Investigating the epidemiology of trypanosomiasis in domestic livestock at the micro-scale in Busia, Kenya

Beatrix von Wissmann

Submitted in fulfilment of the requirements of the degree of

Doctor of Philosophy

The University of Edinburgh

2007

Declaration

I declare that the research described within this thesis is my own work and that this thesis is my own composition and certify that it has never been submitted for any other degree or professional qualification.

<div align="right">

Beatrix v. Wissmann

Edinburgh 2007

</div>

Contents

Acknowledgements

Abstract

Trypanosomiasis in Kenya is no longer viewed as a public health issue, as only sporadic sleeping sickness cases are reported from historic foci such as Busia. Trypanosomiasis is thus mainly perceived as a constraint on livestock production. The responsibility for tsetse and trypanosomiasis control in Kenya has therefore increasingly shifted from the state to individual livestock owners; this drastically reduced the scale of control approaches. This thesis examines the epidemiology of both animal infective and zoonotic trypanosome species in a range of domestic livestock at the micro-scale, in Busia, Kenya. The work is based on a unique cross-sectional census data set of the entire livestock population in two study sites in Busia, employing sensitive molecular tools (PCR) to detect trypanosome infections.

Cattle were the largest reservoir of trypanosomes with an infection prevalence of 20.1%, followed by pigs (11.5%). A low prevalence of infection was detected in small ruminants (3.3%). Human infective trypanosomes (*T. b. rhodesiense*) were detected at a low prevalence in cattle (1.5%) and pigs (2.9%). Key clinical signs for trypanosomiasis infection (anaemia & poor body condition) were only observed in a minority of infected cattle (<20%). Confinement of livestock to the homesteads, instead of grazing in communal grounds and watering at the river did not provide protection from trypanosome infections. An investigation of the micro-geographic variation in the distribution of trypanosome infections over the study population, revealed significant clustering in one of the two study sites. However, there was no significant effect of distance to water features on trypanosomiasis risk at the herd level. A convenience sampling protocol was shown to give a good estimate of overall trypanosomiasis in cattle, but failed to detect the low prevalence of *T. b. rhodesiense*.

The sustainability of small-scale trypanosomiasis control based on trypanocide treatment of visibly diseased cattle is appraised and the feasibility of additional vector control is discussed. Furthermore, the potential human health implications of a livestock reservoir of *T. b. rhodesiense* to the local population are examined.

List of Abbreviations

AAT	African Animal Trypanosomiasis
AHA	Animal Health Assistant
BIIT	Blood Incubation Infectivity Test
bp	Base pairs
bwt	Bodyweight
CAHW	Community Animal Health Worker
CATT	Card Agglutination Trypanosomiasis Test
CCD	Cold Cloud Duration
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide-triphosphate
DVO	District Veterinary Officer
EDTA	Ethylene Diamine Tetra Acetate
EU	European Union
FAO	Food and Agricultural Organization of the United Nations
FITCA	Farming in Tsetse Controlled Areas
ftd	Flies per trap per day
G. f. fuscipes	*Glossina fuscipes fuscipes*
G. pallidipes	*Glossina pallidipes*
GIS	Geographic Information System
GPS	Global Positioning System
HAT	Human African Trypanosomiasis

HIV/AIDS	Human Immunodeficiency Virus / Acquired Immunodeficiency Syndrome
IBAR	Interafrican Bureau for Animal Resources
ITS	Internally Transcribed Spacer
KETRI	Kenya Trypanosomiasis Research Institute
NDVI	Normalised Difference Vegetation Index
NGO	Non-Governmental Organisation
O/E	Observed/ Expected Cases
OAU	Organisation of African Unity
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PLC	Phospholipase C
RMS	Root Mean Square Error
RNA	Ribonucleic Acid
SRA	Serum Resistance Associated Protein
UTM	Universal Transverse Mercator
VAT	Variable Antigenic Types
VO	Veterinary Officer
WHO	World Health Organization

List of Figures

List of Tables

1 Chapter 1   Introduction

1.1 Introduction

Tsetse transmitted African trypanosomiases, which can infect both animals and humans, have a severe socio-economic impact throughout sub-Saharan Africa. There are two human infective trypanosome subspecies, *Trypanosoma brucei rhodesiense*, which cause Rhodesian sleeping sickness, and *Trypanosoma brucei gambiense*, which cause Gambian sleeping sickness. In 1998, the number of cases affected by human African trypanosomiasis (HAT) was estimated as 300,000 to 500,000, resulting in 100,000 deaths a year (WHO, 1998; Cattand *et al.*, 2001). By 2005, active surveillance activities had been reinforced, leading to a reduction in newly infected cases (WHO, 2006). Currently, the cumulative number of sleeping sickness cases is estimated to range between 50,000 and 70,000 by the WHO, the majority of which are caused by *T. b. gambiense* (WHO, 2006). As a livestock disease, African animal trypanosomiasis (AAT), or nagana, reduces productivity and in areas of high tsetse challenge it prohibits livestock-keeping altogether, unless control measures are put into place. Losses due to unrealised cattle production in terms of meat and milk yield in Africa's tsetse belt have been estimated at over US$ 1.3 billion annually (Kristjanson *et al.*, 1999).

Busia District in Western Province, Kenya is part of a historic Rhodesian sleeping sickness focus, but at present the level of this disease is low in Busia, with only sporadic cases being reported (WHO, 2006). Animal trypanosomiasis however is a serious constraint to productivity in this poor rural area, which is vitally dependent on agriculture. An estimated 70% of the potential labour force of the district is engaged in subsistence farming (Government of Kenya, 2001). Trypanosomiasis related losses include both direct livestock out-put (weight-loss, decrease in milk, decreased reproductive rate) as well as lost opportunity in terms of integration of livestock into crop production and the potential for crop-improvement (loss of draught power and manure) (Kristjanson *et al.*, 1999; FITCA, 2005).

Until the late 1980s, large scale aerial and ground-spraying campaigns had been used by public agencies as the main stay of tsetse and thus trypanosomiasis control (Kamuanga, 2003). Over the last two decades, ongoing cuts in the budget of the Veterinary Department, concentrated the remaining available funds on the provision of public-goods services (Holden, 1999). Trypanosomiasis was no longer perceived as a concrete human health risk in Busia, but it was viewed as a livestock production disease, the control of which was in the interest of the individual livestock-owners. This shift in responsibility radically changed the scale of control efforts, from area wide programmes to small-scale community based interventions. However, little is known about the epidemiology of livestock trypanosomiasis at the local scale at which control is now envisaged. It is the aim of this thesis to investigate whether findings from large-scale studies can be directly transferred and applied to the epidemiology of the disease at the micro-scale.

The next section of the introduction provides general background information on both human and animal African trypanosomiasis, focussing on the relevance of this disease for livestock production and the difficulties in diagnosis and control facing the rural population. The final section of the introduction gives an overview of the history of sleeping sickness in Kenya and the changes in approaches to trypanosomiasis control, leading up to the present situation.

1.2 The Trypanosomiases

1.2.1 Trypanosome classification

The African trypanosomiases are part of an important group of animal and human diseases, caused by a parasitic protozoa of the genus *Trypanosoma* (Barrett *et al.*, 2003). Trypanosomes are unicellular, flagellated protozoa, forming part of the order of Kinetoplastida (Levine *et al.*, 1980). Trypanosomes can be broadly divided into stercoraria and salivaria according to the mechanism of vector transmission.

1.2.1.1 Stercoraria

Stercoraria undergo development in their vector to form infective metacyclic trypomastigotes in the posterior part of its gastro-intestinal tract. The stercorarian trypanosomes are transmitted through the faeces of the vector, which are deposited when the insect is feeding. The trypanosomes in the faeces of the insect cross the skin barrier of the host through minor abrasions when the insect bite is scratched (Prata, 2001). The stercocarian group includes *Trypanosoma cruzi* (transmitted by bugs of the subfamily *Triatominae*), which causes Chagas disease in humans and puts approximately 18 million humans in Latin America at risk (Pays, 1998). The group also includes *Trypanosoma theileri*, which has a cosmopolitan distribution among domestic cattle. It is generally believed to be non-pathogenic, although it may cause disease in conjunction with other infections (Hoare, 1972).

1.2.1.2 Salivaria

The pathogenic African trypanosomes belong to the salivarian trypanosomes, which are transmitted cyclically through inoculation by the mouthpart of their vector. The only exception is *Trypanosoma equiperdum*, which is transmitted venereally in equids (Barrowman *et al.*, 1994).

There are three sub-genera of pathogenic African trypanosomes: *Nannomonas*, *Dutonella* and *Trypanozoon* (Figure 1.1). African animal trypanosomiasis, also called nagana, is caused by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei* senso lato (Uilenberg, 1998). The clinical picture of nagana is characterised by anaemia, depression, intermittent pyrexia, enlarged lymphnodes, starring coat and chronic weightloss, to the point of extreme emaciation (section 1.6.1) (Eisler *et al.*, 2007). *T. congolense* is restricted to the vasculature, whereas both *T. brucei* s.l. and *T. vivax* can invade the extravascular space, although this is less common for *T. vivax* (Taylor & Authié, 2004). The pathogenicity of *T. brucei* s.l. in livestock can be highly variable (Wilde & French, 1945; Wellde *et al.*, 1989e; Wellde *et al.*, 1989d) although it is generally reported to cause mild clinical signs in

domestic animals (Uilenberg, 1998). However two of its subspecies, *T. brucei rhodesiense* and *T. brucei gambiense* are the human infective trypanosomes (Dutton, 1902; Stephens & Fantham, 1910). *T. brucei rhodesiense* has long been known to be zoonotic (Heisch *et al.*, 1958), whereas there are fewer reports of *T. brucei gambiense* in animals (Gibson *et al.*, 1978; Jamonneau *et al.*, 2003).

* zoonotic

** human infective

t recently reclassified as *T. simiae tsavo* (Gibson *et al.*, 2001)
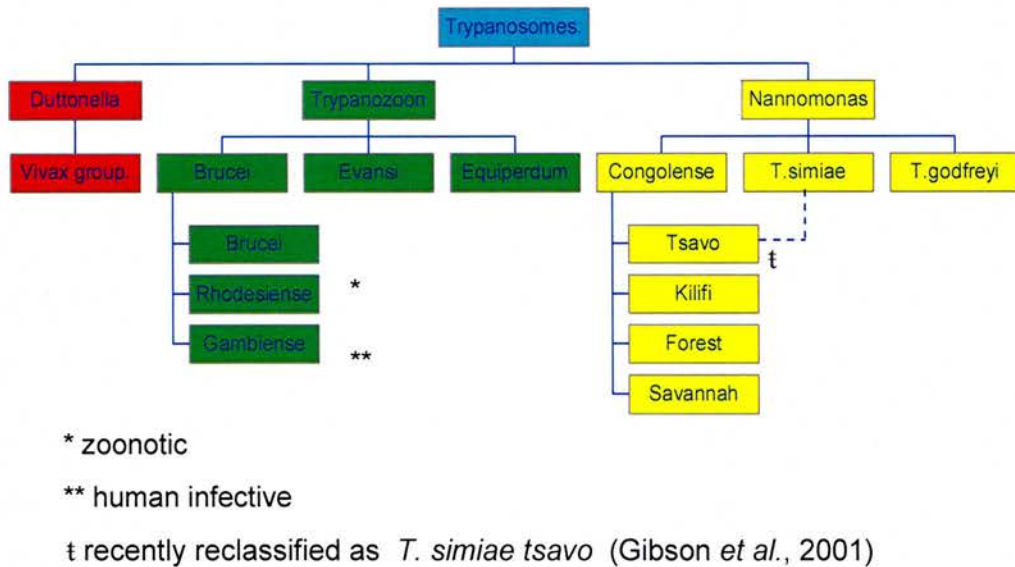
Figure 1.1: Classification of pathogenic African trypanosomes (reproduced with kind permission by K. Picozzi)

1.2.1.2.1 Geographical distribution

T. congolense, T. brucei s.l. and *T. vivax* are cyclically transmitted by the tsetse fly (*Glossina* ssp.), i.e. part of their lifecycle takes place within the vector (section 1.3). Tsetse flies are found exclusively in Africa in a belt that stretches between 14°N to 29°S of the equator (Barrett *et al.*, 2003). Due to specific conditions of temperature, humidity, elevation and vegetation required for tsetse survival, tsetse are restricted to habitat pockets, rather than being present uniformly throughout the tsetse belt (Pepin & Meda, 2001), which is therefore also true for the trypanosome species which are solely transmitted by tsetse.

T. vivax can additionally be transmitted mechanically by tabanids (without maturation within the vector) (Desquesnes & Dia, 2003) and thus also occurs out with the African sub-Saharan tsetse belt, in Central and South America (Jones & Davila, 2001). *Trypanosoma evansi*, a member of the *Trypanozoon* sub-genus, is best known for causing "surra" associated with weightloss and abortion in camels, although it is also infective to other livestock (Schwartz *et al.*, 1983; Wilson *et al.*, 1983). It is thought to have arisen from *T. brucei* s.l., but has adapted to mechanical transmission, and no longer undergoes cyclical transmission in tsetse (Hoare, 1972). *T. evansi* has the widest geographical distribution of all the trypanosomes and is found in Africa, South and Central America as well as Asia and Australia (Davila & Silva, 2000; Reid, 2002).

1.3 Cyclical transmission of African trypanosomiasis

African trypanosomes are transmitted cyclically by tsetse flies, which refers to the parasite completing part of its lifecycle within the vector. The majority of tsetse flies are resistant to trypanosome infection, in fact the rate of mature infection rates in tsetse even in the presence of abundant infected hosts may be as low as 0.1-1% (Snow, 1984; Okoth & Kapaata, 1986). This resistance to trypanosomes has been linked to a lectin, which is produced in the tsetse midgut in response to a bloodmeal and kills trypanosomes entering the midgut (Maudlin & Welburn, 1987). This mechanism affects *T. brucei* s.l. and *T. congolense*, which have to establish infection in the tsetse midgut, but not *T. vivax*, which stay in the tsetse proboscis throughout their development (Maudlin & Welburn, 1988). Tsetse susceptibility to trypanosomes is promoted by the build up of lectin-inhibitory oligo-saccharides, produced by the endochitinasae activity of a tsetse midgut symbiont which degrades chitin, during pupation and predisposes the teneral fly (fly prior to first bloodmeal) to trypanosome midgut infection (Welburn *et al.*, 1993). Normal activity of these symbionts (*Sodalis glossinidius*) is insufficient for significant lectin inhibition, thus explaining the age-dependency of trypanosome susceptibility observed in tsetse

(Welburn & Maudlin, 1999). Recent work suggests that other factors might also be involved in trypanosome killing, including reactive oxygen species (Macleod *et al.*, 2007) and microbial peptides (Hu & Aksoy, 2006), and it may be that tsetse have several mechanisms to defend against trypanosomes.

In susceptible tsetse, trypanosomes taken up with the bloodmeal can establish an infection in the ectoperitrophic space of the midgut. The blood stream form develops into the procyclic form, in the process losing the Variant Surface Glycoprotein (VSG) coat needed for immune evasion in the vertebrate host, replacing it with procyclin as the dominant surface glycoprotein (Roditi & Pearson, 1990). Once established in the midgut, procyclics must undergo maturation to become infective to the vertebrate host. This involves return to the endoperitropic side of the midgut to move to the mouthparts in the case of *T. congolense*, or the salivary glands in the case of *T. brucei* s.l. where they develop into dividing epimastigotes and finally the infective metacyclics (Maudlin & Welburn, 1994). The maturation of an established midgut infection takes an average of 12 days for *T. brucei* s.l., but in *T. congolense* maturation can take as little as 3 days (Welburn & Maudlin, 1989). *T. vivax* undergoes all its developmental stages in the tsetse mouthparts within approximately 5-7 days. Once a mature trypanosome infection is established, tsetse remain infected for the rest of their life-span (Maudlin & Welburn, 1994).

1.4 *Variant Surface Glycoprotein (VSG): evading the immune system*

The African trypanosomes differ from many other protozoa in that they remain in the bloodstream or intercellular space during the course of the infection in their vertebrate hosts, remaining unusually exposed to the host's immune system (Taylor & Rudenko, 2006). The most abundant protein expressed by trypanosomes on their surface in their bloodstream form is the variant surface glycoprotein (VSG) which will be detected by the host's immune system. The VSG forms a single layer which protects underlying proteins from detection by the host immune system (Vickerman, 1969). Periodically trypanosomes switch to a new VSG expressed on their surface

(Cross *et al.*, 1998). These VSGs show vast differences in their sequence, therefore ensuring that they are not recognized by the same antibodies (Cross, 1996) and infection can thus be maintained by proliferation of the clone expressing the "new" VSG.

1.5   Human African Trypanosomiasis

The two subspecies of *T. brucei* s.l., causing Human African Trypanosomiasis (HAT), commonly referred to as sleeping sickness, are *T. brucei rhodesiense* and *T. brucei gambiense*. They differ in pathogenesis as well as treatment required, making distinction between the two forms of sleeping sickness vital. To date, *T. b. gambiense* and *T. b. rhodesiense* occur in geographically separate areas (Figure 1.2) and diagnostic techniques used are not suited to differentiate between the two morphologically identical subspecies. Human African trypanosomiasis is fatal in 100% of cases if left untreated.
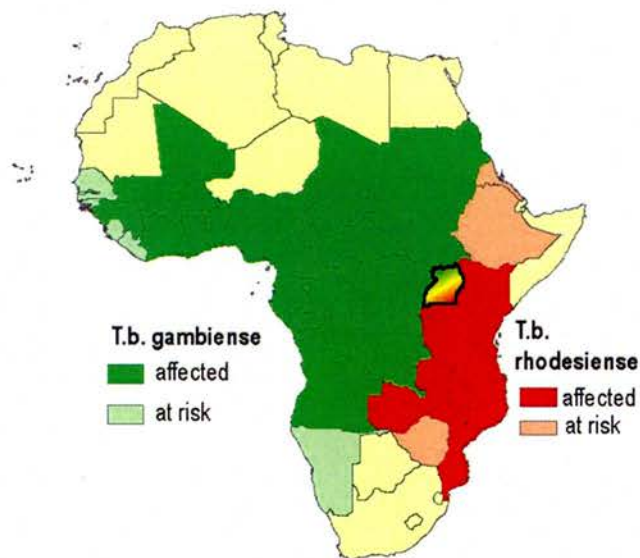
Figure 1.2: Countries affected and at risk of Gambian and Rhodesian sleeping sickness (with Uganda highlighted as the only country with foci of both diseases)

1.5.1 Pathogenesis

1.5.1.1 Rhodesian sleeping sickness

Infections with *T. brucei rhodesiense* are frequently described as acute sleeping sickness. The infective tsetse bite is followed by febrile illness in the first stage of the disease, with rapid progression to the second stage, defined by the parasite invading the central nervous system. In the absence of treatment, death in over 80% of cases occurs within 6 months of illness (Odiit *et al.*, 1997). Underreporting of Rhodesian sleeping sickness is a severe problem. Quantification of underreporting in Uganda estimated eleven deaths caused by this disease occurring in the field, for every death of a hospitalised sleeping sickness case (Odiit *et al.*, 2005).

1.5.1.2 Gambian sleeping sickness

The first phase of Gambian sleeping sickness (also called chronic sleeping sickness) is prolonged, with episodes of fever, headaches and malaise. Parasitaemia is usually low and the disease can remain initially unrecognized (Chappuis *et al.*, 2004). Invasion of the central nervous system may not occur for years, but eventually the parasite crosses the blood-brain barrier resulting in meningo-encephalitis. The autoimmune response of the central nervous system leads to personality changes and general deterioration until death occurs, similar to Rhodesian sleeping sickness (Stich *et al.*, 2003).

1.5.2 Diagnosis

1.5.2.1 Rhodesian sleeping sickness

As parasitaemia is usually high, diagnosis of Rhodesian sleeping sickness relies on microscopic examination of the peripheral blood, in the first stage of the disease (Smith *et al.*, 1998). The technique only confirms the presence of trypanosomes, and there is no morphological distinction between subspecies of *T. brucei* s.l.. Therefore, diagnosis of *T. brucei rhodesiense* infections relies on geographical distinction from *T. brucei gambiense* foci, the gap between which is becoming smaller (Picozzi *et al.*, 2005).

Diagnosis of the second stage of sleeping sickness for both *T. b. gambiense* and *T. b. rhodesiense* is confirmed by examination of cerebrospinal fluid (CSF) obtained by lumbar puncture. Visualisation of trypanosomes is sufficient, however parasite levels may be low, and diagnosis may be based on an increase in CSF leucocyte count (>5 cells/µl) or total protein (>300mg/l) (Lejon *et al.*, 1998; Lejon *et al.*, 2002).

1.5.2.2   Gambian sleeping sickness

In *T. b. gambiense* infections parasitaemia is usually low and it is difficult to isolate trypanosomes. Lymphadenopathy is common, and swelling of the cervical lymphnodes (Winterbottom's sign) is a typical sign for Gambian sleeping sickness (Balfour, 1904). Aspiration and microscopy of lymph fluid are common diagnostic tools but a negative result is not conclusive and patients may need to be tested repeatedly over a period of time before the parasite can be isolated (Simarro *et al.*, 1999).

Currently the Card Agglutination Test for Trypanosomes (CATT) is used for routine screening for *T. b. gambiense*. This test is based on the formation of *T. b. gambiense* specific antibodies by infected individuals, which are identified by binding to the antigen preparation on the card, when a blood sample of the infected individual is applied (Magnus *et al.*, 1978). Diagnosis of sleeping sickness through CATT must be confirmed parasitologically, as the presence of antibodies in the CATT-positive individual may be residual of past infections (Chappuis *et al.*, 2004). The second stage of Gambian sleeping sickness is diagnosed in the same fashion as in Rhodesian sleeping sickness by CSF examination (section 1.5.2.1).

1.5.3   Treatment

With the exception of eflornithine (registered in 1990) no new drugs have been developed for sleeping sickness in 60 years (Pepin, 2007). Those that are in use may have serious side effects and can even be lethal. Hospitalisation is required as intense and skilled treatment is required.

1.5.3.1 1st stage treatment

The drug of choice for treatment of *T. b. rhodesiense* infections is suramin. The drug is water soluble and must be given in repeated slow intravenous injection as subcutaneous or intramuscular injection results in a strong inflammatory response (Docampo & Moreno, 2003). Renal toxicity is the most common side effect of suramin although it is usually mild (Jagielski *et al.*, 2006). Penetration of the central nervous system is poor, and suramin alone is insufficient to cure late stage trypanosomiasis (Jennings *et al.*, 2002). Pentamidine, which is used for treatment of Gambian sleeping sickness, is soluble in water and is given intramuscularly. Injection sites are very tender and sterile gluteal abscesses may occur. Like suramin, pentamidine does not cross the blood brain barrier in sufficient concentration to treat late stage infections (Burchmore *et al.*, 2002).

1.5.3.2 2nd stage treatment

Second stage *T. b. rhodesiense* and *T. b. gambiense* infections are treated with melarsoprol, an arsenic derived compound. The drug is poorly soluble in water, ether or alcohol and is administered intravenously dissolved in propylene glycol, which is extremely painful. Melarsoprol is a highly toxic substance and in 5-10% of patients reactive encephalitis ensues which results in death of 10-50% of those affected. The overall melarsoprol relapse rate in the past 50 years was 5-8 % (Pepin *et al.*, 1994), but in recent years the figures have risen dramatically in all epidemic *T. brucei gambiense* areas: North Uganda: 30% (Legros *et al.*, 1999), Sudan: 16-21% (Brun *et al.*, 2001), Angola and Congo: 25% (Stanghellini & Josenando, 2001).

Eflornithine is the second drug, which can be used for late stage sleeping sickness. However it is ineffective against *T. b. rhodesiense* and is usually only used for *T. b. gambiense* infections resistant to melarsoprol, as eflornithine is prohibitively expensive (Docampo & Moreno, 2003). However, due to high levels of drug resistance to melarsoprol, eflornithine has recently been made first line of treatment

choice in several Gambian sleeping sickness foci, including South Sudan and the Republic of the Congo (Chappuis *et al.*, 2005; Balasegaram *et al.*, 2006b). It is a trypanostatic rather than a trypanocidal drug and is not useful in immunodepressed patients (Balasegaram *et al.*, 2006a).

1.5.4 Animal reservoir

The wildlife reservoir of *T. b. rhodesiense* was recognized when the parasite was first isolated from a bushbuck, and the infectivity to humans was demonstrated using a volunteer (Heisch *et al.*, 1958). Domestic animals were also suspected to be susceptible, and *T. b. rhodesiense* was isolated from cattle soon after (Onyango *et al.*, 1966). Control of the animal reservoir is an important consideration in Rhodesian sleeping sickness epidemics (Welburn *et al.*, 2001a). In the absence of significant numbers of wildlife species, cattle has been shown to be the most important reservoir of *T. b. rhodesiense* in south-west and central Uganda (Hide *et al.*, 1994; Hide *et al.*, 1996). Up to 18% of cattle were shown to be infected with *T. b. rhodesiense* during an epidemic of sleeping sickness in Soroti (Welburn *et al.*, 2001b). But as *T. brucei* s.l. infections are largely asymptomatic (Taylor & Authié, 2004), infected cattle are likely to remain unrecognized and thus untreated. Whilst molecular tools to detect *T. b. rhodesiense* have been developed (Hide *et al.*, 1994; Xong *et al.*, 1998), there is no pen-side test that can distinguish the sub-species of *T. brucei* s.l. and targeted treatment of the cattle reservoir is thus not possible. However, a mathematical model demonstrated that mass treatment of 86% of the cattle population with trypanocides in affected areas would be sufficient to interrupt the transmission of *T. b. rhodesiense* and eliminate human sleeping sickness (Welburn *et al.*, 2006).

T. brucei gambiense is generally not thought to have an important reservoir in animals, despite reports of the parasite having been isolated from pigs and a variety of wildlife in West Africa (Gibson *et al.*, 1978; Penchenier *et al.*, 1999; Nkinin *et al.*, 2002; Jamonneau *et al.*, 2003). It remains controversial however, whether *T. b. gambiense* isolated from animals (in particular pigs) are the same strains seen in human cases of Gambian sleeping sickness (Penchenier *et al.*, 1997; Penchenier *et*

al., 1999; Nkinin *et al.*, 2002), or whether different strains are transmitted in separate pig-tsetse-pig and human-tsetse-human cycles (Jamonneau *et al.*, 2003; Jamonneau *et al.*, 2004). Control of Gambian sleeping sickness at present focuses on active detection of human cases and limited vector control and is successful in reducing the incidence of new cases (Moore *et al.*, 1999; Moore & Richer, 2001), indicating humans are the main reservoir of importance.

1.6 *African Animal Trypanosomiasis*

1.6.1 Pathogenesis in domestic livestock

Infection of livestock with *T. congolense*, *T. vivax* or *T. brucei* s.l. is referred to as African animal trypanosomiasis or nagana. After an infective tsetse bite, the trypanosomes proliferate at the site of inoculation, frequently forming a painful swelling. After an incubation period (4 days to > 3 weeks) in which the trypanosomes proliferate in their metacyclic form the trypanosomes assume their bloodstream form (Uilenberg, 1998). The early acute phase of the disease is characterised by a continuously detectable parasitaemia (10^3-10^8/ml) (Taylor & Authié, 2004). Anaemia develops with the onset of parasitaemia (Paling *et al.*, 1991) and is one of the most pronounced clinical signs of nagana, which may be observed by pallor of the mucous membranes (Eisler *et al.*, 2007). The severity of anaemia is influenced by the virulence of the infective trypanosome as well as host factors including breed, age, nutritional status and immune status as well as co-infection with other diseases (Murray *et al.*, 1982). Pyrexia fluctuates with waves of parasitaemia (Taylor & Authié, 2004), which characterise the chronic phase of the infection, in which parasites are only intermittently detectable in the host's blood (Paling *et al.*, 1991). Other clinical manifestations include enlargement of lymph nodes, staring coat, weakness, depression and loss of condition and productivity is frequently further reduced by decrease in milk yield, abortion and impaired fertility (Connor, 1994).

1.6.1.1 Cattle

The clinical severity of trypanosome infections in cattle to some extent depends on the invading trypanosome species. *T. congolense* infections tend to be less acute than *T. vivax*, but both can be ultimately fatal (Stephen, 1986). Haemorrhagic *T. vivax* stocks have been reported in East Africa, which cause a hyperacute disease defined by high parasitaemia, severe anaemia and haemorrhages related to intravascular disseminated coagulation (Mwongela *et al.*, 1981). *T. brucei* s.l. infections are generally less pathogenic in cattle than either *T. vivax* or *T. congolense* and may not result in clinical signs (Uilenberg, 1998). In areas endemic for Rhodesian sleeping sickness cattle may thus harbour the zoonotic *T. brucei rhodesiense*, without displaying overt signs of disease.

Manifestation of trypanosomiasis in cattle ranges from hyperacute, with death occurring within 3-4 weeks of infection, to a chronic form, which may last for years. Very rare cases of self-cure have also been reported in the literature (Paling *et al.*, 1991). In cattle indigenous to areas endemic for trypanosomiasis, the chronic manifestation is most common. Trypanotolerance, defined as the ability to gain weight and remain productive under trypanosome challenge is mainly associated with the ability to control anaemia (Naessens, 2006), and has been reported in the West African *Bos taurus* cattle (Murray *et al.*, 1982), and to a certain extent in some East African *Bos indicus* breeds (Paling *et al.*, 1991). Exotic breeds or grade cattle on the other hand are more susceptible than indigenous breeds. In general animals under stress (e.g. poor nutrition, lactation, ploughing) are less able to tolerate trypanosome infections (Murray *et al.*, 1982).

1.6.1.2 Small ruminants

Small ruminants are commonly viewed as being more resistant to trypanosomiasis than cattle (Taylor & Authié, 2004). However whilst small ruminants have been reported to be able to harbour low grade chronic trypanosome infections without overt clinical signs (Karib, 1961), such trypanosome infections were shown

experimentally to still be transmissible to other livestock including cattle, in which they induced severe pathology (Mahmoud & Elmalik, 1977). Small ruminants may thus provide a silent and thus untreated reservoir for trypanosome infections for more susceptible species (Gutierrez et al., 2006).

Other studies revealed trypanosome induced pathogenesis in small ruminants, resulting in most clinical signs also observed in cattle, including reduced growth rate and fertility and in some cases death (Katunguka-Rwakishaya et al., 1997; Osaer et al., 1999a; Osaer et al., 1999b). As is the case with cattle, the extent of trypanotolerance in small ruminants is breed related (Murray et al., 1982). However, even small ruminant breeds generally regarded as trypanotolerant and able to withstand mild to moderate tsetse exposure will succumb to severe trypanosome challenge (MacLennan, 1970).

1.6.1.3 Pigs

Periodic outbreaks of *T. simiae* have been reported in pigs, causing acute or hyperacute trypanosomiasis associated with high parasitaemia and which may result in death within 2 days to 4 weeks (Taylor & Authié, 2004). Pigs are moderately susceptible to *T. congolense* and *T. brucei* s.l., which are mainly associated with abortion, anaemia, weightloss, and reduced fertility (Onah, 1991; Waiswa, 2005). Clinical signs are observed in some but not all infected pigs (Onah, 1991). Other pig infective trypanosome species include *T. suis* (very rare) and *T. godfreyi* (Gibson et al., 2001).

Whilst *T. brucei* s.l. infections in pigs may remain sub-clinical (Schütt & Mehlitz, 1981), the pig reservoir of *T. brucei* s.l. has provoked interest in sleeping sickness endemic areas both in East and West Africa from a human health perspective. In West Africa, *T. b. gambiense* has been isolated from pigs (Jamonneau et al., 2003; Simo et al., 2006). But it remains controversial whether the strains found in pigs are the same that were isolated from human cases (Penchenier et al., 1999) or whether

different *T. b. gambiense* strains circulate in separate transmission cycles in the human and porcine population (Jamonneau *et al.*, 2004). Pigs have been identified as a reservoir of the zoonotic *T. b. rhodesiense*, in areas endemic for Rhodesia sleeping sickness in East Africa (Waiswa *et al.*, 2003; Ng'ayo *et al.*, 2005). Mathematical modelling has shown the mass treatment of the cattle reservoir to be a potentially effective measure for the control of Rhodesian sleeping sickness outbreaks, as long as coverage of at least 86% of the cattle population is achieved (Welburn *et al.*, 2006). The question of inclusion of pigs in such treatment efforts is now being raised (Waiswa *et al.*, 2003), as the extent to which pigs may potentially contribute to the *T. b. rhodesiense* reservoir and Rhodesian sleeping sickness epidemics is as yet unknown.

1.6.2 Diagnosis

1.6.2.1 Clinical diagnosis

The clinical signs of animal trypanosomiasis include anaemia, weightloss, depression, intermittent pyrexia, starring coat and enlarged lymphnodes as described above are not specific to trypanosomiasis. Differential diagnoses include a range of diseases such as fasciolosis, schistosomosis and haemonchosis (Catley *et al.*, 2001). Clinical diagnosis of animal trypanosomiasis can therefore be difficult, particularly as the animal may be affected by other diseases concurrently. Nevertheless, clinical diagnosis is frequently the only available option for pen-side diagnosis in poor rural areas, as more sophisticated diagnostic methodology is not affordable for the subsistence farmer. Additionally, animal health assistants, who are a mainstay of animal health services in such remote rural areas as Busia, are not usually equipped with nor necessarily sufficiently trained to use diagnostic tools such as microscopy. Treatment is thus frequently administered based on a tentative diagnosis, which is only confirmed if the animal improves subsequently.

A simple decision support card, which ranks clinical signs according to importance for a range of endemic cattle diseases, has recently been developed to serve as

memory support and aid pen-side diagnosis (Magona *et al.*, 2003a; Eisler *et al.*, 2007). This tool has yet to be fully validated in the field situation. Mixed infections with several endemic diseases, as well as sub-clinical infections will remain difficult to be diagnosed on the basis of clinical signs alone. It may thus be of interest to investigate and establish epidemiological factors that can be recorded as part of a case history, which may determine the likelihood of an animal being infected with trypanosomiasis. Such information may be inherent to the individual animal, such as age, breed and sex, or factors related to the likely exposure of an animal to tsetse habitats due to management. The literature on the effect of these factors on trypanosomiasis are reviewed and investigated in detail in Chapter 4.

1.6.2.2 Diagnostic techniques

Microscopy of wet blood films is the most straightforward diagnostic technique applied in the field. Alternatively thick or thin dry smears can be obtained, which can be stored, stained and examined at a later point in time. Levels of parasitaemia in the affected animal may be low, particularly in chronic infections and sensitivity of microscopy can be increased by concentration methods (Kalau *et al.*, 1986). Micro haematocrit centrifugation achieves concentration of trypanosomes in the layer between erythrocytes and lymphocytes called buffy coat in the capillary tubes used (Woo technique) (Woo, 1970). In an improved version of this technique, the buffy coat is expressed onto a microscope slide for examination (Murray *et al.*, 1977). These standard microscopy methods require trypanosome levels of 1000 parasites/ml for detection of an infection (Solano et al., 1999).

More sophisticated techniques for diagnosis of trypanosome infections include sub-innoculation of an uninfected animal, culture, immunological techniques and molecular tools, which have been reviewed in detail elsewhere (Eisler *et al.*, 2004). But due to time and expenses involved, these are usually reserved for research rather than veterinary diagnostic purposes.

In the present study the polymerase chain reaction (PCR) was used as the most sensitive tool available for the detection of trypanosome infections. Less than the genetic material of a single trypanosome is required to detect the presence of *T. brucei* s.l., for example (Moser *et al.*, 1989). PCR can increase the number of cases of trypanosomiasis detected at least two fold when compared directly to the microscopy results from the same sample set of cattle (Clausen *et al.*, 1998, Solano *et al.*, 1999). Due to its high specificity with respect to trypanosome speciation, PCR can also be of use in diagnostic differentiation between Gambian (Radwanska *et al.*, 2002) and Rhodesian Human African Trypanosomiasis in humans (Welburn *et al.*, 2001b; Welburn & Odiit, 2002; Picozzi *et al.*, 2005).

1.6.3 Treatment and control

1.6.3.1 Chemotherapy

Animal trypanosomiasis can be treated by chemotherapy, usually with a single intramuscular injection. However, there are only a limited number of drugs currently available. Curative drugs licensed to treat trypanosomiasis include diminazene aceturate administered at 3.5-7mg/kg bodyweight (bwt) and homidium (bromide and chloride) given at a dose rate of 1mg/kg bwt (Holmes *et al.*, 2004).

The third compound, isometamidium chloride (0.25-1.mg/ kg bwt), is generally used as a prophylactic drug, protecting cattle from re-infection for up to three months. Intramuscular injection of isometamidium generally forms a sterile abscess, from which the drug is released gradually into the tissue, prolonging the protection from infection (Connor, 1992). A curative drug level is therefore maintained in the animal's bloodstream for a longer period of time, during which new trypanosome infections can not take a hold.

The length of the prophylactic effect of isometamidium chloride is proportional to the sensitivity of the trypanosome strains to the drug (Peregrine *et al.*, 1991). The higher the level of trypanosome transmission in an area, the more likely is the

occurrence of more resistant strains (Connor, 1992). Maintaining the protection in areas infested heavily by tsetse, may thus require treatment with isometamidium chloride at frequent intervals, and may not be cost-effective for the individual farmer. Additionally cattle treated too frequently with isometamidium may have adverse reaction due to build up of toxic levels of the drug in the liver (Dolan *et al.*, 1992).

Cattle protected from trypanosomiasis with systematic use of trypanocides remain productive under tsetse challenge, and the advantages in weight gain have been shown to make the use of trypanocides cost-effective (Angus, 1996). Furthermore this advantage is greatest when drug treatment is targeted at infected animals rather than blanket treatment of all cattle, when tsetse challenge is low to moderate and trypanosomiasis was diagnosed using parasitological techniques (Munstermann *et al.*, 1992). Due to economic considerations, trypanocidal treatment is usually reserved for cattle, which are the most valuable animals, despite a considerable prevalence of trypanosomiasis in small ruminant such as goats and sheep, as well as pigs (Katunguka-Rwakishaya,1996; Mwendia *et al.* 1997, Waiswa *et al.*, 2003). Any untreated infected animals may ultimately serve as a trypanosome reservoir, leading to potentially rapid re-infection of treated cattle.

1.6.3.2 Vector control at the micro-scale

Over the last twenty years, responsibility for tsetse and trypanosomiasis control has increasingly shifted from the state to the individual farmer and local communities. This has led to increased interest in small-scale control options, for which an overview is provided here. However, whilst technology applicable at the micro-scale may be available, the cost-efficiency and sustainability of these operations remains debatable (Hargrove *et al.*, 2000). It must be remembered that one simple issue of small-scale tsetse control is that the smaller the controlled area, the larger the circumference to area ratio, which translates into a higher chance of tsetse re-invasion.

1.6.3.2.1 Zero grazing

There are several options of trypanosomiasis control that are based on interrupting tsetse and host contact. Mechanical interruption of this contact can be achieved by zero-grazing, where food and water is provided to cattle in their enclosure. However, tsetse may still enter such closed units, putting the animals at risk of trypanosome infections. Additional protection from tsetse can be provided by insecticide treated netting around these enclosures. If the technique is used by a number of farmers in an area, this technique will also depress the vector population as the insecticide treated nets simultaneously work like tsetse targets resulting in the death of flies alighting on them (Bauer *et al.*, 2006). However initial and maintenance costs of the netting are considerable, and zero-grazing management of animals is labour intensive.

1.6.3.2.2 Live-bait

Since the advent of synthetic pyrethroids with residual effects of up to 4 weeks, insecticides can be directly applied to cattle to control tsetse (Bauer *et al.*, 1992). This does not necessarily protect the individual animal from tsetse bites or trypanosome infections. The technique relies on sufficient cattle in an area to be treated, to depress the vector population to decrease or even interrupt transmission (Vale *et al.*, 1999). Synthetic pyrethroids are available as pour-on formulation, which are applied to small areas of the skin (along the spine of the animal from poll to tail), are absorbed and emitted gradually. Alternatively water-soluble spray/dip formulation are available. Pour-on pyrethroids are expensive due to the drug-vehicle, whereas spray formulations are more affordable. Research into predilection sites of tsetse found that the majority of these flies target the belly and legs of cattle, and spraying of the insecticide onto these restricted sites (restricted application), confers adequate vector control whilst simultaneously considerably reducing the amount of insecticide needed per animal (Vale *et al.*, 1999; Torr *et al.*, 2007). As this technique still allows (limited) tick attachment, it also reduces the threat to endemic stability of tick-borne diseases (Torr *et al.*, 2007).

1.6.3.2.3 *Community-participation: traps and targets*

Traps and targets, made of pyrethroids impregnated cloths, which kills tsetse on contact, are important tools in the control of tsetse. Due to the low reproductive rate of tsetse, odour-baited targets evenly spaced at a density of $4/km^2$ have been successfully used to depress and in some cases seemingly eradicate tsetse in the space of two years (Vale *et al.*, 1988; Dransfield *et al.*, 1990; Willemse, 1991). Traps and targets require regular maintenance and re-impregnation, to avoid loss of use due to damage, theft or decline in effectiveness.

Tsetse programmes involving deployment of traps and insecticide treated targets based on community participation have previously been initiated for local vector control. Examples include programmes in Kenya (Busia) (Echessah *et al.*, 1997), the Congo (Gouteux & Sinda, 1990) and South Sudan (Joja & Okoli, 2001) to name but a few. A strong incentive for tsetse control has been observed where sleeping sickness epidemics have occurred within the living memory of the local population (Kamuanga, 2003). However to date, long term sustainability of some of these projects has been low, with many of them collapsing when external support and input is withdrawn after the initial start-up period, as communities frequently do not take ownership of these projects (Barrett & Okali, 1998).

1.7 Historical changes in the approach to control of trypanosomiasis in Western Kenya

Over the last century, control of trypanosomiasis in Western Kenya has undergone a series of changes not only reflecting the advent of new technologies but also the changes in political perception of the problem. The following section explores the historical shift from sleeping sickness being viewed as a human health risk warranting large scale, state funded intervention strategies, to the perception of trypanosomiasis as a livestock production disease, which is the responsibility of the individual affected farmer.

1.7.1 The 1901 epidemic

At the turn of the last century, an epidemic of sleeping sickness affected the population of the shores of Lake Victoria in Uganda. This epidemic extended into Kenya by 1901, spreading further southwards along the Lake and reaching the Tanzanian border in 1902 (Christy, 1903). Around 250,000 people or more were thought to have died of sleeping sickness in this epidemic, the majority of them in Uganda (Smith *et al.*, 1998), but there are few accurate records of the number of cases available from this period. At the peak of the epidemic, up to 50% of the population along parts of the Kenyan shore of Lake Victoria were estimated to be infected. By 1908, the disease had spread approximately 25 miles inland from the lakeshore along some of the river systems (Kuja and Migori rivers, South Nyanza) (Milne, 1908). *Trypanosoma* were identified as the causative agent of sleeping sickness in West Africa in 1902 (Dutton, 1902), and shortly thereafter Colonel Bruce theorized that trypanosomes were the cause of sleeping sickness in Uganda, and that, because of its distribution, the disease was probably transmitted by the tsetse fly *Glossina fuscipes fuscipes* (Bruce & Nabarro, 1903). But no effective drugs to cure the disease were available at that time (Lumsden, 1964). Control efforts in Kenya were limited to isolation of infected cases in sleeping sickness camps, and movement restriction for the population to prevent the spread of the disease further inland and across the border to Tanzania (Government of Kenya, 1940). Unlike in Uganda, in

Kenya the population was not evacuated from the Lake shores, but many people moved away from infected areas of their own accord (Morris, 1960).

Figure 1.3: Map of areas affected by sleeping sickness within Kenya (current Busia District outline).

1.7.2 The second wave - active screening and prophylactic treatment

By 1912, this first wave of sleeping sickness was abating in Western Kenya, but over the next three decades the disease remained endemic with focal outbreaks interrupting periods of quiescence (Wellde *et al.*, 1989c). In the initial period, fighting the vector of the disease, mainly by bush clearing, was the only available method to combat the disease (Lumsden, 1964). In 1923, Bayer 205 (suramin) was introduced to Kenya, which alongside tryparsamide was used as a curative drug, but was also used for sleeping sickness prophylaxis, in combination with evacuation from affected areas (Wellde *et al.*, 1989c). The mid 1920s and early 1930s were

characterised by a flurry of sleeping sickness control activities, in response to the threat of sleeping sickness flaring up with renewed force, as indicated by a rise in reported cases (McLean, 1931). Control measures included large-scale active screening programmes in Central and South Nyanza, prophylactic drug treatment with suramin, and extensive tsetse surveys. Tsetse control measures included the manual destruction of tsetse habitats as well as tsetse trapping, but were only temporarily successful due to problems of reinvasion, particularly in heavily infested areas (Morris, 1960).

1.7.3   Third wave – residual insecticides

Following the control of the outbreaks in Central and South Nyanza in the 1920s and early 1930s, a period of low endemicity ensued until fresh epidemic started building up in the mid 40s (Morris, 1960) (Figure 1.4). The end of the 1940s were marked by the introduction of residual insecticides, which could be used effectively for the control of tsetse (Lumsden, 1964). Hand spraying of DDT was combined with bush clearing activities to control the various sleeping sickness foci in South and Central Nyanza. In the mid 50s, DDT was replaced by the more efficient insecticide Dieldrin in large scale spraying operations. As a result, sleeping sickness looked to be in recession by the mid 1960s (Wellde et al., 1989c). In South Nyanza, sleeping sickness had been largely exterminated except around the mouth of the Olambwe river, by the treatment of G. fuscipes habitats with insecticide between 1955 and 1957 (Glover, 1962). Between 1964 and 1967 little was done in South Nyanza as the tsetse control teams participated in the operations in Alego, Central Nyanza where an outbreak of Rhodesian sleeping sickness had erupted in 1964 (Wellde et al., 1989c) (Figure 1.4).
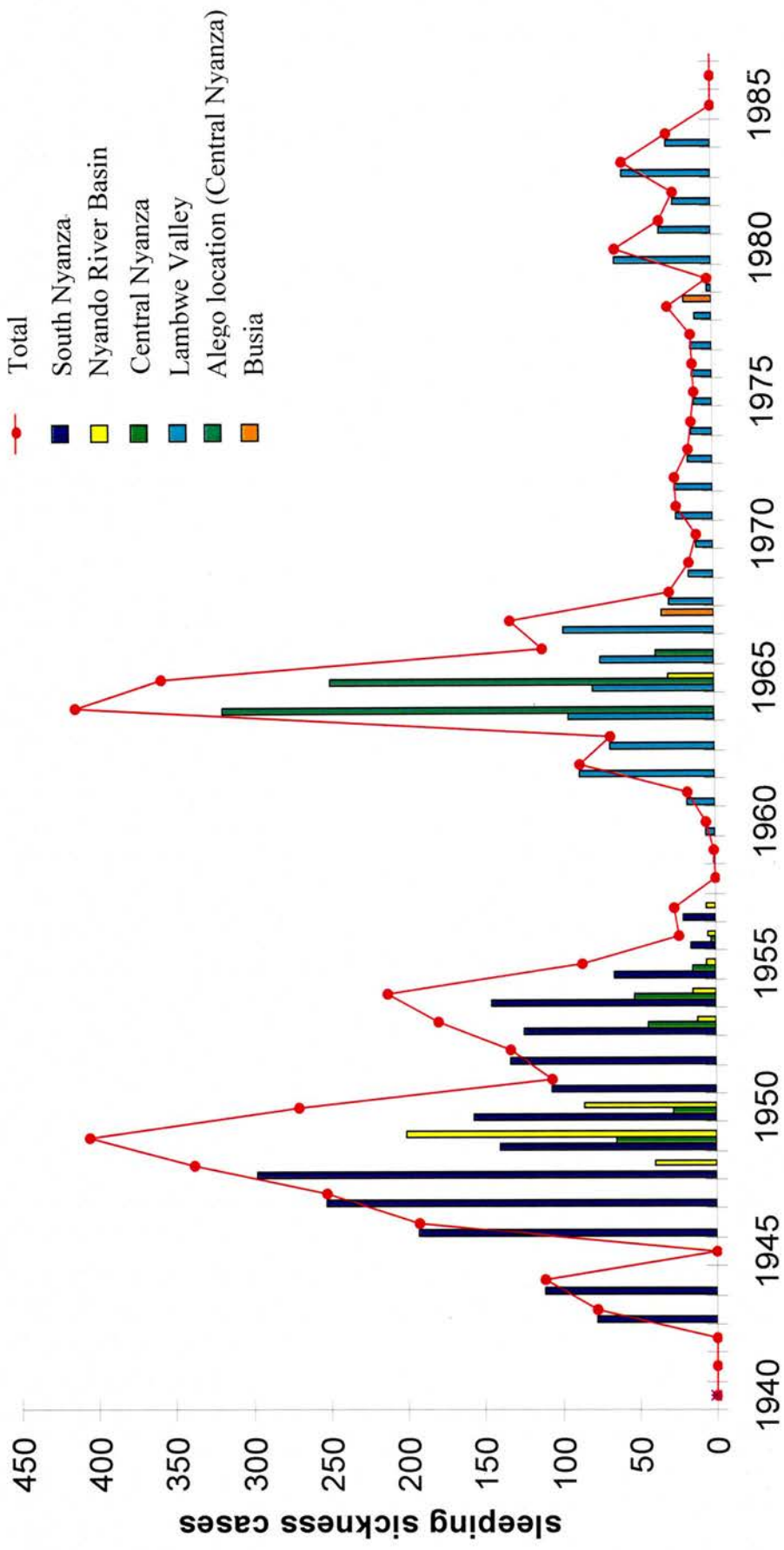
Figure 1.4: Sleeping sickness outbreaks in Kenya between 1940-1986, number of cases/year summarised by geographical location as recorded in (Morris, 1960; Baldry, 1972; Wellde et al., 1989a; Wellde et al., 1989b).

1.7.4 Gambian or Rhodesian sleeping sickness?

The early epidemic of sleeping sickness in Kenya and Uganda at Lake Victoria (Busoga focus) were believed to have been caused by *T. b. gambiense*, with *T. b. rhodesiense* being introduced from Zambia in the 1940s (Baldry, 1972). But in the 1940s, the resistance to arsenical drugs was the only criteria by which *T. b. rhodesiense* was differentiated from *T. b. gambiense* (Lumsden, 1964). In fact, when the 1900 epidemic first arose, *T. b. gambiense* was the only trypanosome known to infect man, as *T. b. rhodesiense* was not described until 1910 (from what is now Zambia) (Stephens & Fantham, 1910). More recent re-examination of the available evidence, suggested that *T. b. rhodesiense* has been the causative agent of sleeping sickness in the Busoga focus all along (Koerner *et al.*, 1995; Fèvre *et al.*, 2004). Factors taken into consideration included the social and political history in the Busoga region, the clinical course of sleeping sickness as recorded by case reports and the relative ease of cyclical transmission of the causative trypanosome, to name but a few, all of which pointed towards *T. b. rhodesiense* (Koerner *et al.*, 1995; Fèvre *et al.*, 2004).

1.7.5 The Alego outbreak –recognition of cattle as a reservoir of *T. b. rhodesiense*

After a ten-year period of few reported cases in Central Nyanza, a sizeable outbreak of sleeping sickness occurred in Alego, starting in 1964, which was attributed to *T. b. rhodesiense* (van Hoeve *et al.*, 1967) (Figure 1.4). The vector, *G. f. fuscipes* was reported to have colonised peri-domestic bush thickets in the Alego area, away from its usual riverine habitat (Bertram, 1969), and the resulting increase in tsetse-human contact was believed to have exacerbated transmission (Baldry, 1972). During this outbreak, *T. b. rhodesiense* was isolated from domestic cattle and shown to be infective to man, providing evidence for a cattle reservoir of Rhodesian sleeping sickness in a field situation for the first time (Onyango *et al.*, 1966).

T. b. rhodesiense was known to be zoonotic and had previously been isolated from a bushbuck (Heisch *et al.*, 1958). Livestock had long been suspected as a reservoir of

sleeping sickness (Bruce *et al.*, 1911; Kleine & Eckard, 1913; Duke, 1928; Van Hoof, 1947) and the maintenance of human-infectivity of *T. b. rhodesiense* after repeated passageing through livestock had been demonstrated (Corson, 1936; Fairbairn & Burtt, 1946; Ashcroft *et al.*, 1959), but *T. b. rhodesiense* had never before been isolated from cattle in the field (Onyango *et al.*, 1966). This was later confirmed by Robson and colleagues who detected human infective *T. b. rhodesiense* in over 10% of *T. brucei* s.l. infected cattle, using the Blood Incubation Infectivity Test (BIIT) (Rickmann & Robson, 1970; Robson *et al.*, 1972). Consequently, control of the Alego outbreak, for the first time, included the mass-treatment of all cattle in the area with the trypanocide Berenil to target the livestock reservoir, in addition to the established bush-clearing and ground-spraying activities targeting the vector (Wellde *et al.*, 1989c).

1.7.6 Sleeping sickness in the Lambwe Valley – WHO aerial spraying programme

As previously mentioned, sleeping sickness had been largely exterminated from Southern Nyanza, except around the mouth of the Olambwe river, through insecticide spraying campaigns between 1955 and 1957. Some cases of Rhodesian sleeping sickness were reported from the Lambwe Valley in 1959 and 1960, and this outbreak accelerated over the following years (Wellde *et al.*, 1989b). The number of cases declined in 1968 in response to extensive control efforts (Figure 1.4). As part of the programme of research on aerial spraying for control or eradication of *G. pallidipes*, the WHO Trypanosomiasis Project targeted some of the key habitats of the vector in the Lambwe Valley (Willett, 1972). It was thus considered to have been the main factor in controlling sleeping sickness in the lower Lambwe Valley.

Control was re-enforced by a ground-spraying programme in 1968-69 executed by the Kenyan Ministry of Agriculture, Tsetse Survey and Control Division, which also implemented bush clearing in 1969 and 1970 (Baldry, 1972). More strict enforcement of the game reservoir, established in 1966, also served to break the close tsetse-man contact. Throughout the 1970s the Lake shore was included in the

spraying programmes. But in the early 1980s control efforts concentrated on the Lambwe Valley, which was the only persistent focus in Kenya (Figure 1.4), where after a period of quiescence a renewed outbreak started in 1980 (Wellde *et al.*, 1989b). Sporadic cases in Busia District along the Kenyan-Ugandan border were perceived as spill over from Uganda (Ogada, 1979).

1.7.7 Decentralisation in the 1980 – trypanosomiasis control as farmer responsibility

In the post-independence era, the Kenyan government had assumed a dominant role in provision of agricultural and veterinary services (Smith, 2001), which was also expressed in trypanosomiasis control. Until the mid-1980s, tsetse fly control had been achieved using mainly bush-clearing and insecticide spraying, implemented by public agencies, with little or no involvement of the local population (Kamuanga, 2003). This was primarily aimed at control of human sleeping sickness, but simultaneously served to control livestock trypanosomiasis. When cattle were recognised as a reservoir for *T. b. rhodesiense* (Onyango *et al.*, 1966), drug treatment of cattle was included into sleeping sickness control measures (Wellde *et al.*, 1989c). The high cost and complexity of these operations proved to be beyond the scope of national organisations without extensive technical and financial external support (Kamuanga, 2003).

In addition to concerns over environmental safety and natural resource conservation, the financial crisis that affected most countries in sub-Saharan Africa in the wake of deceleration of world economic growth in the 1970s and 80s, put traditional veterinary systems under severe pressure, necessitating cuts in the operating budgets of delivery systems (Leonard, 1993). In the 1970s, the World Bank had placed a lot of emphasis on poverty alleviation, expressed in substantial projects lending in areas such as rural development for small farmers (Smith, 2001). But at the end of the decade increasing concern over long-term development and sustainability placed the need for structural re-adjustment into the spot-light which led to a switch from project based lending to policy-based lending, tied to structural reforms (Smith,

2001). Reforms necessary to lower government spending and conform to lending-stipulations, also forced Kenya to prioritize expenditure for core obligations in the public sector (Holden, 1999).

Transfer of economic functions from the public to the private sector were instigated in the delivery of animal health services in general (Leonard, 1993; Umali et al., 1994) and trypanosomiasis in particular (Kamuanga, 2003). Significant and ongoing reductions in the budget of the Veterinary Department, alongside considerations of efficiency of delivery of veterinary services, resulted in a shift towards privatization of veterinary services in Kenya (Holden, 1997). The decrease in funding automatically reduced the services that could be provided by the Veterinary Department, which had to focus on the provision of what was considered to be public goods (de Haan & Umali, 1992).

Livestock trypanosomiasis was regarded as endemic and by the end of the 1980s sleeping sickness was no longer considered a major human health issue in Kenya as compared to malaria or HIV/AIDS (Bourn, 2001). Thus large-scale trypanosomiasis control was no longer state-funded, neither under the veterinary nor the human health aspect. Trypanosomiasis was deemed to be a livestock-production disease, the control of which was in the interest of the individual affected farmer. This was reflected by research in Kenya in the 1980s and early 90s with many studies focusing on the cost-efficiency of trypanosomiasis treatment and prophylaxis using trypanocides and pour-on insecticides (Wellde & Chumo, 1983; Wilson et al., 1986; Peregrine et al., 1991; Munstermann et al., 1992; Kamau et al., 2000) as well as breed specific genetic resistance to trypanosomiasis (Murray & Black, 1985; Njogu et al., 1985). The advent of tsetse traps and targets appeared to provide the technology necessary for small-scale community based interventions for local tsetse control, which could replace spraying and bush clearing campaigns (Kamuanga, 2003). The responsibility for trypanosomiasis control had thus shifted from the government to the livestock owners and local communities.

1.7.8 Control efforts in Busia, Western Kenya - illustration of shift in responsibility

Busia, Teso and Bungoma Districts in Western Province, situated on the border to Uganda, were the only areas in Kenya that still reported cases of sleeping sickness in the 1990s (WHO, 2006) (Figure 1.5). Prior to 1990, tsetse control in Busia was carried out on an *ad hoc* basis, involving bush clearance and spraying in and around villages where sporadic cases of sleeping sickness had occurred. In the wake of the sleeping sickness outbreak in 1989/90, a vector control programme of systematic trapping was initiated. It reduced the apparent tsetse density in Busia District by up to 99%, between 1990 and 1994, as recorded by the Tsetse Control Section of the Veterinary Department Busia (Angus, 1996).
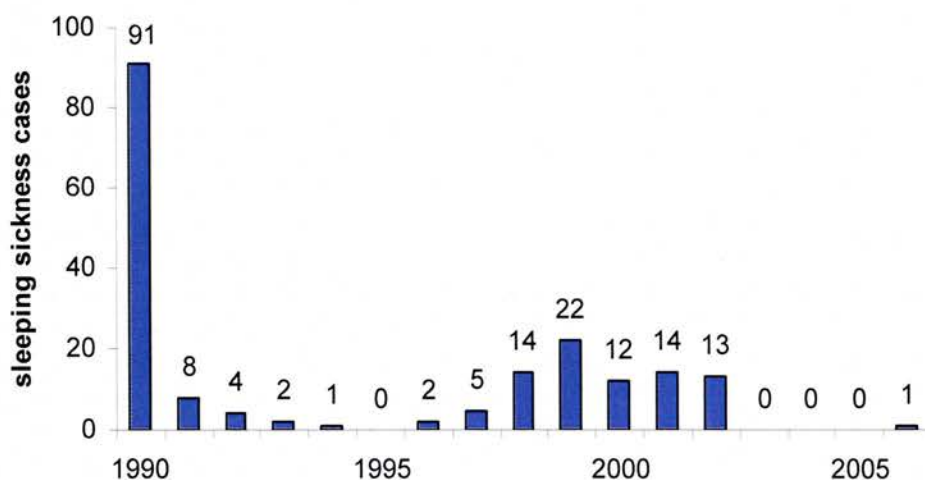
Figure 1.5: Sleeping sickness cases reported from Busia, Teso and Bungoma Districts, Western Province, Kenya between 1990 and 2006 (WHO, 2006).

The Organization of African Unity (OAU) became involved in 1991, to improve cross border cooperation of tsetse control departments between Uganda and Kenya (FITCA, 2005). Through its Interafrican Bureau for Animal Resources (IBAR) office in Nairobi, it supplied pour-on insecticide treatments for cattle, to facilitate tsetse control. However due to the high cost of the pour-on this method of control was not sustainable (FITCA, 2005). Sleeping sickness had returned to low endemic levels in

Busia and neighbouring districts by the mid-1990s (Figure 1.5), reducing the urgency for tsetse control from a human health point of view. Budget reductions in the Veterinary Department limited the resources for vector control to combat trypanosomiasis in livestock (FITCA, 2000). Increasingly the state regarded trypanosomiasis as an endemic livestock production disease, the control of which was considered private good (Holden, 1999). Control was to be achieved on the micro-scale through treatment of individual animals with trypanocides purchased through the private sector at the farmer level, or vector control at the community level by use of pour-on insecticides or dip tanks. However, farmers appeared to be inadequately equipped to deal with the shift in responsibility for trypanosomiasis control, both in financial terms and in terms of expertise.

Between 1992 and 1995, the Kenyan Trypanosomiasis Research Institute (KETRI) initiated a tsetse trapping programme based on community participation. Considerable effort was put into a feasibility study (Echessah *et al.*, 1997) and a pilot project was initiated successfully in two study villages. However, the project was later regarded as a research rather than a control programme. Due to lack of community ownership of the project, no further actions were taken, once KETRI withdrew (Kamuanga, 2003). The impact of trypanosomiasis on livestock production in Busia seemed to increase over the following years. Cattle mortality, particularly amongst exotic or grade cattle (which were being increasingly encouraged as part of a breeding programme) was high, and farmers' understanding of animal health was found to be a major constraint (FITCA, 2000). The Kenyan project of the Farming in Tsetse Controlled Areas Programme (FITCA-K) attempted to address this problem through farmer education and training (FITCA, 2005).

1.7.9 FITCA- Kenya

In 1999, an EU funded programme, Farming in Tsetse Controlled Areas (FITCA), was implemented in 5 tsetse infested districts in western Kenya including Busia, Bondo, Siaya, Teso and Bungoma. FITCA was initiated as a regional programme including Uganda and Tanzania in response to the regional characteristics of the

tsetse and trypanosomiasis problem, but each country programme operated largely independently (Mosele *et al.*, 2002). As sleeping sickness in western Kenya was at a low endemic level, the main objective of FITCA Kenya was to improve the welfare of the people in the area by improving livestock production. The long term aim in western Kenya was to facilitate sustainable tsetse control, operated and financed by the livestock keeper (FITCA, 2005).

Initial tsetse surveys, conducted between February and April 2000, concentrated on areas infested with *G. pallidipes*. Based on mature infection rates, *G. pallidipes* was recognised to be more efficient than *G. f. fuscipes* in transmitting livestock trypanosomiasis in Busia, even though the apparent density of *G. pallidipes* was considerably lower (Karanja, 2006). Towards the end of the 1990s, *G. pallidipes* had increasingly been reported as a problem from Busia and Teso, in areas that were previously considered to have been infested with *G. f. fuscipes* only (FITCA, 2005). Apparent density of *G. pallidipes* was highest in Teso District, with over 1000 tsetse flies/trap/day (ftd) recorded at some sites. FITCA therefore instigated intensive tsetse control measures employing traps and odour-baited insecticide-impregnated target in Teso District, successfully reducing the *G. pallidipes* prevalence by over 95% over a two year period (Politzar, 2001). A similarly intense, though shorter programme (6 months) was also initiated in Bondo District, after investigation of increased reported livestock mortality revealed tsetse densities of over 80ftd. Busia District had a comparatively modest tsetse population and was therefore not included in these intensive programmes (FITCA, 2005).

The FITCA programme envisaged tsetse control in Busia District to operate on the basis of insecticide-treatment of cattle, which does not necessarily protect the individual animal from tsetse bites, but reduces the overall tsetse challenge if a sufficient proportion of host animals is treated (Bauer *et al.*, 1992; Vale & Torr, 2004). The only synthetic pyrethroids registered for sale in the project districts at the start of the project in 1999, were expensive pour-on formulations (FITCA, 2005). FITCA liaised with the Veterinary Department achieving registration of the spray/dip

formulation of deltamethrin for use in the project districts, which is considerably cheaper for the end user. Farmers were encouraged to build crush pens and form groups to raise 40% of the money for a spray pump. The FITCA project covered the remaining cost of the pump and supplied one litre of deltamethrin for the start up of each group. Crush pens were then to be operated on a cost-recovery basis (Mosele *et al.*, 2002).

Despite best efforts to train committee members on management, a high number of spraying groups ceased working once initial supplies were used up. A survey conducted in 2003, involving the initial 193 spraying groups, found that only 20% of groups set up in Busia were still working, which was the lowest proportion in all project districts (over 40% of groups set up were still operating in Bondo, around 30% in Siaya, Teso and Bungoma) (FITCA, 2005). It was estimated that only 3000 cattle were sprayed at two weekly intervals in the project districts, 1200 of them in Bondo District. This was nowhere near the necessary 5-10% of the cattle population that was required to be insecticide treated in order to achieve tsetse control, or even maintain tsetse suppression that had been achieved through use of targets in Teso and Bondo (FITCA, 2005).

1.7.10 Present state of trypanosomiasis control in Busia

The FITCA-Kenya project drew to a close in 2004. In a final feedback report, FITCA-Kenya stated that the extreme levels of poverty in the project districts had been underestimated, and that it would take a more long-term input than the five years planned for the FITCA project, to achieve significant changes in the area (FITCA, 2005). The lack of sustainability of regular insecticide spraying through community groups, funded through cost-recovery, was one indicator of this lack of up-take. Further evidence for the problems that FITCA encountered in improving knowledge on trypanosomiasis was provided by a survey in Butula and Funyula. This study was conducted in Funyula and Butula Division in July 2002, three years after the initiation of the FITCA programme in Busia, and only one out of 267 respondents named FITCA as a source of information on trypanosomiasis (Machila-

Eisler, 2005). These results highlighted the poor penetration of FITCA's awareness and extension programmes in the two divisions of Butula District, which are also the study sites for this thesis. In workshops conducted in Busia in 2002, many livestock owners appreciated the link between tsetse and trypanosomiasis, but the prevailing opinion appeared to be that only the state had the necessary tools and the money to tackle this problem (Machila-Eisler, 2005). This attitude demonstrated the vast discrepancy between government policy and farmer perception of trypanosomiasis control, which may be detrimental to the success and sustainability of future community based control projects unless farmers can be convinced that tsetse and trypanosomiasis control is within their scope.

1.8 Objective

With decentralization and privatization of veterinary services it is left to farmers to control trypanosomiasis, which is viewed primarily as a livestock disease in Western Kenya, even though sporadic cases of sleeping sickness serve as a reminder of the ongoing threat to the human population. Prophylactic and curative drug treatment has been shown to be an efficient means to combat the effects of trypanosomiasis on livestock production (Munstermann *et al.*, 1992). Furthermore the benefits of treatment of infected animals in terms of increased productivity have been shown to financially outweigh the initial cost of the drug (Angus, 1996). However, increasingly reports of drug resistance in trypanosomes across East Africa (although so far not from Busia District) (Eisler *et al.*, 2000) implicate that caution is necessary in the unregulated use of trypanocides in livestock. In particular as cross resistance in drugs has become an issue (Mäser *et al.*, 2003), extensive use of trypanocides in cattle based on a tentative diagnosis or for prophylactic purposes increases the possibility of selecting for drug resistant human infective parasites in livestock. If control at the micro-scale is to be sustainable in the long term, vector control will have to be integrated into the approach. Donor organizations and governments increasingly pin their hopes (and funds) to small-scale vector control operations, which seem progressively more feasible with the advent of trapping and live-bait

technology, although the effectiveness of such localized interventions in the face of tsetse re-invasion has been called in to question (Hargrove *et al.*, 2000).

Many previous studies investigated risk factors and the spatial distribution of tsetse and trypanosomiasis, clinical indicators of disease and important reservoirs of the disease at a country or eco-system wide scale, which was appropriate to determine target areas for large scale control or eradication projects. Whilst over recent years both government and NGOs have stepped away from large scale top-down trypanosomiasis control programmes, leaning towards individual control or community based projects (Kamuanga, 2003), little is known about the epidemiology of trypanosomiasis at the micro-scale at which control is now envisaged.

This thesis examines the extent to which insights gained on livestock trypanosomiasis at the macro-scale are transferable to the micro-scale. Sensitive molecular tools are used to explore trypanosomiasis prevalence in Busia, Kenya. The work is based on a unique cross-sectional data set, collected by census sampling of the entire livestock population of two study sites. The investigation is approached under the following aspects:

- Chapter 4 explores the importance of different livestock species as reservoirs of animal as well as human infective trypanosomes. Furthermore, the effect of animal inherent factors and management practise on trypanosomiasis risk is analysed.

- Chapter 5 employs GIS to examine the geographical distribution of trypanosome infected livestock and investigates spatial clustering, as well as testing the association of household herd infection risk with proximity to hypothesised tsetse habitats.

- Chapter 6 evaluates the accuracy of a convenience sampling technique commonly used for rapid assessment of cattle trypanosomiasis by comparing it to the gold standard results obtained by census sampling.

An in depth background of the study area, exploring the importance of agriculture and the constraints to livestock production in Busia, Kenya, is given in Chapter 2, followed by study design and general methodology, which are outlined in Chapter 3.

2 Chapter 2   Description of study area

2.1 Introduction

This chapter aims to introduce the area involved in this study namely Busia District, with particular emphasise on Funyula and Butula, the two divisions of Busia District in which the study villages were situated. The selected villages were well established field sites, characterised in terms of their socio-economic background, livestock dynamics and veterinary care seeking behaviour which has been researched in detail previously (Machila-Eisler, 2005; Thuranira, 2005). An overview of this information is given in the following sections to provide a relevant background to this study. Originally, these field sites were chosen, as sub-locations with a trypanosomiasis prevalence in cattle of at least 6% as detected by microscopy in a cross-sectional survey in 1997 (INCO-DC, 2000; Machila-Eisler, 2005; Thuranira, 2005). Building on the ample available background information, the current study could concentrate on the distribution of trypanosomiasis in the sampling villages.

2.2 Study area Busia: general description

2.2.1 Location and administration

Busia District, is one of six districts of Western Province, Kenya, located between latitudes 0°1'36'' south and 0°33' north and longitudes 33°54'32'' and 34°25'24'' east. It is bordered by Teso and Bungoma Districts to the north, Butere District to the east, Siaya District of Nyanza Province to the South and Uganda and Lake Victoria to the west. Eleven per cent of its total area of 1262km^2 are permanently covered by the waters of Lake Victoria.

Busia District has six administrative divisions: Busia Township, Budalangi, Butula, Funyula, Matayos and Nambale, which are further sub-divided into 30 locations and 99 sub-locations, with individual villages within the sub-locations making up the smallest administrative units. This study included nine villages, from three adjacent sub-locations within Funyula Division (Bukhulungu, Sigulu and Wakhungu Sub-

locations) and ten villages, in three adjacent sub-locations of Butula Division (Bujumba, Ikonzo and Nawitsula Sub-locations) (Figure 2.1).
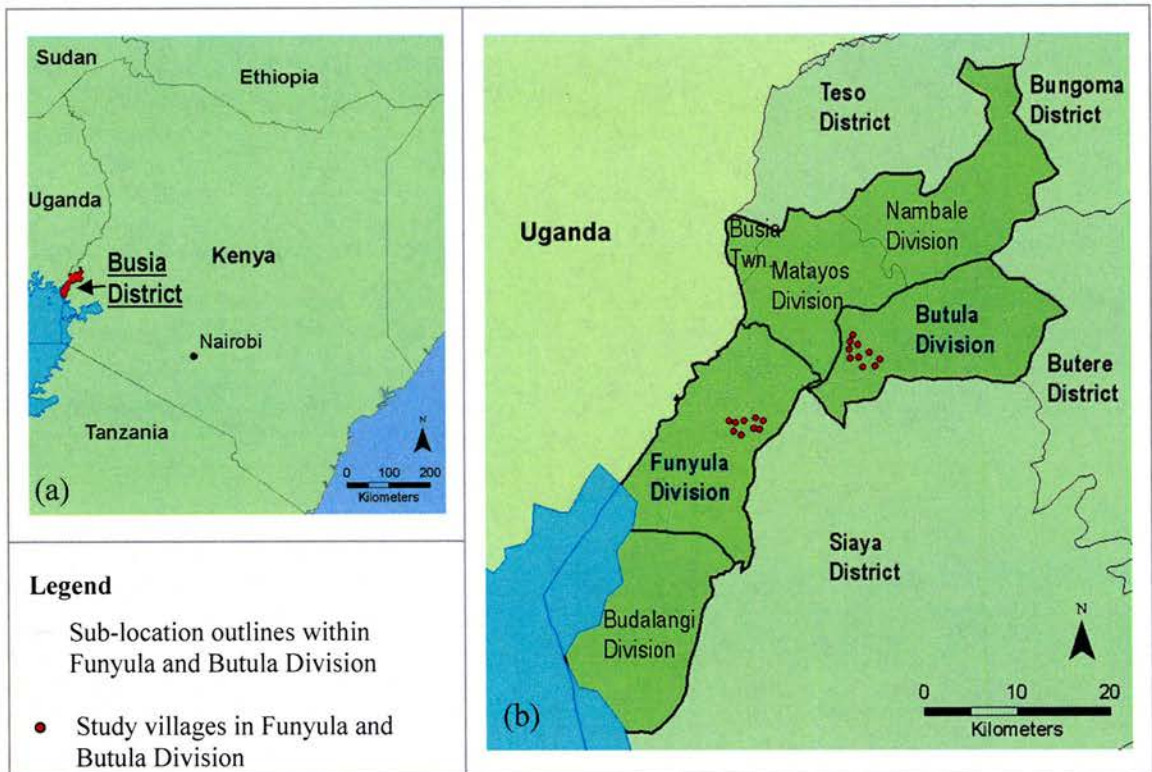
Figure 2.1: Map of study area. (a) Geographical location of Busia District. (b) Geographical location of study villages in Funyula and Butula Division within Busia District.

2.2.2 Human demography

The population of Busia District consists mainly of Luhya speaking (Bantu) people as well as some Luo speaking (Nilotic) people. Intermarriage between the two groups is common. Busia Township has representatives of most ethnic groups of Kenya and some of the Ugandan people. At the last population census in 1999, Busia District had a total population of 370,608. With an annual growth rate of 2.89%, the population is predicted to increase to 485,047 by 2008 (Government of Kenya, 2001). There were close to 82,000 households in the district. Funyula and Butula Division accounted for 22% and 26% of the population of Busia District, respectively (Government of Kenya, 2001). This could be attributed to their relatively large surface area as well as medium to high agro-ecological potential (see

Section 2.2.4). With a mean number of 9 household members, household size in the study villages was considerably larger than the average of 4.5 for the whole of Busia District (Thuranira, 2005).

Enrolment for primary school education was relatively high at over 90% for both boys and girls as reported in 2001. Enrolment to secondary school was drastically lower, barely reaching 20% (Government of Kenya, 2001). Literacy rate in the older population of the study area was low with only approximately 65% of over 35 year olds having received formal education (Thuranira, 2005).

The sex ratio of females to males was 100:89 overall in the district. There were considerable differences in this ratio according to age group. Whilst in the 10-14 year age group the ratio was 100:103 female to male, the ratio droped to 100:73 in the 20-69 year old group. The drop in the male population of working age was generally attributed to a high number of men migrating outside the district for employment (Government of Kenya, 2001). However, it seemed likely that the staggering rates of HIV/AIDS in the district (33% of the total population), contributed to the disproportional decline in the male population (Government of Kenya, 2001). HIV/AIDS was observed to impact on productivity in the labour intensive agricultural sector, indirectly denying children the opportunity of education by increasing the need for child labour to compensate for the affected adults (Government of Kenya, 2001).

2.2.3 Climate

Temperatures in Busia District range from annual mean minimum temperatures between 14°C and 18°C and maximum temperatures between 26°C and 30°C. The rainfall is bi-modal, the long rains beginning in March and last into May. After a short relatively dry hiatus in June and July, the short rains begin in August and continue into October, followed by the dry season from November until February. The mean annual rainfall in Busia is 1500mm, with most areas (including Butula

Division) receiving between 1270 and 1790mm a year. The driest parts of the district are found along the shores of Lake Victoria, with only 760 to 1015mm precipitation a year. The southern regions of the district (which include Funyula Division) have approximately 1020 to 1270mm rainfall annually. Due to the proximity of Lake Victoria, mean evaporation ranges between 1800 and 2000mm a year, resulting in relatively high levels of humidity. Climate and topography are ideally suited for the riverine tsetse species *Glossina fuscipes fuscipes* (*G. f. fuscipes*) and Busia District forms part of the continuous tsetse fly belt with the sleeping sickness areas of south-east Uganda (Ford & Katando, 1973). The drier undulating southern parts of the district additionally provide a suitable habitat for the savannah tsetse species *Glossina pallidipes* (*G. pallidipes*) (FITCA, 2005). The study villages in Funyula were located in close proximity to the Samia Hills, which provide good potential habitat for pockets of *G. pallidipes*.

2.2.4 Agriculture

In 1999, over 70% of the potential labour force of 174,854 people between the ages of 15 and 64, were not in formal employment. The majority of this labour force was engaged in agricultural activities on family farms. The remaining 30% were distributed over economic activities such as fishing, trading and employment in the formal and informal sector. Despite over 70% of the work force being involved in agriculture, this sector contributed on average only 35.4% to the mean monthly household income of KSh 5,141.8 (~US$ 74) in the district in 2001 (Government of Kenya, 2001). This highlighted the limited productivity of the mixed crop-livestock agricultural system in Busia District. There were an estimated 44,000 farm holdings in Busia, with an average size of 2.5ha. The main crops include maize, sorghum, sweet potatoes, soya beans, cowpeas, beans, cassava, avocados, oranges and bananas. Where the soil is sufficiently fertile (parts of Butula and Nambale), cash crops such as sugar cane are grown. However the majority of households are forced to practice subsistence farming, with limited surplus crops sold at local markets (Government of Kenya, 1997).

In the study villages in Funyula and Butula the main income generating activities of households were crops (71%), livestock (1%), mixed crop-livestock farming (25%). Off-farm activities, including activities such as working as casual labour, rope and basket weaving, beer brewing or making charcoal or bricks, only provided the main income in 3% of households, emphasizing that the vast majority of rural households in the study areas were vitally dependant on subsistence farming for their livelihoods (Thuranira, 2005).

2.2.5 Constraints to crop production

Busia District is amongst the poorest districts in Kenya, with 65.9% of the population living in poverty with an income of under US$1/day. Busia is one of five districts in Kenya, in which more than 50% of the population live in hardcore poverty, defined as the inability to afford the minimum recommended food-energy requirements even if a household devotes its entire income to food (Government of Kenya, 2001). Reasons for the high level of poverty include low utilization of agricultural land. Use of superior technology for land preparation is negligible and a majority of the hard manual labour is performed by women. The use of animal traction fell drastically after large cattle losses in Busia District in the early 1990s due to disease (presumably trypanosomiasis) (FITCA, 2005). Poor soil fertility and low levels of use of manure, artificial fertilizers or certified seeds result in low crop yields. Inaccessibility of credit diminishes the possibilities of investment into crop or livestock improvements. Collapse of the Kenyan textile industry destroyed the market value of cotton, which was formerly an important cash crop in the drier south of the district. A low number of organized markets for farm and off-farm produce, combined with a poor road network and limited transport provide insufficient access of farmers to markets and thus the ability to generate cash (FITCA, 2005).

2.2.6 Livestock

The last complete livestock census in Western Province was performed in 1960. Figures appearing as livestock census data from 1999 in the year 2000 District reports were largely projections based on the numbers collected in 1960, and as such

only of limited value (FITCA, 2005). More reliable figures available were based on predictions made from a census of 72% of homesteads in Busia District in 2000/2001 as part of the baseline data collection for the Farming in Tsetse Controlled Areas – Kenya programme (FITCA- K) (Table 2.1) (Mosi & Nyandega, 2002).

Table 2.1: Livestock in Busia District in 2000/2001 based on predictions from census of 72% of households (Mosi & Nyandega, 2002).

Division	zebu cattle	grade cattle	sheep	goats	pigs	donkey	rabbits	local chickens	exotic chickens
Budalangi	9823	17	2704	9483	812	77	1628	77941	635
Butula	23350	235	9175	12582	2853	184	4577	165452	826
Funyula	7118	89	2666	8738	2481	57	1861	89340	583
Matayos	11248	115	4705	6526	5618	178	2352	81213	1439
Nambale	12648	88	4082	4115	4050	1091	2697	83800	952
Township	10631	1163	4862	8697	5466	531	3699	95254	13312
total	74818	1707	28194	50141	21280	2118	16814	593000	17747

Despite a high proportion of mixed crop-livestock systems, integration of livestock into crop production is limited in Busia District. Manure is used to improve soil fertility and animal power is used for traction, but the number of draught oxen available in the district is low (Thuranira, 2005). On the whole, livestock are perceived as a kind of savings account or source of ready cash and are bought when surplus cash is available. Sheep and goats are frequently sold to cover expenses such as school fees and uniforms or for the purchase of other household goods, whereas cattle is only sold when unforeseen family circumstances require large amounts of cash (Thuranira, 2005). Less than 5% of cattle in Busia District are grade cattle (zebu improved by crossing with exotic cattle breeds) and the daily milk yield of zebu cattle rarely exceeds an average of one liter (Machila-Eisler, 2005). Milk is thus used for home consumption, or sold on to neighbours but only few farmers sell to milk cooperatives, which require a regular supply of at least one liter per day (Thuranira, 2005).

Pig rearing, whilst still relatively new to the district, is increasingly popular. The pig population of Busia District was estimated to have risen from 7,000 in 1991 to 11,000 in 1995 (Government of Kenya, 1997). Recent work in the study areas in Funyula and Butula showing a highly significant increase of 153% in the number of pigs between 2001 and 2002, compared to cattle, sheep and chickens increasing by approximately 10% over the same period (Thuranira, 2005). Pigs are perceived as low input livestock, which can be fed on household scraps and are rarely ill, as Busia District has not yet been affected by epidemics of diseases such as African Swine Fever. There is also a ready market for pigs at local butcheries. Nearly 95% of households keep chickens, which provide eggs for sale and home consumption as well as meat (Thuranira, 2005).

2.2.7 Constraints to livestock production

Livestock keeping provides a valuable source of income to farmers in Busia District, however there are a number of factors which limit the potentially achievable output.

2.2.7.1 Grazing

Traditionally, cattle and small ruminants are either tethered around the farm compound for grazing, or taken to communal land. In Busia, Kenya, animals are not grazed together as a village herd, as is the case in neighbouring districts in Uganda. Instead each household manages its animals individually. The average size of farm in the study villages in Busia District is only 4.1 acres. In the study area of Funyula and Butula, surveyed households could spare under 15% of their land for grazing and less than 1.5% for cultivating fodder plants (Thuranira, 2005). Poor nutritional status not only reduces the productivity of animals in terms of live weight gain, milk yield, draught power and fertility, but also renders them more susceptible to disease (Osaer *et al.*, 1999a; Murray *et al.*, 2004). There is insufficient traction available to open more land for grazing and existing communal land is frequently overgrown, located in swampy areas or along streams and rivers, which provide ideal tsetse habitat. Livestock is thus exposed to high risk of trypanosomiasis infection which hinders restocking of draught animals, bringing the vicious circle full turn (FITCA, 2005).

2.2.7.2 Disease

There is a wide range of endemic diseases that affect livestock in Busia District and reduce productivity. Trypanosomiasis, tick borne diseases and heleminthosis were perceived as the most important diseases by the farmers, but other endemic diseases include pneumonia, lumpy skin disease, foot and mouth disease and Newcastle disease (in chickens) (Machila *et al.*, 2003; Karanja, 2006). A cross-sectional survey on the prevalence of tick borne diseases in cattle in Busia (blood and lymph node aspirate microscopy), found an infection rate of 6.9% of *Theileria parva*, 16.4% *Anaplasma* and 4.8% *Babesia* infections (cattle: n=2827). Detailed background data on trypanosomiasis in Busia District can be found in section 2.4.2.

Unlike traditionally pastoralist tribes in northern Kenya, farmers in Busia District may move in and out of livestock keeping according to their financial circumstances, and knowledge of livestock husbandry and diseases is frequently limited (Thuranira, 2005). A survey among cattle owners in Butula and Funyula Division in 1999, found that 56.2% of perceived disease episodes in cattle remained unidentified or were identified incorrectly (Machila *et al.*, 2003). In cattle, disease related mortality rates between 6.5 % and 13.5% a year were reported from sampling areas in Butula and Funyula. It was estimated that animal deaths due to disease resulted in losses of 80% of the total output generated by livestock per household, thus reducing the average gain from livestock keeping to less than US$7 annually per household (Thuranira, 2005).

2.2.7.3 Availability of veterinary services

The administrative structure of government veterinary services in Kenya is hierarchical headed by the Director of Veterinary Services at Nairobi in charge of the Provincial Veterinary Offices which is in turn are in control of the District Veterinary Offices (DVO) (Kajume, 1998). Each of the six divisions within Busia, has a Divisional Veterinary Officer (VO) or Livestock Officers (diploma qualification) supported by animal health assistants, hide and skin inspectors, artificial

inseminators and lab technicians. Major constraints to the veterinarians and other frontline staff, include lack of transport and obligatory meat inspections stretching the capacity of the VO for other veterinary commitments (Machila-Eisler, 2005). Significant and ongoing reductions in the budget of the Veterinary Department, since the world recession in the 1980s, resulted in a shift towards privatization of veterinary services in Kenya (Mlangwa & Kisauzi, 1994; Holden, 1999). However farmers frequently can not afford veterinary treatment for their animals, hence rendering private veterinary practices in rural areas un-viable (Holden, 1997; FITCA, 2005). Animal health assistants (AHA) and agrovet traders seek to fill the created void by providing animal health advice and or veterinary drugs in these marginal areas, but the quality of the health care available through unqualified or partially trained providers is questionable (Machila *et al.*, 2003).

In the study villages, the majority of drugs purchased were bought at agrovet stores (Machila-Eisler, 2005). Whilst two third of treatments, were administered by AHAs, the remaining one third were administered by the livestock owners or neighbours and friends despite insufficient knowledge on animal diseases (Machila-Eisler, 2005). 17.9% of cattle perceived to be ill were treated with trypanocides in Busia. However 40% of these animals were thought to have conditions not associated with trypanosomiasis, highlighting a basic lack of understanding of cattle owners for the use of trypanocides (Machila *et al.*, 2003).

2.3   *Background of trypanosomiasis in Busia*

2.3.1   Perception of trypanosomiasis

The presence of tsetse and trypanosomiasis was ranked as the biggest problem in terms of livestock diseases in Busia District in focus groups held in Funyula and Butula in 1999 as well as by a cross-sectional questionnaire survey performed across Busia District in October 2002 (Machila-Eisler, 2005; Karanja, 2006). Reasons given for the perceived gravity of the problem included the presence of too many tsetse flies due to lack of trapping and spraying of tsetse habitats. Trypanosomiasis was

regarded as having severe effects on the animals and being difficult to treat. Tsetse were said to be present constantly and "hard to get rid of, only government has means to control by using pour-on medicines". Ticks on the other hand were thought to be controllable by available drugs (Machila-Eisler, 2005). The attitude of livestock owners reflects that tsetse and trypanosomiasis control was deemed to be the responsibility of the state until very recently.

2.3.2 Trypanosomiasis in livestock

There are only limited recent data available on trypanosomiasis prevalence in livestock in Busia District. Employing microscopy as well as inoculation of immuno-suppressed mice, Angus (1996) reported an average annual prevalence of 14.6% in cattle (5091 samples from 767 cattle), 12.1% in pigs (58 samples from 33 pigs), 2.3% in goats (391 samples from 113 goats) and 1.9% in sheep (479 samples from 113 sheep) as established through repeated sampling of untreated livestock over a 12 month period from Busia and neighbouring Teso District. The predominant trypanosome species detected in cattle was *T. vivax*, followed by *T. brucei* s.l.. The infection prevalence in pigs was very variable between sampling villages, but all infections in pigs were identified as *T. brucei* s.l.. Only a minority of infections were identified as *T. congolense*: <3% in cattle and close to 0% in small ruminants (Angus, 1996). Strain typing of mouse passaged *T. brucei* s.l. stocks identified the human infective *T. b. rhodesiense* in at least one sample isolated from cattle. It was the same strain as stocks isolated from human patients from Busia District, Kenya and Tororo District, Uganda some five years previously (Angus, 1996).

Also employing microscopy, Karanja conducted a cross-sectional survey in Busia District in November 2002 and reported a considerably lower prevalence of trypanosomiasis in cattle at 4.7% (n=2827). Of these infections, the majority (40.6%) were attributed to *T. vivax*, followed by *T. congolense* and *T. brucei* s.l.. The prevalence detected specifically in cattle from the two Divisions of interest in the current study were 6.4% in Funyula and 4.0% in Butula (Karanja, 2006).

A recent study in Busia District (Funyula and Budalangi Divisions) concentrated on trypanosome infections in small livestock, and recorded a prevalence of 19.2% in pigs (n=52), 25.3% in sheep (n=95) and 20% in goats (n=255) by PCR (Ng'ayo *et al.*, 2005). The prevalence in small ruminants reported by Ng'ayo was approximately ten fold higher than that detected by Angus (1996) previously. This was surprising even considering the highly sensitive nature of PCR which has been reported to detect two to three times the number of infections in a herd as compared to microscopy (Solano *et al.*, 1999). *T. b. rhodesiense* was identified in one sheep, one goat and one pig sample respectively, confirming Angus' observation of the presence of human infective trypanosomes in the animal reservoir in Busia District (Angus, 1996; Ng'ayo *et al.*, 2005).

2.3.3 Tsetse density and infection rate

Apparent tsetse densities in Busia District were recorded in two studies over the last 15 years. Angus (1996) reported mean apparent tsetse densities of 0.2 flies per trap day (ftd), ranging between 0.9ftd in the long rains and 0.05ftd in the dry period in sampling villages situated in low lying swampy areas in Funyula, Busia District and neighbouring south Teso. Over the same study period (May 1993 - April 1994) even lower apparent densities were recorded in the hilly areas of south Teso, where tsetse were only recorded in one out of the twelve month, at a density of less than 0.1ftd. All tsetse caught were *G. f. fuscipes* and no *G. pallidipes* were trapped, despite reports by the Kenya Trypanosomiasis Research Institute (KETRI) in 1994 of pockets of *G. pallidipes* in Busia District (KETRI, 1994; Angus, 1996).

A longitudinal tsetse survey was conducted in Budalangi and Funyula Divisions from April to December 2003, reporting the presence of both *G.f. fuscipes* and *G. pallidipes* at higher densities (Table 2.2). It was of interest that whilst the apparent density of *G. fuscipes* was higher, *G. pallidipes* had a higher rate of mature trypanosome infections, suggesting that it was the more efficient vector of the two (Karanja, 2006).

Table 2.2: Mean tsetse density and infection rate from longitudinal survey in Funyula and Budalangi Divisions, Busia District (April-December 2003) (Karanja, 2006)

Division	Tsetse species	Mean apparent tsetse density (ftd)	No. tsetse	Mature infection rate (%)
Funyula	*G.f. fuscipes*	9.2	780	1.5
	G. pallidipes	5.8	474	3.8
Budalangi	*G.f. fuscipes*	10.1	930	1.8
	G .pallidipes	4.2	389	3.3

The major source of tsetse blood meals in Funyula was ruminants, whereas in Budalangi, situated on the shores of Lake Victoria, a considerable proportion of blood meals was derived from crocodiles, highlighting that tsetse feeding behaviour seemed to be opportunistic. However only a minority of blood meals were identified to be of human origin (Table 2.3) (Karanja, 2006).

Table 2.3: Source of tsetse blood meals from longitudinal survey in Funyula and Budalangi Divisions, Busia District (April-December 2003) (Karanja, 2006)

Division	tsetse	samples	ruminant	pig	human	crocod.	m. lizard	mixed
Funyula	*G.f.f*	46	88%	0%	4%	4%	0%	4%
	G.p.	17	70%	6%	0%	0%	12%	12%
Budalangi	*G.f.f*	35	48%	6%	3%	40%	0%	3%
	G.p.	12	42%	8%	0%	33%	0%	17%

(*G.f.f*= *G. f. fuscipes*, *G.p*=*G. pallidipes*, crocod= crocodylidae, m.lizard = monitor lizard)

2.3.4 Sleeping sickness

Busia District is endemic for sleeping sickness. The causative parasite, *Trypanosoma brucei rhodesiense*, has an important reservoir in domestic cattle in particular where wildlife is largely absent (Hide *et al.*, 1994; Hide *et al.*, 1996), inextricably linking control of human sleeping sickness to control of trypanosomiasis in livestock (Welburn *et al.*, 2006). Throughout the last century three sleeping sickness epidemics

occurred in the Busoga focus of Uganda, each of which spilled over into western Kenya (Hide, 1999). A detailed account of the history of sleeping sickness in Kenya can be found in Chapter 1. In the last ten years, sleeping sickness cases have only been reported sporadically from Busia District. The maximum number of cases in that time span was 4, in 1999, with no or only one case in all other years (Figure 2.2) (Batchelor, 2004; WHO, 2006). All active screening in Busia, was terminated in 2002, as its cost and labour intensity was considered no longer in proportion with the low number of sleeping sickness cases (FITCA, 2005). The latest case from Busia District which probably was infected towards the end of 2005 was reported in early 2006 (WHO, 2006).
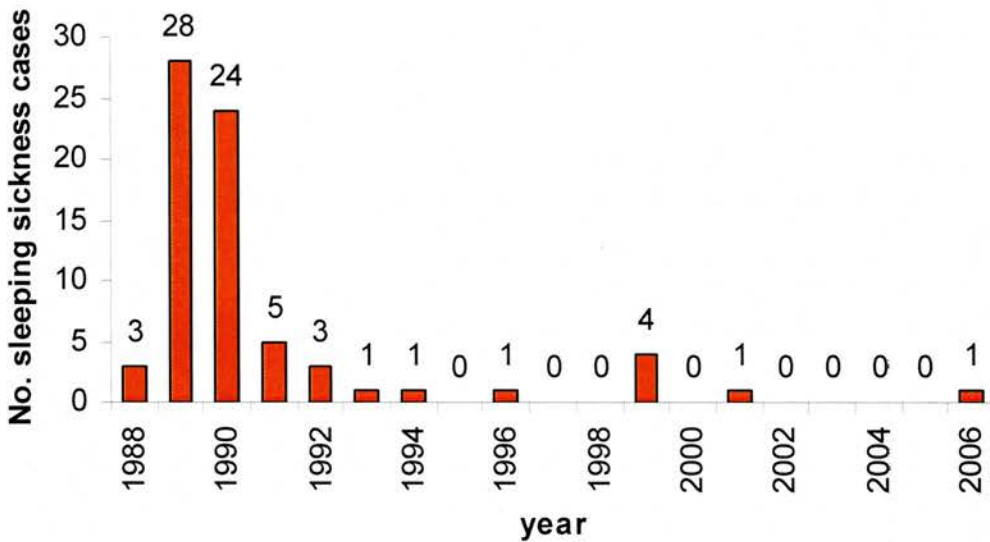
Figure 2.2: Annual reported sleeping sickness cases in Busia District (defined by current district demarcation i.e. does not include cases from what is now Teso District) (Batchelor, 2004; WHO, 2006)

Awareness for sleeping sickness and its causes was low in western Kenya. In a survey of community believes 34.7% of respondents attributed sleeping sickness to witchcraft and another 32.9% listed HIV/AIDS as its cause. Only 20.8% of respondents associated sleeping sickness with the bite of the tsetse fly (Bukachi *et al.*, 2005). In the Busoga sleeping sickness focus in Uganda it was estimated that for

every one patient with sleeping sickness dieing in hospital, eleven more die at home undiagnosed , but the level of underreporting of sleeping sickness in Busia District, Kenya is unknown (Odiit *et al.*, 2005).

2.4 Summary

In summary, the observed trypanosomiasis infection rates in cattle, small ruminants and pigs varied considerably throughout Busia between and within studies, according to location, tsetse challenge and sensitivity of diagnostic methodology employed (Angus, 1996; Ng'ayo *et al.*, 2005; Karanja, 2006). Busia District is affected by extremely high levels of poverty, with the livelihood of the majority of its population dependant on subsistence farming. Despite the prevalence of mixed crop-livestock farms, the integration of livestock into the system is sub-optimal and livestock outputs are small (Thuranira, 2005). However farmers have little scope for investment to improve livestock and crop outputs, due to limited cash and poor access to credit facilities. The diagnostic skills of livestock keepers with respect to endemic diseases of their animals were insufficient (Machila *et al.*, 2003). Extension programmes to raise levels of knowledge and change livestock management to prevent disease, have shown poor penetration and sustainability, partly due to comparatively short time available to the programmes, and partly due to an underestimation of the level of poverty in rural Busia limiting the scope of farmers to adopt new methodologies that require initial investment (FITCA, 2005).

Animal trypanosomiasis is endemic in Busia, limiting the productivity of livestock, and control is seen as the responsibility of the affected farmer. Chemotherapy as well as new tsetse control technologies potentially allow intervention at the farmer level (Hargrove *et al.*, 2000). However, whilst large scale studies have provided a wealth of knowledge on the epidemiology of trypanosomiasis, it needs to be confirmed that these findings translate to the local scale of the disease before the limited resources of farmers are used for the implementation of new control strategies based on and

targeted according to such findings. It was thus of interest to investigate the epidemiology of trypanosomiasis in livestock at the micro-scale in Busia, Kenya.

The next chapter outlines the general methodology of this study, detailing the study design, the sampling protocol and the laboratory analysis performed.

3 Chapter 3   General Methodology:
Study Design & Laboratory Analysis

3.1 Study design

3.1.1 Study area

The study described in this thesis was performed in Busia District, Western Province, Kenya. Within Busia District, two geographically separate sites were included in the study, one located in Funyula Division and the other in Butula Division (Figure 3.1). In Funyula Division the sampling site consisted of nine adjacent villages, situated in three sub-locations; in Butula Division the site comprised ten adjacent villages in three sub-locations (Figure 3.2). These two sampling areas were established field site, originally chosen on the basis of a cattle trypanosomiasis prevalence of at least 6%, as established by a cross-sectional survey in 1997 (INCO-DC, 2000) and well characterised in terms of livestock-keeping dynamics and veterinary care seeking behaviour (Machila-Eisler, 2005; Thuranira, 2005). A detailed description of Busia District, and the selection of study sites was provided in Chapter 2, which also included a summary of recent data on human and animal trypanosomiasis in Busia.

3.1.2 Sampling approach

The sampling approach was two-pronged:

1. Central sampling - a sub-set of the cattle population from each of the two study areas (Funyula and Butula) was sampled on a convenience basis. Sampling was conducted on a convenience basis using two crush pens per site (Figure 3.2). The central sampling protocol is detailed in section 3.1.3.

2. Census sampling - all cattle, pigs, sheep and goats were sampled at every livestock-keeping homestead within the two sampling areas. This included 191 households from the nine sampling villages in Funyula and 358 households from the ten sampling villages in Butula. The census sampling protocol is detailed in section 3.1.4.

An overview of the sampling approach is given in Table 3.1.
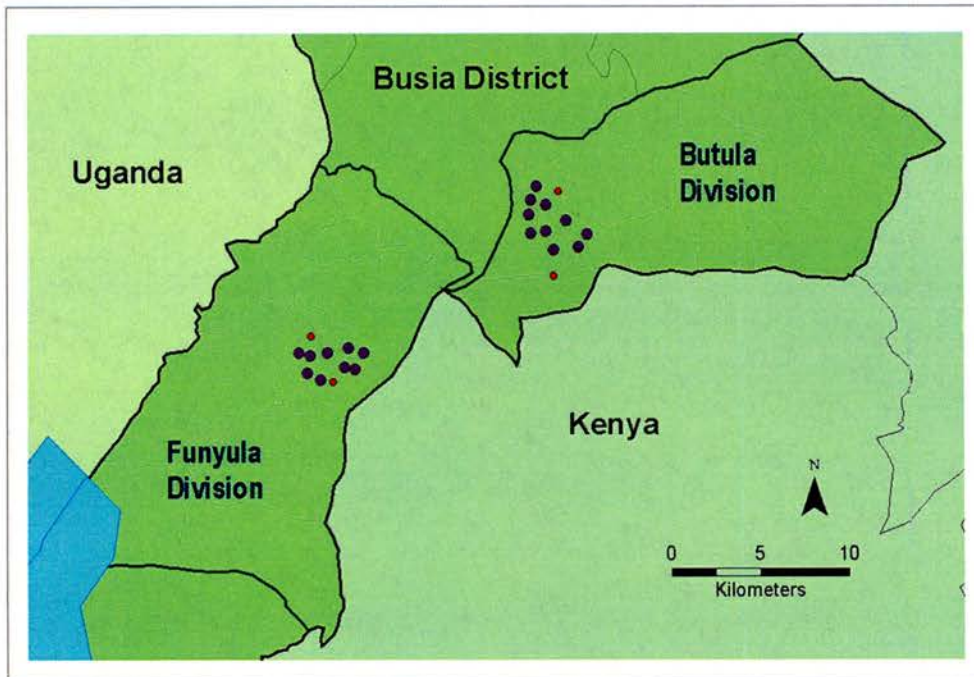
Figure 3.1: Map of sampling sites showing location of study villages (🟣) and central sampling points (🔴). (light green: Kenya; grey lines: district outlines; green: Busia District, black lines: outlines of divisions within Busia; light grey lines: outlines of sub-locations within Busia (1989); beige: Uganda; blue: Lake Victoria)
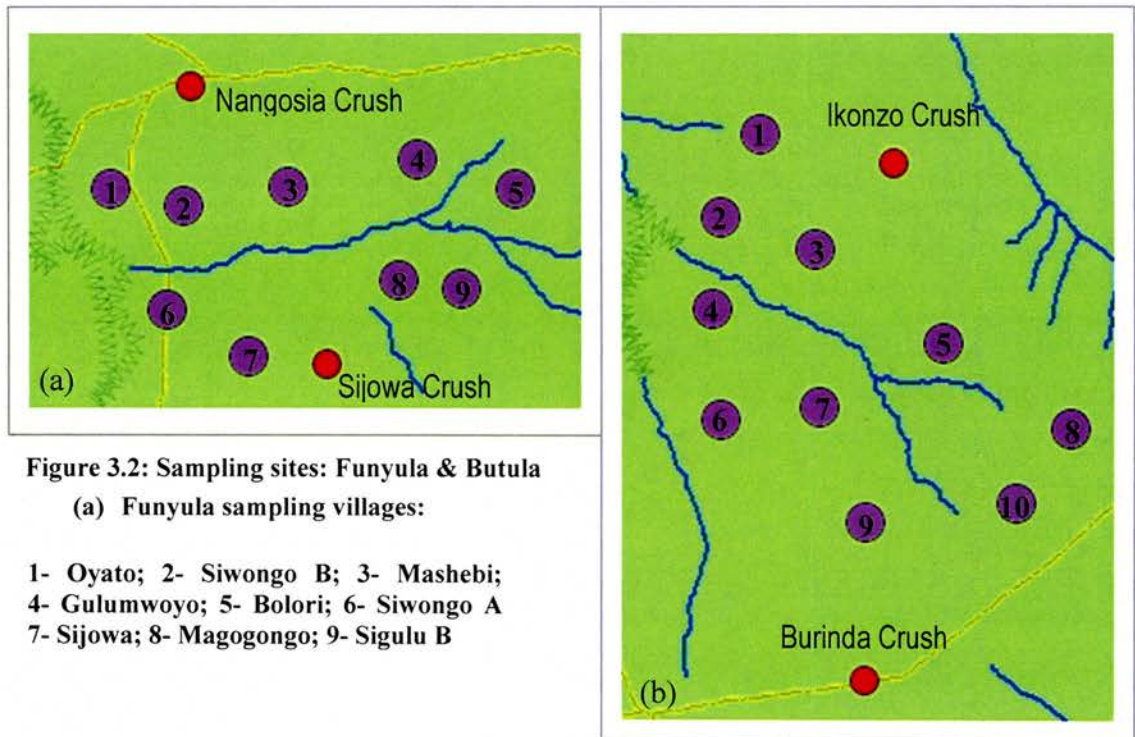
Figure 3.2: Sampling sites: Funyula & Butula
    (a) Funyula sampling villages:

1- Oyato; 2- Siwongo B; 3- Mashebi;
4- Gulumwoyo; 5- Bolori; 6- Siwongo A
7- Sijowa; 8- Magogongo; 9- Sigulu B

🟣 location of sampling villages ; 〽 swamp
🔴 location of central sampling sites; 〰 river
.... dirt road

(b) Butula sampling villages:

1- Bukhulumi; 2- Siroba B; 3- Ikonzo B;
4- Kengo; 5- Khwikali; 6- Bukhwako
7- Bujumba; 8- Sirikhaya B; 9-Nebolola
10- Sirikhaya A

Table 3.1: Overview of study design

Sampling approach	Date	Division	Sampling frame	Livestock sampled
Central point	June 2004	Funyula	Nangosia crush	cattle attending crush (n=78)
Central point	June 2004	Funyula	Sijowa crush	cattle attending crush (n=123)
Central point	June 2004	Butula	Burinda crush	cattle attending crush (n=144)
Central point	June 2004	Butula	Ikonzo crush	cattle attending crush (n=126)
Homesteads (census sampling)	July 2004	Funyula	All 191 livestock keeping homesteads in the 9 sampling villages Oyato (20) Sijowa (23) Siwongo A (21) Siwongo B (20) Magogongo (22) Mashebi (26) Sigulu B (20) Bolori (23) Gulumwoyo (16)	All livestock (cattle: n=446, pigs: n=109, goats: n=378, sheep: n=148)
Homesteads (census sampling)	October 2004	Butula	All 358 livestock keeping homesteads in the 10 sampling villages Bujumba (40) Bukhwako (52) Kengo (46) Khwikali (24) Nebolola (39) Siroba (19) Ikonzo B (32) Sirikhaya A (42) Sirikhaya B (25) Bukhulumi (39)	All livestock (cattle: n=814, pigs: n=203, goats: n=390, sheep: n=285)

3.1.3 Central sampling points

3.1.3.1 Location and date

In both Funyula and Butula, two existing community crushes were selected as sites for central sampling. In Funyula, Nangosia crush and Sijowa crush were used (Figure 3.2a); in Butula, Burinda crush and Ikonzo crush were selected (Figure 3.2b). Local veterinary staff revealed these specific crushes were located at the centre of any livestock related activities in the study areas, and easily accessible to farmers from the surrounding villages. Thus these crush pens were chosen as central sampling points, despite appearing to be geographically peripheral to the study areas. Central sampling was performed in June 2004. One day was allocated per central sampling point:

8. June 2004 - Sijowa crush (Funyula)

9. June 2004 - Nangosia crush (Funyula)

10. June 2004 - Burinda crush (Butula)

11. June 2004 - Ikonzo crush (Butula)

3.1.3.2 Selection of animals for central sampling

Farmers were asked to present their cattle for sampling at the central point most conveniently located to their village. Information on the sampling dates was distributed via the village elders of the individual sampling villages. Free anthelmintics were offered to cattle attending the sampling as an incentive to farmers. Only the 19 villages that were also going to be included in the census sampling were mobilized. In some cases, farmers from neighbouring villages which were not mobilized officially, learned about the sampling taking place by word of mouth. Central points were thus also attended by cattle owners from villages not included in the initial sampling frame. As this phenomenon is common for central sampling of cattle, no attempts were made to exclude these cattle from the sampling, but the number of cattle sampled from each village was recorded. Between 70 and 150 cattle were sampled at each of the central points to obtain a total of at least 200

cattle blood samples for the Funyula and Butula sampling area each. For an estimated population size of 600 cattle in each of the two sampling areas and an expected trypanosomiasis prevalence between 10% and 20% a sample size of 200 cattle was calculated to provide an estimate of trypanosome prevalence accurate to within plus/minus 5% of the true prevalence at a 95% confidence level (calculation WinEpiscope 2.0).

3.1.4 Census sampling

3.1.4.1 Location and sampling dates

Census sampling on the household level was performed in the 19 villages included in the study comprising a total of 549 livestock-keeping homesteads. All samples were collected in the presence of the author.

In Funyula, a total of 191 livestock-keeping homesteads in nine villages situated in three neighbouring sub-locations were sampled: Oyato, Sijowa, Siwongo A and Siwongo B villages in Bukhulungu sub-location, Magogongo, Mashebi and Sigulu B villages in Sigulu sub-location and Bolori and Gulumwoyo villages in Wakhungu sub-location. The study area in Funyula was demarcated by a dirt road to the north, a swamp to the west and Odidi and Sirima Hills to the south. A permanent stream, the Wakhungu River and several smaller tributaries, traversed the sampling area. Sampling in the selected villages in Funyula took place between the 21st and the 31st July, 2004, with one sampling day per village.

In Butula, a total of 358 livestock-keeping homesteads in ten villages from three adjacent sub-locations were sampled: Bujumba, Bukhwako, Kengo, Khwikali, Nebolola and Siroba villages in Bujumba sub-location, Ikonzo B, Sirikhaya A and Sirikhaya B villages in Ikonzo sub-location and Bukhulumi village in Namwitsula sub-location. The sampling area in Butula was demarcated by a swamp to the west and permanent streams to the south-west and north-east. Another permanent stream traversed the centre of the sampling area. The south-west of the sampling area was

marked by a dirt road. Sampling in Butula was performed between the 5th and the 22nd October, 2004, with one to two sampling days allocated per village, depending on the number of homesteads to be visited.

3.1.4.2 Selection of sampling animals for census sampling

The aim of the census approach was to sample the entire local population of cattle, goats, sheep and pigs in each of the two sampling areas. Village elders identified all homesteads that owned any of these animals in their respective villages. All of those homesteads were then visited under the guidance of the appropriate village elder. The study objectives were explained to the respective homeowners, they were questioned on the number of animals they owned and permission to take blood samples from all of his or her animals was sought.

Animals were only sampled in the presence of a responsible adult from the respective households. If the owner was absent, all efforts were made to revisit the homestead later in the day. Due to time constraints, visits could not be repeated on a different day but only a small number of livestock-keeping homesteads had to be excluded as a result (proportion of total livestock keeping households not included: Funyula: 5/196 (2.6%); Butula 9/367 (2.5%)). Sampling of ear-veins was attempted in all animals but those under two weeks of age. However, ear vein puncture failed to draw sufficient blood for laboratory analysis in a number of animals, mainly goats and sheep, due to small or collapsing ear veins. A number of pigs had to be excluded as owners were reluctant to give permission for sampling of sows close to term or lactating sows and their piglets, for fear of stress causing abortion or cessation of lactation. Table 3.2 summarises the number of animals sampled as a proportion of the entire livestock population.

Table 3.2: Summary of samples collected and total livestock population in sampling sites

Livestock species	N° livestock sampled	Total livestock population *	Proportion of population sampled
	(Funyula & Butula combined)		
cattle	1260	1347	93.5%
pigs	312	495	63%
goats	768	877	87.6%
sheep	433	530	81.7%
total	2773	3249	85.3%

* accurate livestock numbers from excluded households could not be obtained and were thus not included

3.1.5 Sample collection and storage

After lancing of the ear vein of the animal, whole blood was collected into a heparinised capillary tube and applied directly onto an FTA- card (Whatman, Maidstone, Kent, UK) (Figure 3.3). One circle, (approximately 100µl of blood) was filled for each animal sampled (Figure 3.3). Once all four circles of each card were filled, they were left to dry un-assisted, for approximately 1 h or until the bloodspots were completely dry. Cards were then placed together with desiccant into airtight Multi-Barrier Pouches (Whatman), until processed in the laboratory.
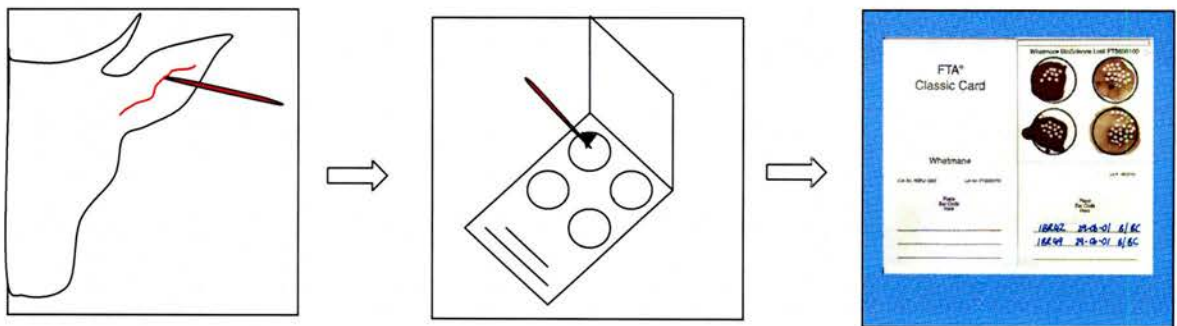
Figure 3.3: Diagram of sample collection by ear vein puncture and transfer to FTA-cards

FTA-cards are composed of a filtration matrix impregnated with a patented formulation of protein denaturants, a chelating agent and a free- radical trap, thus entrapping and protecting nucleic acids (United States Patent: 5, 756, 126). This rapidly inactivates organisms, such as blood borne pathogens, offering user safety and allowing legal import of samples by mail into the UK. Samples on FTA-cards can be stored at room temperature for over 10 years without degrading as long as samples are kept dry with the help of desiccants.

3.1.6 Sample associated data

3.1.6.1 Animal level data

For each blood sample taken the following information was recorded:

- homestead of origin (where applicable: i.e: for samples collected during household level census sampling but not at central points)

- animal species (cattle, pigs, sheep, goats)

- sex of animal (male, female)

For cattle samples additional information on each animal was recorded:

- Age category as identified by tooth eruption pattern (category a: milk teeth, under 18 months of age; category b: one pair of permanent incisors, between 18 months and 3 years of age; category c: more than one pair of permanent incisors, over 3 years of age)

- Body condition score of the animal on a scale of nine categories. A complete description of the categories can be found elsewhere (Nicholson & Butterworth, 1986). Briefly, animals are assessed as lean (L), medium (M) or fat (F), with each of these categories being subdivided into three classes, for example M-/M/M+ according to muscle mass and extent of fat deposition. Repeatability of this method of condition scoring is high between trained scorers (Nicholson & Sayers, 1987b) and scoring was performed consistently by the same experienced veterinary clinician throughout the whole study.

Condition score is not affected by size of animal, parturition, pregnancy alterations in rumen, bladder and gastrointestinal content which may affect alternative measures such as weight and heart girth, and restraint of animals is not required (Nicholson & Sayers, 1987b; Nicholson & Sayers, 1987a). Due to the low number of animals in some of the categories, e.g. only eight animals were scored to be F, and even fewer were scored as L or L- (four and one respectively), the initial nine categories were collapsed to the three main categories (L,M,F) for statistical analysis.

- Anaemia score was categorised as normal, slightly anaemic or severely anaemic as assessed by the veterinarian according to the colouration and perfusion of the mucus membranes in eyes and mouth of the animal. Anaemia scoring in cattle by visual assessment of the mucous membranes of the mouth and eyes has been shown to have a good correlation with blood haemoglobin levels (Machila-Eisler, 2005). Due to the low number of animals in some of the categories, these were collapsed to normal (N) or anaemic (N+) for statistical analysis.

3.1.6.2 Homestead level data

Each homestead visited was assigned a unique number used consistently for recording any associated data. The administrative units recorded for each homestead were location, sub-location and village. A hand held Garmin Global Positioning System (GPS) was used to determine and record the geographical co-ordinates of each homestead. The time of day at which sampling was begun at each homestead was noted. Additionally data on animal management was recorded:

3.1.6.2.1 Grazing and watering management

An experienced enumerator filled out a questionnaire (Appendix A) according to the information provided by the homeowner or a responsible adult member of the household. The respondent was asked which livestock species (cattle, pigs, sheep,

goats) were owned by the household, and where these animals were grazed and watered. For statistical analysis, the response on the grazing and watering management was assigned to one of two categories (home/ away) separately for each livestock species in the household and was recorded separately for feeding and watering practices:

- *Home*: animals of the species in question were fed / watered within the immediate compound of the homestead in question, either through grazing within the confines (usually tethered), or through feed/ water being brought to the animals

- *Away*: animals of the species in question were taken outside the confines of the homestead compound for grazing/ watering.

3.1.6.2.2 *Overall management*

The overall management of a particular species of livestock within a homestead was categorised into "home" or "away" as follows:

- *Home*: Both feed and water were provided within the confines of the homestead.

- *Away*: If animals of that species were taken outside the confines of the compound for either one or both feeding and watering the overall management of that species of livestock within that household was classified as "away".

When grazing and watering or overall management practices were analysed jointly for all animals kept within a homestead, the relevant management practice was categorised as "*home*" only when all animals belonging to that homestead, regardless of species, where fed/ watered at home. If any one of the animals left the homestead for grazing or watering the management practice was categorised as "*away*" for that homestead.

3.2 Laboratory analysis

3.2.1 Sample screening approach

All samples were screened for trypanosomes by Polymerase Chain Reaction (PCR). Each sample was screened twice, the first, ITS-PCR, detecting and differentiating between the important pathogenic African trypanosome species affecting livestock (Cox *et al.*, 2005). The second PCR employed was specific for *Trypanosoma brucei* s.l. (Moser *et al.*, 1989), and had a higher number of target copies (10,000 target copies/ trypanosome) as compared to ITS-PCR (200 target copies/trypanosome). Any sample that was positive by PCR for *T. brucei* s.l. (by one or both ITS-PCR and/ or Trypanozoon specific PCR), was further screened by PCR in pentaplicate for the presence of the human infective subspecies *T. brucei rhodesiense* (Picozzi *et al.*, in press) and Southern blotting was performed for all *T. b. rhodesiense* specific PCRs to increase sensitivity. All laboratory analysis was performed by the author.

3.2.2 Sample purification

To purify samples for use as a PCR template, discs of 2mm diameter were cut out from each FTA Card sample. Discs were cut out with a Harris Micro Punch ™ Tool, and transferred into an eppendorf tube. Discs cut from the same sample were deposited into the same eppendorf tube for the washing steps. Between cutting discs from different samples, the punch was cleaned by cutting discs from blank FTA Cards, in order to prevent carry over of residual material between samples. Care was taken to cut each sample on a clean, unused space on the cutting mat, thus avoiding contamination between samples.

The sample discs were washed twice for 15min in 200µl of FTA Purification Reagent (Whatman) per disc to remove PCR inhibitors such as haemoglobin from the sample; the DNA remained bound to the disc. Subsequently the discs were washed twice for 15min in 200 µl of 1x concentrated Tris-EDTA per disc (TE) (10mM Tris HCl pH 8; 1mM EDTA), to remove FTA Purification Agent from the discs, which is a PCR inhibitor. After the second wash with TE individual discs were

transferred into PCR- tubes, using one disc as a template for each 25μl reaction. To detect any cross-contamination that may have occurred during sample washing, discs of a blank FTA-card were washed with each batch of samples processed, and used as negative controls in the PCR.

3.2.3 Polymerase chain reaction (PCR) for African animal infective trypanosomes

3.2.3.1 ITS-PCR

ITS-PCR was used for initial screening of all samples. This PCR can simultaneously detect and differentiate a range of African animal trypanosomes including *T. brucei* s.l., *T. vivax*, *T. congolense* (Forest, Kilifi, Savannah, Tsavo), *T. simiae* and *T. theilerie* (Cox *et al.*, 2005). ITS-PCR is a nested PCR, targeting the internal transcribed spacers (ITS) of the ribosomal RNA genes, of which there are 200 copies per trypanosome genome.

Using one sample disc per reaction as template, the first round of the ITS-PCR was performed in a standard volume of 25μl per reaction under the following conditions: 10mM Tris-HCl, pH 9.0, 1.5mM $MgCl_2$ 50mM KCl, 0.1% Triton X-100 and 0.01% (w/v) stabiliser (Super-Taq PCR Buffer, HT Biotechnologies, Cambridge, UK), 1mM total dNTPs (Bioline, London, UK), 1.25 Units of Biotaq per reaction (Bioline, London, UK) and 0.2μM of each outer primer ITS1 and ITS2 (Table 3.3). One positive control (genomic DNA) and one negative control (blank FTA disc) were run with each PCR. Reactions took place in a DNA Engine DYAD^{TM} Peltier Thermal Cycler. Cycling was preceded by a 7min step at 95°C to ensure complete denaturation of the template. For cycling conditions, see Table 3.3.

For the second round reaction, 1μl of the PCR product from the first round was transferred into a fresh tube. The second round reaction was performed in a standard volume of 25μl per reaction under the same conditions as the first round, with the exception of the substitution of the primers (inner primers: ITS3 and ITS4) (Table

3.3). Cycling was preceded by a 5min step at 95°C to ensure complete denaturation of the template. Cycling conditions were the same as for the first round (Table 3.3). PCR products were stored at 4°C until electrophoresis.

3.2.3.2 *Trypanozoon* specific PCR

An additional PCR, specific to *T. brucei* s.l. (10,000 target copies/ trypanosome) (Moser *et al.*, 1989), was performed on each sample. The PCR was performed in a standard volume of 25μl per reaction under the following conditions: 16.0mM $(NH_4)_2SO_4$, 67mM Tris-HCl (pH8.8 at 25°C) 0.01% Tween 20 (NH$_4$ Buffer, Bioline, London, UK), 1.5mM Mg^{2+}, 800μM of total dNTPs, 0.7 Units of BIOTAQ RED™ DNA Polymersase per reaction (Bioline, London, UK) and 0.4μM of each of the primers TBR1 and TBR2 (Table 3.3). One positive control (genomic DNA) and one negative control were run with each PCR. Reactions took place in a DNA Engine DYAD™ Peltier Thermal Cycler. Cycling was preceded by a 3min step at 94°C, to ensure complete denaturation of the template. For cycling conditions see Table 3.3. PCR products were stored at 4°C until evaluation by electrophoresis.

3.2.4 Polymerase chain reaction (PCR) for African human infective trypanosomes: *T. b. rhodesiense*

A multiplex PCR specific for the detection of *T. brucei rhodesiense* was performed on samples positive for *Trypanozoon* (by both or just one of the ITS-PCR and/ or Trypanozoon PCR) (5 reactions per sample) (Picozzi *et al.*, in press). The two sequences amplified in parallel code for the Serum Resistance Associated (SRA) gene and Phospholipase C (PLC), respectively. SRA, which is specific to *T. brucei rhodesiense*, is a single copy gene. PLC is also a single copy gene found in *T. brucei* s.l.. A PCR positive for the PLC sequence, but negative for SRA, proves that sufficient genetic material of *T. brucei* s.l. is in the sample to detect SRA, if it was present in the sample.

The multiplex PCR was performed in a volume of 25µl per reaction under the following conditions: PCR Buffer (Qiagen, Crawley, UK) containing a combination of KCl and $(NH_4)_2SO_4$ and a final concentration of 2.5 mM $MgCl_2$, 200µM of each of the 4 dNTPs, 1.5u of HotStarTaq®DNA Polymerase (Qiagen) and 0.2µM of each of the primers were used per reaction (primer sequences Table 3.3). Reactions took place in a DNA Engine DYAD™ Peltier Thermal Cycler. Cycling was preceded by a 15min step at 94°C for activation of the enzyme. For cycling conditions see Table 3.3. One negative and one positive control (genomic DNA) were run with each PCR. Products were stored at 4°C until evaluation by electrophoresis and Southern blotting.

Table 3.3: PCR primers, cycling conditions and amplicon sizes

PCR and Primer sequence (5' to 3')	Specific amplicon sizes
ITS-PCR (Cox *et al.*, 2005)	2. Round products
1. Round (outer primers) ITS1: GAT TAC GTC CCT GCC ATT TG ITS2: TTG TTC GCT ATC GGT CTT CC	*T. congolense Forest*: 1501bp *T. congolense Kilifi*: 1430bp *T.congolense Savannah*: 1408bp
2. Round (inner primers) ITS3: GGA AGC AAA AGT CGT AAC AAG G ITS4: TGT TTT CTT TTC CTC CGC TG	*T. congolense Tsavo*: 951bp *T. brucei* s.l.: 1215bp *T. simiae*: 847bp *T. vivax*: 620bp *T. theilerie*: 998bp

35 cycles: 94°C for 60s, 55°C for 60s, 72° for 120s

Trypanozoon (Moser *et al.*, 1989)	
TBR1: CGA ATG AAT ATT AAA CAA TGC GCA GT TBR2: AGA ACC ATT TAT TAG CTT TGT TGC	*T. brucei* s.l. : 177bp

30 cycles: 94°C for 60s, 55°C for 60s, 72°C for 30s; final extension 72°C for 5min

SRA-PLC Multiplex PCR (Picozzi *et al.*, in press)	
SRA	SRA
SRAf : GAA GAG CCC GTC AAG AAG GTT TG SRAr: TTT TGA GCC TTC CAC AAG CTT GGG	*T. brucei rhodesiense*: 669bp
PLC	PLC
PLCf : CGC TTT GTT GAG GAG CTG CAA GCA PLCr: TGC CAC CGC AAA GTC GTT ATT TCG	*T. brucei* s.l.: 325bp

42 cycles: 94°C for 30s, 63°C for 90s, 72°C for 70s; final extension 72°C for 10min

3.2.5 Agarose gel electrophoresis

PCRs were evaluated by agarose gel electrophoresis. At neutral pH, DNA molecules have one negative charge per phosphate and therefore travel towards the anode, when loaded at the cathode. Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the $\log_{10}$ of their molecular weight; i.e. longer DNA fragments travel a shorter distance than short fragments during the same period of time, leading to separation of bands during electrophoresis.

A 1.5% (w/v) agarose gel of $100cm^3$ was prepared with 1x Tris Borate EDTA Buffer (TBE Buffer: 89mM Tris Borate, pH approximately 8.3, containing 2mM EDTA, Sigma-Aldrich, Poole, Dorset, UK), 5μM Ethidium Bromide. PCR products were loaded into the wells (ITS-PCR: 12.5μl/ sample; Trypanozoon PCR: 25μl/ sample) and gels were run in 1xTBE, 5μM Ethidium Bromide at 100V (ITS-PCR: 60-90min; *Trypanozoon* PCR: 30min). Superladder-Mid 100bp Ladder (ABgene, Epsom, Surrey, UK) containing a mixture of particular sized DNA fragments (100-2000bp, in 100bp increments) was loaded alongside the PCR products to assess the product band sizes. Ethidium Bromide intercalates between DNA bases and fluoresces under UV-light. An ultraviolet transilluminator (Gel-Doc 2000, Bio-Rad) was used for UV-exposure of the agarose gels and visualisation of DNA. Exposure was optimised and pictures were stored with the aid of Bio-Rad Software (Quantity One, Bio-Rad). PCR results were only accepted when the negative control remained clean and the positive control showed a band of the expected size. Samples were accepted as positive for a trypanosome species/ subgroup when they displayed a band of the expected product size during gel electrophoresis (Table 3.3). Examples of agarose gel electrophoresis of PCR products are given in Figures 3.4-3.6.
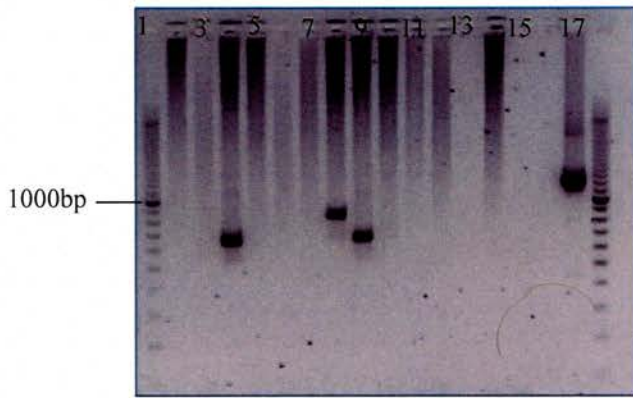
Figure 3.4: Example of agarose gel of second round of the ITS- PCR (Cox *et al.*, 2005): Lane 1 & 18: molecular marker in 100 bp intervals, lane 4 & 9: *T. vivax* positive samples (620bp), lane 8: *T. simiae* positive sample (850bp), lane 16: negative control, lane 17: positive control for *T. brucei* s.l. (1215bp), remainder of lanes: negative samples.
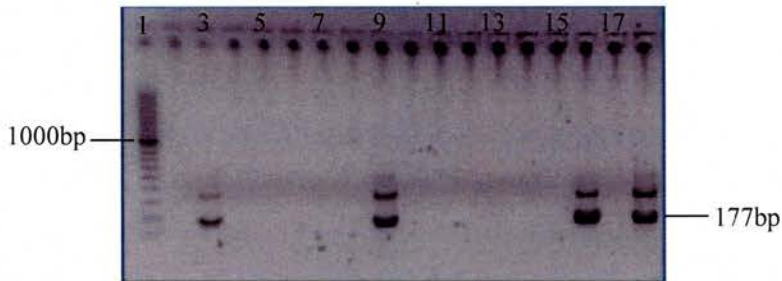
Figure 3.5: Example of agarose gel of *T. brucei* s.l. specific PCR (Moser *et al.*, 1989): Lane 1: molecular marker in 100 bp intervals, lane 2: negative control, lane 3: positive control, (*T. brucei* s.l. specific band at 177bp, laddering effect due to multiple repeats of target gene), lane 9, 16 & 18: *T. brucei* s.l. positive samples, remainder of lanes: *T. brucei* s.l. negative samples.
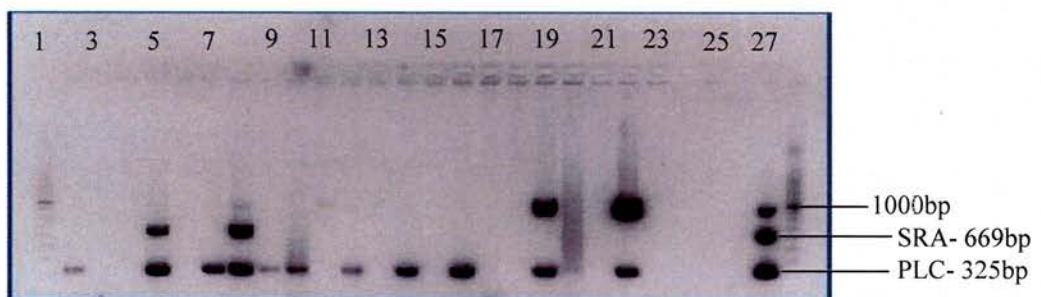
Figure 3.6: Example of agarose gel of multiplex PCR (Picozzi *et al.*, in press). Lane 1& 28: molecular marker; lane 27: positive control, lane 26: negative control, lane 5& 8 samples positive for PLC&SRA, lane 2,7,9, 10,12,14,16,19&22 samples positive for PLC, additional band in positive control and lanes 19&22: VSG

3.2.6 Southern blotting

Southern blotting was performed to increase sensitivity of detection of *T. brucei rhodesiense*. Southern blotting refers to the transfer of DNA from a gel to a positively charged nylon membrane. For DNA to be accessible to a probe it must first be denatured so that it is single stranded. This denaturation takes place under alkaline conditions whilst the DNA is still in the agarose gel. High pH denatures DNA by altering the tautomeric state of the bases and disrupting specific hydrogen bonding. Labelled probes of the desired sequence are introduced to the membrane under strict conditions and areas of hybridisation, showing the presence of the desired PCR product, are visualised through label activation.

3.2.6.1   Probe

To obtain probe, genomic DNA of *T. brucei rhodesiense* (LIRI024) (PLC and SRA) was amplified by multiplex PCR as described above. After separation of bands by gel electrophoresis, DNA bands were gel extracted with a MiniElute Gel Extraction Kit (Qiagen) according to manufacturer's instructions.

Briefly, bands of DNA were visualised under UV-light and cut from the remainder of the gel with a scalpel blade. Gel slices were dissolved in QG-Buffer (gel: buffer ratio= 1:3 (w/v)) at 50°C and the resulting solution was mixed thoroughly with isopropanol at a ratio of 4:1 (v/v). The sample was applied to a MiniElute Spin Column, and centrifuged for binding of DNA to the spin column membrane. The membrane was washed by consecutive addition of QG-Buffer (0.5ml) and PE-Buffer (0.75ml) and centrifugation of the spin column. The DNA was eluted off the spin column membrane by incubation for 15 in EB-Buffer (10µl) and harvested by centrifugation into a clean 1.5ml eppendorf tube. All buffers were provided in the MiniElute Gel Extraction Kit. The eluted DNA was stored at 4°C.

A total volume of 16μl of DNA eluate estimated to contain between 10ng and 3μg of DNA was heat denatured in a boiling water bath for 10min and immediately chilled on ice. 4μl of DIG-High Prime labelling mixture (Roche, Mannheim, Germany) was added and incubated at 37°C for a minimum of 1h. The reaction was stopped by heating to 65°C.

3.2.6.2 Probe yield estimation

A dilution series was prepared ranging from 1ng μl^{-1} to 0.1pg μl^{-1} of DIG-labelled control DNA as provided by the manufacturer. After estimation of approximate probe yield, a 10x dilution series in a similar range was prepared from the newly labelled probe. One μl of each dilution of probe and control was spotted onto a piece of positively charged nylon membrane and allowed to air dry, prior to permanent fixation to the membrane through a 5min exposure to UV-light (Figure 3.7).
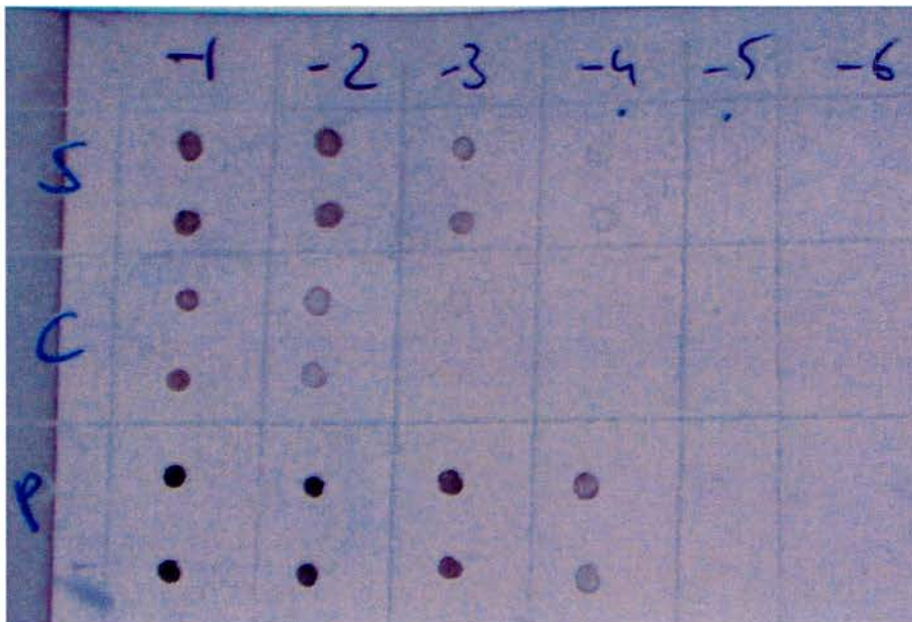
Figure 3.7: Probe yield estimation: Ten times dilution series of DIG-labelled control DNA (C) as compared to ten x dilution series of DIG-labelled SRA probe (S) and PLC probe (P). The SRA probe was estimated to be between 10 and 100 times more concentrated than the control, the PLC probe was estimated to be 100 times more concentrated than the control.

All solutions used in the following steps were provided by Roche. The membrane was washed briefly in 1x Washing buffer (0.1 maleic acid, 0.15M NaCl: pH 7.5, 0.3% (v/v) Tween ® 20) before a 30 min incubation at room temperature in 1x Blocking solution (1g of blocking reagent in 100cm^3 of maleic acid buffer 0.1 maleic acid, 0.15M NaCl: pH 7.5). This was followed by a 30 min incubation at room temperature with Anti-DIG-alkaline phosphatase, diluted 1/5000 in blocking solution. The membrane was then washed twice for 15min in 1x Washing buffer, before transfer into Detection buffer (0.1M Tris-HCl at pH 9.5, 0.1M NaCl) to equilibrate. The membrane was introduced to colour substrate solution (80µl NBT/BCIP in 10 cm^3 detection buffer) and colour development was carried out in the dark, without shaking. The reaction was stopped by a 5min wash in H$_2$O. Probe yield was estimated by visual comparison of colour development of control and probe dilutions.

3.2.6.3 Transfer

After agarose gel electrophoresis of samples the gel was soaked in denaturing solution (0.5M NaOH, 1.5M NaCl) for 20min, followed by a 20min wash in neutralising solution (0.5 M Tris-HCl at pH 7.5, 3M NaCl).

Transfer of the DNA from the gel onto the nitrocellulose membrane, was performed on a vacuum blotter (QBiogene, Cambridge, UK). For the transfer, the nitrocellulose membrane was soaked in 20x SSC (3M NaCl, 0.3M sodium citrate pH 7) which inhibits re-naturation of the DNA. The nitrocellulose membrane was placed on top of a carrier filter paper (Whatman, 3mm) (also soaked in 20x SSC) and placed between the vacuum- blotting machine and the gel (Figure 3.8). Negative pressure of 65mbar was applied for an hour. Throughout the transfer drying-out was avoided by ensuring the gel surface retained a covering of 20xSSC. The position of the wells was marked on the nitrocellulose membrane by punching through the wells with a pipette tip.

After transfer the nitrocellulose membrane was placed onto filter paper (Whatmann 3mm) soaked in 10xSSC to avoid drying out of the membrane during the 5min UV exposure which permanently cross-links the DNA to the membrane. After UV-cross-linking the membrane was washed in double distilled water and allowed to air dry. The membrane could be stored dry at this point at 2-8°C.

1. vacuum blotter

2. filterpaper

3. nitrocellulose membrane

4. vacuum seal

5. gel
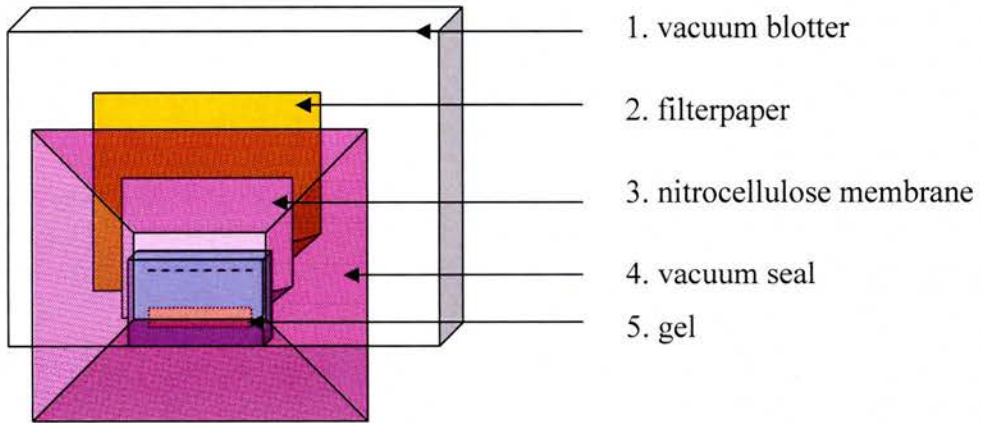
Figure 3.8: Arrangement for vacuum blotting

3.2.6.4 Hybridisation

The membrane was pre-hybridised in standard hybridisation buffer (Roche) at 42°C for 1h. This prepared the membrane by blocking any non-specific nucleic acid-binding sites. The DNA probes (SRA and PLC) were denatured by boiling for 10min and were directly chilled on ice. The probes were then diluted to 25ng cm^{-3} in preheated hybridisation buffer and introduced to the membrane. Hybridisation took place overnight at 42°C. The membrane was then washed twice for 5 min in 2x wash solution (0.3M NaCl; 30mM sodium citrate, pH7, 0.1% (w/v) SDS) at room temperature to remove unbound probe and the washed twice for 15min at 68°C in 0.5x wash solution (75mM NaCl; 7.5 mM sodium citrate at pH 7, 0.1% SDS). Regions of hybridisation were detected as described for estimation of probe yield (Section 3.2.6.2)

3.3 *Data analysis*

The data collected is analysed in three data chapters under the following aspects:

Chapter 4 – Animal inherent indicators and management related risk factors for livestock trypanosomiasis

The laboratory results on trypanosomiasis infections in all livestock samples obtained through census sampling in Funyula and Butula, are analysed in conjunction with the sample associated data collected (detailed in section 3.1.6): animal level factors (species, gender, age, condition, anaemia score) and management factors (grazing and watering regime).

Chapter 5 - Spatial distribution of livestock trypanosomiasis

The distribution of trypanosomiasis infections in the livestock population of the sampling sites in Funyula and Butula (assessed by census sampling), is analysed for spatial clustering, with respect to the geographical location of the respective homesteads (section 3.1.6.2). Furthermore, the infection status of each household herd is analysed in relation to its spatial proximity to hypothesised preferred tsetse habitats (streams and swamps).

Chapter 6 – Central point versus census sampling to assess cattle trypanosomiasis

The validity of central point sampling as a tool for rapid estimation of cattle trypanosomiasis in an area of interest is assessed by comparing the demography and trypanosomiasis prevalence of cattle sampled at central points to the population in the catchment area as determined by census sampling.

3.3.1 Statistical analysis

The relevant statistical analysis is detailed in the individual methodology section of each data chapter.

4 Chapter 4   Inherent and management risk factors for trypanosome infections in domestic livestock

4.1 Introduction

Busia, Kenya is a resource poor district, where mixed crop-livestock farming predominates and trypanosomiasis places severe constraints on animal productivity (Angus, 1996; FITCA, 2005). Traditionally, control of trypanosomiasis in Kenya was state-run, but significant budget cuts in the Veterinary Department of Kenya since the 1980s and increasing privatisation of veterinary services have shifted this responsibility to the individual livestock owners (Holden, 1999). The need for farmer-based control raised interest in identifying aspects associated with livestock trypanosomiasis in Busia at the local level. This chapter aims to establish risk factors for trypanosomiasis at the micro-scale to identify parasite reservoirs and to aid diagnosis of diseased animals. The effect of animal inherent characteristics, specifically livestock species, gender and age on trypanosomiasis prevalence at the animal level are investigated. Furthermore, the dependability of two clinical signs, anaemia and condition score, as diagnostic indicators for trypanosome infections in cattle, is tested. Finally, the impact of grazing and watering practices on the prevalence of livestock trypanosomiasis, is assessed at the herd level.

The following sections of the introduction explore the literature to put factors that are investigated in this chapter into context. Factors under consideration are broadly split into two categories: firstly the animal level factors (host species, gender, cattle age, cattle anaemia and cattle condition) and secondly the herd level, factors (grazing and watering management).

4.1.1 Animal level factors

4.1.1.1 Host species

Animal trypanosomiasis is a well known constraint to livestock production throughout the Lake Victoria Crescent (Wain *et al.*, 1970). Nevertheless thousands of cattle survive along the shores of the lake under constant tsetse challenge; small ruminants are maintained throughout this area and the number of pigs kept in the

study area of Busia is increasing rapidly (Cunningham, 1966; Thuranira, 2005). Livestock owners chiefly recognize and treat trypanosomiasis in cattle, with small ruminants as only incidental beneficiaries of left over products, the level of input reflecting the relative value of the different species of livestock (Machila *et al.*, 2003). However the role of small ruminants in the epidemiology of livestock trypanosomiasis as a potential reservoir and source of re-infection for cattle, remains unclear, in particular as small ruminants have been shown to carry trypanosome infections without necessarily displaying clinical signs (Mahmoud & Elmalik, 1977). Increasingly, the role of pigs as a reservoir for trypanosomiasis in East Africa is recognized, and their inclusion in treatment programmes is being advocated, although mainly in conjunction with efforts to reduce reservoirs of the human infective *T.b. rhodesiense* (Waiswa *et al.*, 2003; Waiswa, 2005).

Studies comparing trypanosomiasis in cattle and smaller livestock conducted in Western Kenya and neighbouring Uganda employing microscopy, reported a prevalence of around 15% in cattle, and under 5% in small ruminants whilst the prevalence in pigs was the most variable, fluctuating between 2 and 20% depending on the district (Angus, 1996; Waiswa *et al.*, 2003; Waiswa, 2005). A study in Busia District, focusing on small livestock only and employing PCR for the detection of trypanosome infections, reported a much higher prevalence of between 20-25% in small ruminants (Ng'ayo *et al.*, 2005). On the other hand a recent study focusing on different endemic diseases of cattle in Busia, Kenya, reported an overall prevalence of only 4.7% trypanosomiasis in cattle, employing buffy coat microscopy (Karanja, 2006). PCR is a much more sensitive tool than microscopy and the low prevalence of trypanosomiasis detected in some of the studies in small ruminants and cattle by microscopy may either be a truthful reflection of lower prevalence or may be a reflection of parasitaemia below the detection threshold of microscopy.

4.1.1.2 Gender

Most literature available on the effect of host gender on the prevalence of trypanosomiasis concentrates on cattle. A number of studies reported a higher prevalence in male cattle, which has been ascribed to a range of factors including larger size of bulls and olfactory cues being attractive to tsetse (Vale, 1977; Torr & Mangwiro, 2000). However, the apparent effect of cattle gender on trypanosomiasis prevalence may have been confounded by management practices (Torr *et al.*, 2006). Increased stress due to traction work has been described as an occupational hazard making male cattle more susceptible to infections, and movement of hired out oxen between villages has been suggested to increase exposure to tsetse (Rowlands *et al.*, 1993; Angus, 1996).

4.1.1.3 Cattle age

Cattle age has been shown to have a significant effect on the prevalence of trypanosomiasis, with a lower prevalence consistently detected in cattle under 18 months of age (Angus, 1996; Rowlands *et al.*, 2001). The cause of this age effect has not been demonstrated conclusively, but yet again, both exposure and attractiveness to tsetse appear to play a role: Calves are commonly restrained at the homestead whilst the rest of the herd is taken for grazing, which has been implicated in reducing exposure of young cattle to tsetse and therefore decreasing the likelihood of infection (Angus, 1996; Rowlands *et al.*, 2001). Attractiveness of adult cattle to tsetse due to size and olfactory cues may provide an alternative explanation to age dependant differences in infection prevalence (Vale, 1974; Torr & Mangwiro, 2000; Torr *et al.*, 2006). Another study, reported pre-weaning calves grazing with their dams to have considerable protection from *T. vivax* and *T. congolense* infections as compared to their dams and as compared to their own post-weaning situation (Trail *et al.*, 1994). Trail and colleagues cited higher resistance to trypanosome infections through better nutrition as one possible cause for this observation. Cattle age was thus shown to influence the likelihood of exposure to tsetse and the likelihood of trypanosome infections through a range of intrinsic and management factors, which may interact to produce the observed results.

4.1.1.4   Anaemia

Even though anaemia is associated with a range of livestock diseases endemic to sub-Saharan Africa, this clinical sign has been reported to be of particular relevance to trypanosomiasis and was ranked as an important diagnostic indicator for this disease (Magona *et al.*, 2003a; Eisler *et al.*, 2007). Severe anaemia associated with trypanosomiasis can be observed by pallor of the visible mucous membranes (Taylor & Authié, 2004). The onset of anaemia in trypanosome infected cattle coincides with the onset of parasitaemia in the early acute phase, however in the later chronic stage of the disease, levels of parasitaemia and anaemia dissociate (Paling *et al.*, 1991; Naessens *et al.*, 2003). Control of anaemia, more so than control of parasitaemia, has been shown to be associated with the ability to gain weight and remain productive in the face of trypanosome challenge, commonly described as trypanotolerance (Murray & Trail, 1984; Trail *et al.*, 1991a; Trail *et al.*, 1991b; Naessens, 2006). Trypanotolerance in cattle is usually associated with the West and Central African *Bos taurus* cattle breeds such as N'Dama (Cunningham, 1966), however there is evidence that decreased susceptibility may also be found to varying degrees in the East African *Bos indicus* breeds (Mwangi *et al.*, 1998), although most will require regular treatment to survive severe tsetse challenge (Murray *et al.*, 2004). It is thus uncertain whether visual assessment of anaemia status is an adequate tool for the diagnosis of trypanosomiasis in cattle in an endemic area, where animals exposed to repeated infections may only develop limited anaemia in response (Murray *et al.*, 2004; Taylor & Authié, 2004).

4.1.1.5   Condition

Alongside anaemia, weight loss is ranked as an important clinical sign particularly prominent in trypanosome infected cattle (Taylor & Authié, 2004; Eisler *et al.*, 2007), and the diagnostic value of cattle condition scores (Nicholson & Butterworth, 1986) in an endemic area is of interest. Whilst weight loss is a symptom of trypanosomiasis, poor nutrition in itself, along side other physiological stressors such as pregnancy and lactation as well as hard labour, has been shown to predispose cattle and small ruminants to trypanosome infections (Murray *et al.*, 1982; Osaer *et*

al., 1999a). The interaction between stressors and trypanosome infections in turn affected their ability to maintain bodyweight and control anaemia (Agyemang *et al.*, 1991; Katunguka-Rwakishaya *et al.*, 1997).

4.1.2 Herd level factors

4.1.2.1 Livestock management: grazing and watering regime

The effect of livestock management on trypanosomiasis has been mentioned in this chapter where management was tightly interlaced with animal inherent factors such as cattle age and gender. Furthermore, grazing and watering regimes have been shown to affect trypanosomiasis risk at the herd level: Up to ten-fold variation in tsetse exposure has been observed in cattle herds stemming from the same village depending on the grazing routes used (Wacher *et al.*, 1994). Reduced infection prevalence was reported for tethered and zero-grazed animals as opposed to free-grazing cattle (Karanja, 2006). Small ruminants grazing together with cattle had an increased likelihood of trypanosome infections, compared to those tethered close to the homestead (Waiswa *et al.*, 2003). Management risk factors associated with high trypanosomiasis prevalence in cattle included the frequenting of natural watering sites, which were identified as transmission hotspots in terms of tsetse infection rates (de La Rocque *et al.*, 1999; Michel *et al.*, 2002). The size of the cattle herds served as a confounding factor, as larger herds were preferentially watered at natural sites, rather than being watered at home (Michel *et al.*, 2002). Thus maintaining animals within the confines of the homestead compound, by providing feed and water, rather than taking animals to likely tsetse habitats such as semi-overgrown communal grazing areas, swampland or the river may have a protective effect with respect to the likelihood of animals acquiring trypanosome infections.

It is questionable however, whether the amount of feed and water provided within the compound would be necessarily sufficient to maintain adequate nutritional status of the livestock. In the study area, Busia District, crop production was seen to take priority in terms of cash resources and man power over animal management and

health and little money was spent on feed supplements for cattle, and even less so on small ruminants or pigs (Machila-Eisler, 2005; Thuranira, 2005). During the course of this study, cattle were observed to be fed with grass cuttings, pigs with household waste and small ruminants generally simply grazed tethered within the compound if livestock was not taken out with the compound for grazing. Adjusting grazing and watering of animals according to crop cycle and available man power, has been shown to be detrimental to the nutrition levels of livestock, predisposing them to trypanosomiasis (Osaer *et al.*, 1999a).

4.1.3 Aims

Using the comprehensive data set obtained from visiting all livestock owning households in the two study areas within Busia district, this chapter aimed to investigate a range of factors potentially associated with an increased risk of trypanosomiasis. Blood samples and associated data from almost the entire livestock population of cattle, pigs, goats and sheep of the 19 study villages were collected for this purpose. The objective of this census sampling design was to gain unique insights at the micro-scale into the relative importance of animal inherent factors (species, gender, cattle age) and management practices (grazing and watering practices) on the prevalence as well as the diagnosis (anaemia, condition score) of trypanosomiasis. These insights related to the animal pathogenic trypanosome species but also extended to the reservoir of the human infective sub-species *T. b. rhodesiense*, as the sampling area is endemic for both livestock and human trypanosomiasis.

4.1.4 Null-Hypotheses

1. H_0 - There is no significant effect of animal inherent factors including species, gender, cattle age, anaemia or condition score on the prevalence of trypanosomiasis detected.

2. H_0 - There is no significant effect of grazing and watering management practices on the prevalence of trypanosomiasis detected in livestock.

4.2 Methodology

4.2.1 Samples

A detailed description of study design, sample collection, storage and laboratory analysis is given in the General Methodology (Chapter 3). Briefly, census sampling was performed in the two sampling areas, located in Funyula (July 2004) and Butula Division (October 2004) of Busia District, Kenya. All livestock owning households within the 19 study villages were visited and a whole blood sample from every animal (cattle, pigs, sheep, goats) was collected by ear vein lancing and stored on FTA-cards. A total of 2773 animals (cattle n=1260, pigs n=312, goats n=768, sheep n=433) from 549 households were sampled.

All samples were analysed in the laboratory by PCR for the following African livestock-infective trypanosome species: *T. brucei* s.l., *T. vivax*, *T. congolense* and *T. simiae* (Moser *et al.*, 1989; Cox *et al.*, 2005). Any sample positive for *T. brucei* s.l. was additionally screened for the presence of the human infective subspecies *T. b. rhodesiense*, by specific PCR and Southern blot (Picozzi *et al.*, in press).

4.2.2 Factors under investigation

Additional information was systematically collected for all samples, on the factors potentially associated with trypanosomiasis, which are under investigation in this chapter.

4.2.2.1 Animal level factors

In addition to the homestead of origin, the following information was recorded for each blood sample collected:

- Animal species
- Gender of animal

For each cattle sample, the following additional information was recorded:

- Age category

- Body condition score

- Anaemia score

(Details on the categorisation of each of these factors are described in the General Methodology, section 3.1.6.1)

4.2.2.2 Herd level factors

For each homestead, the questionnaire respondent was asked how many animals of each livestock species (cattle, pigs, sheep, goats) were owned by the household, and where these animals were grazed and watered (Questionnaire see Appendix A). The factors of interest at the herd level were thus:

- Grazing regime

- Watering regime

- Overall management (combined grazing and watering regime)

This information was categorised for the household herd in general as well as separately for each livestock species kept by a household, for analysis in livestock species specific models (categorization in General Methodology, section 3.1.6.2)

4.2.3 Statistical analysis

All factors under investigation in this chapter, were analysed for their effect on the prevalence of trypanosome infections detected, using mixed effect models computed with the statistical software package R (R: copyright 2004, The R foundation for Statistical Computing Version 2.0.1, available free on the internet at http://CRAN.R-project.org). The analysis was broadly divided into two different approaches, depending on whether the factor under investigation was collected at the animal level (species, gender, age, condition, anaemia score: section 4.2.3.1) or at the herd level (grazing, watering, overall management: section 4.2.2.2).

4.2.3.1 Animal level factors

The effect of livestock species, gender, cattle age, cattle condition and cattle anaeamia score on the presence of trypanosomiasis were analysed using mixed effect models for binary data.

Response variable: Trypanosome infection status of each sample was used as a binary response variable, each sample having been classified as positive (1) or negative (0) for overall trypanosomiasis as well as specifically for the animal infective trypanosome species *T. brucei* s.l. , *T. vivax* and *T. simiae*, and the zoonotic *T.b. rhodesiense* according to the PCR results. In addition to these trypanosome species overall trypanosomiasis also included *T. congolense* infections, which were not analysed in separate models, due to the low prevalence (total 7 out of 2773 samples *T. congolense* infected).

Random effect: The village of origin of each animal was included as the random effect in each model, to take into account potential pseudo-replication or any non-independence issues that may have been caused by sampling multiple animals from the same village and any village level factors not considered. As no village specific data had been collected the exact effect of each village was not of interest. Ideally, in order to account for the influence of household on animal level data, household would have been included as an additional random effect, nested within the village random effect. Unfortunately, a considerable proportion of all livestock keeping households (76 out of 549) owned only one animal, and of all households keeping cattle approximately one quarter (102/407) owned only one cow. This precluded the use of household as random effect, in models considering all livestock, as well as those considering only cattle (age, condition, anaemia score), due to lack of variation within the one animal households. In order to try and consider any potential pseudo-replication due to multiple animal households, all analyses at the animal level were run with the number of animals per household in the model fitted as a covariate as

first fixed effect. But as there was no significant effect of the number of animals in any of the models, this factor was subsequently excluded from the analysis and the model was run with the factor under investigation as the only fixed effect.

Fixed effect: The factors under investigation (livestock species, gender, age, condition and anaemia) were included as fixed effects in separate univariate models.

4.2.3.1.1 Model structure for factors under investigation for all livestock

1. Host species: a four level factor accounting for cattle (C), pigs (P), goats (G) and sheep (Sh).

```
(a) >summary(glmmPQL(tryps01~herdsize+hostspecies,random=~1|village,
data=BusiaAllsamples, family="binomial"))

(b) >summary(glmmPQL(tryps01~hostspecies,random=~1|village,
data=BusiaAllsamples, family="binomial"))
```

Figure 4.1: Example of R-command line for initial model including number of animals per herd as first fixed effect (a) and final reduced model (b), factor of interest: host species, response variable in this example overall trypanosome infection status of each sample

2. Gender: a two level factor differentiating male (m) and female (f). The effect of gender on overall trypanosome prevalence was analysed in separate models for the four different host species. Visual inspection of the data did not reveal any strong trends in the data, hence the effect of host gender on the different trypanosome species was not analysed separately.

```
>summary(glmmPQL(tryps01~gender,random=~1|village,data=BusiaPigs,
family="binomial"))
```

Figure 4.2: Example of R-command line for univariate model with gender as fixed effect, overall trypanosome infection status of each pig as response variable.

4.2.3.1.2 Model structure for factors under investigation for cattle only

For each of the following models the number of cattle was initially included as a first fixed effect to try and account for any possible influence of pseudo-replication, if the effect of number of cattle was non-significant, it was excluded from the model, which was then re-run with the factor of interest as the only fixed effect.

1. Age category, a three level factor: a: <18months, b: 18months-36 months, c: >36months

```
>summary(glmmPQL(tbrucei01~age,random=~1|village,data=BusiaCattle,
family="binomial"))
```

Figure 4.3: Example of R-command line for univariate model with cattle age as fixed effect, and *T. brucei* s.l. infection status of each cattle sample as response variable.

2. Condition score, included as a three level factor: L=lean, M=medium, F=fat

```
>summary(glmmPQL(tvivax01~condition,random=~1|village,data=
BusiaCattle, family="binomial"))
```

Figure 4.4: Example of R-command line for univariate model with cattle condition score as fixed effect, and *T. vivax* infection status of each cattle sample as response variable.

3. Anaemia score, included as a two level factor: N=normal, N+=anaemic

```
>summary(glmmPQL(SRA01~anaemia,random=~1|village,data=BusiaCattle,
family="binomial"))
```

Figure 4.5: Example of R-command line for univariate model with cattle anaemia score as fixed effect, and *T. b. rhodesiense* infection status of each cattle sample as response variable.

4.2.3.2 Herd level factors

The effect of the management factors collected at the household level on the prevalence of trypanosomiasis were assessed using mixed effect models, again including village of origin as the *random effect* to account for village dependant variation in the data.

Response variable: Ideally, the effect of household level factors would have been analysed, using a two-vector response variable, comprising the number of successes (infected animals) and the number of failures (non-infected animals) per household, bound together into a single object (using a function within R called cbind), thus accounting for the proportional nature of the data (Crawley, 2002). However diagnostic plots showed poor fit of that type of model for the data collected, due to high number of households with only one or two animals, in particular when separate models for the different livestock species were constructed. Even when all species of livestock were considered, 164 out of the total 549 households owned only one or two animals, making exclusion of these households to persevere with a proportional data model unfeasible without unduly reducing the power of the model. Additionally, there were concerns over management of animals varying according to herd size resulting in a biased analysis should small herds be excluded.

It was thus decided to use a binary response variable, with households owning at least one infected animal of the species under consideration being assigned positive status (1) and only households with no infected animals of that species considered negative (0). As a higher number of animals in a given homestead would increase the probability that at least one of the animals was infected purely by chance, the number of animals in the homestead (total herdsize or number of the livestock species under consideration when separate models were constructed) was included into each model as the first fixed effect, before the management factor of interest. If interaction between herdsize and the factor of interest did not at least approach significance ($p<0.06$), the interaction term was excluded from the final model.

Each model was constructed first considering all animal species, followed by separate models considering (a) only infection status of the cattle in any herd and (b) only the infection status of small ruminant in any herd, respectively. These separate models for all cattle and all small ruminants were constructed as management frequently differed between animals of different species within the same homesteads, which could not be incorporated into a single model at the household level. Pigs were not considered in separate models, as the vast majority of pig keeping households confined them at home (162 out of 177). Models including all animals or cattle only, were constructed for overall trypanosomiasis as well as *T. brucei* s.l., *T. vivax*, *T.simiae* and *T. b. rhodesiense* separately.

Sheep and goats were considered together as small ruminants, as there was no significant difference in trypanosome prevalence between these two livestock species and management never differed between the two if they were kept within the same household. The small ruminant models were only constructed for the effects of management on overall trypanosomiasis, *T. brucei* s.l. and *T. vivax*. No separate small ruminant models for *T. simiae* or *T. b. rhodesiense* were constructed as only one infection respectively was detected in small ruminants in total.

4.2.3.2.1 Model structure for management factors under investigation

1. Grazing management: two level factor defined as "home" for households confining animals at the homestead, or "away" for animals being taken elsewhere for grazing (detailed definition in General Methodology)

```
>summary(glmmPQL(home_smR_tryps01~number_smR*grazing,random=~1|vil
lage,data=BusiaHomesSmR, family="binomial"))
```

Figure 4.6: Example of R-command line, factor of interest (2nd fixed effect): grazing management of small ruminants, adjusted for the number of small ruminants in the household (1st fixed effect), response variable in this example: binary trypanosome infection status of homesteads keeping small ruminants

2. Watering management: two level factor defined as "home" for households watering animals at the homestead, or "away" for animals being taken to the river for grazing (detailed definition in General Methodology)

```
>summary(glmmPQL(home_Cattle_tbrucei01~number_C*watering,random=~1
|village,data=BusiaHomesCattle, family="binomial"))
```

Figure 4.7: Example of R-command line, factor of interest (2nd fixed effect): grazing management of cattle, adjusted for the number of cattle in the household (1st fixed effect), response variable in this example: binary *T. brucei* s.l. infection status of each cattle keeping homestead

3. Combined management: two level factor combining grazing and watering management: if animals leave the homestead for either "*away*" status was assigned, only when animals remain within the immediate vicinity of the homestead at all times "*home*" status was assigned (detailed definition in General Methodology).

```
>summary(glmmPQL(home_All_tbrucei01~herdsize*management,random=~1|
village,data=BusiaHomesAll, family="binomial"))
```

Figure 4.8: Example of R-command line, factor of interest (2nd fixed effect): combined management of all animals, adjusted for the number of animals in the household (1st fixed effect), response variable in this example binary: *T. brucei* s.l. infection status of each homestead

4.2.4 Model output

The summary of each model run, revealed the effect of the individual factor levels on the logit of the odds of a positive outcome, relative to the effect of the first factor level. When factors with more than two levels were under investigation, the order of factor levels within the model could be changed to establish the relative effect between any two particular factor levels of interest. Degrees of freedom were noted as subscripts of the test statistic (t). The significance of the overall variation of

factors under investigation in all models throughout this chapter were established by running analysis of variance (ANOVA) summaries on each fitted model. Degrees of freedom were noted as subscripts of the F statistic.

All analysis was at first performed separately for the samples from the two different sampling areas within Busia District, Funyula and Butula Divisions, as samples were collected from geographically separate areas at different times of the year. But as the data from both Funyula and Butula Division in general followed the same trend with reference to the potential risk factors explored in this chapter, the data from the two sampling areas from within Busia District were subsequently combined to increase the sample number and lend additional power to the analysis. For the sake of brevity, only the results from the joint analysis for Busia District were reported, unless the results diverge (significant opposing effects) between sampling areas, in which case results are additionally reported separately for Funyula and Butula.

Multiple comparisons were performed, due to a number of outcome measures (individual trypanosome species) being evaluated, and the analysis of several sub-groups within the trial (livestock species). Multiple comparisons may lead to spurious results being declared significant, as the experiment-wise error rate is often much larger than the error rate applied to each individual analysis (5%). One of the simplest adjustments, the Bonferroni correction, stipulates change of the significance level to : $p \leq 0.05/$ number of tests conducted (Bland & Altman, 1995). However, this results in a very conservative estimate of the statistical significance of each evaluation, which is appropriate for strict experimental designs. As the study was based on samples collected through fieldwork rather than obtained from a tightly controlled experiment, the Bonferroni correction was considered too severe and statistical significance was accepted at $p \leq 0.01$.

4.2.5 Overview

Table 4.1 gives an overview of the factors under investigation in this chapter.

Table 4.1: Factors of interest considered

Level of data	Factor of interest (factor levels)	Individual host species models	Trypanosome species considered individually
Animal level	Host species (C, P, G, Sh)	all animals	Overall tryps, *T. brucei* s.l., *T. vivax, T. simiae, T.b. rhodesiense*
	Gender (M, F)	separate models for C, P, G, Sh	Overall tryps
Animal level (cattle only)	Age (a, b, c)	cattle	Overall tryps, *T. brucei* s.l., *T. vivax, T. simiae, T.b. rhodesiense*
	Condition (L, M, F)	cattle	Overall tryps, *T. brucei* s.l., *T. vivax, T. simiae, T.b. rhodesiense*
	Anaemia (N, N+)	cattle	Overall tryps, *T. brucei* s.l., *T. vivax, T. simiae, T.b. rhodesiense*
Homestead level	Grazing (Home, away)	all animals	Overall tryps, *T. brucei* s.l., *T. vivax, T. simiae, T.b. rhodesiense*
	Watering (Home, away)	cattle	Overall tryps, *T. brucei* s.l., *T. vivax, T. simiae, T.b. rhodesiense*
	Combined management (Home, away)	small ruminants	Overall tryps, *T. brucei* s.l., *T. vivax,*

4.3 Results

4.3.1 Samples

The number of samples collected in the two sampling sites in Busia District (Funyula and Butula Division) was summarised in Table 4.2. The total population for each livestock species was calculated from the number of animals which household respondents claimed the household owned. In total, over 85% of the livestock population was sampled.

Table 4.2: Summary of samples collected and total livestock population in sampling sites

Livestock species	N° livestock sampled			Total livestock population	Percentage of population sampled
	Funyula	Butula	total		
cattle	446	814	1260	1347	93.5%
pigs	109	203	312	495	63%
goats	378	390	768	877	87.6%
sheep	148	285	433	530	81.7%
total	1081	1692	2773	3249	85.3%

4.3.2 Animal level factors

4.3.2.1 Host species

The effect of host species on the prevalence of overall trypanosomiasis for all samples collected in Busia was statistically significant ($F_{3,2751}=42.4$, $p<0.001$) with village of origin included in the model as random effect. The prevalence in cattle was significantly higher than that in pigs ($t_{2751}=-3.3$, $p=0.001$), goats ($t_{2751}=-9.0$, $p<0.001$) and sheep ($t_{2751}=-7.2$, $p<0.001$) (Table 4.3). The difference in prevalence between pigs and goats was significant ($t_{2751}=4.6$, $p<0.001$), but there was no significant difference between sheep and goats ($t_{2751}=-1.2$, $p=0.224$).

Table 4.3: Total trypanosomiasis prevalence according to livestock species in Busia

	Cattle (n=1260)	Pigs (n=312)	Goats (n=768)	Sheep (n=433)
Total tryp. prevalence*	**20.1%**	**11.5%**	**3.8%**	**2.5%**
(95% CI)	(18-22.4)	(8.5-15.6)	(2.6-5.4)	(1.4-4.5)

* total prevalence includes *T. congolense* infections, mixed trypanosome infections were detected in 17/253 infected cattle samples and 1/36 pig samples

When looking at the two divisions separately, it was of interest to note that in the samples collected in Funyula, there was no significant difference in prevalence of overall trypanosomiasis detected between cattle and pigs (t_{922}=-0.3, p=0.774) (Figure 4.9a). In Butula however, the overall trypanosome prevalence in cattle was significantly higher than in pigs (t_{1679}=-3.7, p<0.001) (Figure 4.9 b), which remained the case when samples from Funyula and Butula were combined for analysis.
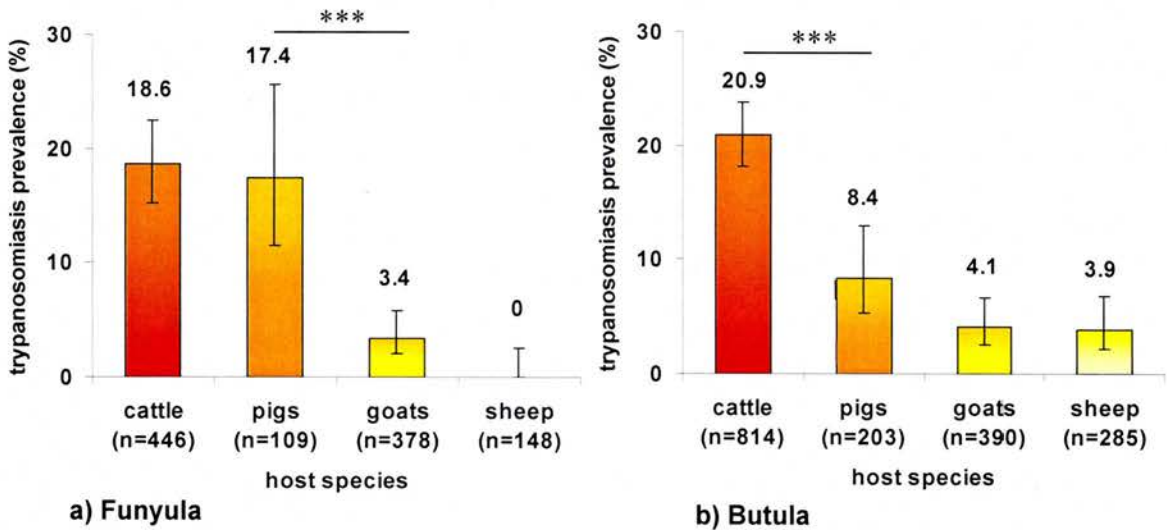
Figure 4.9: Overall trypanosome prevalence according to host species for Funyula and Butula (level of significance ***: p<0.001)

When the different species of trypanosomes were analysed separately, the effect of host species on the prevalence of *T. brucei* s.l. ($F_{3,2751}=17.4$, $p<0.001$) and *T. vivax* ($F_{3,2751}=23.1$, $p<0.001$) was significant, but the effect on *T. simae* ($F_{3,2751}=2.1$, $p=0.103$) was not significant (Figure 4.10). The effect of host species on the prevalence of *T. b. rhodesiense* approached statistical significance ($F_{3,2751}=3.8$, $p=0.011$), with a prevalence of 1.5% (19/1260; 95% CI: 1-2.3%) detected in cattle, 2.9% (9/312; 95% CI: 1.5-5.4%) in pigs, 0.1% (1/768; 95%CI: 0-0.7%) in goats and 0% (0/433; 95% CI: 0-0.9%) in sheep.
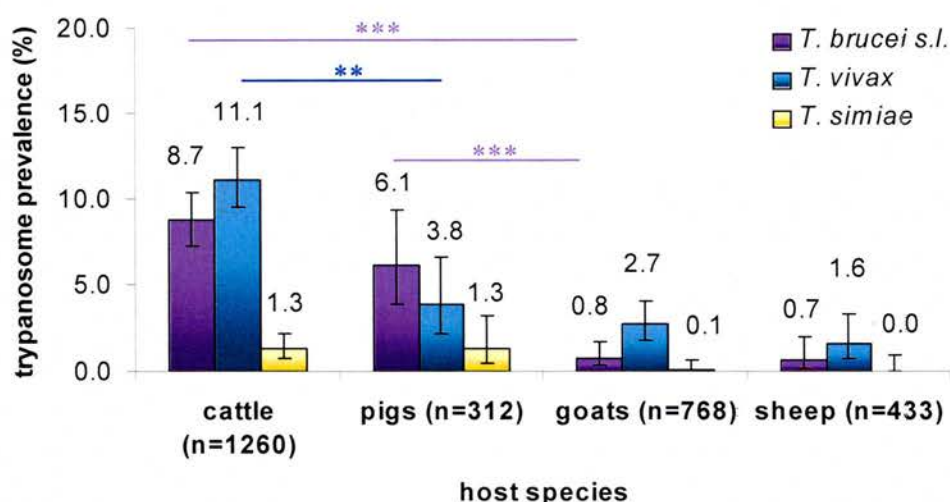
Figure 4.10: Prevalence of animal infective trypanosome species by host species in Busia (level of significance: **: p<0.01;   ***: p<0.001)

4.3.2.2 Gender

There was no significant effect of gender on overall trypanosomiasis in cattle ($F_{1,1240}=0.4$, $p=0.551$) or goats   ($F_{1,745}=0.4$, $p=0.517$) but the difference of trypanosome infection prevalence between males and females approached significance in pigs ($F_{1,291}=6.0$, $p=0.015$) (Table 4.4). No model was run for sheep, as there were no trypanosome infections detected in male sheep.

Table 4.4: Gender effect on trypanosome prevalence by host species in all samples from Busia

gender	Trypanosome prevalence by gender in %; fraction (95% CI)			
	cattle (n=1260)	pigs (n=311*)	goats (n=765*)	sheep (n=431*)
female	20.6; 184/895 (18-23.3)	8.8; 20/227 (5.8-13.2)	4.1; 23/567 (2.7-6)	3.1; 11/351 (1.8-5.5)
male	18.9; 69/365 (15.2-23.2)	19.0; 16/84 (12.2-28.7)	3.0; 6/198 (1.4-6.5)	0.0; 0/80 (0-4.6)

* no gender was noted for a number of samples (1pig, 3goats and 2 sheep) which therefore had to be excluded from this analysis

As visual inspection of the prevalence of individual trypanosome species by host species and gender did not reveal any convincing trends (e.g. higher prevalence of *T. brucei* s.l. in female cattle whereas the prevalence of *T. vivax* was higher in male cattle and 95% confidence intervals overlap in all male-female comparisons) no separate models were run (Figure 4.11).
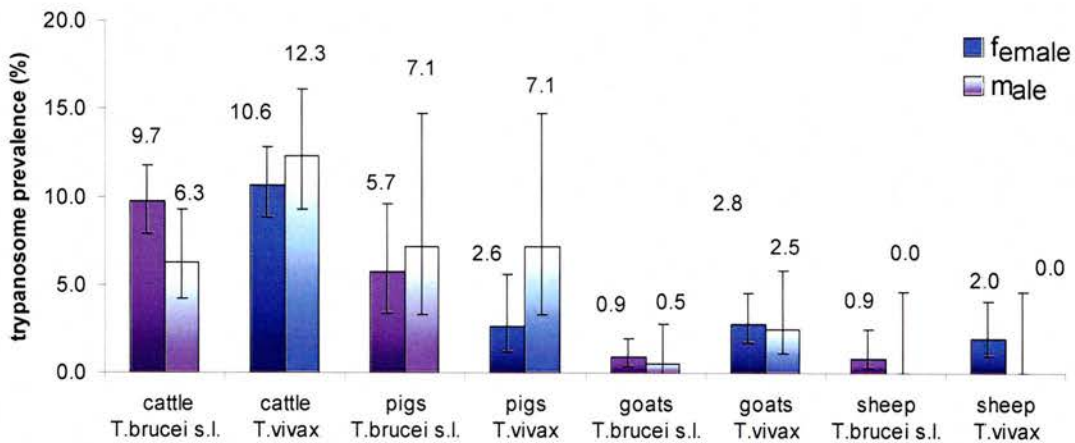
Figure 4.11: Prevalence of *T. brucei* s.l and *T. vivax* by host species and gender for Busia

4.3.3 Animal level factors recorded for cattle only

4.3.3.1 Age group

A total of six cattle samples were excluded from the age group analysis as no age group was recorded for these samples. There was a significant effect of age group on the prevalence of trypanosome infected cattle samples from Busia ($F_{2,1233}$=8.6, p<0.001), with the lowest prevalence detected in the youngest age group a (<18 months) (13%; 53/407; 95% CI: 10.1-16.6%), followed by age group b (18-36 months) (18.4%; 39/207; 95% CI: 13.7-24.2%) and age group c (>36 months) (25.2%; 161/640; 95% CI: 21.9-28.7%)[1]. The difference between age groups a and c was significant (t_{1233}=5.3, p<0.001), whereas the difference between the intermediate age group b and the other two age groups, age group a (t_{1233}=2.4, p=0.016) and age group c (t_{1233}=1.7, p=0.091) respectively, only approached statistical significance.
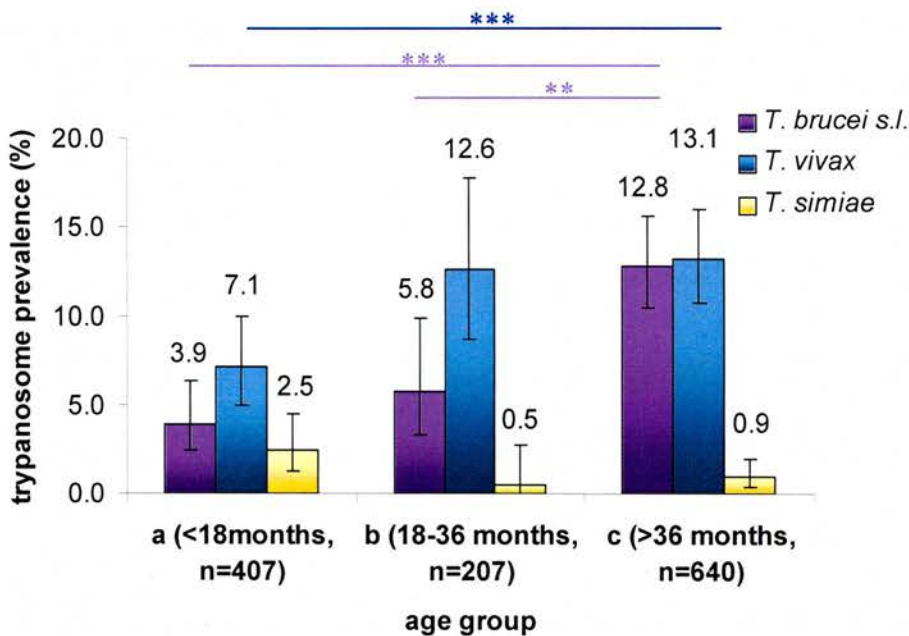
Figure 4.12: Prevalence of trypanosome infections in cattle in Busia by age group (level of significance: **: p<0.01; ***: p<0.001)

---

[1] Mixed species trypanosome infections were detected in 3/53, 2/39 and 12/161 of the infected samples from age group a, b and c respectively.

When separate models were constructed for the different trypanosome species, the effect of age group on the prevalence of *T. brucei* s.l. ($F_{2,1233}=15.1$, $p<0.001$) and on *T. vivax* ($F_{2,1233}=5.7$, $p<0.01$) was significant, with the lowest prevalence always found in the youngest group: a (Figure 4.12). This was not the case for *T. simiae*, where the highest prevalence was found in age group a, but the effect of age group only approached significance ($F_{2,1233}=3.6$, $p=0.028$) (Figure 4.12). The prevalence of *T. b. rhodesiense* detected in cattle was highest in age group c (2.3%; 15/640; 95% CI: 1.4-3.8%), followed by group b (1%; 2/207; 95% CI: 0.3-3.5%) and lowest in group a (0.5%; 2/407; 95%CI: 0.1-1.8%), but the difference between age groups was not significant ($F_{2,1233}=2.6$, $p=0.071$).

4.3.3.2 Condition score

A total of ten cattle samples were excluded from the analysis as no condition score was recorded for these samples. There was no significant effect of the condition score category recorded on the overall trypanosome prevalence detected in the cattle samples, (lean: 23.8%; 10/42; 95% CI: 13.5-38.5%; medium: 19.4%; 221/1138; 95% CI: 17.2-21.8%; fat: 25.7%; 95% CI:16.9-37%) ($F_{2,1229}=0.6$, $p=0.525$)[2].

When separate models were constructed for different trypanosome species, there was no significant effect of condition score on the prevalence of infection detected in the samples for *T. brucei* s.l. ($F_{2,1229}=2.6$, $p=0.076$) and *T. vivax* ($F_{2,1229}=0.1$, $p=0.882$). No model was run for *T. simiae* as infections were only found in the "medium" condition score category (Figure 4.13). There was a significant effect of condition score on the prevalence of *T. b. rhodesiense*, with the prevalence detected in the "medium" category (1.1%; 12/1138; 95%CI: 0.6-1.8%) lower than in either the "lean" (4.8; 2/42; 95% CI: 1.3-15.8) or "fat" category (5.7%; 4/70; 95% CI: 2.2-13.8%) ($F_{2,1229}=5.4$, $p<0.01$).

---

[2] Mixed infections were detected in 17/221infected samples in the condition score category "medium"
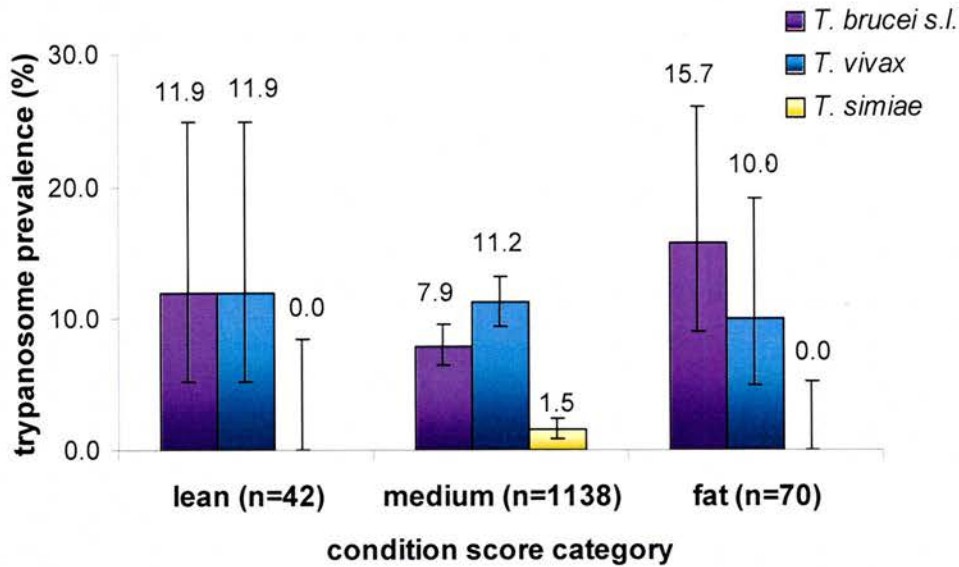
Figure 4.13: Trypanosome prevalence in cattle in Busia by condition score category

4.3.3.3 Anaemia score

Twenty-seven out of the total 1260 cattle samples were excluded from the analysis as no anaemia scores had been recorded. The prevalence of overall trypanosomiasis was higher in the group of samples classified as anaemic (25.4%; 46/181; 95% CI: 19.6-32.2%) than in the group classified as normal (18.9%; 199/1052; 95% CI: 16.7-21.4%), however this difference only approached statistical significance ($F_{1,1213}$=5.3; p=0.021)[3]. When only samples from Butula were considered the effect of anaemia score on trypanosome prevalence was significant ($F_{1,780}$=7.1; p<0.01), with a higher prevalence in anaemic (29.1%; 30/103; 95% CI: 21.2-38.5%) than in the normal cattle (19.2%; 132/688; 95%CI: 16.4-22.3%). The same trend was seen in Funyula with a higher prevalence in anaemic (20.5%; 16/78; 95% CI: 13-30.8%) than in normal cattle (17%; 62/364; 13.5-21.2%) but the difference was not significant ($F_{1,432}$=0.2; p=0.669).

---

[3] Mixed trypanosome infections were detected in 15/199 and 2/46 infected samples in the "normal" and "anaemic" category respectively.

There was no significant effect of anaemia score on the prevalence of *T. brucei* s.l. ($F_{1,1213}=1.1$; p=0.228) or *T. simiae* ($F_{1,1213}=0.9$; p=0.355) detected for all cattle samples from Busia, but the effect on the prevalence of *T. vivax* approached significance ($F_{1,1213}=4.0$; p=0.045) (Figure 4.14). There was no significant effect of anaemia score of samples on the prevalence of *T. b. rhodesiense* detected ($F_{1,1213}=2.0$; p=0.159), with a prevalence of 2.8% (5/181; 95% CI: 1.2-6.3%) in the anaemic group and 1.3% (14/1052; 95% CI: 0.8-2.2) in the group considered normal.
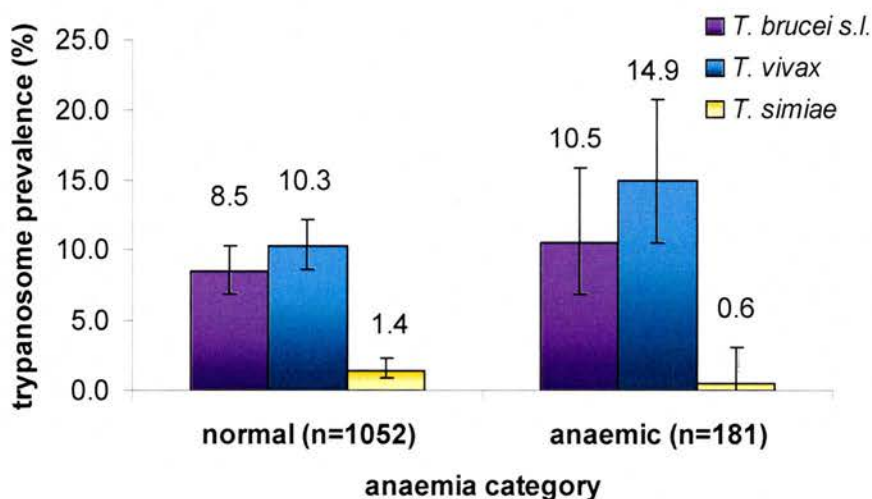
Figure 4.14: Trypanosome prevalence according to anaemia category

4.3.3.4 Herdsize

The number of animals per herd was initially included into each animal level model fitted as a covariate as first fixed effect. As the number of animals in each homestead did not have a statistically significant effect on the response variable (trypanosome infection status of each individual animal) in any of the separate models (p>0.2 throughout) herdsize was not included in the final models, the results of which were reported here.

4.3.4 Summary of animal level factors

1. **Host species** had a **significant** effect on trypanosome prevalence, with the highest prevalence detected in cattle, followed by pigs and a low prevalence detected in small ruminants.

2. There was **no significant effect of gender** on the prevalence of trypanosomiasis within each host species.

3. In **cattle, age group** had a **significant** effect with the highest prevalence of trypanosome infections detected in the oldest group (>36 months).

4. In **cattle, condition score** had **no significant** effect on trypanosome prevalence detected, with the exception of *T.b. rhodesiense*, which was predominantly found in the "fat" category.

5. In cattle, there was a trend for a higher trypanosome prevalence in **anaemic cattle**, however this only **approached significance**.

4.3.5 Household level data concerning animal management

Of a total of 549 households sampled across the 2 sampling areas (in Funyula and Butula Division) within Busia District, information on grazing and watering regimes were available for 529 households which were included in the analysis. Cattle were present in 387 of these households and small ruminants were present in 325 of these households. Any household keeping cattle and any household keeping small ruminants were included in the separate analyses for these livestock species. The fraction of household herds from which at least one trypanosome infection was detected, was summarized in Table 4.5, according to trypanosome species and host species considered.

Table 4.5: Prevalence of infected households according to trypanosome species and host species

| Trypanosome species | Prevalence of households with at least one infected animal | | |
	All livestock (households: n=529)	Cattle (households: n=387)	Small ruminants (households: n=325)
T. brucei s.l.	**18.5%** (15.4-22.1)	**20.9%** (17.2-25.3)	**2.8%** (1.5-5.2)
T. vivax	**24.2%** (20.7-28)	**27.1%** (22.9-31.8)	**7.4%** (5-10.8)
T. simiae	**4.0%** (2.6-6.0)	**4.1%** (2.6-6.6)	**0.3%** (0-1.7)
T.b. rhodesiense	**4.9%** (3.4-7.1)	**4.4%** (2.8-6.9)	**0.3%** (0-1.7)
Total tryps.	**38.4%** (34.8-42.6)	**43.4%** (38.6-48.4)	**10.5%** (7.6-14.3)

4.3.5.1 Grazing regime

All livestock: Of all 529 homesteads from Busia included in the study for which management information was available, 431 households (81.5%), accounting for 1867 samples (70.7%), did not take their livestock out with the confines of the homestead compound for grazing, whereas the remainder did. There was no significant effect of the grazing regime used on the prevalence of infected households ($F_{1,508}$=1.3, p=0.257), when the model was adjusted for number of animals per household ($F_{1,508}$=41.5, p<0.001) (Figure 4.15).
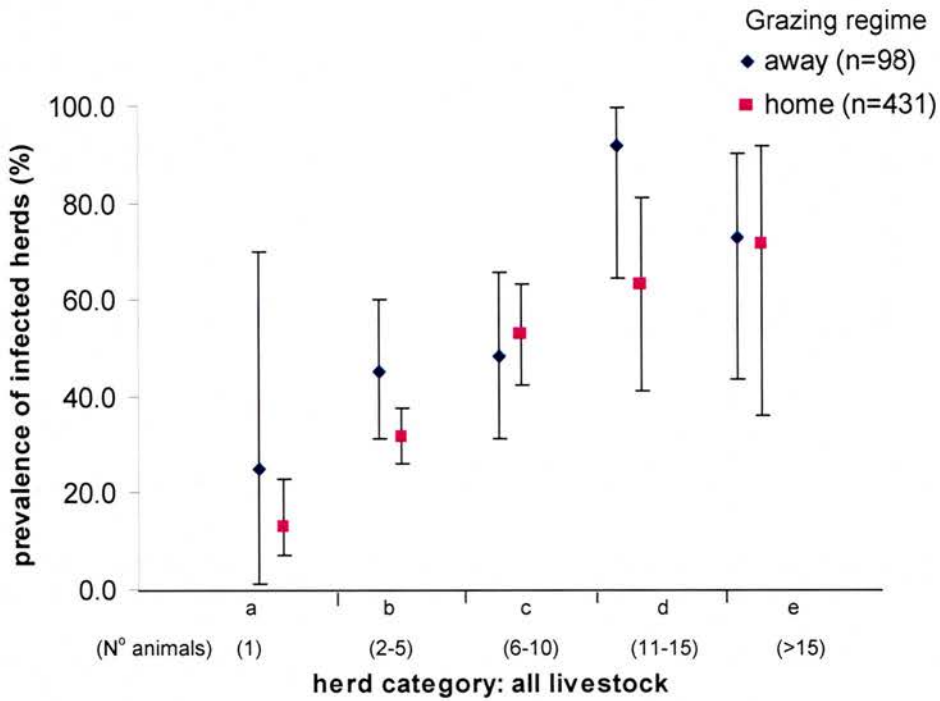
Figure 4.15: Fraction of herds infected with trypanosomiasis according to grazing regime (home/away) considering all livestock (herd size was categorised for display purpose).

When *T. brucei* s.l. infections were considered, there was no significant effect of grazing regime on the prevalence of trypanosome infections when the model included all households from Busia District (Table 4.6). However, when only households from within the sampling area in Butula Division were considered, the effect of grazing regime on the prevalence of households with at least one *T. brucei* s.l. infected animal (adjusted for herdsize, $F_{1,331}=15.3$, $p<0.001$) approached significance ($F_{1,331}=4.6$, $p=0.034$) with a lower likelihood of infection for households, which confined their animal at home for grazing. In the households sampled in Funyula Division, the opposite was observed, with a higher number of households confining their animals at home, owning at least one animal infected with *T. brucei* s.l. , however this effect, adjusted by herdsize ($F_{1,175}=14.6$, $p<0.001$) was not significant ($F_{1,175}=0.2$, $p=0.633$). There was no significant effect of grazing management on the prevalence of household herds infected with *T. vivax, T. simiae* or *T. b. rhodesiense* (Table 4.6).

Table 4.6: Effect of grazing regime for all livestock on the risk of infection at the household level in Busia (output from mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number of livestock per household: 1st fixed effect	Effect of grazing regime (home/away): 2nd fixed effect
T. brucei s.l	$F_{1,508}=30.3$, p<0.001	$F_{1,508}=1.7$, p=0.189
T. vivax	$F_{1,507}=45.4.$, p<0.001*	$F_{1,507}=0.0$, p=0.866*
T. simiae	$F_{1,508}=16.0$, p<0.001	$F_{1,508}=0.2$, p=0.681
T.b. rhodesiense	$F_{1,508}=17.5$, p<0.001	$F_{1,508}=1.4$, p=0.241
Total trypanosomiasis	$F_{1,508}=41.5$, p<0.001	$F_{1,508}=1.29$, p=0.257

* interaction effect: $F_{1,507}=4.2$, p=0.04

Cattle: Of 387 cattle keeping households with grazing details, 295 (76.2%) accounting for 801 out of the 1192 cattle samples (67.2%), fed their cattle within their compound. There was no effect of the grazing regime employed for cattle on the likelihood of a household having at least one infected cow/bull regardless of whether the model was constructed for overall trypanosomiasis or individual trypanosome species (Table 4.7).

Table 4.7: Effect of grazing regime for cattle on the risk of infection at the household level in Busia (output from mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number of cattle per household: 1st fixed effect	Effect of grazing regime (home/away): 2nd fixed effect *
T. brucei s.l	$F_{1,366}=21.5$, p<0.001	$F_{1,366}=0.451$, p=0.502
T. vivax	$F_{1,366}=23.9$, p<0.001	$F_{1,366}=0.267$, p=0.605
T. simiae	$F_{1,366}=7.5$, p<0.01	$F_{1,366}=1.939$, p=0.165
T.b. rhodesiense	$F_{1,366}=18.5$, p<0.001	$F_{1,366}=3.091$, p=0.08
Total tryps	$F_{1,365}=25.1$, p<0.001	$F_{1,365}=0.03$, p=0.864

* There was no significant interaction effect of 1st and 2nd fixed in any of the models, hence only the outputs of the reduced models were presented.

Small ruminants: Of 325 households keeping small ruminants with grazing details recorded, 265 households accounting for 877 out of the 1140 small ruminants (76.9%), grazed these animals within their compound. The effect of grazing regime of small ruminants employed by a household on the risk of at least one trypanosome infection being detected in one of their goats or sheep, approached statistical significance, when the model was adjusted for the number of small ruminants per herd (Table 4.8), with a higher risk for households which confined their small ruminants within the compound.

Table 4.8: Effect of grazing regime for small ruminants (smR) on the risk of infection at the household level in Busia (output: mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number smR per household: 1st fixed effect	Effect of watering regime (home/away): 2nd fixed effect
T. brucei s.l	$F_{1,303}=7.9$, p<0.01*	$F_{1,303}=0.0$, **p=0.907***
T. vivax	$F_{1,304}=7.9$, p<0.01	$F_{1,304}=6.8$, **p<0.01**
Total trypanosomiasis	$F_{1,304}=9.5$, p<0.01	$F_{1,304}=3.7$, **p=0.057**

* interaction: $F_{1,303}=4.0$, p=0.047

The effect of grazing regime of small ruminants on the likelihood of *T. brucei* s.l. infections for the household herd of small ruminants was non-significant (Table 4.8), whereas the effect on the likelihood of being affected by *T. vivax* was significant (Table 4.8) with a higher prevalence being detected amongst households which confined the small ruminants to the immediate compound (Figure 4.16). No separate models were constructed for *T. simiae* or *T. b. rhodesiense* as only one infection of each was detected in small ruminants in total.
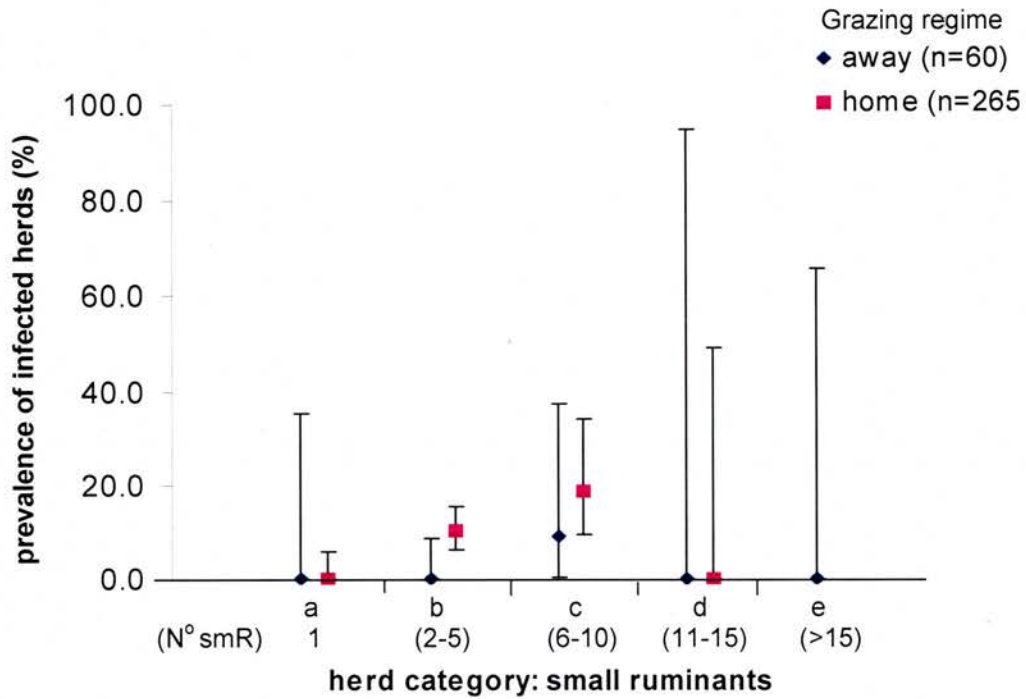
Figure 4.16: Fraction of herds infected with *T. vivax* according to grazing regime (home/away) considering only small ruminants (smR) (herd size was categorised for display purpose).

4.3.5.2 Watering regime

All livestock: Of all 529 households with information on the watering regime employed, 415 households (78.4%), accounting for 1805 out of the 2642 livestock samples (68.3%), watered their animals within their compound. Including all livestock keeping households from the two study areas, the watering regime recorded had an effect on the risk of trypanosomiasis in the household herd which approached statistical significance (Table 4.9) with a lower risk of infection for households which water their animals at home rather than taking them away to the river.
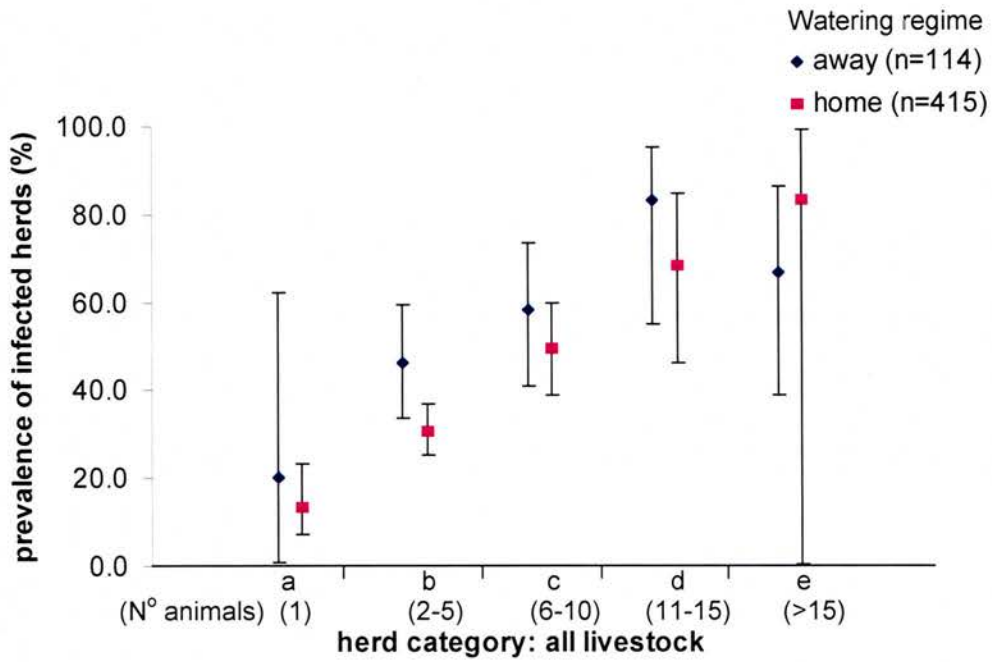
Figure 4.17: Fraction of herds infected with trypanosomiasis according to watering regime (home/away) considering all livestock (herd size was categorised for display purpose).

When only *T. brucei* s.l. infections were considered in the model, the effect of watering regime on the risk of infection in the household herd, adjusted for herd size, also approached significance, with a lower risk of infection at home (Table 4.9). However this effect was not consistent across both sampling areas within Busia District. Considering only households sampled in Butula Division, the effect of watering on prevalence of households with at least one *T. brucei* s.l .infected animal was significant, again with a lower risk for households that watered their animal at home (herdsize: $F_{1,330}=14.9$, $p<0.001$; watering: $F_{1,330}=12.7$, $p<0.001$), whereas in Funyula this was inverted, with a higher proportion of households that watered their animals at home, having at least one *T. brucei* s.l. infected animal, but this effect in Funyula was not significant (herdsize: $F_{1,175}=14.4$, $p<0.001$; watering: $F_{1,175}=5.0$, $p=0.191$).

The effect of watering regime, adjusted for herdsize, on the prevalence of *T. vivax* infected households also approached significance (Table 4.9), with a higher infection risk for households taking their animals away from the homestead for watering. This trend in risk of *T. vivax* infections was consistent for households from both Funyula and Butula, but the effect also only approached significance in Butula (herdsize: $F_{1,330}=22.6$, $p<0.001$; watering: $F_{1,330}=4.1$, $p=0.043$; interaction: $F_{1,330}=9.8$, $p<0.01$), and was non significant in Funyula (herdsize: $F_{1,175}=17.2$, $p<0.001$; watering: $F_{1,507}=0.7$, $p=0.417$). There was no effect of watering regime on either the risk of *T. simiae* infections or the risk of *T. b. rhodesiense* infections (Table 4.9).

Table 4.9: Effect of watering regime for all livestock on the risk of infection at the household level in Busia (output from mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number of livestock per household: 1st fixed effect	Effect of watering regime (home/away): 2nd fixed effect	interaction
T. brucei s.l	$F_{1,507}=29.8$, $p<0.001$	$F_{1,507}=5.0$, $p=0.025$	$F_{1,507}=3.9$, $p=0.049$
T. vivax	$F_{1,507}=42.5$, $p<0.001$	$F_{1,507}=4.1$, $p=0.044$	$F_{1,507}=8.4$, $p<0.01$
T. simiae	$F_{1,508}=15.1$, $p<0.001$	$F_{1,508}=1.3$, $p=0.259$	N/A
T.b. rhodesiense	$F_{1,508}=16.8$, $p<0.001$	$F_{1,508}=0.0$, $p=0.98$	N/A
Total tryps	$F_{1,507}=44.0$, $p<0.001$	$F_{1,507}=5.0$, $p=0.026$	$F_{1,507}=8.8$, $p<0.01$

Cattle: 276 out of 387 cattle keeping households considered (71.3%), accounting for 758/1192 (63.6%) of cattle samples, watered their cattle at home. There was no significant effect of watering regime on the proportion of households with trypanosome infections, when cattle were the only livestock species considered, regardless of whether trypanosome species were analysed jointly or separately (Table 4.10).

Table 4.10: Effect of watering regime for cattle on the risk of infection at the household level in Busia (output from mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number of cattle per household: 1st fixed effect	Effect of watering regime (home/away): 2nd fixed effect
T. brucei s.l	$F_{1,366}=21.1$, p<0.001	$F_{1,366}=2.9$, p=0.089
T. vivax	$F_{1,365}=20.5$, p<0.001*	$F_{1,365}=0.2$, p=0.648 *
T. simiae	$F_{1,366}=6.2$, p=0.013	$F_{1,366}=0.6$, p=0.452
T.b. rhodesiense	$F_{1,366}=14.0$, p<0.001	$F_{1,366}=0.1$, p=0.745
Total trypanosomiasis	$F_{1,366}=24.5$, p<0.001	$F_{1,366}=0.8$, p=0.376

* interaction effect: $F_{1,365}=8.0$, p<0.01

Small ruminants: Of the 325 households with small ruminants, 273 households (84%), accounting for 925/1140 (81%) of sheep and goats watered them at home. There was no significant effect of watering regime on the proportion of households with trypanosome infections, when sheep and goats were the only livestock species considered, regardless of whether trypanosome species were analysed jointly or separately (Table 4.11).

Table 4.11: Effect of watering regime for small ruminants on the risk of infection at the household level in Busia -output from mixed effect model with village of origin as random effect.

Trypanosome species	Effect of number smR per household: 1st fixed effect	Effect of watering regime (home/away): 2nd fixed effect *
T. brucei s.l	$F_{1,304}=5.5$, p=0.02	$F_{1,304}=0.8$, p=0.379
T. vivax	$F_{1,304}=6.5$, p=0.011	$F_{1,304}=0.3$, p=0.573
Total trypanosomiasis	$F_{1,304}=8.9$, p<0.01	$F_{1,304}=1.3$, p=0.263

* no significant interactions between number of small ruminants and watering regime

4.3.6 Overall management

All livestock: Considering the overall management of livestock within each household, 70.1% (371/529) of households, accounting for 68.3% (1805/2642) of livestock samples, kept their animals within the confines of the compound. The risk of having a trypanosome infected animal in the herd was significantly lower for these

households, when adjusted for herdsize (herdsize: $F_{1,507}=45.055$, $p<0.001$; management: $F_{1,507}=9.925$, $p<0.01$; interaction: $F_{1,507}=5.36$, $p=0.021$) (Figure 4.18).
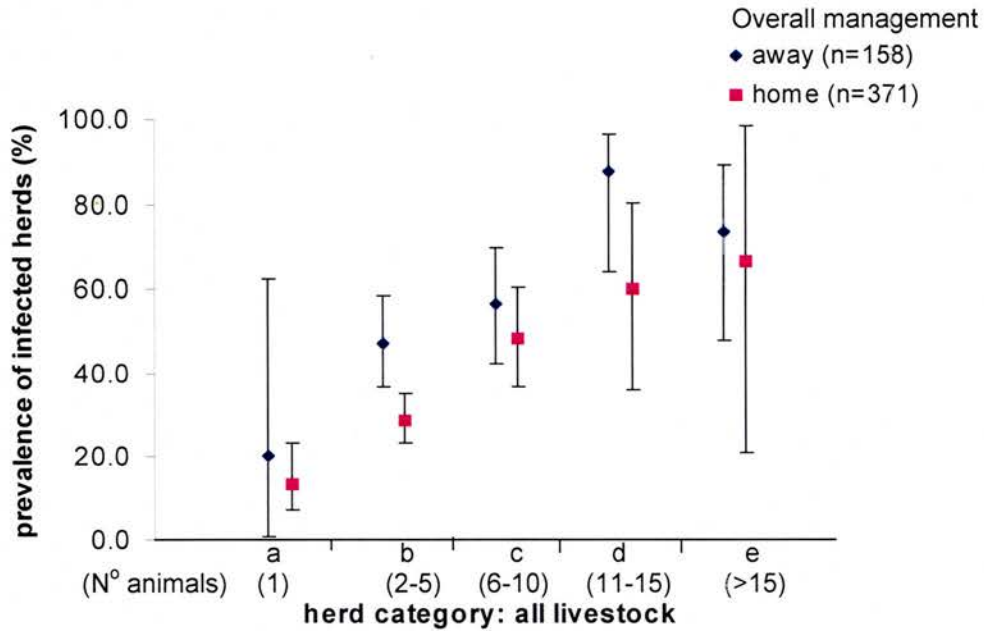
Figure 4.18: Fraction of herds infected with trypanosomiasis according to overall management (home/away) considering all livestock (by herd size category).

This effect was also seen when only *T. brucei* s.l. infections or only *T. vivax* infections were considered in the model (Table 4.12). However, for *T. brucei* s.l., the trend was not consistent across both sampling areas within Busia, with households in Funyula that maintained all their livestock at home having a marginally higher risk of infection, even though this effect was not significant (herdsize: $F_{1,175}=14.673$, $p<0.001$; management: $F_{1,175}=0.529$, $p=0.468$), whereas such households had a significantly lower risk of *T. brucei* s.l. infections in the Butula sampling area (herdsize: $F_{1,331}=14.748$, $p<0.001$; management: $F_{1,331}=13.423$, $p<0.001$).

There was no significant effect of overall management of livestock on the likelihood of infection with *T. simiae* or *T. b. rhodesiense* for a household herds (Table 4.12).

Table 4.12: Effect of overall management of all livestock on the risk of infection at the household level in Busia (output from mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number of livestock per household: 1st fixed effect	Effect of grazing regime (home/away): 2nd fixed effect	interaction
T. brucei s.l	$F_{1,508}=29.6$, $p<0.001$	$F_{1,508}=7.4$, $p<0.01$	N/A
T. vivax	$F_{1,507}=43.1$, $p<0.001$	$F_{1,507}=7.1$, $p<0.01$	$F_{1,507}=6.2$, $p=0.013$
T. simiae	$F_{1,508}=15.7$, $p<0.001$	$F_{1,508}=0.2$, $p=0.697$	N/A
T.b. rhodesiense	$F_{1,508}=16.8$, $p<0.001$	$F_{1,508}=0.2$, $p=0.638$	N/A
Total tryps	$F_{1,507}=45.1$, $p<0.001$	$F_{1,507}=9.9$, $p=0.01$	$F_{1,507}=5.4$, $p=0.021$

Cattle: Of all 387 cattle keeping households which provided information on the management of their animals, 237 (61.2%), accounting for 50.1% of cattle sampled, maintained their cattle within the confines of the compound for both watering and grazing. There was no significant effect of overall management on the prevalence of trypanosomiasis infected households when cattle were the only livestock species considered in the model (herdsize: $F_{1,366}=24.5$, $p<0.001$; management: $F_{1,366}=1.2$, $p=0.279$). The effect on the individual trypanosome species considered separately was summarized in Table 4.13.

Table 4.13: Effect of overall management of cattle on the risk of infection at the household level in Busia (output from mixed effect model with village of origin as random effect).

Trypanosome species	Effect of number of cattle per household: 1st fixed effect	Effect of overall management (home/away): 2nd fixed effect
T. brucei s.l	$F_{1,366}=20.7$, $p<0.001$	$F_{1,366}=4.3$, $p=0.039$
T. vivax	$F_{1,365}=23.1$, $p<0.001$*	$F_{1,365}=0.3$, $p=0.57$*
T. simiae	$F_{1,366}=7.5$, $p<0.01$	$F_{1,366}=0.2$, $p=0.689$
T.b. rhodesiense	$F_{1,366}=14.1$, $p<0.001$	$F_{1,366}=0.2$, $p=0.657$
Total trypanosomiasis	$F_{1,366}=24.5$, $p<0.001$	$F_{1,366}=1.2$, $p=0.279$

* interaction: $F_{1,365}=3.7$, $p=0.054$

A lower prevalence of *T. brucei* s.l. infected herds were detected amongst households that kept their cattle within the compound than those that took cattle out with the compound for either grazing or watering, however this effect only approached significance (Table 4.13). This effect was also not consistent across both sampling areas within Busia. In Funyula, there was a trend for a higher proportion of infected households amongst those that maintained their cattle within the compound, but this trend was not statistically significant (herdsize: $F_{1,117}=7.9$, p<0.01; management: $F_{1,117}=0.1$, p=0.727), whereas in Butula the prevalence of *T. brucei* s.l. infections was significantly higher for households taking their cattle outside their compound for either grazing or watering (herdsize: $F_{1,247}=13.8$, p<0.01; management: $F_{1,247}=6.7$, p<0.01). There was no significant effect of overall management of cattle in any other of the models considering individual trypanosome species (Table 4.13).

Small ruminants: A total of 239 out of the 325 households with small ruminants (73.5%) providing information on grazing and watering, accounting for 793 of the total of 1140 of these animals (70%), managed their sheep and goats within the compound. There was no significant effect of the overall management of small ruminants, on the prevalence of infected households, regardless of which trypanosome species was considered (Table 4.14).

Table 4.14: **Effect of overall management of small ruminants on the risk of infection at the household level in Busia -output from mixed effect model with village of origin as random effect.**

Trypanosome species	Effect of number smR per household: 1st fixed effect	Effect of overall management (home/away): 2nd fixed effect
T. brucei s.l	$F_{1,303}=7.6$, p<0.01*	$F_{1,303}=0.1$, p=0.716*
T. vivax	$F_{1,304}=6.2$, p=0.013	$F_{1,304}=2.4$, p=0.121
Total trypanosomiasis	$F_{1,304}=9.2$, p<0.01	$F_{1,304}=1.9$, p=0.17

* interaction: $F_{1,303}=4.8$, p=0.03.

4.3.7 Summary of management factors at the household level

The **number of animals** per household, included as first fixed effect in all models, was a **significant** predictor of household infection status.

Grazing regime

6. **All livestock** - there was **no significant** effect of grazing regime.

7. **Cattle** - there was **no significant** effect of grazing regime.

8. **Small ruminants** - effect **approached significance**, with a higher trypanosome prevalence in households grazing their sheep and goats within confines of compound.

Watering regime

- **All livestock** - there was a **lower prevalence** of trypanosome infection amongst households watering their animals at home, with the effect **approaching significance.**

- **Cattle** - there was **no significant** effect of watering regime.

- **Small ruminants** - there was **no significant** effect of watering regime.

Overall management

- **All livestock** - there was a **significantly lower risk** of infection for animals managed at **home** for both grazing and watering.

- **Cattle** – there was **no significant** effect of overall management.

- **Small ruminants** - there was **no significant** effect of overall management.

4.4 Discussion

4.4.1 Study design

Cross-sectional designs such as used in this study, by definition capture a snapshot in time and the data obtained can only be used to find permanent associations between disease and the factors in question rather than being able to establish causal relationships or identify disease triggers (Dohoo *et al.*, 2001). However data obtained from cross-sectional studies can be used to investigate the association of infection status of a host and the simultaneous presence of clinical signs (Thrusfield, 1995). Furthermore, a cross-sectional design was deemed appropriate for this study as the other factors under investigation were of a permanent nature (host species, gender) or were thought to have a cumulative effect (exposure to tsetse related to management routine) (Rowlands *et al.*, 1993). Despite some restrictions of cross-sectional study frames, useful information on a variety of potential risk factors and indicators for trypanosomiasis could thus be gained for mixed livestock systems. The extensive cross-sectional data set collected through census sampling, achieved a coverage of over 85% of the total livestock population of the sampling areas and allowed a detailed analysis of the trypanosome reservoir at the micro-scale.

4.4.2 Animal level factors

4.4.2.1 Host species

At the animal level, livestock species was a significant factor determining the risk of animal trypanosomiasis as detected by PCR. The highest prevalence of around 20% overall trypanosomiasis infections was found in cattle. The lowest overall prevalence, at under 4%, was found in small ruminants, with no significant differences in prevalence between sheep and goats. The level of trypanosomiasis found in pigs was the most variable between the two sampling areas within Busia District, with a prevalence of 17.4% detected in Funyula Division as opposed to only 8.4% in Butula Division resulting in an overall prevalence of 11.5%. These results confirm other studies in Western Kenya and Eastern Uganda, comparing the level of trypanosomiasis in different species of livestock albeit using microscopy (Angus,

1996; Waiswa *et al.*, 2003). In these studies cattle were identified as the most important reservoir of trypanosomiasis. Very low levels of infection were detected in small ruminants and highly variable infection prevalence depending on sampling sites were seen in pigs (2-20%) (Angus, 1996; Waiswa *et al.*, 2003).

A variety of possible explanations for the differences in trypanosome prevalence detected between different host species have been proposed. Inherent reduced susceptibility of small ruminants to trypanosome infections resulting in low or transient parasitaemia has been suggested (Murray *et al.*, 1982). Also, tsetse have been shown to be opportunistic feeder with the predominant source of their bloodmeal reflecting the fauna of their habitat (Staak *et al.*, 1986). In Busia, Kenya and Uganda the major sources of bloodmeals of both *Glossina fuscipes fuscipes* and *Glossina pallidipes* identified by ELISA were cattle, reptiles and pigs (Okoth & Kapaata, 1988; Welburn *et al.*, 1995; Wamwiri *et al.*, 2007). The lower prevalence of trypanosomiasis in small ruminants may thus be attributed to a low attractiveness to tsetse or due to a lower total biomass as compared to other potential hosts such as cattle or pigs (Staak *et al.*, 1986).

Looking at individual trypanosome species, under 0.3% of all animals were infected with *T. congolense*. These were included in the overall trypanosome infection prevalence but were not analysed separately. Other studies conducted in Busia, corroborate low *T. congolense* infection rates of under 3% in cattle and close to 0% in small ruminants and pigs (Angus, 1996; Karanja, 2006). In cattle and small ruminants, *T. vivax* was the most prevalent trypanosome species detected. In pigs, *T. brucei* s.l. infections predominated, with a similar prevalence to that found in cattle. *T. simiae* prevalence was low (<1.5%) in all host species.

4.4.2.2 *T. b. rhodesiense* reservoir

The prevalence of the human infective subspecies *T. b. rhodesiense* detected in pigs (2.9%) was not significantly different to that detected in cattle (1.5%). In Eastern Uganda, cattle has long been recognised as the most important reservoir of *T. b. rhodesiense* (Hide *et al.*, 1994; Hide *et al.*, 1996), with up to 18% of cattle infected in an epidemic focus (Welburn *et al.*, 2001b). The comparatively low prevalence of *T. b. rhodesiense* detected in cattle and pigs in Busia, Kenya during the current study nevertheless poses a threat to human health, as was demonstrated by a case of sleeping sickness reported from Busia in early 2006. In fact, a similarly low prevalence of *T. b. rhodesiense* was found by the author in cattle sampled recently in three districts newly affected by sleeping sickness in central Uganda, where over 300 sleeping sickness cases have been reported between August 2004 and 2006 (data unpublished). It remains uncertain which mechanisms create the difference in transmission to humans, between a low endemic area with only occasional human cases such as Busia, Kenya and a sleeping sickness outbreak such as the one currently observed in central Uganda (Berrang-Ford *et al.*, 2006).

Being infective to humans in addition to animals should confer a competitive advantage to *T. b. rhodesiense* over *T. b. brucei* according to a mathematical model of vector-borne transmission of multi-host trypanosomes (Coleman & Welburn, 2004). However, field observations have shown that *T. b. brucei* was by far more prevalent in the animal reservoir than *T. b. rhodesiense*. This implied that the trait of human serum resistance was associated with fitness costs to *T. b. rhodesiense* outside the human host (Coleman & Welburn, 2004). On average the ratio of *T. b. rhodesiense*: *T. b. brucei* in the animal reservoir was 1 in 3 (Coleman & Welburn, 2004). In the current study the ratio of *T. b. rhodesiense* to *T. b. brucei* in cattle was lower than the expected average at approximately 1 in 5. However, the ratio in pigs was considerably higher, with *T. b. rhodesiense* being detected in 1 out of 2 animals with *T. brucei* s.l. infections. This apparent imbalance of the human infective parasite in the animal reservoir, may be a function of Busia being located at the fringe of the Busoga sleeping sickness focus. Pigs have become increasingly popular in Busia

over recent years, with an increase in the pig population of over 150% being recorded within the span of 1 year in 2002 (Thuranira, 2005). As *T. b. rhodesiense* appeared to have less of a disadvantage to *T. b. brucei* in the pig reservoir than in the cattle reservoir, a continued increase in pig numbers may increase the risk of sleeping sickness to the human population in Busia.

4.4.2.3 Gender

Previous studies suggested that trypanosomiasis infections were more prevalent in male cattle, due to increased odour and size related attractiveness to tsetse (Vale, 1974; Torr *et al.*, 2006) and increased occupation related exposure to tsetse (Rowlands *et al.*, 1993; Angus, 1996). However, gender did not significantly affect trypanosome prevalence in cattle or any of the other livestock species investigated in the present study. This may be a reflection of few farmers making use of traction power in Busia, Kenya at present and thus only few of the large adult oxen being present in the sampling frame which would be at risk of increased tsetse challenge (Thuranira, 2005).

4.4.2.4 Cattle age

The effect of age on the prevalence of trypanosomiasis was only investigated for cattle as no data had been collected regarding age for small ruminants or pigs. Trypanosome prevalence in cattle was shown to increase with age, with the lowest prevalence (13%) in animals under 18 months of age, as compared to over 25% in cattle over 36 months of age. This increase was observed for both *T. brucei* s.l. and *T. vivax*. *T.b. rhodesiense* was also predominantly detected in the oldest group. The low prevalence of trypanosomiasis, observed in young cattle, has previously been explained by either an inherent resistance to trypanosome infections (Trail *et al.*, 1994), or lower tsetse challenge due to tsetse feeding preferences (Torr & Mangwiro, 2000; Torr *et al.*, 2006) and management of calves separate from the rest of the herd (Rowlands *et al.*, 2001). However, as animals remain infected unless treated, the probability of having a trypanosome infection may simply increase cumulatively

with time spent exposed to tsetse. Nonetheless, age could provide a powerful indicator of the likelihood of trypanosome infection in an animal.

4.4.2.5 Condition and anaemia score

Anaemia status judged by pallor of mucous membranes and condition score of cattle are measures readily available to animal health staff or even livestock owners in a resource poor rural area, and are considered predominant clinical signs of trypanosome infections (Magona et al., 2003b; Eisler et al., 2007). However, neither condition score nor anaemia score provided a good indicator for the presence of trypanosome infections in the present study. No association of low condition score with overall trypanosomiasis prevalence was detected. It was not surprising that *T. brucei* s.l. was not associated with poor body condition as this trypanosome species is widely thought to cause only mild pathology in livestock (Taylor & Authié, 2004). *T. vivax* on the other hand, is generally associated with severe pathology in cattle, but there was no significant effect of body condition score on *T. vivax* prevalence detected. Whilst there was a trend for a higher prevalence of trypanosome infected animals in the anaemic than in the normal category, less than 20% of trypanosome infected cattle were actually classified as anaemic according to the colour of their mucous membranes. This may either be attributed to a certain degree of trypanotolerance in zebu cattle in Busia, resulting in sub-clinical infections, or insufficient sensitivity of visual examination of mucous membranes to detect anaemia as has been reported in human studies (Shulman et al., 2001).

Whilst future studies could investigate the relationship between trypanosome infections and level of anaemia in cattle using more sophisticated methodology, e.g. pen-side tests determining haemoglobin values, such expensive diagnostic tests will not be available to veterinary clinicians or animal health workers in Busia. With anaemia and weightloss judged the most predominant signs of cattle trypanosomiasis (Eisler et al., 2007), but these signs only present in a minority of infected animals, a large number of cattle carrying trypanosome infections may not be identified as such. PCR, used in the present study, is very sensitive and may detect trypanosome

infections in animals in which the parasitaemia is too low to be of clinical relevance, however tsetse feeding on these animals may nevertheless become infected (Van den Bossche *et al.*, 2004). Hence, an untreated reservoir of trypanosomes may be preserved providing the source of re-infection for treated and healthy animals, thus preventing long-term impact on trypanosomiasis prevalence.

4.4.3 Herd level factors

4.4.3.1 Limitations of herd level analysis

In rural Busia (Kenya) mixed crop-livestock farming is the predominant form of livelihood and a high proportion of homesteads included in the sampling frame only owned one or two animals of a particular species, or even in total. As these types of households were considered quintessential to the sampling area, the author was reluctant to exclude such households from the analysis. Exclusion would have been necessary to ensure adequate fit of mixed effect models for proportion data at the household level. Instead, herd infection rates were collapsed to the binary level, with herds described as infected or non-infected. This resulted in a loss of detail in data from within each herd, but this was deemed acceptable as it allowed inclusion of all livestock owning homes, thus avoiding bias towards wealthier households owning more animals. The number of animals per household was accounted for by inclusion as first fixed effect and was shown to have a highly significant effect on the probability of herd infection.

4.4.3.2 Grazing and watering regime

Previous studies have demonstrated that the trypanosome prevalence detected in livestock varied significantly according to the grazing routes used and the type of watering places frequented. Natural watering sites at rivers were shown to be transmission hotspots (Wacher *et al.*, 1994; de La Rocque *et al.*, 1999) and cattle and small ruminants tethered for grazing within the village had a lower probability of becoming infected (Waiswa *et al.*, 2003; Karanja, 2006). In the current study the majority of households (70.1%), accounting for 68.3% of livestock, claimed to

confine their animals to the immediate surrounding of their compound, providing the animals with feed and water *in situ*. There appeared to be a significant protective effect of this strategy, with herds maintained at home having a lower likelihood of being infected with trypanosomiasis, when herdsize was taken into account. However, management strategy was confounded by the livestock species owned. 95% of households that took their animals away for grazing or watering owned cattle, whereas over 35% of households that maintained their animals on their compound did not own cattle. As cattle in general had a higher infection prevalence than the other livestock species, herds including cattle had a higher chance of being infected. The higher likelihood of infection for herds taken outside the confines of the homestead was most likely due to a higher proportion of these herds incorporating cattle. When the effect of overall management was assessed separately for cattle there was no longer a significant protective effect of confinement to the homestead.

Similarly, for small ruminants, there was no punitive effect of grazing away from the homestead compound. This did not agree with observations that the low prevalence of trypanosomiasis often reported in small ruminants was solely attributable to a lower exposure to tsetse habitats, and that small ruminants sharing the grazing grounds with cattle had a higher trypanosomiasis prevalence (Vale, 1977; Waiswa *et al.*, 2003). In fact, when grazing regimes were considered separately, small ruminant herds that were confined to the compound had a higher probability of trypanosome infections, than those taken out for grazing, an effect which was significant for *T. vivax* infections and approached significance for overall trypanosome infections. It is possible that the level of feed provided for some of the small ruminant herds within the household compounds was insufficient, and that the nutritional stress predisposed them to trypanosome infections (Osaer *et al.*, 1999a).

Overall, the data collected on management regimes in the present cross-sectional study did not provide convincing evidence that confining animals within the homestead compounds decreased the likelihood of animals becoming infected. In

fact, of a total of 313 livestock samples detected to be trypanosome infected over 50% (173) belonged to animals which did not leave the immediate vicinity of their homestead. This suggested that trypanosome transmission to livestock in Busia occurred in the peri-domestic environment at considerable frequency. Many households were observed to be surrounded by artificially planted and/or maintained hedges, which demarcated boundaries, and provided shade as well as convenient tethering points for domestic livestock. These hedges may also provide shade and resting places required for a suitable habitat for tsetse, in close vicinity to easily available hosts (Okoth, 1986). In many cases, the shrubs surrounding homesteads were recognised to be *Lantana camara*, plants which have been reported to be particularly attractive to tsetse as resting places (Syed & Guerin, 2004).

Close interaction with tsetse not only puts livestock, but also humans at risk of trypanosomiasis, in particular as the human infective *T. b. rhodesiense* has been identified in the livestock reservoir in this study, albeit at a low prevalence. Peri-domestic transmission of sleeping sickness has previously been identified as a problem in West African sleeping sickness foci (Gouteux *et al.*, 1993). *G. pallidipes*, which is present at low apparent densities in Busia (Karanja, 2006), is a savannah tsetse species, which can spread through areas of scattered thicket, woodland and even open country in mild conditions (Pollock, 1982). The predominant tsetse species in Busia, *G.f. fuscipes* , has previously been reported to have colonised peri-domestic bush thickets in Western Kenya (Alego, Central Nyanza), away from its usual riverine habitat (Bertram, 1969). At the time, the high number of sleeping sickness cases reported during the Alego outbreak, was attributed to this peri-domestic infestation increasing the tsetse-man contact and thus also transmission (Baldry, 1972).

4.5 Conclusion

Of all livestock species investigated, cattle were the predominant reservoir of trypanosome infections. However, the infection prevalence in pigs was also

substantial, and inclusion of pigs as targets for trypanocidal treatment should be seriously considered, even if the monetary value of individual pigs does not seem to warrant such input, to reduce re-infection of cattle. Small ruminants appear to be less significant reservoirs of trypanosomiasis. The human infective *T.b. rhodesiense* was detected to similar extent in both cattle and pigs, which adds a human health dimension to trypanosomiasis, which is currently merely regarded as a livestock production constraint in Busia, Kenya, for which the individual farmer carries responsibility.

Within the cattle population, age was the most important factor affecting trypanosome prevalence. Anaemia status as assessed by pallor of visible mucous membranes and condition score were un-suitable indicators, as the majority of trypanosome infections in cattle (~80%) were not associated with these clinical signs. This suggested that a high percentage of infected animals potentially carry sub-clinical trypanosome infections, which remain undiagnosed and hence untreated. Such a "silent" reservoir would make depression of trypanosomiasis through targeted treatments unfeasible in Busia, as a reservoir for re-infection would be maintained. For a long-term sustainable decrease in livestock trypanosomiasis, a blanket approach eliminating the sub-clinical reservoir and/or targeting the tsetse vector would be required, rather than only limiting production losses by treating acute cases.

The management practise of maintaining livestock within the immediate vicinity of the homestead rather than taking animals for grazing on communal land and watering at the river is widespread in the sampling areas of Busia, in particular for small herds. However such management appeared to provide only a very limited protective effect against trypanosome infections in livestock. Evidence of a considerable proportion of infections having been acquired by livestock maintained on homestead compounds, pointed towards an important element of peri-domestic transmission in the epidemiology of trypanosomiasis in Busia.

5 Chapter 5   Spatial distribution of trypanosome infections

5.1 Introduction

5.1.1 Spatial epidemiology of infectious diseases

Spatial analysis is an important tool in epidemiological investigations of infectious diseases. Infectious diseases have different modes of transmission but are in general less likely to be transmitted the further the distance between hosts. Spatial dimension are therefore of immediate importance to the epidemiology of infectious disease (Ostfeld *et al.*, 2005). In its simplest form, mapping of the geographical location of cases can aid in visualising the spatial dimension of a disease and recognising patterns that may help identifying causes or risk factors. One of the earliest examples of the application of spatial analysis, is John Snow's investigation into the causes of the 1854 London cholera outbreak. Famously, mapping of cholera cases allowed identification of a contaminated public water pump in Broad Street located centrally to the cluster of cases as the source of the outbreak (Snow, 1855; Eyler, 2001).

In the 1930s, Pavlovsky developed concepts of landscape epidemiology, in which he stated that most diseases are restricted in their geographical extent and that this spatial variation in disease occurrence could be attributed to variations in the underlying physical or biological conditions, which are required to support the pathogen. If these biotic and abiotic factors were known and could be delineated, it would be possible to predict current and future distribution of a disease (Pavlovsky, 1966). Technology for remote sensing of environmental data has become increasingly available and the advent of Geographic Information Systems (GIS) facilitated integration of any type of data with a spatial component into increasingly complex models. With these advances, landscape epidemiology has been rediscovered as an invaluable tool for the construction of risk maps (Kitron, 1998). The concept of environmental risk maps for the prediction of disease are of particular relevance to vector borne disease which occur only where the appropriate habitat for vector, host and pathogen overlap (de La Rocque *et al.*, 1999), although such an appropriate habitat by no means ensures the presence of all three.

Disease maps on a global, continental or countywide scale, whether based on reported cases of disease, or constructed from predictions of disease risk based on host and/or vector distribution, can be used in the allocation of health resources, for treatment or prevention of the disease in question (Robinson, 1998; de La Rocque *et al.*, 2004; Courtin *et al.*, 2005). As previously mentioned, disease by no means occurs in all geographic habitats which may be conducive to the disease, and at the same time the spatial occurrence of a disease may change as environmental conditions alter (Rogers & Randolph, 2003). Investigation of heterogeneity of disease prevalence on a micro-scale may give insights into disease dynamics, which could be of particular relevance in unstable disease foci.

The study area, Busia, Kenya is endemic for animal trypanosomiasis, and has a history of human African trypanosomiasis (Wellde *et al.*, 1989c), with sporadic cases occurring at present, the most recent case having been reported in 2006 (WHO, 2006). Conditions are thus potentially appropriate for the local transmission of sleeping sickness in humans. An investigation into the spatial distribution of infected animal hosts could pinpoint protective factors or risk factors, influencing disease transmission. The aims of this chapter are:

(1) to identify any heterogeneity of infection rates of animal trypanosomiasis on a local scale

(2) to investigate the spatial distribution of the animal reservoir of the human infective trypanosome sub-species *T. brucei rhodesiense* and

(3) to analyse the influence of the proximity of homesteads to water features such as streams and swamps, which have been indicated as risk factors for animal and human trypanosomiasis in previous studies (de La Rocque *et al.*, 1999; Michel *et al.*, 2002; Odiit *et al.*, 2006) on the prevalence of trypanosomiasis in the homestead herd.

The following section provides an overview of the applications and findings of spatial analysis in the field of trypanosomiasis to date. The second section

specifically discusses the background to spatial cluster detection, which forms the basis of a major part of the statistical analysis of this chapter.

5.1.2 Application of spatial analysis in vector borne diseases with particular reference to trypanosomiasis

5.1.2.1 Vector distribution

A popular approach to investigating the spatial epidemiology of vector borne diseases is to map the distribution of the vector itself. Entomological surveys provide the necessary data for constructing these maps. Accumulation of such survey data, frequently acquired from anonymous contributors, forms the basis of tsetse distribution maps, which are still in use today (Ford & Katando, 1973). The work and time effort required is prohibitive for regular entomological surveys on a large scale, necessary to obtain country or continent wide distribution maps, in particular as vector populations are by no means static.

Whilst arthropod vectors are known to be sensitive to climatic conditions, prediction of the likely occurrence and abundance of vectors such as the tsetse fly, requires meteorological data at a precision, which can not necessarily be provided by meteorological stations, of which there is only a total of 362 (or 1 per 28,000km^2) in African tsetse belt (Rogers & Randolph, 1991). Remote sensing can provide high resolution climatic and vegetation data from satellite imagery. Remote sensing has first been applied in studies on schistosomiasis (Malone *et al.*, 1997), malaria (Beck *et al.*, 1994), tick-borne diseases (Lessard *et al.*, 1990; Kitron *et al.*, 1992) and trypanosomiasis (Rogers & Randolph, 1991) followed by other vector borne diseases. Satellites providing global data on environmental conditions are reviewed in detail elsewhere (Rogers & Randolph, 2003). Briefly, remotely sensed data is available at varying temporal and spatial resolutions. Rainfall data can be deduced from indices such as the Cold Cloud Duration (CCD), whereas the Normalised Difference Vegetation Indices (NDVIs) is a popular source for vegetation data (Thomson *et al.*, 1997). Good association between various NDVI measures and tsetse presence and abundance, as determined through entomological surveys, has

been recorded in the literature (Rogers & Randolph, 1993; Kitron *et al.*, 1996; Hendrickx *et al.*, 1999b; Kitron, 2000; Hendrickx *et al.*, 2001; Bouyer *et al.*, 2006). However specific measures yielding the best predictions vary between tsetse species and between countries for which the various models were constructed. Remotely sensed data can thus complement field based entomological surveys, extending available data through interpolation, but can, as yet, not replace ground collected data.

5.1.2.2 Anthropogenic factors influencing the spatial distribution of trypanosomiasis

Whilst remote sensing has thus proven to be an excellent tool in the prediction of tsetse, it is less accurate in the prediction of trypanosomiasis. For example a model on seasonal trypanosomiasis prevalence in cattle in the Gambia, which captured 93% of the seasonal patterns in the tsetse population, could only account for 62% of variation in trypanosomiasis prevalence detected through field surveys (Rogers, 2000). Considering the extra layers of complexity involved in parasite transmission, ranging from vector infection rate to temporal and spatial host vector interaction, this steep decline in prediction accuracy from vector to disease abundance was not unexpected (Rogers & Randolph, 2003). In Togo, country wide vector and disease mapping for trypanosomiasis with subsequent prediction exercises yielded the most accurate results for tsetse predictions, with less accurate predictions for cattle parasitaemia and packed cell volume (PCV) (Hendrickx *et al.*, 1999a; Hendrickx *et al.*, 2000). Satisfactory results for PCV as a measure of pathogenic trypanosome infections in cattle, were only obtained, once anthropogenic variables linked to herd management, cattle breed and land use were combined with ground measured and remotely sensed environmental predictors (Hendrickx *et al.*, 2001).

Studies, concentrating on smaller geographic areas and incorporating more detailed information at the herd level were able to identify important factors determining spatial heterogeneity in trypanosomiasis prevalence in cattle: Field surveys in the Gambia identified distinct seasonal peaks in trypanosomiasis prevalence in cattle,

which could not be explained solely by the timing of peak tsetse challenge (Rawlings *et al.*, 1991). However migratory patterns, as well as fodder quality and availability were also identified as explanatory factors for trypanosomiasis prevalence (Rawlings *et al.*, 1991).

In a study in Burkina Faso including over 2000 head of cattle, the serological trypanosomiasis prevalence of individual herds could be predicted from livestock management factors and from their spatial location (Michel *et al.*, 2002). The hydrological network and land occupation patterns played a key part in determining trypanosomiasis distribution transmitted by *G. tachinoides* and *G. palpalis gambiensis*. The risk factors associated with high trypanosomiasis prevalence were as follows: (a) proximity to the hydrographic network (<2km), (b) frequentation of natural watering sites and (c) larger herds (which were more likely to frequent natural watering sites) (Michel *et al.*, 2002). In an investigation of spatial heterogeneity of trypanosomiasis prevalence in cattle, differences in infection rates according to geographical location were also identified by de La Rocque and colleagues. A negligibly low trypanosomiasis prevalence was detected in cattle raised far removed from the hydrological network (de La Rocque *et al.*, 1999). River watering sites were identified as trypanosomiasis transmission hotspots, with high infection rates in trapped tsetse and in cattle herds grazing in the vicinity and frequenting the watering sites (de La Rocque *et al.*, 1999). As opposed to a multitude of studies on the spatial distribution of trypanosomiasis in cattle, no such studies on small ruminants or pigs were found in the published literature at the time of writing.

5.1.2.3 Spatial epidemiology of human African trypanosomiasis

There are few published studies, investigating risk factors for the spatial distribution human trypanosomiasis in East Africa and the results of these studies partly contradict each other. In Uganda, Odiit and colleagues used remote sensing and GIS to identify villages at high risk for Rhodesian sleeping sickness in south-east Uganda. Amongst other risk factors (including low population density and vicinity to the sleeping sickness hospital) vicinity of villages to long vegetation swamps was

identified as a significant risk factor for sleeping sickness (Odiit *et al.*, 2006). In Busia, Kenya, vicinity to the Ugandan border was identified as the only risk factor significantly associated with the occurrence of Rhodesian sleeping sickness, whereas vicinity to swamps actually appeared to have a protective effect from sleeping sickness risk, once the model was adjusted for vicinity to the Ugandan border (Batchelor, 2004).

In West Africa, Courtin and colleagues could relate land-use patterns associated with heterogeneity of tsetse prevalence to the distribution of cases of Gambian sleeping sickness in a focus in Côte d'Ivoire. Areas with a history of intense agricultural use reported a much lower number of sleeping sickness cases than less intensely utilised regions, which provided a better tsetse habitat as proven by a higher apparent tsetse density. These areas were consequently to be targeted for vector control and active screening of the human population (Courtin *et al.*, 2005).

The expansion of Rhodesian sleeping sickness into previously unaffected districts within Uganda was investigated, employing temporospatial cluster analysis (Fèvre *et al.*, 2001). Cattle had long been identified as the most important reservoir of *T.b. rhodesiense* in south-west Uganda (Hide *et al.*, 1994; Hide *et al.*, 1996), and movement of infected cattle had been suspected to be involved in the spread of the parasite. The new disease focus in Soroti could be linked to a particular cattle market, where *T. b. rhodesiense* infected cattle, imported from sleeping sickness endemic areas in south-east Uganda were traded (Fèvre *et al.*, 2001). The continuing northwards spread of Rhodesian sleeping sickness within Uganda (Fèvre *et al.*, 2005; Picozzi *et al.*, 2005) demonstrates the precarious balance of geographical disease delimitations. In the instance of Uganda, areas outside the historical sleeping sickness focus combined suitable vector habitat, and indeed the presence of a suitable vector, *Glossina fuscipes fuscipes*, with the presence of a potential reservoir host, cattle. To halt the northwards spread of Rhodesian sleeping sickness in Uganda an emergency intervention programme was recently instigated in the newly affected Districts (Kabasa, 2007). The project aimed to interrupt transmission of the disease,

by treating 86% or more of the cattle population with trypanocides, thus depressing the reservoir of *T. b. rhodesiense* below the threshold prevalence required for maintenance of the parasite in a population (Welburn *et al.*, 2006).

In a more recent study, records of sleeping sickness cases reaching back to 1970 were compiled and used to track the rapid propagation of sleeping sickness from its epicentre in Iganga District and its spread north into new districts (Berrang-Ford *et al.*, 2006). The study concluded that two dominant processes were involved in the spatial epidemiology of Rhodesian sleeping sickness in Uganda: Firstly, in regions were the disease is endemic, localised outbreaks were triggered by changes in vector-human interaction or vector density, which pushed the probability of infection over the threshold level. Secondly, spread of the disease into previously unaffected districts was driven by movement of infected livestock as previously demonstrated for the Soroti outbreak (Fèvre *et al.*, 2001; Berrang-Ford *et al.*, 2006).

5.1.3   Cluster detection

5.1.3.1   Cluster detection in spatial epidemiology

A disease cluster can be described as an aggregation of disease events in space and/ or time in excess of the number of events expected relative to the distribution of the population at risk (Sankoh & Becher, 2002). Detection of spatial clustering of a disease is of interest in identifying areas of high risk and to determine the associated underlying factors resulting in this increased risk. Cluster detection can also be used as a decision support tool in prioritizing control efforts to the areas most severely affected by a particular disease. It must be emphasized however that a discernible cluster may be a result of random distribution of events across the population at risk (Sankoh & Becher, 2002). A range of statistical tests has hence been developed to test for clustering and to determine the significance of any clusters detected. As different tests are tailored to different purposes, the following section gives a brief outline of the available approaches and the test selected as appropriate for detecting spatial clustering of trypanosomiasis cases over the study areas.

5.1.3.2 Classification of disease cluster tests

Clustering in point patterns can be determined using distance based tests, which compare the distance between cases with the distance between controls. Alternatively clustering may be defined as variability of case counts in a certain area, called quadrat. Cluster tests may be classified as follows (Sankoh & Becher, 2002):

Global Tests: test for overall clustering or random distribution and are appropriate for answering questions on the infectious nature of a disease, but do not give the spatial location of clusters.

Local Test: identify the location of possible clusters

Focused Test: identify the presence or absence of a cluster around a suspected risk factor

5.1.3.3 Choice of cluster test

As this chapter aims to identify the location of trypanosomiasis cluster within the study area, without prior knowledge of particular risk factor a local cluster test was most appropriate. Original descriptive local tests (Openshaw *et al.*, 1987; Openshaw *et al.*, 1988) involved multiple testing and thus lacked the power to detect significance. To improve upon Openshaw's method, Besag and Newell attempted to address significance testing (Besag & Newell, 1991). Turnbull and colleagues succeeded in designing a test determining the location of clusters and the associated level of significance, but only for a predetermined cluster population (Turnbull *et al.*, 1990).

The test used in this chapter, Kulldorff's Scan Statistic, is based on Turnbull's approach. The test is descriptive and inferred (Kulldorff & Nagarwalla, 1995). The application of Kulldorff's Scan Statistic using the SaTScan software is described in detail in the next section.

5.1.4 SaTScan

SaTScan version 5.1.3 (available at http://www.satscan.org), which is based on Kulldorff's Spatial Scan Statistic (Kulldorff, 1997), was employed to test for spatial clustering of cases of trypanosomiasis throughout the animal population of the two sampling areas in Busia, western Kenya. A detailed explanation of the theory behind Kulldorff's Spatial Scan Statistic can be found elsewhere (Kulldorff & Nagarwalla, 1995; Kulldorff, 1997). Briefly, the Spatial Scan Statistic is a local cluster test, which detects the location of the most likely cluster of events in a data set, and infers its statistical significance using Monte Carlo replications. A grid of points, referred to as centroids, is superimposed on top of the sampling area. Around each centroid, circular zones with a continuously varying radius are constructed. The likelihood ratio test statistic for each zone, which is based on the population and number of cases included within that zone, is calculated. The total number of zones around each centroid, although infinite in theory due to the continuous variation of the radius, is in fact limited by the possible combinations of population and case numbers. Usually the zones are restricted in size to include a maximum of 50% of the population, as "high rate" clusters larger than this in actual fact signify a cluster of unusually low rate of events in the remaining population. When space-time clusters are under investigation, a third dimension is added to the model, with time as the varying height of the now cylindrical zones. The most likely cluster is the zone associated with the maximum value of the likelihood ratio test statistic. Monte Carlo hypothesis testing is employed to infer the statistical significance of the most likely cluster, based on the null-hypothesis that there is no significant spatial cluster.

The null-hypothesis is based on one of two basic underlying distributions. The **Bernoulli distribution**, otherwise known as binomial distribution, is used to consider a finite number of individuals with binary (1/0) variables. This may be applied to explore the distribution of cases versus non-cases, or two different stages of a disease, for example. Cases and controls may be drawn from a larger population, or may together make up the entire population of the sampling area. As an example of the former application of the Bernoulli model, spatial clustering of malaria was

investigated in the western highlands of Kenya, using one to two age matched non-infected controls per malaria case identified (Brooker *et al.*, 2004). The study on the spatial distribution of trachoma in a Tanzanian village (Polack *et al.*, 2005), is an example for the latter, with all inhabitants of the village (population of the study area) being included either as a trachoma case or as a non-affected control.

The second basic option is the **Poisson distribution**. The Poisson distribution is used where the rate of events under investigation is known for an inhomogenous population. Under the null-hypothesis the expected number of cases in each area is proportional to its population size or to the person years in that area. With the Poisson model it is possible to adjust for covariates such as age or sex for example, if these are confounding factors influencing the rate of disease. In a study of the geographic distribution of breast cancer in Massachusetts (Sheehan *et al.*, 2004), for example, the age distribution of the population was included as a covariate, which affects expected breast cancer prevalence.

5.1.4.1 Multivariate Scan Statistic

Animal species was shown to have a significant effect on the prevalence of trypanosome infections in Chapter 4. The Bernoulli model does not allow for covariates, with the help of which the expected frequency of cases can be adjusted. To avoid detection of apparent clusters of trypanosome infections, which were due to clustering in the distribution of animal species in the underlying population, data was grouped into separate sets according to animal species. However, multiple testing of separate data sets leads to a loss in statistical power. Additionally, clusters formed together by infected animals of different species may be obscured if sets are tested separately.

The Multivariate Scan Statistic is designed to circumvent these problems by simultaneously evaluating single data sets and various combinations of multiple data sets (Kulldorff *et al.*, 2005).

This is achieved as follows:

1. Circular zones with continuously varying radius, encompassing up to 50% of the total population, are constructed as previously described for the analysis of a single data set.

2. For each zone, the log likelihood ratios are calculated for each data set. It is noted whether the observed number of cases within the zone is larger or smaller than expected for each data set.

3. For each zone, the log likelihood ratios for the data sets, with more than the expected number of cases, is summed up (detection of high rate clusters). Equally, the log likelihood ratios for the datasets with less than the expected number of cases are summed up for each zone (detection of low rate clusters).

4. The maximum of all the log likelihood ratios, taken over all the zones, constitutes the most likely cluster, and its statistical significance is inferred through Monte Carlo replications of the model.

5.1.5 Objectives

This Chapter investigates spatial heterogeneity of trypanosomiasis in domestic animals on the micro-scale in Busia, Kenya, which is endemic for human sleeping sickness. Firstly, SaTScan software is applied to census type data on the trypanosomiasis prevalence detected by PCR in all cattle, pigs, sheep and goats, georeferenced by the location of the respective homesteads, to identify significant clusters of human and animal infective trypanosome infections.

Secondly, the effect of distance of homesteads to water features defined as streams or swamps on the prevalence of trypanosomiasis in the household herd is determined employing logistic regression.

5.1.6 Null Hypotheses

1. H_0 - Animal pathogenic trypanosome infections and human infective *T. brucei rhodesiense* infections are randomly distributed over the underlying population of domestic animals without the occurrence of statistically significant spatial clustering.

2. H_0 – There is no effect of the distance of a homestead to the nearest water feature on the likelihood of the domestic livestock of that homestead being infected with animal pathogenic or human infective trypanosomes.

5.2 Methodology

5.2.1 Samples

This Chapter utilizes the data obtained from census sampling of all livestock in the two study sites. Details of study design, sampling and laboratory analysis are given in Chapter 3. Briefly, whole blood samples were collected from all domestic animals including cattle, goats, sheep and pigs of all animal keeping homesteads in the study sites in Funyula (191 households) and Butula (358 households). All samples were stored on FTA-cards and analysed in the laboratory for the African animal infective trypanosome species *T. brucei* s.l. , *T. vivax* , *T. congolense* and *T. simiae* by PCR (Moser *et al.*, 1989; Cox *et al.*, 2005). All *T. brucei* s.l. positive samples were further screened for the presence of the human infective *T. b. rhodesiense* by PCR and Southern Blot (Picozzi *et al.*, in press).

Each animal keeping homestead visited was assigned a unique number used consistently for recording any associated data including identification of the animal samples collected at the corresponding homestead. A hand held Garmin Global Positioning System (GPS) was used to determine and record the geographical co-ordinates of each homestead.

5.2.2 Maps

5.2.2.1 Georeferencing and digitising of paper maps

Paper maps at a scale of 1:50,000 were obtained for the study area, which included the sheets for Busia (101/1), Mumias (101/2) and Samia (101/3) of the D.O.S. 423 (Series Y731) published by the Directorate of Overseas Survey for the Kenya in 1970. These maps are in the Universal Transverse Mercator (UTM) projection in zone 36N, datum: Arc1960. Details of the projection are found in Table 5.1. Maps were digitally scanned and georeferenced using the ArcView GIS Image Warp function (Kenneth R. McVay, available at http://arcscripts.esri.com). Scanning distortions of the topographic maps were corrected using eight ground control points

for each map, taken at gridline intersections. The root mean square errors (RMS) on the x-axis and on the y-axis were below 10 (between 0.36 and 6.73) for all three maps sheets (RMS in map units). The hydrographic network, swamps and roads were digitized from the georeferenced maps in ArcView3.2 and saved as shape files.

Table 5.1: Projection details of paper maps of Busia District

Grid	U.T.M Zone 36N
Projection	Transverse Mercator
Spheroid	Clark 1880 (modified)
Unit of Measurement	meter
Meridian of Origin	33°00' East of Greenwich
Latitude of Origin	Equator
Factor at Origin	0.9996
False Coordinates at Origin	500,00m Easting, 0m Northing
Datum	Arc 1960

5.2.2.2 Extraction of distance to water features

The coordinates for all sampled homesteads were imported into ArcView3.2 as a database file (DBF4) and converted into shapefiles for further use. In the spatial analyst extension of ArcView3.2 (ESRI, Redlands, CA, USA) a grid of distance to hydrographic network was produced (1x1m grid squares) and the distance of each homestead to the nearest stream was extracted. Distance of each homestead to the nearest swamp was established in the same manner and the two data sets were consolidated to establish distance of each homestead to the nearest water feature (stream or swamp).

5.2.2.3 Presentation maps

All maps used to present the distribution of trypanosome infected animals over the respective homesteads and the location of clusters of infection were produced by manipulation of the relevant datasets in ArcGIS9 (ESRI, Redlands, CA, USA).

5.2.3 Statistical analysis

5.2.3.1 Cluster detection

SaTScan version 5.1.1 software by Martin Kulldorff freely available at http://www.satcan.org was used for cluster detection. Analysis was performed separately for the two study areas (Funyula and Butula), as they are geographically separate (see Figure 3.1 in the General Methodology). To investigate the spatial distribution of trypanosomiasis cases over the homesteads in the two study areas in Busia, the Bernoulli model was appropriate. The data was collected from a finite number of individuals, the location of the homestead to which each of the animals belonged was known and the variable of interest was binary as animals could be classed as either non-infected (control) or infected with trypanosomes as detected by PCR (case). Together cases and controls made up the entire population of the domestic animals under investigation in the study areas. As the study was cross-sectional rather than longitudinal, cluster detection was purely spatial and did not include time as a variable. Clustering with respect to the distribution of the livestock population was investigated for trypanosome infections overall as well as for the different trypanosome species detected separately: *T. brucei* s.l., *T. vivax*, *T. simiae* and *T. b. rhodesiense*. Even though *T. congolense* infection were included in the total trypanosome infections, no separate cluster analysis was run for T. congolense, due to the low number of infections detected (total 7 out of 2773 samples *T. congolense* infected). The spatial distribution of *T. b. rhodesiense* in Butula was additionally tested for spatial clustering with respect to the distribution of *T. brucei* s.l. infected animals (only those samples that tested positive for the *T. brucei* s.l. specific control gene PLC, in the multiplex PCR performed to detect *T. b. rhodesiense* were used as the background population for this analysis).

To account for host species specific variation in trypanosome prevalence and heterogenous distribution of the different host species across the study area, separate datasets were created for cattle, pigs, goats and sheep and the multivariate model was applied for cluster analysis. In Funyula, no trypanosome infections were detected in sheep (Figure 4.9a), thus no sheep dataset was included in the multivariate cluster analyses for Funyula. The analysis performed was purely spatial, scanning for "high rate" clusters, i.e. areas where a higher number of cases than expected aggregated. The number of Monte Carlo replications was set to 9999, as was recommended for small data sets. The maximum size of cluster admissible was restricted to 50% of the total population in the study area, and no geographical overlap of clusters was permitted.

All cluster analyses that detected a significant cluster were repeated, restricting the maximum permitted cluster size to 30% of the total population, and allowing limited overlap, in that no pairs of centroids were allowed to be each in each others cluster. Multiple small partly overlapping clusters could potentially define a high risk space that was non-circular in shape (e.g. elongated along a river) better than one large cluster. Thus, this second cluster analysis was performed in an attempt to refine the space defined by large high risk clusters.

Due to multiple cluster testing for individual trypanosome species the level of statistical significance was adjusted. The Bonferroni correction stipulates adjustment of the significance level to $p \leq 0.05/$ number of tests conducted (Bland & Altman, 1995). However as the study did not have a tightly controlled experimental design, due to the field nature of the study design, a less severe adjustment was made and statistical significance was accepted at $p \leq 0.01$.

5.2.3.2 Distance to water features

Logistic regression, computed using the statistical software package R (R: copyright 2004, The R foundation for Statistical Computing Version 2.0.1), was used to

analyse the effect of distance of households to water features on the probability of the herd of the household to be affected by trypanosomiasis. The distance of homesteads to the water feature in question was categorised into 250m distance intervals. Analyses for the effect of distance to streams and the effect of distance to swamps were performed separately, followed by analysis of the effect of distance to the nearest of either of these water features: stream or swamp. Where the effect of distance interval to water feature approached significance, the model was repeated with varying distance intervals (100m intervals and exact distance) to test robustness of the model. The overall significance of the distance effect under investigation in all models throughout this chapter was established by running analysis of variance (ANOVA) summaries on each model. Degrees of freedom were noted as subscript of the χ^2 statistic.

Response variable: Ideally, the effect of distance to water features would have been analysed, using a two-vector response variable, comprising the number of successes (infected animals) and the number of failures (non-infected animals) per household, bound together into a single object (using a function within R called cbind), thus accounting for the proportional nature of the data (Crawley, 2002). However diagnostic plots showed poor fit of that type of model for the data collected, due to high number of households with only one or two animals, in particular when separate models for the different livestock species were constructed.

It was thus decided to use a binary response variable, with households owning at least one infected animal of the species under consideration being assigned positive status (1) and only households with no infected animals of that species considered negative (0). Separate models were run for overall trypanosomiasis as well as specifically for the animal infective trypanosome species *T. brucei* s.l. , *T. vivax* and *T. simiae*, and the zoonotic *T. b. rhodesiense* according to the PCR results. In addition to these trypanosome species overall trypanosomiasis also included *T. congolense* infections, which were not analysed in separate models, due to the low prevalence.

As a higher number of animals in a given homestead would increase the probability that at least one of the animals was infected purely by chance, the number of animals in the homestead (total herdsize or number of the livestock species under consideration when separate models were constructed) was included into each model as the first predictor, before the distance to the water feature of interest (Figure 5.1).

```
anova(glm(homeCtbrucei~C+distance250_streams,data=FunyulaH2O,subset=cat
tle_present!="0",family="binomial"),test="Chisq")
```

Figure 5.1: Example of R command line: response variable – *T. brucei* s.l. infection status of herds, considering only cattle in the model; predictors: distance to streams categorised in 250m intervals,, adjusted for the number of cattle per herd as first predictor.

Each model primarily included all host animals, followed by analysis of separate models accounting for cattle only and pigs only (Figure 5.1). Due to the low prevalence of trypanosomiasis detected in small ruminants (Table 4.3), no separate small ruminant models were run. Due to multiple testing the level of statistical significance was adjusted to $p \leq 0.01$.

5.2.4 Overview

Table 5.2 and Table 5.3 provide an overview of the two sections of statistical analysis that were performed for this chapter.

Table 5.2: Overview of multivariate cluster analysis to detect spatial clustering of trypanosomiasis

Maximum clustersize	Study area	Trypanosome species considered individually	Background population sets for respective multivariate analyses
50% of the pop., no overlap	Funyula	Total tryps, *T. brucei* s.l.,*T. vivax*, *T. simiae*, *T.b. rhodesiense*	Cattle, pigs & goats
	Butula	Total tryps, *T. brucei* s.l.,*T. vivax*, *T. simiae*, *T.b. rhodesiense*	Cattle, pigs, goats & sheep
		T.b. rhodesiense	*T. brucei* s.l. positive samples detected by PLC
30% of the pop., partial overlap*	Butula	Total tryps, *T. brucei* s.l.,	Cattle, pigs, goats & sheep

*(no pairs of centroids allowed to be each in each others cluster)

Table 5.3: Overview of logistic regression at the homestead level, to investigate effect of distance to waterfeature on the infection status of the household herd

Study area	Individual host species considered	Trypanosome species considered individually	Factor of interest
Funyula	All livestock, cattle, pigs individually	Total tryps, *T. brucei* s.l. *T. vivax, T. simiae, T.b. rhodesiense*	Distance to streams Distance to swamps Distance to nearest water feature (either streams or swamps)
Butula	All livestock, cattle, pigs individually	Total tryps, *T. brucei* s.l. *T. vivax, T. simiae, T.b. rhodesiense*	Distance to streams Distance to swamps Distance to nearest water feature (either streams or swamps)

5.3 Results

5.3.1 Spatial clustering of trypanosomiasis: maximum cluster size 50% of population, no cluster overlap

5.3.1.1 Funyula: Clustering of trypanosome infected animals

5.3.1.1.1 *Overall trypanosome infections*

Using the Multivariate Scan Statistic to adjust for host species, and allowing for the maximum cluster size to include 50% of the population, no significant spatial clustering of trypanosome infected animals occurred in the Funyula sampling area. The most likely cluster reported for the spatial distribution of trypanosome infections observed, was not statistically significant (observed/expected cases (O/E) cattle=1.814; O/E pigs=0.688, O/E goats= 1.454; log likelihood ratio=6.895; p=0.374). The distribution of trypanosome infections with relation to the underlying population distribution is displayed in Figure 5.2.
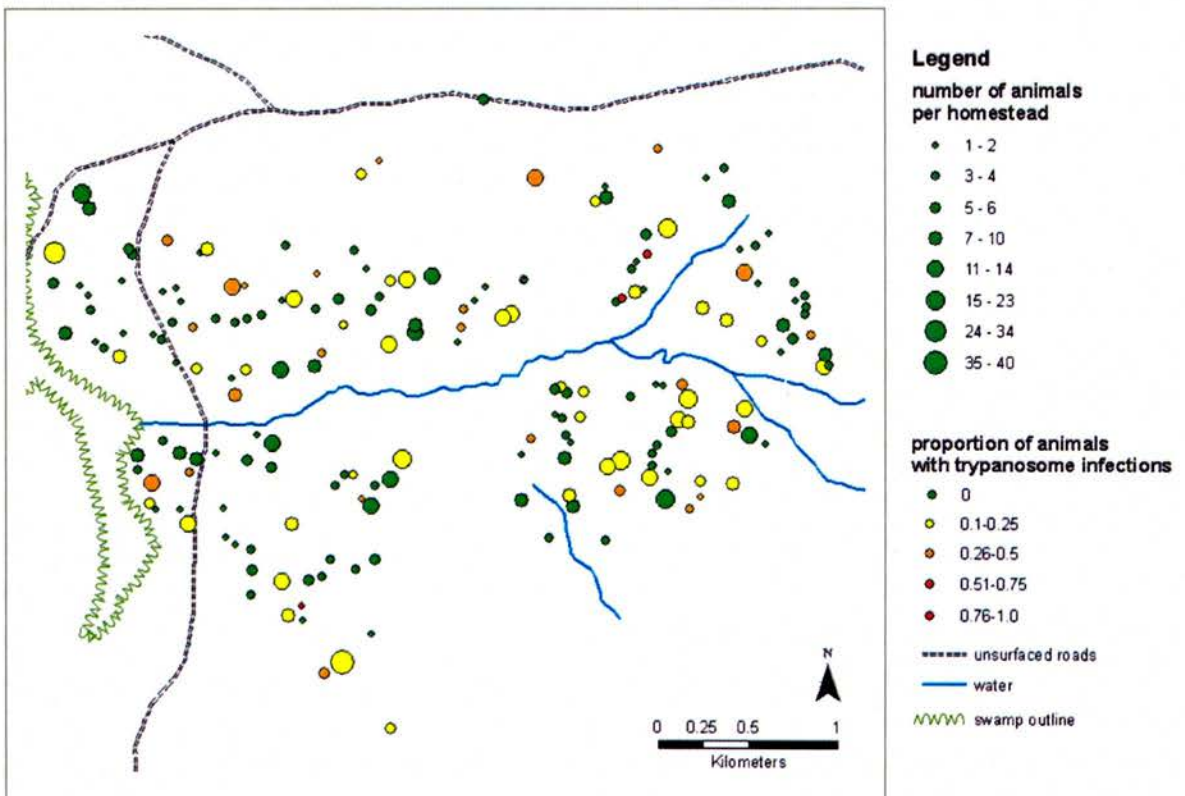
Figure 5.2: Funyula: Distribution of trypanosome infected domestic animals

5.3.1.1.2 Individual trypanosome species

There was no significant clustering detected when the spatial distribution of infected animals was analysed separately for the individual trypanosome species (Table 5.4). Only the most likely cluster detected in the distribution of *T. brucei* s.l. approached statistical significance, which was attributed to a high relative risk of *T. brucei* s.l. infections in cattle (O/E cattle=4.931; O/E pigs=0.583; O/E=0; log likelihood ratio=9.5; p=0.055). As only the risk ratio for cattle was increased within this most likely cluster of *T. brucei* s.l. infected animals, the location of the cluster was displayed with relation to the geographical distribution of *T. brucei* s.l. infected cattle (Figure 5.3)

Table 5.4: Most likely clusters detected in the spatial distribution of trypanosome infections in Funyula using the Multivariate Scan Statistic adjusted for host species (maximum clustersize: 50% of population, no overlap allowed)

Trypanosome species	Most likely cluster: centre* (radius)	p-value	observed/expected number of cases			Log likelihood ratio
			Cattle	Pigs	Goats	
T. brucei s.l.	0.273398 N 34.115589 E (0.56km)	0.055	4.931	0.583	0	9.5
T. vivax	0.268341 N 34.116371 E (0.74km)	0.115	2.393	0.826	2.418	8.491
T. simiae	0.271242 N 34.093571 E (0km)	0.634	0	0	189.0	5.547
T.b. rhodesiense	0.269069 N 34.105 E (0.44km)	0.455	1.820	8.175	N/A**	5.344
Total trypanosome infections	0.267611 N 34.116051 E (0.82km)	0.374	1.814	0.688	1.454	6.895

* Cluster centres: Grid: lat/lon hddd.ddddd°, Datum: WGS84

** N/A: no infected animals; host species was thus not included in analysis for this trypanosome species/ subspecies
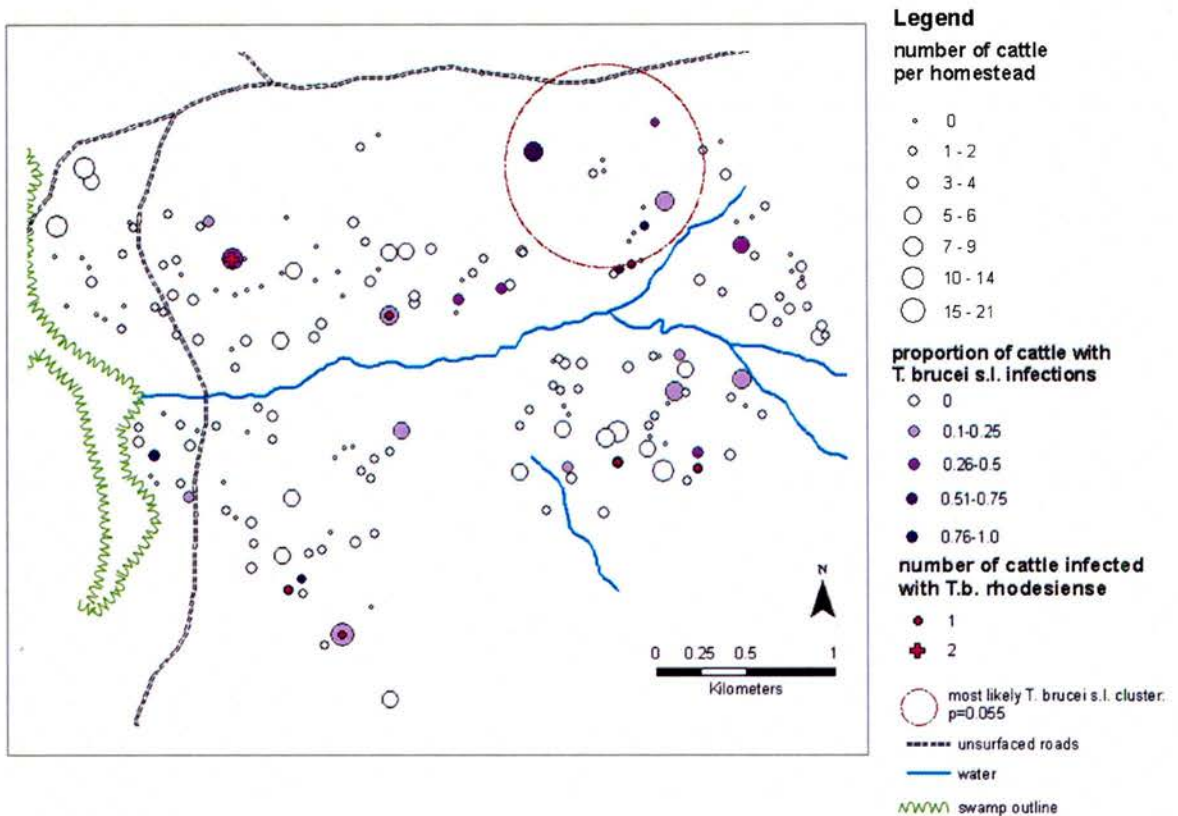
Figure 5.3: Funyula: Distribution of *T.brucei* s.l. infected cattle, location of most likely cluster of *T. brucei* s.l., which only approaches significance (p=0.055) (as determined by multivariate analysis with relation to the spatial distribution of host species) and the distribution of *T. b. rhodesiense* infected cattle.

5.3.1.2 Butula: Clustering of trypanosome infected animals

5.3.1.2.1 Overall trypanosome infections

Using the Multivariate Scan Statistic to adjust for host species, and allowing for the maximum cluster size to include 50% of the population, one significant spatial cluster of trypanosome infected animals was detected in Butula (O/E cattle=1.417, O/E pigs=1.706, O/E goats 1.168, O/E sheep=1.524, log likelihood ratio=20.951; p<0.0001), with a radius of 1.81km which included 45.5% (163/358) of homesteads in the study area accounting for 45% (762/1692) of the total population of domestic animals. The spatial distribution of trypanosome infected domestic animals and the location of the significant cluster was displayed in Figure 5.4.
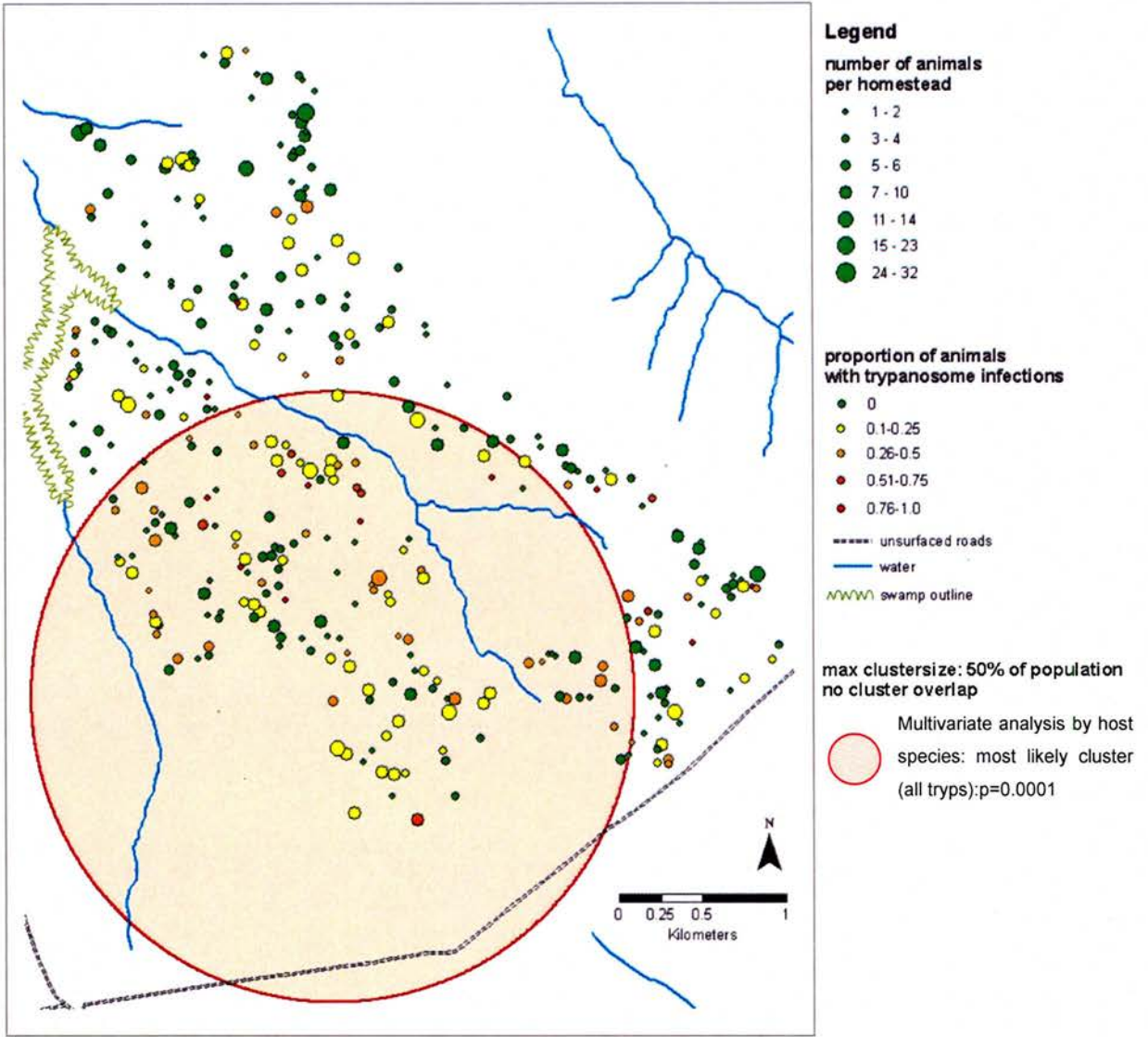
Figure 5.4: Butula: Distribution of trypanosome infected domestic animals and the location of the most likely cluster (p=0.0001) as determined through multivariate analysis with relation to the spatial distribution of host species.

5.3.1.2.2 Individual trypanosome species

When the spatial distribution of infected animals was analysed separately for the different trypanosome species, a significant cluster was detected for *T. brucei* s.l. infected animals (O/E cattle=1.789; O/E pigs=1.977; O/E goats=1.258; O/E sheep=1.348; log likelihood ratio=21.612; p≤0.001), with a radius of 1.6km which encompassed 153 of the 358 homesteads (42.7%) in the study area, accounting for

42.7% (722/1692) of the total population of domestic animals. The location of the *T. brucei s.l.* cluster can be seen in Figure 5.5. No significant spatial clusters were detected in the distribution of any of the other trypanosome species (Table 5.5). But the most likely cluster in the distribution of *T. vivax* infections approached significance (O/E cattle=1.341; O/E pigs= 2.476; O/E goats=1.419; O/E sheep=1.404; log likelihood ratio= 11.146; p=0.046) (Figure 5.6). This most likely *T. vivax* cluster spatially overlaps with the significant *T. brucei* s.l. cluster, with 61.6% (77/125) homesteads found in the *T. vivax* cluster also included in the *T. brucei* s.l. cluster (Figure 5.9).

Table 5.5: Most likely clusters detected in the spatial distribution of trypanosome infections in Butula using the Multivariate Scan Statistic adjusted for host species (maximum clustersize: 50% of population, no overlap allowed)

Trypanosome species (infected animals in relation to host species population)	Most likely cluster: centre* (radius)	p-value	observed/expected number of cases				Log likelihood ratio
			Cattle	Pigs	Goats	Sheep	
T. brucei s.l.	0.323247 N 34.213501E (1.60km)	0.0002	1.789	1.977	1.258	1.348	21.612
T. vivax	0.318367 N 34.219250 E (1.73km)	0.046	1.341	2.476	1.419	1.404	11.146
T. simiae	0.340923 N 34.209309 E (0.89km)	0.609	3.625	0	N/A**	N/A**	5.634
T.b. rhodesiense	0.317752 N 34.216621 E (1.79km)	0.037	2.062	2.859	2.583	N/A**	9.984
T.b. rhodesiense over PLC+ population	0.323851 N 34.213551 E (0.63km)	0.805	1.11	0.0	No controls	N/A**	3.856
Total trypanosome infections	0.320816 N 34.216358 E (1.81km)	0.0001	1.417	1.706	1.168	1.524	20.951

* Cluster centres: Grid: lat/lon hddd.ddddd°, Datum: WGS84, ** N/A: no infected animals; host species was thus not included in analysis for this trypanosome species/ subspecies

The most likely cluster in the distribution of *T. brucei rhodesiense* infected animals also approached statistical significance (O/E cattle=2.062; O/E pigs=2.859; O/E goats=2.583; log likelihood ratio=9.985; p=0.037). The significant *T.brucei* s.l. cluster and the most likely *T.b. rhodesiense* cluster were geographically approximately concomitant (see Figure 5.5). When the animal samples in which *T. brucei* s.l. infections were detectable with the Multiplex PCR were taken as the background population, there was no significant spatial clustering of *T. b. rhodesiense* infected animals within the *T. brucei* s.l. infected population (Table 5.5).
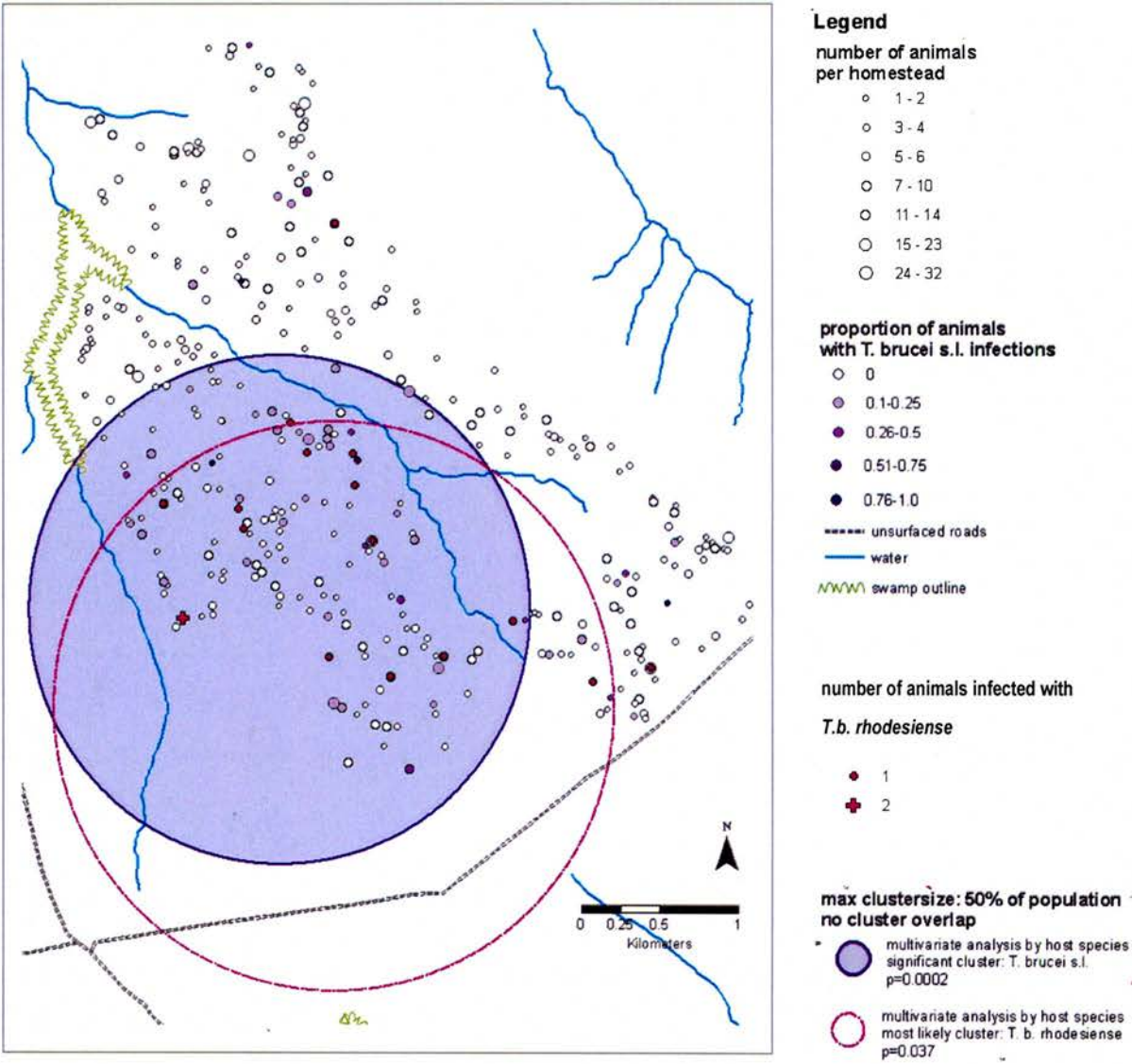
Figure 5.5: Butula: Distribution of *T. brucei* s.l. infected animals with location of significant cluster (purple) (p=0.0002) and distribution of *T. b. rhodesiense* infected animals with most likely cluster (pink), which approaches significance (p=0.037).
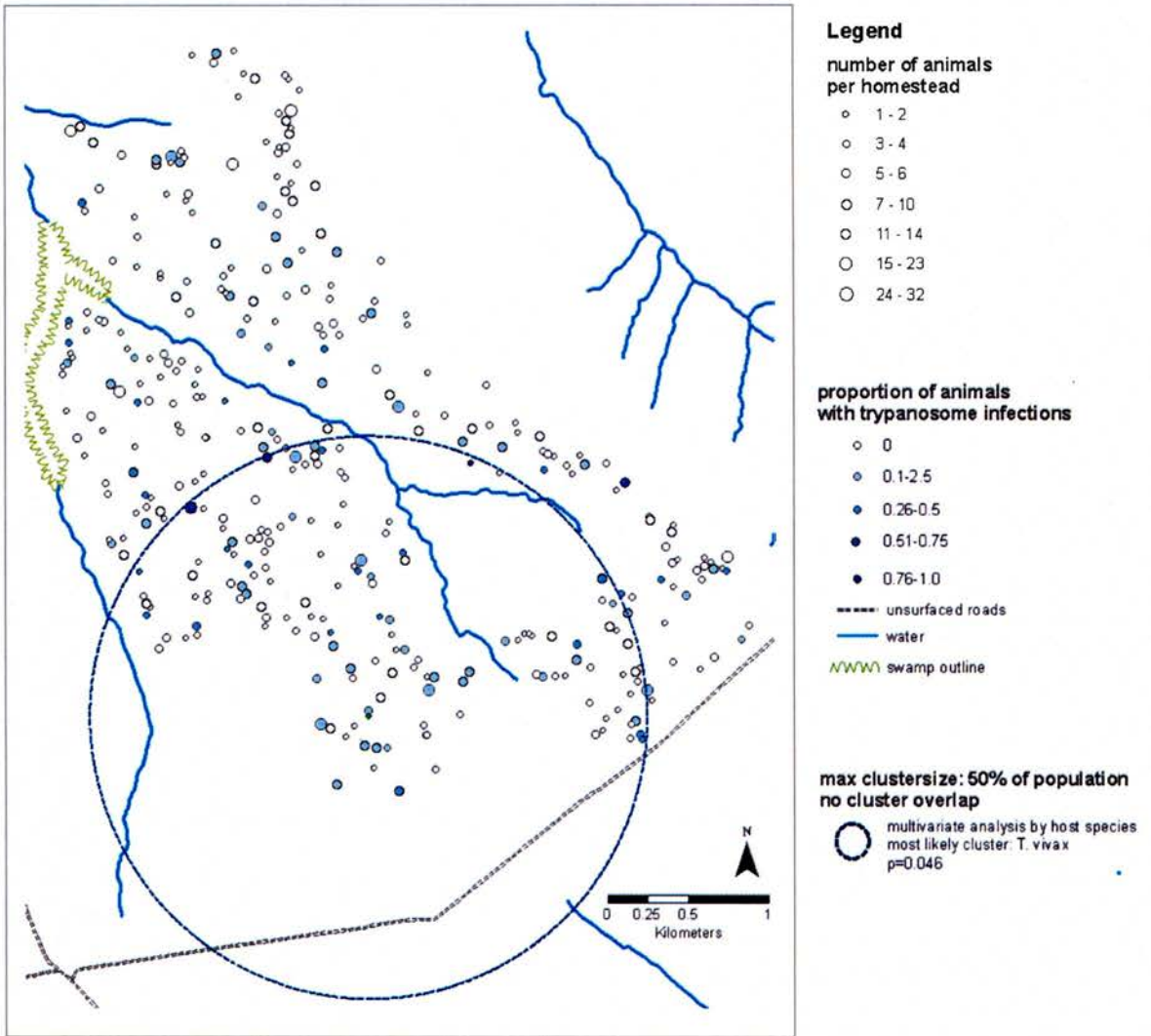
Figure 5.6: Butula: Distribution of *T. vivax* infected animals, with location of most likely cluster, which approaches significance (p=0.037).

5.3.2 Spatial clustering of trypanosomiasis: maximum cluster size 30% of population, limited overlap

5.3.2.1 Funyula

As no significant clusters were detected in Funyula allowing a maximum cluster size to include 50% of population, no restricted cluster tests further were performed.

5.3.2.2 Butula

In Butula, restriction of permitted maximum cluster size to 30% of the population, whilst simultaneously allowing limited overlap of clusters (no pairs of centroids allowed both in each other's clusters), resulted in no significant clusters when overall trypanosomiasis was considered. However the four most likely clusters detected approached significance ($p<0.05$) (Table 5.6). All four were located within the same area as the cluster detected when a maximum cluster size of 50% of the population was permitted (section 5.3.1.2.1) (Figure 5.7).
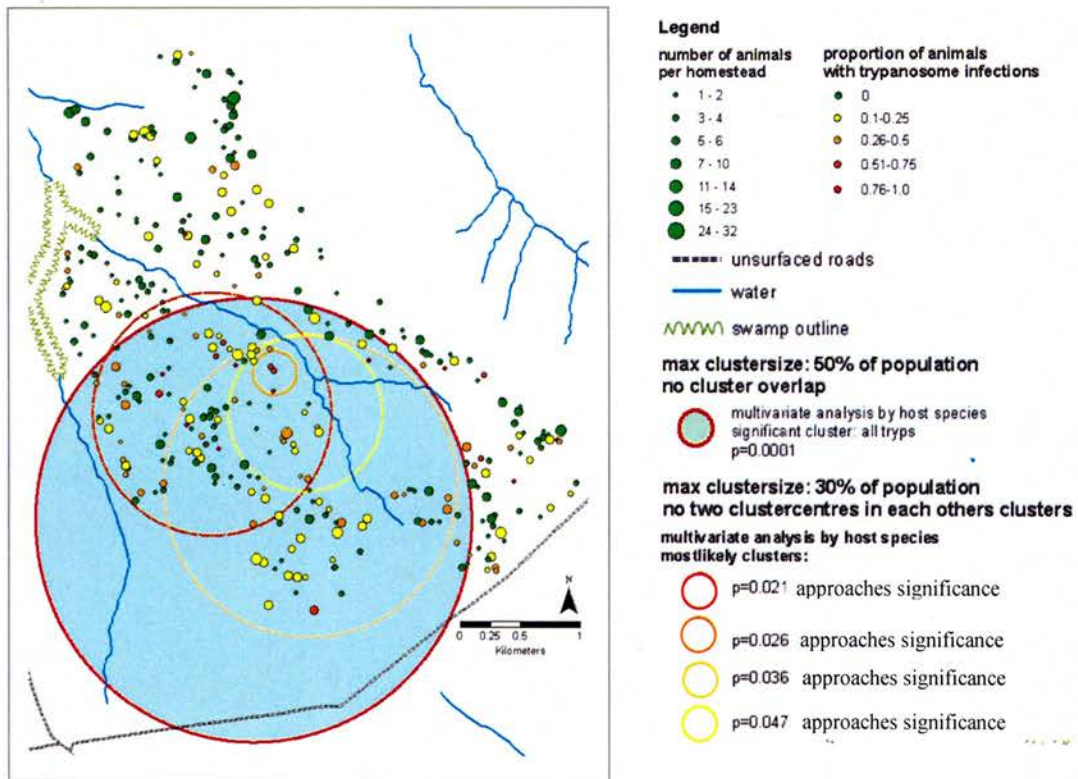
Figure 5.7: Butula: Distribution of trypanosomiasis infected animals with location of most likely clusters for maximum cluster size restricted to 30% of population, limited overlap.

When trypanosome species were considered separately, no significant clusters were detected for *T. vivax* (Table 5.6). For *T. brucei* s.l. restricted cluster size (max 30% of population) with limited overlap allowed, revealed two significant clusters ($p<0.001$) and two clusters approaching significance ($p<0.05$), all of which were located within the large significant cluster detected with a maximum cluster size of 50% of the population was allowed (section 5.3.1.2.2) (Figure 5.8). When the maximum cluster size was restricted to 30% of the population, no significant cluster of *T. b. rhodesiense* was detected, with the most likely cluster detected ($p=0.056$) being located within the *T. brucei* s.l. cluster (Table 5.6).
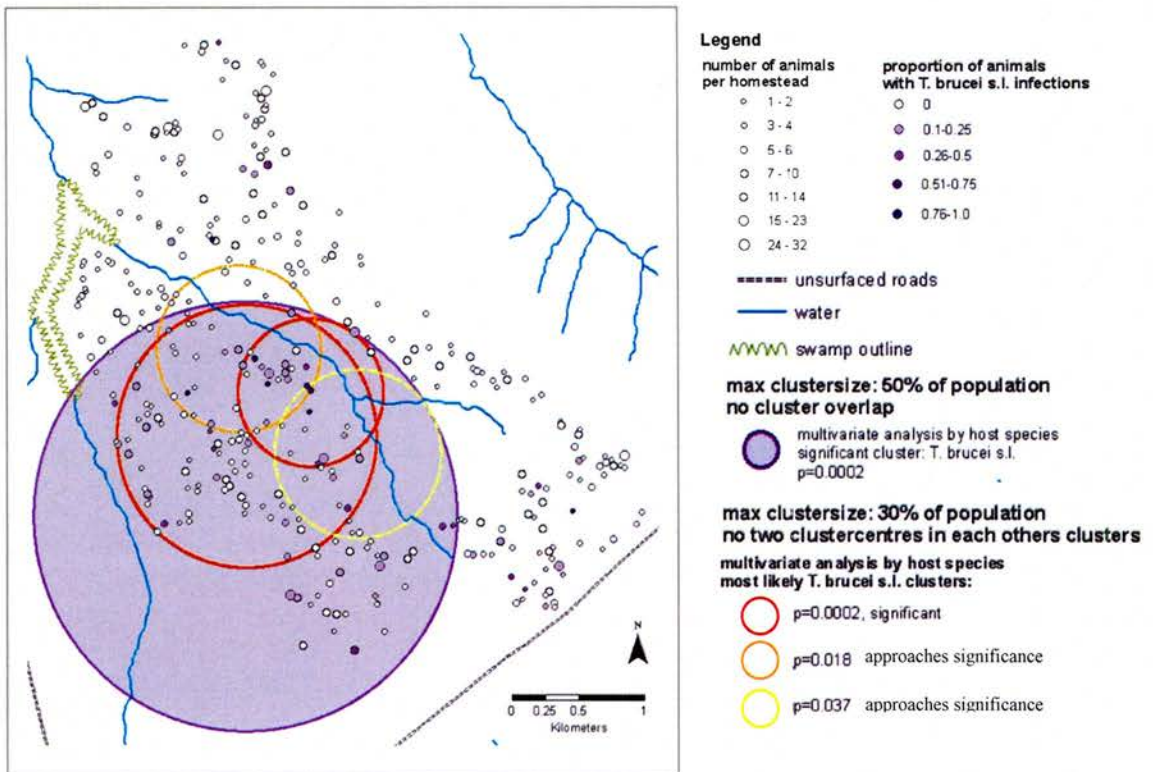
Figure 5.8 Butula: Distribution of *T. brucei* s.l. infected animals with location of most likely clusters for maximum cluster size restricted to 30% of population, limited overlap.

Table 5.6: Most likely clusters detected in the spatial distribution of trypanosome infections in Butula using the Multivariate Scan Statistic adjusted for host species (maximum clustersize: 30% of population, no limited overlap: no pairs of centroids each in each other's clusters)

Trypanosome species		Most likely cluster: centre* (radius)	p-value	observed/expected number of cases				Log likelihood ratio
				Cattle	Pigs	Goats	Sheep	
T. brucei s.l.:	1	0.331613 N 34.217941E (0.55km)	0.0002	3.513	3.172	0	0	18.954
	2	0.328649 N 34.213600 E (0.98km)	0.0002	2.085	1.952	1.857	0	17.956
	3	0.334550 N 34.213070 E (0.62km)	0.018	2.465	5.438	0	0	12.166
	4	0.327456 N 34.221149 E (0.63km)	0.037	3.636	1.812	0	0	11.202
T.b. rhodesiense		0.322202 N 34.210098 E (0.55km)	0.056	0.707	12.688	14.444	N/A**	9.358
Total trypanosome infections	1	0.328649 N 34.213261E (0.99km)	0.021	1.492	1.769	1.342	1.205	12.025
	2	0.331613 N 34.217941 E (0.18km)	0.026	4.788	3.980	0	0	11.843
	3	0.323247 N 34.220570 E (1.22km)	0.036	1.374	4.44	4.55	3.47	11.385
	4	0.328649 N 34.220211 E (0.64km)	0.047	2.294	1.706	2.708	3.701	11.100

* Cluster centres: Grid: lat/lon hddd.ddddd°, Datum: WGS84

** N/A: no infected animals; host species was thus not included in analysis for this trypanosome species/ subspecies

5.3.3 Summary: Spatial clusters

Funyula

There were **no significant spatial clusters** of trypanosome infected animals.

The most likely cluster of *T. brucei* **s.l.** infected animals (higher than expected number of infected cattle) only **approached significance**.

Butula

There was a **significant cluster of overall trypanosome** infections in livestock.

There was a **significant cluster of *T. brucei* s.l.** infected livestock

The most likely cluster of *T. vivax* infected livestock **approached significance**.

The most likely cluster of *T. b. rhodesiense* infected livestock **approached significance**.

All trypanosome species specific clusters spatially overlapped to a high degree (Figure 5.9)

Reducing the maximum size of reported clusters from 50% to 30% of the total livestock population, refined the space described as high risk for trypanosomiasis.
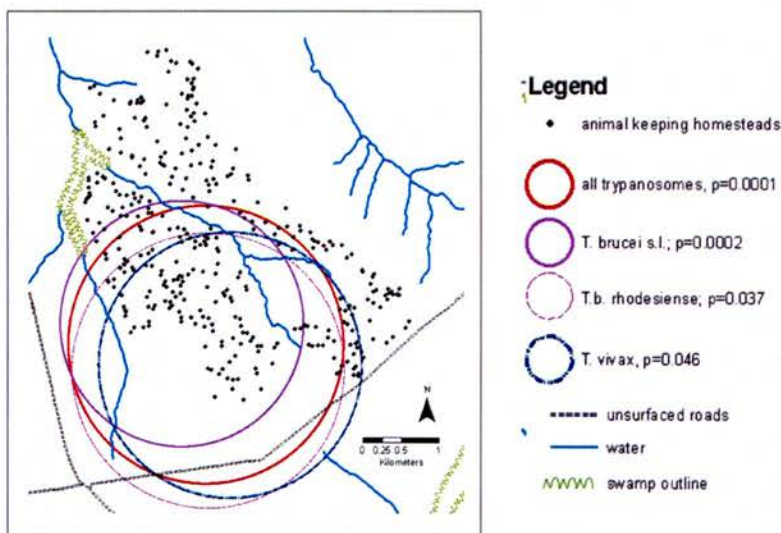
Figure 5.10: Summary: Spatial location of clusters of trypanosome infected livestock in Butula

5.3.4 Distance to water features

5.3.4.1 Funyula

5.3.4.1.1 Funyula: all livestock

In Funyula, there was no significant effect of the distance of homesteads to the nearest water feature on the probability of homesteads having at least one trypanosome infected animal in their herd when the model was adjusted for the number of animals in each herd. This was true, regardless whether the distance to a swamp or the distance to a stream was considered separately or jointly, both in separate models for the individual trypanosome species and in models considering all trypanosome infections together (Table 5.7).

When the distance of homesteads to the nearest water feature, either swamp or streams, was considered, the effect on the probability of household herds to be affected by *T. vivax*, approached significance (χ^2_1=3.8, p=0.051) when the model was adjusted for size of each herd (χ^2_1=26.0, p<0.001). This was attributed to only a low number of herds in the two highest distance brackets (4 and 3 respectively) a high proportion of which were infected (3/4 and 2/3 respectively) (Figure 5.11). When the distance to the nearest water feature was considered in 100m intervals (χ^2_1=3.0, p=0.082) or as the exact distance (χ^2_1=3.4, p=0.067), the effect on the probability of herds to be *T. vivax* infected no longer approached significance.
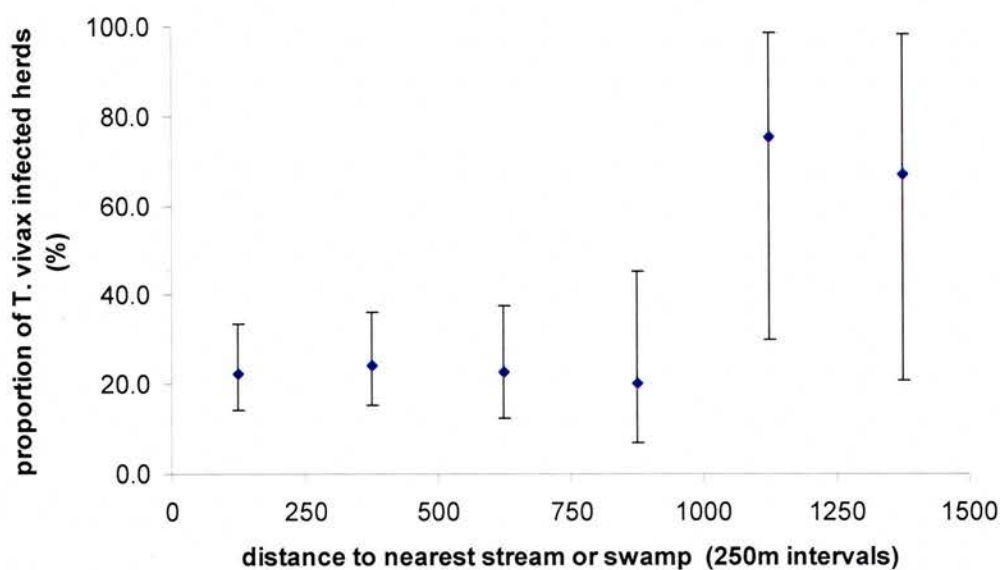
Figure 5.11: Proportion of herds with at least one animal infected with *T. vivax* with respect to distance to the nearest water feature: stream/swamp (in 250m intervals).

Table 5.7: All livestock: Effect of distance to water feature on the proportion of household herds with at least one infected animal (distance in 250m intervals) Summary of three models (1. distance to streams, 2. distance to swamps, 3. distance to nearest stream/swamp), each model adjusted for herdsize as first effect.

Trypanosome species	herdsize (1st predictor in each model)	distance to respective water feature (2nd predictor in 3 separate models)		
		1. streams	2. swamps	3. nearest feature (stream/swamp)
T. brucei s.l.	$X^2_1=24.4$, p<0.001	$X^2_1=0.3$, p=0.592	$X^2_1=1.0$, p=0.329	$X^2_1=0.6$, p=0.434
T. vivax	$X^2_1=26.0$, p<0.001	$X^2_1=0.6$, p=0.437	$X^2_1=1.6$, p=0.202	$X^2_1=3.8$, p=0.051
T. simiae	$X^2_1=9.1$, p<0.01	$X^2_1=0.1$, p=0.78	$X^2_1=2.8$, p=0.095	$X^2_1=0.1$, p=0.793
T.b. rhodesiense	$X^2_1=26.0$, p=0.013	$X^2_1=0.6$, p=0.411	$X^2_1=0.1$, p=0.783	$X^2_1=2.6$, p=0.109
Total tryps.	$X^2_1=32.8$, p<0.001	$X^2_1=0.6$, p=0.425	$X^2_1=1.4$, p=0.244	$X^2_1=3.2$, p=0.076

5.3.4.1.2 Funyula: cattle

When the probability of herds to be infected was analysed considering only cattle (adjusted for the number of cattle per herd), no effect of distance to water features was seen in any of the models constructed, regardless of whether distance to swamp, distance to stream or distance to the nearer of the two was considered (Table 5.8).

Table 5.8: Cattle: Effect of distance to water feature on the proportion of household herds with at least one infected cow (distance in 250m intervals) Summary of three models (1. distance to streams, 2. distance to swamps, 3. distance to nearest stream/swamp), each model adjusted for number of cattle per herd as first effect.

Trypanosome species	Number of cattle per herd (1st predictor in each model)	distance to respective water feature (2nd predictor in 3 separate models)		
		1. streams	2. swamps	3. nearest feature (stream/swamp)
T. brucei s.l.	X^2_1=6.8, p<0.01	X^2_1=0.4, p=0.526	X^2_1=1.9, p=0.166	X^2_1=0.0, p=0.881
T. vivax	X^2_1=12.0, p=0.001	X^2_1=0.0, p=0.934	X^2_1=1.5, p=0.219	X^2_1=0.4, p=0.515
T. simiae	X^2_1=8.5, p<0.01	X^2_1=0.0, p=0.924	X^2_1=0.4, p=0.538	X^2_1=0.3, p=0.617
T.b. rhodesiense	X^2_1=8.5, p<0.01	X^2_1=0.6, p=0.454	X^2_1=0.0, p=0.966	X^2_1=2.3, p=0.131
Total tryps.	X^2_1=16.0, p<0.001	X^2_1=0.0, p=0.99	X^2_1=2.3, p=0.128	X^2_1=0.5, p=0.481

5.3.4.1.3 Funyula: pigs

When the probability of homesteads to own trypanosome infected pigs was analysed as a function of the distance of the homestead to water features in Funyula, there was no significant effect of the distance to water, the distance to swampland or the distance to the nearest of either of the two. All models were adjusted for the number of pigs per homestead for consistency between models, even though the number of pigs per homestead did not significantly affect the proportion of herds with at least one infected pig (Table 5.9).

The effect of distance to water measured in 250m intervals, on the probability of households having *T. brucei* s.l. infected pigs approached significance ($X^2_1=3.4$, p=0.06), which remained the case when the exact distance ($X^2_1=4.2$, p=0.039) or 100m distance intervals ($X^2_1=4.6$, p=0.032) were considered.

The effect of distance to water features, regardless of whether water or swamps were considered separately or jointly, on the probability of herds to have *T. simiae* infected pigs, approached significance (Table 5.9). The geographical proximity of the only three homesteads with *T. simiae* infected pigs (Figure 5.12), would always place them into the same or neighbouring distance brackets resulting in a significant or close to significant effect of distance to any feature on the prevalence of *T. simiae* in pigs. As this cluster of *T. simiae* infected pigs was not reported to be statistically significant (see section 5.3.1.1.2), these effects were reported but not investigated any further.
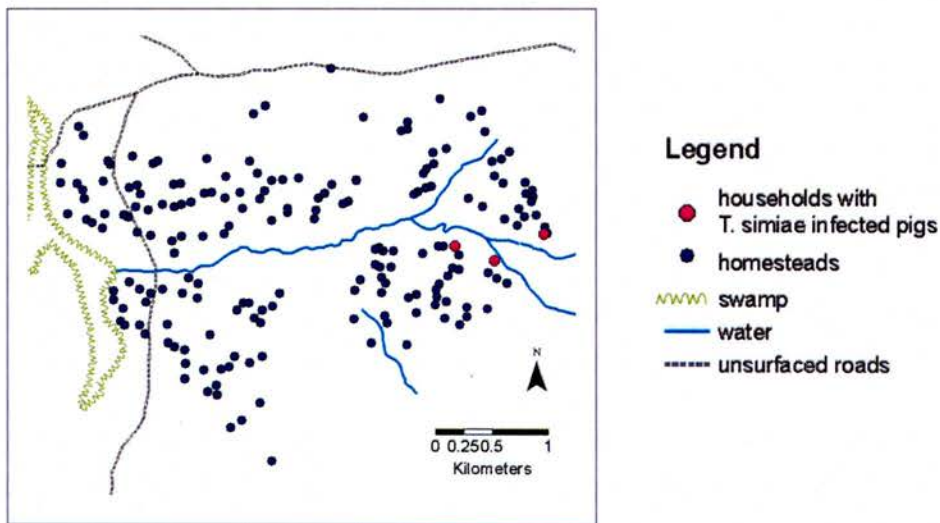
Figure 5.12: Funyula: Geographical proximity of homesteads with *T. simiae* infected pigs

Table 5.9: Pigs: Effect of distance to water feature on the proportion of household herds with at least one infected pig (distance in 250m intervals) Summary of three models (1. distance to streams, 2. distance to swamps, 3. distance to nearest stream/swamp), each model adjusted for number of pigs per herd as first effect.

Trypanosome species	Number of pigs per herd (1st predictor in each model)	distance to respective water feature (2nd predictor in 3 separate models)		
		1. streams	2. swamps	3. nearest feature (stream/swamp)
T. brucei s.l.	$X^2_1=3.2$, p=0.074	$X^2_1=3.4$, p=0.06	$X^2_1=3.3$, p=0.067	$X^2_1=0.0$, p=0.966
T. vivax	$X^2_1=0.7$, p=0.4	$X^2_1=0.4$ p=0.526	$X^2_1=0.2$ p=0.678	$X^2_1=0.0$ p=0.988
T. simiae	$X^2_1=6.2$, p=0.013	$X^2_1=6.0$, p=0.015	$X^2_1=3.6$, p=0.056	$X^2_1=5.1$, p=0.025
T.b. rhodesiense	$X^2_1=3.8$, p=0.051	$X^2_1=0.8$ p=0.368	$X^2_1=0.4$ p=0.512	$X^2_1=2.49$ p=0.115
Total tryps.	$X^2_1=4.6$, p=0.032	$X^2_1=0.6$, p=0.422	$X^2_1=0.9$, p=0.344	$X^2_1=0.12$, p=0.729

5.3.4.2   Butula

5.3.4.2.1 Butula: all livestock

In Butula, there was no significant effect of distance of homesteads to the nearest water feature on the probability of homesteads to include at least one trypanosome infected animal in their herd, in a model adjusted for the number of livestock per herd. This held true regardless of whether trypanosome infection were considered by species, or together, for all three approaches of analysis- distance to streams, distance to swamps or distance to the nearest of the two (streams/ swampland) (Table 5.10).

Table 5.10:All livestock: Effect of distance to water feature on the proportion of household herds with at least one infected animal (distance in 250m intervals) Summary of three models (1. distance to streams, 2. distance to swamps, 3. distance to nearest stream/swamp), each model adjusted for herdsize as first effect.

Trypanosome species	Number of animals per herd (1st predictor in each model)	distance to respective water feature (2nd predictor in 3 separate models)		
		1. streams	2. swamps	3. nearest feature (stream/swamp)
T. brucei s.l.	$X^2_1=12.3$, p<0.001	$X^2_1=0.64$, p=0.42	$X^2_1=1.8$, p=0.18	$X^2_1=0.1$, p=0.75
T. vivax	$X^2_1=28.8$, p<0.001	$X^2_1=1.15$, p=0.28	$X^2_1=2.4$, p=0.12	$X^2_1=0.68$, p=0.41
T. simiae	$X^2_1=1.6$, p=0.209	$X^2_1=1.9$, p=0.173	$X^2_1=0.889$, p=0.346	$X^2_1=2.8$, p=0.09
T.b. rhodesiense	$X^2_1=4.5$, p=0.033	$X^2_1=0.0$, p=0.952	$X^2_1=1.7$, p=0.193	$X^2_1=0.1$, p=0.801
Total tryps.	$X^2_1=26.4$, p<0.001	$X^2_1=2.2$, p=0.14	$X^2_1=1.8$, p=0.18	$X^2_1=1.3$, p=0.26

5.3.4.2.2 Butula: cattle

When only cattle were considered, there was a significant effect of the distance of homesteads to the nearest stream on the probability of households to own at least one infected cow, with the model adjusted for the number of cattle per herd (Table 5.11). This was attributed to a very low proportion of cattle herds affected by trypanosome infections at a distance of 500 to 750m from the nearest stream (Figure 5.13). When the effect of distance to streams was analysed using 100m distance intervals ($X^2_1=4.8$, p=0.03) and the exact distance ($X^2_1=5.3$, p=0.02) respectively, the effect on proportion of infected cattle herds still approached significance.
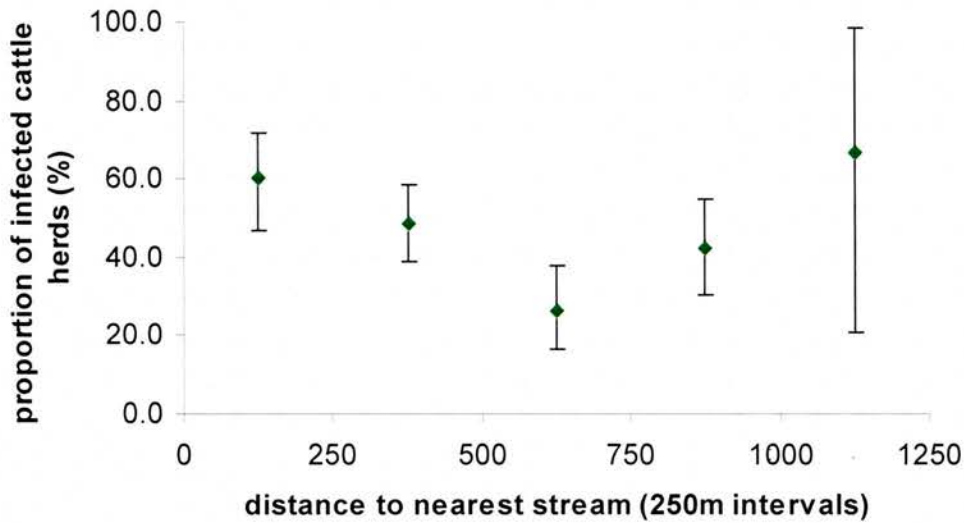
Figure 5.13: Proportion of cattle herds with at least one trypanosome infected cow with respect to distance to the nearest stream (in 250m intervals).

There was no significant effect of distance to swamp on the probability of infection in cattle herds. But the effect of the distance to the nearest water feature (swamp/ stream) approached significance (X^2_1=5.4, p=0.02), which was also the case when exact distance (X^2_1=5.0, p=0.03) or 100m distance intervals (X^2_1=4.5, p=0.03) were used. No significant effects of distance to streams, swamps or both were seen when the probability of infection in cattle herds were analysed separately for the individual trypanosome species (Table 5.11).

Table 5.11: Cattle: Effect of distance to water feature on the proportion of household herds with at least one infected cow (distance in 250m intervals) Summary of three models (1. distance to streams, 2. distance to swamps, 3. distance to nearest stream/swamp), each model adjusted for number of cattle per herd as first effect.

Trypanosome species	Number of cattle per herd (1st predictor in each model)	distance to respective water feature (2nd predictor in 3 separate models)		
		1. streams	2. swamps	3. nearest feature (stream/swamp)
T. brucei s.l.	X^2_1=8.1, p<0.01	X^2_1=2.2, p=0.138	X^2_1=1.4, p=0.244	X^2_1=1.2, p=0.264
T. vivax	X^2_1=16.5, p<0.001	X^2_1=2.0, p=0.16	X^2_1=0.2, p=0.65	X^2_1=1.9, p=0.17
T. simiae	X^2_1=0.0, p=0.925	X^2_1=1.8, p=0.185	X^2_1=2.2, p=0.142	X^2_1=2.8, p=0.093
T.b. rhodesiense	X^2_1=4.0, p=0.047	X^2_1=0.0, p=0.928	X^2_1=2.2, p=0.139	X^2_1=0.0, p=0.847
Total tryps.	X^2_1=10.1, p<0.01	X^2_1=6.5, p=0.01	X^2_1=0.3, p=0.58	X^2_1=5.4 p=0.02

5.3.4.2.3 Butula: pigs

When pigs were considered separately in Butula, there was no significant effect of distance of homesteads to the nearest water feature on the probability of homesteads to have at least one trypanosome infected pig in their herd, in a model adjusted for the number of pigs per herd. This held true regardless of whether trypanosome infection were considered by species, or together, for all three approaches of analysis- distance to streams, distance to swamps or distance to the nearest of the two (streams/ swampland) (Table 5.12). Even though the effect of the number of pigs per herd on the probability of infection of the herd was not significant, number of pigs was included as the first effect in all models for consistency (Table 5.12).

Table 5.12: Pigs: Effect of distance to water feature on the proportion of household herds with at least one infected pig (distance in 250m intervals) Summary of three models (1. distance to streams, 2. distance to swamps, 3. distance to nearest stream/swamp), each model adjusted for number of pigs per herd as first effect.

Trypanosome species	Number of pigs per herd (1st predictor in each model)	distance to respective water feature (2nd predictor in 3 separate models)		
		1. streams	2. swamps	3. nearest feature (stream/swamp)
T. brucei s.l.	$X^2_1=0.2$, p=0.67	$X^2_1=0.0$, p=0.828	$X^2_1=0.0$, p=0.827	$X^2_1=0.0$, p=0.952
T. vivax	$X^2_1=2.7$, p=0.1	$X^2_1=0.0$, p=0.954	$X^2_1=1.9$, p=0.169	$X^2_1=0.0$, p=0.97
T. simiae	$X^2_1=2.5$, p=0.111	$X^2_1=0.1$, p=0.724	$X^2_1=0.7$, p=0.398	$X^2_1=0.1$, p=0.743
T.b. rhodesiense	$X^2_1=0.3$, p=0.605	$X^2_1=0.7$, p=0.396	$X^2_1=0.0$, p=0.879	$X^2_1=0.5$, p=0.475
Total tryps.	$X^2_1=3.2$, p=0.072	$X^2_1=0.0$, p=0.994	$X^2_1=0.0$, p=0.827	$X^2_1=0.0$, p=0.968

5.3.5 Summary: Effect of distance to water features on trypanosome infection status of herds

5.3.5.1 Funyula

All livestock - cattle separately - pigs separately

- There was **no significant effect** of distance of homesteads to **streams**, distance to **swamps** or distance to the **nearest of the two features** (streams or swamps) on the probability of a herd to be infected for all livestock considered together, or cattle and pigs considered separately respectively.

5.3.5.2 Butula

All livestock & pigs separately

- There was **no significant effect** of distance of homesteads to **streams**, distance to **swamps** or distance to the **nearer of the two water features** (swamp or stream), on the probability of a herd to be infected for all livestock considered together or when pigs were considered separately.

Cattle

- There was a **significant effect** of the **distance of homesteads to streams** on the probability of at least one **cow** from within the herd to be infected with trypanosomes when only cattle were considered (lowest probability of infection at intermediate distance interval, 500-750m)

- There was **no significant effect** of distance of homesteads to **swamps** or of the distance to **the nearest water feature** (swamp or stream) on the probability of at least one cow from within the herd to be infected with trypanosomes when only cattle were considered.

5.4 Discussion

Whilst spatial analysis can quite literally add three dimensions to epidemiological data, the user must be aware of the pitfalls and limitations of the approach. Spatial analysis can never prove causal links to a disease but detection of patterns of disease or factors associated with disease occurrence can serve to raise, strengthen or disprove hypotheses on the causes of disease (Openshaw, 1996).

The analysis in Chapter 4, showed a significant effect of host species on trypanosomiasis prevalence, with an overall prevalence close to 20% in cattle and a prevalence under 5% in small ruminants in both Funyula and Butula. The trypanosomiasis prevalence in pigs varied between sampling areas. In Funyula the prevalence in pigs (17.4%) was similar to that in cattle, whereas in Butula the trypanosomiasis prevalence detected in pigs was under 10%. Therefore multivariate cluster analysis adjusted for the spatial distribution of the individual host species was required in this chapter, in order to avoid detection of clusters solely due to clustering of host species within the sampling area.

Spatial heterogeneity not only refers to the variables that describe the data but also to the nature of the spatial unit themselves which may be arbitrary in relation to the disease under investigation (Kitron, 1998). For example describing trypanosome prevalence according to administrative units may have little relevance, as tsetse challenge will not cohere to these boundaries, even if host movements may be influenced by them. Village boundaries within the sampling areas were observed to be relatively fluid in nature. Villages were located close to each other, and it was not possible to determine village boundaries visually on a map without prior knowledge as to which homesteads belonged to each village. During sampling there were even occasional disputes between village elders about which village a borderline homestead belonged to. It was thus decided not to differentiate between villages for spatial analysis, even though the effect of village on trypanosomiasis prevalence was taken into consideration for the risk factor analysis in Chapter 4.

5.4.1 Absence of trypanosomiasis clusters in Funyula

There were interesting differences in the pattern of the spatial distribution of trypanosome infected animals that emerged from the two sampling areas. In Funyula, allowing a maximum cluster size of 50% of the total animal population, no significant clustering was detected in the distribution of overall trypanosomiasis. Of the trypanosome species assessed separately, only the most likely cluster of *T. brucei* s.l. approached statistical significance. Within the circumference of this most likely cluster, only cattle (but none of the other host species) had a higher than expected *T. brucei* s.l. infection prevalence. Interestingly, *T. b. rhodesiense* infected animals appeared to be distributed randomly across the sampling area. In fact, not a single *T. b. rhodesiense* infection detected in either cattle or pigs coincided spatially with the most likely *T. brucei* s.l. cluster.

5.4.2 Significant clustering of trypanosome infected livestock in Butula

The spatial distribution of trypanosome infected animals within the Butula study area provided an entirely different picture. Allowing a maximum cluster size to include 50% of the total animal population, a significant cluster of overall trypanosomiasis infections was detected, including 45.5% of homesteads in the sampling area, accounting for 45% of the total animal population. The close association between percentage of homesteads and percentage of animal population included in the cluster signified that the cluster wasn't preferentially composed of homesteads with herds of below or above average size. The high proportion of animals included within the cluster and the positioning of the cluster indicates that the study area seems to contain a high risk area located to the southwest, with the majority of the high risk cluster located between the two streams traversing the study area, and a low risk area to the north and east. The ratio of observed/expected number of trypanosome infected animals was raised for all 4 host species within this significant cluster indicating that all species were at increased risk of trypanosomiasis within as compared to out with the cluster.

Separate analysis of the spatial distribution of the individual trypanosome species in Butula, revealed a large significant cluster of *T. brucei* s.l. infected animals, in the same general location as the cluster for overall trypanosome infections (Figure 5.10). In the case of Butula, a cluster approaching statistical significance was also detected in the distribution of *T. b. rhodesiense* infected animals. This cluster largely coincided with the location of the high risk *T. brucei* s.l. cluster. In the *T. brucei* s.l. cluster, the risk of infection was raised above average for all four host species, as indicated by the ratio of observed/expected number of cases. In the most likely *T. b. rhodesiense* cluster, this ratio was raised for cattle, pigs and goats. Out of a total of 17 *T. b. rhodesiense* infection (12 cattle; 4 pigs & 1 goat) 15 were within the circumference of the cluster (10 cattle, 4 pigs & 1goat), which approached statistical significance. Sheep were excluded from this analysis, as no *T. b. rhodesiense* was detected in this species. When the distribution of *T. b. rhodesiense* infected animals was analysed against a background population of *T. brucei* s.l. infected animals, no clustering of *T.b. rhodesiense* was detected, indicating that *T. b. rhodesiense* infected animals were randomly distributed amongst *T. brucei* s.l. infected animals within this locality.

A cluster approaching statistical significance, was also detected for the spatial distribution of *T. vivax*, with a raised risk for all four host species. This cluster largely included the same homesteads as the *T. brucei* s.l. cluster, indicating that the same factors are associated with higher risk of infection for both these trypanosome species. No significant clustering of *T. simiae* infected animals was detected, which may be attributed to the low total number of *T. simiae* infections detected (13/1692). No separate cluster analysis was run for *T. congolense* as the number of infections detected was even lower (5/1692).

5.4.3 Considerations of scale of data and cluster shape

The scale of data needs to be taken into consideration for spatial analysis. The better the geographical resolution of data, the greater the danger that patterns depicted in the maps are artefacts representing a mixture of real patterns and variation in data

precision. Additionally, variation in results may occur when data are considered under different spatial scales, which is referred to as the modifiable area unit problem (Openshaw, 1996). Size of study area has been identified as a factor influencing the outcome of cluster analysis, with smaller maximum cluster sizes permitted (default: maximum cluster size to include 50% of the population) identifying a higher number of differentiated clusters (Gregorio *et al.*, 2006). Exploratory cluster detection, allowing different maximum cluster sizes has been suggested as a tool to determine ideal maximum cluster size for the size of study area and study population (Gregorio *et al.*, 2006).

Allowing a maximum cluster size of 50% of the total population, maximises the potential of the test to detect clusters of high prevalence, as all cluster sizes up to the maximum are "tried out" (a high rate cluster including over 50% of the population rather being indicative of a significant low rate cluster, in the remainder of the population). If this setting does not detect significance clustering, as was the case in the Funyula sampling area, restricting the maximum permitted cluster size would not reveal any additional results.

However, when a significant cluster including a high proportion of the population is detected, restricting the maximum permitted cluster size, whilst simultaneously allowing limited overlap of clusters reported, may serve to improve the definition of high risk areas. The combined area of partially overlapping smaller clusters would allow the high risk area to be defined as an area which is not necessarily circular. When this was applied on the data set from Butula, restriction of the maximum permitted cluster size to 30% whilst allowing limited overlap of clusters, revealed four cluster of overall trypanosome infections approaching statistical significance, all located within the circumference of the previously detected large significant cluster (Figure 5.7). These four clusters together encompass nearly all of the homesteads included in the larger, significant cluster, bar 8 homesteads to the north of the streams. However, visually the smaller clusters refine the high risk space, excluding the large area that does not contain any household at the edge of the study area.

This effect became even clearer when this mode of analysis was applied to *T. brucei* s.l. infections separately. Two significant clusters, and two clusters approaching significance were detected, which were all basically located within the circumference of the larger cluster detected in previous analysis. But within that circumference a number of homesteads in the very south of the study area were excluded from the high risk *T. brucei* s.l. space by the smaller clusters.

SaTScan is limited by using only circular scan windows, which may not be ideal to reflect clustering patterns that are of different shape such as oblong due to risk associated with structures like rivers, for example. A flexibly shaped scan statistics to avoid this problem has been published, however it was only appropriate for sample sets of up to 30 points, as a more efficient algorithm is required to deal with bigger sampling sizes (Tango & Takahashi, 2005). The exploratory analysis used here, demonstrated how the restrictions of SaTScan to circular scan windows can be circumvented, by additional analysis allowing crystallisation of differently shaped composite high risk spaces. However, the need for repeated exploratory testing reduces the statistical relevance of the detected clusters.

5.4.4 Differences in trypanosomiasis distribution: Funyula versus Butula

The differences in degree of heterogeneity of trypanosomiasis space between the two study areas Funyula and Butula within the same district were striking. The high risk clusters detected in Butula were situated between the two streams traversing the study area, prompting questions of possible associations with the riverine habitat preferences of *G. fuscipes fuscipes* which is the prominent tsetse species in Busia. But visual inspection did not reveal any obvious pattern of a higher prevalence of trypanosome infections following the course of the streams. Neither did statistical analysis detect any consistent effects of the distance of homesteads to any water feature, be it stream or swamps, on the likelihood of any trypanosome infections in the household herd. This was the case in the study areas both in Butula as well as

Funyula. Studies in the scientific literature have previously associated proximity of herds to the hydrographic network and to long vegetation swamps, with an increased risk of both animal and human trypanosomiasis (de La Rocque *et al.*, 1999; Michel *et al.*, 2002; Odiit *et al.*, 2006). In all these studies however, the risk space was divided into a distance of above or below 2km of the nearest swamp/river, as the distance which tsetse are perceived to travel (Michel *et al.*, 2002). All homesteads in the present study were located within 1500m of the nearest stream, thus such a separation of risk space was not possible in the current study, which may indicate that the study area was too small in scale to assess the influence of proximity to swamps and rivers on the prevalence of animal trypanosomiasis. Nevertheless, heterogeneity in trypanosome space was detected in Butula but not in Funyula.

A second species of tsetse, *G. pallidipes*, which is a savannah species, has been reported to occur at low apparent densities in Busia District, with hilly areas as preferred habitats (Angus, 1996; Karanja, 2006). The Funyula study area is located in close proximity of the Funyula Hills. Presence of a suitable habitat for both riverine and savannah species may explain the apparent absence of heterogeneity in trypanosome prevalence across this study area. The heterogenous distribution of trypanosomiasis risk in Butula, may also have been associated to sugar cane which was observed to be planted throughout the Butula but not the Funyula sampling area. As sugar cane is a cash crop it warrants higher input, and the use of insecticides may affect the distribution of tsetse in Butula. However, neither of these two possible explanations could be confirmed as tsetse trapping as well as analysis of satellite imagery for vegetation cover, which may have served to explain the heterogeneity in trypanosome risk in Butula, was beyond the scope of this study.

5.5 Conclusion

The importance of spatial epidemiology of infectious diseases has long been recognised (Pavlovsky, 1966). Recent advances in computer technology and reduction in computing times have made spatial analysis widely accessible and have led to renewed interest in this field. Development of new techniques applied in spatial analysis are still very much in progress, and comparison of a variety of approaches available to detect clustering of disease cases, on artificial data sets, showed that there is as yet no "one size fits all" approach available. The current study showed how the circular SaTScan statistic, could detect high risk cluster and further exploratory analysis (using overlapping smaller maximum permitted cluster sizes) could refine the area of interest to a composite high risk space, no longer strictly bound to a circular shape.

Spatial analysis, using cluster analysis, revealed interesting patterns of the geographic distribution of trypanosome infected animals. The pattern was different between study areas, with a relatively homogenous distribution in Funyula as compared to division into high and low risk areas in Butula. The level of trypanosome infections was different between host species, however within each of the study areas, the pattern of spatial trypanosome distribution held true across host species. In Butula, the significant clusters detected represented increased risk of trypanosome infection for all host species considered (cattle, pigs and small ruminants), whereas in Funyula, no significant clusters of trypanosome infections were detected for any of the host species. This was the first study to investigate the spatial distribution of trypanosomiasis in pigs and small ruminants, in addition to cattle.

The distribution of trypanosomiasis infections could not be explained by the effect of proximity to water features in the current study, as had previously been demonstrated in a range of studies on cattle trypanosomiasis (de La Rocque et al., 1999; Michel et al., 2002) and Rhodesian sleeping sickness (Odiit et al., 2006). This illustrated that

insights on risk factors for the spatial distribution of trypanosomiasis gained from large-scale studies could not be directly transferred to the micro-scale. Furthermore, these results strengthened the suggestion of a more dispersed tsetse challenge throughout the study area, which was previously formed in Chapter 4. Analysis of exposure of livestock herds to presumed preferred tsetse habitats through grazing and watering management showed no consistent effect on trypanosomiasis risk in herds in Chapter 4.

6 Chapter 6   Central versus census sampling

6.1 Introduction

Busia, Kenya is an area endemic for animal trypanosomiasis, and simultaneously the human infective parasite *Trypanosoma brucei rhodesiense* is present in the livestock reservoir. To investigate the actual prevalence of trypanosomiasis a range of strategies for cross-sectional sampling are available, all of them aiming to estimate the infection level in the population as accurately as possible. The various methodologies differ with respect to the amount of background information required, the sample size needed and the time and manpower involved. Sampling tactics directly affect the confidence with which resulting data can be extrapolated to the whole of the study population (Fraser *et al.*, 2006). Nevertheless, the sampling approach of a study may have to be selected out of necessity by the constraints of the study. This chapter introduces the standard sampling methodologies and their prerequisites, before comparing two cattle sampling strategies from opposing ends of the scale of required input: convenience sampling versus near census sampling.

Chapters 4 and 5 of this thesis have detailed the prevalence of trypanosomiasis in two study areas in Busia, Kenya. In these study areas all livestock was sampled. However, this type of sampling is not feasible for many studies. Therefore this chapter compares the detailed household census sampling strategy with the easier to implement convenience sampling at central points. Convenience sampling, which requires the lowest level of prior input, has frequently been used in the literature to estimate trypanosome prevalence (Van den Bossche & Rowlands, 2001; Sinyangwe *et al.*, 2004; Fèvre *et al.*, 2006; Fyfe, 2007), and was also conducted in both of the sampling areas of this study, prior to census sampling. It is the aim of this chapter to asses whether such a convenience sample is representative for the study population. For that purpose, the samples acquired by these two strategies are compared with respect to demographic data as well as the obtained results for trypanosomiasis prevalence in cattle, both animal and human infective species.

6.1.1 Sampling strategies

6.1.1.1 Census

A census, which, by definition, surveys the complete study population, is the only way to establish the true prevalence of a disease, assuming 100% sensitivity and specificity of the diagnostic test employed (Dohoo *et al.*, 2003). A census is commonly performed to obtain demographic data, such as number of citizens, age distribution, employment status and income. In Britain, such a population census is administered every ten years, the last of which took place in 2001 and was made obligatory by law to optimise return rates (National Statistics Online, Census 2001). However, constraints such as time, manpower and cost of diagnosis usually do not allow for census data collection to determine disease prevalence. Nevertheless, some studies achieve census or near census sampling of the study population. For example 2,400 out of 2,800 farms in California were surveyed to determine the prevalence of mycoplasmal mastitis in dairy cattle (Jasper *et al.*, 1979).

In many cases, census type data on diseases can only be collected for a small population, due to the high costs involved. The prevalence and spatial distribution of trachoma in humans for example was investigated by eye examination of the complete population of a single village in Tanzania (Polack *et al.*, 2005). In this case the study population equaled the village population, and data was collected for all individuals. The study provided evidence for a significant within household effect, which could be explained by infected flies mechanically transmitting the disease from one member of the household to the next. It would have been unfeasibly time consuming to collect such data on trachoma prevalence in the entire population of Tanzania. Instead a subset of the population can be tested, and provided that the sample is representative, the data thus collected can be extrapolated to the rest of the study population (Stovner, 2006). Different sampling strategies that aim to obtain such a representative sample are introduced in the following paragraphs.

6.1.1.2   Random sampling

6.1.1.2.1 Simple random sampling

A random sample of the study population is least likely to be biased, according to the laws of probability. The strategy ensures an equal chance for each member of the study population to be selected (Fraser *et al.*, 2006). For that purpose a list of the entire study population, usually referred to as the sampling frame, must be available prior to sample selection, performed for example by computer generation of random numbers. Population census data or administrative databases are frequently useful to provide such sampling frames. For example, census data of the Mulungu do Morro municipality in the state of Bahia, northeastern Brazil, collected by the Prefecture in 1998, was used by Gomes *et al.*, to randomly select households from this municipality for inclusion in a study on the prevalence of taeniasis and cysticercosis in humans in search for an explanation of the high prevalence of epilepsy in this municipality (Gomes *et al.*, 2002). As long as the sampling frame equals the study population, this method is least likely to produce a biased sample; however assembling said sampling frame may prove work intensive and not always feasible, particularly when little or no background data on the study population is available or census statistics are outdated (Milligan *et al.*, 2004).

6.1.1.2.2 Systematic sampling

For systematic sampling the study population is divided into groups at regular intervals, for example 100 individuals per group. The sample from the first group is selected at random, the following samples are collected at the same regular intervals. This sampling selection method does not require a complete sampling frame, only an estimate of the complete population size (Dohoo *et al.*, 2003). Due to the set regular intervals between samples, they are more evenly spaced throughout the study population than is usually achievable through simple random sampling. Systematic sampling is frequently applied for batch quality control in manufacturing. However, systematic sampling may be biased, if periodicity occurs in the study population

(Dohoo *et al.*, 2003). For example a study investigating the prevalence of tuberculosis in beef cattle, by sampling all cattle delivered to the local abattoirs at regular intervals on a certain day of the week, may be severely biased towards farmers that are in the habit of bringing their animals on those days. Equally the method can not be applied, if the study population does not filter through a certain "gateway" such as the abattoir, which allows data collection (Thrusfield, 1995).

6.1.1.2.3 Stratified sampling

For stratified sampling, the study population is divided into groups (strata) according to characteristics which influence disease prevalence such as age, sex or income bracket, for example. Within each of these groups samples are then selected at random. Usually the number of samples per stratum is allocated proportionally according to the number of individuals in that group. The obtained sample is thus evenly distributed with respect to population characteristics and allows adjustment for confounding factors (Rothman & Greenland, 1998; Shepherd *et al.*, 2005). For example, Gitau *et al.*, investigated the burden of tick-borne diseases in dairy cattle in Murang'a District Kenya, stratifying the sub-locations (administrative units) by agro-ecological zones and randomly sampled the same proportion of farms within each agro-ecological zone. Thus the study could differentiate between management factors such as grazing strategies or tick control which affected tick-borne disease prevalence, and confounding environmental factors such as vegetation and climate typical for each of the agro-ecological zones, that would influence tick burden (Gitau *et al.*, 1997).

Stratified sampling is a highly effective strategy but is dependent on the quality of information available for the complete sampling frame (Dunn & Ferri, 1999). For example, in a study on the influence of management factors on trypanosomiasis prevalence, cattle age and breed would have been of interest as factors that were suspected to confound trypanosome prevalence in cattle. However, as opposed to European countries, where such information is collected routinely for all cattle, with

each animal identifiable by a unique ear tag number associated with full breeding and movement records (DEFRA, 2006), the same type of data is near impossible to obtain on a large scale in East African countries and requires large resources in terms of man-power.

6.1.1.2.4 Cluster sampling

For cluster sampling, the study population is divided into non-overlapping groups, usually according to geographic limits. As opposed to stratified sampling, where the strata are selected to be homogenous with respect to characteristics affecting the disease under investigation, cluster sampling relies on within cluster distribution to be as heterogeneous as possible reflecting the variation displayed in the complete population (Hoshaw-Woodard, 2001). There is no need to construct a sampling frame for the whole of the study population, only information on the level of the primary clusters, say districts, is required at first (Dhand *et al.*, 2005).

For example, three stage cluster sampling, was performed to investigate the prevalence of tick-borne diseases in cattle in Western Kenya (Okuthe & Buyu, 2006). The study was conducted in the Uasin Gishu District in the northern part of the Rift Valley Province, Kenya. In the first stage, sub-locations (smallest administrative unit within the district) were selected at random, in the second stage two villages from within each of the selected sub-locations and in the third stage 30 farms from within each of the selected villages were selected. Thus it was not necessary to list all farms within the Uasin Gishu District, which would have been extremely time consuming, but only farms in the selected villages of the selected sub-locations needed to be identified.

If sufficient data is lacking to even select primary stage clusters, sampling can be performed using grid references. This method was employed to investigate the prevalence of tick-borne diseases, heleminthosis and trypanosomiasis in cattle by

microscopy in Busia, Western Kenya (Karanja, 2006). A grid was placed over a map of the geographic area to be sampled and sampling locations were selected by random generation of grid coordinates. Twenty randomly selected ten by ten km grids (out of a total of 39 grid squares), representing 35 villages, were selected and up to a maximum of three cattle per farmer were sampled. For grid-sampling it is important that the margins of the selected grids are observed strictly and no cattle outside these margins are sampled, even if no cattle are present within it. This is necessary to avoid overrepresentation of samples from sparsely populated areas (Thrusfield, 1995).

Cluster sampling is appropriate where the compilation of a data frame for the complete study population is unfeasible (Dhand *et al.*, 2005; Ngondi *et al.*, 2005; Shepherd *et al.*, 2005; Karimurio *et al.*, 2006; Okuthe & Buyu, 2006). However as each individual cluster is rarely representative of the whole study population, between cluster variance is usually larger than within cluster variance. The estimated population prevalence of a disease obtained through cluster sampling is thus less accurate than stratified or systematic sampling for the same sample size (Shepherd *et al.*, 2005). This is accounted for by the calculation of a confidence interval which incorporates inter-cluster variance as a measure of the heterogeneity of the study population and is usually a lot wider than the confidence interval of a random sample of the same size (Klar *et al.*, 1995).

6.1.1.3 Limitations of random sampling

Random sampling requires knowledge of the study population prior to sampling. Whilst there is no need to list all individuals of the study population for approaches such as cluster sampling, this approach still requires knowledge of the size of the population in each cluster unit, to be able to assure proportional probability of cluster selection (Hoshaw-Woodard, 2001). Even this type of information may be extremely difficult to gather.

For example, cattle numbers for Busia published by the Kenyan government in 2001 (Government of Kenya, 2001) were predictions based on the last credible livestock census which was carried out in Kenya between 1967 and 1969 as part of the UNDP/FAO East African Livestock survey. A FITCA census performed in Busia and Teso Districts in 2002, covered 72% of households, the quality of some of the data was criticised as doubtful in terms of validity and accuracy by the editors of the report (Mosi & Nyandega, 2002). This data was nevertheless included as it was deemed not to be grossly misleading. It was unfortunate that the livestock survey did not specify which data was deemed to be robust, and obvious incongruity in some tables throws doubts on the care with which the data was compiled. It would have been impossible to compile a frame sufficiently robust for random sampling for the whole of Busia, with the resources available for the study presented here. Constraints in time and money frequently necessitate the use of non-random sampling methods to collect data (Thrusfield, 1995).

6.1.2 Non-random sampling

6.1.2.1 Purposive sampling

Purposive sampling refers to the purposeful selection of individual animals to cover all strata with reference to characteristics that are known or thought to influence the disease under investigation (Dohoo *et al.*, 2003). This process is notably different to stratified sampling, where the study population is stratified prior to proportional random sampling within each strata. Whilst purposive sampling has the same aim of achieving a sample which reflects the variations of the study population, the person performing the selection has been shown to frequently severely bias the sample (Thrusfield, 1995).

Depending on the investigation, purposive sampling may even include only visibly diseased animals. For example in a study which aimed to investigate the infectivity of *T. congolense* to tsetse flies in relation to the degree of drug resistance of the different parasite strains, cattle were "short listed" for parasitological diagnosis on

the basis of clinical signs and subsequently included in the study if found to be infected with *T. congolense* (Van den Bossche *et al.*, 2006). Selecting visibly diseased cattle thus increased the chances of finding cattle with a *T. congolense* infection, minimizing costs in identifying study subjects. However this approach excluded animals that carried a chronic infection with low parasitaemia biasing the study towards virulent strains of the parasite. Purposive sampling may be useful for pilot studies to give a first indication of the effect of a number of factors, prior to more in depth studies, which would employ random sampling.

6.1.2.2 Convenience sampling

Convenience sampling refers to selection of a sample at convenient time points and locations (Shepherd *et al.*, 2005). This type of sampling is frequently used where the study population is poorly accessible in its usual environment (Muhib *et al.*, 2001). This is the case with the cattle population in Busia, Western Kenya, where infrastructure is poor, herd sizes are extremely small, with an average of two to three animals per households (Thuranira, 2005), and farmers graze their animals separately instead of merging their animals to big herds which would make them easier to access. As previously discussed there was no available census data from which households could be selected at random for sampling. Markets are one example previously employed in a study in Uganda as a site for convenience sampling of cattle (Fèvre *et al.*, 2006). Local markets take place on a weekly basis, at set locations, which are usually accessible by road and sufficient animals converge to allow sampling of reasonable number of cattle in a short period of time. Other sites for convenience sampling may include abattoirs or cattle dips and spray groups where these exist.

Whilst convenience sampling is thus cost and time effective, the sample obtained is less likely to be representative of the study population, as cattle will attend these points for a other reasons. For example, cattle owners are more likely to try and sell poorly rather than healthy cattle, to avoid veterinary costs or losses envisaged by the death of the animal (Thuranira, 2005). Similarly, there may be a high proportion of

young animals will be for sale at markets as fewer people would be interested in buying old and spent cattle. As the population sampled at convenience points is thus not the same as the intended study population, the results obtained from the sample can only be extrapolated to the study population with extreme caution.

6.1.2.2.1 Central point sampling

Central point sampling is in essence a type of convenience sampling. When target villages have been selected, whether through random selection or a more purposeful approach, the village population can be mobilized to bring animals to a central point. Usually some type of small incentive is offered, such as free treatment with anthelmintics, to compensate farmers for their time and effort (Fyfe, 2007).

Central point sampling has been applied to assess prevalence in studies investigating a variety of different aspects of trypanosomiasis, including the distribution of drug resistant trypanosome strains (Sinyangwe *et al.*, 2004), the correlation of trypanosome prevalence in cattle to herd health parameters (Van den Bossche & Rowlands, 2001), the prevalence and distribution of the human infective *T. b. rhodesiense* in the cattle reservoir (Fèvre *et al.*, 2005; Fèvre *et al.*, 2006), the effect of intervention strategies on animal health (Brownlow, 2007) and for impact assessment of a large scale trypanosomiasis control programme employing trypanocides (Fyfe, 2007). The central point approach avoids sampling of a subset of the population which assembled for a purpose other than the study in question. Nevertheless this type of sampling does not ensure that the cattle attending the central point are representative of the cattle population of the village and any extrapolations to the study population must be performed with great care taking into account the likely sources of bias (Shepherd *et al.*, 2005).

6.1.3 Likely sources of bias in non-random samples

Any factor that influences the prevalence of the disease under investigation can be a source of bias, when the sample is not representative of the study population with respect to that characteristic. These characteristics may be intrinsic to the individual animal or may be imposed by the owner such as use of the animal (ploughing, breeding, milking). Similarly the location of sampling may influence the distribution of the sample depending on the intention of the cattle owners attending. Factors that have previously been shown to affect the probability of trypanosomiasis infections include cattle age (Rowlands *et al.*, 2001), size (Vale, 1974), host defensive behaviour against tsetse and nuisance flies (Torr & Mangwiro, 2000), sex (Rowlands *et al.*, 1993; Torr *et al.*, 2006) as well as management factors related to tsetse exposure (de La Rocque *et al.*, 1999; Michel *et al.*, 2002) and level of veterinary care (Bett *et al.*, 2004). These factors were reviewed in detail in Chapter 4. The distribution of such factors in a non-random sample may be different to that in the target population, thus biasing the results on trypanosomiasis prevalence obtained. For example, the age distribution of central point samples may be skewed, as calves are frequently kept at home and thus less likely to be included than the remainder of the herd, which can conveniently be taken for sampling during their daily grazing routine.

Owner intention may also significantly bias samples that can be obtained at convenience points. As previously discussed, markets may have a high proportion of sick animals as owners will sell poorly animals first in order to minimize losses (Thuranira, 2005). A study conducted in Uganda, comparing trypanosomiasis prevalence in cattle sampled at local markets, with the cattle sampled at central points in the surrounding villages showed a significantly higher prevalence of trypanosomiasis in the samples obtained at the market. Only in a district epidemic for sleeping sickness at the time of sampling (Soroti), was the prevalence of trypanosomiasis equally high in village as in market samples (Fèvre *et al.*, 2006).

Motivation of farmers to attend central sampling point may also result in bias. It stands to reason that owners, with a higher interest in their cattle would be more likely to invest time and bring their cattle to a central sampling point, in particular when incentives such as free anthelmintics are offered. But it remains uncertain whether motivated owners attending central sampling points would lead to the cattle sample obtained at these points being biased towards well managed and thus healthier cattle, or whether this would be counterbalanced by farmers being more likely to bring sicker animals in the hope of free treatment.

6.1.4 Addressing sampling constraints

Non-random approaches are unlikely to provide an unbiased sample representative of the study population. Nevertheless researchers were frequently forced to resort to convenience strategies, when data frames for random sampling of the population in question were too costly to obtain, in terms of time and manpower required. Central point sampling at convenient sites, such as village crush pens, has been applied in a number of studies, investigating trypanosomiasis prevalence at the herd level. Study design varied in approach to cattle age and other factors that may bias the sample. Some of these studies only sampled adult cattle, thus eliminating potential bias due to different age demographics between sites, but simultaneously ignoring the role of young cattle as a trypanosome reservoir in the herd (Van den Bossche *et al.*, 1999; Van den Bossche & Rowlands, 2001). Another study, investigating the prevalence of trypanosomiasis in relation to drug resistance in Zambia, sampled cattle from a range of different groups, recording age, weight, sex and treatment history, but did not report the effect of these factors in the analysis (Sinyangwe *et al.*, 2004).

Central point sampling of cattle in individual villages was also applied to establish the prevalence of trypanosomiasis in the village herd with special interest in the extent of the reservoir of the human infective *T. brucei rhodesiense* in Uganda (Fèvre *et al.*, 2005; Fèvre *et al.*, 2006). Central point sampling in villages was deemed to give a more accurate estimate of trypanosomiasis prevalence in cattle than sampling at local markets, which was observed to generally result in a higher trypanosomiasis

prevalence estimate, possibly as a result of tendencies to preferentially sell poorly animals.

6.1.5 Aims

To date no assessment has been carried out as to whether cattle samples obtained at a central point were representative of the cattle population of the respective villages. It is the aim of this chapter to evaluate the extent of systematic bias in central point samples, when compared to the cattle population in the villages of the catchment area. For this purpose, central point samples were compared to near census sample of the cattle population of the surrounding villages, achieved by visiting all cattle owning households within the study villages in two different divisions within Busia District, Kenya. Extent of bias was assessed with respect to the demographic distribution of sex and age as well as the proportion of cattle attending the central point from the respective villages. Finally the accuracy with which trypanosomiasis prevalence of the study population could be estimated from the central point samples was evaluated by comparison with the prevalence established through near census sampling.

6.1.6 Null-Hypotheses

1- H_0: The cattle presented at central points are representative of the cattle population in the catchment area (distribution with respect to sex, age and in terms of proportion per study village herd).

2. H_0: There is no significant difference in trypanosomiasis prevalence in the cattle sampled at central points and the cattle population of the surrounding villages as established through homestead sampling.

6.2 Methodology

6.2.1 Samples

A detailed description of study design and sample collection is provided in Chapter 3. This chapter utilizes the data on the cattle samples collected by convenience sampling at two central sampling points within each of the two sampling areas in June 2004: Nangosia Crush and Sijowa Crush in Funyula and Burinda Crush and Ikonzo Crush in Butula (Figure 6.1). Additionally the data on the cattle samples collected during census sampling in July and October 2004 in Funyula and Butula respectively, were used.

(a) Funyula sampling villages

1- Oyato; 2- Siwongo B; 3- Mashebi;
4- Gulumwoyo; 5- Bolori; 6- Siwongo A
7- Sijowa; 8- Magogongo; 9- Sigulu B

● homesteads of sampling villages ; /M swamp
△ location of central sampling sites; ⌐ river
······· dirt road

(b) Butula sampling villages:

1- Bukhulumi; 2- Siroba B; 3- Ikonzo B;
4- Kengo; 5- Khwikali; 6- Bukhwako
7- Bujumba; 8- Sirikhaya B; 9-Nebolola
10- Sirikhaya A
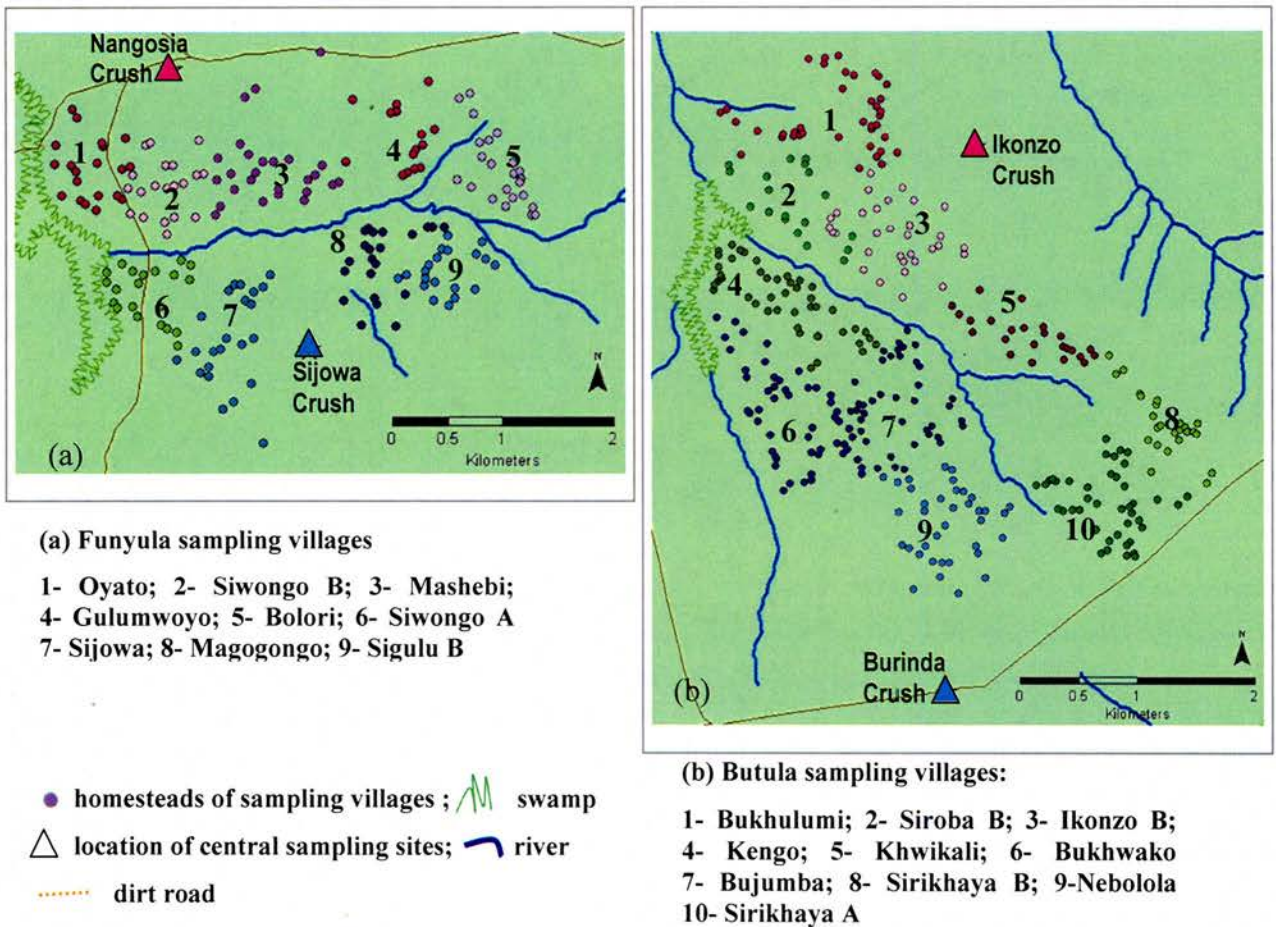
Figure 6.1: Map of sampling villages with reference to the catchment area of the respective central sampling sites (blue villages attend blue central sampling crushes, pink villages attend pink central sites, green villages did not attend central sampling)

Whole blood samples were collected from ear veins and stored on FTA cards. All samples were analysed in the laboratory for the animal infective African trypanosome species *T. brucei* s.l., *T. vivax*, *T. simiae* and *T. congolense* by PCR (Moser *et al.*, 1989; Cox *et al.*, 2005). All *T. brucei* s.l. positive samples were additionally screened for the human infective *T. b. rhodesiense* by PCR and Southern Blot (Picozzi *et al.*, in press). Data on cattle age group (a:<18 months, b: 18-36months, c >36 months) and sex (m: male, f: female) were systematically recorded for all samples. For all cattle attending central points, village of origin was also recorded.

6.2.2 Statistical analysis

Prevalence of trypanosomiasis as detected by PCR within any given group of samples was calculated as a percentage. The 95% confidence interval was computed using the exact binomial interval (R, version 2.0.1). The 95% confidence interval was displayed as error bars on the appropriate bar graphs.

Sample groups were tested for statistically significant differences in the prevalence of trypanosomiasis infection (or any other variable in question) using a binomial proportion test (χ^2) (R, version 2.0.1). (Degrees of freedom were noted as subscripts to the χ^2 statistics). Where frequencies were low (under 5 infected animals) the Fisher's Exact test (Fe) (R, version 2.0.1) was used to test for statistically significant differences in prevalence. Samples collected at central points and at the homesteads were assumed to be independent of each other. As was the case throughout this thesis, the level of statistical significance was adjusted to $p \leq 0.01$, due to multiple testing.

6.2.3 Overview

Table 6.1 provides an overview of the comparisons made between census samples and central point convenience samples in this chapter.

Table 6.1: Overview of comparisons between census and central point cattle samples

Factor of interest	Level of comparison
Sample demography	
Cattle sex (m, f)	Overall central (Nangosia, Sijowa, Burinda & Ikonzo Crush) versus Overall census (all cattle samples from Funyula & Butula)
Cattle age group (a,b,c)	Funyula central (Nangosia & Sijowa Crush) versus Funyula census (all cattle samples from Funyula)
Village of origin	Butula central (Burinda & Ikonzo Crush) versus Butula census (all cattle samples from Butula)
Proportion of sample with trypanosome infections	
	Central point sampling versus census sampling Overall central (Nangosia, Sijowa, Burinda & Ikonzo Crush) versus Overall census (all cattle samples from Funyula & Butula)
	Funyula central (Nangosia & Sijowa Crush) versus Funyula census (all cattle samples from Funyula)
	Butula central (Burinda & Ikonzo Crush) versus Butula census (all cattle samples from Butula)
Total tryps, *T. brucei* s.l., *T. vivax,* *T. simiae* *T. congolense,* *T.b. rhodesiense*	**Within sampling area variation** Funyula central Nangosia Crush versus Funyula central Sijowa Crush
	Butula central Burinda Crush versus Butula central Ikonzo Crush
	Individual central points versus catchment area census sample Funyula central Nangosia Crush only versus Funyula census samples from villages in catchment area of Nangosia Crush
	Funyula central Sijowa Crush only versus Funyula census samples from villages in catchment area of Sijowa Crush
	Butula central Burinda Crush only versus Butula census samples from villages in catchment area of Burinda Crush
	Butula central Ikonzo Crush only versus Butula census samples from villages in catchment area of IkonzoCrush

6.3 Results

6.3.1 Samples collected

6.3.1.1 Census sampling

A total of 1260 cattle blood samples from 407 cattle owning households were collected. Of these, 446 samples were obtained from 133 cattle owning households in the 9 sampling villages in Funyula and 814 cattle samples from 274 households from the 10 sampling villages in Butula. in October 2004. Sampling of ear-veins was attempted in all animals but those under two weeks of age. However, ear vein puncture failed to draw sufficient blood for laboratory analysis in a number of animals, due to small or collapsing ear veins. Nevertheless, when compared to the number of cattle that people stated they owned in the accompanying questionnaires, the sampled animals represented 92.9% and 93.9% of the cattle population in the Funyula and Butula sites, respectively (Table 6.2).

Table 6.2: Number of cattle sampled as proportion of cattle owned (as stated in questionnaire) by sampling area.

Sampling site	stated as owned	sampled	proportion sampled (%)
Funyula	480	446	92.9
Butula	867	814	93.9

6.3.1.2 Central sampling

In Funyula, a total of 201 cattle blood samples were collected from the two central sampling points, 123 at Sijowa crush and 78 at Nangosia crush. In Butula, 270 cattle samples were obtained at the two central points, 144 at Burinda crush and 126 at Ikonzo crush.

6.3.2 Comparison of demographics of cattle population of census sample versus central point sample

6.3.2.1 Sex distribution

Overall, there was a high female to male ratio in sampled cattle, with **72.6%** (342/471; 95% CI: 68.3-76.6%) of all cattle sampled at central points and **71%** (895/1260; 95% CI: 68.4-73.5%) of cattle sampled at homesteads being female. There was no significant difference in the proportion of female cattle sampled at central points as compared to the proportion of female cattle sampled at the homesteads during census sampling (χ^2_1=0.35, p=0.557) when samples from Funyula and Butula were combined. Equally, there was no significant difference in the proportion of cattle that were female between central points and the census sample, when samples from Funyula and Butula were assessed separately. In Funyula, **71.1%** (143/201; 95% CI: 64.4-77.3%) and **71.7%** (320/446; 95% CI: 67.3-75.9%) of the central point sample and the census sample respectively were female (χ^2_1=0.004, p=0.949). In Butula, **73.7%** (199/270; 95% CI: 68-78.9%) and **70.6%** (575/814; 95% CI: 67.4-73.7%) of the cattle at central points and in the census sample respectively were female (χ^2_1=0.79, p=0.375). There was no significant difference in the proportion of female cattle sampled between Funyula and Butula, at central points (χ^2_1=0.26, p=0.609) and at the homestead level during census sampling (χ^2_1=0.12, p=0.726).

6.3.2.2 Age category distribution

There were significant differences in the distribution of cattle over the age categories between central points and the census of the sampling areas (Table 6.3). Overall, the proportion of cattle in the youngest age category (category a) was significantly lower at central points (54/471; **11.5%**; 95% CI: 8.7-14.7%) than in the census (407/1260; **32.3%**; 95% CI: 29.7-35%) (χ^2_1=75.12, p<0.001) and reversely the proportion of cattle in the oldest category (category c) was significantly higher in samples from central points (324/471; **68.8%**; 95% CI: 64.4-73%) than in samples from the census (640/1260; **50.8%**; 95% CI: 48-53.6%) (χ^2_1=44.27, p<0.001). There was no significant difference in the overall proportion of cattle in age category b between

central point (89/471; **18.9%**; 95% CI: 15.5-22.7%) and the samples from the census (207/1260; **16.4%**; 14.4-18.6%) (χ^2_1=1.30, p=0.254).

Analysed separately, the same basic pattern in distribution over age categories was seen in samples obtained from Funyula and Butula (Table 6.3). The proportion of cattle in age category a was significantly lower in samples from central points than in the census sample in both Funyula (χ^2_1=7.62, p<0.01) and Butula (χ^2_1=66.93, p<0.001), whereas age category c was represented to a significantly greater extent in central point samples than in the census samples in Funyula (χ^2_1=0.9.17, p<0.01) and Butula (χ^2_1=34.63, p<0.001). There was no significant difference in proportion of age category b between central point samples and census samples in Funyula (χ^2_1=1.78, p=0.182), whereas in Butula a significantly higher proportion of samples in category b were sampled at central points than obtained through homestead sampling (χ^2_1=6.83, p<0.01).

Table 6.3: Distribution of cattle samples over age categories in Funyula and Butula

age category	Funyula		Butula	
	central point	census	central point	census
category a:	**10.9%** (22/201)[x]	**20.2%** (90/446)[y]	**11.9%** (32/270)[x]	**38.9%** (317/814)[z]
	(7-16.1%)	(16.6-24.2%)	(8.2-16.3%)	(35.6-42.4%)
category b:	**18.9%** (38/201)[x]	**24.0%** (107/446)[x]	**18.9%** (51/270)[x]	**12.3%** (100/814)[y]
	(13.7-25%)	(20.1-28.2%)	(14.4-24.1%)	(10.1-14.7%)
category c:	**68.2%** (137/201)[x]	**55.2%** (246/446)[y]	**69.2%** (187/270)[x]	**48.4%** (394/814)[y]
	(61.2-74.5%)	(50.4-59.8%)	(63.4-74.7%)	(44.9-51.9%)
not recorded	2% (4/201)	0.7% (3/446)	0% (0/270)	0.4%(3/814)

Proportions with different superscript (x,y,z) in the same row are significantly different

Comparing cattle sampled at central points between Funyula and Butula, there was no significant difference in the proportion of cattle in each of the age categories (category a: χ^2_1=0.03, p=0.87; category b: χ^2_1=0, p=1, category c: χ^2_1=0.02, p=0.88).

On the other hand, the age distribution of the cattle population as determined by census sampling, differed significantly between the two sampling areas. While the proportion of cattle in the youngest age category (category a) was significantly higher in Butula (χ^2_1=45.54, p<0.001), the proportion of cattle in the two older categories were higher in Funyula which was significant in category b (χ^2_1=27.91, p<0.001) but only approached significance in category c (χ^2_1=4.99, p=0.025) (Table 6.3).

6.3.2.3 Village of origin

Viewed by village of origin, the number of cattle examined at central sampling was not representative of the number of cattle presented and sampled in the corresponding village during census sampling (Figure 6.2 and Figure 6.3). Some of the villages of the sampling area were not represented at central points at all, such as Siwongo A in Funyula and Kengo, Sirikhaya A and B and Siroba in Butula. On the other hand, cattle from villages neighbouring the actual sampling area, but not part of the sampling frame and thus not sampled at the homestead level during census sampling, were included at central points (Nangosia, Namasumbi and Nandi in Funyula, Figure 6.2; Edadila, Ikonzo A, Mundaya, Namwitsula in Butula, Figure 6.3).
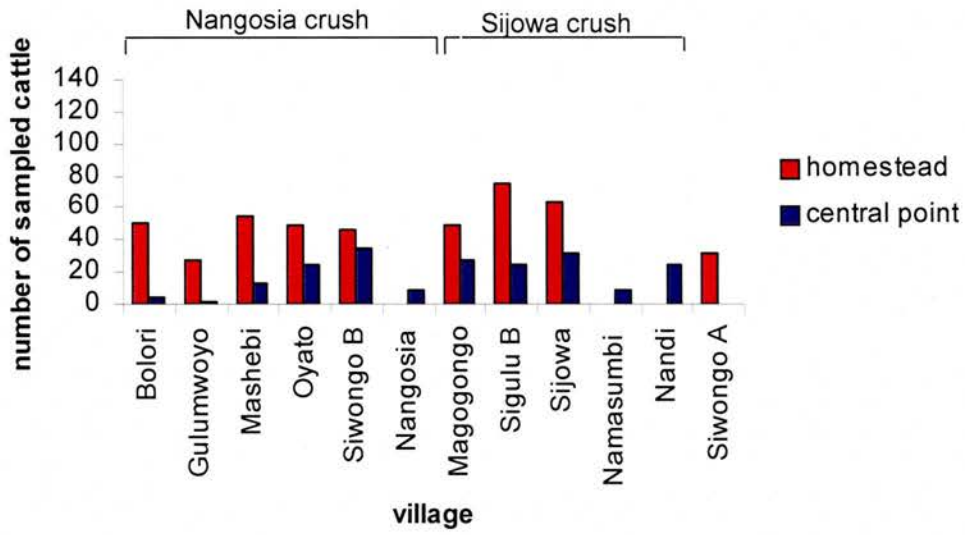
Figure 6.2: Distribution of cattle samples according to village of origin in Funyula: central versus census sampling
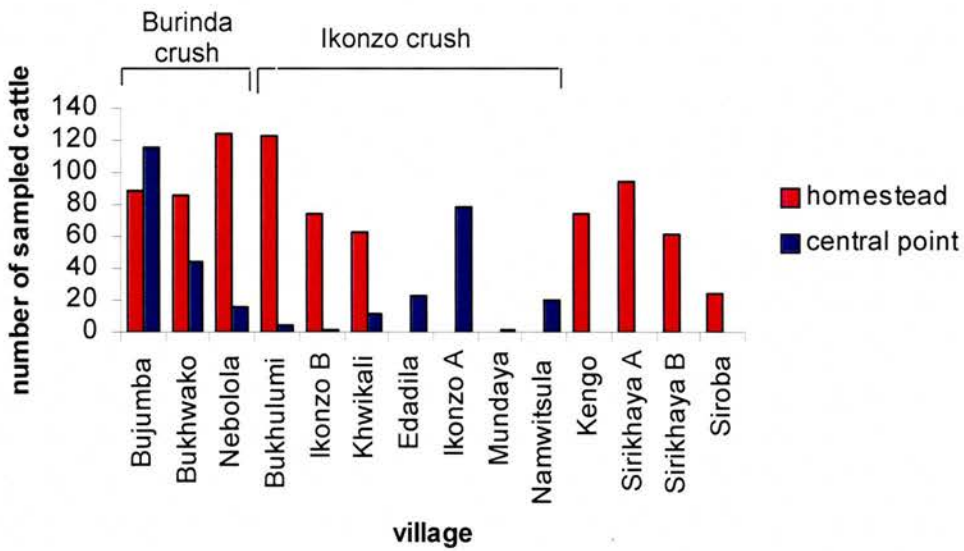
Figure 6.3: Distribution of cattle samples according to village of origin in Butula: central versus census sampling

6.3.3 Trypanosome prevalence in cattle samples

6.3.3.1 Funyula & Butula combined: central point versus homestead sampling

6.3.3.1.1 Total trypanosome prevalence

Of the 1260 cattle blood samples obtained in total through census sampling in Funyula and Butula, 253 samples tested positive by PCR for at least one pathogenic trypanosome species (*T. brucei* s.l., *T. vivax*, *T. simiae*, *T. congolense*) (**20.1%**, 95% CI: 17.9-22.4%). Of a total of 471 cattle blood samples collected at the four central points in Funyula and Butula, 77 were detected to be positive for pathogenic trypanosome infections, when screened by PCR (**16.3%**, 95% CI: 13.1-20%). There was no significant difference in overall prevalence of pathogenic trypanosomes between cattle samples from central points and in samples collected during census sampling (χ^2_1=2.85, p=0.091).

6.3.3.1.2 Individual trypanosome species

There was no significant difference in the overall prevalence of *T. brucei* s.l., *T. vivax*, *T. simiae* or *T. congolense* between central point cattle samples and census cattle samples (Table 6.4).

There was no significant difference in the overall prevalence of the zoonotic sub-species *T. b. rhodesiense* between cattle samples from central points (3/471; **0.6%**; 95% CI: 0.1-1.9%) and cattle samples from the census (19/1260; **1.5%**; 95% CI: 0.9-2.3%) (χ^2_1=1.44, p=0.231).

Figure 6.4: Prevalence of trypanosomiasis at central points (combined for all 4 central points) versus prevalence established through census sampling (combined for Funyula & Butula)

Trypanosome species	Prevalence (proportion) (95% CI)		χ^2_1	p
	Overall central point sample	Overall census sample		
T. brucei s.l.	**7.9%** (37/471) (5.6-10.7%)	**8.7%** (110/1260) (7.2-10.4%)	0.23	0.63
T. vivax	**8.1%** (38/471) (5.8-10.9%)	**11.1%** (140/1260) (9.4-13%)	3.12	0.08
T. simiae	**0.8%** (4/471) (0.2-2.2%)	**1.3%** (17/1260) (0.8-2.2%)	0.36	0.55
T. congolense	**0%** (0/471) (0-0.8%)	**0.2%** (3/1260) (0-0.7%)	n.a.*	0.57
Total infected cattle	**16.3%** (77**/471) 13.1-20%	**20.1%** (253***/1260) (17.9-22.4%)	2.85	0.09

*Fisher's exact test

** 2 mixed infections (2x *T. brucei* s.l.& *T. vivax*),

*** 17 mixed infections (14x *T. brucei* s.l.& *T. vivax*, 1x *T. brucei* s.l.& *T. congolense*, 1x *T. simiae* & *T. vivax*, 1x *T. simiae* & *T. congolense*)

6.3.3.2 Central points versus homestead sampling: comparison of trypanosome prevalence within each sampling area

6.3.3.2.1 Funyula: central point versus homestead sampling

There was no significant difference in total trypanosome prevalence in cattle in Funyula as established through central point sampling (38/201; 18.9%; 95% CI: 13.7-25%) as compared to census sampling (83/446; 18.6%; 95% CI: 15.1-22.5%) (χ^2_1=0, p=1).

When trypanosomiasis prevalence was broken down by species, there was no significant difference in the prevalence of the individual animal infective trypanosome species in cattle as between the central point sample compared to the

census sample (*T. brucei* s.l.: χ^2_1=0.02, p=0.90; *T. vivax*: χ^2_1=0.03, p=0.86; *T. simiae*: Fe p=0.67; *T. congolense*: Fe p=1) (Figure 6.3).

In Funyula, there was no significant difference in the prevalence of the zoonotic *T. b. rhodesiense* in cattle as established by central point sampling (1/201; **0.5%**; 95% CI: 0-2.7%) as compared to census sampling (7/446; **1.6%**; 95% CI: 0.6-3.2%) (Fe p=0.45).
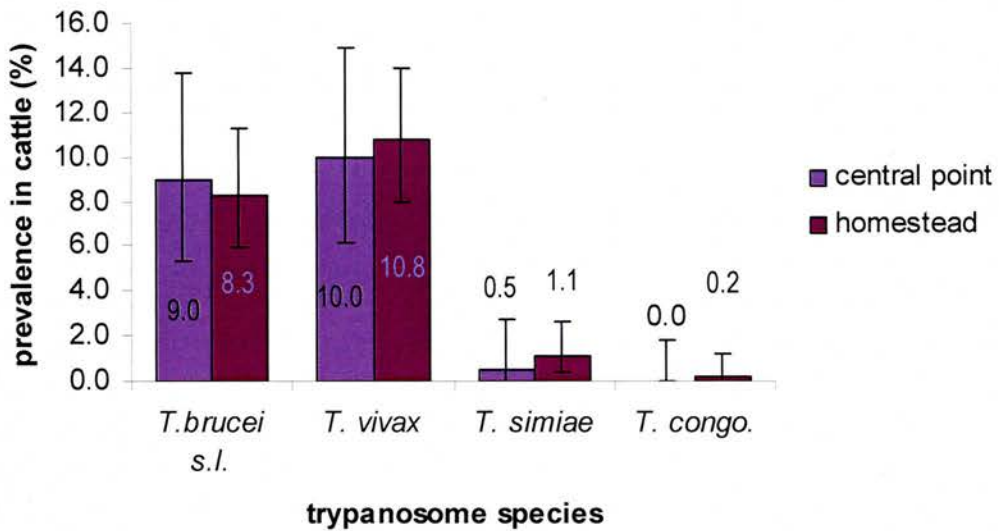
Figure 6.5: Prevalence of trypanosome species in cattle in Funyula: central point versus homestead sampling

6.3.3.2.2 *Butula: central point versus homestead sampling*

The prevalence of trypanosomiasis in Butula was higher in the cattle population sampled at the homestead level during census sampling (170/814; **20.9%**; 95% CI: 18.2-23.8%) than in cattle sampled at the central points (39/270; **14.4%**; 95% CI: 10.5-19.2%) with the difference approaching statistical significance (χ^2_1=5.0, p=0.03).

Broken down by individual trypanosome species, the prevalence of *T. vivax* in Butula was higher in cattle sampled at homestead level during census sampling than in the samples obtained at the central points with the difference approaching statistical significance ($\chi^2_1 = 4.28$, p=0.04). Although there was a tendency for a higher prevalence to be detected in the cattle samples from the census, there was no significant difference in the prevalence of any of the other trypanosome species tested for, between central point and census samples (*T. brucei* s.l.: $\chi^2_1 = 0.74$, p=0.39; *T. simiae*: Fe p=1; *T. congolense*: Fe p=1) (Figure 6.6).

In Butula, there was no significant difference in the prevalence of *T. b. rhodesiense* in cattle as established by central point sampling (2/270; **0.7%**; 95% CI: 0.1-2.7%) as compared to the census sampling (12/814; **1.5%**; 95% CI: 0.8-2.6%) (Fe p=0.54).
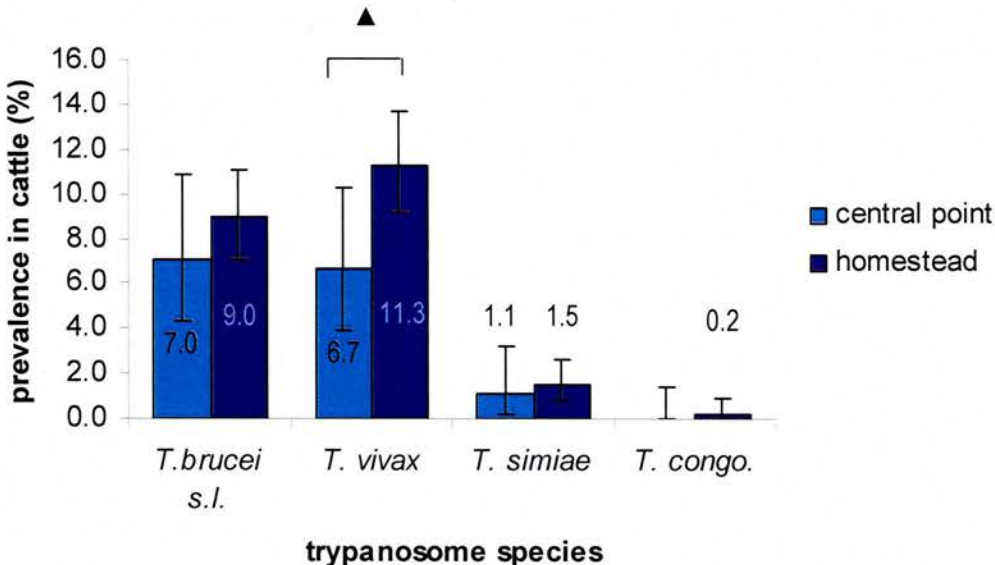
Figure 6.6: Prevalence of trypanosome species in cattle in Butula: central point versus homestead sampling, ▲ difference approaching statistical significance, p<0.05

6.3.3.3 Comparison of prevalence of trypanosomiasis between the two central points within each sampling area

6.3.3.3.1 Funyula: Nangosia central point versus Sijowa central point

In Funyula, there was no significant difference in the overall prevalence of cattle infected with trypanosomes between the two central sampling points (Nangosia: 15/78; **19.2%**; 95% CI: 11.2-29.7%; Sijowa: 23/123; **18.7%**; 95% CI: 12.2-26.7%) (χ^2_1=0, p=1). There was also no difference in the prevalence of any of the trypanosome species detected when analysed separately, between the two central points in Funyula (Table 6.5).

Table 6.4: Prevalence of trypanosome species at the two central point sites in Funyula: Nangosia versus Sijowa crush

Trypanosome species	Prevalence (proportion) (95% CI)		χ^2_1	p
	Nangosia crush	Sijowa crush		
T. brucei s.l.	**10.3%** (8/78)	**8.1%** (10/123)	0.07	0.79
	(4.5-19.2%)	(4-14.4%)		
T. vivax	**10.3%** (8/78)	**9.8%** (12/123)	0	1
	(4.5-19.2%)	(5.1-16.4%)		
T. simiae	**0%** (0/78)	**0.8%** (1/123)	n.a.*	1
	(0-4.6%)	(0-4.4%)		
T. congolense	**0%** (0/78)	**0%** (0/123)	-	-
	(0-4.6%)	(0-3%)		
Total infected cattle	**19.2%** (15**/78)	**18.7%** (23/123)	0	1
	11.2-29.7%	(12.2-26.7%)		

* Fisher's Exact Test used due to low frequency

** includes one sample with mixed infection (*T. brucei* s.l. & *T. vivax*)

6.3.3.3.2 Butula: Burinda central point versus Ikonzo central point

In Butula, there was a higher prevalence of trypanosome infected cattle at the Burinda crush central point (27/144; **18.8%**; 95% CI: 12.7-26.1%) than at the Ikonzo

crush central point (12/126; **9.5%**; 95% CI: 5-16%) with the difference approaching statistical significance (χ^2_1=3.91, p=0.05). The prevalence of *T. brucei* s.l. was significantly higher in samples collected at Burinda crush than at Ikonzo crush (χ^2_1=6.55, p=0.01). There was no significant difference in prevalence of any of the other trypanosome species between the two central points in Butula (Table 6.5). No *T. congolense* was detected in samples from either of the central points.

Table 6.5: **Prevalence of trypanosome species at the two central point sites in Butula: Burinda versus Ikonzo crush**

Trypanosome species	Prevalence (proportion) (95% CI)		χ^2_1	p
	Burinda crush	Ikonzo crush		
T. brucei s.l.	**11.1%** (16/144)	**2.4%** (3/126)	6.55	0.01
	(6.5-17.4%)	(0.5-6.8%)		
T. vivax	**8.3%** (12/144)	**4.8%** (6/126)	0.86	0.35
	(4.4-14.1%)	(1.8-10.1%)		
T. simiae	**0%** (0/144)	**2.4%** (3/126)	n.a.*	0.1
	(0.2.5%)	(0.5-6.8%)		
T. congolense	**0%** (0/144)	**0%** (0/126)	-	-
	(0-2.5%)	(0-2.9%)		
Total infected cattle	**18.8%** (27**/144)	**9.5%** (12/126)	3.91	0.05
	12.7-26.1%;	5 -16%		

* Fisher's Exact Test used due to low frequency

** includes one sample with mixed infection (*T. brucei* s.l. & *T. vivax*)

6.3.3.4 Individual central points compared to the respective catchment areas

The trypanosomiasis prevalence detected in cattle at the four central points was compared with the prevalence of trypanosomiasis as detected by census sampling in the villages of the sampling area that attended the respective central point (Figure 6.2 & Figure 6.3).

6.3.3.4.1 Nangosia crush central point (Funyula) versus catchment area samples

The villages from within the Funyula sampling area that attended Nangosia central point sampling were Bolori, Gulumwoyo, Mashebi, Oyato and Siwongo B (Figure 6.2). The prevalence of cattle with trypanosome infections from these 5 villages as established through census sampling, (40/227; **17.6%**, 95% CI: 12.9-23.2%) was not significantly different to the prevalence of cattle with trypanosome infections detected in the central point samples collected at Nangosia crush (15/78; **19.2%**, 95% CI: 11.2-29.7%) (χ^2_1=0.02, p=0.88). One mixed infection (*T. brucei* s.l. & *T. vivax*) was detected in the cattle samples from the central point, Nangosia crush, and four mixed infections (all *T. brucei* s.l. & *T. vivax*) were found in the cattle samples collected at the homesteads of the catchment area.

When trypanosome prevalence was broken down by individual species, there was no significant difference in the prevalence of *T. brucei* s.l. detected, between samples from Nangosia central point (8/78; **10.3%**; 95% CI: 4.5-19.2%) and in the census samples from the 5 villages in the catchment area (19/227; **8.4%**; 5.1-12.8%) (χ^2_1=0.08, p=0.78). There was no significant difference in the prevalence of *T. vivax* detected, between samples from Nangosia central point (8/78; **10.3%**; 95% CI: 4.5-19.2%) and the census samples from the 5 villages in the catchment area (25/227; **11%**; 95% CI: 7.3-15.8%) (χ^2_1=0, p=1). No *T. simiae* or *T. congolense* infections were detected in any of the samples at either the central point or in the catchment area (Figure 6.7a).

6.3.3.4.2 Sijowa crush central point (Funyula) versus catchment area samples

The villages from within the Funyula sampling area that attended Sijowa central point sampling were Magogongo, Sigulu B and Sijowa (Figure 6.2). The prevalence of cattle with trypanosome infections from these 3 villages as established through

census sampling, (37/187; **19.8%**; 95% CI: 14.3-26.2%) was not significantly different to the prevalence of trypanosomiasis detected in the central point samples collected at Sijowa crush (23/123; **18.7%**; 95% CI: 12.2-26.7%) (χ^2_1=0.01, p=0.92). [Five mixed infections were detected in the central point samples (4x *T. brucei* s.l. & *T. vivax*, 1x *T. brucei* s.l. & *T. congolense*)].

There was no significant difference in prevalence of individual trypanosome species between samples collected at Sijowa central point and samples collected during census sampling at the homestead level in the 3 villages of the catchment area of Sijowa crush (*T. brucei* s.l.: χ^2_1=0, p=1; *T. vivax*: χ^2_1=0.05; p=0.82; *T. simiae*: Fe p=0.4; *T. congolense*: Fe p=1) (Figure 6.7b).
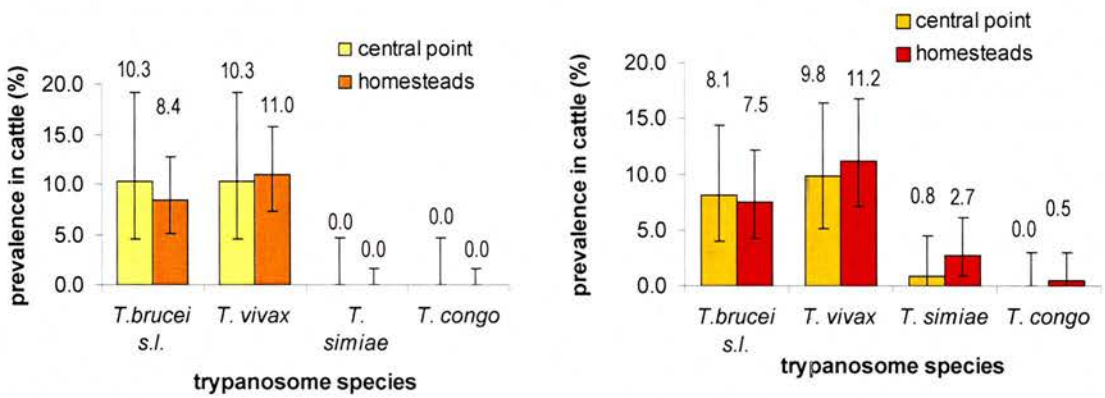
a) Nangosia crush versus census sample from villages in catchment area (Bolori, Gulumwoyo, Mashebi, Oyato and Siwongo

b) Sijowa crush versus census sample from villages in catchment area (Magogongo, Sigulu B and Sijowa villages)

Figure 6.7: Trypanosomiasis prevalence at the two central sampling points in Funyula versus prevalence in the census samples of the respective catchment areas

6.3.3.4.3 Burinda crush central point (Butula) versus catchment area samples

The villages from within the Butula sampling area that attended Burinda central point sampling were Bujumba, Bukhwako and Nebolola (Figure 6.3). There was no significant difference in prevalence of trypanosome infected cattle from these 3 villages as established through census sampling, (79/299; **26.4%**, 95% CI: 21.5-31.8%) and through the central point sampling at Burinda crush (27/144; **18.8%**; 95% CI: 12.7-26.1%) (χ^2_1=2.74, p=0.1). [One mixed infection (*T. brucei* s.l & *T. vivax*) was detected in the central point samples and three mixed infections (3x *T. brucei* s.l. & *T. vivax*) were detected in the cattle samples collected at the homestead level in the catchment area.]

There was no significant difference in prevalence of individual trypanosome species between samples collected at Burinda central point and samples collected during census sampling in the 3 villages of the catchment area of Burinda crush. (*T. brucei* s.l.: χ^2_1=0.27, p=0.6; *T. vivax*: χ^2_1=1.92; p=0.17; *T. simiae*: Fe p=1). No *T. congolense* infections were detected in any of the samples (Figure 6.8a).

6.3.3.4.4 Ikonzo crush central point (Butula) versus catchment area samples

The villages from within the Butula sampling area that attended Ikonzo central point sampling were Bukhulumi, Ikonzo B and Khwikali (Figure 6.3). The prevalence of trypanosomiasis in cattle from these 3 villages as established through census sampling, (26/261; **10%**; 95% CI: 6.6-14.3%) was not significantly different to the prevalence of trypanosomiasis detected in the central point samples collected at Ikonzo crush (12/126; **9.5%**; 95% CI: 5-16%) (χ^2_1=0, p=1). [Two mixed infections (2x *T. brucei* s.l. & *T. vivax*) were detected in the cattle samples collected during census sampling in the catchment area.]

There was no significant difference in prevalence of individual trypanosome species between samples collected at Ikonzo central point and samples collected during census sampling in the 3 villages of the catchment area of Ikonzo crush (*T. brucei* s.l.: p=1; *T. vivax*: χ^2_1=0.21; p=0.65; *T. simiae*: Fe p=0.34; *T. congolense*: Fe p=1) (Figure 6.8b).
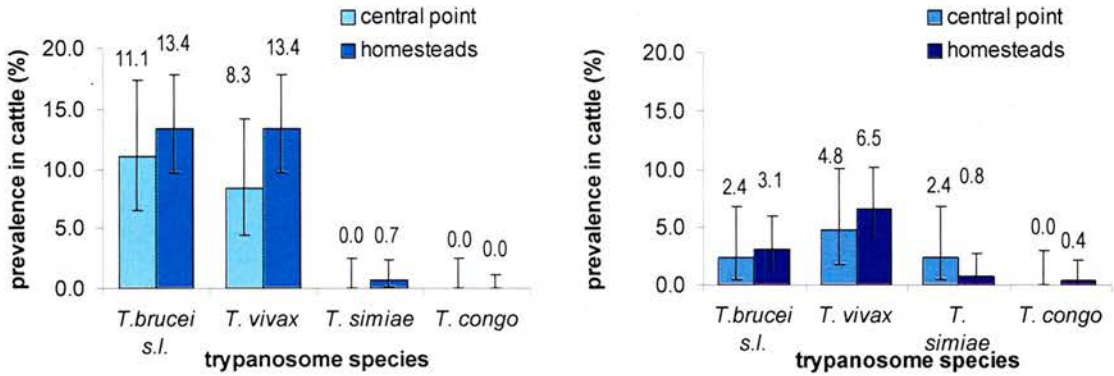
a) Burinda crush versus census sample from villages in catchment area (Bujumba, Bukhwako and Nebolola villages)

b) Ikonzo crush versus census sample from villages in catchment area (Bukhulumi, Ikonzo B and Khwikali villages)

Figure 6.8: Trypanosomiasis prevalence at the two central sampling points in Butula versus prevalence in census samples of the respective catchment areas.

6.3.4 Summary

6.3.4.1 Demography

- The central point samples were representative of the census of the cattle population in terms of gender distribution but not in terms of age distribution, with the cattle at central points being significantly older.

- The number of cattle attending the central points from each village were not representative of the number of cattle in each of the villages in the census sampling area.

6.3.4.2 Trypanosome prevalence

- Overall there was no significant difference in trypanosome prevalence between the central points (all 4 sites combined) and the census samples (combined for Funyula & Butula)

Funyula

- there was no significant difference in trypanosome prevalence between the central point samples and the census sample.

- there was no significant difference in trypanosome prevalence between the two central points

- there was no significant difference in trypanosome prevalence between the individual central point and the census sample of the villages in the respective catchment areas

Butula

- the prevalence of trypanosomiasis in the cattle population as determined by census sampling was higher than in the central point sample, with the difference approaching significance for overall trypanosome prevalence and for *T. vivax* prevalence

- the difference in overall trypanosome prevalence between the two central points approached significance. The difference in *T. brucei* s.l. prevalence between the two central points was significant

- there was no significant difference in trypanosome prevalence between the individual central point and the census sample of the villages in the respective catchment areas

6.4 Discussion

6.4.1 Coverage

Census sampling achieved coverage of over 90% of the cattle population of the villages in both study areas, Funyula and Butula. A complete census with 100% coverage is rarely achievable, even when participation is enforced by law (National Statistics Online, Census 2001). Voluntary participation and cooperation of cattle owners throughout the study were impressive, allowing achievement of a high coverage. Cattle were only omitted from homestead sampling for two reasons. Sampling was not attempted in very young calves under two weeks of age, as sampling from their small ear veins would have been extremely difficult and was deemed to potentially cause unnecessary distress to the calves at a vulnerable age. The other reason for exclusion of cattle from sampling was unavailability due to absence of owners. Whilst any data acquired through census sampling was still an estimate of the study population rather than exact data for the study population, the data was sufficiently close to a complete census to justify using the estimate as gold standard for the purpose of evaluation of the central point sample.

6.4.2 Statistical analysis

To assess whether central point samples were representative of the study population, one would ideally analyse whether there was a significant difference in the parameters in question between the sample obtained at the central point as compared to the rest of the study population, that did not attend the central point. This would fulfil the assumption of sample independence of the Chi-square test. For future studies a concerted effort should be undertaken to uniquely identify cattle at central points, in order to facilitate such a distinction.

For the present study, samples were collected opportunistically at central points, prior to homestead sampling. This order of sampling was necessary in order to avoid atypically good mobilisation for central sampling, as a response to a higher awareness, that would have been created, had census sampling taken place first. With

reference to discussions with local veterinary staff it was not deemed possible to uniquely identify cattle at central points, without alienating cattle owners and thus jeopardising cooperation during census sampling. Cattle marked in conjunction with a trypanosomiasis survey would easily be conjured to be marked as "diseased" rather than "sampled" by the local population, thus compromising the value of the animal in the eyes of the owner and any potential buyer. It was therefore not possible to identify which part of the population covered during census sampling had attended the central sampling point. Thus, the data presented here compared a subset of the population, the central point samples, with the complete population. Despite a reported high turnover rate of cattle in the study area and perceivable changes in trypanosomiasis infection status of individual animals between the time point of central sampling and census sampling, these two samples could not be considered independent and the assumptions of the Chi-square test were not fulfilled. Results therefore had to be interpreted with caution, in particular where p-values approached the significance threshold of 0.01 from either side. Nevertheless, the data was thought to be well worth presenting, as no evaluation of the extent of bias in a convenience sample had previously been presented in any of the studies employing such sampling methodologies.

6.4.3   Bias of central point samples as compared to homestead samples

6.4.3.1   Village of origin

The number of cattle that attended central points from each of the study villages was not proportional to the total cattle population of the respective villages. Whilst close to all cattle from some villages attended the central points, other villages were barely represented at all. In the instance of Bujumba village, Butula, more cattle attended the central point than were still found in this village three months later, when the census sampling took place, illustrating the high turnover in the local cattle population through trade or disease (Figure 6.3).

All villages that were part of the sampling frame were mobilised by informing the village chairman of the proposed sampling date and place and the incentive offered. Disproportional attendance from the different villages may have been due to differences in motivation and efficiency of the chairman, in passing on the information to the farmers. Other priorities of the village community on the sampling day, such as market day, or a funeral, can also severely reduce attendance. Close spatial proximity to the central sampling site may be expected to increase the proportion of the cattle population of a village attending, although this effect was not consistent (e.g. low attendance from Nebolola village, which was closest to the Burinda central site Figure 6.1).

Interestingly, farmers from villages that were not part of the sampling frame, and thus were not directly mobilised, also attended the central sampling points. These farmers may have been attracted to attend by word of mouth by friends and family in the study villages, expecting benefits of free de-worming. Animals from these villages were also included in the central point sampling, so as not to offend and jeopardise the good will of the local community. Low correspondence between the size of cattle population of mobilised villages and the attendance at the central points from these villages as well as non-mobilised villages, illustrated the difficulties in establishing the actual area, which was supposedly represented by the central sample.

6.4.3.2 Gender and age demographics

The demographic distribution of cattle samples collected at central points was shown to be unrepresentative of the study population of cattle in the surrounding villages with respect to certain parameters. The demographic parameters analysed were age and gender of cattle, as these were straightforward to determine, and were previously shown to affect trypanosomiasis prevalence (Rowlands *et al.*, 1993; Torr *et al.*, 2006).

There was no bias in terms of the distribution of gender, with approximately 70% of cattle being female, both at central points and as established through homestead sampling in Funyula as well as Butula. However, the age structure of the cattle sampled at central points was not representative of the age structure of the herd in the surrounding villages. Of the three age categories (a: 0-18 months; b: >18months -3 years and c: >3years), the oldest category c was represented to a significantly higher proportion at central points (68.8% of cattle in category c) than in the census sample of the study villages (50.8% of cattle in category c). Conversely young animals in category a, were significantly underrepresented at central points (11.5%) when compared to the age structure observed during census sampling (32.3% of cattle in category a). The same bias in age distribution towards older animals at central points was seen both in Funyula and Butula.

The discrepancy in age structure observed between central sampling and census sampling may be explained by cattle management practices in Busia. Pre-weaning calves were frequently kept separate from their dams for most of the day, to prevent suckling and improve milk yield for the owner (Angus, 1996; Rowlands et al., 2001). Calves under a year of age thus usually remained tethered at the homesteads, whilst the rest of the herd was taken for grazing . Farmers frequently attended central points with the same cattle that were taken for grazing, and hence fewer young animals would be seen at central points than when each homestead was visited individually. Mature cattle were also perceived as more valuable than very young animals (Thuranira, 2005), which were frequently observed to be sickly during homestead sampling, and could not necessary be expected to survive. Farmers thus may have preferentially brought mature animals to central points, in expectation of free de-worming, as they expected more certain returns in terms of milk yield and draught power from these animals if kept in good health. As cattle age has previously been shown to have a significant effect on the likelihood of trypanosome infection (Rowlands et al., 1993; Torr & Mangwiro, 2000), the skewed age distribution observed at central points was a potentially significant bias for the trypanosomiasis prevalence detected.

6.4.4 Trypanosomiasis prevalence

6.4.4.1 Sampling strategy

The combined trypanosomiasis prevalence detected in cattle samples from the study areas in Funyula and Butula was higher when determined through census sampling (20.1%) than when determined through central point samples (16.3%), although this difference was not statistically significant. It was however of interest that all individual trypanosome species tested for had a higher prevalence in the census than in the central samples by between 0.2 and 3 percent points (although this was not statistically significant). This was in contrast to predictions of a higher trypanosomiasis prevalence expected from the central point samples due to the higher proportion of mature animals included, which according to the literature, should have been more likely to carry trypanosome infections (Trail *et al.*, 1994; Rowlands *et al.*, 2001). It is possible that central sampling was attended by farmers generally more interested in the well-being of their cattle, resulting in the obtained sample being skewed towards better managed and healthier cattle.

6.4.4.1.1 Detection of human infective trypanosomes

In central point samples a lower proportion of *T. brucei* s.l. infected animals was detected to carry the human infective sub-species *T. b. rhodesiense* (3/37; 8.1%), as compared to homestead samples (19/110; 17.3%). Whilst this difference was not statistically significant (p>0.2), it illustrated that central point sampling may underestimate the extent of the animal reservoir of *T. b. rhodesiense* in Busia, Kenya, which is endemic for Rhodesian sleeping sickness. Rare diseases generally require a higher sample size to prove presence or absence in a study population (Cannon & Roe, 1982; Conraths *et al.*, 2003). In an area like Busia, Kenya where reports of sleeping sickness were rare, but the prevalence at which the parasite was maintained in the cattle reservoir was uncertain, more comprehensive sampling than can be achieved through central sampling may be advisable to assess the situation.

6.4.4.2 Effect of sampling strategy within different study areas

To further investigate the difference in trypanosomiasis prevalence observed between central point samples and census samples collected at the homestead level, the sampling areas in Funyula and Butula were analysed separately. In Funyula, the trypanosomiasis prevalence as estimated from the central point sample was representative of the population prevalence as established through census sampling.

In Butula on the other hand, the difference in trypanosomiasis prevalence as established by the two different sampling methods, approached statistical significance (p=0.03) with the overall trypanosomiasis prevalence detected in the central point samples being lower than in the homestead samples. This difference in effect of sampling methods between Funyula and Butula may have been caused by the increased time delay between central point sampling and homestead sampling in Butula (three months) as opposed to Funyula (one month). However, it was suspected that the difference in effect of sampling methodology on trypanosomiasis prevalence detected was at least partly a result of differences in the spatial distribution of trypanosomiasis infections within the two sampling areas, previously observed in Chapter 5.

6.4.4.3 Heterogeneity in trypanosome distribution within study areas

In Funyula, there was no significant difference in trypanosomiasis prevalence detected at the two separate central points (19.2% and 18.7%). An absence of significant clustering of trypanosomiasis in Funyula was already observed in Chapter 5. The relatively even distribution of trypanosomiasis infections over the cattle population of the study villages, thus made trypanosomiasis prevalence observed at central points independent of the village of origin of the cattle attending the central points.

In Butula on the other hand, the difference in overall trypanosomiasis prevalence detected at the two different central points (18.8% and 9.5%) approached

significance (p=0.05). The difference in *T. brucei* s.l. prevalence was statistically significant (p=0.01), with 11.1% of cattle attending Burinda crush being infected as opposed to *T. brucei* s.l. infections only being detected in 2.4% of cattle sampled at Ikonzo crush. Similarly the prevalence of *T. vivax* was higher at Burinda (8.3%) than at Ikonzo (4.8%), although not significantly so. The disparity in trypanosomiasis prevalence between central point samples in Butula suggests that the infections were not homogenously distributed over the cattle of this study area. Significant clustering in the distribution of trypanosomiasis, seemingly separating the sampling area into a high risk and a low risk part, has already been demonstrated in Chapter 5. Consequently, in Butula the area of origin of the cattle may have influenced the trypanosomiasis prevalence detected at the central points. As the attendance at sampling point was not representative of the number of cattle in the individual villages, this resulted in a skewed sample, with a trypanosome infection prevalence different to that observed through census sampling.

6.4.4.4 Central point sample representative immediate catchment area

There was no significant difference in trypanosomiasis prevalence between individual central point samples, and the cattle population in the immediate catchment area of these points. This was consistent with the theory of heterogeneity in trypanosome distribution over the whole of the study area directly affecting the prevalence detected at central points. Only at one of the four central points, Burinda in Butula, there was a trend for a higher prevalence of trypanosomiasis in the cattle population of the catchment area than in the central point sample (p=0.1). However, considering that the number of cattle attending the central points was not proportional to the cattle population in the individual villages, and central points were also attended by cattle from villages that were not part of the sampling frame for homestead samples, this disparity was still unexpectedly small.

6.5 Conclusions

In summary, central sampling was shown to give a good approximation of the trypanosomiasis prevalence in cattle in a given area in a time efficient manner. However, the results must be interpreted with due caution, as the cattle attending central points were not representative of the cattle population in terms of demographic parameters. It may be appropriate to stratify and analyse central samples according to age group, in order to adjust for discrepancies in age distribution as a potential source of bias and allow a more accurate comparison between the trypanosome prevalence obtained at different sampling sites.

Simultaneously, significant inaccuracies in trypanosomiasis prevalence as detected by central point samples could occur due to heterogeneity in distribution of trypanosome prevalence over the study area. This was suspected to be the cause of disparities in Butula where the overall trypanosomiasis prevalence as detected through the cumulative results of the two central points, was not representative of the trypanosomiasis prevalence of the study population as established through census sampling. This effect was reduced when the individual central point samples were only compared with the study population in their immediate catchment area.

As central point samples may only reflect the trypanosomiasis prevalence in their most immediate surrounding, calculation of trypanosomiasis prevalence for a wider area should treat samples collected at several different central points as cluster samples, rather than simple random samples (Thrusfield, 1995). The calculation of the associated confidence intervals for cluster samples takes into account the variability in trypanosomiasis prevalence that is likely to exist between the groups that constitute the clusters, when trypanosomiasis is not distributed homogenously across the study area of interest.

It was worth pointing out however that rare events, like the occurrence of *T. b. rhodesiense* infections in cattle, in an area endemic for sleeping sickness at a low

level, may be underestimated through central sampling. The maintenance of *T. b. rhodesiense* in the animal reservoir in Busia is indicative of a local transmission cycle and poses a human health risk. Transmission appears to be low at present, with only sporadic cases of sleeping sickness recorded. However the level of underreporting is significant in sleeping sickness endemic areas in neighbouring Uganda (Odiit *et al.*, 2005), which is also perceivable in Kenya although no specific studies on this have been published to date. Monitoring of changes in the *T. b. rhodesiense* reservoir in the animal population may help to predict any increase in risk to the human population. However, time-consuming census sampling may be warranted to assess this animal reservoir with the necessary accuracy.

7 Chapter 7   General discussion

7.1 Objectives

Trypanosomiasis in Kenya is no longer viewed as a public health issue but merely a constraint to livestock production, and livestock production is now considered a private enterprise. The responsibility for tsetse and trypanosomiasis control in Kenya has thus increasingly shifted from the state to the individual livestock owners, drastically reducing the scale of control approaches. This thesis examined the epidemiology of both animal infective and zoonotic trypanosome species in a range of domestic livestock at the micro-scale, in Busia, Kenya. The work was based on a unique cross-sectional census data set of the entire livestock population in the two study sites, employing sensitive molecular tools to detect trypanosome infections. Chapter 4 explored animal inherent indicators and potential management related risk factors affecting the probability of infection. Chapter 5 investigated the micro-geographic variation in the distribution of trypanosome infections over the study population and assessed the impact of distance to water features on trypanosomiasis risk at the herd level. Chapter 6 compared the census data with a convenience sample, to evaluate a protocol for rapid trypanosomiasis assessment.

7.2 Identifying the trypanosome reservoir – implications for control strategies

One of the unique features of infectious disease epidemiology is the fact that a case can simultaneously constitute a risk factor to the remainder of the susceptible population (Giesecke, 2002). In the context of the present investigation this means that any group of animals identified to be at high risk of trypanosome infections potentially also provides a source of infection for tsetse flies, thus perpetuating transmission. As wildlife was largely absent in the densely populated agro-pastoral study district of Busia, domestic livestock posed the only significant trypanosome reservoir. Cattle were the livestock species with the highest prevalence of trypanosome infections as detected by PCR (20.1%), followed by pigs (11.1%), whereas the prevalence in small ruminants was below 5%. As cattle are the livestock

species with the highest economic value, protection of cattle from pathogenic trypanosome infections is at the centre of productivity-motivated control strategies.

The majority of trypanosome infections in pigs were due to *T. brucei* s.l. (>50%). This potentially reduces the economic importance of the pig-based trypanosome reservoir for infecting tsetse (and thus indirectly infecting cattle), as *T. brucei* s.l. is generally considered to be only mildly pathogenic in cattle (Uilenberg, 1998). However, trypanosome infections should not be considered in isolation. In Busia, infections with the tick borne pathogens *Theileria parva, Anaplasma* and *Babesia* were detected in local zebu cattle at a prevalence of 6.9%, 16.4% and 4.8% respectively in a recent microscopy study (Karanja, 2006) and the District is also endemic for foot and mouth and lumpy skin disease. Mixed pathogen infections are therefore likely to occur. As interaction of pathogens in mixed infections may exacerbate the clinical condition of zebu cattle beyond the additive effect expected of the individual infections (Hofmann-Lehmann *et al.*, 2004; Tossas i Auguet, 2007), the importance of *T. brucei* s.l. for cattle health and productivity should not be underestimated.

Irrespective of the relative importance of *T. brucei* s.l. infections, the role of pigs as compared to cattle as a trypanosome reservoir in Busia remains uncertain. *G.f. fuscipes* the most prevalent tsetse species in Busia, is an opportunistic feeder and the proportion of *G.f. fuscipes* bloodmeals was reported to reflect the relative availability of hosts in studies in south east Uganda (Okoth & Kapaata, 1988) and western Kenya (Karanja, 2006). Due to their smaller size and lower density (~500 pigs as opposed to ~1350 cattle in the two study sites), pigs contribute a smaller proportion of the total livestock biomass in Busia compared to cattle. Additionally, turnover of pigs is higher preventing a build up of trypanosome infections over time. However, pig keeping is becoming increasingly popular in Busia, with an increase of the pig population by 150% having been recorded in the span of only two years (as compared to changes between -3.5% and +11.7% in small ruminant and cattle numbers over the same time period) (Thuranira, 2005). Pigs should therefore not be

neglected in future consideration of trypanosomiasis control strategies in Busia, Kenya, in particular as they were also observed to harbour the human infective *T.b. rhodesiense*, albeit at a low prevalence (2.9%).

At present the control of trypanosomiasis in Busia relies chiefly on the administration of trypanocidal drugs to cattle, suspected to be infected with trypanosomes (Machila-Eisler, 2005). However, two of the key indicators commonly used for the clinical diagnosis of trypanosomiasis, namely anaemia as judged by the colouration of mucous membranes and poor body condition (Eisler *et al.*, 2007), performed inadequately in the present study. There was no significant difference in trypanosome prevalence according to cattle condition score and even though the prevalence of trypanosomiasis was higher in anaemic than normal cattle, over 80% of trypanosome infected cattle did not display pallor of mucous membranes. With very low average profit margins on livestock production in Busia (Thuranira, 2005), there is no financial scope for more sophisticated diagnostic procedures, nor blanket treatment of all cattle. Drug treatment of only visibly ill cattle, as practised at present, only limits immediate economic losses, but will not efficiently reduce the reservoir or transmission of the parasite, which would be required for sustainable control.

Tsetse control has the potential to reduce trypanosome transmission, independently of having to identify the entire animal reservoir of the parasite. Vector control using traps and targets has previously been attempted in Busia (Echessah *et al.*, 1997), but lacked sustainability as community based activities were suspended as soon as outside support was withdrawn (Kamuanga, 2003). The cattle density in Busia is sufficiently high to facilitate sustainable depression of the tsetse population if a consistent proportion of cattle (5-10% according to FITCA estimates) were regularly sprayed with insecticide. However, the up-take of cost recovery based cattle spraying groups initiated by FITCA-Kenya in Busia was poor, with the majority of groups being disbanded once the start-up material was used up (FITCA, 2005). Research into restricted application of insecticides has shown that the amount of spray required per cow can be reduced by up to 80% (to 0.5l/cow) by only applying it to

the tsetse predilection sites of cattle belly and legs (Torr et al., 2007), making this technology very affordable. At the same time restricted application aids in maintaining endemic stability of tick borne diseases (Eisler et al., 2003), as limited tick attachment on sprayed cattle is still possible. However, spraying of cattle by farmers on an individual basis is still constrained by the price of the smallest unit of synthetic pyrethroids available for purchase (20 ml bottles- providing 20l of solution ready for use). In general, subsistence farmers do not have the cash flow resources to buy in bulk or stock pile, which makes single use sachets (from ketchup to shampoo) very popular in developing countries (Prahalad, 2007). The up-take of spraying by the community could be greatly improved by marketing of deltamethrin in smaller sachets, enabling farmers to spray their own animals (average of 2.3 cattle per livestock keeping household) when cash resources are available thus making spraying and vector control an attractive alternative to trypanocide treatments.

7.3 Micro-geographic variation in trypanosomiasis risk

Investigations of geographical variation in disease risk at the micro-scale, within the action radius of the local population, has given interesting insights into a wide variety of human infectious diseases including trachoma (Polack et al., 2005), malaria (Brooker et al., 2004), heleminth infections (Raso et al., 2006), and sexually transmitted diseases (Bernstein et al., 2004), to name just a few. For example, an investigation of the geographic distribution of Schistosoma mansoni and malaria infections in a Kenyan village served to explain co-infection patterns and resulting immune responses in local schoolchildren (Booth et al., 2004). But fewer studies at the local scale have been conducted with respect to animal infective diseases. The present study was the first to investigate the micro-geographic variation in animal trypanosomiasis risk taking into consideration the distribution of all livestock species affected.

Potential problems of small area studies include "background noise" resulting from random variations in infection prevalence and a lack of power to detect significant

clusters due to small sample sizes (Booth & Dunne, 2004). With a total of 2773 livestock samples from 549 household herds included in the present study, sample size was not an issue and spatial analysis detected significant clustering of trypanosome infections in one of the two study sites, Butula (Figure 5.10). The significant high risk clusters (overall trypanosomiasis as well as *T. brucei* s.l. considered individually) included approximately 45% of the total study population, with the case number within the cluster increased by a factor of 1.3-2 in all livestock species, compared to what would have been expected from a random distribution of cases. No significant clustering of trypanosomiasis was observed in the other study site, Funyula. The cluster of trypanosome infected livestock in Butula appeared to be located in between the two streams traversing the study area, however when this was investigated specifically, there was no significant association of trypanosomiasis risk to a herd and the proximity of the respective household locality to streams or swamps in either of the two sampling sites.

The significant trypanosomiasis clustering in Butula was thus suspected to be related to a clustered tsetse distribution independent of the classic riverine or swamp habitats associated with the main vector in Busia, *G. f. fuscipes* (Pollock, 1982). In large scale studies, proximity to rivers and long vegetation swamps has previously been identified as risk factors for cattle trypanosomiasis (Michel *et al.*, 2002) and human sleeping sickness respectively (Odiit *et al.*, 2006). Interestingly, the risk space for cattle trypanosomiasis was divided into distance under or over 2km from the river, 2km being the distance that the riverine tsetse species involved (*G. palpalis gambiense* and *G. tachinoides*) were perceived to span (Michel *et al.*, 2002). Within the present study all households were located within 2 km of swamps or streams, so that the apparent dispersed trypanosomiasis risk throughout the study area may be attributed to a dispersed tsetse challenge within the study area. This demonstrated that findings on spatial risk factors for trypanosomiasis can be scale sensitive, and heterogeneity of trypanosomiasis risk observed at the local scale may have to be incorporated into the bigger picture, to allow optimum interpretation of results (de La Rocque *et al.*, 2005).

Small scale studies also have to take into consideration the movement patterns and exposure times of the population to potential risk areas (Booth & Dunne, 2004). Previous studies reported identification of individual natural watering sites regularly frequented by cattle as trypanosomiasis transmission hotspots, with a localised high infection rate in tsetse attributed to a tight trypanosome transmission cycle between host and vector (de La Rocque et al., 1999). In contrast, confinement of livestock to the homesteads, instead of grazing in communal grounds and watering at the river did not provide a protective effect from trypanosomiasis infections in the present study, with over 50% of detected trypanosome infections occurring in livestock that were reported not to leave the homesteads. This strengthened the suspicion of dispersed tsetse challenge throughout the study area, with transmission apparently occurring in the peri-domestic space.

Peri-domestic transmission would also result in intense tsetse-man contact, putting the human population of Busia at high risk of contracting *T. b. rhodesiense* infections (Baldry, 1972), as the zoonotic parasite was demonstrated in the livestock reservoir in the present study. However, the low number of sleeping sickness cases reported from Busia over the last ten years (Figure 3.2) (WHO, 2006) does not support the supposition of such close human-tsetse interaction. These low numbers of reported sleeping sickness cases in Busia do not necessarily reflect the true risk to the human population. In sleeping sickness endemic areas in neighbouring Uganda, the level of underreporting of the disease is significant, with an estimated eleven people dying undiagnosed in the field for every sleeping sickness related death in hospital (Odiit et al., 2005). The level of underreporting in Busia, Kenya has not been investigated, but awareness for the disease is low, with a high proportion of people attributing clinical signs of the disease to HIV/AIDS or even witchcraft (Bukachi et al., 2005). Future work is required to investigate the apparent independence of trypanosomiasis risk from immediate proximity to riverine tsetse habitats in Busia and the postulated peri-domestic transmission, which could place considerable risk to human health. This would include entomological studies but also expansion of the work on animal

management and incorporate the exact grazing routes and time spent in each type of vegetation (of those animals not confined within the homesteads) into the spatial analysis as has previously been demonstrated by a number of studies in West Africa (Wacher *et al.*, 1994; Michel *et al.*, 2002).

7.4 Central sampling for the rapid assessment of trypanosomiasis

Whilst census sampling provided a precise picture of the distribution of trypanosome infections, census sampling proved to be extremely work, time and cost intensive, and is thus not feasible for larger scale studies without significant financial support. Compared to the "gold-standard" set by census sampling, convenience sampling at central points was shown to provide a time-efficient alternative, giving a good estimation of the trypanosome prevalence in cattle in the respective catchment areas. Of course, care must be taken to consider the limitations of convenience sampling, when the data from this sub-set is extrapolated to the remainder of the population. With a view to the bias in age groups and the difference in attendance between study villages observed in the present study, data analysis should account for these potential sources of bias by sample stratification.

However, whilst central sampling was found to be suitable for the rapid assessment of overall trypanosomiasis risk, this sampling technique underestimated the presence of the human infective parasite *T. b. rhodesiense*. This was likely to be due to the low overall prevalence of the parasite in the cattle population (1.5%). In general, a larger sample size is required to prove the absence of a rare disease (Cannon & Roe, 1982; Conraths *et al.*, 2003). The detection of *T. b. rhodesiense* in the animal reservoir is of critical importance to monitor the risk of sleeping sickness to the human population. Central point sampling not only underestimated the human infective parasite in the cattle reservoir, but as central sampling rarely includes other livestock species, it would also not pick up on *T. b. rhodesiense* detected in the pig population (2.9%) by census sampling. Where decisions on intervention strategies against human sleeping sickness are based on the presence or absence of the human infective parasite in the

animal reservoir, it may be advisable to employ a more intensive sampling strategy than central point sampling. At the very least, a larger sample should be obtained to account for a potentially low prevalence in *T. b. rhodesiense* in the cattle reservoir.

7.5 *The animal reservoir of* T. b. rhodesiense *in endemic and epidemic foci*

Busia, Kenya is situated at the south eastern perimeter of the historic Busoga sleeping sickness focus. In Busia, only sporadic cases of Rhodesiense sleeping sickness occurred over the last ten years, and it was thus not surprising to find that the zoonotic *T. b. rhodesiense* was only present at a low prevalence in the local animal reservoir (1.5% in cattle and 2.9% in pigs). These apparently stable conditions with respect to sleeping sickness in Busia, Kenya contrasted with the situation observed in Uganda, where the geographic extent of Rhodesian sleeping sickness has spread far beyond the classical Busoga focus over the last 20 years (Picozzi *et al.*, 2005; Berrang-Ford *et al.*, 2006). In areas newly affected by sleeping sickness, with a high number of human cases, up to 18% of cattle were shown to be infected with *T.b. rhodesiense* (Welburn *et al.*, 2001b).

The northwards spread of Rhodesian sleeping sickness within Uganda has continued and since August 2004 more than 300 cases of sleeping sickness have been reported from 3 newly affected Ugandan districts, Kaberamaido, Dokolo and Lira (Picozzi *et al.*, 2005). Recent investigations by the author (data unpublished) sampling a considerable number of cattle (n=1800) in these districts in central Uganda, revealed the presence of *T. b. rhodesiense* in the cattle reservoir. But contrary to expectations, the prevalence of *T. b. rhodesiense* detected in the cattle reservoir in these three newly epidemic districts was much lower (<2%) than the proportion of *T. b. rhodesiense* infected cattle previously reported from epidemic areas in Uganda (up to 18%) (Welburn *et al.*, 2001b). Despite this low prevalence of *T. b. rhodesiense* in the animal reservoir, the high number of sleeping sickness cases in these newly affected districts provides testimony for significant transmission of the parasite to

humans. These observations raised the following questions: If the animal reservoir of *T. b. rhodesiense* in an area with a current sleeping sickness epidemic is as low as the animal reservoir detected in Busia, Kenya, where apparently only sporadic human cases occur, which other factors drive the increased level of transmission to humans in the epidemic focus? Secondly, is there a danger of the balance of such factors changing in Busia, Kenya leading to an increase in human cases?

7.5.1 Future work

The course an infectious disease takes in a population can be estimated by its basic reproductive rate R_0 which is determined by the contact rate between infected and susceptible individuals and the probability of infection on contact (Dohoo *et al.*, 2003). This basic concept can be used to identify factors, which may influence the rate of *T. b. rhodesiense* transmission to humans, causing the difference between low endemic and epidemic foci.

The most prevalent tsetse species both in Busia, Kenya and in central Uganda is *G. f. fuscipes* (Ford & Katando, 1973), however differences in vector density and distribution may result in increased tsetse-human contact in central Uganda. Historically, sleeping sickness epidemics have commonly been associated with political upheaval (Koerner *et al.*, 1995), and the resulting changes in human-tsetse contact. Busia, Kenya has been consistently politically stable with little population movement in contrast to the situation in Uganda where the population has been subjected to continuing civil conflict associated with internal displacement of large parts of the population in central and northern Uganda (IDMC, 2006). Quantification of differences in vegetation and land-use patterns may consider the proportion of land under cultivation, as well as the proportion of overgrown shrub-land, which may provide suitable tsetse habitats, influencing tsetse density. Simultaneously population movement leading to re-cultivation of overgrown farmland, and the resulting increase in contact between tsetse and humans requires exploration. Furthermore, differences in the circulating strains of *T .b. rhodesiense* may influence infectivity to tsetse or vertebrate hosts and may elucidate differences in transmission patterns, even

though previous investigations revealed little differences in strains circulating during endemic and epidemic periods in the Busoga focus (Hide *et al.*, 1998).

7.6 *Outlook*

The work presented in this thesis investigated the epidemiology of trypanosomiasis in domestic livestock at the micro-scale in Busia, Kenya and demonstrated that subsistence farmers in a poor rural area are at present inadequately equipped to control livestock trypanosomiasis in a sustainable fashion. Treatment is administered to visibly sick animals to reduce economic losses but reservoirs of the disease are difficult to identify based on clinical signs. The blanket treatment of all livestock required to depress the trypanosome reservoir and reduce re-infection is not within most farmers' means. Limiting exposure of animals to supposed preferred tsetse habitats also did not have a protective effect from trypanosome infections and peri-domestic transmission was suspected.

Furthermore, the presence of the human infective *T. b. rhodesiense* in the animal reservoir demonstrated the continued threat of local transmission of sleeping sickness to humans in Busia. Even though the prevalence of *T. b. rhodesiense* in the animal reservoir in Busia was low, it was not significantly different to the prevalence recently detected in districts of Uganda newly affected by an epidemic of sleeping sickness. This highlighted the continuing risk to the human population in Busia. The danger of Rhodesian sleeping sickness lies in the devastation that can be caused by sudden epidemics of the disease, but the tipping point responsible for a change from endemicity with only sporadic cases of the disease to a full blown epidemic is still obscure. It would appear to be tempting fate to leave tsetse and trypanosomiasis control to be regulated purely by the market forces of livestock productivity, in an area such as Busia with a history of human sleeping sickness outbreaks. New and affordable concepts of tsetse control, such as restricted application of synthetic pyrethroids to cattle, could easily be brought within the financial reach of individual livestock owners, by the marketing of smaller units of the product. However,

government and donor organisations may have to consider providing support in the form of extension programmes and education of livestock owners, to ensure uptake of such techniques.

References

Agyemang, K., Dwinger, R.H., Little, D.A., Leperre, P., & Grieve, A.S. (1991). Interaction between physiological status in N'Dama cows and trypanosome infections and its effect on health and productivity of cattle in Gambia. *Acta Tropica,* **50**, pp. 91-99.

Angus, S.D. (1996). *The epidemiology of trypanosomiasis in village livestock in an endemic sleeping sickness area of western Kenya.* University of Glasgow, Glasgow.

Ashcroft, M.T., Burtt, E., & Fairbairn, H. (1959). The experimental infection of some African wild animals with *Trypanosoma rhodesiense, T. brucei* and *T. congolense. Annals of Tropical Medicine and Parasitology,* **53**, pp. 147-161.

Balasegaram, M., Dejene, S., Tinnemann, P., Perkins, S., & Davidson, R. (2006a). Examples of tropical disease control in the humanitarian medical programmes of MSF and Merlin. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **100**, pp. 327-334.

Balasegaram, M., Harris, S., Checchi, F., Ghorashian, S., Hamel, C., & Karunakara, U. (2006b). Melarsoprol versus eflornithine for treating late stage Gambian trypanosomiasis in the Republik of the Congo. *Bulletin of the World Health Organization,* **84**, pp. 783-791.

Baldry, D.A. (1972). A history of Rhodesian sleeping sickness in the Lambwe Valley. *Bulletin of the World Health Organization,* **47**, pp. 699-718.

Balfour, A. (1904). Trypanosomiasis in the Anglo-Egyptian Soudan. Preliminary Note. *The British Medical Journal,* **1904**, pp. 1455-1456.

Barrett, K. & Okali, C. (1998). Partnerships for tsetse control- community participation and other options. *World Animal Review, FAO,* **90**, pp.

Barrett, M.P., Burchmore, R.J.S., Stich, A., Lazzari, J.O., Frasch, A.C., Cazzulo, J.J., & Krishna, S. (2003). The trypanosomiases. *The Lancet,* **362**, pp. 1469-1480.

Barrowman, P., Stoltz, W.H., van der Lugt, J.J., & Williamson, C.C. (1994). Dourine. In J.A.W. Coetzer, M.C. Thomson, & R.C. Tustin (Eds.), *Infectious Diseases of Livestock* (Vol. 1). Oxford: Oxford University Press.

Batchelor, N. (2004). *An assessment of spatial risk factors determining the distribution of Rhodesian sleeping sickness in a low endemnicity area of western Kenya.* MSc Thesis, London School of Hygiene and Tropical Medicine, London.

Bauer, B., Gitau, D., Oloo, F.P., & Karanja, S.M. (2006). Evaluation of a preliminary title to protect zero-grazed dairy cattle with insecticide-treated mosquito netting in Western Kenya. *Tropical animal health and production,* **38**, pp. 29-34.

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

Beck, L.R., Rodriguez, M.H., Dister, S.W., Rejmankova, E., Ulloa, A., Meza, R.A., Robberts, D.R., Paris, J.F., Spanner, M.A., Washino, R.K., Hacker, C., & Legters, L.J. (1994). Remote sensing as a landscape epidemiologic tool to identify villages at risk for malaria transmission. *American Journal of Tropical Medicine and Hygiene,* **51**, pp. 271-280.

Bernstein, K.T., Curriero, F.C., Jennings, J.M., Olthoff, G., Erbelding, E.J., & Zenilman, J. (2004). Defining core gonorrhea transmission utilizing spatial data. *American Journal of Epidemiology,* **160**, pp. 51-58.

Berrang-Ford, L., Berke, O., Abdelrahman, L., Waltner-Toews, D., & McDermott, J. (2006). Spatial analysis of sleeping sickness, southeastern Uganda, 1970-2003. *Emerging Infectious Diseases,* **12**, pp. 813-820.

Bertram, D.S. (1969). Tsetse and trypanosomiasis control in Nyanza Province, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **63**, pp. 125.

Besag, J. & Newell, J. (1991). The detection of clusters in rare diseases. *Journal of the Royal Statistical Society, Series A,* **154**, pp. 143-155.

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

Bland, J.M. & Altman, D.G. (1995). Multiple significance tests: the Bonferroni method. *British Medical Journal,* **310**, pp. 170.

Booth, M. & Dunne, D.W. (2004). Spatial awareness in parasite immuno-epidemiology. *Parasite Immunology,* **26**, pp. 499-507.

Booth, M., Vennervald, B., Kenty, L.C., Butterworth, A., Kariuki, H., Kadzo, H., Ireri, E., Amaganga, C., Kimani, G., Mwatha, J., Otedo, A., Ouma, J., Muchiri, E., & Dunne, D. (2004). Micro-geographical variation in exposure to Schistosoma mansoni and malaria, and exacerbation of splenomegaly in Kenyan school-aged children. *BMC Infectious Diseases,* **4**, pp. 13.

Bourn, D. (2001). *Case studies of environmental change and trypanosomiasis control in Kenya.* Oxford: Environmental Research Group Oxford.

Bouyer, J., Guerrini, L., Desquesnes, M., de La Rocque, S., & Cuisance, D. (2006). Mapping African animal trypanosmiasis risk from the sky. *Veterinary Research,* **37**, pp. 633-645.

Brooker, S., Clarke, S., Njagi, J.K., Polack, S.R., Mugo, B., Estambale, B., Muchiri, E., Magnussen, P., & Cox, J. (2004). Spatial clustering of malaria and associated risk factors during an epidemic in a highland area of western Kenya. *Tropical Medicine and International Health,* **9**, pp. 757-766.

Brownlow, A. (2007). *Evaluation of a novel method for controlling bovine trypanosomiasis. A longitudinal study in south-east Uganda.* PhD Thesis, University of Edinburgh, Edinburgh.

Bruce, D., Hammerton, A.E., Baterian, H.R., Mackie, F.P., & Bruce, L. (1911). *Report of the Sleeping Sickness Commission of the Royal Society-1911.*

Bruce, D. & Nabarro, D. (1903). Progress report on sleeping sickness in Uganda. *Royal Society Reports: Sleeping Sickness Commission*, **1**, pp. 11-88.

Brun, R., Schumacher, R., Schmid, C., Kunz, C., & Burri, C. (2001). The phenomenon of treatment failures in Human African Trypanosomiasis. *Tropical Medicine & International Health*, **6**, pp. 906-914.

Bukachi, S., Wandibba, S., & Nyamongo, I. (2005). *The socio-economic impact of human African trypanosomiasis in western Kenya.* Paper presented at the 28th meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Addis Ababa.

Burchmore, R.J.S., Ogbunande, P.O., Enanga, B., & Barrett, M.P. (2002). Chemotherapy of human African trypanosomiasis. *Current Pharmaceutical Design*, **8**, pp. 256-267.

Cannon, R.M. & Roe, R.T. (1982). *Livestock disease surveys: a field manual for veterinarians*. Canberra: Australian Bureau of Animal Health.

Catley, A., Okoth, S., Osman, J., Fison, T., Njiru, Z., Mwangi, J., Jones, B.A., & Leyland, T.J. (2001). Participatory diagnosis of a chronic wasting disease in cattle in southern Sudan. *Preventive Veterinary Medicine*, **51**, pp. 161-181.

Cattand, P., Jannin, J., & Lucas, P. (2001). Sleeping sickness surveillance: an essential step towards elimination. *Tropical Medicine & International Health*, **6**, pp. 348-361.

Chappuis, F., Stivanello, E., Adams, K., Kidane, S., Pittet, A., & Bovier, P.A. (2004). Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of Human African Trypanosomiasis (*Trypanosoma brucei gambiense*) among serologically suspected individuals in Southern Sudan. *American Journal of Tropical Medicine and Hygiene*, **71**, pp. 313-317.

Chappuis, F., Udayraj, N., Stietenroth, K., Meussen, A., & Bovier, P.A. (2005). Eflornithine is safer than Melarsoprol for the treatment of second-stage *Trypanosoma brucei gambiense* Human African Trypanosomiasis. *Clinical Infectious Diseases*, **41**, pp. 748-751.

Christy, C. (1903). The distribution of sleeping sickness, *Filaria perstans* etc. in East Equatorial Africa. *Report of the Sleeping Sickness Commission of the Royal Society*, **3**, pp. 2-8.

Coleman, P. & Welburn, S.C. (2004). Are fitness costs associated with resistance to human serum in *Trypanosoma brucei rhodesiense*? *Trends in Parasitology*, **20**, pp. 311-315.

Connor, R.J. (1992). *The diagnosis, treatment and prevention of animal trypanosomiasis under field conditions*. Rome: FAO.

Connor, R.J. (1994). The impact of nagana. *Onderstepoort Journal of Veterinary Research*, **61**, pp. 379-383.

Conraths, F.J., Staubach, C., & Tackmann, K. (2003). Statistics and sample design in epidemiological studies of *Echinococcus multilocularis* in fox populations. *Acta Tropica,* **85**, pp. 183-189.

Corson, J.F. (1936). Experimental transmission of *Trypanosoma rhodesiense* by *Glossina morsitans* from man to sheep and back to man. *Journal of Tropical Medicine and Hygiene,* **39**, pp. 125-126.

Courtin, F., Jamonneau, V., Oké, E., Coulibaly, B., Oswald, Y., Dupont, S., Cuny, G., Doumenge, J.-P., & Solano, P. (2005). Towards understanding the presence/absence of Human African Trypanosomiasis in a focus of Côte d'Ivoire: a spatial analysis of the pathogenic system. *International Journal of Health Geographics,* **4**, pp.

Cox, A., Tilley, A., McOdimba, F., Fyfe, J., Eisler, M.C., Hide, G., & Welburn, S.C. (2005). A PCR based assay for detection and differentiation of African trypanosome species in blood. *Experimental Parasitology,* **111**, pp. 24-29.

Crawley, M.J. (2002). *Statistical Computing: An introduction to data analysis using S-Plus.* Chichester, West Sussex: John Wiley & Sons Ltd.

Cross, G.A.M. (1996). Antigenic variation in trypanosomes: secrets surface slowly. *BioEssays,* **18**, pp. 283-291.

Cross, G.A.M., Wirtz, L.E., & Navarro, M. (1998). Regulation of vsg expression site transcription and switching in Trypanosoma brucei. *Molecular and Biochemical Parasitology,* **91**, pp. 77-91.

Cunningham, M.P. (1966). Immunity in bovine trypanosomiasis. *East African Medical Journal,* **43**, pp. 394-397.

Davila, A.M. & Silva, R.A. (2000). Animal trypanosomiasis in South America. Current status, partnership and information technology. *Annals New York Academy of Sciences,* **916**, pp. 199-212.

de Haan, C. & Umali, L.D. (1992). Public and private sector roles in the supply of veterinary services. In J.R. Anderson & C. de Hahn (Eds.), *Public and private sector roles in agricultural development. Proceedings of the 12th Agricultural Sector Symposium* (pp. 125-137). Washington DC: The World Bank.

de La Rocque, S., Bengaly, Z., Michel, J.F., Solano, P., Sidibé, I., & Cuisance, D. (1999). Importance des interfaces spatiales et temporelles entre les bovins at les glossines dans la transmission de la trypanosomose animal en Afrique de l'Ouest. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux,* **52**, pp. 215-222.

de La Rocque, S., Michel, J.F., Bouyer, J., De Wispelaere, G., & Cuisance, D. (2005). Geographical Information Systems in parasitology: a review of potential applications using the example of animal trypanosomosis in West Africa. *Parassitologia,* **47**, pp. 97-104.

de La Rocque, S., Michel, V., Plazanet, D., & Pin, R. (2004). Remote sensing and epidemiology: examples of applications for two vector-borne diseases.

Comparative Immunology, Microbiology and Infectious Diseases, **27**, pp. 331-341.

DEFRA. (2006). *Regulatory impact assessment (RIA) for England on proposed amendments and consoliadtion of cattle identification regulations 1998.* London: DEFRA.

Desquesnes, M. & Dia, M.L. (2003). *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis.* *Experimental Parasitology*, **103**, pp. 35-43.

Dhand, N.K., Gumber, S., Singh, B.B., Aradhana, Bali, M.S., Kumar, H., Sharma, D.R., Singh, J., & Sandhu, K.S. (2005). A study on the epidemiology of brucellosis in Punjab (India) using Survey Toolbox. *Review Sci Tech*, **24**, pp. 879-885.

Docampo, R. & Moreno, S.N.J. (2003). Current chemotherapy of human African trypanosomiasis. *Parasitology Research*, **90**, pp. 10-13.

Dohoo, I.R., Martin, W., & Stryhn, H. (2003). *Veterinary epidemiologic research.* Charlottetown, Canada: AVC Inc.

Dohoo, I.R., Tillard, E., Stryhn, H., & Faye, B. (2001). The use of multilevel models to evaluate sources of variation in reproductive performance in dairy cattle in Reunion Island. *Preventive Veterinary Medicine*, **50**, pp. 127-144.

Dolan, R.B., Stevenson, P.G.W., Alushula, H., & Okech, G. (1992). Failure of chemoprophylaxis against bovine trypanosomiasis on Galana Ranch in Kenya. *Acta Tropica*, **51**, pp. 113-121.

Dransfield, R.D., Brightwell, R., Kyorku, C., & Williams, B. (1990). Control of tsetse fly (*Diptera: Glossinidae*) populations using traps at Nguruman, south-west Kenya. *Bulletin of Entomological Research*, **80**, pp. 265-276.

Duke, H.L. (1928). *Studies on the bionomics of the polymorphic trypanosomes of man and ruminants.* Geneva: Final Report of the League of Nations International Commission on Human Trypanosomiasis.

Dunn, J. & Ferri, C.P. (1999). Epidemiological methods for research with drug misusers: review of methods for studying prevalence and morbidity. *Revista de Saude Publica*, **33**, pp. 206-15.

Dutton, J.E. (1902). Preliminary note on thte trypanosomes occuring in the blood of man. *British Medical Journal*, **2**, pp. 141-147.

Echessah, P.N., Swallow, B.M., Kamara, D.W., & Curry, J.J. (1997). Willingness to contribute labor and money to tsetse control: Application of contingent valuation in Busia District, Kenya. *World Development*, **25**, pp. 239-253.

Eisler, M.C., Dwinger, R.H., Majiwa, P.A.O., & Picozzi, K. (2004). Diagnosis and epidemiology of African animal trypanosomiasis. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The trypanosomiases.* Wallingford, Oxfordshire: CABI Publishing.

Eisler, M.C., Magona, J.W., Jonsson, N.N., & Revie, C.W. (2007). A low cost decision support tool for the diagnosis of endemic bovine infectious diseases

in the mixed crop-livestock production system of sub-Saharan Africa. *Epidemiology and Infection,* **135**, pp. 67-75.

Eisler, M.C., Ndung'u, J.M., Murilla, G.A., Mdachi, R.M., Mbwambo, H., Sinyangwe, L., Machila, N., Delespaux, V., Geerts, S., Peregrine, A.S., McDermott, J., & Holmes, P.H. (2000). Area wide appraisal of drug resistance in trypanosomes infecting cattle in East and Southern Africa. *ICPTV Newsletter,* **2**, pp. 16-18.

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

Eyler, J.M. (2001). The changeing assessments of John Snow's and William Farr's cholera studies. *Sozial un Praeventivmedizin,* **46**, pp. 225-232.

Fairbairn, H. & Burtt, E. (1946). The infectivity to man of a strain of *Trypanosoma rhodesiense* transmitted cyclically by *Glossina morsitans* through sheep and antelope: evidence that man requires a minimum infective dose of metacyclic trypanosomes. *Annals of Tropical Medicine and Parasitology,* **40**, pp. 270-313.

Fèvre, E.M., Coleman, P.G., Odiit, M., Magona, J.W., Welburn, S.C., & Woolhouse, M.E.J. (2001). The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda. *The Lancet,* **358**, pp. 625-628.

Fèvre, E.M., Coleman, P.G., Welburn, S.C., & Maudlin, I. (2004). Reanalyzing the 1900-1920 sleeping sickness epidemic in Uganda. *Emerging Infectious Diseases,* **10**, pp. 567-573.

Fèvre, E.M., Picozzi, K., Fyfe, J., Waiswa, C., Odiit, M., Coleman, P.G., & Welburn, S.C. (2005). A burgeoning epidemic of sleeping sickness in Uganda. *The Lancet,* **366**, pp. 745-747.

Fèvre, E.M., Tilley, A., Picozzi, K., Fyfe, J., Anderson, I., Magona, J.W., Shaw, D.J., Eisler, M.C., & Welburn, S.C. (2006). Central point sampling from cattle in livestock markets in areas of human sleeping sickness. *Acta Tropica,* **97**, pp. 229-232.

FITCA. (2000). *Technical Assistance Annual Report, February 1999-February 1999.* Farming in Tsetse Controlled Areas (Kenya component).

FITCA. (2005). *Farming in Tsetse Controlled Areas. Kenya Project 1999-2006. Lessons Learned.*

Ford & Katando. (1973). The distribution of tsetse flies (*Glossina*) in Africa, 1973. Sheet 1: *Palpalis* Group; Sheet 2: *Morsitans* Group; Sheet 3: *Fusca* Group. In S.a.T.R.C.O.S. Organisation of African Unity (Ed.). London: Cook Hammond & Kell Ltd.

Fraser, A., Leibovici, L., Reeves, D.S., & Johnson, A.P. (2006). Back to the basics: epidemiology in the Journal of Antimicrobial Chemotherapy. *Journal of Antimicrobial Chemotherapy,* **58**, pp. 489-91.

Fyfe, J.C. (2007). *The impact and outcomes of recent control interventions against trypanosomiasis in eastern Uganda.* PhD Thesis, University of Edinburgh, Edinburgh.

Gibson, W., Mehlitz, D., Lanham, S.M., & Godfrey, D.G. (1978). The identification of *Trypanosoma brucei gambiense* in Liberian pigs and dogs by isoenzymes and by resistance to human plasma. *Tropenmedizin und Parasitologie,* **29**, pp. 335-345.

Gibson, W.C., Stevens, J.R., Mwendia, C.M., Ngotho, J.N., & Ndung'u, J.M. (2001). Unravelling the phylogenetic relationships of African trypanosomes of suids. *Parasitology,* **122**, pp. 625-31.

Giesecke, J. (2002). *Modern Infectious Disease Epidemiology.* (2nd ed.). London: Arnold.

Gitau, G.K., Perry, B.D., Katende, J.M., McDermott, J.J., Morzaria, S.P., & Young, A.S. (1997). The prevalence of serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a District, Kenya; a cross-sectional study. *Preventive Veterinary Medicine,* **30**, pp. 95-107.

Glover, P.E. (1962). Paper presented at the 9th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Conakry.

Gomes, I., Veiga, M., Embirucu, E.K., Rabelo, R., Mota, B., Meza-Luca, A., Tapia-Romero, R., Carrillo-Becerril, B.L., Alcantara-Anguiano, I., Correa, D., & Melo, A. (2002). Taeniasis and cysticercosis prevalence in a small village from northeastern Brazil. *Arq. Neuro-Psiquiatr.,* **60**, pp.

Gouteux, J.P., Kounda Gboumbi, J.C., D'Amico, F., Wagner, C., Noutoua, L., & Bailly, C. (1993). An epidemiological survey to discover the probable places of infection with sleeping sickness in the Central African Republic. *Bulletin of the World Health Organization,* **71**, pp. 605-614.

Gouteux, J.-P. & Sinda, D. (1990). Community participation in the control of tsetse flies: Large scale trials using the pyramid trap in the Congo. *Tropical Medicine and Parasitology,* **41**, pp. 49-55.

Government of Kenya. (1940). *Brief outline of work on G. palpalis and human trypanosomiasis in Kenya.* Kenya Medical Department.

Government of Kenya. (1997). *Busia District development plan 1997-2001.* Nairobi: Ministry of Finance and Planning.

Government of Kenya. (2001). *Busia District Development Plan 2002-2008. Effective management for sustainable economic growth and poverty reduction.* Nairobi: Ministry of Finance and Planning.

Gregorio, D.I., Samociuk, H., DeChello, L., & Swede, H. (2006). Effects of study area size on geographic characterizations of health events: prostate cancer incidence in Southern New England, 1994-1998. *International Journal of Health Geographics,* **5**, pp.

Gutierrez, C., Coreba, J.A., Morales, M., & Buscher, P. (2006). Trypanosomiasis in goats: current status. *Annals New York Academy of Sciences*, **1081**, pp. 300-310.

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

Heisch, R.B., McMahon, J.P., & Mansonbahr, P.E. (1958). The isolation of *Trypanosoma rhodesiense* from a bushbuck. *British Medical Journal*, **15**, pp. 1203-1204.

Hendrickx, G., de La Rocque, S., Reid, R., & Wint, W. (2001). Spatial trypanosomiasis management: from data layers to decision making. *Trends in Parasitology*, **17**, pp. 35-41.

Hendrickx, G., Napala, A., Dao, B., Batawui, D., Bastiaensen, P., De Deken, R., Vermeilen, A., Vercruysse, J., & Slingenbergh, J.H.W. (1999a). The area-wide epidemiology of bovine trypanosomiasis and its impact on mixed farming in sub-humid west Africa: a case study in Togo. *Veterinary Parasitology*, **84**, pp. 13-31.

Hendrickx, G., Napala, A., Dao, B., Batawui, D., De Deken, R., Vermeilen, A., & Slingenbergh, J.H.W. (1999b). A systematic approach to area-wide tsetse distribution and abundance maps. *Bulletin of Entomological Research*, **89**, pp. 231-244.

Hendrickx, G., Napala, A., Slingenbergh, J.H.W., De Deken, R., Vercruysse, J., & Rogers, D.J. (2000). The spatial patterns of trypanosomiasis predicted with the aid of satellite imagery. *Parasitology*, **120**, pp. 121-134.

Hide, G. (1999). History of sleeping sickness in East Africa. *Clinical Microbiology Reviews*, **12**, pp. 112-125.

Hide, G., Angus, S.D., Holmes, P.H., Maudlin, I., & Welburn, S.C. (1998). *Trypanosoma brucei*:Comparison of circulating strains in an endemic and an epidemic area of a sleeping sickness focus. *Experimental Parasitology*, **89**, pp. 21-29.

Hide, G., Tait, A., Maudlin, I., & Welburn, S.C. (1996). The origins, dynamics and generation of *Trypanosoma brucei rhodesiense* epidemics in East Africa. *Parasitology Today*, **12**, pp. 50-55.

Hide, G., Welburn, S.C., Tait, A., & Maudlin, I. (1994). Epidemiological relationships of *Trypanosoma brucei* stocks from South East Uganda: evidence for different population structures in human infective and non-human infective isolates. *Parasitology*, **109**, pp. 95-111.

Hoare, C.A. (1972). *The trypanosomes of mammals. A zoological monograph*: Blackwell Scientific Publications.

Hofmann-Lehmann, R., Meli, M.L., Dreher, U.M., Goenczi, E., Deplazes, P., Braun, U., Engels, M., Schuepbach, J., Joerger, K., Thoma, R., Griot, C., Staerk, K.D.C., Willi, B., Schmidt, J., Kocan, K.M., & Lutz, H. (2004). Concurrent infections with vector-borne pathogens associated with fatal hemolytic

anemia in a cattle herd in Switzerland. *Journal of Clinical Microbiology,* **42**, pp. 3775-3780.

Holden, S. (1997). *Comunity Based Animal Health Workers in Kenya: An example of private delivery of animal health services to small-scale farmers in marginal areas.* Kenya: DFID.

Holden, S. (1999). The economics of the delivery of veterinary services. *Revue Scientifique et Technique de l'Office International des Epizooties,* **18**, pp. 425-439.

Holmes, P.H., Eisler, M.C., & Geerts, S. (2004). Current chemotherapy of animal trypanosomiasis. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases.* Wallingford, Oxfordshire: CABI Publishing.

Hoshaw-Woodard, S. (2001). Description and comparison of the methods of cluster sampling and lot quality assurance sampling to assess immunization coverage, *WHO document WHO/V&B/01.26.* Geneva: WHO.

Hu, C. & Aksoy, S. (2006). Innate immune responses regulate trypanosome parasite infections of the *Glossina morsitans morsitans. Molecular Microbiology,* **60**, pp. 1194-1204.

IDMC. (2006). *Only peace can restore the confidence of the displaced- Update on the implementation of the recommendations made by the UN Secretary-General's representative on Internally Displaced Persons following his visit to Uganda.* Geneva: Internal Displacement Monitoring Centre, Norwegian Refugee Council, Refugee Law Project.

INCO-DC. (2000). *Novel approaches to the epidemiology of resistance to drugs used in the control of bovine trypanosomiasis in East Africa. Final Report.* Glasgow: International Cooperation with Developing Countries (1994-1998).

Jagielski, A.K., Kryskiewicz, E., & Bryla, J. (2006). Suramin-induced reciprocal changes in glucose and lactate synthesis in renal tubules contribute to its hyperglycaemic action. *European Journal of Pharmacology,* **537**, pp. 205-209.

Jamonneau, V., Barnabe, C., Koffi, M., Sane, B., Cuny, G., & Solano, P. (2003). Identification of *Trypanosoma brucei* circulating in a sleeping sickness focus in Cote d'Ivoire: assessment of genotype selection by the isolation method. *Infection, Genetics and Evolution,* **3**, pp. 143-149.

Jamonneau, V., Ravel, S., Koffi, M., Kaba, D., Zeze, D.G., Ndiri, L., Sane, B., Coulibaly, B., Cuny, G., & Solano, P. (2004). Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Côte D'Ivoire. *Parasitology,* **129**, pp. 693-702.

Jasper, D.E., Dellinger, J.D., Rollins, H., & Hakanson, H.D. (1979). Prevalence of mycoplasmal bovine mastitis in California. *American Journal of Veterinary Research,* **40**, pp. 1043-1047.

Jennings, F.W., Rodgers, J., Bradley, B., Gettinby, G., Kennedy, P.G.E., & Murray, M. (2002). Human African trypanosomiasis: Potential therapeutic benefits of

an alternative suramin and melarsoprol regimen. *Parasitology International,* **51**, pp. 381-388.

Joja, L.L. & Okoli, U.A. (2001). Trapping the vector: Community action to curb sleeping sickness in Southern Sudan. *American Journal of Public Health,* **91**, pp. 1583-1585.

Jones, T.W. & Davila, A.M.R. (2001). *Trypanosoma vivax* - out of Africa. *Trends in Parasitology,* **17**, pp. 99-101.

Kabasa, J.D. (2007). Public-private partnership works to stamp out sleeping sickness in Uganda. *Trends in Parasitology,* **23**, pp. 191-192.

Kajume, J.K. (1998, 14-18. December). *Delivery of animal health services in East Africa- Kenya.* Paper presented at the Conference on Delivery of animal health services in Eastern Africa, Arusha, Tanzania.

Kalau, A.U., Edeghere, H.U., & Lawani, F.A. (1986). Comparison of diagnostic techniques during sub-clinical single infections of trypanosomiasis in goats. *Veterinary Parasitology,* **22**, pp. 37-47.

Kamau, S.W., Omukuba, J., Kiragu, J., Masika, P., Ndung'u, J.M., Wachira, P., & Mehlitz, D. (2000). Financial analysis of animal trypanosomiasis control using cypermethrin pour-on in Kenya. *Preventative Veterinary Medicine,* **44**, pp. 231-246.

Kamuanga, M. (2003). *Socio-economic and cultural factors in the research and control of trypanosomiasis.* Rome: FAO.

Karanja, S.M. (2006). *Epidemiology and importance of trypanosomiasis, heleminthosis and tick borne diseases on the performance of cattle in Busia District, Kenya.* PhD Thesis, Freie Universitaet Berlin, Berlin.

Karib, A.A. (1961). Animal trypanosomiasis in the Sudan. *The Sudan Journal of Veterinary Science and Animal Husbandry,* **2**, pp. 39-46.

Karimurio, J., Gichangi, M., Ilako, D.R., Adala, H.S., & Kilima, P. (2006). Prevalence of trachoma in six districts of Kenya. *East African Medical Journal,* **83**, pp. 63-68.

Katunguka-Rwakishaya, E., Murray, M., & Holmes, P.H. (1997). The influence of supplementation with cotton seed cake on the resistance of Ugandan goats to primary and secondary challenges with *Trypanosoma congelense* and on their response to treatment. *Veterinary Parasitology,* **70**, pp. 67-76.

KETRI. (1994). *Tsetse fly trapping in Busia District May 1994. Internal Report.* Kenya: Kenya Trypanosomiasis Research Institute.

Kitron, U. (1998). Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. *Journal of Medical Entomology,* **35**, pp. 435-445.

Kitron, U. (2000). Risk Maps: Transmission and burden of vector-borne diseases. *Parasitology Today,* **16**, pp. 324-325.

Kitron, U., Jones, C.J., Bouseman, J.K.S., Nelson, G.S., & Baumgartner, D.L. (1992). Spatial analysis of the distribution of *ixodes dammini* on white tailed

deer in Ogde country, Illinois. *Journal of Medical Entomology,* **29**, pp. 259-266.

Kitron, U., Otieno, L.H., Hungerford, L.L., Odulaja, A., Brigham, W.U., Okello, O.O., Joselyn, M., Mohamed-Ahmed, M.M., & Cook, E. (1996). Spatial analysis of the distribution of tsetse flies in the Lambwe Valley, Kenya, using landsat TM satellite imagery and GIS. *Journal of Animal Ecology,* **65**, pp. 371-380.

Klar, N., Gyorkos, T., & Donner, A. (1995). Cluster randomization trials in tropical medicine: a case study. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **89**, pp. 454-9.

Kleine, F.K. & Eckard, B. (1913). Ueber die Bedeutung der Haustiere und des Wildes fuer die Verbreitung der Schlafkrankheit. *Zeitschrift fuer Hygiene und Infectionskrankheiten,* **75**, pp. 118-122.

Koerner, T., de Raadt, P., & Maudlin, I. (1995). The 1901 Uganda sleeping sickness epidemic revisited: A case of mistaken identity? *Parasitology Today,* **11**, pp. 303-306.

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

Kulldorff, M. (1997). A spatial scan statistic. *Communications in Statistics: Theory and Methods,* **26**, pp. 1481-1496.

Kulldorff, M., Mostashari, F., Duczmal, L., Yih, K., Kleinmann, K., & Platt, R. (2005). Multivariate scan statistics for disease surveillance, *SaTScan Bibliography Manuscript http://www.satscan.org/papers/k-M2005.pdf.*

Kulldorff, M. & Nagarwalla, N. (1995). Spatial disease clusters: detection and inference. *Statistics in Medicine,* **14**, pp. 799-810.

Legros, D., Evans, S., Maiso, F., Enyaru, J.C.K., & Mbulamberi, D. (1999). Risk factors for treatment failure after melarsoprol for *Trypanosoma brucei gambiense* trypanosomiasis in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **93**, pp. 439-442.

Lejon, V., Buscher, P., Magnus, E., Moons, A., Wouters, I., & Van Meirvenne, N. (1998). A semi-quantitative ELISA for detection of *Trypanosoma brucei gambiense* specific antibodies in serum and cerebrospinal fluid of sleeping sickness patients. *Acta Tropica,* **69**, pp. 151-164.

Lejon, V., Lardon, J., Kenis, G., Pinoges, L., Legros, D., Bisser, S., N'Siesi, X., Bosmans, E., & Buscher, P. (2002). Interleukin (IL)-6, IL-8 and IL-10 in serum and CSF of *Trypanosoma brucei gambiense* sleeping sickness patients before and after treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **96**, pp. 329-333.

Leonard, D.K. (1993). Structural reform of the veterinary profession in Africa and the new institutional economics. *Economic Development and Cultural Change,* **24**, pp. 227-267.

Lessard, P., L'Eplattenier, R., Norval, R.A.I., Kundert, K., Dolan, T.T., Croze, H., Walker, J.B., Irvin, A.D., & Perry, B.D. (1990). Geographical information systems for studying the epidemiology of cattle disease caused by *Theileria parva*. *Veterinary Record*, **89**, pp. 255-266.

Levine, N.D., Corliss, J.O., Cox, F.E.G., Deroux, G., Grain, J., Honigberg, B.M., Leedale, G.F., Loeblich, A.R., Lom, J., Lynn, D., Merinfeld, E.G., Page, F.C., Poljansky, G., Sprague, V., Vavra, J., & Wallace, F.G. (1980). A newly revised classification of the Protozoan. *Journal of Protozoology*, **27**, pp. 37-58.

Lumsden, W.H. (1964). Changing Patterns of Trypanosomiasis Research in East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **58**, pp. 97-135.

Machila, N., Wanyangu, S.W., McDermott, J., Welburn, S.C., Maudlin, I., & Eisler, M.C. (2003). Cattle owners' perceptions of African bovine trypanosomiasis and its control in Busia and Kwale Districts of Kenya. *Acta Tropica*, **86**, pp. 25-34.

Machila-Eisler, N. (2005). *Improved targetting and appropriate use of trypanocidal drugs for the control of African bovine trypanosomiasis in tsetse endemic areas of western and coastal Kenya within the context of primary veterinary care*. PhD Thesis, University of Edinburgh, Edinburgh.

MacLennan, K.J.R. (1970). The epizootiology of trypanosomiasis in livestock in West Africa. In H.W. Mulligan (Ed.), *The African trypanosomiases* (pp. 751-765). London: Allen and Unwin.

Macleod, E.T., Maudlin, I., Darby, A.C., & Welburn, S.C. (2007). Antioxidants promote establishment of trypanosome infections in tsetse. *Parasitology*, **136**, pp. 827-831.

Magnus, E., Vervoort, T., & Van Meirvenne, N. (1978). A card-agglutination test with stained trypanosomes (CATT) for the serological diagnosis of *T. b. gambiense trypanosomiasis*. *Annales de la Societe Belge de Medecine Tropical*, **58**, pp. 169-176.

Magona, J.W., Anderson, I., Olaho-Mukani, W., Jonsson, N.N., Revie, C.W., & Eisler, M.C. (2003a). Diagnosis of endemic diseases in village cattle herds in southeast Uganda: a low technology decision support system. *ICPTV Newsletter*, **8**, pp. 43-46.

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

Mahmoud, M.M. & Elmalik, K.H. (1977). Trypanosomiasis: Goats as a possible reservoir of *Trypanosoma congolense* in the Republik of Sudan. *Tropical Animal Health and Production*, **9**, pp. 167-170.

Malone, J.B., Abdel-Rahman, M.S., El Bahy, M.M., Huh, O., Shafik, M., & Bavia, M. (1997). Geographic information systems and the distribution of *Schistosoma mansoni* in the Nile Delta. *Parasitology Today,* **13**, pp. 112-119.

Mäser, P., Luscher, A., & Kaminsky, R. (2003). Drug transport and drug resistance in African trypanosomes. *Drug Resistance Updates,* **6**, pp. 281-290.

Maudlin, I. & Welburn, S.C. (1987). Lectin mediated establishment of midgut infections of *Trypanosoma congolense* and *Trypanosoma brucei* in *Glossina morsitans. Tropical Medicine and Parasitology,* **38**, pp. 167-170.

Maudlin, I. & Welburn, S.C. (1988). Tsetse immunity and the transmission of trypanosomiasis. *Parasitology Today,* **4**, pp. 109-111.

Maudlin, I. & Welburn, S.C. (1994). Minireview: Maturation of trypanosome infections in tsetse. *Experimental Parasitology,* **79**, pp. 202-205.

McLean, N. (1931). *East African Medical Journal Kenya,* **4**, pp. 386.

Michel, J.-F., Dray, S., de La Rocque, S., Desquesnes, M., Solano, P., De Wispelaere, G., & Cuisance, D. (2002). Modelling bovine trypanosomosis spatial distribution by GIS in an agro-pastoral zone of Burkina Faso. *Preventive Veterinary Medicine,* **56**, pp. 5-18.

Milligan, P., Njie, A., & Bennett, S. (2004). Comparison of two cluster sampling methods for health surveys in developing countries. *International Journal of Epidemiology,* **33**, pp. 469-76.

Milne, A. (1908). *Report of the East African Protectorate to the Secretary of State, Enclosure Number 1.*

Mlangwa, J.E. & Kisauzi, D.N. (1994). Systems approach to animal health services delivery in sub-Saharan Africa: the case of privatisation. *Revue Scientifique et Technique de l'Office International des Epizooties,* **13**, pp. 673-685.

Moore, A. & Richer, M. (2001). Re-emergence of epidemic sleeping sickness in southern Sudan. *Tropical Medicine and International Health,* **6**, pp. 342-347.

Moore, A., Richer, M., Enrile, M., Losio, E., Roberts, J., & Levy, D. (1999). Resurgence of sleeping sickness in Tambura County, Sudan. *The American Journal of Tropical Medicine and Hygiene,* **61**, pp. 315-318.

Morris, K.R.S. (1960). Studies on the epidemiology of sleeping sickness in East Africa: II Sleeping sickness in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **54**, pp. 71-86.

Mosele, L., Leroy, E., Mattosovich, D., Carugi, C., & Cattand, P. (2002). *OAU/IBAR: Farming in Tsetse Controlled Areas Programme (FITCA), mid-term review.* Rome: Development Researchers' Network.

Moser, D.R., Cook, G.A., Ochs, D.E., Baily, C.P., McKane, M.R., & Donelson, J.E. (1989). Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. *Parasitology,* **99**, pp. 57-66.

Mosi, R.O. & Nyandega, I.A. (2002). *FITCA-Kenya Project: Report of the 2000/2001 Livestock census*. Nairobi: FITCA Kenya.

Muhib, F.B., Lin, L.S., Stueve, A., Miller, R.L., Ford, W.L., Johnson, W.D., & Smith, P.J. (2001). A venue-based method for sampling hard-to-reach populations. *Public Health Reports*, **116 Supplement 1**, pp. 216-22.

Munstermann, S., Mbura, R.J., Maloo, S.H., & Lohr, K.F. (1992). Trypanosomiasis control in Boran cattle in Kenya: a comparison between chemoprophylaxis and a parasite detection and intravenous treatment method using isometamidium chloride. *Tropical animal health and production*, **24**, pp. 17-27.

Murray, M. & Black, S.J. (1985). African trypanosomiasis in cattle: working with nature's solution. *Veterinary Parasitology*, **18**, pp. 167-182.

Murray, M., d'Ieteren, G.D.M., & Teale, A.J. (2004). Trypanotolerance. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiasis* (pp. 461-477). Wallingford, Oxfordshire: CABI Publishing.

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

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

Murray, M. & Trail, J.C.M. (1984). Genetic resistance to animal trypanosomiasis in Africa. *Preventive Veterinary Medicine*, **2**, pp. 541-551.

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

Mwongela, G.N., Kovatch, R.M., & Frazil, M.A. (1981). Acute Trypanosoma vivax infection in dairy cattle in Coast Province, Kenya. *Tropical animal health and production*, **13**, pp. 63-69.

Naessens, J. (2006). Bovine trypanotolerance: A natural ability to prevent severe anaemia and haemophagocytic syndrome? *International Journal for Parasitology*, **36**, pp. 521-528.

Naessens, J., Leak, S.G.A., Kennedy, D.J., Kemp, S., & Teale, A.J. (2003). Responses of haemopoitic chimera twin cattle from susceptible and tolerant genetic background to experimental infection with *Trypanosoma congolense*. *Veterinary Parasitology*, **111**, pp. 125-142.

National Statistics Online. (Census 2001). http://www.statistics.gov.uk/census2001.

Ng'ayo, M.O., Njiru, Z.K., Kenya, E.U., Muluvi, G.M., Osir, E.O., & Masiga, D.K. (2005). Detection of trypanosomes in small ruminants and pigs in western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology and Disease*, **4**, pp. 5.

Ngondi, J., Onsarigo, A., Adamu, L., Matende, I., Baba, S., Reacher, M., Emerson, P., & Zingeser, J. (2005). The epidemiology of trachoma in Eastern Equatoria

and Upper Nile States, southern Sudan. *Bulletin of the World Health Organization*, **83**, pp.

Nicholson, M.J. & Butterworth, M.H. (1986). *A guide to condition scoring of zebu cattle*. Addis Ababa: International Livestock Centre for Africa.

Nicholson, M.J. & Sayers, A.R. (1987a). Relationship between body weight, condition score and heart girth changes in Boran cattle. *Tropical animal health and production*, **19**, pp. 115-120.

Nicholson, M.J. & Sayers, A.R. (1987b). Repeatability, reproducibility and sequential use of condition scoring of *Bos Indicus* cattle. *Tropical animal health and production*, **19**, pp. 127-135.

Njogu, A.R., Dolan, R.B., Wilson, A.J., & Sayer, P.D. (1985). Trypanotolerance in East African Orma Boran cattle. *Veterinary Record*, **117**, pp. 632-636.

Nkinin, S.W., Njiokou, F., Penchenier, L., Grebaut, P., Simo, G., & Herder, S. (2002). Characterization of *Trypanosoma brucei* s.l. subspecies by isoenzymes in domestic pigs from the Fontem sleeping sickness focus of Cameroon. *Acta Tropica*, **81**, pp. 225-232.

Odiit, M., Bessell, P.R., Fèvre, E.M., Robinson, T., Kinoti, J., Coleman, P.G., Welburn, S.C., McDermott, J., & Woolhouse, M.E.J. (2006). Using remote sensing and geographic information systems to identify villages at high risk for rhodesiense sleeping sickness in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **100**, pp. 354-362.

Odiit, M., Coleman, P.G., Liu, W.-C., McDermott, J.J., Fèvre, E.M., Welburn, S.C., & Woolhouse, M.E.J. (2005). Quantifying the level of under-detection of *Trypanosoma brucei rhodesiense* sleeping sickness cases. *Tropical Medicine and International Health*, **10**, pp. 840-849.

Odiit, M., Kansiime, F., & Enyaru, J.C.K. (1997). Duration of symptoms and case fatality of sleeping sickness caused by *Trypanosoma brueci rhodesiense* in Tororo, Uganda. *East African Medical Journal*, **74**, pp. 792-795.

Ogada, T. (1979). The remaining foci of human sleeping sickness in Kenya-Homa Bay District and Busia District- 1968-1979. *International Scientific Committee for Trypanosomiasis Research and Control*, **111**, pp. 125-137.

Okoth, J.O. (1986). Peridomestic breeding sites of *Glossina fuscipes fuscipes* Newst. in Busoga, Uganda, and epidemiological implications for trypanosomiasis. *Acta Tropica*, **43**, pp. 283-286.

Okoth, J.O. & Kapaata, R. (1986). Trypanosome infection rates in *Glossina fuscipes fuscipes* Newst. in the Busoga sleeping sickness focus, Uganda. *Annals of Tropical Medicine and Parasitology*, **80**, pp. 459-461.

Okoth, J.O. & Kapaata, R. (1988). Hosts of *Glossina fuscipes fuscipes* in Busoga, Uganda. *Annals of Tropical Medicine and Parasitology*, **80**, pp. 517-518.

Okuthe, O.S. & Buyu, G.E. (2006). Prevalence and incidence of tick-borne diseases in smallholder farming systems in the western-Kenya highlands. *Veterinary Parasitology*, **141**, pp. 307-312.

Onah, D.N. (1991). Porcine trypanosomiasis in Nigeria: infection in local and exotic pigs in the Nsukka area of Anambra State. *Tropical animal health and production*, **23**, pp. 141-146.

Onyango, R.J., Van Hoeve, K., & De Raadt, P. (1966). The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in Alego location, Central Nyanza, Kenya. I. Evidence that cattle may act as reservoir hosts of trypanosomes infective to man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **60**, pp. 175-182.

Openshaw, S. (1996). Geographical information systems and tropical diseases. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **90**, pp. 337-339.

Openshaw, S., Charlton, M., Wymer, C., & Craft, A.W. (1987). A mark 1 analysis machine for the automated analysis of point data sets. *International Journal of Geographical Information Systems*, **1**, pp. 335-358.

Openshaw, S., Craft, A.W., Charlton, M., & Birch, J.M. (1988). Investigation of leukemia clusters by use of a geographical analysis machine. *Lancet*, **1**, pp. 272-273.

Osaer, S., Goossens, B., Kora, S., Gaye, M., & Darboe, L. (1999a). Health and productivity of traditionally managed Djallonke sheep and West African dwarf goats under high and moderate trypanosomosis risk. *Veterinary Parasitology*, **82**, pp. 101-119.

Osaer, S., Goossens, B., Kora, S., & Jeffcoate, I. (1999b). Effects of *Trypanosoma congolense* infection and diet on puberty, age at first lambing and haematology changes in Djallonke ewe lambs. *Veterinary Parasitology*, **80**, pp. 215-230.

Ostfeld, R.S., Glass, G.E., & Keesing, F. (2005). Spatial epidemiology: an emerging (or re-emerging) discipline. *Trends in Ecology & Evolution*, **20**, pp. 328-336.

Paling, R.W., Moloo, S.K., Scott, J.R., Gettinby, G., McOdimba, F.A., & Murray, M. (1991). Susceptibility of N'Dama and Boran cattle to sequential challenges with tsetse transmitted clones of *Trypanosoma congolense*. *Parasite Immunology*, **13**, pp. 427-445.

Pavlovsky, E.N. (1966). *Natural nidality of transmissble diseases: with special reference to the landscape epidemiology of zooanthroponoses*. Illinois: University of Illinois Press.

Pays, A. (1998). American human trypanosomiasis 90 years after its discovery by Carlos Chagas, I: epidemiology and control. *Med Trop (Mars)*, **58**, pp. 391-402.

Penchenier, L., Grebaut, P., Ebo'o Eyenga, V., Bodo, J.M., Njiokou, F., Binzouli, J.J., Simarro, P., Soula, G., Herder, S., & Laveissiere, C. (1999). Le foyer de trypanosome humaine de Campo (Cameroun). Historique et situation de l'endémie en 1998. *Bulletin de la Societe de pathologie exotique*, **92**, pp. 85-90.

Penchenier, L., Mathieu-Daude, F., Brengues, C., Banuls, A.L., & Tibayrenc, M. (1997). Population structure of *Trypanosoma brucei* s.l. in Côte D'Ivoire assayed by multilocus enzyme electrophoresis: epidemiological and taxonomic considerations. *Journal of Parasitology*, **83**, pp. 19-22.

Pepin, J. (2007). Combination therapy for sleeping sickness: A wake-up call. *Journal of Infectious Diseases*, **195**, pp. 311-313.

Pepin, J. & Meda, H.A. (2001). The epidemiology and control of human trypanosomiasis. *Advances in Parasitology*, **49**, pp. 71-132.

Pepin, J., Milord, F., Khonde, A., Niyonsenga, T., Loko, L., & Mpia, B. (1994). Gambiense trypanosomiasis: frequency of, and risk factors for, failure of melarsoprol therapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **88**, pp. 447-452.

Peregrine, A.S., Moloo, S.K., & Whitelaw, D.D. (1991). Differences in sensitivity of Kenyan *Trypanosoma vivax* populations to the prophylactic and therapeutic actions of isomethamidium chloride in Boran cattle. *Tropical Animal Health Production*, **23**, pp. 29-38.

Picozzi, K., Carrington, M., & Welburn, S.C. (in press). A multiplex PCR that discriminates between *Trypanosoma brucei brucei* and *T.b. rhodesiense*. *Experimental Parasitology*, pp.

Picozzi, K., Fèvre, E.M., Odiit, M., Carrington, M., Eisler, M.C., Maudlin, I., & Welburn, S.C. (2005). Sleeping sickness in Uganda: a thin line between two fatal diseases. *British Medical Journal*, **331**, pp. 1238-1241.

Polack, S.R., Solomon, A.W., Alexander, N.D.E., Massae, P.A., Safari, S., Shao, J.F., Foster, A., & Mabey, D.C. (2005). The household distribution of trachoma in a Tanzanian village: an application of GIS to the study of trachoma. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **99**, pp. 218-225.

Politzar, H. (2001). Present status of the FITCA Project. *ICPTV Newsletter*, **3**, pp. 22-23.

Pollock, J.N. (1982). Ecology and behaviour of tsetse, *Training manual for tsetse control personnel* (Vol. 2). Rome: Food and Agricultural Organization of the United Nations.

Prahalad. (2007). Democratising commerce: The challenge for the 21st century, *Times MBA Podcast Week 6*: Timesonline (http://www.timesonline.co.uk/tol/audio_video/podcasts/mba).

Prata, A. (2001). Clinical and epidemiological aspects of Chagas disease. *Lancet Infectious Diseases*, **1**, pp.

Radwanska, M., Claes, Y., Magez, S., Magnus, E., Perez-Morga, D., Pays, E., & Buscher, P. (2002). Novel primer sequences for polymerase chain reaction - based detection of *T. brucei gambiense*. *The American Journal of Tropical Medicine and Hygiene*, **67**, pp. 289-295.

Raso, G., Vounatsou, P., Singer, B.H., N'Goran, E.K., Tanner, M., & Utzinger, J. (2006). An integrated approach for risk profiling and spatial prediction of Schistosoma mansoni-hookworm coinfection. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, pp. 6934-6939.

Rawlings, P., Dwinger, R.H., & Snow, W.F. (1991). An analysis of survey measurements of tsetse challenge to trypanotolerant cattle in relation to aspects of analytical models of trypanosomiasis. *Parasitology*, **102**, pp. 371-377.

Reid, S.A. (2002). *Trypanosoma evansi* control and containment in Australasia. *Trends in Parasitology*, **18**, pp. 219-224.

Rickmann, L.R. & Robson, J. (1970). The testing of proven *Trypanosoma brucei* and *T. rhodesiense* strains by the blood incubation infectivity test. *Bulletin of the World Health Organization*, **42**, pp. 911-916.

Robinson, T.P. (1998). Geographic information systems and the selection of priority areas for control of tsetse-transmitted trypanosomiasis in Africa. *Parasitology Today*, **14**, pp. 457-461.

Robson, J., Rickman, L.R., Allsopp, R., & Scott, D. (1972). The composition of the *Trypanosoma brucei* subgroup in nonhuman reservoirs in the Lambwe Valley, Kenya, with particular reference to the distribution of *T. rhodesiense*. *Bulletin of the World Health Organization*, **46**, pp. 765-770.

Roditi, I. & Pearson, T.W. (1990). The procyclin coat of African trypanosomes. *Parasitology today*, **6**, pp. 79-82.

Rogers, D.J. (2000). Satellites, space, time and the African trypanosomiases. *Advances in Parasitology*, **47**, pp. 130-173.

Rogers, D.J. & Randolph, S.E. (1991). Mortality rates and population density of tsetse flies correlated with satellite imagery. **351**, pp. 739-741.

Rogers, D.J. & Randolph, S.E. (1993). Distribution of tsetse and ticks in Africa: Past, present and future. *Parasitology Today*, **9**, pp. 266-271.

Rogers, D.J. & Randolph, S.E. (2003). Studying the global distribution of infectious diseases using GIS and RS. *Nature Reviews Microbiology*, **1**, pp. 231-237.

Rothman, K.J. & Greenland, S. (1998). *Modern Epidemiology*. (second ed.). Philadelphia: Lippincot-Raven Publisher.

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

Rowlands, G.J., Mulatu, W., Authie, E., d'Ieteren, G.D.M., Leak, S.G.A., Nagda, S.M., & Peregrine, A.S. (1993). Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 2. Factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence of recurrent infections. *Acta Tropica*, **53**, pp. 135-150.

Sankoh, O.A. & Becher, H. (2002). Disease cluster methods in epidemiology and application to data on childhood mortality in rural Burkina Faso. *Ruprecht-Karls University, Heidelberg, Sonderforschungs Bereich (SFB) 544 (Kontrolle Tropischer Infectionskrankheiten): Discussion Paper Series*, **04**, pp.

Schütt, I.D. & Mehlitz, D. (1981). On the persistance of human serum resistance and isoenzyme patterns of *Trypanozoon* in experimentally infected pigs. *Acta Tropica*, **38**, pp. 367-373.

Schwartz, H.J., Dolan, R.B., & Wilson, A.J. (1983). Camel production in Kenya and its constraints. *Tropical animal health and production*, **15**, pp. 169-178.

Sheehan, J.T., DeChello, L., Kulldorff, M., Gregorio, D., Gershman, S., & Mroszczyk, M. (2004). The geographic distribution of breast cancer incidence in Massachusetts 1988 to 1997, adjusted for covariates. *International Journal of Health Geographics*, **3**, pp. 17.

Shepherd, B.E., Rossini, A.J., Soto, R.J., de Rivera, I.L., & Mullins, J.I. (2005). Sampling designs for HIV molecular epidemiology with application to Honduras. *AIDS Research and Human Retroviruses*, **21**, pp. 907-914.

Shulman, C.E., Levene, M., Morison, L., Dorman, E., Pershu, N., & Marsh, K. (2001). Screening for severe anaemia in pregnancy in Kenya, using pallor examination and self-reported morbidity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **95**, pp. 250-255.

Simarro, P.P., Ruiz, J.A., Franco, J.R., & Josenando, T. (1999). Attitude towards CATT-positive individuals without parasitological confirmation in the African Trypanosomiasis (*T.b. gambiense*) focus of Quicama (Angola). *Tropical Medicine and International Health*, **4**, pp. 858-61.

Simo, G., Asonganyi, T., Nkinin, S.W., Njiokou, F., & Herder, S. (2006). High prevalence of *Trypanosoma brucei gambiense* group 1 in pigs from the Fontem sleeping sickness focus in Cameroon. *Veterinary Parasitology*, **139**, pp. 57-66.

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

Smith, D.H., Pepin, J., & Stich, A. (1998). Human African trypanosomiasis: an emerging public health crisis. *British Medical Bulletin*, **54**, pp. 341-355.

Smith, D.L. (2001). *Reform and decentralisation of agricultural services: a policy framework*. Rome: Food and Agricultural Organization of the United Nations.

Snow, J. (1855). *On the mode of transmission of cholera*. (2nd ed.). London: Churchill.

Snow, W.F. (1984). Tsetse feeding habits in an area of endemic sleeping sickness in southern Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78**, pp. 413-414.

Solano, P., Michel, J.F., Lefrancois, T., de La Rocque, S., Sidibe, I., Zoungrana, A., & Cuisance, D. (1999). Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso. *Veterinary Parasitology*, **86**, pp. 95-103.

Staak, C., Kaempe, U., & Korkowski, G. (1986). Species identification of bloodmeals from tsetse flies (Glossinidae): Results 1979-1985. *Tropical Medicine and Parasitology*, **37**, pp. 59-60.

Stanghellini, A. & Josenando, T. (2001). The situation of sleeping sickness in Angola: a calamity. *Tropical Medicine & International Health*, **6**, pp. 330-334.

Stephen, L.E. (1986). *Trypanosomiasis: a veterinary perspective*. Oxford: Pergamon Press.

Stephens, J.W.W. & Fantham, H.B. (1910). On the peculiar morphology of a trypanosome from a case of sleeping sickness and the possibility of its being a new species (*T. rhodesiense*). *Annals of Tropical Medicine and Parasitology*, **4**, pp. 343-350.

Stich, A., Barrett, M.P., & Krishna, S. (2003). Waking up to sleeping sickness. *Trends in Parasitology*, **19**, pp. 195-197.

Stovner, L.J. (2006). Headache epidemiology: how and why? *Journal of Headache and Pain*, **7**, pp. 141-4.

Syed, Z. & Guerin, P.M. (2004). Tsetse flies are attracted to the invasive plant *Lantana camara*. *Journal of Insect Physiology*, **50**, pp. 43-50.

Tango, T. & Takahashi, K. (2005). A flexibly shaped spatial scan statistic for detecting clusters. *International Journal of Health Geographics*, **4**, pp. 11.

Taylor, J.E. & Rudenko, G. (2006). Switching trypanosome coats: what's in the wardrobe? *Trends in Genetics*, **22**, pp. 614-620.

Taylor, K. & Authié, E. (2004). Pathogenesis of animal trypanosomiasis. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases* (pp. 331-353). Wallingford, Oxfordshire: CABI Publishing.

Thomson, M.C., Connor, S.J., Milligan, P., & Flasse, S.P. (1997). Mapping malaria risk in Africa: What can satellite data contribute. *Parasitology Today*, **13**, pp. 313-318.

Thrusfield, M. (1995). *Veterinary Epidemiology*. (2. Edition ed.). Cambridge: Blackwell Science Ltd.

Thuranira, C.M. (2005). *Socio-economic factors influencing livestock keeping dynamics in a smallholder crop-livestock system in western Kenya*. PhD Thesis, University of Edinburgh, Edinburgh.

Torr, S.J., Mangwiro, T.N., & 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**, pp. 71-84.

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

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

Tossas i Auguet, O. (2007). *Interactions amongst the community of endemic pathogens of African cattle; a longitudinal study in south east Uganda.* PhD Thesis, University of Edinburgh, Edinburgh.

Trail, J.C.M., d'Ieteren, G.D.M., Feron, A., Kakiese, O., Mulungo, M., & Pelo, M. (1991a). Effect of trypanosome infection, control of parasitaemia and control of anaemia development on productivity of N'Dama cattle. *Acta Tropica*, **48**, pp. 37-45.

Trail, J.C.M., d'Ieteren, G.D.M., Maille, J.C., & Yangari, G. (1991b). Genetic aspects of control of anaemia development in trypanotolerant N'Dama cattle. *Acta Tropica*, **48**, pp. 285-291.

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

Turnbull, B., Iwano, E.J., Burnett, W.S., Howe, H.L., & Clark, L.C. (1990). Monitoring for clusters of disease: application to Leukemia incidence in upstate New York. *American Journal of Epidemiology*, **132**, pp. 136-143.

Uilenberg, G. (1998). *A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis*. Rome: Food and Agricultural Organization of the United Nations.

Umali, D.L., Feder, G., & de Haan, C. (1994). Animal health services: finding the balance between public and private delivery. *The World Bank Research Observer*, **9**, pp. 71-96.

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

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

Vale, G.A., Lovemore, D.F., Flint, S., & Cockbill, G.F. (1988). Odour-baited targets to control tsetse flies, *Glossina* spp. (*Diptera: Glossinidae*) in Zimbabwe. *Bulletin of Entomological Research*, **78**, pp. 31-49.

Vale, G.A., Mutika, G., & Lovemore, D.F. (1999). Insecticide-treated cattle for controlling tsetse flies (*Diptera: Glossinidae*): some questions answered, many posed. *Bulletin of Entomological Research*, **89**, pp. 569-578.

Vale, G.A. & Torr, S.J. (2004). Development of bait technology to control tsetse. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiasis.* Wallingford, Oxfordshire: CABI Publishing.

Van den Bossche, P., Akoda, K., Kubi, C., & Marcotty, T. (2006). The transmissibility of *Trypanosoma congolense* seems to be associated with its level of resistance to isometamidium chloride. *Veterinary Parasitology*, **135**, pp. 365-367.

Van den Bossche, P., De Deken, R., Brandt, J., Geerts, S., Geysen, D., & Berkvens, D. (2004). The transmission of mixed *Trypanosoma brucei brucei/T. congolense* infections by tsetse (*Glossina morsitans morsitans*). *Veterinary Parasitology*, **119**, pp. 147-153.

Van den Bossche, P., Mudenge, D., Mubanga, J., & Norval, A. (1999). The parasitological and serological prevalence of tsetse-transmitted bovine trypanosomosis in the Eastern Caprivi (Caprivi District, Namibia). *Onderstepoort J Vet Res*, **66**, pp. 103-10.

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

van Hoeve, K., Onyango, R.J., Harley, J.M., & De Raadt, P. (1967). The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in Alego Location, Central Nyanza, Kenya. II. The cyclical transmission of *Trypanosoma rhodesiense* isolated from cattle to a man, a cow and to sheep. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **61**, pp. 684-687.

Van Hoof, L.M.J. (1947). Observation on trypanosomiasis in the Belgian Congo. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **40**, pp. 728-754.

Vickerman, K. (1969). On the surface coat and flagellar adhesion in trypanosomes. *Journal of Cell Science*, **5**, pp. 163-193.

Wacher, T.J., Milligan, P.J.M., Rawlings, P., & Snow, W.F. (1994). Tsetse-trypanosomiasis challenge to village N'Dama cattle in The Gambia: field assessments of spatial and temporal patterns of tsetse-cattle contact and the risk of trypanosomiasis infection. *Parasitology*, **109**, pp. 149-162.

Wain, E.B., Sutherst, R.W., Burridge, M.J., Pullan, N.B., & Reid, H.W. (1970). Survey for trypanosome infections in domestic cattle and wild animals in areas of East Africa. 3. Salivarian trypanosome infections in cattle in relation to the *Glossina* distribution in Busoga District, Uganda. *British Veterinary Journal*, **126**, pp. 634-641.

Waiswa, C. (2005). Porcine trypanosomiasis in southeastern Uganda: prevalence and assessment of therapeutic effectiveness. *Bulgarian Journal of Veterinary Medicine*, **8**, pp. 59-68.

Waiswa, C., Olaho-Mukani, W., & Katunguka-Rwakishaya, E. (2003). Domestic animals as reservoirs for sleeping sickness in three endemic foci in south-eastern Uganda. *Annals of Tropical Medicine and Parasitology*, **97**, pp. 149-155.

Wamwiri, F.N., Nkwengulila, G., & Clausen, P.-H. (2007). Hosts of *Glossina fuscipes fuscipes* and *G. pallidipes* in areas of western Kenya with endemic sleeping sickness, as determined using an egg-yolk (IgY) ELISA. *Annals of Tropical Medicine and Parasitology,* **101**, pp. 225-232.

Welburn, S.C., Arnold, K., Maudlin, I., & Gooday, G.W. (1993). Rickettsia-like organisms and chitinase production in relation to transmission of trypanosomes by tsetse flies. *Parasitology,* **107**, pp. 141-145.

Welburn, S.C., Coleman, P.G., Maudlin, I., Fèvre, E.M., Odiit, M., & Eisler, M.C. (2006). Crisis, what crisis? Control of Rhodesian sleeping sickness. *Trends in Parasitology,* **22**, pp. 123-128.

Welburn, S.C., Fèvre, E.M., Coleman, P.G., Odiit, M., & Maudlin, I. (2001a). Sleeping sickness: a tale of two diseases. *Trends in Parasitology,* **17**, pp. 19-24.

Welburn, S.C. & Maudlin, I. (1989). Lectin signalling of maturation of *T. congolense* infections in tsetse. *Medical and Veterinary Entomology,* **3**, pp. 141-145.

Welburn, S.C. & Maudlin, I. (1999). Tsetse-trypanosome interactions: Rites of passage. *Parasitology Today,* **15**, pp. 399-403.

Welburn, S.C., Maudlin, I., & Milligan, P.J.M. (1995). *Trypanozoon*: Infectivity to humans is linked to reduced transmissibility in tsetse : I. Comparison of human serum-resistant and human serum-sensitive field isolates. *Experimental Parasitology,* **81**, pp. 404-408.

Welburn, S.C. & Odiit, M. (2002). Recent developments in human African trypanosomiasis. *Current Opinion in Infectious Diseases,* **15**, pp. 477-484.

Welburn, S.C., Picozzi, K., Fèvre, E.M., Coleman, P.G., Odiit, M., Carrington, M., & Maudlin, I. (2001b). Identification of human-infective trypanosomes in animal reservoirs of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. *The Lancet,* **358**, pp. 2017-2019.

Wellde, B.T. & Chumo, D.A. (1983). Persistence of Berenil in cattle. *Tropical animal health and production,* **15**, pp. 149-150.

Wellde, B.T., Chumo, D.A., Reardon, M.J., Nawiri, J., Olando, J., Wanyama, L., Awala, J., Koech, D., Siongok, T.A., & Sabwa, C. (1989a). Diagnosis of Rhodesian sleeping sickness in the Lambwe Valley (1980-1984). *Annals of Tropical Medicine and Parasitology,* **83**, pp. 63-71.

Wellde, B.T., Chumo, D.A., Reardon, M.J., Waema, D., Smith, D.H., Gibson, W.C., Wanyama, L., & Siongok, T.A. (1989b). Epidemiology of Rhodesian sleeping sickness in the Lambwe Valley, Kenya. *Annals of Tropical Medicine and Parasitology,* **83 Suppl 1**, pp. 43-62.

Wellde, B.T., Chumo, D.A., Waema, D., Reardon, M.J., & Smith, D.H. (1989c). A history of sleeping sickness in Kenya. *Annals of Tropical Medicine and Parasitology,* **83 Suppl 1**, pp. 1-11.

Wellde, B.T., Reardon, M.J., Chumo, D.A., Muriithi, R.M., Towett, S., & Mwangi, J. (1989d). Experimental infection of cattle with *Trypanosoma brucei rhodesiense*. *Annals of Tropical Medicine and Parasitology*, **83**, pp. 133-150.

Wellde, B.T., Reardon, M.J., Chumo, D.A., Muriithi, R.M., Towett, S., & Mwangi, J. (1989e). Experimental infection of goats with *Trypanosoma brucei* ssp. and effects of treatment with suramin and Mel-B. *Annals of Tropical Medicine and Parasitology*, **83**, pp. 161-169.

WHO. (1998). *WHO expert committee on control and surveillance of African trypanosomiasis*. No 881. Geneva: WHO.

WHO. (2006). Human African Trypanosomiasis (sleeping sickness): epidemiological update. *Weekly epidemiological record*, **81**, pp. 71-80.

Wilde, J.K.H. & French, M.H. (1945). An experimental study of *Trypanosoma rhodesiense* infections in zebu cattle. *Journal of Comparative Pathology*, **55**, pp. 206-228.

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**, pp. 351-357.

Willett, K.C. (1972). An outline of the findings of the recent WHO-UNDP trypanosomiasis project in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**, pp. 331-332.

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

Wilson, A.J., Schwartz, H.J., Dolan, R.B., & Olaho-Mukani, W. (1983). A simple classification of different types of trypanosomiasis occuring in four camel herds in selected areas of Kenya. *Tropenmedizin Und Parasitologie*, **34**, pp. 220-224.

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

Xong, H.V., Vanhamme, L., Chamekh, M., Chimfwembe, C.E., Van Den Abbeele, J., Pays, A., Van Meirvenne, N., Hamers, R., De Baetselier, P., & Pays, E. (1998). A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell*, **95**, pp. 839-46.

Appendix A

Household questionnaire on livestock management administered during census sampling in Funyula and Butula

Date:

Time:

Name of enumerator:

GPS reference: North:_____   East:_____

Name (Head of Household):_____

Number of   cows:_____

pigs:_____

goats:_____

sheep:_____

Where do animals graze?   Where are animals watered?

cows _____   _____

pigs _____   _____

goats _____   _____

sheep _____   _____