsetse fly, now the vector of the parasite, a series of changes take place in the trypanosome before transformation to the infecting metatrypanosome occurs. In the course of the next blood meal, the infective metatrypanosomes (metacyclic forms) will be transmitted to another host. The life cycle of livestock trypanosomosis is shown in figure 1.1.

**Figure 1.1.** The tsetse-transmitted trypanosomosis transmission cycle




In mechanical transmission, the parasite does not undergo cyclic development in the vector; the parasite is merely picked by haematophagus insects (including tsetse flies) on their probosces and in a vey short time transferred and deposited into susceptible host through the bite of the insect.

## 1.4. Epidemiology of livestock trypanosomosis

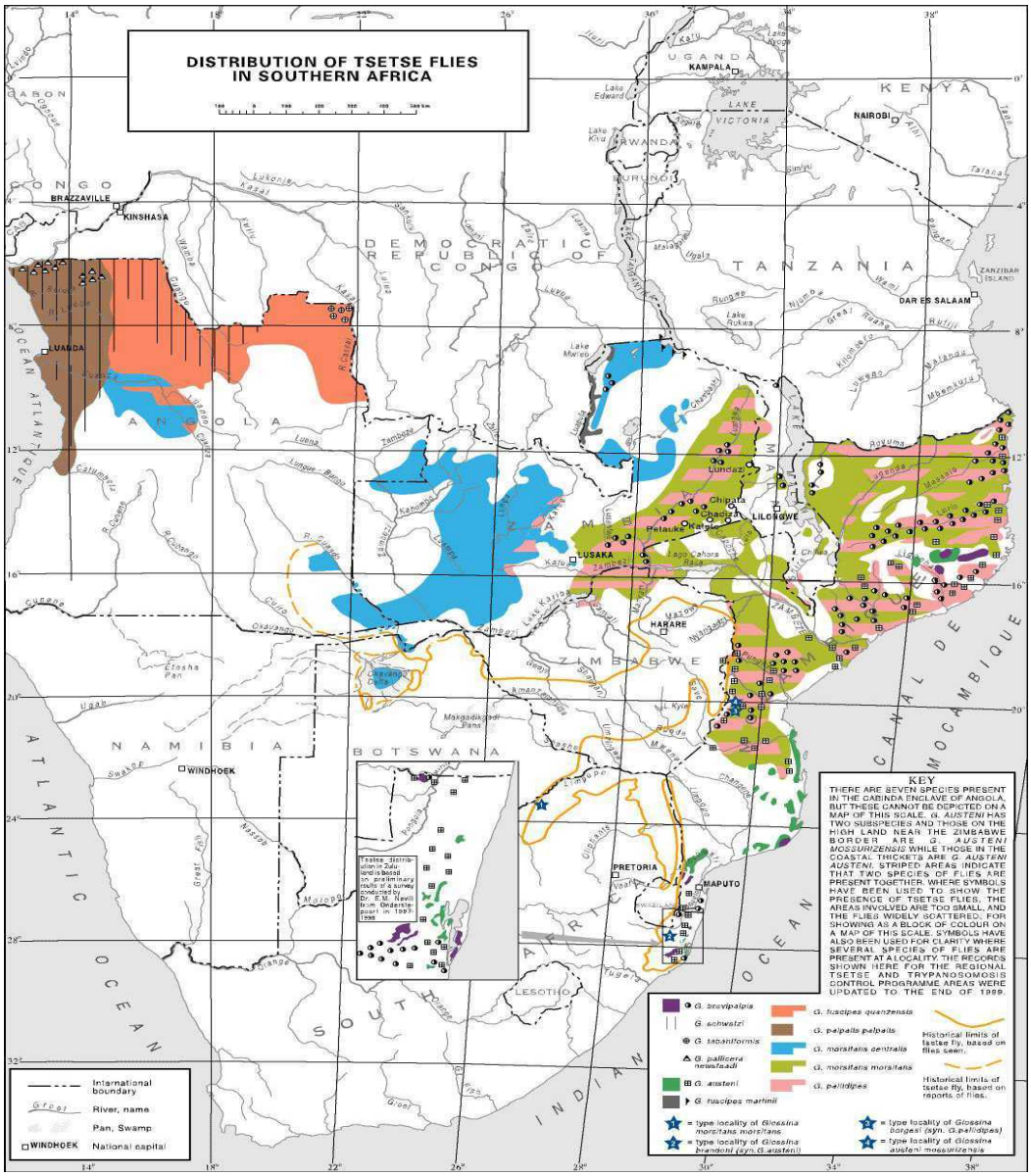
### 1.4.1. Epidemiological settings

The geographic distribution, the incidence of the disease and its economic impact on livestock production are under the influence of many factors, such as density of vectors and of the hosts (both domestic and wild animals) and local husbandry practices such as seasonal transhumance. All these factors intervene in the epidemiological situation and drive the method(s) used for disease and vector control. A major factor in the epidemiology of trypanosomosis is the susceptibility of the host to acquire the parasite; this is a function of the trypanosome-vector-host interactions. In general, the higher the densities of infected tsetse flies in the vicinity of a susceptible host on which tsetse feed, the higher the probability of that host to become infected. Hence and simplifying, the relative densities of tsetse, the trypanosome infection rates in their population and the proportion of feeds that they take from animals determine the trypanosomosis challenge (Murray *et al*., 1981; Leak *et al*., 1990).

It appears evident that a thorough knowledge of the local epidemiologic situation is a key component for planning effective tsetse control intervention(s). Improvement of existing and/or implementation of new control programs must be based on information on the regional geographic distribution and density of the vectors, the prevalence and/or incidence of the disease in livestock and the economic impact of trypanosomosis to the livestock industry in the targeted area.

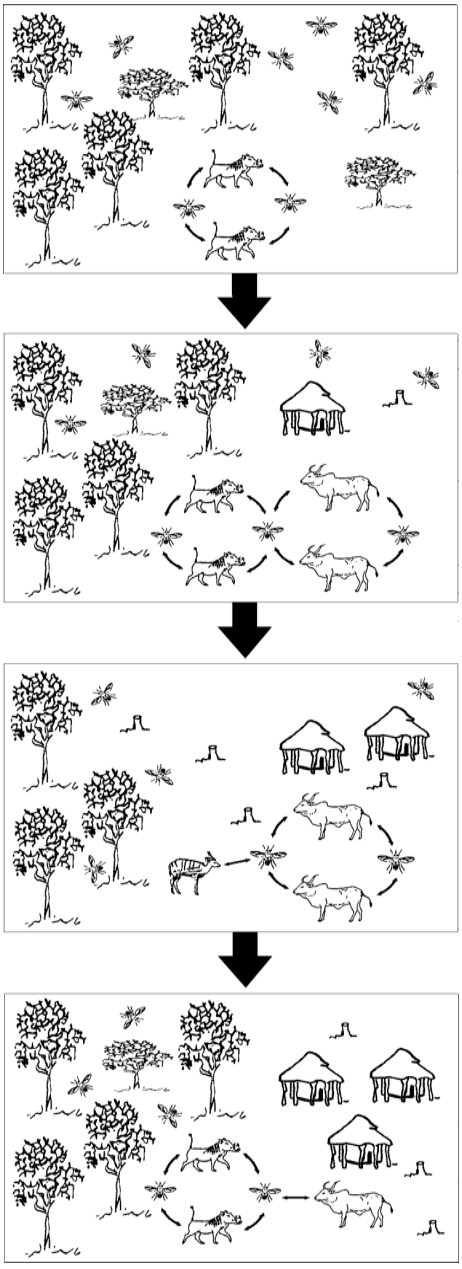
The common tsetse fly belt of southern Africa (Figure 1.2) stretches from Zimbabwe into Zambia and Mozambique and into Malawi (Connor, 1989; Van den Bossche and Vale, 2000). In this vast area, four main agroecological and epidemiological conditions can be depicted (Van den Bossche, 2001):

**Figure 1.2.** Distribution of tsetse flies in Southern Africa (source: Van den Bossche and Vale, 2000)




(i) areas where livestock are absent; (ii) zones where livestock have been introduced in game areas with game still abundant and representing a major source of food for tsetse; (iii) areas where, due to human interference, the density of game animals is low with livestock as the main source of food for tsetse and finally, (iv) areas where livestock occupy the edge of tsetse-infested wildlife zones (game/livestock interface) (Fig. 1.3).

**Figure 1.3.** The epidemiological situations in southern Africa: from top to bottom- wildlife zones; livestock introduced into a wildlife area; endemic trypanosomosis; interface trypanosomosis. Source: (Van den Bossche, 2001).



These epidemiological situations represent various degrees of interaction between tsetse flies, domestic and/or wild hosts and, thus, different levels of challenge. Although the various epidemiological settings occur in geographically different areas, they are the result of one overriding process, i.e. the encroachment of people and their livestock into tsetse-infested wildlife zones and the subsequent

alteration of the environment as a result of anthropogenic changes (e.g. clearing of vegetation for settlements, agricultural practices). Such changes have significant repercussions on the density and spatial distribution of tsetse, wildlife and domestic animals and hence on the interaction between those components of the epidemiology of tsetse-transmitted trypanosomosis. They also influence the effectiveness of tsetse control measures and tools.

*Areas where livestock is absent* -- These areas constitute large protected areas (e.g. game reserves, national parks, forest reserves) e.g in Zambia these are mainly situated in the Luangwa and Zambezi Valleys. Livestock is absent and wildlife constitutes the host and reservoir of trypanosomes. Tsetse density is usually high.

*Areas where livestock have been introduced in game areas but where game is still abundant and constitutes the major source of food for tsetse* -- This epidemiological setting is a consequence of the recent introduction of livestock (usually cattle) into a tsetse-infested wildlife area, as large zones of Mozambique where cattle are being introduced as part of the national restocking programme. The density of livestock, as compared to game animals, is usually low. Therefore, tsetse flies are largely dependent on game animals for blood meals but also feed on livestock. In these areas, livestock trypanosomosis has an epidemic character with significant impacts on livestock production (Doran, 2000). A study conducted in the Matutuine District of Maputo Province in southern Mozambique, revealed a high prevalence and incidence of trypanosomal infections in cattle that were introduced. Moreover, the average packed cell volume (PCV) of the herds was low suggesting a substantial impact of the trypanosomal infection on the health of cattle (Sigauque *et al*., 2000). Trypanosome infections are associated with low PCVs (Van den Bossche and Rowlands, 2001), which indicates the presence of anaemia in animals and hence impact on health.

*Areas where the density of game animals is low and livestock constitute the main source of food for tsetse* -- These areas are characterised by high human and livestock population density with substantial alterations of the environment as a result of human activity. The areas represent extensive zones where mixed crop/livestock agriculture is practiced. In such areas, wildlife (mainly large animal species) is almost absent and livestock is the main source of blood for tsetse flies. This epidemiological situation occurs on the plateau of eastern Zambia and is considered a trypanosomosis endemic area. The zone is an important livestock production area

despite a high prevalence of trypanosomal infections in cattle (Van den Bossche and Vale, 2000). Surprisingly, however, the impact of the disease on cattle production is rather low (Doran, 2000).

*Areas where livestock occur at the edge of tsetse-infested wildlife zones* -- Trypanosomosis at the game/livestock interface occurs in areas where tsetse flies are restricted to protected zones but are, as a result of clearing of suitable habitats, absent in the areas adjacent to the protected areas. In southern Africa, those interfaces are found mainly in Malawi and South Africa. For example, in Malawi, bovine trypanosomosis occurs along the tsetse-infested Nkothakota Game Reserve and the Kasungu National Park (Van den Bossche *et al*., 2000). Outside those protected areas, flies are almost absent. Tsetse flies, usually occurring at high density, feed mainly on wildlife and occasionally on livestock present along the interface and accessible to tsetse. In South Africa, bovine trypanosomosis is almost restricted to the areas adjacent to the tsetse-infested wildlife zones such as the Hluhluwe-iMfolozi Park in the Kwa-Zulu Natal Province (Van den Bossche *et al*., 2006). Although the incidence of trypanosomal infection can vary substantially between sites, the impact of the disease on livestock is often severe.

### 1.4.2. Endemic livestock trypanosomosis

Areas where livestock constitute the main source of food for tsetse and where livestock is the main reservoir of trypanosomes, such as the plateau area of the Eastern Province of Zambia, are considered trypanosomosis endemic zones.

In these trypanosomosis endemic zones, in absence of concerted national or regional efforts to control the disease and considering the limited resources available to veterinary services, trypanosomosis control is usually the responsibility of the livestock owner.

## 1.5. Livestock trypanosomosis in the Eastern Province of Zambia

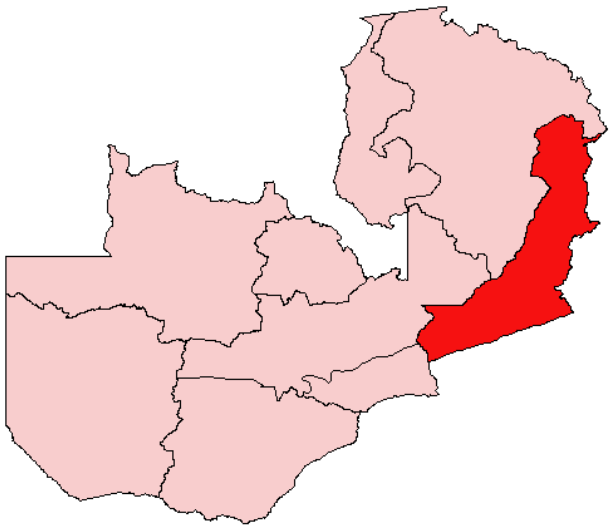
### 1.5.1. General information on the Eastern Province of Zambia

The Eastern Province of Zambia (figure 1.4) is located between latitude 10-15º S and longitude 30-33º E and covers an area of about 60,000 km$^2$. It has an estimated farming population of 151,300 farm families with a total crop area of about 250,000 hectares of which 58% is ploughed by hand (MAFF, 2002). Rainfall ranges from 400-

1000mm per annum. Farming is the most widespread activity. Pressure on forests for agricultural land is increasing rapidly due to the rapid population increase and declining soil fertility in cultivated lands.

Cattle, goats and pigs are the main livestock species present. The local cattle are of zebu type known as Angoni. They are short horned trypanosusceptible zebus found in Zambia, Malawi and Mozambique. The Angoni is larger than the Barotse and Tonga zebu cattle (Maule, 1990). In Zambia, the Angoni were originally kept by the Ngoni in areas around Lundazi and Chipata in the Eastern Province. The Angoni comprise about 22% of the indigenous cattle of Zambia (Challens, 1972). They are well adapted to the local conditions of the Eastern Province. However, although cattle production is important, productivity is low, in part due to the poor nutritive value of natural pastures (Kulich and Nambayo, 1988).

**Figure 1.4**. Map of Zambia showing the location of the Eastern Province (colored). Source: http://en.wikipedia.org/wiki/Image:ZM-Eastern.png.



In Zambia, goats (small east African breed) are important in the rural areas and are widely distributed throughout the country. However, over 60% of the goats are found in river valleys and semi-arid regions (CSO, 1997), characterized by poor

crop production. In these zones, cattle do not thrive because of trypanosomosis and feed scarcity (DAPH, 1993; Ahmadu *et al*, 2000). The numbers in the national flock are not accurately known. However, despite the presence of trypanosomosis and their susceptibility to trypanosomal infections, the number of goats present in the Eastern Province is substantial.

Indigenous pigs are not widely distributed in Zambia. They are largely confined to the eastern part of the country where they contribute to the household food security as sources of meat and income. These pigs are better adapted to extensive outdoor conditions. All indigenous pigs are relatively small in body size and have low growth rates with modest litter sizes and high fat carcasses.

### 1.5.2. Trypanosomosis in Eastern Province of Zambia: history and current situation

After the rinderpest epizootic of the 1890s, eastern Zambia was largely free of tsetse and, towards the end of the nineteenth century, cattle were reared successfully in the Luangwa Valley (Vail, 1977). However, the quick repopulation of wildlife species 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 new outbreaks of bovine trypanosomosis after rinderpest epizootic. 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 to embarking on an extensive programme of bush clearing with a view to reduce the density and spread of tsetse.

Over the years, the distribution of *G. m. morsitans* on the eastern plateau has undergone substantial advances and recessions. Progressive clearing of land for cultivation or settlement and the ever increasing human population have resulted in a gradual decrease in the number of game animals making tsetse more dependent on livestock for their survival. Since the mid-1940s, the plateau has been subject to human encroachment and large parts are currently cultivated. At the moment, tsetse flies are found on vast areas of the plateau, such as in most of Nyimba, Petauke, Katete and Lundazi Districts. Cattle, goats and pigs are the main livestock species present. Game animals are scarce. Trypanosomosis, mostly due to *T. congolense*, is

present in the areas of the Eastern Province where tsetse flies are present (Machila *et al*., 2001; Hopkins *et al*., 1998).

### 1.5.3. Trypanosomosis control in Zambia: history and current situation

#### *1.5.3.1. Introduction*

Vector control involves a number of techniques which may include insecticidal spraying (either ground spraying or aerial spraying), use of traps and targets, the application of insecticides to livestock, bushclearing and sterile insect technique (SIT). In the past tsetse populations were targeted by eliminating wild animals that were considered main hosts of tsetse (Cockerbill, 1971; Chorley, 1947; Robertson and Bemacca, 1958; Potts and Jackson, 1952). The main problems associated with controlling tsetse are the possibility of re-invasion of flies into cleared areas resulting in massive epidemics and the possible adverse effects of tsetse control on the environment.

The parasite can be targeted by using curative or prophylactic trypanocidal drugs. Chemotherapy is currently the major method for control of trypanosomosis in livestock. The main drugs used include isometamidium chloride, homidium and diminazene aceturate. Isometamidium and homidium are used for prophylaxis. The commonly used chemotherapeutic drug is diminazene aceturate. The problem with chemotherapeutics is the possible development of drug resistant trypanosomes (Peregrine, 1994; Sinyangwe *et al*., 2004). Drug resistance develops through: (i) under-dosing, which may occur for a number of reasons, such as underestimation of animal body weight, over-diluted solutions of trypanocides, deliberately under-dosing or incorrectly calculated dose volume, (ii) incorrect injection and (iii) an incorrect strategy of drug use (Peregrine, 1994).

#### *1.5.3.2. Tsetse control in Zambia*

The control of tsetse in Zambia, as elsewhere in southern Africa, has gone through various phases much determined by the development of more effective, and economically and environmentally acceptable technologies.

*Game elimination and fencing* -- The close association between game, tsetse and livestock was recognised by the Zambian Government in the 1940s. This resulted in the creation, in 1942, of the Department of Game and Tsetse Control (Vaughan-

Jones, 1948). After initial trials, the method of game clearing or destruction of the main host of tsetse was adopted as a technique for the large-scale control of tsetse in the country (Evison and Kathuria, 1984). Not surprisingly, however, the large-scale shooting of game resulted in public opposition and the method was abolished in the 1960s. In the 1950s, game fences were introduced in an attempt to preclude wild animals to enter into reclaimed tsetse free land and, thus, reduce the chance of fly re-invasion. 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 tsetse freed 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.

*Ground and aerial spraying* -- The use of insecticides for the control of insects of veterinary and medical importance was practiced for many years before it could be used to control tsetse. It was only after the discovery of persistent and relatively cheap chlorinated hydrocarbon insecticides that these chemical compounds started to be largely used in the control of tsetse. The first extensive use of insecticides for the control of tsetse populations was the campaign carried out in Zululand (South Africa) between 1945 and 1954 (Du Toit, 1954; Du Toit *et al.,* 1954).

In Zambia, chemical control of tsetse was operated in two phases; (i) the application of insecticides to vegetation and (ii) the use of stationary and mobile baits treated with insecticides. As soon as modern insecticides (synthetic pyrethroids such as Deltamethrin, Cypermethrin, Flumethrin and Cyfluthrin) with sufficient toxicity to tsetse became available, the control of tsetse by application of those compounds to the vegetation became possible. 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 and enter in contact with the chemical. This was achieved through the use of persistent, highly toxic chlorinated hydrocarbons such as dichlorodiphenlyltrichloroethane (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 chlorinated hydrocarbon to become readily available was DDT.

In the 1950s, the Zambian Government introduced ground spraying with DDT and dieldrin (Evison, 1980). The method was used up to the 1970s. A degree of mechanization in insecticide application was achieved by carrying out the less selective ground spraying of lower parts of the vegetation from four-wheel-drive vehicles (Unimog).

The aerial application of insecticides has gone through various stages of development since its first use. Early work in Zululand and Zimbabwe used 4% hexachlorocyclohexane (HCH) (formerly benezene hexachloride (BHC)) as a thermal aerosol or smoke. 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 fewer insecticide could be used to cover wider areas. Only dieldrin and endosulfan have been used widely with this technique although some trials have been carried out with synthetic pyrethroids (Spielberger *et al.,* 1979).

In Zambia, aerial spraying was initiated in 1968 to halt 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).

*Bait technology* -- In the mid-1970s, analyses of the tsetse's behaviour suggested that, with the use of appropriate baits it was possible to attract high numbers of tsetse and, eventually, kill them (Vale, 1974). Systematic research into the various components of the tsetse's response to baits (Vale, 1982, 1993) led to the development of traps and later of simple "targets" coated with a persistent insecticide that killed the flies following contact with impregnated target devices. Further studies on the attractiveness of targets (Vale, 1993) 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). Since the initial field trials (Vale *et al.,* 1986; Vale *et al.,* 1988) and assessment of their effect on the environment (Nagel, 1995), odour-baited (e.g. acetone, octenol and phenols), insecticide-treated targets have been used extensively in tsetse control operations in southern Africa and elsewhere (Slingenbergh, 1992). In the Western Province of Zambia, approximately 8 000km$^2$ of land was cleared of *G. m. centralis* (Willemse, 1991; Knols *et al.,* 1993). In Katete District of eastern Zambia, odour-baited targets

have proven to be highly effective in controlling *G. m. morsitans* (Van den Bossche, 1997).

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). 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 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 infection rates 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). A trial conducted on 2000 $km^2$ of Petauke District (Eastern Province) proved the method to be highly effective in controlling tsetse on a large scale (Van den Bossche *et al*., 2004).

#### *1.5.3.3. Trypanocidal drug use in Zambia*

Since the 1960s, the curative compound diminazene aceturate and the prophylactic compound isometamidium chloride have been used to maintain the productivity of cattle under tsetse challenge both in commercial and communal management systems in most tsetse-infested African countries. Unfortunately, multiple drug resistant trypanosome strains have been demonstrated for all economically important trypanosome species (Geerts and Holmes, 1998).

The control of bovine trypanosomosis in eastern Zambia has, for the past 45 years, relied heavily on the use of chemoprophylaxis and chemotherapy (Van den Bossche *et al.*, 2000). It was only after the discovery of early trypanocidal compounds that the Government gained the upper hand against tsetse in areas of Katete and

Petauke Districts that were settled and later 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. The implementation of these drug driven 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.

## 1.6 . The control of livestock trypanosomosis in endemic areas

### 1.6.1. Introduction

Although it is theoretically possible to eradicate tsetse flies from the African continent, it may take a long time before complete eradication is achieved. This will leave large areas, including important trypanosomosis endemic areas and where the disease constitutes a significant economic burden, infested with tsetse flies for the foreseeable future. With the governments' restrictions on the provision of animal health assistance to farmers, resource poor livestock holders often remain largely responsible for the management of the diseases and the problems that it causes to livestock-agricultural development. Thus, it appears that livestock keepers in trypanosomosis endemic areas will continue to rely on the currently available tsetse and trypanosomosis control tools. In this regard, the most important tools are trypanocides for the control of the parasite and the insecticide-treatment of livestock to control tsetse flies.

### 1.6.2. The use of insecticide-treated livestock to control tsetse flies

#### *1.6.2.1. General overview*

The use of insecticide-treated livestock as a tsetse control method dates back to the 1980s when it was shown that pyrethroid-treated cattle might be a cost effective

technique to control tsetse (Thomson, 1987). The principle of using insecticide-treated livestock is that the tsetse flies feeding on an insecticide-treated host pick up a lethal dose of insecticide during the blood meal on an insecticide-treated animal. In many areas of sub-Saharan Africa, treating cattle with insecticide has become an important means of controlling tsetse flies since all the financial obligations associated with interventions against trypanosomosis are now a responsibility of livestock owners (Eisler *et al*., 2003). In this regard, it is desired that less expensive methods of tsetse fly control that can be applied by livestock keepers themselves will become available and widely adopted by the livestock-rearing communities. The original technique of treating cattle with insecticide relied on plunging cattle in dip tanks containing acaricides that were meant for controlling ticks. However, because of the constraints associated with maintaining communal dip tanks under the prevailing socio-economic conditions, the dip tanks are no longer sustainable infrastructures for tsetse or tick control in the impoverished regions of southern Africa. Thus, the methods used more often include hand spraying and pour-on preparations applied on cattle by the farmers themselves. The control of tsetse flies using insecticide-treated cattle has been successfully done in a number of operations (Chizyuka and Liguru, 1986; Thompson *et al*., 1991, Thomson and Wilson, 1992; Bauer *et al*., 1992, 1995) although it has also failed to achieve the desired objectives in other places (Fox *et al*., 1993; Leak *et al*., 1995; Warnes *et al*., 1999). However, insecticide-treated cattle are a powerful weapon for tsetse control if applied appropriately (Hargrove *et al*, 2003) and, especially, if used in areas where cattle are relatively numerous and where tsetse flies take the majority of their blood meals from cattle or where cattle are the preferred host of tsetse (Van den Bossche and Vale, 2000).

#### *1.6.2.2. Host preference of tsetse flies*

There is abundant information in the literature that describes host preference in tsetse flies. Generally speaking and based on the fact that tsetse flies have limited energy reserves for short periods of daily activity (Bursell and Taylor, 1980), potential hosts should be present in the same habitats of the flies or in their vicinity. The animals that are rarely fed upon are usually found in open grass country

Many surveys to determine the host preference of tsetse flies have been conducted (Weitz and Glasgow, 1956; Weitz, 1963; Okiwelu, 1977; 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; Küpper, 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, opportunistic feeding behaviour, including man. Although this grouping has been criticized (Moloo, 1993) it can be used to make assumptions as to the potential feeding habits in an area where potential host animals are known.

The feeding habits of *G. m. morsitans* and *G. pallidipes* have been studied extensively in Zimbabwe (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* took approximately 62% of its feeds from suids (mainly warthog) (Okiwelu, 1977). 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 environments and/or new hosts that were either previously not present or not considered as preferential 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, such as cattle and donkeys. 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).

#### *1.6.2.3. Negative aspects of the use of insecticide-treated livestock*

In addition to the high cost associated with the use of insecticide/acaricide compounds to treat cattle, there are also other important negative aspects that must be considered. Most of the tsetse-infested cattle-rearing areas of southern Africa are also infested with ticks and livestock can suffer from tick-transmitted diseases, such as tropical theileriosis, anaplasmosis, babesiosis, cowdriosis and or tick-associated diseases, like dermatofilosis. Ticks are susceptible to the insecticides used for controlling tsetse flies. Although this may seem the ideal situation for the impecunious livestock holders of southern Africa, it however presents a problem. In order to effectively control tsetse flies with insecticides, routine treatments of cattle are required. Such routine treatments of cattle with insecticides or routine dipping with acaricides may be undesirable due to the risk of inducing acaricide resistance in tick populations (Bruce and Wilson., 1998) and reductions in the invertebrate fauna associated with the breakdown of cattle dung (Vale and Grant, 2002; Vale *et al*., 2004). More importantly, the regular application of insecticides to the herd may result in the disruption of endemic stability for certain tick-borne diseases. Endemic stability is an epidemiological state in which overt clinical disease is rare in a given animal population despite its high rate of infection. Endemic stability arises if two conditions are met: (1) disease is more likely, or more severe, in the older than the younger susceptible animals and (2) after one infection, the probability that subsequent infections result in overt disease is reduced (Coleman *et al*., 2001). This phenomenon is prevalent in livestock of poor communities of Africa who generally keep indigenous breeds of cattle which appear resistant to several tick-borne diseases or where enzootic stability has established. This resistance can therefore, be considered as an equilibrium between the micro-organism challenge and the ability of the animal to control the development of disease and is acquired by cattle following repeated contacts with tick-borne micro-organisms from early life. However, this condition of endemic stability can be disrupted by frequent treatment of cattle with acaricides or insecticides for tsetse control (Van den Bossche and Mudenge, 1999; Eisler *et al*., 2003). The most important tick-borne diseases in eastern Zambia are theileriosis (East

coast fever or ECF), anaplasmosis (Gall sickness) and babesiosis (Red water), (Mataka *et al*., 2003). Theileriosis in eastern Zambia is considered to be endemically unstable because of the less favorable climatic conditions for the vector *Rhipicephalus appendiculatus*. On the other hand bovine babesiosis and anaplasmosis exist in a state of endemic stability as described above. The majority of the cattle in eastern Zambia are undoubtedly exposed to *Babesia bovis* and *Anaplasma marginale*, the causative agents of babesiosis and anaplasmosis, respectively, but do not develop overt disease (Mataka *et al*., 2003).

#### *1.6.2.4. Strategic use of insecticide-treated livestock*

Considering the negative externalities of the frequent use of insecticides to control tsetse, efforts should be made to reduce the treatment frequency and/or the fraction of the herd that receives treatment. This can be achieved by the selective treatment of animals and/or the restricted application of particular parts of the body and restricting the treatment to periods of high challenge (Torr et al., 2007).

Evidence accrued from various entomological studies conducted on tsetse fly feeding behavior suggests that the tsetse fly's feeding behaviour operates at three levels: (i) interspecies selectivity, i.e. tsetse flies preferentially feed on a specific animal species. (ii) within or intraspecies selectivity, i.e. tsetse flies feed preferentially on certain categories of animals within a specific livestock species and, finally, (iii) body part selectivity, i.e. tsetse flies have preferential feeding sites on animal's body. In case of cattle herds, it has been shown that, under experimental conditions, tsetse flies feed preferentially on the larger members such as oxen (Torr *et al*., 2001; Torr *et al*., 2007) and that the belly region and the legs, especially the forelegs, are the most favoured feeding sites (Torr *et al*., 2007; Torr and Hargrove., 1998; Vale *et al*., 1999; Torr *et al*., 2001). It is thus expected that the application of insecticides only to the legs and belly region of oxen should be able to drastically reduce the cost, decrease the risk of disrupting tick-borne disease endemic stability and lessen the adverse environmental effects in trypanosomosis endemic areas. However, this practice may speed up induced insecticide/acaricide resistance in tick populations.

Application of insecticide could also be restricted to periods of highest trypanosomosis challenge. The estimation of trypanosomosis challenge has been the subject of much research work. The importance of estimating the challenge stems

from the need to assess the losses associated with trypanosomosis and to estimate the effectiveness and economic benefits of control measures. The assessment of challenge is also needed for planning control strategies. Trypanosomosis challenge is defined as the number of infective bites received from tsetse per animal in a given time period (Smith and Rennison, 1958). The important components determining trypanosomosis challenge are tsetse density, trypanosomosis infection rates, biting rates and infective biting rates, and livestock-tsetse contact. All these, when assessed together, provide an estimate of the challenge in a given area. This challenge may vary spatially and temporarily. Tsetse and/or trypanosomosis control operations can be optimized by focusing the intervention on periods of highest challenge or areas where challenge is highest.

### 1.6.3. The use of trypanocidal drugs to control the parasite

#### *1.6.3.1. General overview*

The most common method to control livestock trypanosomosis used by livestock owners/kepers is chemotherapy. A major problem associated with the use of chemotherapeutics is the cost associated with routine treatments both therapeutically and prophylactically and the development of drug resistant trypanosomes (see section 1.5.3.1). An increasing number of reports on drug resistance in trypanosome populations (mainly *T. congolense*) are being published. Currently, there are 17 African countries in which single or multiple trypanocidal drug resistance has been reported and the problem is increasing and spreading (Delespaux *et al.*, 2008a, b).

#### *1.6.3.2. Strategic use of trypanocidal drugs*

Diagnosis and curative and prophylactic treatments of livestock with trypanocides have for long been the responsibilities of the African governments. However, in the post-privatization era the situation has changed drastically. Government's withdrawal from providing trypanosomosis diagnostic and treatment services has left the livestock keepers to implement and finance those activities on their own. The sustainability of this approach is threatened by the costs incurred as a result of the regular applications of trypanocides and, perhaps far more important, the development and spread of trypanocidal drug resistance. It, thus, seems that the prolonged, continued use of trypanocidal drugs should be based on rational and

strategic drug scheme(s). This involves the curative treatment of infected animals and the strategic, selective use of prophylactic drugs in, possibly, those animals submitted to high challenge and/or during the peaks of tsetse-trypanosomosis challenge.

#### *1.6.3.3. Diagnosis of trypanosomosis*

The field diagnosis of trypanosomosis and, hence, the identification of infected animals that require treatment is notoriously difficult. Clinical and post-mortem signs of trypanosomosis are not pathognomonic of the disease. Clinical signs include intermittent fever, anaemia, oedema, enlarged lymph nodes and emaciation. Post-mortem examinations reveal generalized carcass emaciation, enlarged lymph nodes, enlarged liver, excessive fluid in the body cavities and petechial haemorrhages of the serosal membranes, especially in the peritoneal cavity. Therefore, diagnosis must rely on laboratory techniques that confirm the presence in blood of trypanosomes either by microscopic visualization, by molecular tests or by indirect serological techniques.

*Parasitological diagnosis* - Parasitological detection methods of blood circulating trypanosomes have high specificity but suffer from low sensitivity (Paris *et al.,* 1982). The body fluid most commonly examined is blood. Lymph, aspirated from a punctured superficial lymph node provides useful supplementary diagnostic material. The simplest parasitological diagnostic techniques are the examination of wet or Giemsa-stained thick or thin films of fresh blood. Wet blood films are simple to prepare and to examine; they are relatively inexpensive and provide immediate results when compared to other more sophisticated laboratory techniques, i.e serological techniques for antitrypanosome antibody detection or molecular tests. According to the trypanosome size and parasite's movements a presumptive trypanosome species-specific diagnosis can be made. The Giemsa-stained thin smear permits accurate determination of the species of the parasites involved. With this technique, the trypanosome species are identified by morphological characteristics.

The diagnostic sensitivity can be improved by increasing the volume of blood to be examined and through trypanosome concentration. For example, the microhaematocrit centrifugation technique or Woo-method (Woo, 1970) is more sensitive than previously described parasitological methods, but trypanosome species identification could be 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 methods are particularly useful in that the haematocrit or packed cell volume (PCV) can be assessed after blood centrifugation.

*Xenodiagnosis* - Xenodiagnosis or the subinoculation of blood into rodents such as mice or rats, can be used to detect *T. brucei* and *T. congolense* infections (Uilenberg, 1998). However, since rodents are refractory to *T. vivax* and not all *T. congolense* and *T. brucei* infections become established in the rodents, this method has limitations. Mixed trypanosomal infections may also remain undetected. The mini-anion exchange centrifugation technique (mAECT), the most sensitive parasitological test, is seldom applied in animals due to its high cost (Lumsden *et al*., 1977).

*Molecular-based diagnosis* - In alternative to parasitological tests, DNA-detection, based on Polymerase Chain Reaction (PCR), can be used. This molecular test and its variations usually possess both high sensitivity and specificity. Several DNA trypanosome-specific primers have been identified. Moreover, different molecular tests, such as DNA probing, PCR associated to DNA probing (Desquesnes and Davilla, 2002) and PCR-RFLP analysis (Geysen *et al*., 2003), are now available and have the capacity to differentiate between trypanosomes species of the *Trypanozoon* subgroup, *T. congolense* and *T. vivax*. Other more sensitive and more specific tools such as the loop-mediated isothermal amplification (LAMP) have been recently developed (Thekisoe *et al*., 2007) and are still undergoing further refinement for use in the diagnosis of both livestock and human trypanosomes.

*Serological-based diagnosis* - The development of anti-trypanosomal antibody detection techniques has been a major improvement in the serodiagnosis of Nagana. The indirect fluorescent antibody test (IFAT) (Luckins and Mehlitz 1978) and different enzyme-linked immunosorbent assay (ELISAs) such as the antibody- ELISA systems (Luckins, 1977, Greiner *et al*., 1997; Hopkins *et al*., 1998) have been evaluated and are still used in the field. Unfortunately, the antibody-ELISAs are not species-specific because of strong cross-reactions between the pathogenic *Trypanosoma* species (Desquesnes *et al.*, 2001). Moreover, no distinction can be made between past and present infections restricting their usefulness to measuring challenge.

Although a range of diagnostic tests are available with varying sensitivity and specificity, the identification of infected animals in the field still requires a considerable logistical input. Easy to use and affordable penside test are not yet available. Hence, an appropriate strategic use of trypanocides in trypanosomosis endemic areas would be supported greatly by improved tools to identify (with a reasonable level of accuracy) animals infected with trypanosomes.

### 1.7. Conclusions

It seems, from the above, that despite the range of available livestock trypanosomosis control tools, there is still room for adapting and, at the same time, improving the implementation of control interventions in trypanosomosis endemic areas. Based on the epidemiological characteristics in a given endemic area, e.g. where livestock is the main host of tsetse flies, and the fact that control of trypanosomosis is the responsibility of the livestock owner, two main control approaches can be suggested: (i) the application of insecticides to the main livestock tsetse host and (ii) the strategic use of prophylactic and/or curative trypanocidal drugs. The costs associated with these control interventions and the negative externalities as result of large-scale and intensive use of both insecticides on livestock and trypanocides require a more rational approach. It seems, from the foregoing observations, that with regard to the use of insecticides on livestock/herds substantial improvements are possible through the identification of the main host and within the host species the identification of the main group on which tsetse flies feed. As far as the curative use of trypanocides is concerned, a simple method to improve the diagnosis of trypanosomosis will facilitate the identification of infected animals and rationalize the use of trypanocides. The prophylactic application of trypanocides can also be improved through the identification of the animals in the herd that are exposed to the highest challenge. Finally, trypanosomosis control in endemic areas would benefit from rationalizing control in time by focusing control interventions on periods of highest challenge.

## 1.8 References

Ahmadu, B., Lovelace, C.E.A. and Samui, K.L. (2000) Goat keeping under village production system in semi-arid river valley areas in Zambia. *7th International Conference on Goats, France*, 15-21 May, 2000. pp 528-530.

Baldry, D.A.T. (1980) Local distribution and ecology of *Glossina palpalis* and *G. tachinoides* in forest foci of west African human trypanosomosis, 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. and Bushrod, F.M. (1987) Preliminary observations on the feeding habits of *Glossina morsitans centralis* Machado in Mumbwa district, Zambia. *International Scientific Council for Trypanosomosis Research and Control (ISCTRC)*, 17th meeting, Arusha, pp 419-422.

Bauer, B., Amsler-Delafosse, S., Clausen, P.-H., Kabore, I. and 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 Samorogoun, Burkina Faso. *Tropical Medicine and Parasitology*, **46**, 183-189.

Bauer, B., Kabore, I. and Petrich-Bauer, J. (1992) The residual effect of deltamethrin spoton when tested against *Glossina palpalis gambiensis* under fly chamber conditions. *Tropical Medicine and Parasitology*, **43**, 38-40.

Boyt, W.P. (1978) Food hosts of tsetse. *The Rhodesia Science News,* **12**, 141-143.

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., Mackenzie, P.K.I. and Pilson, R.D. (1972) The importance of the donkey (*Equinus asinus*) as a source of food and a reservoir of trypanosomes for *Glossina morsitans* Westwood. *The Rhodesia Science News,* **6**, 18-20.

Boyt, W.P., Mackenzie, P.K.I. and 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.

Bruce, D. and Wilson, A. (1998) A trial to control or eradicate *Amblyomma hebraeum* ticks and heartwater on three ranches in Zimbabwe. *Annals of the New York Academy of Science*, **849**, 381-383.

Bursell, E. and Taylor, P. (1980) An energy budget for *Glossina*. *Bulletin of Entomological Research,* **70**, 187-197.

Buxton, P.A. (1955) The natural history of tsetse flies. *Memoirs of the London School of Hygiene and Tropical Medicine*, **10,** Lewis, London, 816 pp.

Challens, B. (1972) *Cattle husbandry in Zambia: A guide for extension workers.* Zambia College of Agriculture, Monze.

Chitambo, H. and 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. and 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. and Luguru, S.M.K. (1986) Dipping to control vectors of cattle parasites. *Parasitology Today,* **2**, 123

Chorley, J.K. (1947) Tsetse fly operations in southern Rhodesia. *Rhodesian Agricultural Journal*, **44**, 520-531.

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

Cockbill, G.F. (1971) The control of tsetse and trypanosomosis in Rhodesia. *Bulletin de l'Office international des Epizooties*, **76**, 347-352.

Coleman, P.G., Perry, B.D. and Woolhouse, M.E.J. (2001) Endemic stability-A veterinary idea applied to human public health. *The Lancet*, **357**, 1284-1286.

Connor, R.J. (1989) *Final report of the Regional Trypanosomosis Expert*. Regional Tsetse and Trypanosomosis Control Programme (RTTCP), Harare, 123 pp.

CSO (1997) Central office of Statistics. Agricultural Census in Zambia. Unpublished Report.

Dagnogo, M., Lohuirignon, K. and 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.

DAPH (1993) Department of Animal Production and Health. Ministry of Agriculture, Food and Fisheries. Annual Report, Zambia.

Delespaux, V., Geysen, D., Van den Bossche, P., and Geerts, S. (2008a) Molecular tools for the rapid detection of trypanocidal drugs resistance in animal trypanosomes. *Trends in Parasitology*, **24**, 236-242.

Delespaux, V., Dinka, H., Masumu, J., Van den Bossche, P., and Geerts, S. (2008b) Five fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven years period in Eastern Zambia. *Drug Resistance Updates*, **11,** 205-209.

Doran, M. (2000) *Socio-economics of trypanosomosis. Implications for control strategies within the Common Fly-Belt of Malawi, Mozambique, Zambia and Zimbabwe. Bovine trypanosomosis in Southern Africa*, **Vol. 3**. Harare, Zimbabwe, Regional tsetse and trypanosomosis control project for Southern Africa. 156 pp.

Desquesnes, M. Bengally, Z., Millogo, L., Meme, Y. and Sakande, H. (2001) The analysis of the cross-reactions occuring in antibody-ELISA for the detection of trypanosomes can improve identification of the parasite species involved. *Annals of Tropical Medicine and Parasitology*, **95**, 141-155.

Desquesnes, M and Davila, A.M.R. (2002) Application of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. *Veterinary Parasitology*, **109**, 213-213.

Du Toit, R. (1954) Trypanosomosis 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., and Fiedler, D.G.H. (1954) The eradication of *Glossina pallidipes* from Zululand by chemical means. *International Scientific Council for Trypanosomosis Research and Control (ISCTRC)*, 5th meeting, Leopoldville, p. 141.

Eisler, S., Torr, S.J., Coleman, P.G., Machila, N. and Morton, J.F. (2003) Integrated control of vector-borne diseases of livestock-pyrethroids: poison or panacea? *Trends in Parasitology*, **19**, 341-345.

Evison, C. (1980) *Development of tsetse control in Zambia, 1942 to 1979 and its relation to animal trypanosomosis 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, G. and Kathuria, K.D.S. (1984) *Survey of the distribution of Glossina spp. and factors influencing their control in the territory of Northern Rhodesia [Zambia]*. AGRIS record, Food and Agriculture Organisation (FAO).

Fox, R.G.R., Mbando, S.O., Fox, M.S. and Wilson, A. (1993) Effect on herd health and productivity of controlling tsetse and trypanosomosis by applying deltamethrin to cattle. *Tropical Animal Health and Production*, **25**, 203-214.

Geerts, S. and Holmes, P.H. (1998) *Drug management and parasite resistance in bovine trypanosomosis in Africa.* Food and Agriculture Organization (FAO), Rome, 19 pp.

Geysen, D., Delespaux, V. and Geerts, S. (2003) PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Veterinary Parasitology,* **110**, 171-180.

Gledhillm J.A. and 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 Trypanosomosis 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.

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. and 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.

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

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.

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

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.

Hopkins, J.S., Chitambo, H., Machila, N., Luckins, A.G., Rae, P.F., Van den Bossche, P. and 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.

Kappmeier, K., Nevill, E.M. and Bagnall, R.J. (1998) Review of tsetse and trypanosomosis in South Africa. *Onderstepoort Journal of Veterinary Research,* **65**, 195-203.

Knols, B.G.J., Willemse, L., Flint, S. and 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.

Kulich, J. and Nambayo, G. S. (1988) Advances in pasture research and development in Zambia. In: I. Haque, S. Jutzi and P.J.H. Neate (eds), *Potentials of forage legumes in farming systems of sub-Saharan Africa*. Proceedings of a workshop held at ILCA, Addis Ababa, Ethiopia, 16-19 September 1985. ILCA, Addis Ababa, pp. 306.

Küpper, W., Staak, C., Kröber, T. and Späth, J. (1990) Natural hosts of *Glossina tachinoides* in northern Côte d'Ivoire. *Tropical Medicine and Parasitology,* **41**, 217-218.

Laveissiere, C., Vale, G.A. and Gouteux, J-P. (1990) Bait methods for tsetse control. In: Curtis, C.F. (ed) *Appropriate Technology in Vector Control*. CRC Press, Boca Raton, Florida, pp. 47-74.

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., Corlardelle, C., Coulibaly, L., Dumont, P., Feron, A., Hecker, P., D'Ieteren, G.D., Jeannin, P., Minengu, M., Minja, S., Mulatu, W., Nankodaba.,

G., Ordner, G., Rowlands, G.J., Sauveroche, B., Tikubet, G. and Trail, J.C.M. (1990) Relationship between tsetse challenge and trypanosome prevalence in trypanotolerant and susceptible cattle. *Insect Science and its Application*, **11**, 293-299.

Leak, S.G.A., Woudyalew Mulatu, Rowlands, G.J., D'Ieteren, G.D.M. (1995) A trial of cypermethrin 'pour-on' insecticide to control *Glossina pallidipes*, *G. fuscipes fuscipes* and *G. morsitans submorsitans* (Diptera: Glossinidae) in southwest Ethiopia. *Bulletin of Entomological Research,* **85**, 241-251.

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. (1988) *Trypanosoma evansi* in Asia. *Parasitology Today*, **4**, 137-142.

Luckins, A.G. and Mehlitz, D. (1978) Evaluation of an indirect fluorescent antibody test, enzyme-linked immunosorbent assay and quantification of immunoglobulins in the diagnosis of bovine trypanosomosis. *Tropical Animal Health and Production.* **10**, 149-159.

Lumsden, W.H., Kimber, C.D. and Strange, M (1977) *Trypanosoma brucei*: detection of low parasitaemias in mice by miniature anion-exchange/centrifugation technique. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, **71**, 421-424.

Machila, N., Sinyangwe, L., Mubanga, J., Hopkins, J.S., Robinson, T. and Eisler, M.C. (2001) Antibody-ELISA seroprevalence of bovine trypanosomosis in Eastern Province of Zambia. *Preventive Veterinary Medicine* **49**, 249-57.

Madubunyi, L.C. (1978) Relative frequency of reproductive abnormalities in a natural population of *Glossina morsitans* Westwood (Diptera: Glossinidae) in Zambia. *Bulletin of Entomological Research,* **68**, 437-442.

MAFF (2002) Ministry of Agriculture, Fisheries and Food. Agricultural Commercialization Programme (ACP). 2002-2005. Republic of Zambia.

Makumi, J.N., Green, C.H. and 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.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P. and Van den Bossche, P. (2006) Comparison of the virulence of *Trypanosoma congolense* strains isolated from cattle in a trypanosomosis endemic area of eastern Zambia. *International Journal for Parasitology,* **36**, 497-501.

Mataka, L.H., Mangani, P., Fujisaka, K. And Nagasama, H. (2003) The current status of major tick borne diseases in Zambia. *Veterinary Research*, **34**, 27-45.

Maule, J.P. (1990) The Cattle of the Tropics. University of Edinburgh. Centre for Tropical Veterinary Medicine. Redwood Press Limited. UK.

Moloo, S.K. (1993) The distribution of *Glossina* species in Africa and their natural hosts. *Insect Science and its Application,* **14**, 511-527.

Murray, M., Clifford, D.J., Gettinby, G., Snow, W.F. and McIntyre, W.I.M. (1981) Susceptibility to African trypanosomosis of N'Dama and Zebu cattle in an area of *Glossina morsitans submorsitans* challenge. *The Veterinary Record*, **109**, 503-510.

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

Nagel, P. (1995) *Environmental monitoring handbook for tsetse control operations*. Magraf Verlag, Weikersheim, 323 pp.

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.

Okiwelu, S.N. (1977) 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. and 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. and Kapaata, R. (1988) The host of *Glossina fuscipes fuscipes* in Busoga, Uganda, and epidemiological implications for trypanosomosis. *Annals of Tropical Medicine and Parasitology,* **82**, 517-518.

Paris, J., Murray, M. and McOdimba, F. (1982) A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomosis in cattle. *Acta Tropica,* **39**, 307-316.

Peregrine, A.S. (1994) Chemotherapy and delivery systems: haemoparasites. *Veterinary Parasitology*, **54**, 223-248.

Pilson, R.D. and Harley, J.M.B. (1959) The importance of cattle as a host of *Glossina morsitans* in Ankole. *East African Trypanosomosis Research Organization report,* **58**, 45-46.

Pilson, R.D., Boyt, W.P. and 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.

Potts, W.H. and Jackson, C.H.N. (1952) The shinyanga game destruction experiment. *Bulletin of Entomological Research*, **43**, 365-374.

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. and Bernaca, J.P. (1958) Game elimination as a tsetse control measure in Uganda. *East African Agricultural and Forestry Journal*, **23**, 254-261.

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.

Sasaki, H., Kang'ethe, E.K. and 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.

Sigauque, I., Van den Bossche, P., Moiana M., Jamal S., Neves L. (2000) The distribution of tsetse (Diptera: Glossinidae) and bovine trypanosomosis in Mututuine district, Maputo Province, Mozambique. *Onderstepoort Journal of Veterinary Medicine*, **3**, 167-72

Sinyangwe, L., Delespaux,V., Brandt, J., Geerts, S., Mubanga, J., Machila, N., Holmes, P.H. and Eisler, M.C. (2004) Trypanocidal drug resistance in eastern province of Zambia. *Veterinary Parasitology*, **119**, 125-135.

Slingenbergh, J. (1992) Tsetse control and agricultural development in Ethiopia. *World Animal Review,* **70-71**, 30-36.

Smith, I.M. and Rennison, B.D. (1958) Some factors concerned in trypanosome challenge. *ISCTRC/CCTA*, *7th meeting Bruxelles 1958*, pp. 63-66.

Snow, W.F. and 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.

Spielberger, U., Na'isa, B.K., Koch, K., Manno, A., Skidmore, P.R., and 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.

Steel, W.S. and 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.

Symes, C.B., Hadaway, A.B., Barlow, F., and 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. and Williamson, D.L. (1981) Feeding preference of Glossina in Northeast Tanzania. *International Scientific Council for Trypanosomosis Research and Control (ISCTRC),*17th meeting, Arusha, pp. 415-418.

Thekisoe Oriel M.M., Kuboki Noritaka, Nambota Andrew, Fujisaki Kozo, Sugimoto Chihiro, Igarashi Ikuo, Yasuda Jun, Inoue Noboru. (2007) Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. *Acta Tropica*, **102**, 182-189.

Thompson, J.W., Mitchell, M., Rees, R.B., Shereni, W., Schoenefeld, A., and 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.

Thomson, J.W. and Wilson, A. (1992) The control of tsetse flies and trypanosomosis by the application of deltamethrin to cattle. *Bulletin of Animal Health and Production in Africa*, **40**, 5-8.

Thomson, M. (1987) The effect on tsetse flies (*Glossina* spp) of Deltamethrin applied to cattle either as a spray or incorporated into eartags. *Pest Management*, **33,** 329-335.

Torr, S.J. and Hargrove, J.W. (1998) Factors affecting the landing and feeding responses of the tsetse fly *Glossina pallidipes* to a stationary ox. *Medical and Veterinary Entomology,* **12**, 196-207.

Torr, S.J. and Mangwiro, T.N.C. (2000) Interactions between cattle and biting flies: effect on the feeding rate of tsetse. *Medical and Veterinary Entomology,* **14**, 400-409.

Torr, S.J., Maudlin, I. and Vale, G.A. (2007) Less is more: restricted application of insecticide to cattle to improve the cost and efficiency of tsetse control. *Medical and Veterinary Entomology*, **21**, 53-64.

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

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

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., and Grant, I.F. (2002) Modelled impact of insecticide-contaminated dung on the abundance and distribution of dung fauna. *Bulletin of Entomological Research*, **92**, 251-263.

Vale, G.A. (1982) The trap orientated behaviour of tsetse flies and other diptera. *Bulletin of Entomological Research,* **72**, 71-93.

Vale, G.A. (1993) Development of baits for tsetse flies (Diptera: Glossinidae) in Zimbabwe. *Journal of Medical Entomology*, **30**, 831-842.

Vale, G.A. and 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., Grant, I.F., Dewhurst, C.F. and Aigreau, D. (2004) Biological and chemical assay of pyrethroids in cattle dung. *Bulletin of Entomological Research*, **94**, 273-282.

Vale, G.A., Hargrove, J.W., Cockbill, G.E., and 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. and Cockbill, G.F. (1988) Odour-baited targets to control tsetse flies, *Glossina* spp., in Zimbabwe. *Bulletin of Entomological Research,* **78**, 31-49.

Vale, G.A., Mutika, G. and Lovemore, D.F. (1999) Insecticide-treated cattle for controlling tsetse (Diptera: Glossinidae): some questions answered, many posed. *Bulletin of Entomological Research*, **89**, 567-577.

Van den Bossche , P . ( 1997 ) The control of *Glossina morsitans morsitans* (Diptera: Glossinidae) in a settled area in Petuake District (Eastern Province, Zambia), using odour-baited targets . *Onderstepoort Journal of Veterinary Research*, **64**, 251 – 257 .

Van den Bossche, P., (2001) Some general aspects of the distribution and epidemiology of bovine trypanosomsosis in southern Africa. *International Journal for Parasitology*, **31**, 592-598.

Van den Bossche, P., Chigoma, D., and Shumba, W. (2000) The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge. *Acta Tropica*, **77**, 263-270.

Van den Bossche, P., Esterhuizen, J., Nkuna, R., Matjila, T., Penzhorn B., Geerts S. and Marcotty. T. (2006) An update on the bovine trypanosomosis situation at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal Province, South Africa. *Onderstepoort Journal of Veterinary Research*, **73**, 77-79.

Van den Bossche, P. and Mudenge, D. (1999) The effect of short-interval deltamethrin application, to control tsetse, on the prevalence of babesiosis. *Tropical Animal Health and Production*, **31**, 215-222.

Van den Bossche, P., Musimbwe, J., Mubanga, J., Jooste, R. and Lumamba, D., (2004) A large scale trial to evaluate the efficacy of a 1% pour-on Formulation of Cyfluthrin (cylence, Bayer) in controlling Bovine Trypanosomosis in Eastern Zambia. *Tropical Animal Health and Production*, **36**, 33-43.

Van den Bossche, P. and Vale, G.A. (2000) *Tsetse and trypanosomosis in southern Africa. Bovine trypanosomosis in Southern Africa*, Vol. 2. Harare, Zimbabwe, Regional tsetse and trypanosomosis control project for Southern Africa. 140 pp.

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.

Warnes, M.L., Van den Bossche, P., Chihiya, J., Mudenge, D., Robinson, T.P., Shereni, W., and 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.

Weitz, B. (1963) The feeding habits of *Glossina*. *Bulletin of the World Health Organization,* **28**, 711-729.

Weitz, B. and 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.

Whiteside, E.F. (1949) An experiment in control of tsetse with DDT-treated oxen. *Bulletin of Entomological Research,* **40**, 123-134.

Wiersma, E., and Schoonman, L. (1992) *Lusu spoton trial Sesheke.* Department of Veterinary Services Zambia, Mongu, 14 pp.

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.

Woo, P.T.K. (1970) The haematocrit centrifuge technique for the diagnosis of African trypanosomosis. *Acta Tropica,* **27**, 384-386.

## OBJECTIVES OF THE THESIS

The general objective of this thesis was to clarify the epidemiology of tsetse-transmitted livestock trypanosomosis in a trypanosomosis endemic area of the Eastern Province of Zambia.

To achieve the above general objective, the study comprised of the following specific objectives:

*To determine the comparative role of cattle, goats and pigs in the epidemiology of trypanosomosis on the plateau area of Eastern Province of Zambia*. The main livestock species present on the plateau of eastern Zambia are cattle, goats and pigs. Cattle are considered to be the economically most important species and, therefore, receive most of the trypanocidal drug treatments. However, tsetse flies can feed on goats and pigs. Consequently, these two livestock species can also play an important epidemiological role as reservoir of trypanosomes and their productivity may be affected as a result of the diseases. Although it is known that on the plateau of eastern Zambia tsetse flies take a large proportion of their bloodmeals from cattle, the potential role of goats and pigs in the epidemiology of trypanosomosis needs to be clarified. In this respect, a study was conducted comparing the prevalence of trypanosomal infections in the three livestock species using highly sensitive diagnostic tools.

*To determine the incidence of trypanosomosis in cattle of different age or sex kept on the plateau area of the Eastern Province of Zambia*. Experimental studies on the tsetse fly's feeding behaviour have shown that tsetse flies do feed preferentially on certain categories of cattle. Especially large animals seem to be more preferred than others. Question remains if this selective feeding pattern influences the incidence of trypanosomosis in animals belonging to the same herd and thus subject to similar challenge. Such differences may have important repercussions for control strategies. Indeed, animals that are fed upon preferentially could receive more regular treatments with prophylactic trypanocidal drugs or could be treated with insecticides to increase the impact of such treatments on the tsetse population.

*To determine the monthly incidence of trypanosomosis in cattle kept on the plateau area of the Eastern Province of Zambia*. From the above-mentioned research

objectives it is clear that depending on the outcome of the research, the control of trypanosomosis on the plateau of eastern Zambia could be improved by focusing on the right livestock species and, within a livestock species (i.e. cattle), targeting those animals that are affected most. Another potential improvement is to intervene strategically during periods of highest challenge. Although data are available on the seasonal variations in the density of tsetse flies on the eastern plateau little is known of variation in challenge i.e. variations in the incidence of infections in livestock. To clarify if seasonal variations in challenge occur, a longitudinal study was conducted and the monthly incidence of trypanosomal infection in cattle determined using sensitive molecular diagnostic tools.

*To investigate whether the PCV-value could be a useful parameter in the diagnosis of bovine trypanosomosis on the plateau area of the Eastern Province of Zambia*. Reliable and affordable diagnosis should be the basis for any sustainable trypanosomosis control programme. A range of tools for the diagnosis of livestock trypanosomosis is available and is being used. These methods vary in their diagnostic sensitivity and specificity and their applicability under field conditions. The identification of the most appropriate diagnostic tool(s) will thus depend on the purpose of the diagnosis and the prevailing circumstances. Especially under the conditions in remote rural areas, typical for extensive areas on the plateau of eastern Zambia, diagnosis of trypanosomosis would benefit from easy indicators of trypanosomal infections that would make it possible for the veterinary official or the livestock owner to identify infected animals and to act accordingly. In this regards, a Bayesian framework was applied to evaluate the use of the PCV or the level of anaemia of an individual animal in the diagnosis of trypanosomal infections in cattle.

The above objectives will help clarifying the epidemiology of the disease in the trypanosomosis endemic area of eastern Zambia and will improve its control by providing the basis for the development and evaluation of more appropriate tools for the diagnosis of the disease in livestock under the conditions prevailing on the plateau area of the Eastern Province and in similar epidemiological settings of tsetse-infsted Africa.

## CHAPTER 2

# The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia

Adapted from H. Simukoko, T. Marcotty , I. Phiri , D. Geysen, J. Vercruysse, P. Van den Bossche (2007). The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia. Veterinary Parasitology, 147, 231-238.

## 2.1. Introduction

The epidemiology of tsetse-transmitted trypanosomosis and its impact on livestock production varies from one locality to another and depends largely on the level of interaction between tsetse, domestic and game animals. The nature of that interaction is subject to spatial and temporal variations. Within the tsetse belts of southern Africa four distinct epidemiological situations can be distinguished: (i) wildlife zones where livestock is absent, (ii) areas where livestock have been recently introduced into wildlife zones, (iii) areas where livestock are present at the edge of wildlife zones (interfaces) and (iv) areas where livestock are kept in tsetse-infested zones and where large game animals are absent (Van den Bossche, 2001). Areas where livestock are kept in a tsetse-infested zone and where livestock constitutes the major host of tsetse are of particular economic importance. Such an epidemiological circumstance is usually the consequence of the gradual encroachment of people and their livestock into tsetse-infested areas and the subsequent disappearance of large game animals as a result of human interference and the clearing of vegetation for cultivation. It is found in large parts of the fertile and cultivated areas of southern Africa such as the plateau of the Eastern Province of Zambia. Since the mid-1940s, the plateau has been subject to human encroachment and large parts are currently cultivated. Cattle, goats and pigs are the main livestock species present. Game animals are scarce. *Glossina morsitans morsitans*, the only tsetse species present, is highly dependent on livestock for its survival (Van den Bossche and Staak, 1997). Although bovine trypanosomosis was considered an important livestock disease on the plateau of eastern Zambia, little was known of the prevalence of trypanosome infections in pigs and goats. Nevertheless, pigs and goats can be suitable hosts for *G. m. morsitans* and are susceptible to infection with trypanosomes. To determine the relative importance of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the eastern plateau of eastern Zambia and to assess the relevance of controlling the disease in livestock species other than cattle, a cross-sectional survey was conducted.

## 2.2. Materials and methods

### 2.2.1. Study area

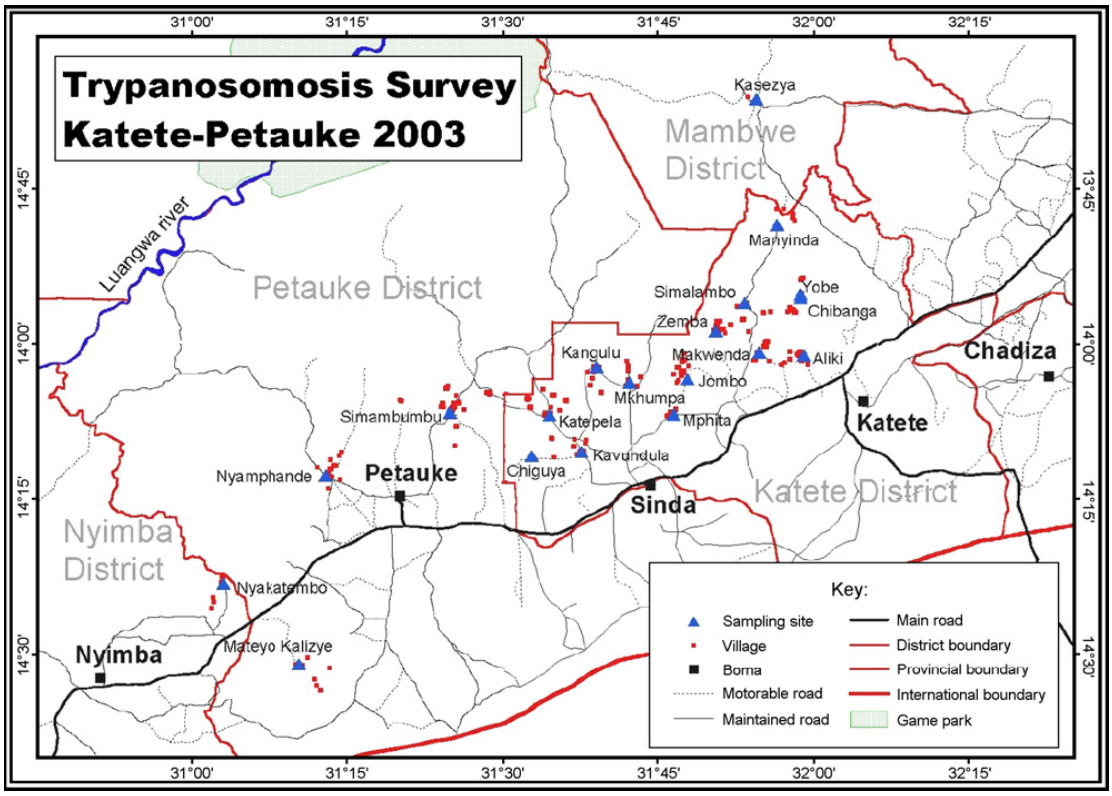
The Eastern Province of Zambia lies between latitudes 10° and 15° S and longitude 30° and 33° E. It borders Malawi to the east and Mozambique to the south and covers an area of 69,000 km2, about 9% of Zambia's total territory. It is divided into eight districts: Chipata, Chama, Lundazi, Chadiza, Mambwe, Nyimba, Katete and Petauke. This study was conducted in the latter two districts. There are three distinct seasons: the warm wet season or agricultural season, from November to April; the cool dry season from May to August and the hot dry season from September to October. The average annual rainfall is about 1000 mm, with most of the rains occurring between December and March. The plateau of the Eastern Province has a flat to gently rolling landscape with altitudes ranging from 900 to 1200 m. The vegetation is Miombo woodlands dominated by tree species such as *Braychystegia* and *Julbernadia* (Van den Bossche and De Deken, 2002). Most of the plateau is highly cultivated and carries a cattle population of approximately 11 animals/km2 in the settled areas (Doran and Van den Bossche, 1999). Maize, groundnuts and cotton are the main crops. A total of 63, 43 and 32% of the households in the study area own pigs, goats or cattle, respectively with an average number of 7 cattle, 5 goats and 5 pigs per owner (Doran, 2000). Goats and pigs roam freely in the vicinity of the villages. Cattle are usually herded but grazing patterns differ between seasons (Van den Bossche and De Deken, 2002). According to the 2003 livestock census, a total number of 49,089 cattle (Angoni breed), 24,211 goats (mainly Small East African breed) and 32,524 pigs were present in Katete district whereas 62,650 cattle, 39,565 goats and 39,142 pigs were present in Petauke district.

### 2.2.2. Sample selection

The cross-sectional survey was conducted at 11 sampling sites (crushpens) in Katete and Petauke districts during the dry season in 2003 (Figure 2.1). The sample sizes at each of the sampling sites were calculated to provide 95% certainty of detecting at least one positive case at a prevalence of 5% (Cannon and Roe, 1982). The calculated sample size was 350 for each livestock species. A proportional stratified random sampling was applied to select cattle at each crushpen. Age and sex

categories were considered as strata. Random sampling was then performed in such a way that the number of samples in each stratum

**Figure 2.1.** Location of the sampling sites in the Eastern Province of Zambia




was proportional to the herd structure described by Doran (2000). This was done to ensure that samples of all strata had the same weight. Villages within a perimeter of at least 2 km from where cattle were sampled were visited for goat and pig sampling. When sampling goats and pigs, a ‘‘home to home visit’’ sampling strategy was adopted in which all the goats or pigs from the homes were sampled to meet the required sample sizes.

### 2.2.3. Blood collection and diagnosis

From each selected animal, jugular blood was colleced in a vacutainer tube with EDTA as anticoagulant. After sampling, the vacutainer tubes were placed in a cool box containing ice packs and transported to the laboratory within four hours of collection. From each vacutainer tube, blood was transferred into three capillary tubes

which were sealed at one end with ‘‘Cristaseal’’ (Hawxley). The capillary tubes were spun in a microhaematocrit centrifuge for 5 min at 9000 rpm. After centrifugation, the packed cell volume (PCV) was determined. 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 at x400 magnification (Murray *et al*., 1977). At least 50 fields were observed before declaring a slide as negative. Blood samples that were positive were further processed as blood smears for trypanosome species identification. Giemsa-stained thick and thin blood smears were examined under x100 oil immersion objective lens (x1000 magnification).The buffy coats of the two remaining capillary tubes were extruded onto a labelled filter paper (Whatman no 3, Whatman1). Filter papers were stored in sealed plastic bags containing silica gel and transferred in a freezer at -18 °C. The samples were further analysed using the PCR-RFLP described by Geysen *et al*. (2003). Briefly the protocol was as follows. Phenol extraction was used to extract the DNA from the filter papers. Standard PCR amplifications were carried out in 25-ml reaction mixtures containing 5-ml unknown sample, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM MgCl2, 200-µl of each dNTP, 20 pmol of each primer and 0.5 U Taq polymerase enzyme (Goldstar, Eurogentec). The reaction mixtures were placed on a heating block in a programmable thermocycler (Techgene, TECHNE DUXFORD, CAMBRIDGE, UK) with a heated lid. After a denaturation step of 4 min at 94 °C each of the 40 cycles consisted of 45 s at 92 °C, 45 s at 58 °C and 60 s at 72 °C. Semi-nested runs were performed in which 0.5-µl of amplification product from the first run was added to 24.5-µl of PCR mix at 84 °C (hot start principle), containing the same ingredients and concentrations except for 25 cycles. A negative control consisting of adding ultrapure water instead of template DNA to the PCR mixture was included in each PCR amplification. A 5-µl volume of each sample was electrophoresed in a 2% agarose gel for 20 min and stained with ethidium bromide for 30 min. A 100 bp DNA ladder (MBI Fermentas, Lithuania) was included in every gel. For further typing of the fragments, RFLP-based methods were used. Primers used. The first amplification was done on the 18S gene using the forward primer 18ST nF2 (CAACGATGACACCCAT GAATTGGGGA) and 18ST nR3 (TGCGCGACCAATAATTGCAATAC) as reverse primer. A semi-nested second amplification was done using the forward primer 18ST nF2 of the first amplification with the reverse primer 18ST nR2 (GTGTCTTGTTCTCACTG ACATTGTAGTG).

RFLP-nested products were digested with *Msp* 1 and *Eco*571 enzymes in buffer Y + /Tango with S-adenosylmethionine according to the manufacturer's specifications (Gibco, UK) using 6-ml of amplified DNA in 15-ml total volume. The reaction was left overnight in awater bath at 37 °C. Four microlitres of restricted sample was then mixed with 2-µl loading buffer and transferred onto a 10% polyacrylamide gel together with a 100 bp DNA ladder (MBI Fermentas, Lithuania) for fragment size determination. DNA fragments were thereafter separated by horizontal electrophoresis in 0.5 x TBE buffer at 100 V for 2.5 h. The gels were stained using a commercial silver stain kit (Silver staining kit DNA plusone, Pharmacia Biotech, Uppsala, Sweden) and mounted for storage.

### 2.2.4. Statistical analysis

Statistical analyses were performed in Stata 9.1 (Sata Corp., 2003). The prevalence of Trypanosome infections (determined using the PCR-RFLP) in the different species (cattle, goats and pigs) was compared in a single model. Explanatory variables were the crushpens, the host species and the interaction between the two. A poison regression specifying the exposure was applied since the prevalence was zero in a number of categories. Simplifications of the model were tested using the likelihood ratio test (cut off: P = 0.05). For each of the three livestock species, the PCV data were analysed using linear regressions. The crushpens and the trypanosome infection status were used as categorical explanatory variables. The stratification of the cattle data was taken into account in the model. Finally, the correlation between the prevalence of *T. congolense* infections in pigs and goats and its prevalence in cattle was analysed using a poisson regression specifying the exposure. The *T. congolense* prevalence in cattle of the respective sampling sites was used as continuous explanatory variable.

## 2.3 Results

A total of 734 cattle, 333 goats and 324 pigs originating from 59 villages surrounding the 11 sampling sites were sampled. According to the results obtained using the microscopical diagnostic method, the proportion of infected cattle, goats and pigs was 13.5, 0 and 0.9, respectively (Table 2.1). Using the PCR-RFLP as diagnostic test, the proportion of infected cattle, goats and pigs was 33.5, 3.3 and 6.5%, respectively (Table 2.1). All parasitological positive animals were also positive on

PCR-RFLP. In goats and pigs, all infections were due to *T. congolense*, whereas in cattle, the majority of the infections (91.2%) were due to this trypanosome species (Table 2.1). The *T. congolense* prevalence of infection, determined using the PCR-RFLP, differed substantially between sampling sites with the highest proportion of infections in animals sampled at sites located in Katete district (Table 2.2). According to the statistical analyses, cattle were significantly more infected with *T. congolense* than goats and pigs ($P < 0.001$ for both). The prevalence of trypanosome infections in goats and pigs did not differ significantly ($P < 0.28$). The proportion of infected cattle at a sampling site was significantly ($P < 0.03$) correlated with the proportion of trypanosome infections in pigs but not in goats ($P < 0.365$). The proportion of infected oxen, cows, young males, young females and calves is summarised in Table 2.3. The differences in the proportion of infected animals belonging to the various age categories and the sexes were statistically not significant ($P = 0.44\%$). The presence of a trypanosome infection in cattle, pigs or goats significantly reduced the PCV. The PCV of infected cattle was on average 8.5% lower ($P < 0.001$) compared to the PCV of non-infected cattle (Table 2.4). For infected goats and pigs, on the other hand, trypanosome infection reduced the PCV on average by 4.8 and 13.6%, respectively ($P < 0.001$ for both goats and pigs).

**Table 2.1.** Observed proportions (± SD) of cattle, goats or pigs infected with trypanosomes, using the PCR-RFLP or parasitological diagnostic tools

| | | PCR-RFLP | | | | Parasitology | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | *T. congolense* (%) | *T. vivax* (%) | Mixed[a] (%) | Total[b] (%) | *T. congolense* (%) | *T. vivax* (%) | Mixed[a] (%) | Total[b] (%) |
| Cattle | 734 | 30.5 ± 1.7 | 2.4 ± 0.6 | 0.5 ± 0.3 | 33.5 ± 1.7 | 10.6 ± 1.1 | 2.2 ± 0.5 | 0.7 ± 0.3 | 13.5 ± 1.3 |
| Goats | 333 | 3.3 ± 1 | 0 | 0 | 3.3 ± 1 | 0 | 0 | 0 | 0 |
| Pigs | 324 | 6.5 ± 1.4 | 0 | 0 | 6.5 ± 1.4 | 0.9 ± 0.5 | 0 | 0 | 0.9 ± 1.6 |

a: *T. congolense* and *T. vivax*; b: *T. congolense*, *T. vivax* and mixed.

**Table 2.2**. Observed and predicted (CI) proportions of cattle, goats, or pigs infected with *T. congolense* at 11 sampling sites on the plateau of eastern Zambia

| Sampling | Cattle | | | Goats | | | pigs | | |
|---|---|---|---|---|---|---|---|---|---|
| | Observed | | | Observed | | | Observed | | |
| site | n | (%) | Predicted (%) | n | (%) | Predicted (%) | n | (%) | Predicted (%) |
| Alick (K) | 77 | 31.2 | 27.6 (18.5-41.3) | 35 | 2.9 | 3.1 (1.5-6.2) | 36 | 8.3 | 4.6 (2.6-8.2) |
| Chiguya (K) | 68 | 30.9 | 27.0 (17.6-41.6) | 35 | 5.7 | 3.0 (1.5-6.1) | 35 | 2.9 | 4.5 (2.5-8.1) |
| Makwenda (K) | 83 | 53 | 47.1 (35.0-63.3) | 31 | 9.7 | 5.2 (2.7-10.0) | 55 | 1.8 | 7.8 (4.7-12.8) |
| Katepela (K) | 60 | 78.3 | 68.8 (51.5-92.0) | 45 | 2.2 | 7.6 (4.0-14.3) | 20 | 20 | 11.3 (6.8-19.1) |
| Nyakatembo (P) | 57 | 1.8 | 4.5 (1.4-14.0) | 54 | 3.7 | 0.5 (0.1-1.7) | 23 | 0 | 0.7 (0.2-2.5) |
| Manyinda (K) | 61 | 47.5 | 46.0 (32.7-64.5) | 34 | 2.9 | 5.1 (2.6-10.0) | 69 | 11.6 | 7.6 (4.6-12.6) |
| Kasero (P) | 59 | 1.7 | 1.6 (0.2-11.2) | 39 | 0 | 0.1 (0.02-1.3) | 0 | | |
| Nyamphande (P) | 66 | 1.5 | 0 | 0 | | | 0 | | |
| Jombo (K) | 70 | 44.3 | 39.6 (27.8-56.5) | 22 | 4.5 | 4.4 (2.2-8.7) | 35 | 5.7 | 6.6 (3.8-11.3) |
| Simabumbu (P) | 62 | 9.8 | 8.9 (4-19.7) | 29 | 0 | 1.0 (0.3-2.6) | 15 | 0 | 1.5 (0.6-3.6) |
| Zemba (K) | 71 | 62 | 55.1 (40.8-74.5) | 9 | 11 | 6.1 (3.1-11.9) | 36 | 5.6 | 9.1 (5.5-15.2) |

(K): Katete; (P): Petauke.

**Table 2.3**. Proportions of cattle belonging to different categories infected with *T. congolense* and sampled at eleven sites on the plateau of eastern Zambia

| Sampling Site | n | Age/sex category | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Calves | n | Young females | n | Young males | n | Cows | n | Bulls | n | Oxen |
| Alick (K) | 9 | 0 | 4 | 50 | 0 | | 28 | 32 | 3 | 33 | 33 | 36. |
| Chiguya (K) | 23 | 22 | 6 | 17 | 4 | 0 | 16 | 31 | 0 | | 19 | 53 |
| Makwenda (K) | 5 | 0 | 11 | 36 | 0 | | 29 | 62 | 3 | 67 | 35 | 57 |
| Katepela (K) | 1 | 0 | 1 | 0 | 5 | 100 | 20 | 70 | 0 | | 33 | 82 |
| Nyakatembo (P) | 3 | 0 | 4 | 0 | 2 | 0 | 17 | 0 | 0 | | 31 | 3 |
| Manyinda (K) | 4 | 50 | 8 | 12 | 0 | | 19 | 53 | 3 | 33 | 27 | 56 |
| Kasero (P) | 22 | 0 | 0 | | 1 | 100 | 6 | 0 | 0 | | 30 | 0 |
| Nyamphande (P) | 0 | | 0 | | 0 | | 34 | 3 | 0 | | 32 | 0 |
| Jombo (K) | 5 | 0 | 10 | 20 | 9 | 22 | 21 | 67 | 0 | | 25 | 52 |
| Simabumbu (P) | 8 | 12 | 2 | 0 | 6 | 17 | 21 | 10 | 1 | 0 | 24 | 80 |
| Zemba (K) | 4 | 75 | 6 | 0 | 13 | 77 | 17 | 53 | 3 | 33 | 28 | 54 |

**Table 2.4**. Observed and predicted (CI) PCVs of infected and non-infected cattle on the plateau of eastern Zambia

| Ampling | Infected | | | Non-infected | |
|---|---|---|---|---|---|
| Site | n | Observed | Predicted (CI) | Observed | Predicted (CI) |
| Alick (K) | 77 | 24.1 | 23.5 (22.5-24.6) | 32.1 | 32.0 (31.1-33.0) |
| Chiguya (K) | 68 | 24.2 | 22.6 (21.5-24.0) | 31.1 | 31.1 (30.1-32.2) |
| Makwenda (K) | 83 | 21.7 | 22.2 (21.2-23.3) | 31.4 | 30.8 (29.7-31.8) |
| Katepela (K) | 60 | 23.7 | 23.4 (22.0-25) | 32.1 | 31.8 (30.3-33.4) |
| Nyakatembo (P) | 57 | 28 | 24.7 (23.3-26.1) | 33.1 | 33.2 (32-34.3) |
| Manyinda (K) | 61 | 22.1 | 21.8 (21.0-22.8) | 30.1 | 30.3 (29.4-31.3) |
| Kasero (P) | 59 | 25 | 22.2 (20.8-23.5) | 30.6 | 30.6 (29.6-31.7) |
| Nyamphande (P) | 66 | 20 | 21.7 (20.4-23) | 30.2 | 30.2 (29.2-31.3) |
| Jombo (K) | 70 | 23.9 | 24.2 (23.1-25.4) | 32.9 | 32.7 (31.7-33.7) |
| Simabumbu (P) | 62 | 23 | 25.5 (24.1-26.9) | 34.2 | 34 (32.7-35.2) |
| Zemba (K) | 71 | 22.8 | 22.6 (21.6-23.5) | 31.2 | 31.1 (30.0-32.1) |

## 2.4. Discussion

Hitherto, parasitological diagnostic methods and the antibody detection ELISA have been used extensively for epidemiological studies in the Eastern Province of Zambia (Sinyangwe *et al*., 2004; Van den Bossche *et al*., 2004; Machila *et al*., 2001; Van den Bossche and Rowlands, 2001). The parasitological prevalence of bovine trypanosomosis observed using the buffy coat method in this study was similar to the prevalence observed during other surveys but was substantially lower than the prevalence determined using a molecular diagnostic tool. This is not surprising considering the low sensitivity of parasitological diagnostic methods (Picozzi *et al*., 2002). This is especially so when the parasitaemia is low and may explain the very low parasitological prevalence of trypanosome infections in goats and pigs (MacLennan, 1970; Omeke, 1994). The antibody detection ELISA, on the other hand, has high sensitivity but detects antibodies against current and past infections (Van den Bossche *et al*., 2000). Hence, the antibody detection ELISA overestimates the actual prevalence of infection. Although the sensitivity of molecular diagnostic tools such as the PCR-RFLP is also affected by the parasitaemia; the outcome of the molecular diagnosis is probably a good representation of the proportion of animals of each of the main livestock species present in the study area that are infected with trypanosomes. The high proportion of *T. congolense* infections is in accordance with observations made in other southern African countries (Van den Bossche, 2001). The absence of *T. vivax* infections in goats is attributed to the overall low prevalence of trypanosome infections in this livestock species. Since *T. vivax* is not infective for pigs, none of the pigs were infected with this trypanosome species. The high trypanosomosis prevalence in Katete district compared to Petauke district is attributed to higher level of cultivation and vegetation clearing resulting in a substantial destruction of suitable tsetse habitat in the latter district. The probability of a host contracting trypanosomosis depends on the rate at which it is fed upon by infected tsetse flies (Rogers, 1988). The attraction of tsetse flies to a host and subsequently the proportion of tsetse that feed and challenge that host is the result of a number of stimuli. The number of tsetse attracted to a host is determined largely by the amount of odour (mainly carbon dioxide and other unidentified kairomones) produced by that host or, in the case of a herd of potential hosts, by that herd (Torr *et al*., 2006). The probability that an attracted tsetse fly feeds on that host is determined

by short-range visual (Vale, 1974) and olfactory stimuli (Warnes, 1995) and the behaviour of the host (Torr and Mangwiro, 2000). Hence, host preference and thus challenge is the result of a range of factors that may differ depending on the ecological conditions. Under the conditions prevailing on the plateau of eastern Zambia where livestock constitutes the potential hosts of tsetse, cattle are the preferred host and undergo the highest level of challenge. There are a number of reasons why cattle are the most preferred tsetse host in the Eastern Province. First, cattle are spread more evenly in the study area and are thus more available, whereas the distribution of goats and pigs is restricted to the vicinity of villages (Van den Bossche and De Deken, 2002). Second, because of the odour plumes produced by individual cattle and the large odour plumes produced by cattle grouped in herds, tsetse flies are expected to be far more attracted to cattle than pigs or goats. Similar low levels of challenge of goats have been observed in other tsetse-infested areas of Zambia (Ahmadu *et al*., 2002). Nevertheless, goats can be an important tsetse host and become readily infected with trypanosomes. This was, for example, the case in the Luangwa Valley of eastern Zambia where the incidence of trypanosome infections in goats was high with a significant impact on goat production (Bealby *et al*., 1996). It thus seems that despite the defensive behaviour of goats and in the absence of alternative more attractive hosts, goats can be fed upon frequently. Although suidae are considered preferred hosts of *G. m. morsitans* (Clausen *et al*., 1998; Torr, 1994; Weitz, 1963), the prevalence of trypanosome infections in domestic pigs was relatively low. This is again attributed to the higher availability and attractiveness of cattle and the restricted distribution of pigs. Nevertheless, the relationship between the proportion of infected cattle and the proportion of infected pigs suggest that when challenge is high, pigs are more readily fed upon. This may indicate a density-dependent feeding success on cattle which has already been suggested by Vale (1977). According to our results, it thus seems that in an area where livestock constitute the main host of tsetse, cattle act as a protective shield by attracting the majority of the tsetse flies and protecting other livestock species from high levels of tsetse challenge. Hence, in the presence of cattle, trypanosomosis seems to be of minor importance in other livestock species. Nevertheless, despite the low proportion of infected pigs, the infection caused severe anaemia in this livestock species. Such high levels of anaemia in pigs infected with *T. congolense* have been reported elsewhere (Omeke, 1994). In goats, on the other hand, the average PCV of infected

animals was only 4.8% lower suggesting a level of tolerance. Such trypanotolerance has been reported in indigenous Small East African (SEA) goats (Geerts *et al.*, 2009; Griffin and Allonby, 1979). In cattle, trypanosome infections caused a variable level of anaemia. Such variations between cattle herds or cattle sampled at various sampling points are attributed mainly to differences in disease management practices and possibly differences in the virulence of circulating trypanosome strains (Masumu *et al*., 2006). Because of human encroachment into tsetse-infested areas, the importance of livestock as host of tsetse flies is likely to increase substantially. The repercussions of this change in host preference on livestock production and productivity will depend largely on the livestock species present and their attractiveness to tsetse flies. Depending on the circumstances, the role of small ruminants and pigs in the epidemiology of livestock trypanosomosis may be minimal. In the absence of cattle, on the other hand, pigs and goats can be suitable hosts and challenge may impact substantially on their productivity. In such situations, control of the disease in those species is advisable.

## 2.5 References

Ahmadu, B., Lovelace, C.E.A., and Samui, K.L. (2002) A survey of trypanosomosis in Zambian goats using haematocrit centrifuge technique and polymerase chain reaction. *Journal of the South African Veteterinary Association.-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging,* **73**, 224–226.

Bealby, K.A., Connor, R.J., and Rowlands, G.J. (1996) *Trypanosomosis in goats in Zambia*. International Livestock Research Institute (ILRI), Nairobi, 88 pp.

Cannon, R.M., and Roe, R.T. (1982) Livestock Disease Surveys: *A Field Manual for Veterinarians*. Australian Government Publishing Services, Canberra.

Clausen, P.H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F., and Staak, C. (1998) Host preferences of tsetse (Diptera: Glossinidae) based on blood meal identifications. *Medical and Veteterinary Entomology,* **2**, 169–180.

Doran, M. (2000) Socio-Economics of Trypanosomosis: Implications for Control Strategies Within the Common Fly-Belt of Malawi, Mozambique, Zambia and Zimbabwe, volume **3**. *Regional Tsetse and trypanosomosis Control Programme for Southern Africa*, p. 156.

Doran, M., and Van den Bossche, P. (1999) An assessment of the socioeconomic impact of bovine trypanosomosis and its control in the southern African region. In: *Proceedings of the 25th Meeting of the ISCTRC*, Mombasa, Kenya, pp. 307–315.

Geysen, D., Delespaux, V., and Geerts, S. (2003) PCR-RFLP using SsurDNA amplification as an easy method for species-specific diagnosis of Trypanosoma species in cattle. *Veterinary Parasitology,* **110**, 171–180.

Griffin, L and Allonby, E.W. (1979) Trypanotolerance in breeds of sheep and goats with an experimental infection of Trypanosoma congolense. *Veterinary Parasitology*, **5**, 97-105.

Machila, N., Sinyangwe, L., Mubanga, J., Hopkins, J.S., Robinson, T., and Eisler, M.C. (2001) Antibody-ELISA seroprevalence of bovine trypanosomosis in eastern province of Zambia. *Preventive Veterinary Medicine,* **49**, 249–257.

MacLennan, K.J.R. (1970) The epizootiology of trypanosomosis in livestock in West Africa. In: Mulligan, H.W. (Ed.), *The African Trypanosomiases*. Gearge Allen and Unwin/Ministry of Overseas Development, London, UK, pp. 751–765.

Masumu, J., Marcotty, T., Geysen, D., Geerts, S., Vercruysse, J., Dorny, P., and Van den Bossche, P. (2006) Comparison of the virulence of Trypanosoma congolense strains isolated from cattle in a trypanosomosis endemic area of eastern Zambia. *International Journal of Parasitology*, **36**, 497–501.

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

Omeke, B.C. (1994) Pig trypanosomosis: prevalence and significance in the endemic mid belt zone of southern Nigeria. *Revue d'Elevage et de Medecine v´terinaire des Pays tropicaux*, **47**, 381–386.

Picozzi, K., Tilly, A., Fe´vre, E.M., Coleman, P., Magona, J.W., Odiit, M., Eisler, M.C., and Welburn, S. (2002) The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk. *African Journal of Biotechnology,* **1**, 39–45.

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

Sinyangwe, L., Delespaux, V., Brandt, J., Geerts, S., Mubanga, J., Machila, N., Holmes, P.H., and Eisler, M.C. (2004) Trypanocidal drug resistance in eastern province of Zambia. *Veterinary Parasitology*, **119**, 125–135.

Stata Corp. (2003) Stata Statistical Software: Release 9.1. Stata Corporation, Texas.

Torr, S.J. (1994) Responses of tsetse flies (Diptera: Glossinidae) to warthog Phacochoerus aethiopicus Pallas). *Bulletin of Entomological Research*, **84**, 411–419.

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

Torr, S.J., and Mangwiro, T.N.C. (2000) Interactions between cattle and biting flies: effects on the feeding rate of tsetse. *Medical and Veterinary Entomology*, **14**, 400–409.

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

Vale, G.A. (1977) Feeding response of tsetse flies (Diptera: Glossinidae) to stationary hosts. *Bulletin of Entomological Research*, **67**, 635–649.

Van den Bossche, P. (2001) Some general aspects of the distribution and epidemiology of bovine trypanosomsosis in southern Africa. *International Journal of Parasitology*, **31**, 592–598.

Van den Bossche, P., Chigoma, D., and Shumba, W. (2000) The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge. *Acta Tropica,* **77**, 263–270.

Van den Bossche, P., and De Deken, R. (2002) Seasonal variations in the distribution and abundance of the tsetse fly, Glossina morsitans morsitans in eastern Zambia. *Medical and Veterinary Entomology*, **16**, 170–176.

Van den Bossche, P., Musimbwe, J., Mubanga, J., Jooste, R., and Lumamba, D. (2004) A large scale trial to evaluate the efficacy of a 1% pour-on Formulation of Cyfluthrin (cylence, Bayer) in controlling Bovine Trypanosomosis in Eastern Zambia. *Tropical Animal Health and Production*, **36**, 33–43.

Van den Bossche, P., and Rowlands, G.J. (2001) The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume. *Acta Tropica,* **78**, 163–170.

Van den Bossche, P., and Staak, C. (1997) The importance of cattle as a food source for *Glossina morsitans morsitans* in Katete district, Eastern Province, Zambia. *Acta Tropica,* **65**, 105–109.

Warnes, M.L. (1995) Field studies on the effect of cattle skin secretions on the behaviour of tsetse. *Medical and Veterinary Entomology*, **9**, 284–288.

Weitz, B. (1963) The feeding habits of Glossina. *Bulletin of the World Health Organisation,* **28**, 11–729.

## CHAPTER 3

# Heterogeneity in the trypanosomosis incidence of Zebu cattle of different ages and sex on the plateau of eastern Zambia

Adapted from H. Simukoko, T. Marcotty , I. Phiri , D. Geysen, J. Vercruysse, P. Van den Bossche (2007). Heterogeneity in the trypanosomosis incidence of Zebu cattle of different ages and sex on the plateau of Eastern Zambia. Acta Tropica, 103, 98-101.

## 3.1. Introduction

Over the years, a variety of methods to control livestock trypanosmosis have been developed and used successfully for the area-wide or localised control of tsetse flies or the control of the parasite in susceptible animals. Currently, the control of animal trypanosomosis in Eastern Zambia relies mainly on the curative or prophylactic treatment of livestock.

The pattern of contact between haematophagous insects, such as tsetse flies, and their hosts is extremely heterogenous and non-random (Kelly, 2001). As a result some host species are challenged substantially more than others and may contribute more to parasite transmission (Woolhouse *et al*., 1997). On the plateau of eastern Zambia, for example, a high proportion of tsetse feed on cattle rather than goats or pigs (Van den Bossche and Staak, 1997). Hence, the prevalence of trypanosome infections is likely to be substantially higher in cattle compared to the other livestock species. Moreover, much progress has been made in describing how some individuals within a species are bitten more than others (Torr *et al*., 2006, Torr *et al*., 2001; Torr, 1994). In cattle, for example, tsetse are attracted most to animals that produce large amounts of host odours. Hence, a host's attractiveness to tsetse flies is correlated with its weight and/or age (Torr *et al*., 2006). Although odours may be important in attracting tsetse flies to individual hosts, they may be a less important determinant of biting risk when animals of all sizes, ages and sexes are aggregated in a herd or in a kraal. This is usually the case under traditional livestock management practices. This study was conducted to determine if under such traditional cattle management practices challenge differs between animals of different age and sex and kept in the same herd.

## 3.2. Materials and methods

### 3.2.1. Study area

The study was carried out in an area situated between 31°47'-31°55' E and between 13°55'-14°12' S in Katete District, Eastern Province, Zambia. It is a highly cultivated area with a cattle population of approximately 8-10 animals/km$^2$ (based on an aerial survey conducted in August 1997). *Glossina morsitans morsitans*, which takes the majority (75%) of its bloodmeals on cattle, is the only tsetse species present (Van den Bossche and Staak, 1997; Van den Bossche and De Deken, 2002). Bovine

trypanosomosis (mainly due to *Trypanosoma congolense*) is endemic with an average herd prevalence of about 30% (personal observation).
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).

### 3.2.2. Animal selection and follow-up

Cattle sampled during the longitudinal study in 2005 were part of 19 herds of which all owners were based in Alick village (31°52´E and 14°06´S). The herds were selected because they grazed in the same grazing areas surrounding the village. A total of 354 animals was identified for the study. This sample size provided 95% certainty of detecting at least 1 positive case at a prevalence of 5% (Cannon and Roe, 1982). To select animals from the 19 herds to be included in the study, a proportional stratified random sampling was applied in which age and sex categories were considered as strata. Random sampling was performed in such a way that the number of samples in each stratum was proportional to the normal herd structure in the study area (Doran, 2000). The animals consisted of approximately 40% oxen, 30% adult females (cows, >48 months), 15% young females and young males (12 to 48 months), 13% calves (< 12 months) and 2% bulls (>48 months) and were kept under traditional livestock management practices. During the study period all categories of cattle (including calves) were herded together. At the start of the experiment, all sampled animals were ear-tagged and injected intramuscularly with a double dose (7.0 mg/kg bw) of diminazene aceturate (Berenil®, Hoechst) to clear all trypanosomal infections. Through offering free diagnosis and treatment farmers were likely to abide by the request not to treat their cattle. Blood was collected from all sampled animals on a monthly basis and the trypanosome infection status of each animal was determined. Blood collection started in March (one and a half months after the diminazene treatment) and continued for three consecutive months (March-May), a period when tsetse challenge in the study area is high (Van den Bossche and De Deken, 2002). Animals infected with trypanosomes were treated with a curative dose of diminazene aceturate (at 3.5 mg/kg bw).

### 3.2.3. Sampling and diagnosis

Blood samples were collected and analysed in the laboratory as described in chapter 2.

### 3.2.4. Statistical analysis

Statistical analyses were carried out in Stata 9.1 (2003). For the survival analysis, a Cox proportional hazards model was used to compare the relative risks of infection in the different age and sex categories. Infected animals were censored. The proportional-hazards assumption was tested on Schoenfeld residuals ($P > 0.05$).

## 3.3. Results

During the three months observation period, a total of 180 sentinel animals became infected with trypanosomes (all *T. congolense*). The monthly incidence of new infections during each month of observation in each category and the relative risks to infection for each category are shown in Table 3.1 and 3.2.

**Table 3.1**. Number of new trypanosome infections in the sentinel animals belonging to different categories according to PCR-RFLP diagnosis.

| Category | Number of animals | Number of new trypanosome infections | | |
|---|---|---|---|---|
| | | Month | | |
| | | 1 | 2 | 3 |
| Calves | 46 | 0 | 3 | 4 |
| Young females | 28 | 7 | 5 | 2 |
| Young males | 28 | 4 | 6 | 3 |
| Cows | 106 | 12 | 15 | 18 |
| Bulls | 6 | 0 | 0 | 1 |
| Oxen | 140 | 27 | 40 | 33 |

The survival analysis showed significant differences in the risk of infection between age categories and sexes (Figure 3.1)

**Figure 3.1**. Kaplan-Meier survival curves for infection with *T. congolense* of cattle of different ages and sex on the plateau of eastern Zambia

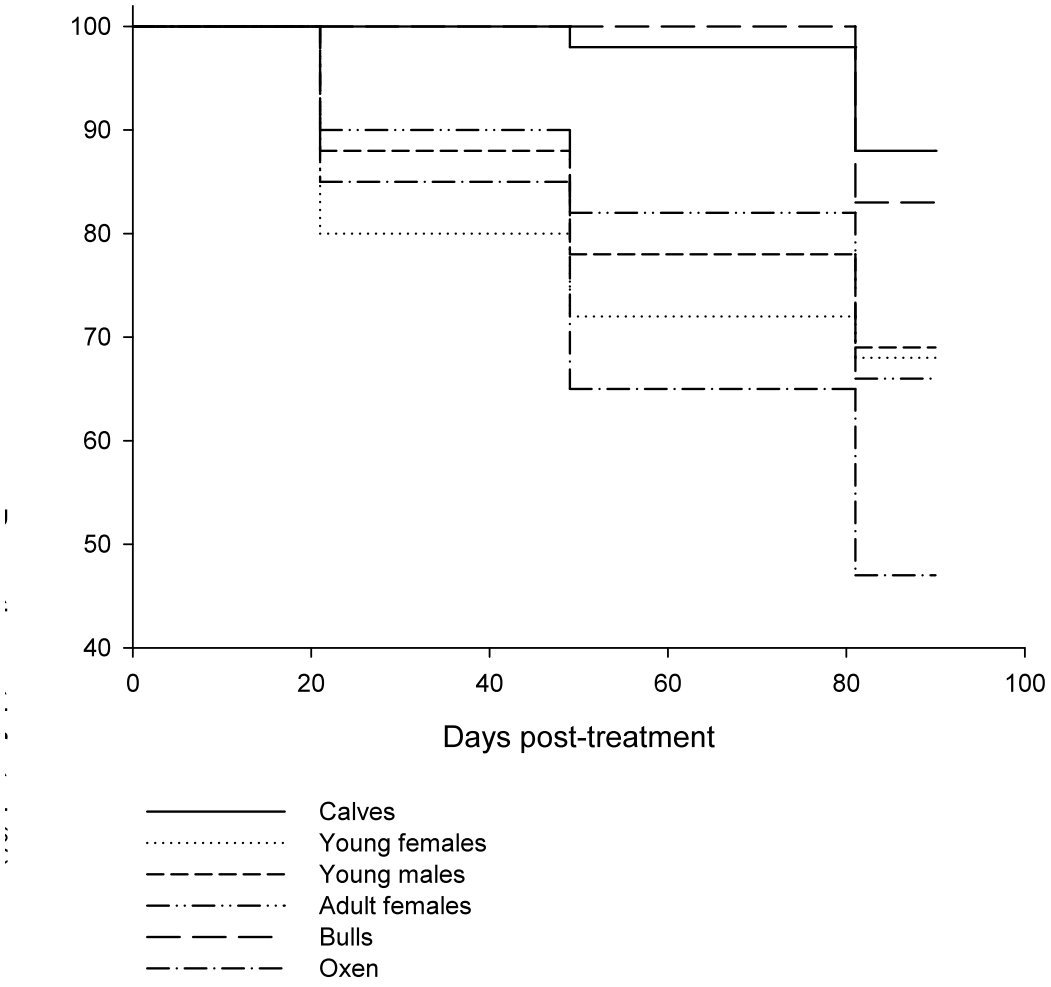



**Table 3.2**. Relative risks of infection with trypanosomes of cattle of different ages and sex on the plateau of eastern Zambia (calves were used as reference category)

| Category | Relative risks (CI) |
|---|---|
| Young females | 3.3 (1.1 - 10.2) |
| Young males | 3.1 (1 - 9.8) |
| Adult females | 3.3 (1.3 - 8.5) |
| Bulls | 1.4 (.16 - 11.8) |
| Oxen | 5.6 (2.3 - 14.1) |

The risk of infection relative to calves was almost six times higher in oxen (P<0.001) and almost twice as high in cows (P=0.01) and young animals (P=0.05) (Table 3.2). The risk of infection in bulls was not significantly different from that in calves (P<0.8). Finally, the risk of infection was lower in cows than in oxen (P=0.01).

### 3.4. Discussion

The results of this longitudinal study show significant heterogeneity of trypanosomosis incidence in the various cattle categories. Although the study is of relative short duration, the timing was chosen because of the high level of trypanosomosis challenge during the rainy season and the communal herding during this time of the year. Moreover, during the observation period (peak of the rainy season) oxen are less used for animal traction and are, most of the time, kept within the herd. The study's results are in accordance with the entomological findings on the attractiveness of different age categories and sexes of cattle to tsetse flies. Indeed, according to Torr *et al*. (2006) and Torr and Mangwiro (2000), tsetse flies are attracted significantly more by odour of large animals (i.e. oxen) and animals that showed less defensive behaviour and least by calves. The latter is especially so when calves are part of a herd. This study suggests that the observed differences in attractiveness also translate in differences in trypanosomosis incidence even when animals are kept in a herd.

A number of studies have shown an effect of age on the incidence or prevalence of trypanosome infections in cattle. Rowlands *et al*. (2001) found a significant effect of age on the incidence of *T. congolense* infections in animals below 15 months of age with calves being the least infected. Similar low infection rates in calves were observed by Trail *et al*. (1994). In both cases, however, calves were kept at the homestead. Furthermore, although those studies confirm part of our observations, the findings suffer from the poor sensitivity of the pararasitological diagnostic tests used to identify trypanosome infections.

The low prevalence and incidence of trypanosome infections in calves has been attributed also to the protective effect of maternal immunity that does not prevent calves from becoming infected but mitigates the adverse effect of such an infection by reducing the parasitaemia to a very low almost undetectable level

(Fiennes, 1970). Although maternal immunity cannot be excluded, the low incidence of trypanosome infections in calves suggests low levels of challenge or low attractiveness to tsetse. The apperant low risk of infection in bulls is attributed to the small sample size.

Heterogeneity of incidence or individual variation in exposure to vector-borne diseases is a well-known phenomenon and may have repercussions for the optimal design of disease control programs (Kelly, 2001; Woolhouse *et al*., 1997). Although the results obtained from this study may not be sufficient to draw general conclusions for the control of livestock trypanosomosis some important observations can be made with regard to the control of bovine trypanosomosis in the study area and perhaps in other areas where epidemiological circumstances are comparable. First, the high risk of trypanosome infection in oxen and the high proportion of oxen in the cattle herd make oxen the most appropriate part of the herd for prophylactic treatment with trypanocidal drugs. Indeed, such targeted drug campaigns will affect the most infected part of the herd, protects the most valuable part of the herd in the mixed-crop livestock production system and may, at the same time, reduce the number of tsetse that will become infected. In addition, diminishing the trypanocidal drug use frequency is likely to reduce the risk of resistance development in trypanosomes. Second, because of the high attractiveness of oxen even when part of a herd, oxen may be the most appropriate cattle category to be treated with insecticides to control tsetse.

### 3.5 References

Cannon, R. M. and R. T. Roe. (1982) *Livestock disease surveys: a field manual for veterinarians*. Australian Government publishing Services, Canberra.

Doran, M. (2000) Socio-Economics of Trypanosomosis: Implications for control strategies within the Common Fly-Belt of Malawi, Mozambique, Zambia and Zimbabwe. Volume 3. *Regional Tsetse and trypanosomosis Control Programme for Southern Africa*. P. 156.

Fiennes, R.N.T.W. (1970) Pathogenesis and pathology of animal trypanosomosis. In: *The African Trypanosomiases*, Mulligan, H.W., Ed., Allen and Unwin, London, 729-750.

Geysen, D., Delespaux, V. and Geerts, S. (2003) PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Veterinary Parasitology*, **110**, 171-180

Kelly, D.W. (2001) Why are some people bitten more than others? Trends *in Parasitology*, **17**, 578-581.

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

Stata Corp. (2003) Stata statistical software: Release 9.1. Stata Corporation, Texas.

Torr, S.J. (1994) Responses of tsetse flies (Diptera: Glossinidae) to warthog (*Phacochoerus aethiopicus Pallas*). *Bulletin of Entomological Research*, **84**, 411-419.

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

Torr, S.J. and Mangwiro, T.N.C. (2000) Interactions between cattle and biting flies: effects on the feeding rate of tsetse. *Medical and Veterinary Entomology,* **14**, 400–409.

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

Trail, J.C.M., Wissocq, N., d'Ieteren, G.D.M., Kakiese, O. and Murray, M. (1994) Patterns of *Trypanosoma vivax* and *T. congolense* infection differ in young N'Dama cattle and their dams. *Veterinary Parasitology*, **55**, 175-183.

Van den Bossche, P. and De Deken, R. (2002) Seasonal variations in the distribution and abundance of the tsetse fly, *Glossina morsitans morsitans* in eastern Zambia. *Medical and Veterinary Entomology,* **16**, 170-176.

Van den Bossche, P. and Staak, C. (1997) The importance of cattle as a food source for *Glossina morsitans morsitans* in Katete district, Eastern Province, Zambia. *Acta Tropica*, **65**, 105-109.

Woolhouse, M.E.J., Dye, C., Etard, J.F., Smith, T., Charlwood, J.P., Garnett, G.P., Hagan, P., Hii, J.L., Ndhlovu, P.D., Quinnel, R.J., Watts, C.H., Chandiwana, S.K. and Anderson, R.M. (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. *Proceedings of The National Academy of Sciences,* **94**, 338-342.

# CHAPTER 4

## Bovine trypanosomosis risk in an endemic area on the eastern plateau of Zambia

Simukoko, H., Marcotty, T., Vercruysse, J., Phiri, I.K., and Van den Bossche, P. Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia. Submitted.

## 4.1. Introduction

The importance of trypanosomosis, a devastating disease affecting livestock populations in large parts of Africa, is often determined by calculating the monthly incidence of infection. The incidence of trypanosomal infections depends to a large extend on the level of challenge animals are subjected to. Trypanosomosis challenge is determined by the tsetse density, the prevalence of trypanosomal infections in tsetse and the proportion of meals taken by tsetse from the host species of interest. On the plateau of eastern Zambia, most of these parameters have been quantified. The monthly average proportion of infected tsetse flies is about 9% (Kubi *et al*., 2007) and the proportion of meals taken from cattle amounts to 75% (Van den Bossche and Staak, 1997). Such a high preference for livestock is not surprising considering the almost complete absence of large game animals from the highly cultivated areas of the eastern plateau. This high preference for cattle is reflected in the low prevalence of trypanosomal infections in other livestock species such as goats and pigs (see chapter 2). Finally, the density of the tsetse population is known to undergo substantial seasonal variations (Van den Bossche and De Deken, 2002). Notwithstanding the high prevalence of trypanosomal infections and the important role of cattle in the epidemiology of livestock trypanosomosis on the eastern plateau, possible seasonal differences in the incidence of infection could be exploited to further focus trypanosomosis control strategies. Determining the incidence of infection is often difficult because of the low sensitivity of parasitological diagnostic tools (see chapter 5) and the presence of trypanocidal drug resistance (Delespaux and De Koning, 2007). The latter is of particular importance on the plateau area of the Eastern Province (Delespaux *et al.,* 2008) and makes it difficult to distinguish new from persisting infections. Molecular diagnostic tools could compensate for the low sensitivity of the parasitological diagnostic methods but results are usually delayed. To overcome the problems associated with resistance and molecular diagnosis, the monthly risk of infection was used as a measure of challenge or incidence. It was calculated as the monthly probability of a primo infection based on molecular diagnosis and used as the basis to improve trypanosomosis control strategies.

## 4.2. Materials and methods

The study was conducted on the plateau of eastern Zambia between April 2004 and December 2005. The study area is situated between 31° 45′ and 32° 00′ E and between 13° 45′ and 14° 00′ S. The area is highly settled and cultivated and carried a cattle population of approximately 11 animals/km² (Doran and Van den Bossche, 1999). Two main vegetation types are present. Miombo woodlands, an open one-storied woodland with tall trees of the genera *Brachystegia* and *Julbernadia*, dominates (Van den Bossche and De Deken, 2002). Most of the villages are located in miombo. Munga woodland, a one or two-storied woodland where the principal tree genera are *Acacia*, *Combretum* and *Terminalia* is found mainly in lower lying areas. 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). The main livestock species reared in the study area are cattle of the Angoni breed, goats, pigs and chickens. Cattle generally graze in the communal grazing areas. However, grazing patterns vary according to season (Van den Bossche and De Deken, 2002). During the rainy season, cattle are mainly found in miombo whereas from June onwards cattle disperse and are found in both munga and miombo. This distribution pattern is in accordance with changes in the management practices of communal cattle in eastern Zambia (De Clercq, 1997).

*Glossina morsitans morsitans* is the only tsetse species present in the area. It takes 75% of its bloodmeals from cattle (Van den Bossche and Staak, 1997). *Trypanosoma congolense* is the most prevalent trypanosome species. The prevalence of infection in cattle is about 30% whereas in pigs and goats the prevalence of trypanosomal infections is low (Simukoko *et al*., 2007).

### 4.2.1. Animal selection

A total of 85 head of cattle, representing five age and sex categories (i.e. oxen, cows, young stock, calves and bulls) were selected randomly from their respective herds. The number of animals in each category was proportional to the normal herd structure in the study area (Doran, 2000). Animals were selected from herds that graze together and thus, theoretically, are subjected to the same tsetse challenge. All sentinel animals

were ear-tagged and, two months before the start of the study, treated with a double dose of diminazene aceturate (i.e. 7 mg/kg BW, Berenil©, Hoechst) to clear any trypanosome infections acquired prior to the study. The animals were followed for a period of 20 consecutive months. Livestock owners whose animals were part of the study were advised not to treat their animals. Confirmed trypanosomosis cases were treated with 3.5mg/kg body weight of diminazene aceturate (Berenil©, Hoechst).

### 4.2.2. Sampling and diagnosis

Sentinel animals were sampled monthly to determine their infection status. From each animal, jugular blood was collected in a vacutainer tube with EDTA as anticoagulant. After sampling, the vacutainer tubes were placed in a cool box containing ice packs and transported to the laboratory within four hours of collection. From each vacutainer tube, blood was transferred into three capillary tubes which were sealed at one end with "Cristaseal" (Hawxley). The capillary tubes were spun in a microhaematocrit centrifuge for 5 minutes at 9,000 rpm. After centrifugation, the packed cell volume (PCV) was determined. The buffy coat and the uppermost layer of red blood cells of one capillary tube were extruded onto a microscope slide and examined for the presence of motile trypanosomes. Samples were examined with a phase-contrast microscope at x 400 magnification (Murray *et al*, 1977). At least 50 fields were observed before declaring a slide as negative. Blood samples that were positive were further processed as blood smears for trypanosome species identification. Giemsa-stained thick and thin blood smears were examined under x 100 oil immersion objective lens (x1000 magnification).

The buffy coats of the two remaining capillary tubes were extruded onto a labelled filter paper (Whatman n$^{o}$ 3, Whatman®). Filter papers were stored in sealed plastic bags containing silica gel at -18°C. The samples were further analysed using the PCR-RFLP described by Geysen *et al.* (2003).

### 4.2.3. Statistical analysis

To analyse the data, use was made of a parametric survival model in Stata 10 assuming an exponential survival distribution. A failure (i.e. an animal becoming infected with trypanosomes) was recorded when an animal was found to be infected using the PCR-RFLP diagnostic tool. Only animals that were PCR-RFLP negative at

the start of the study were included in the sentinel herd. To avoid problems associated with drug resistance (thus excluding trypanosomal infections that were a result of treatment failure rather than tsetse challenge) and considering the delay between sampling and obtaining results from the molecular analyses, only the first infections or primo-infections were taken into account. Hence, once an animal has become infected it was excluded from further analyses. The overall risk of infection was calculated in a model without explanatory variables. The significance of the months as explanatory variable was estimated in a separate model.

The PCV data were analysed using linear regressions in Stata 10. Cross-sectional models were used to account for the repeated sample collection from individual animals. The square-root of PCV values ranging between 0 and 1 were arcsin transformed to assure normality. Discrete explanatory variables were the trypanosome infection status determined by PCR/RFLP and the time of sampling. The interaction between the two explanatory variables was tested and ignored if the likelihood ratio test was not significant ($P > 0.05$). The normal distribution assumption was verified in non cross-sectional models using the same response and explanatory variables. Residual quantiles plotted against the quantiles of a normal distribution (Q-Q plot) were visually assessed and the heteroskedasticity was tested (Breusch-Pagan / Cook-Weisberg test for heteroskedasticity in Stata 10).

### 4.3. Results

A total of 19 monthly samplings was conducted. Three animals died due to suspected trypanosomosis during the study period. One died during the first year of observation while the other two died during the second year. During the samplings, 155 trypanosomal infections were detected when diagnosis was based on the results of the PCR-RFLP technique. Of those 155 trypanosomal infections, only 85 (54.8%) were detected using parastological diagnostic tools (buffy coat method). A total of 143 (92.3%) infections were due to *T. congolense*, 7 (4.5%) to *T. vivax* and 5 (3.2%) to mixed infections with *T. congolense* and *T. vivax*. The majority of the single or mixed *T. vivax* infections (11 out of 13 or 84.6%) were detected during the hot dry seasons. The remaining two *T. vivax* infections were detected during the month of July (cold dry season). The *T. congolense* incidence during the study period is summarised in a Kaplan-Meier survival curve (Figure 4.1). Throughout the observation period the 4 weekly average risk of infection was 6.0% (95% CI: 4.6 –

7.7%). However, the risk of infection varied significantly between months (P = 0.017) with a higher risk between December and February (i.e. the beginning of the rainy season) (Figure 4.2). The effect of age and sex category on the incidence of infection was not significant.

Infection with trypanosomes was significantly correlated with a reduction in the value of the PCV (P<0.001). Monthly average PCV values ranged between 19.5% and 24.9% in infected animals and between 27.7% and 30.8% in uninfected animals (Figure 4.3). In spite of the low amplitude of the monthly variations, the effect of time of sampling on the PCV of infected and uninfected animals was statistically significant (P<0.001, Figure 4.3). However, the interaction between the infection status and the time of sampling was not significant (P=0.52), indicating that seasonal variations was similar in infected and uninfected animals.

**Figure 4.1**. Kaplan-Meier survival curve for infection with *T. congolense* of the sentinel animals

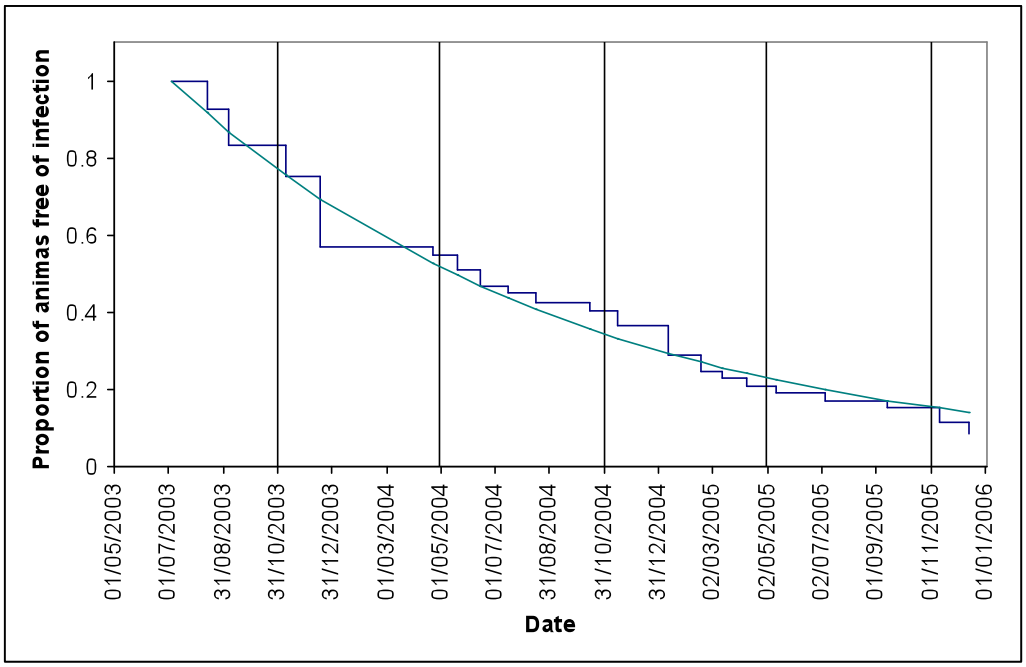


**Figure 4.2**. Variations in predicted monthly risk of trypanosomosis transmission to cattle on the plateau of eastern Zambia (95% confidence intervals) (1-12 = January-December).

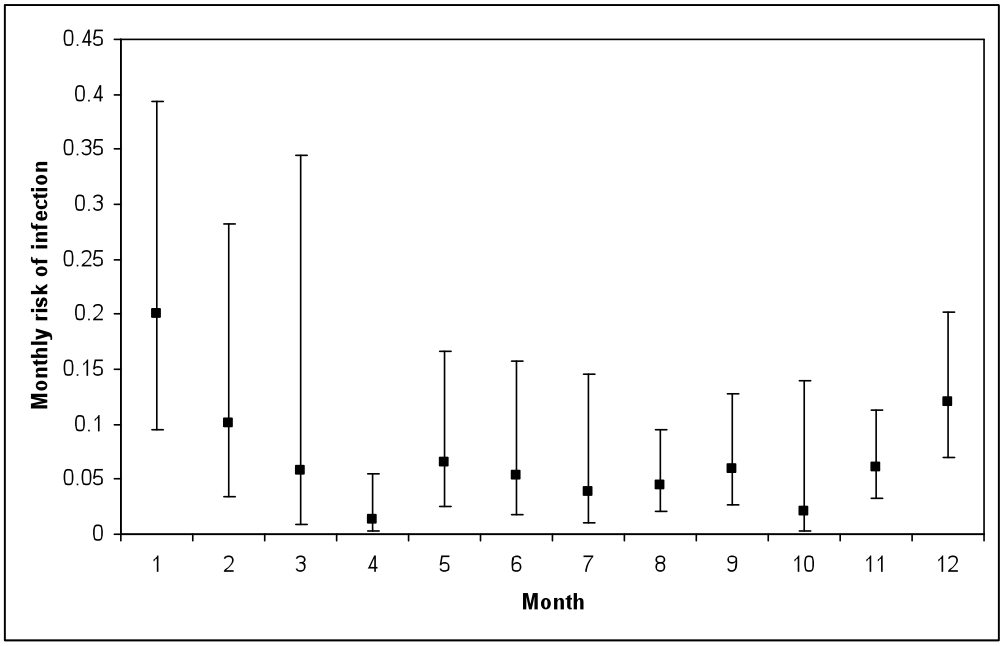
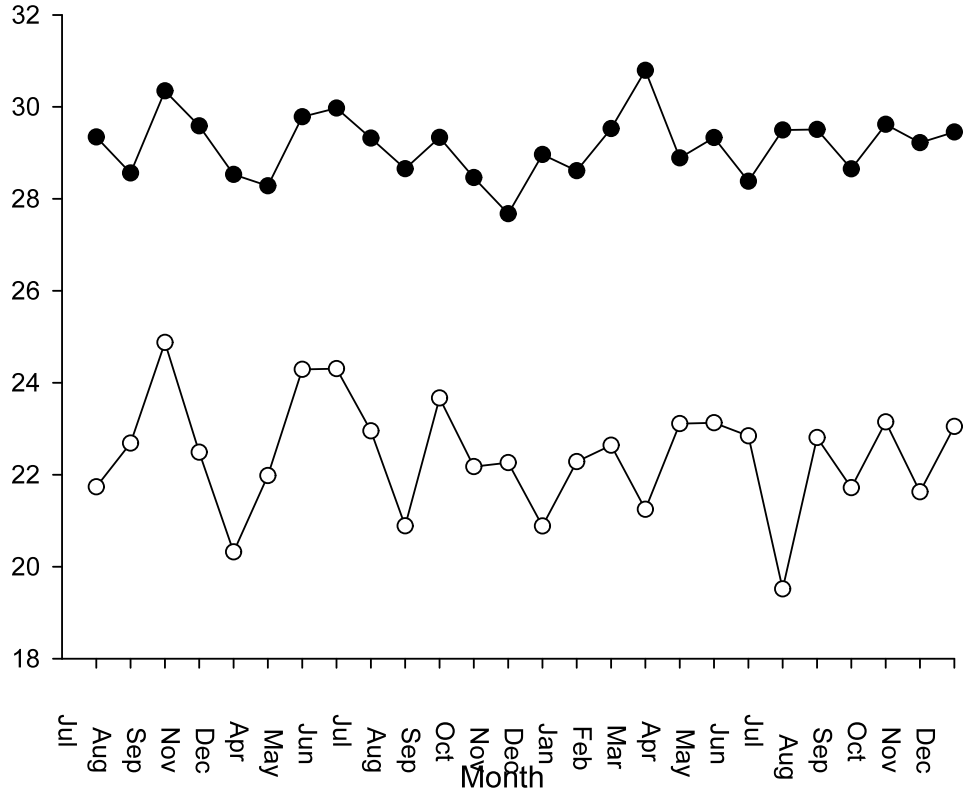


**Figure 4.3**. Monthly average PCV of infected (○) and non-infected (●) sentinel animals




## 4.4. Discussion

The results presented give a good picture of the trypanosomosis challenge livestock undergo on the highly cultivated plateau of Zambia. The area is representative for large tsetse-infested cultivated areas in southern Africa where livestock constitutes the main host of tsetse and the main reservoir of trypanosomes (Van den Bossche, 2001). Presenting challenge as risk of infection with trypanosomes (i.e. infection with *T. congolense*) clearly avoids problems associated with the overestimation of the incidence of infection as a result of trypanocidal drug resistance, the time lag between sampling and the results of the molecular analysis and thus the delay in the treatment of animals that after parasitological diagnosis were false negatives. In this respect, about 50% of the infected animals could not be detected using parasitological diagnostic tools. This lack of sensitivity questions the accuracy of trypanosomosis incidence data based on parasitological diagnosis and stresses the need for diagnostic

tools to improve the field diagnosis of trypanosomal infections in livestock (Marcotty *et al*., 2008).

Although the risk of infection with trypanosomes was constant throughout most of the year it increased significantly during the beginning of the rainy season. Sinyangwe *et al.* (2004) could not detect such seasonality in trypanosomosis incidence. This may not be surprising considering the fact that infections in that study were diagnosed solely based on parasitological diagnosis. Since the infection rate of the tsetse population undergoes little variation (Kubi *et al.,* 2007), the high incidence of trypanosomal infections at the beginning of the rainy season is explained by the high density of tsetse during this time of the year (Van den Bossche and De Deken, 2002). Such a close relationship between tsetse density and incidence of infection is attributed largely to the high proportion of bloodmeals taken from cattle by tsetse (Van den Bossche and Staak, 1997). The higher level of challenge at the beginning of the rainy season is reflected in the high frequency of trypanocidal drug treatments given during this period of the year (Van den Bossche *et al*., 2000). Contrary to previous observations (Chapter 3), no significant effect of age and sex on infection was observed. This is explained by the absence of calves in the sentinel herd, the age category with a significant lower level of challenge.

*Trypanosoma congolense* is the main trypanosome species in the study area but infections with *T. vivax* do occur (Simukoko *et al*., 2007). These *T. vivax* infections seem to be most prevalent during the time of the year when the survival of tsetse flies is lowest and, hence, favouring the development of trypanosome species (such as *T. vivax*) with a short development cycle. The study again confirms the importance of *T. congolense* as the main trypanosome species in livestock in Zambia, in particular, and southern Africa, in general.

An infection with trypanosomes results in a significant decline in the PCV (Murray and Dexter, 1988; Marcotty *et al*., 2008). However, monthly variations in the average PCV of the infected and uninfected cattle did not differ significantly. The latter is in accordance with observations made by Van den Bossche and Rowlands (2001) reflecting that factors such as nutrition affect the PCV of rural cattle.

In conclusion, the outcome of the longitudinal study suggests that further focussing and prioritising of bovine trypanosomosis control is possible. Indeed, more effort could be put in optimizing trypanosomosis control through, for example, prophylactic

treatment, during the period of highest challenge i.e. especially the beginning of the rainy season.

## 4.5 References


De Clercq, K. (1997) Feeding evaluation of the Angoni cattle during the late dry season in Chipata (Zambia). *MSc Thesis*. Ghent University, Ghent.

Delespaux,V., Dinka, H., Masumu, J., Van den Bossche, P. & Geerts, S. (2008) Five fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven years period in Eastern Zambia. *Drug Resistance Updates,* **11,** 205-209.

Doran, M., and Van den Bossche, P. (1999) An assessment of the socio-economic impact of bovine trypanosomosis and its control in the southern African region. *Proceedings of the 25th meeting of the ISCTRC*, Mombasa, Kenya, pp. 307-315.

Doran, M. (2000) Socio-Economics of Trypanosomosis: Implications for control strategies within the Common Fly-Belt of Malawi, Mozambique, Zambia and Zimbabwe. Volume 3. *Regional Tsetse and trypanosomosis Control Programme for Southern Africa*. P. 156.

Kubi C, Billiouw M, and Van den Bossche P. (2007) Age prevalence of trypanosomal infections in female *Glossina morsitans morsitans* (Diptera: Glossinidae) on the plateau area of eastern Zambia. *Onderstepoort Journal of Veterinary Research*, **74**, 223-9

H. Simukoko, T. Marcotty, I. Phiri, D. Geysen, J. Vercruysse, and P. Van den Bossche (2007) The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia. *Veterinary Parasitology,* **147**, 231-238.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N. and Van den Bossche P. (2008). Evaluating the use of Packed Cell Volume as an indicator of trypanosomal infections in cattle in eastern Zambia. *Preventive Veterinary Medicine*, **87**, 288-300.

Sinyangwe, L., Delespaux, V., Brandt, J., Geerts S., Mubanga, J., Machila, N., Holmes P.H. and Eisler, M.C. (2004) Trypanocidal drug resistance in eastern province of Zambia. *Veterinary Parasitology*, **119,** 125-135.

Stata Corp. (2008) Stata statistical software: Release 10. Stata Corporation, Texas.

Van den Bossche, P. and Rowlands, G.J. (2001) The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume. *Acta Tropica,* **78,** 163-170.

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

Van den Bossche, P. (2001) Some general aspects of the distribution and epidemiology of bovine trypanosomsosis in southern Africa. *International Journal for Parasitology*, **31**, 592-598.

Van den Bossche, P. and De Deken, R. (2002) Seasonal variations in the distribution and abundance of the tsetse fly, *Glossina morsitans morsitans* in eastern Zambia. *Medical and Veterinary Entomology*, **16**, 170-176.

Van den Bossche, P. and Staak, C. (1997) The importance of cattle as a food source for *Glossina morsitans morsitans* in Katete district, Eastern Province, Zambia. *Acta Tropica*, **65**, 105-109.

# CHAPTER 5

## Evaluating the use of Packed Cell Volume as an indicator of trypanosomal infections in cattle in eastern Zambia

Adapted from T. Marcotty, H. Simukoko, D. Berkvens, J. Vercruysse, N. Praet & P. Van den Bossche (2008). Evaluating the use of Packed Cell Volume as an indicator of trypanosomal infections in cattle in eastern Zambia. Preventive Veterinary Medicine, 87, 288-300.

T. Marcotty and H. Simukoko contributed equally to the work presented in this paper.

## 5.1. Introduction

Notwithstanding the availability of a number of diagnostic tests, the management of livestock trypanosomosis through diagnosis and treatment remains problematic (Schlater and Van den Bossche, 2004). Because of the often low parasitaemia, simple parasitological diagnostic tools such as the buffy coat method have low sensitivity and, hence, about 50% of the cases are not diagnosed and a large proportion of the infected animals remains untreated (Picozzi *et al*., 2002). Diagnostic problems could be resolved by using molecular diagnostic tests. These tests are highly specific and more sensitive than parasitological diagnosis (Geysen *et al*., 2003). Like parasitological tests, molecular tools detect the presence of the pathogens and may lack sensitivity in chronic cases when parasitaemia is low. Molecular tests require sophisticated infrastructure, are expensive and results become available after a considerable delay. They are thus less appropriate for use in rural areas where communities are mostly interested in identifying infected animals that require treatment at the cheapest price. Serological anti-trypanosomal antibody detection tests, on the other hand, are much cheaper but require laboratory facilities and positive cases are poorly associated with infection because of the persistence of the antibodies even after an infection has been cured (Van den Bossche *et al*., 2000). In addition, serological tests have no use in the early stages of the infection, before the rise of specific antibodies. There is thus a need for simple tools that improve the sensitivity of the parasitological diagnosis, particularly in chronic clinical cases, and hence support the management of trypanosomosis.

In cattle and other susceptible domestic animals, anaemia is a well-recognized and inevitable consequence of an infection with pathogenic trypanosomes, including *T. congolense* and *T. vivax* (Murray and Dexter, 1988). It is measured by determining the packed cell volume (PCV). In the absence of other factors causing anaemia, the PCV gives a reliable indication of the disease status of a trypanosome-infected animal (Trail *et al*., 1993; Grace *et al*., 2007). Hence, in the absence of other factors causing anaemia, the use of PCV and its parallel combination with the parasitological diagnosis could improve the detection of trypanosome-infected animals and ease the decision to treat them with a trypanocidal drug. Parallel combinations of diagnostic tests, for which one single positive test makes a combination positive, should however

be used with caution. While their sensitivity is higher than those of the individual diagnostic tests, their specificity is lower.

The objective of this study was to evaluate the use of PCV and determine its sensitivity and its specificity, in the diagnosis of bovine trypanosomosis, either on its own or in combination with parasitological examination. In the absence of gold standards (100% sensitive and specific tests), Bayesian models are useful to evaluate the disease prevalence and the characteristics of batteries of tests applied on the same sets of samples (Lesaffre *et al*., 2007). These iterative models integrate data and prior information to make estimates. The prior information consists of the conditional probabilities underlying the relationship between the various test results and the disease prevalence and the limits within which these parameters are allowed to vary. Prior information should be sufficiently detailed to allow the convergence of the models but it should be kept in mind that priors have an important effect on their output. Priors are usually based on expert's opinion. It is however acknowledged that such opinion may lack objectivity.

## 5.2. Material and methods

The study was conducted in a trypanosomosis endemic area of eastern Zambia. The area was chosen because of the high prevalence of trypanosomal infections in cattle and the absence of tick-borne diseases that could cause anaemia in animals. The study took place in the Katete and Petauke districts, north of the Great East Road, at an altitude ranging between 900 and 1000 m above sea level. East Coast fever does not occur in the region and, although other tick-borne pathogens such as *Babesia* spp. and *Anaplasma* spp. are present, the diseases are endemically stable, causing virtually no clinical cases in cattle. Trypanosomosis transmitted by *Glossina morsitans morsitans* is also endemic. The prevalence of trypanosome infections differs substantially between livestock species with cattle being the most important reservoir of infection (Chapter 2).

### 5.2.1. Sample selection

A cross-sectional survey was conducted at eleven randomly selected sampling sites (crushpens) during the dry season (Chapter 2). The sampling sites were selected in a lottery from a sampling frame of all the crushpens with more than 100 heads of cattle. The list of crushpens and the cattle population data were provided by the

provincial veterinary office. All animals belonged to the Angoni breed and were maintained in a traditional husbandry system. The animals mainly feed by grazing, assuring appropriate nutritional status (4 or above on the 9 level scale of Nicholson and Butterworth, 1986), including in the dry season during which they are left in free range. The sample size in the sampling sites was calculated to provide 95 % certainty of detecting at least one positive case at a prevalence of 5 % (Thrusfield, 1995). A prevalence of 5 % or less would be considered as marginal. The calculated sample size was 59. In each sampling site, animals were recorded and allocated to the different age and sex classes. Systematic sampling was then performed in such a way that the number of samples in each age and sex category was proportional to the herd structure recorded in the sampling site. This was done to assure a good representativeness of the samples, since age and sex are known to affect the exposure to trypanosomosis (Chapter 3). A total of 734 blood samples were collected (between 57 and 83 in each crushpen): 84 calves (< 1 year old), 52 young females and 40 young males (between 1 and 3 years of age), 228 cows, 317 oxen and 13 bulls (> 3 years).

### 5.2.2. Blood collection and diagnosis

From each selected animal, jugular blood was collected in tubes with EDTA as anticoagulant. After sampling, the tubes were placed in a box containing ice packs and transported to the laboratory within four hours of collection.

From each tube, blood was transferred into three capillary tubes. The capillary tubes were spun in a microhaematocrit centrifuge for 5 minutes at 9,000 rpm. After centrifugation, the packed cell volume (PCV) was determined with Hawxley reader. 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 at x 400 magnification (Murray *et al*., 1977). At least 50 fields were observed before declaring a slide as negative. Blood samples that were positive were further processed as blood smears for trypanosome species identification. Giemsa-stained thick and thin blood smears were examined under x 100 oil immersion objective lens (x 1000 magnification) to obtain a parasitological diagnosis.

The buffy coats of the two remaining capillary tubes were extruded onto a labelled filter paper (Whatman n$^{o}$ 3, Whatman®). Filter papers were stored in sealed

plastic bags containing silica gel and transferred in a freezer at -18°C. The samples were further analysed using the PCR/RFLP described by Geysen *et al*. (2003) to detect *Trypanosoma* spp DNA. This test, which detects 1 genome in 40 µl of blood, allows the identification of *T. congolense, T. brucei, T. vivax* and *T. theileri* with a high degree of specificity (Geysen *et al*., 2003).

### 5.2.3. Data analysis

Parasitological and PCR/RFLP results were considered positive when either *T. congolense* or *T. vivax* were present in a single or mixed infection. The PCV-values were first analysed using a robust linear regression in Stata 9 (StataCorp, 2006) to detect a possible confounding effect of the age and sex category or an effect of trypanosomal infections on the PCV. The indicator used for trypanosomal infection was the PCR/RFLP result since it is currently the most sensitive and specific tool available for the diagnosis of trypanosomosis in cattle (Geysen *et al*., 2003). Explanatory variables were the PCR/RFLP binary results, the age and sex category, and the interaction between the two (PCV = a.PCR + b.category + c.PCR.category). Primary sampling units (sampling sites) and strata (age and sex classes) were taken into account. The significance of grouped explanatory variables was evaluated using the adjusted Wald test. The distribution of the residuals was verified by plotting their quantiles against the quantiles of the normal distribution (Q-Q plot). Heteroskedasticity was tested ($P > 0.05$) in non-robust models using the same explanatory variables.

In view of quickly estimating the PCV-values which could be of any use in the diagnosis of trypanosomosis, PCV-values were transformed in binary data using cut-off values ranging from the smallest to the largest observation. The sensitivity and the specificity of each transformation were calculated, using the PCR/RFLP results as a reference and plotted in a Receiver Operator Characteristic (ROC) curve.

Finally, the binary results of the PCR/RFLP test, the parasitological diagnosis and the PCV (using defined cut-off values) were introduced in a Bayesian model to estimate the sensitivity and the specificity of the three diagnostic tests (Berkvens *et al*., 2006). The Bayesian model was run in Winbugs (http://www.mrc-bsu.cam.ac.uk/bugs). The model (Annex 1) contained conditional probabilities, which were restricted as follows:

- the specificity of the parasitological diagnosis and the PCR/RFLP were set to 1; it is acknowledged that the true specificities of these tests can only be less than 1 but the specificity of both the parasitological (Schlater and Van den Bossche, 2004) and the PCR/REFLP (Geysen *et al*., 2003) tests were reported to be close to 1;
- the sensitivity of the PCR/RFLP was allowed to range between 0.9 and 1; this is in accordance with previously reported observations (Geysen *et al*., 2003) and it reflects the high sensitivity of the technique;
- based on our own experience, the conditional probability that an infected animal testing negative at PCR/RFLP would test positive at the parasitological diagnosis was set to a maximum of 0.1; this is based on the fact that both test sensitivities depend on the level of parasitaemia and on the postulation that an infected animal that is negative at PCR/RFLP is unlikely to be positive at a parasitological examination.

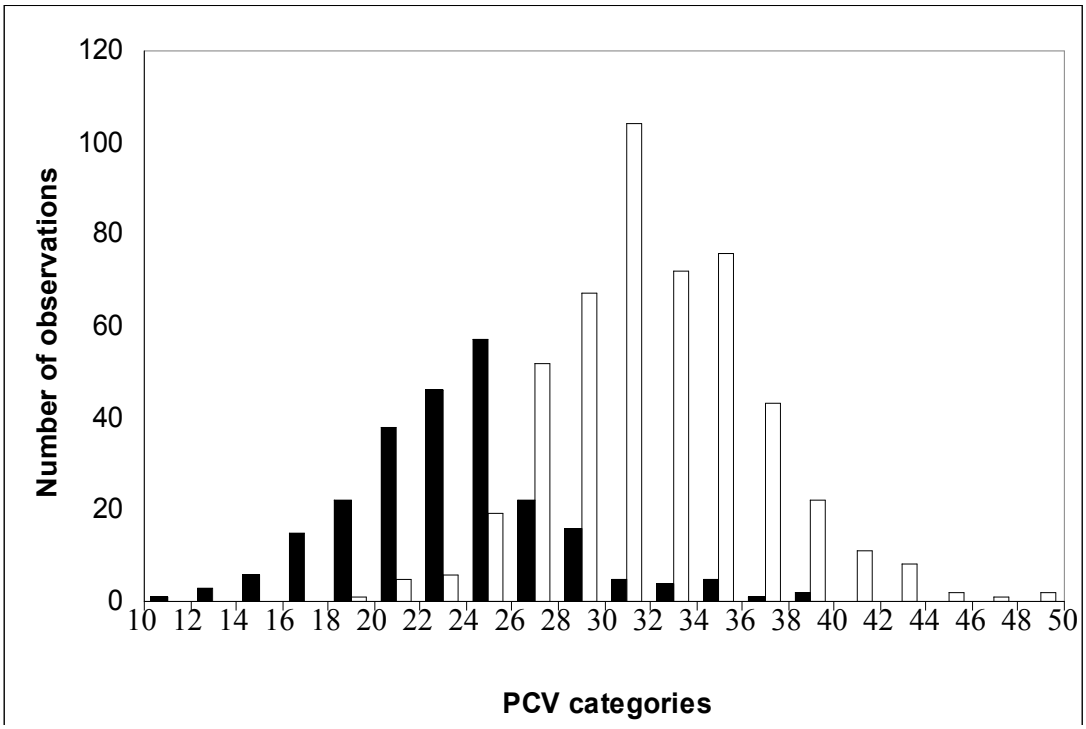
The conformity of the model was evaluated using the criteria proposed by Berkvens *et al*. (2006). In brief, the deviance information criterion (DIC) and the effective number of parameters estimated (pD) were calculated using, on one hand, parent node values in Winbugs and, on the other hand, the posterior means of the multinomial probabilities in R (http://www.r-project.org) (Annex 2). The agreement between the two types of calculations provides assurance on the conformity of the model. A Bayes P value (Gelman *et al*., 2003) close to 0.5 is another indicator of conformity. A high Bayes P value denotes a lack of fit of the model with the data. Each model was run three times in Winbugs. First, a non informative model, without any constraint on the conditional probabilities was run to estimate the DIC and pD. Second, the genuine model with the constraints listed above was run and the Bayes P, DIC and pD values were evaluated. This model provided estimates of the conditional probabilities and credibility intervals. Finally, a validation model, with strict constraints applied around the conditional probability estimates, was run to confirm that the Bayes P values tended towards 0. For each model, 20,000 iterations were used in three chains, following a burn-in of 10,000. Convergence was ensured by examining plots of the variable values against iteration numbers for the 3 chains. The sensitivity and the specificity of the three diagnostic tests as well as those of the combination of the parasitological test and the PCV were estimated.

### 5.3. Results

Clear discrepancies were observed between the results of the parasitological and PCR/RFLP diagnoses. Twenty percent of the samples (149/734) were positive on PCR/RFLP and negative at parasitological examination while none of the parasitologically positive samples were negative on PCR/RFLP. Since the specificity of both tests is assumed to be one, this reflects a significant difference in the sensitivity of the two tests (Fisher exact test: $p<0.001$).

Taking the PCR/RFLP results as a reference, the distribution of the PCV values of positive and negative animals differed substantially (Figure 5.1).

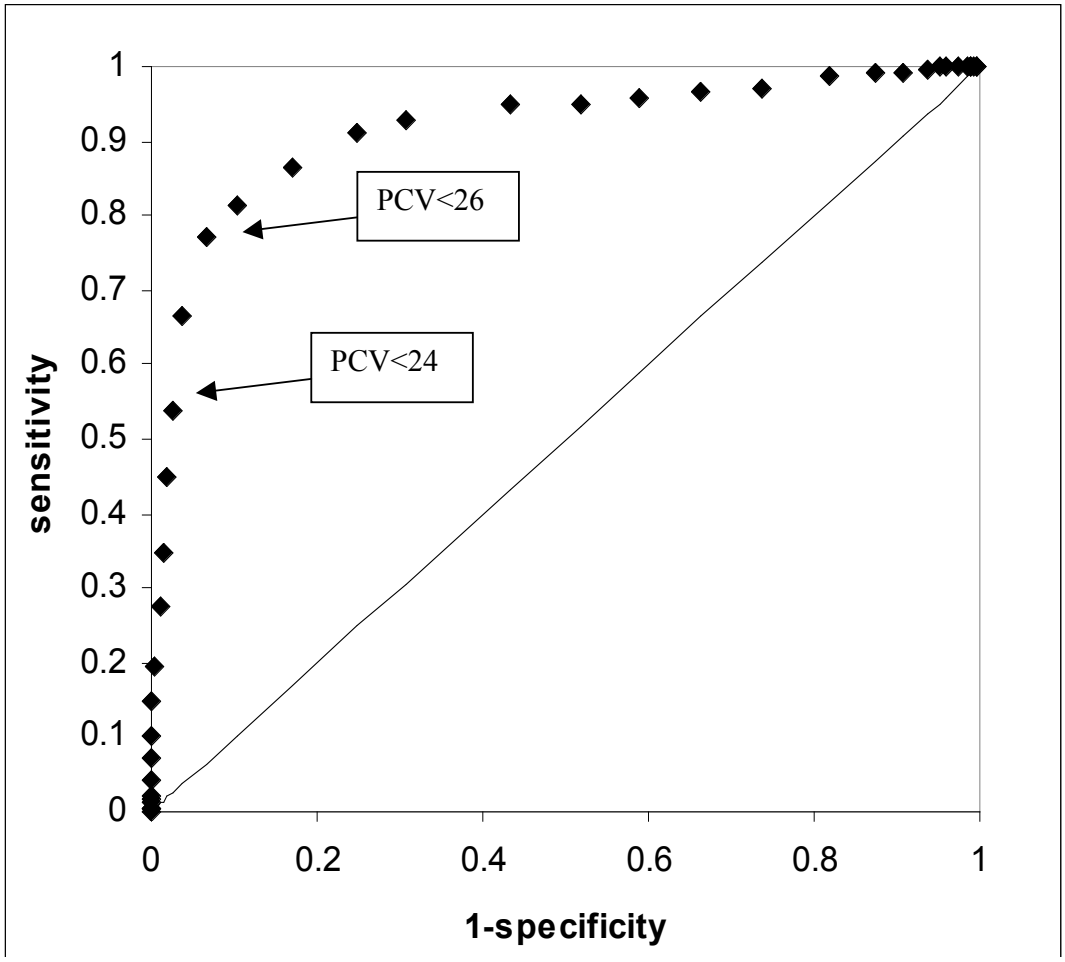
**Figure 5.1**. PCV-distribution of PCR/RFLP positive (■) and negative (□) animals




The linear regression of the PCV-values indicated a significant effect of the PCR/RFLP results ($p<0.001$) but no effect of the age and sex category of cattle from which the samples originated and their interaction with PCR/RFLP (grouped adjusted Wald test: $p=0.87$). The ROC curve (Figure 5.2) shows that, in the study area, the PCV-value that is usually used as a cut-off for anaemia in cattle (<24%) has a high specificity but a rather low sensitivity for identifying *Trypanosoma* spp PCR/RFLP

positive animals (0.97 and 0.54, respectively). Using a cut-off of 26% increases the sensitivity to 0.77 without much affecting the specificity (0.93).

**Figure 5.2**. ROC curve of PCV binary results using various cut-off values (<10 to <48, step 1); the PCR/RFLP test was considered as the reference test




The data used in the two Bayesian models, with 24% and 26% as cut-off values for the transformation of the PCV-values in binomial values, are presented in Table 5.1.

**Table 5.1**. Number of observations recorded in the 8 categories corresponding to a combination of the 3 test results and depending on the PCV cut-off value

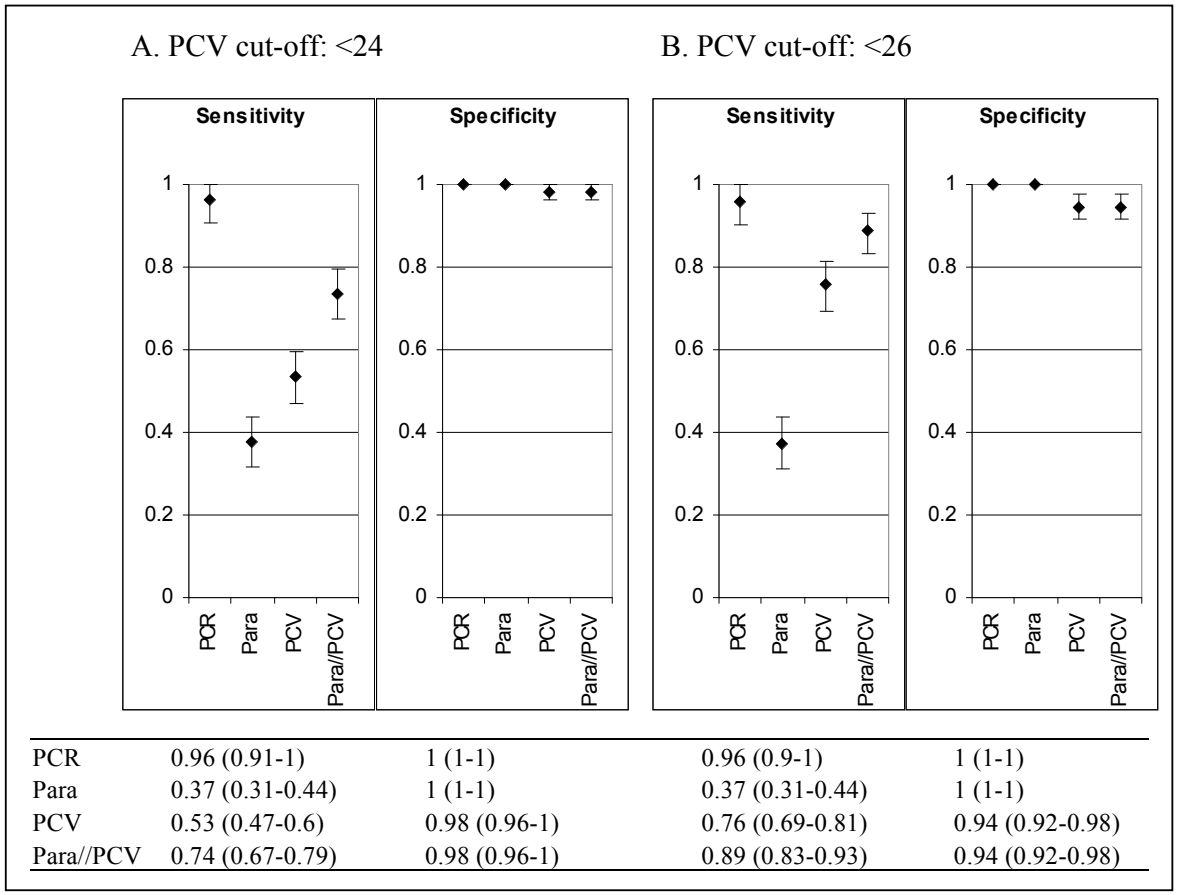
| PCR/RFLP | Parasitology | PCV | Number of observations | |
|---|---|---|---|---|
| | | | PCV < 24 | PCV < 26 |
| - | - | - | 479 | 460 |
| - | - | + | 12 | 31 |
| - | + | - | 0 | 0 |
| - | + | + | 0 | 0 |
| + | - | - | 61 | 22 |
| + | - | + | 88 | 127 |
| + | + | - | 51 | 33 |
| + | + | + | 43 | 61 |
| Total | | | 734 | 734 |

The indicators of the Bayesian analyses are presented in Annexes 3 and 4. They show a good fit of the data with the experts' constraints (Berkvens *et al*., 2006).

The conditional probabilities estimated by the two Bayesian models are listed in Annex 5. Among them, theta[1] (prevalence), theta[4] (parasitology sensitivity related parameter), theta[8] & theta[9] (PCV sensitivity related parameters) and theta[12] (PCV specificity related parameter) were well estimated, with narrow confidence intervals. For the others, the posterior distribution ranged uniformly between the limits of the constrained intervals, either because of the severity of the constraints (e.g.: theta[2], theta[3], theta[5], theta[6] & theta[7]) or because of a lack of data (e.g.: given its high sensitivity, very few infected animals are negative at the PCR/RFLP test, implying that theta[10] and theta[11] are poorly estimated).

According to these models, the prevalence of trypanosomal infections was 34% (95% credibility interval: 31 – 39%). The models confirmed the low sensitivity of the parasitological diagnosis compared to the PCR/RFLP test (37% and 96% respectively). They also indicated that the PCV has a better sensitivity than the parasitological diagnosis, whereas its estimated specificity was over 94% (Figure 5.3).

**Figure 5.3**. Estimated sensitivity and specificity and their 95% credibility intervals for the parasitological test (Para), the PCR/RFLP test (PCR), the PCV and the parallel combination of the parasitological test and the PCV (Para/PCV) using two different PCV cut-off values (24 in A. and 26 in B.)




| | | | | |
|---|---|---|---|---|
| PCR | 0.96 (0.91-1) | 1 (1-1) | 0.96 (0.9-1) | 1 (1-1) |
| Para | 0.37 (0.31-0.44) | 1 (1-1) | 0.37 (0.31-0.44) | 1 (1-1) |
| PCV | 0.53 (0.47-0.6) | 0.98 (0.96-1) | 0.76 (0.69-0.81) | 0.94 (0.92-0.98) |
| Para//PCV | 0.74 (0.67-0.79) | 0.98 (0.96-1) | 0.89 (0.83-0.93) | 0.94 (0.92-0.98) |

A parallel combination of the parasitological diagnosis and the PCV improves diagnostic sensitivity while specificity remains high (Figure 5.3). The estimated sensitivity of test combinations is higher than for independent test combinations [$1-(1-se_{para}).(1-se_{pcv})$ = 0.70 and 0.85 for <24 and <26 cut-offs respectively]. The specificity is lower than that of the parasitological and the PCR/RFLP tests, which are assumed to be 100%.

## 5.4. Discussion

The results of the study confirm the low sensitivity of the routinely used parasitological tests for the diagnosis of trypanosomosis. Despite the concentration of the parasite, the buffy coat method fails to detect 66% of the infected animals in the study area. This finding confirms many earlier observations and is attributed to the low parasitaemia especially in chronic trypanosomosis cases (Picozzi *et al*., 2002). However, in the absence of other diseases causing anaemia in individual animals, the findings of this study suggest that the PCV-value of an individual animal is a good indicator of the presence of a trypanosomal infection. This is especially the case when the PCV-value is used jointly with the results of the parasitological diagnosis. Indeed, by combining the two types of information, the diagnostic sensitivity increases to 74% and even 89% using a PCV cut-off of 24 or 26% respectively, which is considerably higher than the sensitivity of the parasitological diagnosis on its own. These values are surprisingly higher than in independent test combinations. This is related to the fact that the conditional probability theta 8 is lower than theta 9 in Annex 5. This implies a negative dependence between the two diagnostic tests in infected animals: a parasitologically positive animal is less likely to have anaemia than an infected parasitologically negative animal. This could be explained by the fact that early infections are more likely to be detected using parasitological diagnostic tools even before the development of anaemia whereas chronically infected animals, usually presenting low parasitaemia, are more likely to be anaemic.

Although both the sensitivity and the specificity of the parallel combination of the PCV and the parasitological test is lower than that of the PCR/RFLP, the cost of the former is a fraction of the costs involved when conducting PCR/RFLP. Moreover, the equipment required for parasitological diagnosis and PCV measurement should be part of the standard equipment of field veterinary laboratories in trypanosomosis endemic areas. Since under the conditions prevailing in the study area, the PCV-value on its own already has a high diagnostic sensitivity and specificity, one could consider the use of a simple tool to be applied easily by animal health technicians or cattle owners to assess the level of anaemia of an animal. FAMACHA© eye colour chart and Hemoglobin Color Scale tests, which showed evidence of robustness and reliability for the detection of anaemia in cattle in western Africa (Grace *et al*., 2007), could possibly be used as an alternative to PCV

measurement in spite of a probable lower precision. In fact, similar tools have been implemented successfully in the control of haemonchosis in sheep and goats (Bath *et al*., 2001).

The applicability of this simple approach relies heavily on the level of anaemia at which an animal is considered to be infected with trypanosomes. The analysis of a ROC curve, using PCR/RFLP results as a reference should help in selecting the most appropriate cut-off, depending on the required test sensitivity and specificity. The specificity of the parallel combination of PCV and parasitological diagnosis could be affected by infections with *Anaplasma* spp., *Babesia* spp. or *Theileria parva* (Fandamu *et al*., 2007), by gastro-intestinal worm infestations (Grace *et al*., 2007) or by any other factor which could cause anaemia in cattle. Good nutritional status was reported to reduce the impact of trypanosome infection on PCV (Holmes *et al*., 2000; Van den Bossche and Rowlands, 2001). This could reduce the sensitivity of the PCV as indicator of trypanosomal infections in animals with high nutritional status. However, animals' nutritional state is unlikely to affect its specificity since anaemia of nutritional origin can only be caused by severe starvation and nutritional deficiencies (Anon., 2006).

In situations where other causes of anaemia are suspected, additional indicators, specific for these causes (parasitaemia, coprological detection of eggs from helminths that may cause anemia i.e. *Haemonchus, Fasciola…*, fever and other specific clinical signs), might be considered to discriminate their effect on the PCV from that of trypanosomosis. In any case, the specificity of the proposed diagnostic approach should be confirmed and monitored in areas where it is to be used, for instance by comparing a sample of the results to a PCR/RFLP analysis in a ROC curve.

In conclusion, the clinical evaluation of anaemia may be a useful low-cost adjunct to other trypanosomosis management tools. It would certainly complement the parasitological tests and provide the animal health workers that do not have diagnostic tools at their disposal with a relatively objective tool that can be used in identifying animals that require treatment with trypanocidal drugs.

## 5.5 References

Anon, (2006) The Merck Veterinary Manual. Merck and Co., Inc., Whitehouse Station.

Bath, Hansen, Krecek, Van Wyk, Vatta (Eds.), (2001) Sustainable approaches for managing haemonchosis in sheep and goats. *FAO Animal Production and Health Paper*, Rome.

Berkvens, D., Speybroeck, N., Praet, N., Adel, A., and Lesaffre, E. (2006) Estimating disease prevalence in a Bayesian framework using probabilistic constraints. *Epidemiology,* **17**, 145-153.

Fandamu, P., Marcotty, T., Brandt, J.R.A., Duchateau, L., Speybroeck, N., Dolan, T.T., and Berkvens, D. (2007) Red blood cell volume as a predictor of fatal eactions in cattle infected with *Theileria parva* Katete. *Onderstepoort Journal of Veterinary Research*, **74**, 37-43.

Gelman, Carlin, Stern, Rubin (Eds.), (2003) *Bayesian data analysis*. Chapman and Hall/CRC, Boca Raton.

Geysen, D., Delespaux, V., and Geerts, S. (2003) PCR-RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of *Trypanosoma* species in cattle. *Veterinary Parasitology*, **110**, 171-180.

Grace, D., Himstedt, H., Sidibe, I., Randolph, T., and Clausen, P.H. (2007) Comparing FAMACHA eye color chart and Hemoglobin Color Scale tests for detecting anemia and improving treatment of bovine trypanosomosis in West Africa. *Veterinary Parasitology*, **147**, 26-39.

Holmes, P.H., Katunguka-Rwakishaya, E., Bennison, J.J., Wassink, G.J., and Parkins, J.J. (2000) Impact of nutrition on the pathophysiology of bovine trypanosomosis. *Parasitology* **120** Supplement, S73-S85.

http://www.mrc-bsu.cam.ac.uk/bugs

http://www.r-project.org

Lesaffre, E., Speybroeck, N., and Berkvens, D. (2007) Bayes and diagnostic testing. *Veterianry Parasitology*, **148**, 58-61.

Murray, M., and Dexter, T.M. (1988) Anaemia in bovine African Trypanosomosis: a review. *Acta Tropica*, **45**, 389-432.

Murray, M., Murray, P.K., and McIntire, W.M. (1977) An improved parasitological techniques for the diagnosis of African trypanosomosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **71**, 325-326.

Nicholson, Butterworth (Eds.), (1986) *A guide to body condition scoring of zebu cattle*. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia.

Picozzi, K., Tilly, A., Fèvre, E.M., Coleman, P., Magona, J.W., Odiit, M., Eisler, M.C., and Welbum, S. (2002) The diagnosis of trypanosome infections:

applications of novel technology for reducing disease risk. *African Journal of Biotechnology*, **1**, 39-45.

Schlater, J., and Van den Bossche, P. (2004) Trypanosomosis (tsetse-transmitted). *OIE manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees)*. OIE, Paris, pp. 580-588.

StataCorp (Ed.), (2006) Stata Statistical Software: Release 9.2. Stata Corporation, Texas.

Thrusfield (Ed.), (1995) *Veterinary Epidemiology*. Blackwell, O

Trail, J.C.M., D'Ieteren, G.D.M., Murray, M., Ordner, G., Yangari, G., Maille, J.C., oxford. Viviani, P., Colardelle, C., and Sauveroche, B. (1993) Measurements of trypanotolerance criteria and their effect on reproductive performance of N'Dama cattle. *Veterinary Parasitology*, **45**, 241-255.

Van den Bossche, P., Chigoma, D., and Shumba, W. (2000) The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge. *Acta Tropica*, **77**, 263-270.

Van den Bossche, P., and Rowlands, G.J. (2001) The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume. *Acta Tropica*, **78**, 163-170.

## 5.6. Annexes

**Annex 1:** Bayesian model run in Winbugs

```
{
N <-sum(r[])
r[1:8]~dmulti(p[1:8], N)

p[1] <- theta[1]*(1-theta[2])*(1-theta[5])*(1-theta[11])+(1-theta[1])*theta[3]*theta[6]*theta[12]
p[2] <- theta[1]*(1-theta[2])*(1-theta[5])*(theta[11])+(1-theta[1])*theta[3]*theta[6]*(1-theta[12])
p[3] <- theta[1]*(1-theta[2])*theta[5]*(1-theta[10])+(1-theta[1])*theta[3]*(1-theta[6])*theta[13]
p[4]<- theta[1]*(1-theta[2])*theta[5]*(theta[10])+(1-theta[1])*theta[3]*(1-theta[6])*(1-theta[13])
p[5]<-theta[1]*theta[2]*(1-theta[4])*(1-theta[9])+(1-theta[1])*(1-theta[3])*theta[7]*theta[14]
p[6]<-theta[1]*theta[2]*(1-theta[4])*(theta[9])+(1-theta[1])*(1-theta[3])*theta[7]*(1-theta[14])
p[7]<-theta[1]*theta[2]*theta[4]*(1-theta[8])+(1-theta[1])*(1-theta[3])*(1-theta[7])*theta[15]
p[8]<-theta[1]*theta[2]*theta[4]*(theta[8])+(1-theta[1])*(1-theta[3])*(1-theta[7])*(1-theta[15])

theta[1] ~ dunif(0,1)
theta[2] ~ dunif(0.9,1)
theta[3] ~ dunif(1,1)
theta[4] ~ dunif(0,1)
theta[5] ~ dunif(0,0.1)
theta[6] ~ dunif(1,1)
theta[7] ~ dunif(1,1)
theta[8] ~ dunif(0,1)
theta[9] ~ dunif(0,1)
theta[10] ~ dunif(0,1)
theta[11] ~ dunif(0,1)
theta[12] ~ dunif(0,1)
theta[13] ~ dunif(0,1)
theta[14] ~ dunif(0,1)
theta[15] ~ dunif(0,1)

se[1] <- theta[2]
se[2] <- theta[4]*theta[2]+theta[5]*(1-theta[2])
se[3] <- theta[8]*theta[4]*theta[2]+theta[9]*(1-theta[4])*theta[2]+theta[10]*theta[5]*(1-theta[2])+theta[11]*(1-
theta[5])*(1-theta[2])
se[4]<- se[2]+theta[9]*(1-theta[4])*theta[2]+theta[11]*(1-theta[5])*(1-theta[2])

sp[1] <- theta[3]
sp[2] <- theta[6]*theta[3]+theta[7]*(1-theta[3])
sp[3] <- theta[12]*theta[6]*theta[3]+theta[13]*(1-theta[6])*theta[3]+theta[14]*theta[7]*(1-theta[3])+theta[15]*(1-
theta[7])*(1-theta[3])
sp[4]<- sp[2]*sp[3]

r2[1:8] ~ dmulti( p[1:8], N)
for (i in 1:8)
{
        z1[i] <- equals(0,p[i])
        y1[i] <- max(z1[i],p[i])
        x1[i] <- max(r[i],1)
        d[i] <- r[i]*log(x1[i]/(y1[i]*N))

        z2[i] <- equals(0,p[i])
        y2[i] <- max(z2[i],p[i])
        x2[i] <- max(r2[i],1)
        d2[i] <- r2[i]*log(x2[i]/(y2[i]*N))
}
G0 <- 2 * sum(d[])
Gt <- 2 * sum(d2[])
bayesp <- step(G0 - Gt)
}
```

p[1:8] are the number of observations of each of the test result combinations (Table 1); theta[1:15] are the conditional probabilities (see Annex 5) subjected to experts' constraints; se[1:4] are the sensitivity of the PCR/RFLP, the parasitological test, the PCV and the parallel combination of the parasitological test and the PCV; sp[1:4] are the specificity of the same tests.

**Annex 2:** Code for the estimation of DIC and pD using posterior means of the multinomial probabilities in R software

```
doit <- function(results, iterations, bugsmodel)
{
i <- iterations
r <- results
N <- sum(r)
data <- list(r=r)
inits <- NULL
nodes <- c("bayesp", "p", "theta", "se", "sp")
diagtest.sim <- bugs(data, inits, nodes, bugsmodel, n.chains=3, n.iter=i[1], n.burnin=i[2], n.thin=1)
attach.bugs(diagtest.sim)
dbar <- mean(deviance)
numClass <- length(r)

rtheta<-matrix(,15)
for (i in 1:15){
rtheta[i] <- mean(theta[,i])
}
rp<-matrix(,8)

## proportions are calculated from posterior means parent nodes as it is done in Winbugs
rp[1] <- rtheta[1]*(1-rtheta[2])*(1-rtheta[5])*(1-rtheta[11])+(1-rtheta[1])*rtheta[3]*rtheta[6]*rtheta[12]
rp[2] <- rtheta[1]*(1-rtheta[2])*(1-rtheta[5])*(rtheta[11])+(1-rtheta[1])*rtheta[3]*rtheta[6]*(1-rtheta[12])
rp[3] <- rtheta[1]*(1-rtheta[2])*rtheta[5]*(1-rtheta[10])+(1-rtheta[1])*rtheta[3]*(1-rtheta[6])*rtheta[13]
rp[4]<- rtheta[1]*(1-rtheta[2])*rtheta[5]*(rtheta[10])+(1-rtheta[1])*rtheta[3]*(1-rtheta[6])*(1-rtheta[13])
rp[5]<-rtheta[1]*rtheta[2]*(1-rtheta[4])*(1-rtheta[9])+(1-rtheta[1])*(1-rtheta[3])*rtheta[7]*rtheta[14]
rp[6]<-rtheta[1]*rtheta[2]*(1-rtheta[4])*(rtheta[9])+(1-rtheta[1])*(1-rtheta[3])*rtheta[7]*(1-rtheta[14])
rp[7]<-rtheta[1]*rtheta[2]*rtheta[4]*(1-rtheta[8])+(1-rtheta[1])*(1-rtheta[3])*(1-rtheta[7])*rtheta[15]
rp[8]<-rtheta[1]*rtheta[2]*rtheta[4]*(rtheta[8])+(1-rtheta[1])*(1-rtheta[3])*(1-rtheta[7])*(1-rtheta[15])
dhat <- -2*sum(log(seq(N)))
for (i in 1:numClass)
{
z <- max(r[i],1)
dhat <- dhat+2*sum(log(seq(z))) -2*r[i]*log(rp[i])
}
pd <- dbar-dhat
dic <- dhat + 2*pd
print(c("posterior deviance (using parent nodes as done in Winbugs) = ", formatC(dhat, digits=2, format= "f")),quote=FALSE)
print(c("DIC = ", formatC(dic, digits=2, format= "f")), quote = FALSE)
print(c("pD = ", formatC(pd, digits=2, format= "f")), quote = FALSE)

## using posterior means of actual proportions
for (i in 1:8){
rp[i] <- mean(p[, i])
}
dhat <- -2*sum(log(seq(N)))
for (i in 1:numClass)
{
z <- max(r[i],1)
dhat <- dhat+2*sum(log(seq(z))) -2*r[i]*log(rp[i])
}
pd <- dbar-dhat
dic <- dhat + 2*pd

print(c("posterior deviance (using post. means) = ", formatC(dhat, digits=2, format= "f")),quote=FALSE)
print(c("DIC = ", formatC(dic, digits=2, format= "f")), quote = FALSE)
print(c("pD = ", formatC(pd, digits=2, format= "f")), quote = FALSE)

dhat <- -2*sum(log(seq(N)))
for (i in 1:numClass)
{
z <- max(r[i],1)
y <- r[i]/N
if (y==0) y <- 1
dhat <- dhat+2*sum(log(seq(z))) -2*r[i]*log(y)
}
print(c("minimum deviance = ", formatC(dhat, digits=2, format= "f")),quote=FALSE)
format.AsIs(diagtest.sim$summary, digits=5)
diagtest.sim$summary
}
```

**Annex 3**: DIC and pD values estimated using parent nodes and posterior means and obtained from the bayesian models ran on the 2 datasets with different constraints applied on the parameters

| | PCV < 24 | | PCV < 26 | |
|---|---|---|---|---|
| | Using parent nodes | Using posterior means of the multinomial probabilities | Using parent nodes | Using posterior means of the multinomial probabilities |
| Model without constraints | | | | |
| DIC | -365.80 | 40.82 | -299.25 | 41.00 |
| pD | -401.74 | 4.88 | -335.38 | 4.87 |
| Model with experts' constraints | | | | |
| DIC | 38.00 | 38.12 | 38.26 | 38.33 |
| pD | 4.73 | 4.85 | 4.80 | 4.87 |

DIC and pD values of the constrained models are similar whether their estimation is based on parent nodes or on posterior means of the multinomial probabilities, unlike the unconstrained model; in both models restricted by experts' opinion, pD values, which correspond to the number of parameters estimated in Bayesian models, are close to the maximum (estimated in the unconstrained models using posterior means).

**Annex 4:** Bayesp values obtained from the bayesian models ran on the 2 datasets with different constraints applied on the parameters

| | PCV < 24 | PCV < 26 |
|---|---|---|
| Model without constraints | 0.61 | 0.61 |
| Model with experts' constraints | 0.48 | 0.48 |
| Model constrained on estimates | 0.004 | 0.0007 |

Bayesp values indicates too severe constraints when diverting from 0.5; Bayesp value tend towards 0 when appropriate constraints are applied

**Annex 5:** Conditional probabilities (and their 95% credibility intervals) estimated by the Bayesian models

| Code | Conditional probabilities | Constraints | PCV<24 | PCV<26 |
|---|---|---|---|---|
| theta[1] | $\Pr(D^+)$ | | 0.34 (0.31 - 0.38) | 0.35 (0.31 - 0.39) |
| theta[2] | $\Pr(T_1^+ \mid D^+)$ | 0.9 - 1 | 0.96 (0.91 - 1) | 0.96 (0.9 - 1) |
| theta[3] | $\Pr(T_1^- \mid D^-)$ | = 1 | 1 (1 - 1) | 1 (1 - 1) |
| theta[4] | $\Pr(T_2^+ \mid D^+ \text{ ?¿} T_1^+)$ | | 0.39 (0.33 - 0.45) | 0.39 (0.33 - 0.45) |
| theta[5] | $\Pr(T_2^+ \mid D^+ \text{ ?¿} T_1^-)$ | 0 - 0.1 | 0.04 (0 - 0.1) | 0.04 (0 - 0.1) |
| theta[6] | $\Pr(T_2^- \mid D^- \text{ ?¿} T_1^-)$ | = 1 | 1 (1 - 1) | 1 (1 - 1) |
| theta[7] | $\Pr(T_2^- \mid D^- \text{ ?¿} T_1^+)$ | = 1 | 1 (1 - 1) | 1 (1 - 1) |
| theta[8] | $\Pr(T_3^+ \mid D^+ \text{ ?¿} T_1^+ \text{ ?¿} T_2^+)$ | | 0.46 (0.36 - 0.56) | 0.65 (0.55 - 0.74) |
| theta[9] | $\Pr(T_3^+ \mid D^+ \text{ ?¿} T_1^+ \text{ ?¿} T_2^-)$ | | 0.59 (0.51 - 0.67) | 0.85 (0.79 - 0.9) |
| theta[10] | $\Pr(T_3^+ \mid D^+ \text{ ?¿} T_1^- \text{ ?¿} T_2^+)$ | | 0.5 (0.03 - 0.98) | 0.5 (0.03 - 0.98) |
| theta[11] | $\Pr(T_3^+ \mid D^+ \text{ ?¿} T_1^- \text{ ?¿} T_2^-)$ | | 0.46 (0.02 - 0.97) | 0.5 (0.02 - 0.97) |
| theta[12] | $\Pr(T_3^- \mid D^- \text{ ?¿} T_1^- \text{ ?¿} T_2^-)$ | | 0.98 (0.96 - 1) | 0.94 (0.92 - 0.98) |
| theta[13] | $\Pr(T_3^- \mid D^- \text{ ?¿} T_1^- \text{ ?¿} T_2^+)$ | | 0.5 (0.02 - 0.98) | 0.5 (0.03 - 0.98) |
| theta[14] | $\Pr(T_3^- \mid D^- \text{ ?¿} T_1^+ \text{ ?¿} T_2^-)$ | | 0.5 (0.02 - 0.97) | 0.5 (0.02 - 0.97) |
| theta[15] | $\Pr(T_3^- \mid D^- \text{ ?¿} T_1^+ \text{ ?¿} T_2^+)$ | | 0.5 (0.03 - 0.97) | 0.5 (0.02 - 0.98) |

$T_1$, $T_2$ and $T_3$ are the PCR/RFLP, the parasitological test and the PCV respectively

# CHAPTER 6

# Improved control of tsetse-transmitted livestock trypanosomosis.

# How far can we go?

## 6.1. Introduction

For more than a century, the epidemiology and control of livestock trypanosomosis have been the subjects of a large number of research projects and papers published in the scientific literature. Generally, the epidemiology of the disease is relatively known but ongoing changes due to anthropogenic factors (agricultural activities, settlements) affecting landscape and the distribution and density of wild and domestic hosts of tsetse flies require further refinement of the disease's epidemiology to support the development of adapted and more appropriate control strategies. The research presented in this thesis has addressed an important epidemiological setting that occurs in southern Africa (e.g. the plateau of eastern Zambia), namely a situation where tsetse flies have become very dependent on livestock for their survival leading to endemic trypanosomosis conditions. The outcome of the research has resulted in a number of novel findings with regard to the role of cattle and its role in the epidemiology if tsetse-transmitted livestock trypanosomosis and the improved diagnosis of the disease in this livestock species. This final chapter of the thesis discusses these research findings in the broader context of controlling livestock trypanosomosis in endemic areas. Moreover, a simulation model is used to predict the repercussions of implementing some of the findings on the efficacy and cost effectiveness of trypanosomosis control in endemic areas.

## 6.2. The importance of cattle, pigs and goats in the epidemiology of livestock trypanosomosis in the endemic area on the eastern plateau of Zambia

It has long been suspected that, although quite often neglected in trypanosomosis control programmes, goats and pigs could play an important epidemiological role as reservoirs of trypanosomes. Such reservoirs could act as source of trypanosomal infections for tsetse flies that, subsequently, can infect economically more important livestock species, such as cattle. In such a case, the control of trypanosomosis in goats and pigs could possibly contribute to a reduction in the incidence and/or prevalence of the disease in cattle. Moreover, trypanosomal infections in goat and/or pig populations could adversely affect production of these livestock species. Based on the information available from the literature (Van den Bossche and Staak, 1997; also see chapter 2), we postulated that the prevalence of

trypanosomal infections in goats and pigs on the plateau area of eastern Zambia is low. This is attributed to the small proportion of bloodmeals taken from goats and/or pigs by the tsetse fly species, i.e. *G. m. morsitans,* present on the plateau. To verify whether cattle were indeed the main host and the main reservoir of trypanosomal infections, a trypanosomosis survey using sensitive molecular diagnostic tools (PCR-RFLP) was conducted (Chapter 2). The results of the survey supported the hypothesis that cattle consistently have higher trypanosome infection rates compared to goats or pigs. It could be postulated that controlling trypanosomosis in pigs or goats is, from an epidemiological and economic perspective, not adviceable. However, further investigations are needed to confirm or infirm this assumption.

The significant role cattle play in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia has important implications for the control of the vector. Indeed, considering the frequency with which tsetse flies feed on cattle, the exploitation of those animals as moving baits in the control of tsetse is expected to be effective. This was demonstrated by Van den Bossche *et al.* (2004) who managed to control tsetse on an area of about 2000 $km^2$ of the plateau based entirely on the regular application of insecticides to cattle. Moreover, the control of the parasite with trypanocidal drugs could focus mainly on treating those animal species that are the main source of feed for tsetse, i.e. cattle. As these animals also act as reservoir of trypanosomes, it could be envisaged that systematic control of the infection in cattle could lead to a depletion of the source of trypanosomes and, therefore, to a reduction of infection in tsetse fly populations with decreased vector-borne challenge and, hence a reduction in disease challenge, even in conditions of high tsetse pressures.

Although the situation described in this thesis may be specific for the plateau of eastern Zambia, the importance of cattle as host of tsetse flies is likely to be similar in vast zones of tsetse-infested southern Africa. This is driven by the increased human-livestock encroachment into tsetse-infected zones. However, depending on the circumstances, the role of livestock species other than cattle, such as small ruminants and pigs, in the epidemiology of trypanosomosis may be minimal. In absence of cattle, pigs and small ruminants can become preferential hosts of tsetse flies, transmitting the disease and, thus, impact negatively on small ruminant productivity. In such situations, control of the disease in those species could be advisable, also from an economic point of view. However, in areas where small ruminant indigenous

trypanotolerant breeds exist, the impact on small ruminant production may be limited provided the small ruminants are relatively pure breeds and that there is not high crossbreeding with trypanosusceptible breeds (Geerts *et al.*, 2009).

## 6.3. The prevalence of trypanosomal infections in different age and sex categories of cattle in the endemic area on the eastern plateau of Zambia

Considering the importance of cattle in the epidemiology of livestock trypanosomosis on the eastern plateau, it remains questionable if in a cattle herd, (where animals of different ages, sexes and sizes are present) challenge is homogeneously distributed as a result of random selection of hosts by tsetse flies. Entomological studies suggest that, in a herd, tsetse flies are more attracted to, and feed more frequently on the largest animals. According to Torr *et al*. (2006) and Torr and Mangwiro (2000), tsetse flies are attracted significantly more by the odour of large animals (i.e. oxen) and animals that show less defensive behaviour. Hence, the high preference for oxen and the low preference for calves that have well-developed defensive reactions.

The results of our longitudinal study of a sentinel herd composed of a normal distribution of cattle of different ages and sexes (Chapter 3) confirmed that tsetse challenge was not homogeneous but rather heterogenous with the largest animals (i.e. oxen) submitted to the highest challenge resulting in the highest incidence of infections. Although the results of our study may not be definitive to draw general conclusions for the refinement of intervention control strategies for livestock trypanosomosis, some important observations can be made with regard to the control of the disease in the study area with potential extrapolation in comparable agro-epidemiological and ecological areas. The high risk of trypanosome infections in oxen and the high proportion of oxen in the cattle herd (Connor, 2000), make oxen the most appropriate part of the herd for prophylactic trypanocidal treatments. Previous studies on the use of trypanocidal drugs have shown that oxen already receive more treatments with trypanocides than other categories (Van den Bossche *et al.,* 2000). Indeed, of the total number of oxen investigated, 83.8% received at least one diminazene aceturate treatment per year whereas this percentage was only 55.1%, 22.2% and 2.5% for cows, young stock and calves respectively (Van den Bossche *et al.,* 2000). This high percentage of treatment in oxen could be attributed to the higher

level of trypanosome challenge this animal category is submitted to and/or the attention given by the livestock owners to oxen in consideration to the importance oxen play in mixed livestock/farming production systems. Since the majority of the treatments are curative, increased strategic use of prophylactic trypanocides could be a more appropriate option of treatments. Indeed, such a drug strategy will target the most affected or at risk fraction of the herd and may, at the same time, reduce the number of tsetse that will become infected. In addition, it may result in a reduced frequency of chemical treatments and, therefore, in decreased risk of development of drug resistance in trypanosomes. Additionally, in accordance with the strategic insecticide applications on cattle to control tsetse, selective treatment of the "preferred" tsetse hosts will also reduce the number of tsetse flies and, consequently, the vector-disease challenge. Such restricted selective use of insecticide compounds would results in increased economic benefits due to the effect of insecticides on tick infestation(s) with positive outcomes for the establishment and preservation of the endemic stability to tick-borne diseases (Mattioli *et al*., 1999).

## 6.4. Bovine trypanosomosis risk in an endemic area on the eastern plateau of Zambia

The management of bovine trypanosomosis on the eastern plateau of Zambia is usually based on treatment with curative doses of diminazene aceturate of severely sick animals (Van den Bossche *et al*., 2000). This approach aims at reducing the mortality due to the infection but has limited impact on the negative effects of the disease on total herd productivity. In this regard, a better understanding of differences in levels of challenge(s) of various animal categories composing a herd coupled with information on seasonal disease challenge variations would benefit the development and implementation of strategic prophylactic treatment strategies (Chapter 4). In our studies, although distinct monthly challenge trends were not statistically significant, a seasonality of the infections could be evinced, with the incidence peak occurring during the rainy season. On the other hand, the trypanosome infection rate in the tsetse population undergoes little seasonal variation (Kubi *et al.,* 2007). From the above, it could be derived that the high incidence of trypanosomal infections during the rainy season is mainly due to the the increased density of the tsetse population during this period of the year (Van den Bossche and De Deken, 2002). The observed seasonality of infection allows for the temporal prioritization of trypanosomosis

control. Based on our findings, livestock owners could be advised to start mobilizing more financial resources for the control of trypanosomosis when the rainy season approaches. However, the drawback to this recommendation is that in resource poor areas, such as the eastern plateau of Zambia, the pre-rainy season period is also the period when farmers are mobilizing funds and human resources for agricultural activities, for example, maize production. This means that there could be a conflict of interest between resources for crop production and resources for trypanosomosis control. However, the resources for trypanosomosis control does not necessarily need to be highly demanding since farmers can apply strategic prophylactic treatment scheme(s) mainly in oxen considering that this cattle category appears to be the most affected and of high value, particularly during the crop production season.

## 6.5. The diagnostic value of the Packed Cell Volume (PCV) as an indicator of trypanosomal infections in cattle in the endemic area on the eastern plateau of Zambia

Irrespective of the measures taken to control livestock trypanosomosis, the need will remain for an accurate diagnosis of the infections in livestock in order to apply epidemiological knowledge for the establishment of strategic drug treatment regime(s). There is, therefore, a need for simple more senstitive field applicable tools for livestock trypanosomosis diagnosis. Such tools are not yet available; hence improvements of currently available methods could offer an attractive alternative. In cattle and other susceptible domestic animals, anaemia often occurs following infection with pathogenic trypanosomes, including *T. congolense* and *T. vivax* (Murray and Dexter, 1988). The outcome of a Bayesian model applied to data collected in our investigations showed that the clinical diagnostic sensitivity of the PCV value, which is a measure of the level of anaemia, was higher than the diagnostic sensitivity of the parasitological test (buffy coat method) Importantly, the association of the parasitological diagnosis with the measure of the PCV value proved to increase the diagnostic sensitivity (from 37% for buffy coat method alone) to 74% for the parallel use of PCV and parasitological diagnosis with PCR-RFLP as the Gold standard. This finding has important consequences for the management of livestock trypanosomosis on the plateau area of eastern Zambia. Indeed, in the absence of other diseases inducing anaemia in individual animals, the findings of this study suggest that the PCV value of an individual animal is a good indicator of the presence of a

trypanosomal infection. Hence, any tool that can give an estimate of the level of anaemia (such as the FAMACHA-chart used in the diagnosis of haemonchosis in sheep and based on the colour of the mucosa of the eye) could be used by the individual livestock owner to determine the infection status of his animals. It thus seems that the clinical evaluation of anaemia may be a useful low-cost adjunct to other trypanosomosis management tools. It would certainly complement the parasitological tests and provides the animal health workers that do not have diagnostic tools at their disposal with a relatively easy practical method that can be used in the field to identify animals requiring treatment with trypanocidal drugs.

## 6.6. Practical implications of the results for the control of endemic trypanosomosis

Despite long lasting efforts for the control of livestock trypanosomosis in the tsetse-infested areas of Africa in general and on the plateau area of eastern Zambia, in particular, livestock trypanosomosis remains an important veterinary problem that requires attention. Experiences have shown that, despite the availability of several control methods and strategies developed, much remains to be done to ensure adaptation of tsetse and trypanosomosis intervention schemes to the prevailing local epidemiological conditions. Such adaption is likely to increase the effectiveness of the available tools and reduce costs by allowing for a targeted and strategic approach.

The results presented in this thesis show that the trypanosomosis epidemiological setting on the plateau of the Eastern Province of Zambia does allow for considerable improvement of the livestock trypanosomosis control stategies. Indeed, the outcome of the studies presented in the various chapters of this thesis each contributes to better targeting livestock trypanosomosis management and control interventions. First and formost, the comparative study of the prevalence of the disease in different livestock species made it possible to pinpoint cattle as being the livestock species most affected by the disease. Hence, under the conditions prevailing in the study area, allocating resources to control trypanosomosis in other livestock species is not adviseable. Further investigations carried out identified those cattle categories being at high risk/challenge. Although this does not imply that the other cattle classes are not submitted to tsetse challenge, the finding support the implementation of, for example, targeted prophylactic trypanocidal drug strategies or supports the selective application of insecticides to control tsetse flies. The improved

diagnosis of trypanosomosis in cattle, suggested in the last chapter of the thesis, makes it possible to better target interventions by improving the identification of animals that are infected and require drug treatment. It, thus, seems that under the main agro-epidemiological and ecological conditions on the plateau of eastern Zambia, livestock trypanosomosis is not a disease that affects evenly the entire livestock population but mainly cattle and, within a herd, only certain categories and classes of animals. This particular tsetse-trypanosome challenge can be exploited in setting control measures/scheme(s) and strategies specific to local vector-parasite-host epidemiological challenge chain. Moreover, considering the seasonality in the level of challenge, controlling disease in or making use of those "epidemiologically important individuals" can also be focused in time.

Although our studies suggest that substantial improvements can be made to the strategies for the control of livestock trypanosomosis, further studies are required to confirm the validity of our findings with regard to the efficacy and cost of control. In this respect, it is important to note that, with regard to trypanocidal drug use practices, livestock owners already seem to have adapted the most appropriate strategy, i.e. treatment of oxen and concentration of treatments during the rainy season. This could be extrapolated from the perceived higher impact of the disease by livestock owners in work oxen, especially during the rainy season. More studies are, however, required in adapting the insecticide-treatment technology to the findings of our study.

Generally speaking and irrespective of the need for further studies, the foregoing findings place the control of livestock trypanosomis in a complete different perspective and emphasize the importance of distinguishing between various epidemiological scenarios, particualry in consideration of the implications that such different epidemiological settings could have in the planning and related outcomes of livestock trypanosomosis field interventions.

### 6.7 Can trypanosomosis control in endemic areas be improved ?

The findings presented in the various chapters of this thesis suggest some options to improve the control of livestock trypanosomosis using curative and prophylactic trypanocidal drug treatments through:

- focusing on cattle rather than other livestock species present in the study area;
- within the cattle herd, focusing (mainly prophylactic) control efforts on oxen who are challenged most;
- within the cattle herd, focusing (mainly prophylactic) control efforts during periods of high trypanosomosis challenge i.e. the beginning of the rainy season, and;
- within the cattle herd, including PCV as an additional diagnostic tool in the control and management of trypanosomosis

With regard to the use of insecticide-treatments to control tsetse flies, results presented in the thesis suggest a potential improvement of this methods based on the application of the insecticide to the animals that are challenged most i.e. oxen. In this regard, it was postulated that within a herd, the application of insecticides to oxen only would result in a more or less equal effect as applying insecticide to all the age and sex classes in that cattle herd. To test the hypothesis, a simulation model was developed.

The efficacy of applying insecticides to oxen only was compared with the efficacy of this method when applying the insecticide to the entire herd. A simple simulation model was developed in Microsoft Excel aiming to predict the time needed to achieve 99% control of the tsetse population and the costs of achieving this control for various herd compositions (proportion of oxen) relative to treating the entire herd. It was hypothesized that the efficacy and cost of achieving a reduction in the tsetse population of 99% did not differ between insecticide-treatments of oxen only or insecticide-treatment of the entire herd. The input variables of the model were: herd structure (proportion calves, young females, young males, cows, bulls and oxen); herd size; weight of the animals; daily relative risks of a tsetse contacting an animal per age and sex category and pour-on cost (monthly treatment intervals) (Table 6.1). The output variables of interest were: percent infective bites; relative (relative to treatment of the entire herd) cost-benefit ratio for 99% control and the time required to achieve 99% control.

The model was based on a number of important assumptions: (i) the trial area had a size of 1 $km^2$ and was completely isolated (i.e. no immigration or emigration of tsetse flies), (ii) the total livestock population (varying in size) and the tsetse

population (of about 4000 flies) were evenly spread over the trial area, (iii) tsetse flies in the trial area have a probability of 0.22 of contacting cattle on a given day and contact of a fly with treated cattle will result in the death of that respective fly (based on the figure obtained from Hargrove et al., 2003), (iv) the growth rate of tsetse population was 1% and (v) the cost of a pour-on insecticide was proportional to the surface area of the animal. The model was subjected to a sensitivity analysis. The input parameters that were assessed in the sensitivity analysis were the herd structure and the daily relative risks and the output variables of interest were the eradication time and the pour-on cost.

According to the model outputs, 99% of the tsetse population can be killed either by applying insecticides to the entire herd or to oxen only. When treating the entire herd present at a density of 10 animals/km², 99% control can be achieved in about 4 months (116 days) (Table 6.1). When restricting the application to oxen only with oxen constituting 25% of the herd, the time needed to achieve this level of control is four times higher. Considering the higher body surface of oxen and the subsequent higher volume of insecticide required to treat each ox and the time required to achieve 99% control, the relative cost of achieving control by treating oxen only is 1.8 times higher compared to treating the entire herd. The relative cost benefit ratio of treating oxen only compared to treating the entire herd becomes more favourable when the density of cattle increases and the proportion of oxen in the herd increases (Figure 6.2). At high proportions of oxen in the herd (>40%), the cost/benefit ratio of treating oxen alone irrespective of the herd size becomes very similar to treating the entire herd. However, the duration of the control effort is much longer when control is based on the treatment of oxen only (Figure 6.3).

Table 6.1: Input variables and outputs of the simulation model assessing the efficacy and cost-effectiveness of selected insecticide-treatment of oxen (constituting 25% of the herd) to achieve 99% control of a tsetse population in an area of 1 $km^2$ with 10 head of cattle present.

| Class | Calves | Young females | Young males | Cows | Bulls | Oxen | Total |
|---|---|---|---|---|---|---|---|
| a. % in herd (herd structure) | **0.16** | **0.16** | **0.16** | **0.25** | **0.02** | **0.25** | 1.00 |
| b. Number of animals per $km^2$ ($a/b_{total}$) | 1.6 | 1.6 | 1.6 | 2.5 | 0.2 | 2.5 | **10** |
| c. Average weight (kg) | **50** | **200** | **200** | **300** | **400** | **500** | |
| d. Total weight (kg) (b*c) | 82 | 328 | 328 | 738 | 66 | 1250 | 2791 |
| e. Relative total weight ($d/d_{total}$) | 0.03 | 0.12 | 0.12 | 0.26 | 0.02 | 0.45 | 1 |
| f. Relative risk | ***1*** | ***3.3*** | ***3.1*** | ***3.3*** | ***1.4*** | ***5.6*** | |
| g. Daily risk ($f*g_{calves}$) | **0.005** | 0.017 | 0.016 | 0.017 | 0.007 | 0.028 | |
| h. Number of infective bites per day (g if g is low) | 0.005 | 0.017 | 0.016 | 0.017 | 0.007 | 0.028 | 0.0885 |
| i. Weighted number of daily infective bites (a*h) | 0.0008 | 0.0027 | 0.0025 | 0.0041 | 0.0001 | 0.0070 | 0.0172 |
| j. Relative number of infective bites in class ($i/i_{total}$) | 0.048 | 0.157 | 0.147 | 0.235 | 0.007 | 0.406 | 1 |
| k. Relative cost benefit ratio to catch a fly (e/j) | 0.62 | 0.75 | 0.80 | 1.12 | 3.53 | 1.10 | 1 |
| l. Probability a fly is caught in a day ($j*l_{total}$) | 0.0023 | 0.0077 | 0.0072 | 0.0115 | 0.0003 | 0.0198 | ***0.0488*** |
| m. Time (days) needed to kill 99% of fly population (ln(1-0.99)/ln(1-l+0.1)) | | | | 30 | | 466 | 116 |
| n. Relative time needed to kill 99% of fly population ($m/m_{total}$) | | | | 26.4 | | 4.0 | 1 |
| o. Relative eradication cost (n*e) | | | | 7.0 | | 1.8 | 1 |

**Bold: input variables**
***Italic & bold: input variables defined by other studies***

**Figure 6.1**: Tsetse relative eradication cost of applying insecticide to oxen only at different cattle densities (10 – 50 animals/km²) and proportions of oxen (25 – 53%).

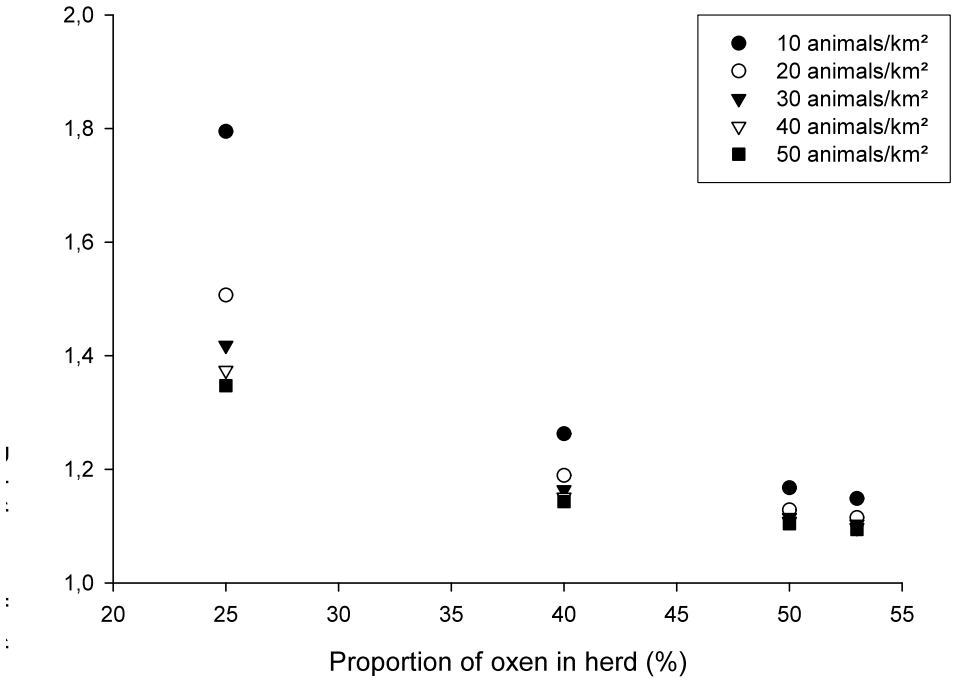
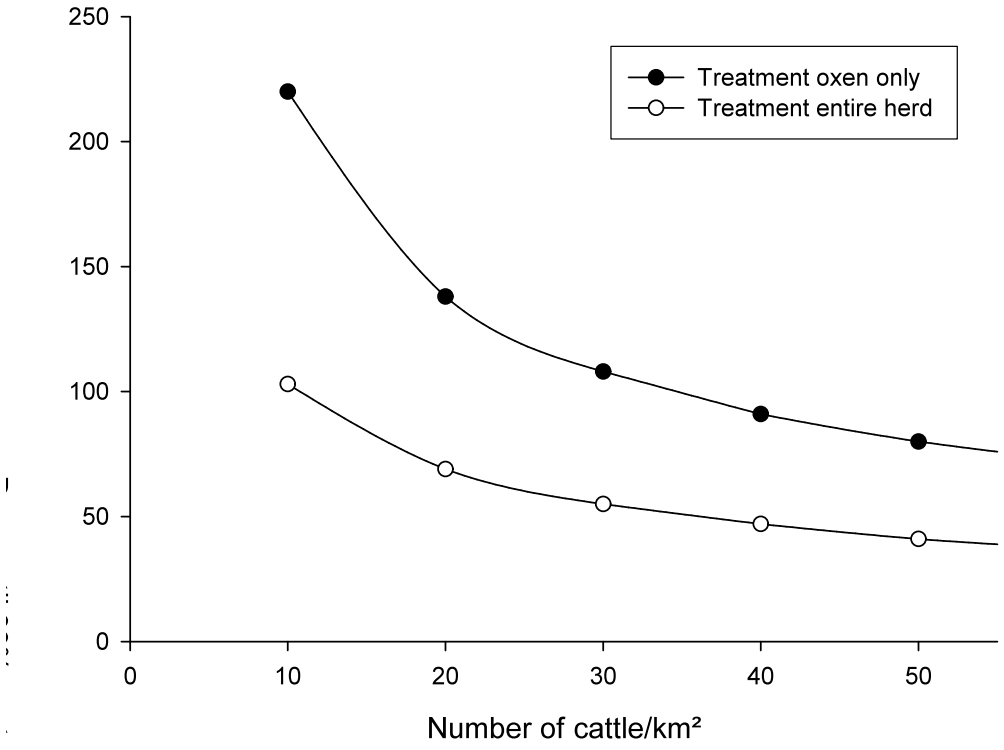


**Figure 6.2**: Number of days required to achieve 99% tsetse control when treatment is based on oxen only or the entire herd and for various densities of cattle.




The simulation model shows that the eradication cost and time of eradication when insecticide is applied to oxen only depends on the density of cattle and the proportion of oxen in cattle herds. Under certain conditions of cattle density and herd structure it is possible to achieve 99% control at comparable cost and time by applying insecticide to oxen only compared to applying to the entire herd. However, this is only possible at high livestock densities and at high proportions of oxen in the herd. For the conditions prevailing on the eastern plateau of Zambia, where cattle are present at a density of 10 animals/km² and oxen constitute 40% of the herd, the relative cost of controlling tsetse through treatment of oxen only is about 1.3 times higher compared to treatments of the entire herd. 99% control will be achieved after 219 days when treating oxen only compared to 104 days when treating the entire

herd. The higher cost when using oxen only is due to the time required to achieve control and the large amounts of insecticide needed to treat an ox. This cost can be reduced by the selective application of the insecticide to, for example, the legs and the belly only. Moreover, the higher cots of applying insecticide to oxen (under the conditions prevailing on the eastern plateau) to achieve tsetse control may be acceptable when considering the costs of the logistics required to treat an entire herd.

Although the simulation model suggests that control can be achieved by each of the two approaches it should be kept in mind that the assumptions made with regard to the tsetse population and the dynamics of the tsetse population were highly hypothetical. The majority of the tsetse populations present on the African continent are not isolated but subjected to substantial invasion of flies. Such invasion pressure will make control much more difficult and may require a better coverage of the insecticide throughout the herd. Indeed, oxen are often used for traction and are not always present in the areas where the tsetse flies are present, thus leaving areas untreated. This will even be worse in areas where the habitat is fragmented and where the mobility of the tsetse flies is restricted and tsetse distribution patchy.

## 6.8 References

De Clercq, K., (1997) Feeding evaluation of the Angoni cattle during the late dry season in Chipata (Zambia). *MSc Thesis*. Ghent University, Ghent.

Delespaux,V., Dinka, H., Masumu, J., Van den Bossche, P. and Geerts, S., (2008) Five fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven years period in Eastern Zambia. *Drug Resistance Updates,* **11,** 205-209.

Delespaux, V. & de Koning, H. P. (2007) Drugs and drug resistance in African trypanosomiasis. *Drug Resistance Updates*, 10: 30-50.

Doran, M., and Van den Bossche, P., (1999) An assessment of the socio-economic impact of bovine trypanosomosis and its control in the southern African region. *Proceedings of the 25th meeting of the ISCTRC*, Mombasa, Kenya, pp. 307-315.

Doran, M., (2000) Socio-Economics of Trypanosomosis: Implications for control strategies within the Common Fly-Belt of Malawi, Mozambique, Zambia and Zimbabwe. Volume 3. *Regional Tsetse and trypanosomosis Control Programme for Southern Africa*. P. 156.

Kubi C, Billiouw M, and Van Den Bossche P., (2007) Age prevalence of trypanosomal infections in female *Glossina morsitans morsitans* (Diptera: Glossinidae) on the plateau area of eastern Zambia. *Onderstepoort Journal of Veterinary Research*, **74**, 223-9.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N., & Van den Bossche, P. (2008) The use of the PCV-value in the diagnosis of trypanosomal infections in cattle. *Preventive Veterinary Medicine*, 87: 288-300.

Murray, M. & Dexter, T. M. (1988) Anaemia in bovine African Trypanosomiasis : a review. *Acta Tropica*, 45: 389-432.

Simukoko, H., Marcotty, T., Phiri, I., Geysen, D., Vercruysse, J., and Van den Bossche, P., (2007) The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia. *Veterinary Parasitology,* **147**, 231-238.

Sinyangwe, L., Delespaux, V., Brandt, J., Geerts S., Mubanga J., Machila N., Holmes P.H. and Eisler M.C. (2004) Trypanocidal drug resistance in eastern province of Zambia. *Veterinary Parasitology*, **119,** 125-135.

Van den Bossche, P. and Rowlands, G.J. (2001) The relationship between the parasitological prevalence of trypanosomal infections in cattle and herd average packed cell volume. *Acta Tropica,* **78,** 163-170.

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

Van den Bossche, P., (2001) Some general aspects of the distribution and epidemiology of bovine trypanosomsosis in southern Africa. *International Journal for Parasitology*, **31**, 592-598.

Van den Bossche, P., and De Deken, R., (2002) Seasonal variations in the distribution and abundance of the tsetse fly, *Glossina morsitans morsitans* in eastern Zambia. *Medical and Veterinary Entomology*, **16**, 170-176.

Van den Bossche, P. and Staak, C., (1997) The importance of cattle as a food source for *Glossina morsitans morsitans* in Katete district, Eastern Province, Zambia. *Acta Tropica*, **65**, 105-109.

***SUMMARY***

In southern Africa, the epidemiology of livestock trypanosomosis can be divided into four main epidemiological settings. Differences between epidemiological settings are caused by differences in the level of encroachment of people and their livestock into tsetse-infested zones. Areas where, because of intensive human and livestock encroachment into wildlife zones, the density of wild animals is low and livestock constitutes the main source of food for tsetse flies, are of particular importance. These areas are characterized by high human and livestock densities and the environment has been altered substantially as a result of human interference (e.g. settlements and agriculture). Most of the areas represent extensive zones where mixed crop/livestock agriculture is executed. This particular epidemiological situation occurs on the plateau of eastern Zambia. The area is an important livestock production area notwithstanding the high prevalence of trypanosomal infections in cattle. Livestock constitute a main source of blood for tsetse flies and trypanosomosis is endemic. The objective of this thesis was to clarify the epidemiology of trypanosomosis in this agriculturally important trypanosomosis endemic area of eastern Zambia.

In the first chapter of the thesis, a review is given of the current knowledge of the epidemiology of trypanosomosis. First, a general review is given of the epidemiology of trypanosomosis followed by a review of the specific epidemiological situations occurring in the southern Africa. Finally, the literature review summarises a number of important questions related to the epidemiology and control of livestock trypanosomosis on the eastern plateau of Zambia that still require investigation.

In the second chapter, a study is described that aims at investigating the role played by the three main livestock species (i.e. cattle, goats and pigs) in the epidemiology of livestock trypanosomosis on the eastern plateau. The study determined the prevalence of trypanosomal infections in each of the three livestock species. Molecular (PCR-RFLP) and parasitological (buffy coat) tools were used to determine the prevalences. The results of this study revealed that cattle had a significantly higher prevalence of trypanosomal infections compared to pigs or goats. The conclusion in this study was that pigs and goats may not be acting as important reservoirs for infections in cattle.

In the third chapter, a study investigating differences in the incidence of trypanosomal infections in different age and sex categories of cattle is presented. This study was initiated because of entomological findings that showed that tsetse flies (and other hematophagous insects) tend to feed more from certain groups within an animal species. Results of this study revealed that challenge is indeed heterogenous with oxen being at a significantly higher risk of infection compared to calves or other categories of cattle.

In the fourth chapter, the seasonality of the incidence of trypanosomosis is investigated. A sentinel herd consisting of 85 heads of cattle was established in a selected area of the eastern province of Zambia. The cattle were followed for a period of 26 months. Results showed that the incidence of trypanosomal infections was highest during the beginning of the rainy season.

The fifth chapter evaluated the usefulness of the PCV as a cost-effective diagnostic tool for use in resource poor countries such as Zambia. Use was made of the data obtained from trypanosmosis prevalence studies conducted on 724 cattle spread over 11 sampling sites. The PCV was evaluated in Bayesian statistical models and ROC in which three diagnostic tools where compared and in which the PCR-RFLP was used as the Gold standard. Results revealed that the PCV could be a reliable and cost-effective diagnostic tool if used in conjunction with the buffy coat technique.

In the last chapter, all the findings are integrated and discussed with regard to their repercussions for the control of trypanosomosis in endemic areas. It is concluded that the various studies suggest that the trypanosomosis control strategies in endemic areas could be improved substantially by (i) focusing the control efforts on the most important livestock species and (ii), within a species, concentrate on the epidemiologically most important group of animals. Futhermore, considering the temporal variations in challenge, control efforts could be altered seasonally. Finally, improved diagnosis by including PCV values as an additional diagnostic tool could be an appropriate approach in support of strategic trypanosomosis control. The last chapter also presents a hypothetical simulation model that evaluated the cost effectiveness and time required to eradicate tsetse flies if oxen only were treated with insecticide as opposed to treating the whole herd. The simulation model showed that the eradication

cost and time of eradication when insecticide is applied to oxen only depends on the density of cattle and the proportion of oxen in cattle herds. Under certain conditions of high cattle density and high proportion of oxen in the herd it is possible to achieve 99% control at comparable cost and time by applying insecticide to oxen only compared to treating to the entire herd.

## SAMENVATTING

De epidemiologie van dierlijke trypanosomiasis in zuidelijk Afrika kan onderverdeeld worden in vier verschillende situaties. De verschillen tussen deze epidemiologische situaties zijn het gevolg van de geleidelijke introductie van mensen en hun vee in gebieden waar tseetsee vliegen aanwezig zijn. Van bijzonder belang zijn gebieden waar, als gevolg van de hoge bevolkingsaangroei, wilde dieren verdwenen zijn en waar het vee de belangrijkste gastheer van de tseetsee vliegen is geworden. In deze gebieden heeft landbouw gewoonlijk de vegetatie grondig gewijzigd. Het oosten van Zambia is een goed voorbeeld van zulk een gebied. De bedoeling van deze thesis is een bijdrage te leveren om de epidemiologie van dierlijke trypanosomiasis in dergelijke belangrijke endemische gebieden, zoals het plateau van oostelijk Zambia waar de studies werden uitgevoerd, te verklaren.

Het eerste hoofdstuk van de thesis geeft een overzicht van wat er tot op heden gekend is over de epidemiologie van dierlijke slaapziekte. Er wordt eveneens een overzicht gegeven van de epidemiologische situaties in zuidelijk Afrika en enkele belangrijke vragen met betrekking tot de epidemiologie van trypanosomiasis in oostelijk Zambia worden aangehaald.

Het tweede hoofdstuk beschrijft een studie waarin de rol van runderen, varkens en geiten in de epidemiologie van dierlijke slaapziekte op het plateau van oostelijk Zambia wordt bepaald. Aan de hand van parasitologische ("buffy coat" methode) en moleculaire (PCR-RFLP) testen werd de prevalentie van de ziekte in elk van deze diersoorten bepaald. De resultaten toonden aan dat de prevalentie significant hoger is in runderen. Varkens en geiten schijnen een eerder bijkomstige rol te spelen in de epidemiologie van dierlijke trypanosomiasis in het studiegebied.

In een derde hoofdstuk werd de incidentie van dierlijke trypanosomiasis vergeleken in runderen van verschillende leeftijd en geslacht binnen eenzelfde kudde. De bedoeling van de studie was om de entomologische bevindingen met betrekking tot de gastheer keuze van tseetsee vliegen te bevestigen. De resultaten toonden aan dat tseetsee vliegen een specifieke voorkeur hebben en proportioneel veel meer voeden op ossen dan op kalveren.

Het vierde hoofdstuk beschrijft een studie over het seizoensgebonden karakter van dierlijke trypanosomiasis op het plateau van oostelijk Zambia. Uit de resultaten bleek dat in een kudde van 85 dieren de incidentie van trypanosoominfecties het hoogst is tijdens het begin van het regenseizoen.

In het vijfde hoofdstuk van de thesis werd geëvalueerd in hoeverre de hematocrietwaarde gebruikt kan worden in de diagnose van dierlijke trypanosomiasis. Gebruik werd gemaakt van gegevens van een prevalentiestudie waarin 724 runderen afkomstig van 11 verschillende locaties waren betrokken. De bruikbaarheid van de hematocrietwaarden in de diagnose van dierlijke slaapziekte werd bepaald door middel van een Bayesiaans model waarin drie verschillende diagnostische methoden werden vergeleken met de PCR-RFLP als gouden standaard. De resultaten van de analyse toonden aan dat de hematocrietwaarde een betrouwbaar en goedkoop diagnosemiddel is wanneer gebruikt in combinatie met de “buffy coat” methode.

Het zesde en laatste hoofdstuk evalueert de gevolgen van de bevindingen voor de controle van dierlijke trypanosomiasis in endemische gebieden. Er wordt besloten dat, in zulke gebieden, de controle kan verbeteren door (i) de interventies te beperken tot de epidemiologisch meest belangrijke diersoort en (ii) binnen de diersoort speciale aandacht te besteden aan de groep die epidemiologisch van het grootste belang is. Bovendien kunnen interventies zich best concentreren tijdens perioden waarin de incidentie van de ziekte het hoogst is. Tenslotte kan de diagnose van dierlijke trypanosomiasis verbeterd worden door eveneens gebruik te maken van de hematocrietwaarde. In de conclusies van de thesis werd een simulatiemodel gebruikt om de doeltreffendheid van de beperkte behandeling van vee met insecticiden na te gaan. Op voorwaarde van een hoge runderpopulatie en een hoge proportie ossen in de kudde zou het toedienen van insecticiden aan ossen alleen voldoende zijn om de populatie tseetsee vliegen te controleren.