The impact and outcomes of recent control interventions against trypanosomiasis in eastern Uganda

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Declaration

I declare that the research described within this thesis is my own work and that this thesis is my own composition

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Contents

Abstract

Trypanosomiasis places a considerable burden on both human and animal health across much of sub-Saharan Africa. Appreciation of the neglected burden of trypanosomiasis led to a European Union funded programme called Farming in Tsetse Controlled Areas (FITCA); within Uganda this programme put in place measures to attempt the control of both animal and human trypanosomiasis.

This thesis begins by examining the history of trypanosomiasis control in Uganda over the past century before exploring a contemporary control treatment programme implemented in four districts of Uganda by FITCA in 2002. The impact of this programme was monitored over the course of a year by the determination of trypanosome prevalence in cattle using molecular diagnostic techniques (PCR) at a number of sites in the treated districts of Uganda. In addition, incidence of sleeping sickness in the human population before and after the intervention programme were monitored by recording and analysing sleeping sickness cases reported to district health centres. A spatial analytical framework was used to investigate disease clustering on a year-by-year basis with reference to FITCA intervention sites.

Prior to the implementation of the control programme, the prevalence of trypanosome infection in cattle across the study area was 16%; post-intervention, trypanosome infection levels had fallen to 9%. In particular, post-intervention levels of *T. brucei* s.l. dropped in all districts and overall the proportion of these infections that were attributed to human infective *T. b. rhodesiense* reduced from 33% to less than 10%. Analysis indicates that the cost per percentage decrease in the prevalence of cattle trypanosomiasis achieved by the programme was US$2,193. Statistical and spatial analysis observed no impact of the FITCA intervention on either the incidence or distribution of reported sleeping sickness cases, although different patterns were observed in epidemic and endemic areas.

Trypanosomiasis remains a neglected disease making it imperative that any resources donated for its control are used wisely by employing optimal control strategies to obtain maximum results at minimal cost. The efficacy of the FITCA Uganda programme is discussed with reference to how the programme may be improved, and whether cattle treatment represents the best long-term strategy for trypanosomiasis control in Uganda.

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Abbreviations

AHO	Animal Husbandry Officer
BIIT	Blood Incubation Infectivity Test
BOD	Burden Of Disease
CATT	Card Agglutination Test for Trypanosomiasis
CBA	Cosy Benefit Analysis
CEA	Cost Effective Analysis
CEPP	Cluster Evaluation Permutation Procedure
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
CTVM	Centre for Tropical Veterinary Medicine
DALY	Disability-Adjusted Life Year
DDT	Dichloro-Diphenyl-Trichloroethane
DFMO	Difluromethylornithine
DNA	Deoxyribose Nuceic Acid
dNTP	Deoxynucleoside triphosphates
DVO	District Veterinary Officer
EATRO	East African Trypanosomiasis Research Organisation
ES	Expression Sites
ESAG	Expression Site Associated Gene
EU	European Union
FITCA	Farming In Tsetse Controlled Areas
GAM	Geographical Analysis Machine
GIS	Geographical Information System(s)

GPI	Glycosyl-Phosphatidy-Inositol
GPS	Global Positioning System
HAT	Human African Trypanosomiasis
HCT	Haematocrit Centrifugation Technique
HSR	Human Serum Resistance
IDP	Internally Displaced Person
ILRI	International Livestock Research Institute
LC	Local Community
LIRI	Livestock Research Institute
LRA	Lords Resistance Army
MGE	Mobile Genetic Elements
NGO	Non-Government Organisation
NUSAF	Northern Uganda Social Action Fund
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PLC	Phospholipase C
QALY	Quality Affected Life Year
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SAT	Sequential Aerosol Technique
SIT	Sterile Insect Technique
SOS	Stamp Out Sleeping sickness
SRA	Serum Resistance-Associated Protein
SRD	Sustained Release Device

TDR	Research and Training in Tropical Diseases
TB	Tuberculosis
TBE	Tris Borate EDTA
TE	Tris EDTA
TIM	Triosephosphate isomerase
UV	Ultraviolet
VAT	Variable Antigen Type
VO	Veterinary Officer
VSG	Variable Surface Glycoprotein
WHO	World Health Organization

1 Chapter 1:

General introduction

1.1 African trypanosomiases

Trypanosomes are unicellular, flagellate, kinetoplastid protozoans that are obligate parasites (Cox, 1993). Although the majority of trypanosomes are non-pathogenic, several members of this genus cause important veterinary and medical diseases (Stevens & Brisse, 2004). In sub-Saharan Africa two sub-species of *T. brucei* s.l. (*T .b. gambiense and T. b. rhodesiense*) cause Human African Trypanosomiasis (HAT, also known as sleeping sickness) whilst several species are the aetiological agents of significant parasitic disease in domestic ruminants, suids, equids and dogs (Table 1.1) and although not pathogenic they can be found in other species.

Trypanosome species	Pathogenic in			
	Ruminants	Suids	Equids	Dogs
T. vivax	+		+	
T. brucei	+		+	+
T. congolense	+		+	
T. simiae		+		
T. evansi			+	+
T. equiperdum			+	
T. suis		+		

Table 1.1 - Pathogenic trypanosome species of significant veterinary importance in sub-Saharan Africa. This table is adapted from (Desquesnes & Davila, 2002)

Tsetse flies (genus *Glossina*, order Diptera) serve as vectors for the transmission of these pathogenic African trypanosomes to their hosts with the exception of *T.evansi* which is mechanically transmitted by biting flies, *T. vivax* which can be transmitted by both tsetse and other biting flies and *T. equiperdum* which is a sexually transmitted disease). As they are transmitted in the saliva of tsetse during haematophagy, African trypanosomes are classified as salivarian (Hoare, 1964). Trypanosomes take approximately 20-40 days to develop in the tsetse host (Dale *et al.*, 1995) and the fly can remain infectious for the rest of its life, around 5-6 weeks in the field (Welburn & Maudlin, 1999). 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),

covering a vast swathe of sub-Saharan Africa. Due to the specific conditions of temperature, humidity and vegetation that are required for their survival, tsetse flies are not present uniformly throughout the 'tsetse belt' but are restricted to habitat pockets (Pepin & Meda, 2001). Consequently, the incidence of trypanosomiasis is spatially-discrete and in HAT approximately 300 separate active disease foci are recognised (Figure 1.1) (Cattand *et al.*, 2001).

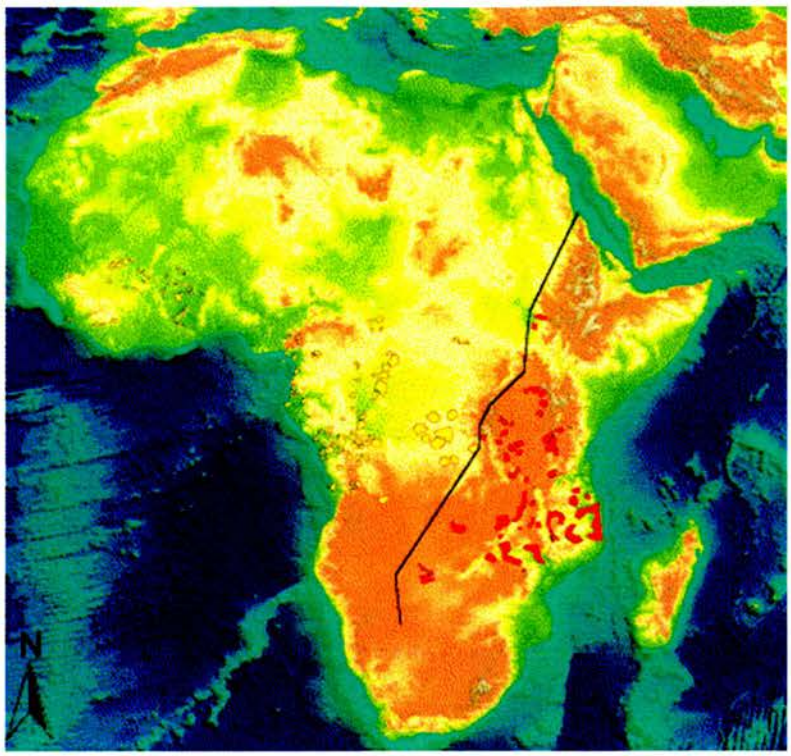


Figure 1.1 – Map of Africa showing the *T. b. rhodesiense* foci (red) and *T. b. gambiense* foci (yellow) geographically separated by the rift valley, shown on a digital elevation model (USCS digital elevation model), image reproduced with kind permission of (Welburn *et al.*, 2001a)

1.2 The impact and importance of human African trypanosomiasis

HAT has a disproportional affect on marginalised and poor rural populations and has been considerably neglected in the recent past (Barrett *et al.*, 2003; Welburn *et al.*, 2006). At the time of large-scale decolonization of Africa in the 1960s, HAT had

been largely brought under control, then, during the second half of the 20th century there was a dramatic increase in the incidence of the disease (Barrett, 1999), and HAT was judged to be a category 1 resurgent disease by WHO/TDR (Remme *et al.*, 2002). In the past few years the number of reported HAT cases has decreased (WHO, 2006). However it is difficult to be certain this is a true reflection of successful control or some other stochastic factor (Fèvre *et al.*, 2006a). There are two distinct forms of sleeping sickness, which are geographically separated by a line that closely approximates to that of the Great Rift Valley (Figure 1.1). In eastern and southern Africa *T. brucei rhodesiense* causes an acute disease whilst in western and central Africa *T. brucei gambiense* causes a chronic disease. Uganda is unique in being the only country in which both forms of the disease are present, with *T. b. gambiense* in the north-west and *T. b. rhodesiense* occurring in the south-east of the country (Welburn *et al.*, 2006) as shown in Figure 1.2. Currently the *T. b. rhodesiense* focus is spreading (see Figure 1.2) leading to concerns that the two foci may merge (Picozzi *et al.*, 2005).

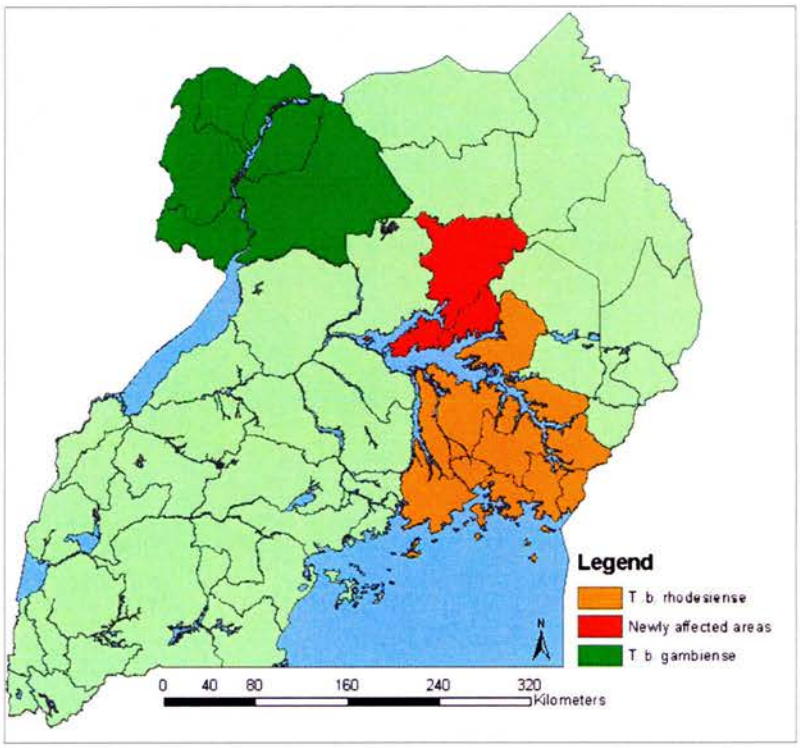


Figure 1.2 – Map of Uganda showing the *T. b. rhodesiense* and *T. b. gambiense* foci within the country. The newly affected areas shown in red are affected by *T. b. rhodesiense* the first case of which was recorded in 2004

It is estimated that 60 million people living in 36 countries in sub-Saharan Africa are at risk from sleeping sickness (World Health Organization, 1998a), a disease which is invariably fatal if left untreated. In 2002, the World Health Organisation (WHO) recorded 48,000 deaths from sleeping sickness (World Health Organization, 2004). However, as most cases of sleeping sickness effect the rural population where levels of screening of the at-risk population are relatively low (<10%), it is assumed that there is substantial under-reporting. In 1998, WHO estimated that only 27,000 out of 300,000 sleeping sickness cases were reported in the Democratic Republic of the Congo, and similarly in Angola where observers expected >100,000 cases only 8,000 were reported (Barrett *et al.*, 2003). Results from a recent study in Uganda have indicated that 12 deaths due to sleeping sickness go undetected for every 1 reported (Odiit *et al.*, 2005). A recent estimate by WHO has suggested that up to half a million people in sub-Saharan Africa may be carrying *T. brucei* s.l. infections

(Barrett *et al.*, 2003), although others have suggested this is an overestimation (Pepin & Meda, 2001). *T. b. rhodesiense* is less significant in terms of numbers than *T. b. gambiense* across sub-Saharan Africa as a whole, but it remains a significant problem at local and regional levels (Fèvre *et al.*, 2006a). Due to the severity of the disease and the greater likelihood of infection of agriculturally active members of rural communities, it has devastating effects of the socio-economic viability of affected families and places a major burden on the rural poor (Fèvre *et al.*, 2005; Odiit *et al.*, 2005). During the intermittent epidemics of sleeping sickness that occur, the disease has severe social and economic effects and can devastate the rural populations of entire regions (Kuzoe, 1993). Sleeping sickness causes loss of 1.6 - 2.05 million disability-adjusted life years (DALYs) (Pepin & Meda, 2001; Welburn *et al.*, 2006), which is an equitable measure of disease burden (Murray, 1994). This places sleeping sickness third behind malaria and schistosomiasis in terms of impact of parasitic diseases in sub-Saharan Africa (Cattand *et al.*, 2001).

1.3 The importance of animal trypanosomiasis

The diseases caused by trypanosome infections of livestock have had a profound effect on human settlement and economic development across large parts of sub-Saharan Africa. Currently, trypanomiasis is one of the major constraints on the development of animal production in sub-Saharan Africa, and causes annual losses of approximately US$1.3 billion due to reduction in livestock product yields and by devaluing farmers investments (Kristjanson *et al.*, 1999). Farmers themselves perceive it as major impediment to keeping livestock (Swallow, 2000). The most socio-economically significant animal trypanomiasis is that of cattle (also known as nagana). It is estimated that 50-70 million cattle are at risk from nagana (Geerts & Holmes, 1998), whilst in some areas the disease has prevented the use of grasslands for cattle rearing. As cattle provide important sources of nutrition and draught power, they are integral to the adoption of efficient mixed agriculturally practices, and in many African societies serve as a 'living bank' that can be sold in times of hardship, nagana has important repercussions for human health and welfare (Geake,

2001; Welburn *et al.*, 2006). Furthermore, as is discussed below, cattle can act as a major reservoir for human infective *T. b. rhodesiense.*

1.4 The zoonotic potential of trypanosomes

Zoonoses are defined as 'disease and their agents which are naturally transmitted between (other) vertebrate species and man' (WHO, 1959). Globally zoonotic disease are responsible for new emerging disease and re-emergence of existing disease (Meslin, 1997), indeed 62% of all human pathogens are classified as zoonotic (Taylor *et al.*, 2001) and many newly emerging and re-emerging diseases (e.g. Rabies, Tuberculosis, Marburg virus, Ebola) are zoonotic.

T. b. gambiense is generally considered to be a non-zoonotic disease although recent work has demonstrated that domestic pigs (Simo *et al.*, 2006) and a variety of wild animals (Njiokou *et al.*, 2006) can harbour the pathogen. In contrast, it is well recognised that *T. b. rhodesiense* is a zoonosis and can infect a wide range of both domestic and wild animals (see Table 1.2).

Wild hosts	Reference
Bushbuck	(Kinghorn, 1925; Heisch *et al.*, 1958)
Hartebeast	(Geigy *et al.*, 1971; Geigy *et al.*, 1975)
Warthog	(Awan, 1971; Awan, 1979)
Hyena	(Awan, 1971; Geigy *et al.*, 1971; Onyango *et al.*, 1973; Geigy *et al.*, 1975; Awan, 1979)
Lion	(Awan, 1971; Geigy *et al.*, 1971; Onyango *et al.*, 1973; Geigy *et al.*, 1975; Awan, 1979)
Impala	(Mulla & Rickman, 1988)
Zebra	(Mulla & Rickman, 1988)
Waterbuck	(Kinghorn, 1925; Awan, 1971; Geigy *et al.*, 1971; Geigy *et al.*, 1975; Awan, 1979)
Domestic hosts	
Cattle	(Onyango *et al.*, 1966)
Pig	(Waiswa *et al.*, 2003)
Dog	(Gibson & Gashumba, 1983)

Table 1.2 – Reservoir hosts of *T. b. rhodesiense*, examples of both wild and domestic reservoir hosts identified by investigation into naturally occurring infections. Table adapted from (Leak, 1999).

In particular cattle are being increasingly identified as an important reservoir host for *T. b. rhodesiense*. Research in Uganda has shown that domestic livestock are a crucial factor in the way the parasite is spread to humans as *T. b. rhodesiense* is 5 times more likely to be transmitted by a cattle-tsetse-human cycle, than a human-tsetse-human cycle (Figure 1.3) (Hide *et al.*, 1996). Work in Uganda has indicated that the recent *T. b. rhodesiense* epidemic in Soroti district originated from the movement of infected cattle (Fèvre *et al.*, 2001) and it has been suggested that the 1900-1920 Ugandan epidemic may also have arisen from large scale movement of

infected cattle (Koerner *et al.*, 1995; Fèvre *et al.*, 2004) which has led to a postulated role for cattle in the eruption of *T. b. rhodesiense* epidemics. Reservoir hosts, either domestic or wild, are thought to be responsible for the inter-epidemic maintenance of the parasite; Fairbairn and colleagues in 1948, made this suggestion when they hypothesised that the HAT epidemics in Tanzania from 1922 – 1948 had probably represented spill over infections from wild animal reservoirs (Fairbairn, 1948).

Figure 1.3 shows the importance of cattle in the lifecycle of *T. b. rhodesiense* in eastern Uganda. The probability that an infected tsetse fly acquires a *T. b. rhodesiense* infection from infected cattle is five times more likely than acquiring it from an infected person (probability from cattle = 0.0026, probability from human = 0.0005). This highlights the importance that cattle play in the lifecycle of *T. b. rhodesiense* in eastern Uganda. Additionally it was also recently demonstrated that despite the chronic nature of *T. brucei* s.l. infections in cattle, *T. b. brucei* can still be readily transmitted (Van den Bossche *et al.*, 2005). Indeed a single trypanosome is sufficient to cause a tsetse infection (Maudlin & Welburn, 1989), although *T. b. rhodesiense* may be less readily transmitted through the vector (Welburn *et al.*, 1995).

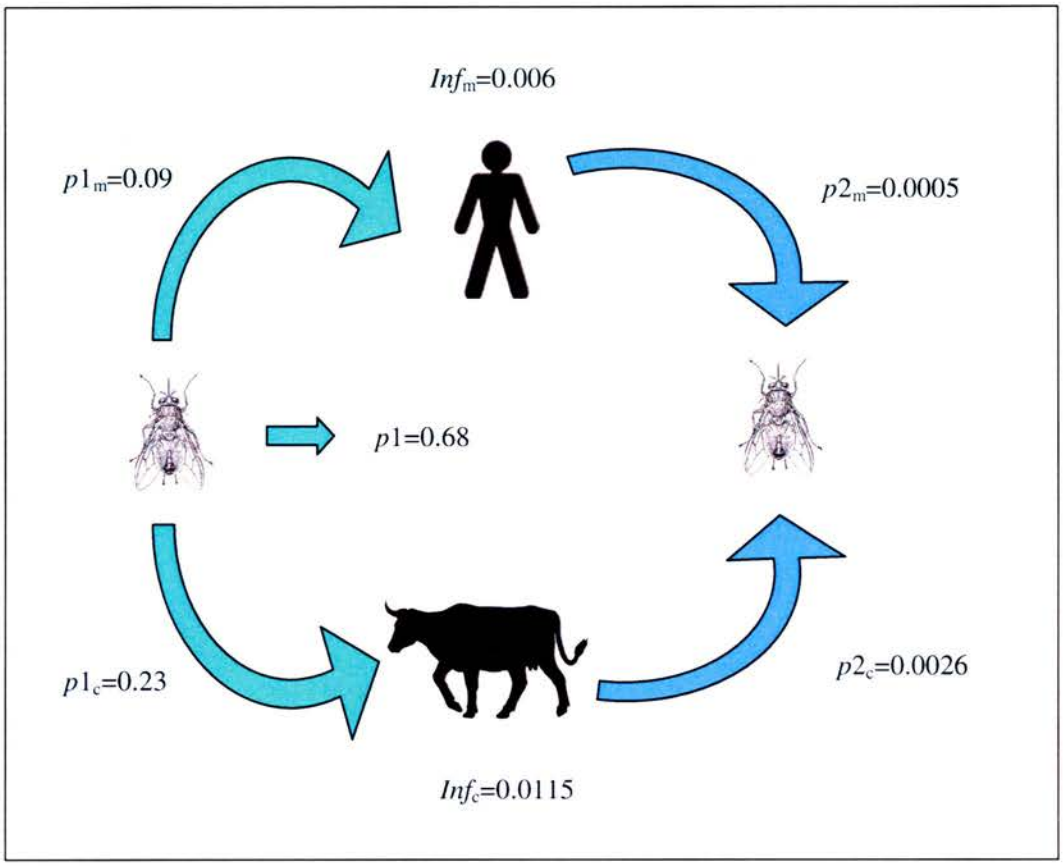


Figure 1.3 – The lifecycle of *T. b. rhodesiense* showing the importance of cattle as a reservoir in Uganda. *p*1m is the probability of tsetse fly feeding on man, *p*1c is the probability of tsetse fly feeding on cattle, the infection rate of *T. b. rhodesiense* in human is *Inf*m and in cattle *Inf*c. *p*2m is the probability that a tsetse fly acquires a *T. b. rhodesiense* infection from a human and *p*2c is the probability that a tsetse fly acquires the infection from cattle. *p*1 is the proportion of tsetse blood meals from other sources. Image adapted from (Hide *et al.*, 1996).

1.5 Pathology

1.5.1 Immune evasion strategy

Trypanosomes, like other pathogens need to evade clearance by the host immune system. Trypanosomes remain extra-cellular and are thus exposed to multiple arms of the immune system. Protection from the host immune response is afforded by variable surface glycoproteins (VSGs) which form a sophisticated evasion mechanism capable of evading both innate and adaptive immune responses. VSGs are glycosylphosphatidylinositol (GPI) anchored molecules that form a dense monolayer of identical proteins (homodimers) protecting against complement mediated lysis (Cox, 1993). There is an exceptional high rate of VSG and surface-membrane recycling which is probably essential for the rapid internalisation of bound antibody and complement (Engstler *et al.*, 2004). Sequencing of the *T. b. brucei* genome is nearly completed and currently there are 1700 VSGs identified (Berriman *et al.*, 2005). The vast majority of VSG genes are clustered in subtelomeric arrays - clusters of 3 - 250 VSG copies (Berriman *et al.*, 2005), however, many are also found at the extremity of telomeres - particularly of mini-chromosomes (Van der Ploeg *et al.*, 1984). It is generally believed that the function of mini-chromosome is to provide a large repertoire of VSGs (Pays, 2006). From the VSG repertoire an individual trypanosome will only express one VSG at any given time, all but one of the VSGs are therefore transcriptionally silent, this is known as mono-allelic expression.

VSGs are only expressed when in specialised telomeric loci called expression sites (ES) (Sheader *et al.*, 2003). The exact number of ES transcription sites remains unknown however, in *T. b. brucei* there are estimated to be approximately 20 (Pays *et al.*, 2001; Becker *et al.*, 2004). Transcription can start in different ES simultaneously but is abortive in all bar one, allowing for mono-allelic expression (Vanhamme *et al.*, 2000). This is due to the recruitment of RNA processing machinery, although how this is regulated remains unknown (Pays *et al.*, 2006). Transcription in ES is polycistronic with up to 20 expression site associated genes

(ESAGs) (such as SRA, a gene that will be discussed further in Chapter 4) in addition to the VSGs. During infection, the VSG being expressed is changed repeatedly. The mechanism regulating VSG switching between ES remains unknown but it has been suggested that it may be linked to cellular stress (Pays *et al.*, 2006). Only a small membrane distal portion of VSGs bearing limited antigenic epitopes are exposed. These exposed epitopes exhibit a high degree of variability between different VSGs. This means that switching VSG expression offers a mechanism to evade any antibody response that the host's immune system may have developed. VSG switching may be due to either transcriptional switching between different ES or switching the resident VSG in ES by DNA recombination (Pays, 2005). There is a semi-predictable hierarchy of VSGs expression, which may be explained by the fact that DNA recombination is more likely between sequences sharing similarity (Morrison *et al.*, 2005).

1.5.2 Pathology of HAT

There are two types of human sleeping sickness caused by different agents and presenting with two distinct clinical syndromes. *T. b. rhodesiense* sleeping sickness is characterised by a rapid progression to severe disease and it is often termed an acute infection. *T. b. gambiense* sleeping sickness is often described as a chronic infection as this form of the disease is characterised by a long asymptomatic stage or with mild clinical symptoms that can persist for many months before progressing to severe disease. Early symptoms in *T. b. gambiense* infection are usually mild and often unnoticed. There are two clinical stages in both forms of sleeping sickness; stage 1 or early and stage 2 or late. In stage 1 the parasites are circulating and proliferating in the blood and lymph of the patient; stage 2 the parasites invade the central nervous system. Then in the absence of treatment the patient will inevitably die. The paragraphs below detail both stage 1 and 2 in *T. b. rhodesiense* and *T. b. gambiense* sleeping sickness.

Stage 1 follows the inoculation of the parasites into the host by the tsetse fly, at the site of the bite there is a local parasite proliferation which can cause an inflammatory nodule or ulceration called a chancre (Moore *et al.*, 2002). This localised inflammation is seen in approximately 50% of *T. b. rhodesiense* cases but rarely in *T. b. gambiense* (Barrett *et al.*, 2003). The chancre resolves three to four weeks after infection. Following inoculation, the parasite spreads to local lymph nodes and into the bloodstream, termed the haemolymphatic stage (Stich *et al.*, 2002) whereby the disease progresses from localised symptoms to a generalised infection. Symptoms include headache, general malaise and an undulating fever. The fevers generally occur in an irregular pattern over many weeks, following the 'waves' of parasite multiplication in the blood. In *T. b. rhodesiense* infections, these early symptoms can be very severe causing pancarditis, pericardial effusion and pulmonary oedema (cardio-pulmonary pathology) and approximately one in ten patients will die unless they have rapid access to treatment (Stich *et al.*, 2002). *T. b. gambiense* infections are insidious in the early stages and often go unrecognised or misdiagnosed. A characteristic sign of this infection is Winterbottom's sign; a posterior cervical lymphadenopathy, this symptom has even been used as an identification tool in some surveillance programmes (Cook & Zumla, 2002). Other more generalised signs of a *T. b. gambiense* infection are a circinate rash, pruritis and generalised oedema.

Over time, the disease proceeds to stage 2 when parasites infect the internal organs and cross the blood brain barrier to invade the central nervous system (CNS). Stage 2 occurs within a few weeks in a *T. b. rhodesiense* infection but after a period of several months or even years in *T. b. gambiense* infection. The parasite invasion into the CNS results in acute encephalopathy. The general symptoms associated with this invasion are headache and mental changes, including reduced higher mental function, difficulties with concentration and the patient's increasing inability to cope with their surroundings. This eventually results in a somnolent state which gave the disease its common name, then seizures, coma and death (Kennedy, 2004). Patients can quickly deteriorate if not treated and in the case of *T. b. rhodesiense* infection

over 80% of deaths occur within 6 months of the onset of illness (Odiit *et al.*, 1997). Essentially, *T. b. gambiense* infections follow the same sequence of infection stages but with a different rate of progression (Sternberg, 2004); progression to the late stage of disease may take several months or longer and late stage CNS infections can last years (Barrett *et al.*, 2003).

There is a spectrum of disease severity associated with *T. b. rhodesiense* between countries in southern East Africa, e.g. Zambia and Malawi and countries further north, e.g. Uganda and Kenya. In the southern area the pathology of the disease has a more chronic type of progression (MacLean *et al.*, 2004). Genetic characterisations of *T. b. rhodesiense* have shown differences between northern and southern isolates (Hide *et al.*, 1991; Gibson, 2002). The difference in severity of the disease has been linked to differences in the host immune responses to the parasites (MacLean *et al.*, 2004).

1.5.3 Pathology of cattle trypanosomiasis

Nagana is the term commonly used to refer to a complex of diseases caused by the *Trypanosoma* spp. transmitted to domestic livestock by *Glossina* species. The distribution of Nagana across Africa corresponds with the distribution of the tsetse fly. *T. congolense* and *T. vivax* are the major pathogenic species in African cattle and although *T. b. brucei* is generally less pathogenic it has been associated with significant disease in exotic cattle (Wellde *et al.*, 1989). Experimental data on trypanosomiasis infection typically shows three clinical stages: acute, stabilisation and chronic, however, in field situations the situation can be more complex. Clinical pathology in cattle can range from acute disease leading to death in three to four weeks to a more common chronic condition lasting for years (Taylor & Authie, 2004). The acute disease phase is associated with a continuous reduction in haemoglobin concentration and packed red cell volume (PCV) resulting in anaemia. It is the anaemia which is correlated to a loss in the productive performance of the

cattle. The clinical signs associated with animal trypanosomiasis include anaemia, weight loss, roughness of hair coat, enlargement of peripheral lymph nodes, pyrexia, abortion, reduced milk yield and eventually death (Eisler *et al.*, 2004). Many African cattle survive the acute phase and develop chronic disease. Chronically infected cattle suffer from cachexia; productivity and reproductive functions are impaired.

1.6 Diagnosis

1.6.1 Diagnosis of HAT

The simplest approach to trypanosome detection is by the direct observation of fresh blood between a cover slip and a slide, using a medium magnification (usually a dry objective of 40x and eye pieces of 5 – 10x). This method is accurate at or above concentrations of 10^4 parasites per ml (Uilenberg, 1998). Trypanosomes are either seen directly or indirectly by the movement of the blood cells. To increase the likelihood of observing a parasite in a blood film various concentration methods can be used of which the simplest is the buffy coat method which can improve detection levels by microscopy to 500 parasites per ml (Uilenberg, 1998). Thick or thin smears can be produced and examined, a thin smear can be used to identify some of the different trypanosome species by morphology but this relies on a highly experienced technician. Determining the stage of disease before treatment is essential as drug regimes vary for first and second stage treatments of both *T. b. rhodesiense* and *T. b. gambiense*. Currently the only way to establish the stage of a patient is to do a lumbar puncture (Bouteille *et al.*, 2003). The sample of cerebral spinal fluid is then examined under a microscope for the presence of trypanosomes; if parasites are identified within this sample, or the white cell blood count is greater than 5 cells per µl, or there is an increased protein count greater than 370 mg/litre (World Health Organization, 1998b) then the patient is deemed to be in stage 2.

Determining whether a human infection is caused by *T. b. rhodesiense* or *T. b. gambiense* is not simple. There is no field test for *T. b. rhodesiense* and diagnosis is based on geographical locale, e.g. a patient with trypanosomiasis in south east Uganda would be treated for *T. b. rhodesiense*. However, in the future this assumption may not be possible as there are concerns that the foci of *T. b. rhodesiense* and *T. b. gambiense* within Uganda may merge (Picozzi *et al.*, 2005). There is a serological test for *T. b. gambiense* (Magnus *et al.*, 1978), known as the card agglutination test for trypanosomiasis (CATT). This is a fast and simple agglutination assay for the detection of *T. b. gambiense* specific antibodies which costs approximately $0.40 per test and can be done in the field without electricity or specialised staff giving results in 10 minutes (Pepin & Meda, 2001). This test is directed against a specific variant antigen (VAT) on the surface, LiTat 1.3 and is now routinely used in mass screening campaigns (Buscher *et al.*, 1999; Robays *et al.*, 2004) However, it has been reported that there are a small number of *T. b. gambiense* variants that do not express the specific test antigen (Enyaru *et al.*, 1998). The CATT test cannot be used to detect *T. b. rhodesiense* due to the absence of LiTat 1.3 from its antigenic reservoir (Enyaru *et al.*, 1999). There can also be a problem in the treatment of individuals which are CATT positive but parasitologically negative; in these situations a CATT titration is performed and a study in Angola recommended the treatment of individuals with an end titre of greater than 1/8 (Simarro *et al.*, 1999). The CATT system is also used for the diagnosis of a different species of trypanosome, *T. evansi*, a major enzoonotic disease of horses (Claes *et al.*, 2002) and camels (Gutierrez *et al.*, 2000). The test is based on the variable antigen type (VAT) RoTat 1.2 which was isolated from a water buffalo in Indonesia in 1982 and has been seen in all type II *T. evansi* which have been isolated (Verloo *et al.*, 2001), however it cannot be used on type I *T. evansi* found in Kenya (Ngaira *et al.*, 2003; Ngaira *et al.*, 2004).

1.6.1.1 Molecular tests for HAT

Molecular tests can demonstrate the presence of DNA sequences specific to the parasites, techniques such as polymerase chain reaction (PCR) can be used for diagnosis and species identification in a research setting, however they are not currently practical for widespread field use (Chappuis *et al.*, 2005). In reality, the equipment needed and the cost of these techniques, make it unlikely that they will be available to any rural African hospital in the near future.

PCR that targets repetitive sequences of DNA can be very sensitive with a detection limit around 0.1 trypanosome per ml of cattle blood (Masake *et al.*, 2002) or 25 trypanosomes per ml of human blood (Kanmogne *et al.*, 1996). The difference observed in the sensitivity between tests carried out in human and cattle blood are the result of factors in human blood that inhibit the PCR (Contamin *et al.*, 1995). In theory, PCR can be applied to any sample that may contain trypanosome DNA; whole blood, buffy coat, lymph or CSF. The discovery of the serum resistance associated (SRA) protein, a VSG type protein which resists lysis by human serum (Xong *et al.*, 1998) has allowed the development of a diagnostic PCR test for *T. b. rhodesiense* (Welburn *et al.*, 2001b; Radwanska *et al.*, 2002a); this diagnostic test is discussed in more detail in Chapter 4. One of the first *T. b. gambiense* specific PCR tests used a gene sequence for the VSG surface protein, AgTat 11.17 (Bromidge *et al.*, 1993). This was effective for isolates taken from Ivory Coast, Nigeria, Congo, Cameroon, Democratic Republic of Congo and Sudan, however later testing of *T. b. gambiense* isolates collected from Moyo in northern Uganda showed that the sequence was absent (Enyaru *et al.*, 1992). The discovery of the *T. b. rhodesiense* specific *SRA* sequence (Xong *et al.*, 1998) which encodes for an atypical VSG, prompted searches for a similar sequence in *T. b. gambiense*. A sequence named *T. b. gambiense* specific glycoprotein (TgsGP) was found to be present in all the tested strains of *T. b. gambiense* and absent from *T. b. rhodesiense* and *T. b. brucei* strains (Berberof *et al.*, 2001) and was used to develop a *T. b. gambiense* specific PCR (Radwanska *et al.*, 2002b).

1.6.2 Diagnosis of cattle trypanosomiasis

Bovine trypanosomiasis is characterised as a production disease which means that diagnosis and treatment are, increasingly, the responsibility of the farmer in decentralised systems (Eisler *et al.*, 2004). This means that the majority of people involved with the diagnosis and treatment of trypanosomiasis rely wholly on physical examination of clinical signs (listed in section 1.5.2). Ultimately a presumptive diagnosis of trypanosomiasis is made on the basis of clinical signs and following the administration of treatment, a positive response is taken as confirmation of the diagnosis (Eisler *et al.*, 2004) and to aid this approach a low cost diagnostic aid has recently been developed (Eisler *et al.*, 2007). However, there are diagnostic tests which can be used. Microscopy, as described in section 1.6.1, can be used although this is not sensitive enough to detect low levels of parasitemia. More advanced methods can also be employed, these include: inoculation of blood from a suspected case into a 'clean' animal, culture, immunological diagnosis and molecular techniques. These methods are in reality only used for research purposes and molecular techniques are used for diagnosis of trypanosomiasis in this thesis (see Chapter 3 and 4). Until a diagnostic method can be developed which costs less than the price of treatment for trypanosomiasis (approximately $1) and is easy to use in the field, they are of little use to a poor rural farmer in Africa.

1.7 Treatment

1.7.1 Treatment of HAT

There are currently four drugs licensed for treatment of HAT, these are pentamidine, suramin, melarsoprol and eflornithine. Three of these drugs were developed in the first half of the twentieth century and given their severe side effects, it is likely that they would not pass current drug safety standards for the development of new drugs (Fairlamb, 1990). The treatment regime for HAT depends on the species causing the

infection and on the stage of disease in the patient. Pentamidine is the stage 1 treatment for *T. b. gambiense* and is administered by an intramuscular injection (Burchmore *et al.*, 2002). The drug was first introduced in 1937 and is associated with some minor side effects, including skin irritation, nausea, vomiting and more rarely serious side effects including hypoglycemia, hypocalcemia and renal failure (Pepin & Milord, 1994). The early stage treatment for *T. b. rhodesiense* is suramin which is administered by slow intravenous injection and can be found in the blood for up to 3 months, giving suramin one of the longest half-life's in human chemotherapy (Pepin & Milord, 1994). Suramin was discovered in 1921 and is an analogue of some of the first drugs used to try to treat sleeping sickness at the turn of the 19th century: trypan blue and trypan red (Docampo & Moreno, 2003). Suramin is generally well tolerated with renal toxicity the most common associated problem, but this is usually mild (Docampo & Moreno, 2003), also there are rare cases of exfoliative dermatitis (May & Allolio, 1991).

Stage 2 of both *T. b. rhodesiense* and *T. b. gambiense* are more difficult to treat; due to the location of parasites in the CNS drugs must cross the blood brain barrier. Melarsoprol is the only drug which can be used to treat stage 2 *T. b. rhodesiense* and is administered by slow intravenous injection. Melarsoprol which was discovered in 1949 is an arsenical derivative and can be used to treat stage 2 *T. b. gambiense*. This drug is associated with severe side effects which include reactive encephalopathy that is fatal in 3-10% of cases (Pepin & Milord, 1994; Braakman *et al.*, 2006) and surviving patients can experience long lasting neurological damage (Kumar *et al.*, 2006). For the treatment of stage 2 *T. b. gambiense* another treatment called eflorinthine or difluromethylornithine (DFMO) was registered for use in 1990. This drug has none of the side effects associated with melarsoprol and would ideally be used as a first line treatment or at least in patients that have a reaction to melarsoprol (Kuzoe, 1993). Unfortunately the use of this drug has been severely restricted by cost and treatment regime. The price to treat one patient, without including medical equipment and fluids, is estimated at $350 (Burri & Brun, 2003).

There have been some reports of drug resistance in the treatment of human African trypanosomiasis, although as patients are hospitalised for the duration of treatment there are less problems of under-dosing or incorrect administration (Matovu *et al.*, 2001). However, adverse reactions to drugs and or the invasive diagnostic techniques, e.g. lumbar puncture can mean that individual patients may refuse to complete the treatment regime or consent to follow up investigations (Bacchi, 1993). Consequently, in recent years reports of treatment failure for melarsoprol have increased. In Uganda, relapse rates of up to 30% for *T. b. gambiense* have been reported (Legros *et al.*, 1999). This is a grave situation as melarsoprol is the first line treatment for late stage *T. b. gambiense* and the only treatment for late stage *T. b. rhodesiense*.

There is an urgent need for the development of new drugs for HAT however, as the market for sleeping sickness drugs is limited to Africa, the prospect of low financial gains has made the pharmaceutical industry reluctant to invest the money necessary for the research and development of new drugs. In fact only two new drugs have emerged for trypanosomiasis in recent years, cymelarsen for the treatment of *T. evansi* and eflornithine for the treatment of *T. b. gambiense* (Anene *et al.*, 2001). However, the high cost involved in producing eflornithine meant that the continuing manufacture of the drug could not be guaranteed, in fact in 1995 the pharmaceutical company Aventis announced it would not continue production (Boseley, 2001). This problem was temporarily solved in 2002 when Aventis agreed to donate the drug to the World Health Organization, who donates it to specialist treatment centres in endemic regions (World Health Organization, 2002) – this has secured its production until 2007. There is, however, some hope that the situation may improve as the Bill and Melinda Gates Foundation recently donated $15.1 million to a consortium of researchers to develop new drugs to fight sleeping sickness (Fuller, 2000).

1.7.2 Treatment of animal trypanosomiasis

There are currently three drugs available on the market for use against trypanosomiasis in domestic livestock: diminazene aceturate, homidium bromide and isometamidium chloride. All of these drugs have been on the market for more than 20 years and remain popular with farmers because they are cheap, widely available and easy to use. Isometamidium chloride exhibits a prophylactic action due to its ability to stay in the circulation for up to 3 months post-treatment (Eisler *et al.*, 2004). In recent years there have been attempts to enhance and extend the prophylactic action of isometamidium chloride using sustained release devices (SRD), which on average sustained protection for three times as long (Geerts *et al.*, 1997). These devices are expensive so in an attempt to reduce costs, field trials were done with a cheaper type of device. These results were promising, showing reduced re-infection rates of trypanosomes in cattle with SRD's, however the tests were carried out on N'Dama cattle and have not yet been tried out on any other breeds (Geerts *et al.*, 1999).

Trypanocidal drugs for animals are a widespread and well established method of trypanosomiasis control in most African countries. It is estimated that 35 million doses are administered each year (Geerts & Holmes, 1998), which shows the use of these drugs has proved to be a sustainable method for trypanosomiasis control for the individual farmer. The benefits of their use has been shown using production parameters, for example, in a study in Kenya lactation yields were shown to be directly related to prophylactic drug use (Itty *et al.*, 1995). Prophylactic drug use against trypanosomiasis in a well managed herd could certainly allow animals to stay healthy and productive. Unfortunately, across much of sub-Saharan Africa, cattle are kept by small scale subsistence farmers who do not have the money or management techniques required to employ this strategy.

The drugs used against animal trypanosomiasis have been in use for a considerable length of time and multiple types of resistance have been demonstrated to these in cattle (Peregrine, 1994) from all the regions where they are commonly used (Matovu *et al.*, 2001). The emergence of drug resistant trypanosomes is considered a very serious problem in trypanosomiasis control (Anene *et al.*, 2001) as the control of bovine trypanosomiasis relies almost entirely on the administration of trypanocidal drugs (Eisler *et al.*, 2004). In the past, the distribution and administration of trypanocidal drugs was strictly controlled by government veterinary departments (Chapter 2). However, with a growing environment of privatisation and deregulation, trypanocides are now more freely available from local pharmacies, drug stores and markets from where they are in many cases purchased and administered by the farmers themselves (Geerts *et al.*, 2001). The availability and low cost of these drugs has led to growing concern that the effectiveness of trypanocides as a control method will be severely affected by rising levels of drug resistance.

A large scale study in Zambia showed almost 50% of tested isolates taken from *T. congolense* infected cattle were resistant to either isometamidium chloride or diminazene aceturate with the majority, 34% resistant to isometamidium chloride (Sinyangwe *et al.*, 2004). The authors suggested that the high prevalence of isometamidium chloride resistance could be attributed to large scale block treatments using isometamidium chloride in the 1980's. In a study in Ethiopia, where trypanosomiasis is considered one of the major constraints to cattle production, 23% of *T. congolense* cattle infections were shown to be resistant to isometamidium chloride at a dose rate of 1.0mg/kg body weight (Afewerk *et al.*, 2000); the authors suggested that the widespread misuse of this drug had contributed to the development of resistance.

1.8 Control of trypanosomiasis

Attempts to control trypanosomiasis began at the start of the twentieth century; in colonial times these were extremely vigorous and included the forced movement of human populations, the destruction of game animals and extensive clearing of tsetse habitat. In some situations these extreme measures were effective and by the early 1950's sleeping sickness had been brought under control (Barrett, 1999). Colonial rule began to dwindle in the late 1950s when many African countries gained independence. Unfortunately, in many cases this strive for independence brought political and social unrest. This coupled with worsening economies meant that there were no longer the resources necessary to continue the types of large scale control programmes implemented under colonial rule (Kuzoe, 1993). This has lead, over the past 50 years, to a resurgence of HAT (Smith *et al.*, 1998; Barrett, 1999) through a recrudescence of old foci and in some areas the spread of disease from these foci into new areas (Fèvre *et al.*, 2001). In many of the countries affected by sleeping sickness the total annual health budget is less that $10 per head of population (Kuzoe, 1993); in Uganda the annual per capita spending on health is only $8 (Wendo, 2002). This means that countries must depend at least partially if not totally on external aid to maintain sleeping sickness control programmes. This situation has lead to many problems; sleeping sickness is a regional disease of the rural poor and although it has the potential to cause devastating epidemics, the annual numbers of reported cases are low compared to other diseases in sub-Saharan Africa which means that sleeping sickness control programmes do not hold much appeal for international donors (Kuzoe, 1993). Additionally, in the 1990's there was a generalised move away from top down, large-scale funded control programmes and a movement toward the idea of private good to public good (Torr *et al.*, 2005), which has resulted in less donor interest in funding large scale intervention programmes administered through government departments.

1.8.1 Vector control

Due to the difficulties of treating hosts, especially humans, for trypanosomiasis the principal approach to controlling disease has been an attempt to halt transmission by the insect vector (Schofield & Maudlin, 2001). The reproductive biology of the tsetse makes it a good control target. Tsetse flies are K-strategists, their low reproductive rate complemented by a high survival rate, adapted for efficient exploitation of their habitat (Hargrove, 2004). Female flies are larviparous, which means that after mating, a single egg develops over a period of 12-14 days in the uterus, and is nourished by 'milk glands'. When fully developed, the larvae weigh more than the female. This large input of resources means a female produces no more than 12 eggs in a lifetime (Molyneux, 1993). To put this low reproductive rate into context, an *Anopheles* mosquito, the vector of malaria, will lay thousands of eggs in a lifetime. Therefore control methods targeted against tsetse flies can quickly reduce the number of vectors, and the low reproductive rate means it can take a long time for the population to recover. One of the first methods used to reduce the tsetse fly population was the destruction of natural tsetse habitat. This focused on clearing large areas of vegetation in an attempt to remove the flies resting sites and general habitat and was done extensively in Uganda around the shores of Lake Victoria (this Ugandan control strategy will be discussed in Chapter 2). Although these methods did have profound effects on tsetse fly and trypanosomiasis distribution in some areas they were devastating to the local ecology.

1.8.1.1 Aerial spraying

The advent of DDT and other persistent insecticides meant that the flies themselves could be targeted with the most successful spraying campaigns completed from the air. Aerial spraying was adopted the 1970's and 1980's (Holmes, 1997) and involved the release of insecticides as aerosols from low flying aircraft. Normal practice is five repeat applications over 60 days to cover the pupal period of the tsetse fly (Allsopp, 2001). Botswana has used aerial spraying as a strategy against

tsetse flies in the Okavango Delta since the early 1970's and although the area was consistently sprayed from 1972 – 1991 tsetse flies were never entirely eliminated (Hargrove, 2003). Until recently it had been very difficult for the spraying planes to fully cover the target areas. The pilots are required to fly in long straight rows over the entire area, usually at night, due to meterological conditions. It is extremely difficult to ensure that no areas are over or under-dosed using traditional navigation tools, however the use of GIS technology allowed precise and accurate spraying over the 16000km^2 target area in the Okavango Delta (Wireless, 2003). This sequential aerosol technique (SAT) was used very successfully in conjunction with odour-bated targets to knock down the tsetse fly population (Mudo, 2003). This method requires skilled personnel, sophisticated equipment and substantial funding. This is a top down approach which is expensive, unsustainable and involves no participation from the affected communities. Additionally, unless the sprayed areas are rigorously surveyed and barriers maintained against re-invasion then the extensive resources needed may be wasted (Hargrove, 2003).

1.8.1.2 Ground spraying

Spraying can also be done on the ground which uses slightly less resources. This focuses on the application of a residual insecticide to specific areas where tsetse flies are known to rest. The chemicals used can remain lethal to tsetse flies for over 60 days, longer than the maximum pupation period (Holmes, 1997). Although DDT and other organochlorides were used in the past to successfully control tsetse flies, it became politically and environmentally unacceptable to continue their use (Curtis & Lines, 2000). However, in 2006 both WHO and USAID have reversed their stance on the use of DDT in the fight against malaria and accepted that it can be used in a controlled manner to great effect (Mandavilli, 2006). Although this is unlikely to herald a change for trypanosomiasis control, the use of DDT against mosquitoes involves limited spraying within homes whereas against the tsetse fly involves widescale spraying over large areas of land. An alternative is the use of synthetic pyrethroids. There are, however, other disadvantages to this type of control method.

Ground spraying requires a degree of country-wide infrastructure, vehicles and spraying equipment need to be maintained, supervision, planning and gaining access to remote areas are all essential to minimise the risk of tsetse re-invasion of a sprayed area (Hargrove *et al.*, 2000). This all requires substantial funding at a national level. Despite these limitations, ground spraying has been used with success in Nigeria, where tsetse flies were eradicated from a 200,000km^2 area (Jordan, 1978). This success makes ground spraying the only proven method of large scale tsetse eradication. The removal of the tsetse fly in this area was combined with an immediate exploitation of the cleared land for agriculture, driven by the expanding population in Nigeria. This land use change significantly reduced the risk of re-invasion as the tsetse flies' natural habitat had been destroyed by development. That situation is probably unique in Africa as population densities in other areas are lower and the demand for land is lower (Jordan, 1978), however this situation is changing as populations and land pressures increase.

1.8.1.3 Tsetse traps

Traps and targets are used as simple vector control methods to reduce the numbers of tsetse flies in the area and can also be used to monitor reductions in tsetse fly numbers using any of the control methods discussed in this section. Early studies showed that tsetse were more often found on dark rather than light surfaces (Saunderson, 1911; Simpson, 1911; Swynerton, 1933; Swynerton, 1936). A better understanding of the role of coloured surfaces in the attraction of tsetse flies was possible by using electric nets (Vale, 1982b; Vale, 1982a). It was Challier and colleages in 1977 that found that a combination of royal blue and black cloth increased the numbers of tsetse trapped within a biconical trap (Challier *et al.*, 1977). Today traps, targets or screens are made from this combination of blue and black materials. The most basic of these are screens which are simply a piece of insecticide treated cloth hung in a tsetse infected area and when the flies land on the screen they pick up a lethal dose of insecticide.

Different size and shapes of traps have been developed over the years to enhance the capture of different tsetse fly species (Leak, 1999). The advantage of using a trap over the simpler screens is that the captured tsetse flies can be counted and this can be used to show local communities that the traps work and can also be used to monitor any impact on the fly population. The first bi-conical traps were developed and shown to be effective against riverine species of *Glossina spp.* (Allsopp, 1984). However, a study around the same time demonstrated that conventional traps failed to catch many of the tsetse flies visiting them and that tsetse flies were attracted to a stationary animal by its odour (Vale, 1980). This led to the use of attractants. Since that time carbon dioxide, acetone, butanone, 1-octen-3-ol, p-cresol, 4-methylphenol, 3-n propylphenol and cow urine have been shown to act as attractants. A comprehensive review of different bait technologies developed for attracting tsetse flies has been published by Green (Green, 1994). The traps treated with attractants still work in the same way but flies attracted to the traps or targets collect a lethal dose of insecticide.

1.8.1.4 Live bait

Another application method for insecticides is on domestic livestock, particularly cattle. Insecticides can be applied to these animals by dips, pour-ons or sprays. The tsetse flies are still attracted to the treated animal and will be killed by the insecticide when they land to feed; this method is termed the live bait technique. In areas where there are large numbers of cattle this is the simplest, cheapest and most effective method of tsetse control (Hargrove, 2003). The principle of this method is similar to baited tsetse traps, previously mentioned, and has been used with varying degrees of success in Burkina Faso (Bauer *et al.*, 1995), Tanzania (Fox *et al.*, 1993) and Ethiopia (Rowlands *et al.*, 2001) to name a few examples. However, there are some difficulties in relying on this as a principal method of tsetse control. Factors which

influence the density, location and movement of cattle have little to do with the needs of a tsetse control programme.

1.8.1.5 Sterile insect technique (SIT)

Finally, a method of tsetse control which has provided heated debate within the trypanosomiasis and tsetse research community over the last few years is the sterile insect technique (SIT). This requires the rearing and subsequent release of sterile male tsetse flies into the environment following reduction of tsetse population using one of the aforementioned methods (Allsopp, 2001). Irradiated sterile males are released into the environment and mate normally with female flies depositing sterile sperm. As female tsetse flies only mate once in their lifetime there is no possibility of any female fertilised by a sterile male, producing any offspring (Hargrove & Williams, 1998), so the fly population can be very quickly affected. There is currently only one example of a successful tsetse fly elimination of *G. austeni* campaign using SIT, a trial on the island of Zanzibar (Bailey, 1998). Although this trial was shown to be effective there are a variety of factors about Zanzibar which differ from mainland Africa. The most obvious and probably the most important of these is that Zanzibar is a small island, geographically isolated and so unlikely to have had any problems with re-infestation (Vale & Torr, 2005). Despite these concerns SIT has been seen by the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) as an essential tool in the eradication of tsetse from Africa (Kabayo, 2002; Kabayo, 2005).

1.8.2 Control of reservoir hosts

In conjunction with early attempts to control tsetse through the destruction of their habitats was the depletion of natural tsetse fly hosts. The animals on which tsetse flies feed include a long list of wild game animals, various species of ungulates, bush pig, warthog, giraffe, buffalo etc. In certain areas these animals were systematically

hunted in an attempt to starve the tsetse into eradication. This was an enthusiastic policy in Uganda where the elimination of these wild hosts was approached with military fervour; this strategy is further discussed in Chapter 2.

1.8.2.1 Treatment of cattle as a reservoir host

As treatments became available for animal hosts it was possible to treat infected or at risk animals rather than have to cull, however these treatments are generally restricted to domestic animals. The treatment of cattle is important not only as means to improve the welfare and productivity of animals by eliminating trypanosomiasis but also as a preventative measure against HAT. The movement of cattle in Uganda has been strongly implicated in the spread of *T. b. rhodesiense* to previously uninfected areas (Koerner *et al.*, 1995; Hide *et al.*, 1996; Welburn *et al.*, 2001a). A new outbreak of sleeping sickness in the late 1990's was linked to the movement of cattle through a specific cattle market (Fèvre *et al.*, 2001). The treatment of cattle with trypanocides is very popular; over 35 million doses are sold each year (Geerts & Holmes, 1997) with treatment mostly carried out by individual farmers rather than part of a larger control strategy. In this way these livestock keepers can use an effective, easy affordable treatment which benefits their own animals and requires no dependence on others (Torr *et al.*, 2005). Treatment of domestic livestock can also be used as part of an integrated control programme against human disease and this is essential in the control of *T. b. rhodesiense*. Mathematical models published by Welburn and colleagues showed the relative effects of a control programme based on the treatment of human cases with no intervention against the cattle reservoir and the effect of chemoprophylaxis on the cattle reservoir (Figure 1.4)(Welburn *et al.*, 2001a). This model showed that in the scenario where the only control measure was the treatment of human cases there would be little effect on the reproduction rate of *T. b. rhodesiense* (Figure 4a). On the other hand, if the intervention efforts were directed towards the animal reservoir chemoprophylactically clearing infection from cattle this would have a substantial effect on the reproductive rate of *T. b. rhodesiense* (Figure 4b). However, the model

shows the most effective intervention against both *T. b. rhodesiense* and *T. b. gambiense* is the removal of the tsetse population (Figure 4c).

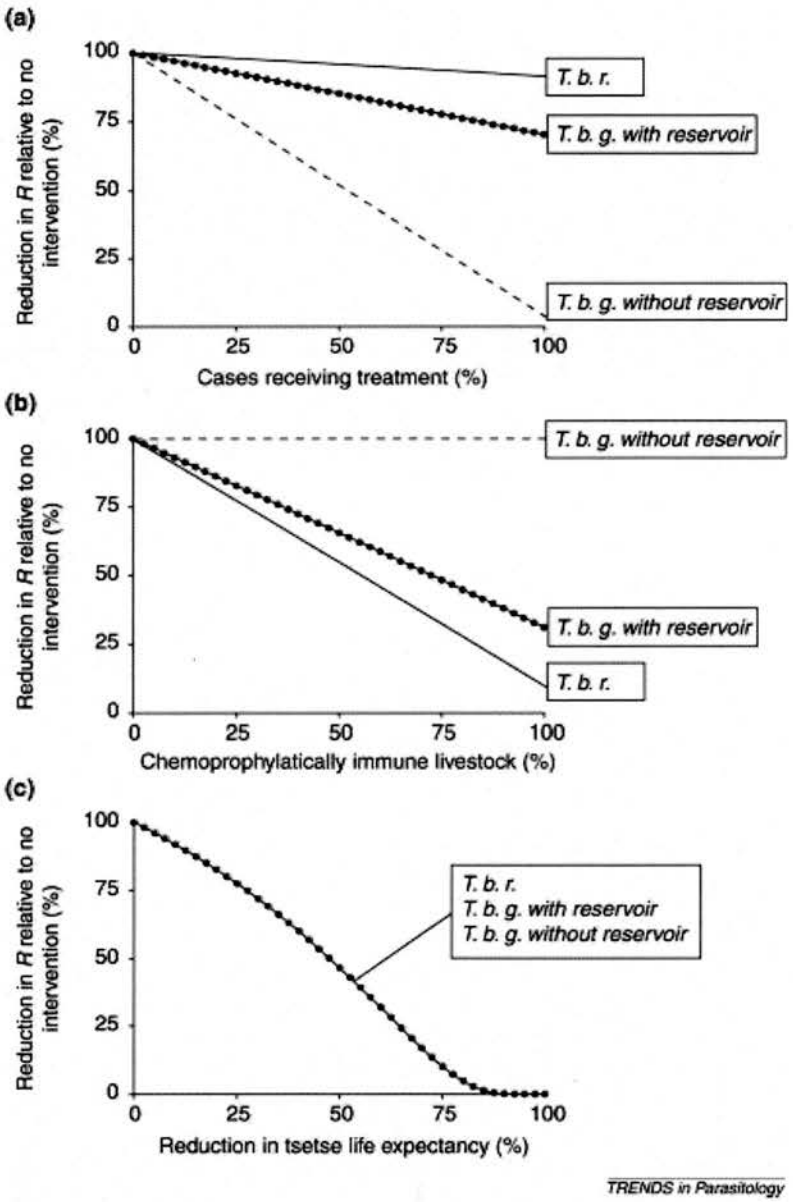


Figure 1.4 – Graphs showing mathematical models of the impact on *R0* of different control strategies on both *T. b. rhodesiense* and *T. b. gambiense* (with and without an animal reservoir). (a) shows the predicted impact of treating only human cases, (b) the impact of treating livestock and (c) the impact of reducing tsetse flies. Figure reproduced with kind permission of (Welburn *et al.*, 2001a)

1.8.2.2 Use of trypanotolerant cattle

A different type of approach to the problem of trypanosomiasis is the use of naturally tolerant breeds of cattle. It has been well recognised that some native African breeds of cattle are more tolerant to trypanosomiasis and can survive a level of infection that would kill a more susceptible breed. The best known of these is the West African N'dama breed, which has been demonstrated to have trypanotolerance (Murray *et al.*, 1981). However, these breeds are not immune to the parasites and will still succumb to very heavy trypanosome infections and as these animals are still infected this approach they would still act as a reservoir for human-infective *T. b. rhodesiense*. The distribution of these breeds is generally limited to West Africa and despite attempts to encourage their rearing in other areas, they remain unpopular due to their small size and lower productivity (Holmes, 1997). Consequently it seems unlikely that the rearing of these cattle will ever be adopted on a larger scale in east Africa therefore this cannot be seen as a practical control option.

1.8.2.3 Surveillance

In terms of a HAT epidemic it is essential to detect the outbreaks early and surveillance is a useful tool to prevent the spread and impact of this disease (Cattand *et al.*, 2001). Surveillance of the human population is important for *T. b. gambiense* because there is a long asymptomatic period of the infection in humans, throughout which time the disease can be transmitted. There are two approaches to surveillance, either passive or active. Passive surveillance is probably the most common, this occurs when a patient who is suspected to have HAT is referred to a specialist centre for diagnosis and treatment. The non-specific nature of the early symptoms of *T. b. gambiense* infection means that active surveillance is necessary to have any impact on the progression of an epidemic. Active surveillance is the active screening of a seemingly healthy population for the disease. This can be achieved by sleeping sickness diagnostic centres operating active case finding initiatives in the

surrounding community; this involves testing healthy individuals in an attempt to find early cases and reduce the probability of transmission. The most effective method of surveillance is the use of mobile case finding units. These units can move throughout the country targeting known risk areas, testing everyone in a village and treating any infected individuals (Cattand *et al.*, 2001). This approach was used very successfully in the Democratic Republic of Congo where at the height of surveillance operations, 250 units covered the main risk areas resulting in a decrease in new cases from 33,502 reported cases in 1930 to 11,837 in 1940 (Ekwanzala *et al.*, 1996). Unfortunately due to the unstable political system this operation collapsed and in the 1990's the numbers of new cases of sleeping sickness in the Congo was thought to have returned back to levels seen pre 1930 (Van Nieuwenhove *et al.*, 2001). Since 1998 control efforts intensified and a concerted effort backed by renewed donor interest has led to a major reduction in the incidence of sleeping sickness within the country, although fears remain over the consequences of any future removal of international aid (Lutumba *et al.*, 2005).

Due to the differing pathology of *T. b. rhodesiense* infections, little can be achieved by extensive active screening of the human population. Passive surveillance is employed for case finding and suspected cases are then referred to a specialist centre for diagnosis and treatment. However the active screening of the villages or focal areas with more than one case of *T. b. rhodesiense* could be very effective in detecting cases and saving lives. This is especially true when HAT is occurring in new areas where people cannot recognise the symptoms; early stages are confused with malaria and late stages with HIV/AIDS (Pers. comm. Dr Alan Mpairwe, Lwala hospital, Kaberamaido district, Uganda). In *T. b. rhodesiense* affected areas it would be useful to be able to screen cattle or other animals hosts to assist with the targeting of control strategies, but unfortunately at this time there is no cheap and easy field diagnostic to show infections with *T. b. rhodesiense*.

1.9 Farming in tsetse controlled areas (FITCA)

Recognising the substantial impact that trypanosomiasis places on the economic output of East Africa, the European Union (EU) in the late 1990s provided substantial funding for a programme called Farming In Tsetse Controlled Areas (FITCA). This was a regional programme of the African Union/Inter African Bureau for animal resources (AU/IBR). The programme consists of three large national projects in Kenya, Uganda, Ethiopia and two smaller projects in Rwanda and Tanzania. FITCA was funded by the European Development Fund (EU) who allocated 20 million Euros (EU Newsletter Uganda, 2004). To combat the movement of infected tsetse flies and prevent the re-infestation of intervention zones, the FITCA programme focused on a large geographical area spanning the borders of numerous countries. The FITCA approach aimed to engage the communities in tsetse control to enable livestock keeping, increase agricultural productivity and enable communities to continue the control after the end of the project (EU Newsletter Uganda, 2004) and as such it was not solely a tsetse/ trypanosomiasis control project.

The overall objective of FITCA was

> *"To contribute to the socio-economic development of the region through coordination of national activities to ensure sustainable rural development to improve the well being of the rural population and the health of livestock through sustainable rural development and to improve the implementation capacity in the countries concerned."*
>
> (EU Newsletter Uganda, 2004)

The chosen approach was an integration of vector surveillance and control, disease surveillance and treatment, as well as the introduction of crop farming and livestock development in the hope that these activities would control and prevent re-infestation by tsetse and trypanosomiasis. National programmes interpreted the overall objective in different ways to suit their development needs and different realities but

for the purposes of this thesis, I will be discussing only the FITCA Uganda programme and specifically those aspects directly concerned with interventions designed to control trypanosomiasis.

1.9.1 The FITCA Uganda programme

FITCA Uganda worked in 12 districts in the eastern part of the country, covering an area of approximately 52,029km^2, 6 million people and 900,000 head of cattle (EU Newsletter Uganda, 2004). This encompassed the traditional Busoga focus of sleeping sickness within the country and includes two areas which have more recently been affected with sleeping sickness – Tororo (from 1988) and Soroti (from 1998). In total the area covered by FITCA covered the following districts: Mukono, Kayunga, Kamuli, Jinja, Mayuge, Bugiri, Busia, Tororo, Mbale and Pallisa and Soroti (Figure 1.5). Soroti was not in the initial list of districts to be involved in this programme, however due to the ongoing human sleeping sickness epidemic (Fèvre *et al.*, 2001) it was latterly included in the programme in a hope to stem the rising incidence of disease.

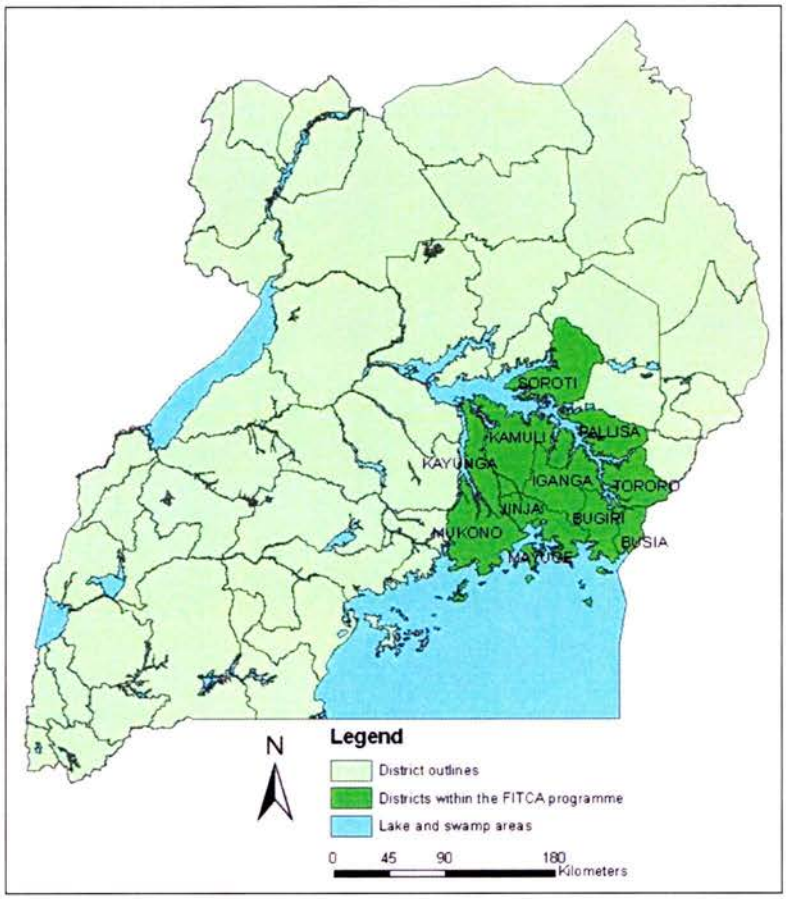


Figure 1.5 – Map of Uganda showing districts involved in the FITCA programme

1.9.1.1 Implementation of the FITCA Uganda programme

The FITCA programme was implemented throughout eastern Uganda using, to a great extent, the existing administrative network and in particular for mass treatments, the district veterinary services. Districts comprise the largest administration unit in Uganda and there are currently 55 districts. Each district is sub divided into counties, sub-counties, parishes and villages (Figure 1.6). Each level of administration fits into a hierarchical management structure with local community (LC) leadership. Leadership is democratically elected by the local population and forms the local government.

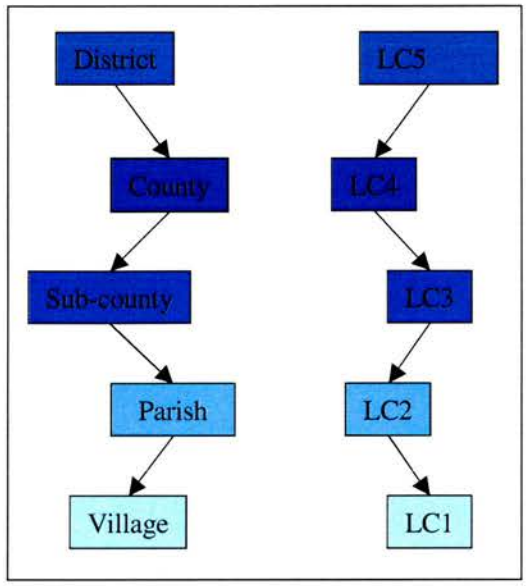


Figure 1.6 – Shows administrative structure of Uganda

Veterinary services are also organised on a district level. Each district has an experienced, veterinary trained individual – the District Veterinary Officer (DVO). The DVO has responsibility for all veterinary care and control of veterinary or zoonotic diseases in that district. On the staff of the DVO are veterinary officers (VOs). These are veterinary trained individuals who are responsible for sub-counties (usually more than one), although this varies with staffing levels. In addition there are animal husbandry officers (AHO) who assist the VOs. These individuals do not have a veterinary degree, but have received some technical training. The structure of the Uganda veterinary service currently allows for comprehensive cover of the veterinary needs in each district however, the effectiveness of this cover does vary from one district to another, and as a rule, the system is under-resourced.

1.9.1.2 FITCA baseline survey

In the initial stages of the project (July-Sept 2001), FITCA Uganda carried out a survey to assess the prevalence and distribution of animal trypanosomes in domestic

cattle herds in southeast Uganda (FITCA Uganda Quarterly report, July 1st – September 30th 2001). This then allowed FITCA to prioritise control activities based on the trypanosomiasis risk assessment (FITCA working paper). The survey was carried out by taking blood samples from a subset of cattle selected randomly in each of the FITCA districts. The survey identified numbers and species of trypanosomes by microscopy techniques. The results from this survey are shown in Table 1.3.

District	**No. of samples**	***T. brucei***	***T. vivax***	***T. congo***	**Mixed infections**	**Total prevalence**
Bugiri	761	0.4%	1.4%	7.1%	1.4%	**10.4%**
Busia	627	0.8%	2.7%	4.9%	0.8%	**9.3%**
Iganga	1795	0.1%	2.0%	7.4%	0.9%	**10.4%**
Jinja	687	2.0%	0.0%	5.2%	0.3%	**7.6%**
Kamuli	1756	1.1%	0.1%	0.8%	0.8%	**2.8%**
Kayunga	1016	1.4%	0.3%	0.2%	0.0%	**1.9%**
Mayuge	897	2.6%	1.0%	5.6%	0.2%	**9.4%**
Mbale	1000	0.0%	1.5%	5.0%	0.6%	**7.1%**
Mukono	1173	0.9%	0.4%	2.5%	0.0%	**3.8%**
Pallisa	1156	0.4%	0.0%	0.3%	1.0%	**1.6%**
Tororo	1400	1.6%	2.6%	3.4%	0.7%	**8.2%**
Total	**12,268**	**1.0%**	**1.1%**	**3.7%**	**0.6%**	**6.3%**

Table 1.3 - Results of FITCA trypanosomiasis survey in cattle by microscopy

Following this initial prevalence survey, data were compared to reported cases of human sleeping sickness from 1996 (FITCA Uganda Quarterly report, October 1st to December 31st 2001). Subsequently, each sub-county within each district in the surveyed area was classified as high, medium or low risk. High risk areas were defined as sub-counties where cases of human sleeping sickness had been found.

Medium risk was an area with a high level of trypanosomes (over 5%) found in cattle but no human sleeping sickness cases. Low-risk referred to areas which had low levels of animal trypanosomes (less than 5%) and no human cases. Areas with no evidence of trypanosomes or where no data had been collected were given a no risk status. A total of 12,268 heads of cattle were surveyed by microscopy over the FITCA area, with the exception of Soroti which was not included in the survey. This data was used to generate a map (Figure 1.7) showing the high risk sub-counties where the trypanosomiasis control interventions were targeted.

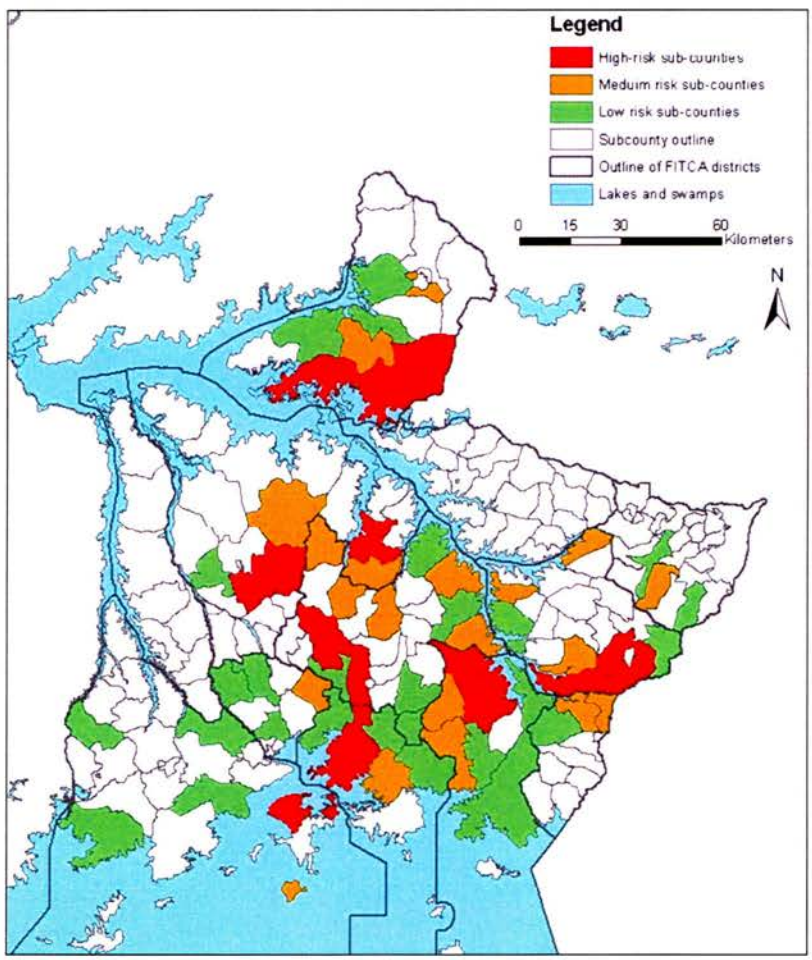


Figure 1.7 – Map showing high, medium and low risk sub-counties within the FITCA area

Within these defined high-risk areas (shown in red on Figure 1.7) FITCA attempted to treat all heads of cattle with a single dose of isometamidium chloride or diminazene aceturate for clinical cases. Isometamidium chloride was preferentially used as it has prophylactic properties whereby it can remain active in the bloodstream of the animal for 3 months (Holmes *et al.*, 2004). In addition to mass treatment, FITCA Uganda planned to distribute insecticide to designated farmers groups which would be used under a cost recovery scheme run by the farmers themselves. The groups were given one litre of insecticide which they were responsible for distributing at cost to farmers, allowing the aim of promoting affordable, sustainable insecticide use within the community.

1.10 Thesis aims

The overall aim of this thesis is to investigate the impact of the large scale trypanosomiasis control programme implemented in Uganda by FITCA in 2002. One of the main methods of trypanosomiasis control implemented by FITCA was a single dose of trypanocide administered to all cattle within designated high-risk areas. The research presented in this thesis is based on a description of the prevalence of trypanosomiasis both pre- and post-intervention within the targeted cattle population. Cattle were sampled from four districts within the FITCA Uganda intervention area and PCR was used as a sensitive diagnostic technique to determine the presence of *T. vivax, T. congolense savannah, T. brucei* s.l. and *T. b. rhodesiense* within samples. The effect of the FITCA intervention programme on reported cases of human sleeping sickness in the intervention districts is also established by monitoring hospital records and examining the incidence and geographical distribution of cases pre- and post- intervention. The outline of this thesis is as follows:

Chapter 2 comprises a historical review of the various approaches that have been used to control sleeping sickness in Uganda since the first recognised major epidemic at the turn of the 19th century. A record of the major discoveries and their impact on the techniques applied to control sleeping sickness and animal trypanosomiasis within Uganda will be discussed. The majority of the data in this Chapter are based on records gathered from veterinary, medical and trypanosomiasis control reports located in the National Archives in Entebbe, Uganda and supplemented by published articles relating to the history of trypanosomiasis in Uganda. This Chapter aims to put into context the ongoing battle against trypanosomiasis in Uganda and examine whether any lessons can be learned from previous attempts to control this disease.

Chapters 3, 4 and 5 switch focus to a contemporary control programme and examine the recent intervention against sleeping sickness and animal trypanosomiasis implemented by FITCA Uganda. Chapter 3 focuses on the impact of the FITCA intervention on animal trypanosomiasis within the targeted cattle population. The prevalence of *T. vivax, T. congolense savannah* and *T. brucei* s.l. in cattle within the FITCA intervention areas are established by molecular diagnostic techniques at three time points: pre-intervention baseline, three months post-intervention, and one year post-intervention. These data constitute one of the largest trypanosome surveillance programmes based on molecular diagnosis to date, with over 3,500 cattle sampled. In addition to establishing the impact of the FITCA programme on cattle trypanosome prevalence, the cost of undertaking such a programme will be estimated and discussed.

In Uganda, cattle are a major reservoir of human infective *T. b. rhodesiense*, making it imperative that the prevalence of this deadly pathogen is accurately assessed. Chapter 4 applies additional molecular analyses to those samples identified as *T. brucei* s.l. in Chapter 3 to establish what portion of *T. brucei* s.l. infections are the human-infective sub-species *T. b. rhodesiense*. The prevalence of *T. b. rhodesiense*

over the course of the intervention is determined and the impact of the FITCA intervention programme will be discussed to establish if zoonotic control as implemented by FITCA can be effective in reducing the risk of human infection.

Chapter 5 continues analysing the impact of the FITCA intervention on human health by monitoring the incidence of reported sleeping sickness cases in two of the districts included in the intervention. The numbers and geographical distribution of newly reported sleeping sickness cases are described over a five and a half year period, between January 2000 and July 2005. Spatial analysis is used to investigate disease clustering on a year-by-year basis. Finally, the number and location of cases reported pre-intervention are compared to those reported post-intervention to determine if any major differences can be observed. The aim of this Chapter is to assess if the FITCA Uganda intervention, aimed at cattle, had any effect on human health by reducing the reported numbers of sleeping sickness cases within intervention areas.

Chapter 6 comprises a final discussion where the overall impact of the FITCA Uganda intervention programme on both animal and human health will be discussed. The FITCA intervention will be discussed with reference to past intervention strategies to establish how the programme may be improved and whether cattle treatment represents the optimal strategy for trypanosomiasis control in Uganda in terms of efficacy. Trypanosomiasis remains a considerable threat to both human and animal health in much of sub-Saharan Africa making it imperative that affordable, sustainable control measures are implemented before the situation worsens.

2 Chapter 2:

Historical review of the control of trypanosomiasis in Uganda

2.1 Introduction

Trypanosomes have had a huge impact on socio-economic development in sub-Saharan Africa (Shaw, 2004) and even before these parasites were discovered, the tsetse fly was associated with disease (Bruce, 1895). The magnitude of the disease burden imposed by trypanosomes first became apparent when various colonial powers tried to venture into the interior of Africa during the second half of the 19th century (Pepin & Meda, 2001). The missionaries and scientists who led these expeditions into the 'dark heart of Africa' encountered many problems; the 'travellers disease' (malaria) killed explorers and the 'tsetse fly disease' killed the pack animals and horses (Lyons, 1992). As a consequence, the tsetse fly and the yet unknown trypanosome delayed the exploration and subsequent colonisation of Africa until the 19th Century (Knight, 1971; Maudlin, 2006). Recognition of the challenges represented by these diseases and the drive to explore and exploit the resources of the African continent prompted the development of many tropical research institutes throughout the main colonial powers in Europe. *"These natural enemies of colonisation had to be defeated and so began an era of intensive research into the African diseases"* (Lyons, 1992).

In her 1992 book "The colonial disease", Lyons argues that colonial attempts to control sleeping sickness lead to the development of vertical health care systems focused on the large scale control of disease; the effects of which influence health care provision in present day sub-Saharan Africa. At independence when the funds were no longer available to support the large institutions that had evolved in the colonial era, health care services broke down and local clinics which could have maintained a more basic level of health care were absent.

In short, the massive investments in sleeping sickness control taken by the colonial authorities could not be maintained by newly independent African states and subsequently sleeping sickness control programmes broke down in the 1960's and

1970's (Barrett, 1999). At this time, the new governments had a myriad of other problems from economic and political, to the ideological issues of nation building in an independent Africa; health care and particularly the control of sleeping sickness were sidelined (Nugent, 2004). Disease control and healthcare in general are moulded by the will of politics, society, and economics, as much as advances in scientific achievements (Maudlin, 2006). Therefore approaches to the control of human African trypanosomiasis and animal trypanosomiasis have been throughout history guided by government systems and economic imperatives as well as scientific development.

2.1.1 Aims of the chapter

In the forthcoming Chapters of this thesis, the impacts of the recent FITCA control programme for both human African trypanosomiasis (sleeping sickness) and cattle trypanosomiasis (nagana) in Uganda are evaluated. Since the discovery of trypanosomes at the turn of the 20th century, various control strategies have been employed to control trypanosomiasis. The aim of this chapter is to provide a historical context for the FITCA programme in Uganda by discussing some of the main control methods implemented in the past 100 years in areas of the country we now know to have been affected with *T. b. rhodesiense* (Koerner *et al.*, 1995).

A large proportion of the information presented in this chapter was compiled using source materials collected from the National Uganda Archives in Entebbe, Uganda, visited by the author for the period of one month in April 2004. The National Archives are the depository of all official documents published by, first the colonial and then the independent government of Uganda. Due to a lack of resources cataloguing of these archives is incomplete and often not well ordered which meant that is was difficult to access all relevant material on the subject matter.

2.2 Discovery of trypanosomiasis

The first accounts of the disease now known as sleeping sickness were made in 1721 by John Atkins, a naval surgeon, in West Africa (Maudlin, 2006). However, the fact that slave traders routinely rejected slaves with swelling of the posterior cervical lymph nodes a clinical symptom associated with HAT in West Africa, (known as Winterbottom's sign following his description in 1792) suggests that the symptoms of this disease had been known about prior to this time (Maudlin, 2006). There is also evidence that the Portuguese were aware from as early as the 15th century of a disease that dramatically reduced the life expectancy of their horses in Africa (Reader, 1997). However, the nature of these diseases was unknown and initial investigations into sleeping sickness did not necessarily consider an infectious agent; diet, exposure to strong sun injuring the brain and lethargy due to racial differences were all cited as potential factors (Davies, 1968). Even as late as the end of the 19th century, the eminent scientist Patrick Manson incorrectly identified a filarial worm (*Filaria perstans* now known as *Mansonella perstans)* as a potential causative agent of sleeping sickness (Bruce & Nabarro, 1903) thereby causing some confusion in the early days of the investigations into sleeping sickness in Africa (Haynes, 2000).

Sir David Bruce identified the first trypanosome in 1894, remarking on the 'curiously shaped objects' found on examination of cattle blood films while investigating an outbreak of nagana in northern Zululand. Injection of blood infected with these 'objects', later termed trypanosomes, into healthy dogs and horses resulted in the development of pathology, confirming Koch's postulates and identifying these parasites as the aetiological agent of the disease (Bruce, 1895). Soon after this in 1901, Everett Dutton identified the first human-infective trypanosome in a European patient in West Africa (Dutton, 1902), and termed it *Trypanosoma gambiense* (now identified as *T. brucei gambiense*, the cause of West African sleeping sickness). Also in 1901, Aldo Castellani identified trypanosomes in the blood and cerebrospinal fluid of a patient with sleeping sickness in Uganda (Castellani, 1903) and termed this parasite *Trypanosoma ugandense.* However, it was subsequently assumed that the parasite present in Uganda was the same species identified by Dutton in West Africa

(Bruce & Nabarro, 1903), and so this name was not adopted. In 1910, Stephens and Fantham described a novel trypanosome species in a sample taken in Northern Rhodesia (Stephens & Fantham, 1910), which they named *Trypanosoma rhodesiense* (now termed *T. brucei rhodesiense*, the causative agent of East African sleeping sickness). Retrospective analysis by Fèvre and colleagues (Fèvre *et al.*, 2004) indicates that the parasite identified by Castellani was not *T. brucei gambiense* but rather *T. brucei rhodesiense* and consequently this species should perhaps be termed *T. ugandense* in honour of Castellani who first identified the new species (Koerner *et al.*, 1995). Discussions of the nomenclature are, however, beyond the scope of this thesis.

2.3 History of sleeping sickness in Uganda

The first reports of a widespread epidemic of an unknown disease in Uganda were received by the British Government in early 1901 (Christy, 1903) with cases admitted to the main missionary run hospital in Mengo (Cook, 1901). However, the number of cases reported rapidly became overwhelming and following appeals to the governing authorities, the Chief Medical Officer of the protectorate was dispatched to investigate the disease. He reported that 20,000 people had already succumbed to the sleeping sickness disease, corroborating the reports by patients from Busoga district that the disease they called 'Mgota' was prevalent (Christy, 1903). The rapidity with which the epidemic had erupted alarmed the authorities and at the instigation of the Foreign Office the Royal Society appointed a Commission to investigate the cause of the disease.

By 1903 the Commission had concluded that (i) sleeping sickness was caused by a species of trypanosome and (ii) trypanosomes were transmitted by a species of tsetse fly (Bruce & Nabarro, 1903). As described above, there was, and remains, some controversy over the species of trypanosome responsible for the 1901 Ugandan sleeping sickness epidemic, with Castellani reporting the presence of a novel trypanosome species in Uganda in 1901 (Castellani et al 1903), whilst Bruce, his

colleague in the Commission, concluded that the causal pathogen was the same as that previously found in West Africa by Dutton (1902). Morphologically *T. b. gambiense* and *T. b. rhodesiense* are identical and it was not until the discovery of SRA and the development of molecular techniques to detect its expression that it was possible to definitively distinguish between the two species (Xong *et al.*, 1998).

The first epidemic continued until 1920 (Langlands, 1967), although this date varies according to different sources. However, the numbers of reported sleeping sickness cases had begun to decline from 1905, when the first annual mortality figures were published and continued to do so except for a resurgence of the disease in 1909 and 1910 (Figure 2.1). The total number of deaths resulting from the epidemic is difficult to determine as in the earliest years of the epidemic many deaths would have been unrecorded and data collection was often very rudimentary. One method described is the presentation by individuals to their chief with a number of twigs to show how many members of their family had perished (Langlands, 1967). Evidence shows that even today there is a substantial underreporting of sleeping sickness cases in Uganda (Fèvre *et al.*, 2005; Odiit *et al.*, 2005), and it must be anticipated that 100 years ago the situation would have been no better (Maudlin, 2006). It has been estimated that the epidemic would have realistically claimed approximately 200,000 lives in the years between 1900 and 1920 (Langlands, 1967).

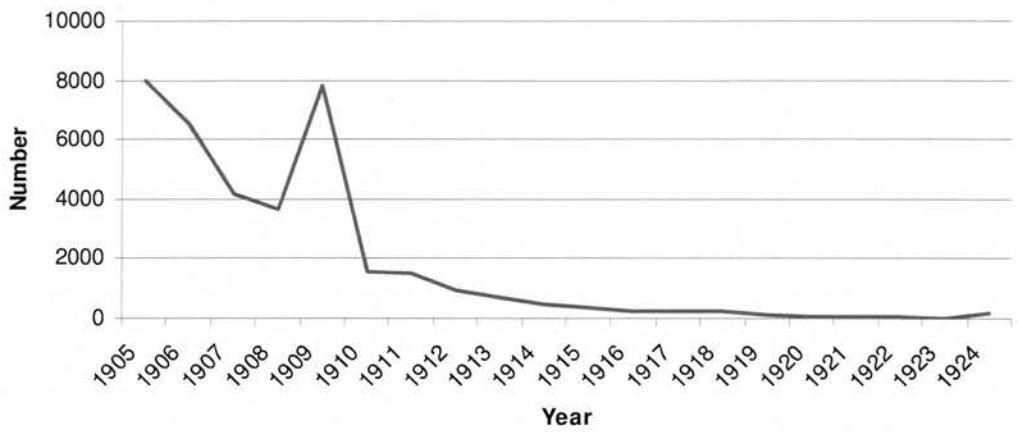


Figure 2.1 – Total numbers of sleeping sickness cases reported from 1905 to 1924, figures taken from Annual Medical records of the Ugandan Protectorate

Further sleeping sickness epidemics occurred in Uganda in 1940-1945 (MacKichan, 1944), 1970- 1989 (Okiria, 1985; Welburn *et al.*, 2006) (Figure 2.2) and 1998-present day (Fèvre *et al.*, 2001). All three of these epidemics have been associated with East African sleeping sickness caused by *T. b. rhodesiense* (Koerner *et al.*, 1995; Fèvre *et al.*, 2004; Maudlin, 2006). During the inter-epidemic periods there have been periods of stability with a focus of disease caused by *T. b. gambiense* in the north-west of the country and another focus of disease caused by *T. b. rhodesiense* in the south and east. A concerning feature of the ongoing epidemic is the encroaching proximity of the two foci and the implications the overlap of the two forms of the disease will have on management of clinical cases (Picozzi *et al.*, 2005).

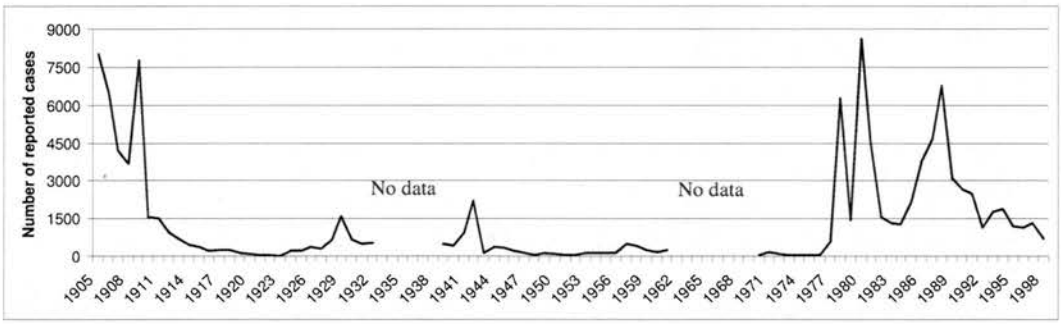


Figure 2.2 – Numbers of recorded cases of sleeping sickness in Uganda from 1905 to 1998, figures taken from national Ugandan archives and WHO country reports

2.4 Response to the first epidemic

The first Royal Society Commission report observed that people involved in agricultural labour were particularly susceptible to contracting the disease (Christy, 1903). Cultivation of cash-crops was seen as integral to the economy of the Ugandan Protectorate and therefore sleeping sickness was perceived to be a significant impediment to economic development because colonial authorities were intending to use the productive lands of Uganda as a source for raw materials (e.g. cotton) (Maudlin, 2006). This may account for the apparently disproportionate devotion of

resources to control of sleeping sickness in comparison to other diseases present in Uganda at the time (Figure 2.3).

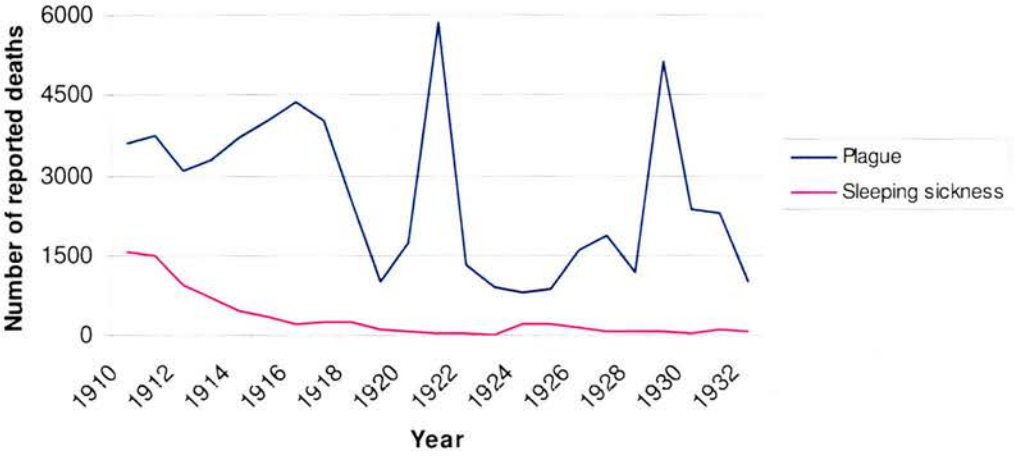


Figure 2.3 – Comparison of the numbers of recorded deaths from sleeping sickness and plague between 1910 and 1932. N.B. this time period is after the highest numbers of cases reported in the first epidemic, but illustrates that after 1910 during the continuing severe control measures against sleeping sickness there were very low numbers of recorded sleeping sickness deaths compared to other diseases. Numbers of reported deaths were taken from Annual Medical Reports of the Protectorate of Uganda.

The early observations made by the Royal Society Commission were fundamental to the development of strategies against the novel pathogen. By 1906 a comprehensive list of sleeping sickness control measures were introduced and strictly enforced in Uganda. Included in these were a suite of measures that attempted to reduce the number of the tsetse fly vector such as (i) the direct killing or catching flies, (ii) the indirect killing of tsetse by destroying their habitats and breeding grounds i.e. by clearing jungle and undergrowth, especially in the various fly-areas connected with human concourse or traffic and (iii) by diminishing or destroying their food supplies in the form of culling game animals (Hodges & Will, 1906).

Other measures introduced attempted to prevent or minimise the opportunity for human/ tsetse contact, for example (i) the removal of huts, villages, markets etc from within the local fly range, (ii) protection of the person by clothing and *"education of the natives to resist and avoid bites of the fly"* (Hodges & Will, 1906), (iii) regulation or selection of hours for certain occupations within the fly range and (iv) protection of employees exposed in their occupation to attack of the fly (Hodges & Will, 1906).

Attempts to restrict the geographical spread of potentially infected people were also reflected in these regulations by (i) surveillance of travellers, traders and emigrants from epidemic area, and especially of their settlement or residence in or in contact with non-infected fly ranges or potential epidemic areas and (ii) the regulation and surveillance of camping places, transport stations etc. on traffic routes by land and water, including effective clearing or diversion where these come in contact with fly ranges (Hodges & Will, 1906). The attitude of the authorities to the contact between local people and tsetse was summed up some years later as *"only persons free from trypanosomes may enter land not free from Glossina"* (Director of Medical and Sanitary services, 1928). However, in the early days of the epidemic the main method of control employed was the segregation of the sick and dying.

2.4.1 Isolation of the sick and dying

Initially local quarantine of sick individuals within their communities was suggested as a form of isolation control. However, with the early clinical stages of disease difficult to identify, and the epidemiology of the disease not definitively characterised this was difficult to effectively maintain. It was therefore decided that sick individuals from every community in direct contact with the tsetse flies would be moved to sleeping sickness camps located in areas free from flies (Hodges, 1905). Removal and segregation of infected persons was, unsurprisingly, met with resistance from the local population (Hodges & Will, 1906).

Four isolation camps located at Busiro, Chegwe, Busu (on the mainland) and Sesse Island had been established by the colonial authorities by December of 1907 (Sleeping sickness news, 1908). Sick individuals were to enter the camps of their *"own free will"* and settlement of the sick *"anywhere outside of the fly-infested districts"* was an acceptable alternative (Sleeping sickness news, 1908). By the end of November 1909 these camps had received 6,619 patients with only 800 patients still surviving (Hodges, 1910a), remembering that at this time there was no curative treatment. Publications concerning the institution of sleeping sickness camps in other countries such as the Belgian Congo (Lyons 1992) and Sudan (Hunt & Bloss, 1945) show that this strategy was not unique to Uganda.

Treatment was administered to the patients within these camps; however there were no known curative agents. Various treatment regimes based on use of organic arsenic were trialled but even the most effective of these secured an apparent success rate of only 2-3% (Hodges, 1910a). It appears that, from a patient's point of view, the most beneficial observations made at this time were that care, cleanliness, good feeding and proper attention may have prolonged life for a considerable period. Consequently, patients interned at the camps inevitably died and, in the absence of any evident protection or cure, attendance of the sleeping sickness camps held little appeal for the local people. In a letter written in 1910 to the Chief medical secretary, Dr Pugh, a medical officer at one of the camps, makes it clear that he believes that the use of isolation camps could no longer be effective. *"Owing to the fact that the majority of the cases which were treated at the former camp have since died, the native have no faith in the treatment, and say that since they cannot be cured they would rather die in their own villages...so great is their objection to leaving their own villages, that I am firmly convinced unless forcible methods are used to make them come here, very little work can be done in this camp."* (Pugh, 1910)

2.4.2 Removal of the population

During his time in Uganda with the Royal Society Sleeping Sickness Commission, Christy observed that the outbreak was concentrated along the northern shores of

Lake Victoria. At this early stage in the epidemic there was no spread inland, in Christy's words *"This area consists of a narrow strip of coast line, at its widest not more than 10 miles, stretching along the shores of Victoria Nyanza for a distance of over 250 miles"* (Christy, 1903). During a later expedition Bruce compiled a map detailing the correlation between prevalence of the disease and proximity to lake shore (Figure 2.4)

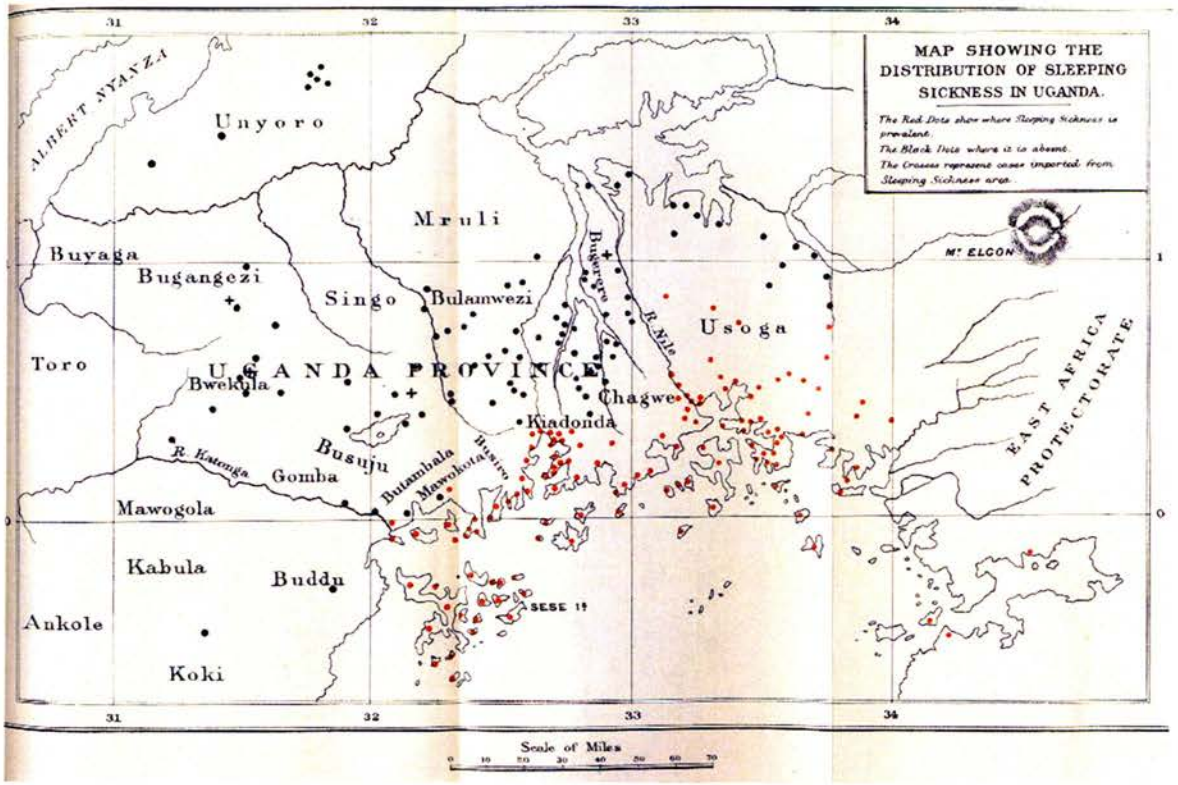


Figure 2.4 – Map showing the location of reported sleeping sickness cases in 1903. The red dots show confirmed sleeping sickness cases and the black dots show areas where surveys were carried out but there were no cases (Bruce & Nabarro, 1903)

In 1906, it was decided to evacuate the entire population from the shore of Lake Victoria and by December 1907 *"all in habitants, infect or not, had been cleared out of a two-mile belt all around the north-west shore of Lake Victoria and allowed to settle inland"* (Sleeping Sickness News, 1908). This evacuation policy was later extended to the islands in Lake Victoria (declaration by Hesketh Bell in 1909, reported by (Hodges, 1910b)) and with the spread of the epidemic, to the populations

on the shores of other lakes and the river Nile (Hodges, 1910b). The scale of population movements enforced by this policy were considerable – in 1909 alone over 24,000 people were re-located (Hodges, 1910b).

In conjunction with the depopulation of the specified areas several regulations were introduced to minimise human entrance into the depopulated areas. This included the prohibition of the collection of rubber within two-miles of Lake Victoria, restriction of fishing activities and canoe traffic on Lake Victoria (and subsequently other Lakes) and the banning of vessels from touching *"the mainland except at an authorised landing-place"* (Hodges, 1910b). A government officer was appointed to maintain the depopulated area and fines and penalties were issued for breach of these regulations (Sleeping sickness News, 1908).

2.4.3 Targeting the tsetse

Christy made numerous observations relating the incidence of sleeping sickness with proximity to wooded or forested areas (Christy, 1903). Notably disease was most common amongst people who worked in the fields whilst people living in villages were less affected by the disease and people resident in *"populous centres were practically exempt from the disease"*. Subsequent studies reported by Bruce and Nabarro (1903) identified that tsetse fly vector was *"only found on the shore of the lake where there is forest"* (Bruce & Nabarro, 1903). Consequently attempts to eliminate the tsetse by destruction of its habitat from the shores of Lake Victoria were incorporated into the sleeping sickness control efforts. Figure 2.5 illustrates the guidelines which were used to clear the lake shores; in 1911 it was emphasised that *"undergrowth, namely shrubs, bushes, vines, creepers and tall grass must all be cleared"* (Anon, 1911). The graduation of severity of vegetation clearing from the Lake shore outwards reflects the observation that the proximity to the shore was correlated with the highest prevalence of the disease.

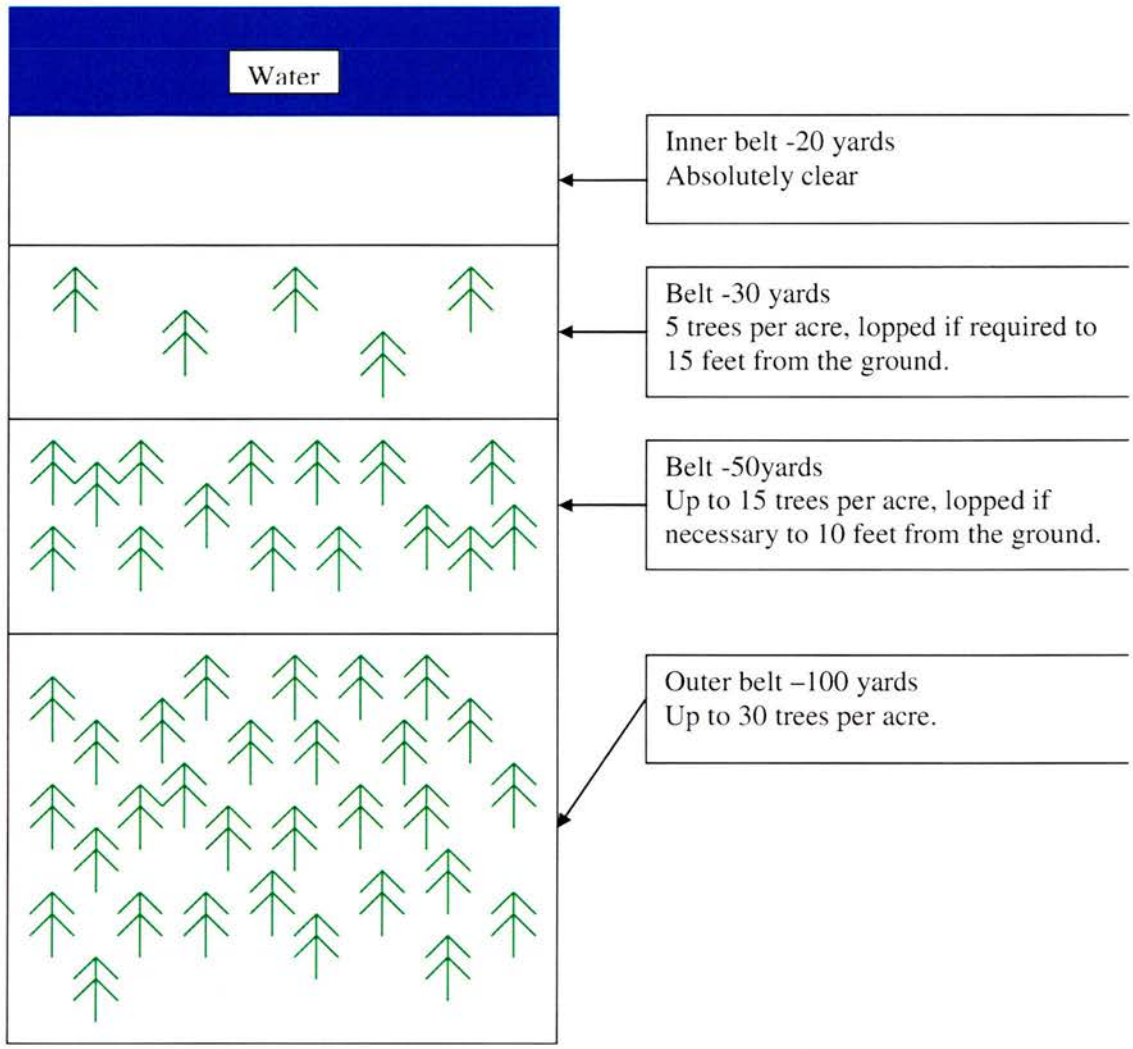


Figure 2.5– Schematic diagram showing the guidelines published in 1911 for clearing the vegetation around Lake Victoria, (Anon, 1911).

Another recommendation made in the 1906 Sleeping Sickness Commission Report was the destruction of the main food supply of tsetse flies in the Ugandan protectorate (Hodges & Will, 1906). At this time the main food source for tsetse flies across the country were game animals, e.g. buffalo, species of antelope, warthog, bushpig etc (Hodges & Will, 1906). Despite the early suspicions that sleeping sickness in east Africa was a zoonotic disease (Bruce *et al.*, 1910), the elimination of game at this time was not an attempt to remove a reservoir host of sleeping sickness but to starve the tsetse vector.

2.5 Continued applications of control strategies from the first epidemic

In the 1913 Annual Medical and Sanitary Report, sleeping sickness was reported to have ceased to be a major cause of mortality. However, it was stated that the decline in the disease was in response to the strict control measures that had been put in place and the Chief Medical Officer observed that, *"these measures are in many ways a serious check to the country, but there appears to be as yet no chance of relaxing them to any extent"* (Annual Medical Report, 1913). In the years and decades following the epidemic many of the control measures implemented were maintained and amended as deemed appropriate by the authorities.

Continued monitoring of the incidence of sleeping sickness cases and the control measures relating to human activity in the post-epidemic period were co-ordinated by the Medical Officer in Charge of Sleeping Sickness. During regular "safaris" to the various infected areas the Medical Officer in Charge of Sleeping Sickness reviewed the current situation, discussed activities with the local government officers, made recommendations regarding control measures and frequently conducted large scale medical examinations. Buxton in 1947 comments on the use of *"African dispensers trained in the use of microscopes"* for active medical surveillance in the Busoga area following the 1940-1945 epidemic (Buxton, 1947): similar surveillance was still being carried out by Sleeping Sickness Orderlies in the early post-colonial era (Abaru, 1985).

During the sleeping sickness epidemic in 1940-1943, the authorities again evacuated people from the shore of Lake Victoria and resurrected the sleeping sickness camp as a means of segregating and accommodating infected people. (MacKichan, 1944).

After the resolution of the first Ugandan epidemic it was considered that in the British possessions in East and Southern Africa *"from the economic point of view, trypanomiases of domestic stock..... immeasurably more important than is trypanomiases of man"* (Yorke, 1920). Consequently much effort was directed towards the control of the tsetse fly as a means of controlling both nagana and sleeping sickness (Maudlin, 2006).

In 1947 a separate Department for Tsetse Control was established to co-ordinate activities and achieve the dual goals of halting the advance of the tsetse fly into the protectorate that had been occurring in the 1930's and early 1940's and of reclaiming newly invaded country from the fly. Previous Reports from this department reveal the militaristic mindset that was adopted with frequent use of terms such as 'salients', 're-inforcements', 'out-flanking' and 'operational areas'. The two main methods of control employed were game slaughter and destruction of the tsetse habitat, which were generally used in conjunction.

Although it was appreciated that game formed the main reservoir host of cattle infective trypanosomes (Buxton, 1947), game slaughter was principally applied to deprive the tsetse fly of its principal food source in areas to be 'cleared' of tsetse and also as a means of preventing tsetse re-invasion by transportation during game movements. Although game clearance as a form of tsetse control was generally regarded as *"distasteful and regrettable"* and even *"morally objectionable"* (Buxton, 1947) the reclamation of 7,000 sq. miles of Ugandan territory from the tsetse fly by 1959 was achieved *"chiefly by use of the game elimination method"* (Annual Tsetse Control Report, 1959). Game destruction was accomplished both by deployment of departmental hunters and by permitting local populations to hunt. The number of game animals killed was recorded in detail in the department's annual reports – by 1955 (i.e. after eight years of operations by the department) 46,167 game animals had been killed (Annual Tsetse Control Report, 1955). Due to the success attributed to game elimination as a means of controlling tsetse it was not abandoned until 1971 (Okoth, 1999).

Habitat clearance was achieved both by department work gangs and by encouraging local populations to do 'late grass burning'. However, the latter often proved unsuccessful and there are complaints in the 1954 Annual Veterinary Report about local people carrying out grass burnings too early in the season, *"despite numerous attempts through local leaders to prevent burning until the grass is very dry"* (Annual Veterinary Report, 1954). Another frequently encountered problem noted in the Tsetse Control Department Reports was the failure to persuade people to settle in cleared areas, making continuing control of bush re-growth in reclaimed areas a serious drain of available resources. Such problems highlight the difficulties encountered during the application of top-down control measures employed by the colonial authorities, and of applying control with little regard for social structures and habits.

In Western Uganda, the East African Trypanomiasis Research Organisation (EATRO) had attempted to use intensified 'discriminative' clearing (i.e. clearing only vegetation directly associated with tsetse) in 1958 (Annual Research Report, 1958). Although a successful discriminative clearing project on the shores of Lake Victoria had been described previously (Buxton, 1947), this project was failing to cope with the expanding problem of tsetse flies in the area and it was decided that the best way to improve the situation was a return to game elimination in the area (Annual Veterinary Report, 1959). However, it was noted in this same year that a more acceptable alternative control alternative should be identified (Annual Veterinary Report, 1959).

Habitat management, although significantly supplanted by the development of insecticides (see section below) continued to be used as a control method into the post-colonial era (Abaru, 1985).

2.6 Development of chemical treatments

2.6.1 Treatment of humans

Upon their development in the 1920's, effective treatments for sleeping sickness were rapidly incorporated in the medical control programmes. After initial trials of *Tryparsamide* (an arsenical based compound) in 1924, its use in treatment of sleeping sickness cases was widely cited the following year and *Tryparsamide* treatment centres established in Uganda (Annual Medical Report, 1925). The availability of an effective treatment offered an opportunity for control to be less reliant on physical separation of humans from trypanosome infected tsetse and it was even suggested that using *Tryparsamide* as a prophylactic could enable people to temporarily re-enter infective areas (Annual Medical Report, 1925). However, it was acknowledged that such a scheme was not possible due to excessive supervision requirements (Annual Medical Report, 1925).

During the 1940-1945 epidemic in Busoga people evacuated from the shore of Lake Victoria and those thought to be at risk from the disease received prophylactic injections of antrypol now known as suramin (MacKichan 1994). Buxton (1947) describes how following this epidemic *"African dispensers capable of treating cases"* were employed in the Busoga region as part of the control effort. Continuing use of human treatments is central to control of sleeping sickness in Uganda and is currently funded by the World Health Organization and drugs are donated by certain pharmaceutical companies (Barrett *et al.*, 2003).

2.6.2 Treatment of cattle

Bovine trypanosomiases were a significant problem impeding the economic development of the livestock in the Ugandan protectorate (Annual Veterinary Report, 1932). A survey conducted on 15,572 cattle in 1923 demonstrated trypanomiasis to be by far the most prevalent bovine disease (Figure 2.6). During the 1930's and 1940's trypanomiases continued to exact a heavy toll on the Ugandan cattle industry with statements such as *"In 1944 some 11,000 cattle in Bugere died of*

trypanomiasis" revealing the extent of the problem (Annual Veterinary report, 1945). However, in the absence of an effective treatment, control of the disease in these decades was reliant on tsetse control measures and quarantine of infected cattle herds.

Following field trials in the late 1940's the trypanocidal drug Antrycide dimethyl sulphate became a core component of a veterinary trypanosomiasis control policy in Uganda. The initial objective of this policy was to eradicate endemic bovine trypanosomiases in the tsetse-free areas by 'block' treatment of herds and in the first three and half years of this policy over ¾ of a million cattle had been treated (Annual Veterinary Report, 1953). Despite early optimism, this objective was never realised and control of bovine trypanomiasis became a persistent problem. In subsequent years treatment of large numbers of animals with Antrycide dimethyl sulphate and later diminazene aceturate, Quinaldines and Phinanthridines (Annual Reports of the department of Veterinary Services and Animal Industry Veterinary 1954 – 1971) was predominantly administered to limit the extents of outbreaks.

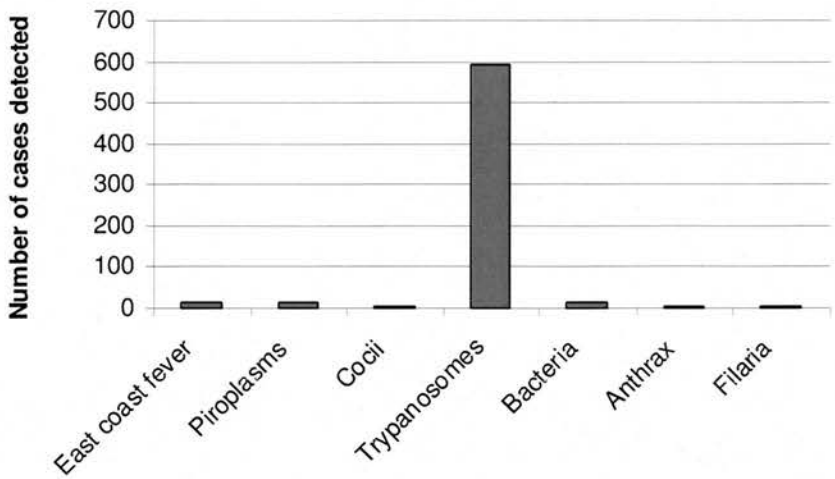


Figure 2.6 – Graph showing the relative importance of trypanosomiasis in cattle in 1923, figures from (Annual Veterinary Report, 1932)

2.7 Insecticides

In Uganda a specialist department for tsetse control was formed in 1947 – prior to this time tsetse control had been the responsibility of the veterinary department (Annual Veterinary Report, 1947). Before insecticides were used, the tsetse control department continued to rely on the clearing of vegetation and the destruction of game. In an attempt to preserve areas which had been cleared of vegetation and game de-flying pickets were established on their boundaries. These were manned stations where tsetse flies would be removed from people or vehicles. In 1953, an example of one such picket in the Northern Province reported that 21 vehicles, 6,677 cyclists and 4,569 pedestrians were examined in a 3 month period and only 20 *G. morsitans* were captured (Annual Tsetse Control Report, 1953). These pickets were still in use into the late 1960's in the south of Uganda near the shores of Lake Victoria (I. Maudlin, pers. comm., University of Edinburgh).

With the advent of insecticides the technology became available to change the approach to tsetse control in Africa. The first insecticide to become widely available was DDT (dichlorodipheyltrichloroethane) which was released for non-military use in 1945 (Allsopp & Hursey, 2004). Although DDT was first synthesised in 1874 its insecticidal properties were not discovered until the 1930's by Paul Muller (West & Campbell, 1950). DDT is cheaply produced and persists in the environment for a long time, which makes it a popular choice for controlling pests but an equally controversial choice for environmental reasons (Turusov *et al.*, 2002). DDT and other organochloridses became an essential part of tsetse and trypanosomiasis control for over 40 years (Allsopp & Hursey, 2004).

2.7.1 Ground Spraying

Ground spraying was adopted in some areas of Uganda in the 1950's (Annual Tsetse Control Report, 1953) in a variety of different ways, one example of this is detailed below.

In Uganda in 1955, a new programme was initiated which was the first attempt in the Protectorate to control tsetse by insecticidal treatments (Annual Tsetse Control Report, 1955). The method used had been development in Kenya in the Nyando river system in Nyanza Province, where a 4.5% DDT solution was sprayed onto riverside vegetation (Annual Tsetse Control Report, 1955). Experiments in Uganda were carried out to establish which of the following methods would be most effective:

(a) Hand spraying of river bank vegetation.

(b) The use of DDT treated hessian curtains (Chorley curtains) strung across an infested river at quarter mile intervals.

The insecticide used was Arkotine a proprietary Shell product which is a miscible oil containing 18% DDT. For both treatments this concentrate was diluted to 4.5% weight/ volume solution of DDT. The method applied for treatment of river bank vegetation was that the sprayer to walk along in the river and spray all the waterside fringing vegetation reached with a four foot spray lance. The predetermined rate of application (based on the work in Kenya) was 20lbs (9.1kg) of DDT per mile of river. Successive applications were made every 2 weeks for a total of 8 weeks.

The method applied for Chorley curtains was to sew the Hessian lengths onto the wire so as to form a T-shaped cross section across the river. It was noted that the river valley was more of a V shape, so the curtains were not drawn tight but allowed to sag to a height of 6 feet from the water. A roof was constructed by sewing sticks into the hessian. Each curtain was sprayed once a week for a period of 9 weeks, including both sides of the curtain and the underside of the roof. Rates of application were 2 gallons per 500sq feet (9.1 litres per 4.6km^2) of spraying. A comparison between the two methods is shown in Figure 2.7.

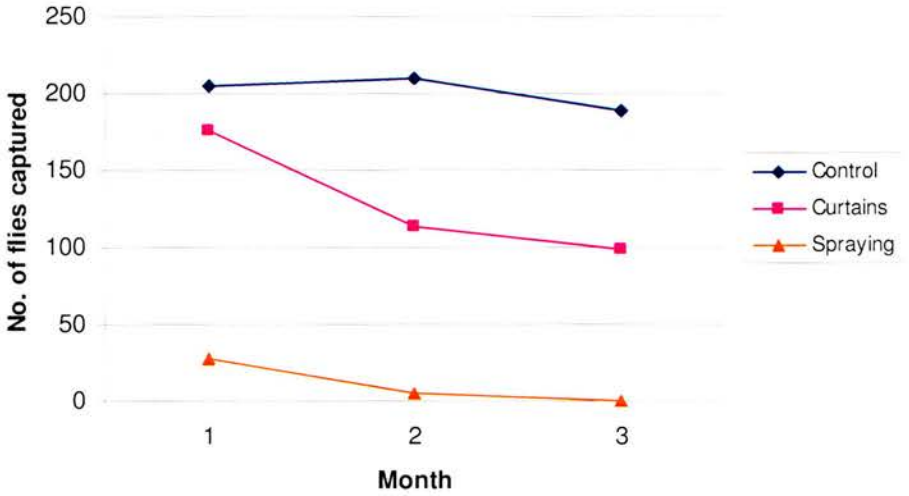


Figure 2.7 – Comparison of the of the different application methods of DDT to eliminate *G. palpalis* (now known as *G. fuscipes)* from riverside vegetation in Uganda. (Annual Tsetse Control Report, 1955)

This experiment was hampered by the fact that the river used in the spraying technique had much lower numbers of flies. However, the report states that "*the rapid and seemingly complete success of the spraying led to the embarkation of a full scale field trial of DDT in the Tororo area*". Costs listed in the 1955 Annual Tsetse Control Department report spraying of infested rivers in Tororo with DDT cost £69 per mile but these were due to the high observation levels required in an experimental applications. They estimated that the actual cost would be £45 per mile.

2.7.2 Aerial spraying

The first field trial of aerial spraying in Uganda took place in 1953, in cooperation with the colonial insecticide unit covering an area in Lango district in the Northern Province (Annual Tsetse Control Report, 1955). The aerial spraying unit operated throughout the year and despite being a field experiment the results were very

promising. However, there are no further reports of aerial spraying in Uganda until the 1980's.

In 1988, large areas of the Busoga region were sprayed with an aerial application of endosulfan, which was repeated in 1990 (Welburn *et al.*, 2006). This was carried out in response to the ongoing sleeping sickness epidemic of the early 1980's in Busoga which was linked to civil disturbances in the 1970's (Berrang-Ford *et al.*, 2006; Welburn *et al.*, 2006). The number of sleeping sickness cases between 1976 and 1992 is shown in Figure 2.8 along with the timings of the aerial applications of insecticide. The aerial spraying carried out in 1980 appears to have had a dramatic effect on the numbers of sleeping sickness cases. However, the number of cases began to increase in 1985 and consequently more aerial spraying was completed in 1988 and 1990.

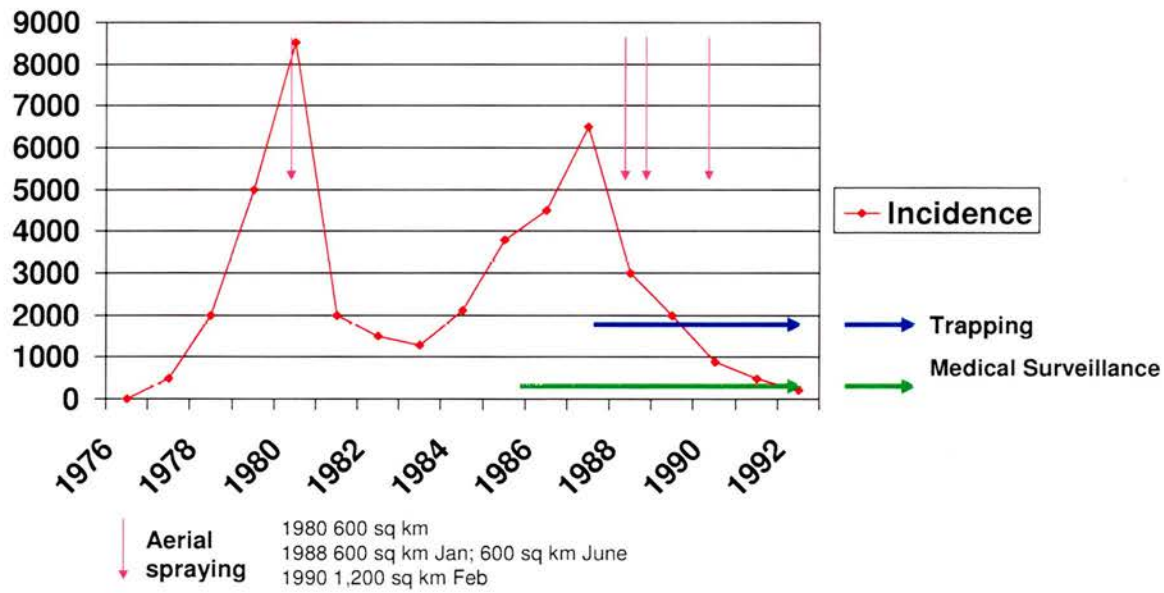


Figure 2.8 – Graph showing the number of reported sleeping sickness cases in south east Uganda during the years in which aerial spraying was undertaken as well as when tsetse trapping and medical surveillance was being undertaken. Image kindly provided by Ian Maudlin.

2.7.3 Traps and bait technologies

The use of bait to assist in the capture of tsetse flies for research purposes was used extensively in Uganda and indeed in a 1952 Annual Tsetse Control Report, there is mention of using a cow as bait for tsetse with 'fly-boys' positioned to catch any attracted tsetse (Annual Tsetse Control Report, 1952). Fly-boys were also employed in surveys to assess the number of tsetse in an area – they used either themselves or an animal as bait and would complete transects through an area collecting as many tsetse as they could. Interestingly in 1938 a vehicle was used as bait for tsetse flies (Annual Veterinary Report, 1938) as a useful tool for eliminating *G. morsitans* from cleared areas in the Ankole region, *"the main problem is eliminating the stragglers which remain after the main concentrations and densities have been broken up. Various methods have been tried but the most successful is using a motor car as a lure. The car moves at slow speed with attendant fly boys at the front and back, when a tsetse lands on the car it is captured by the fly boys. It cannot however be used to any great extent owing to the expense and the strain on the car and driver"* (Annual Veterinary Report, 1938).

By the 1960's the general view was that baits were only useful as survey devices and research tools (Vale & Torr, 2004). However, there was concern about the destructive nature and environmental impact of bush clearing, game destruction, wide scale use of persistent insecticides, and an awareness of the need for alternative methods. Many studies were undertaken examining the basic responses of tsetse flies to various stimuli including: distinguishing visual from olfactory attractants (Vale, 1974), attraction to wavelengths of light from different fabric (Green, 1988) and the effect of different odour attractants (Hall *et al.*, 1984; Vale *et al.*, 1986), although artificial attractants have only proved to be half as effective as natural odour suggesting more research remains to be completed (Hargrove *et al.*, 1995). Different traps have now been developed for different tsetse species (Kappmeier, 2000; Belete *et al.*, 2004) and have been used extensively for both survey and research purposes

(de la Rocque *et al.*, 2005) (Hendrickx *et al.*, 2001) and control (Vale *et al.*, 1988; Lancien, 1991).

Trapping using Lancien traps was carried out extensively in the Busoga region of Uganda between 1987 and 1993 (see Figure 2.8) and reported to have been a great success (Lancien, 1991; Smith *et al.*, 1998), however it is be difficult to determine if the reduction in tsetse was due to trapping or its combination with aerial spraying.

2.7.4 Insecticide treated cattle

In the late 1960's a variety of insecticides were tested on cattle in Zimbabwe, however, none remained effective on tsetse for more than a week after application (Vale & Torr, 2004). In the late 1980's deltamethrin was tested on cattle (Thompson, 1987) leading to large scale field trials (Thompson *et al.*, 1991) and subsequent use. Recent research has shown that only insecticide treating larger cattle within a herd can be an effective way to protect the whole herd from trypanosomiasis (Torr & Mangwiro, 2000). Additionally, feeding studies on cattle have shown that tsetse preferentially feed on the legs and belly so the animal can be effectively protected by treating only these parts with insecticide (Torr *et al.*, 2001). This has the advantage of reducing the cost of insecticide application to individual farmers. This technique has recently been shown to be effective in south east Uganda (Brownlow, 2007).

2.8 Final comments

Since the beginning of the 20th century numerous strategies have been employed in attempts to control disease caused by trypanosomes. The selection of these strategies has depended on a variety of criteria including economics, politics and prioritization as well scientific knowledge.

The scale of the first sleeping sickness epidemic ensured that control of the disease became a priority for the colonial administration. The attitude of the colonial government enabled the implementation of control measures that to the modern perspective appear extreme. However, by wide-scale forced evacuation of populations from tsetse-infested areas, enactment of regulations that placed severe restrictions on the movement and daily occupations of the inhabitants of Uganda and extensive efforts to eradicate tsetse by a combination of tsetse habitat destruction and elimination of game animals, the first epidemic was brought under control. This achievement using relatively rudimentary measures founded on limited scientific understanding of the disease illustrates how strict centralised governance can be effective in controlling disease in the absence of advanced technology. Subsequently, the importance of bovine trypanosomiasis to the economy of the protectorate guaranteed that the colonial authorities continued efforts to control trypanosomes, primarily through the medium of eradicating the tsetse vector. Such expansive efforts required considerable financial support justified by the perceived significance of the disease to successful colonisation.

In the immediate post-colonial era many of the control measures were sustained. However, with the advent of political instability in the early 1970's many, if not all of these measures collapsed. Following the return of relative political stability in the 1980's insufficient financial resources of the government meant that healthcare funding predominantly became the domain of external donor funding. Consequently, donor ideology was imposed on trypanosomiasis control, including environmental considerations and socio-political sensitivities, these limitations also applied to the

FITCA programme discussed in this thesis. Therefore, measures that had been successfully applied in the past, such as bush clearing, game elimination and indiscriminate use of residual insecticides were deemed to be unacceptable. Additionally the rise of the HIV/AIDS epidemic in sub-Saharan Africa meant that the majority of available funds were dedicated to control of this devastating disease.

Changing development ideologies have advocated the use of community based projects which require the active participation of community groups in the implementation of disease control activities (Dransfield & Brightwell, 2004). Trypanosomiasis is increasingly seen primarily as a livestock disease responsible for hindering economic development (Torr *et al.*, 2005). Consequently, there is a trend to promote schemes that use veterinary treatments (e.g. insecticidal spraying of cattle) that have a benefit to individual farmers and which can incorporate an element of cost-recovery from the beneficiaries, rather than large-scale control programmes. However, it is appreciated that centralised programmes may afford the best means of controlling disease during epidemics (Molyneux, 2001b).

Since trypanosomiases were first documented in Uganda over a century ago, numerous strategies have been employed to effect their control. The FITCA intervention programme represents the most recent of the control strategies to be used in Uganda. An evaluation of the impact of this programme is presented in the following Chapters within this thesis.

3 Chapter 3:

Assessment of FITCA Uganda intervention programme on the prevalence of animal trypanosomes

3.1 Introduction

Trypanosomiasis may be the most important constraint to livestock and mixed farming in tropical Africa (Kristjanson *et al.*, 1999). Approximately 50-70 million cattle are at risk from tsetse transmitted trypanosomiasis (Geerts & Holmes, 1998) in addition to millions of sheep, goats, donkeys, camels and horses (Reid *et al.*, 1970). In response to this threat it has been estimated that livestock owners in Africa administer 35 million trypanocide doses annually (Geerts & Holmes, 1997). For a rural African subsistence farmer, livestock, especially cattle, can have an enormous impact on the productivity of their land and therefore the standard of living for the entire family. Cattle can be used directly for milk or meat, ploughing the land for cultivation while their manure can be used to improve the quality of the soil. Consequently trypanosomiasis can impact not only on crop yields and the nutritional status of the individual farmer but also on the total agricultural output of the country. It has been estimated that animal trypanosomiasis costs US$1.3 billion annually to both producers and consumers (Kristjanson *et al.*, 1999).

In Uganda, the major pathogenic trypanosome species found in cattle are *Trypanosoma congolense*, *Trypanosoma vivax* and to a lesser extent *Trypanosoma brucei brucei* (Taylor & Authie, 2004). Whilst these species are important for economic reasons, cattle can also act as a reservoir host for the zoonotic human-infective parasite *Trypanosoma brucei rhodesiense*. *T. b. rhodesiense* leads predominantly to an asymptomatic infection in cattle but is fatal in humans. While sleeping sickness is not a national public health problem in Uganda when compared to malaria and HIV, it does nevertheless have a serious impact in certain regions. It is, therefore, essential to control the animal reservoir in order to improve human health. The impact of the FITCA (Farming In Tsetse Controlled Areas) Uganda programme on human disease will be discussed fully in Chapters four and five. This Chapter will focus firstly on the success of the FITCA Uganda programme in reducing the prevalence of pathogenic animal trypanosomes and secondly examine the cost effectiveness of this programme.

3.1.1 The importance of cost analysis

Economic evaluations of interventions against disease are a very useful tool to aid decisions by policy makers on the effective use of scarce resources. A 2001 review on economic appraisals stated that the published literature does not yet include many examples of applications of economic evaluation on communicable diseases or on the major health problems of developing countries (Hutubessy *et al.*, 2001). The available literature for these topics is focused on interventions relating to HIV, hepatitis and a variety of immunisation programmes (Brenzel & Claquin, 1994). A comprehensive review of the economics of African trypanosomiasis in 2004 states that despite increases in the amount of information available about this disease it remains difficult to ascertain the extent to which trypanosomiasis constrains the pattern of rural development in Africa (Shaw, 2004).

The most common technique used in economic evaluation in areas other than healthcare is cost benefit analysis (CBA), which measures the economic outcomes (benefits) of a system against the costs of implementing this system (Hutubessy *et al.*, 2001). However, human health outcomes are difficult to quantify in monetary terms and there are ethical concerns about the attempt to attribute economic value to a life (Johannesson & Jonsson, 1991). Therefore CBA has not been used extensively in the health sector, which has instead focused on cost effective analysis (CEA) (Hutubessy *et al.*, 2001); this is certainly true in the case of parasitic disease (Walker & Fox-Rushby, 2000). In CEA, health outcomes are measured in physical units, for example, cure rate of TB for different programmes (Saunderson, 1995) or number of years of life saved by HIV infected pregnant women using antiretrovirals (Wilkinson *et al.*, 1998). A review by Walker and Fox-Rushby in 2000 shows a list of a total of 33 different outcomes used in CEA for parasitic disease (Walker & Fox-Rushby, 2000). A sub-set of CEA, known as cost-utility analysis uses both morbidity and mortality as an output measure either as quality adjusted life year gained (QALY) or disability adjusted life year averted (DALY) (Hutubessy *et al.*, 2001).

A study by Kapiriri and colleagues on the use of burden of disease (BOD) as a tool in health planning in Uganda commented that health planners were not free to allocate resources. Most resources for health were found to be available from donors with a pre-set agenda or from central government in the form of grants for specific programmes (Kapiriri *et al.*, 2003). In fact Jeppson noted in 2001 that in Uganda, district officials commonly felt it was more difficult to satisfy the needs of the health sector than of any other sector at the district level. During the financial year of 1997/98 a total of 87% of funding for primary health care was contributed by donors with specific requirements for the resources (Jeppson, 2001). Additional problems can arise from centralised funding; human African trypanosomiasis can often be overlooked at national level but can be a priority at district level (Kapiriri *et al.*, 2003).

3.1.2 Aims and objectives

To examine the effectiveness of the FITCA Uganda programme, prevalence of different trypanosome species were measured in cattle (n = 3920) from four districts. These districts all contained FITCA high-risk areas defined as sub-counties which had high levels of animal trypanosomes by microscopy coupled with cases of human sleeping sickness. Samples were collected from cattle at three time points: baseline, three months post-intervention and one year post-intervention. The three month time point was chosen to coincide with the end of the prophylactic period of the predominantly administered trypanocide, isometamidium chloride, providing an indication of the short-term effectiveness of the programme. Meanwhile, the samples collected one year post-intervention provide an indication of the long-term effectiveness of mass trypanocidal intervention. Prevalence data from the four districts will be examined at different levels of resolution – individual sample site level, district level and entire study area – at both post-intervention time points in order to accurately assess the efficacy of the FITCA programme.

The second part of this chapter addresses the cost of the mass treatment campaign in the four study districts. This is not a full cost-effective analysis but rather an

investigation into the amount of money needed to implement this type of intervention in Uganda in the context of the measured benefits in terms of reduction in prevalence. The analysis will focus on expenditure at the district level in terms of cost per person, per livestock owner, and per percentage reduction of trypanosome prevalence in each area.

3.2 Materials and methods

3.2.1 Study area

The FITCA programme area comprised the following districts: Bugiri, Busia, Iganga, Jinja, Kamuli, Kayunga, Mayuge, Mukono, Pallisa, Soroti and Tororo (section 1.9). Six of these districts (Kamuli, Iganga, Bugiri, Mayuge, Tororo and Soroti) were identified by FITCA as containing at least one high-risk sub-country (section 1.9.1.1) which consequently received mass trypanocide treatment. For the purposes of this thesis all six districts could not logistically be included so the following four districts were selected. Kamuli and Iganga were chosen as they were both part of the original Busoga focus (Abaru, 1985). Similarly Tororo was chosen as it was the site of the 1988-1990 expansion of the original Busoga focus (Hide *et al.*, 1996). Finally, Soroti was chosen as it is currently undergoing a sleeping sickness epidemic (Fèvre *et al.*, 2001). There is a general move towards the decentralisation of Uganda administrative structures, therefore these districts are described as they existed in 2002 (Ugandan Bureau of Statistics, 2005).

3.2.1.1 Iganga district

Iganga district (see Figure 3.1)was originally part of Busoga but gained separate district status in 1975 as South Busoga district before being renamed as Iganga in 1981. Iganga borders another of the districts in this study, Kamuli to the north, and has an area of 4265 km^2. The latest human census figures for Uganda show that the district has a population of 708,690 with 94% of the population residing in rural areas (Ugandan Bureau of Statistics, 2002). The main economic activity in the district is agriculture comprising mainly subsistence farming with cotton, coffee, and rice grown as cash crops. Similarly, industry is primarily agriculture-based encompassing coffee processing, rice milling, and the manufacture of jaggery (unrefined sugar). Aside from agriculture, the other main industry is brick making (Ugandan Bureau of Statistics, 2005). The main administrative centre, Iganga town,

is a growing commercial centre situated on the main overland route from Kenya which has boosted business activity.

3.2.1.2 Kamuli district

Like Iganga, Kamuli was also part of the former Busoga district but gained separate district status in 1975 as North Busoga district before being renamed as Kamuli in 1980. Kamuli district (see Figure 3.1) has an area of 4,302 km^2 and lies to the south of Lake Kyoga. It has a population of 707,332; 98% of whom live in rural areas (Ugandan Bureau of Statistics, 2002). The main economic activity in Kamuli is agriculture comprising mostly of subsistence farming with cotton, coffee and sugarcane grown as cash crops. Small industries are based on rural activities and include the manufacture of jaggery, brick making, maize milling, and cotton ginning (Ugandan Bureau of Statistics, 2005).

3.2.1.3 Soroti district

Soroti district (see Figure 3.1) is a relatively new district, created in 1980 from part of the South Teso district. It covers an area of 3,374 km^2 and borders Lake Kyoga to the South. The latest national human population census for Uganda recorded 369,789 people in Soroti, of which the majority, (89%) live in rural areas (Ugandan Bureau of Statistics, 2002). The main economic activity is subsistence farming where the only cash crop grown is cotton. Small industry is based on rural activities and include milling of oil, flour, and millet, cotton ginning, bread baking, and furniture making (Ugandan Bureau of Statistics, 2005).

3.2.1.4 Tororo district

Tororo district (see Figure 3.1) has been in existence since independence; it was originally named Bukedi and was renamed Tororo in 1980. It covers an area of 1,849 km^2 and borders Kenya to the East. Tororo has a population of 536,888, 93%

of whom live in rural areas (Ugandan Bureau of Statistics, 2002). The main economic activity is agriculture which is mostly subsistence level farming with cotton comprising the main cash crop. Industry in this district includes agricultural based enterprise such as oil milling and cotton ginning as well as other manufacturing industries such as the production of corrugated roofing sheets, cement, fertilizers, fungicides, and soap (Ugandan Bureau of Statistics, 2005). The proximity to the Kenyan border means that the administrative headquarters, Tororo town, has become a small commercial centre.

3.2.2 Sample collection and analysis

The impact of the FITCA programme was assessed by collecting blood samples from cattle within designated sample sites at three time points: baseline, three months post-intervention and one year post-intervention. Baseline sampling coincided with administration of trypanocidal drugs by FITCA in November 2002.

3.2.2.1 Sample sites

Sampling sites were selected in each of the four study districts to monitor any change in the prevalence of trypanosomes, during the mass intervention programme. As previously mentioned, three species of trypanosome were monitored: *T. vivax, T. congolense savannah,* which are the important animal trypanosomiases in this area and *T. brucei* s.l., a sub-species of which is responsible for Rhodesian sleeping sickness. The sampling locations were chosen, where possible, to be representative of the selected districts. FITCA intervention sites had in all cases already been selected, and as such a true randomised cluster sampling protocol (Thrusfield, 2005) was not possible. In addition within the FITCA designated high-risk zones, individual District Veterinary Officers (DVOs) were responsible for deciding on a timetable for the local interventions. Due to the scale of the FITCA interventions, cattle in the high-risk areas could not be treated simultaneously. The situation was further complicated by the fact that the trypanocidal drugs used in the intervention programmes (isometamidium chloride and diminazene aceturate) were distributed

quarterly in 2002 and 2003. Consequently, village sampling sites had to be compatible with the pre-existing intervention timetable and were, therefore, selected with the assistance of the DVO. Ultimately in each district the sample sites were located in a single high-risk sub-county. Figure 3.1 shows the location of the sample sites.

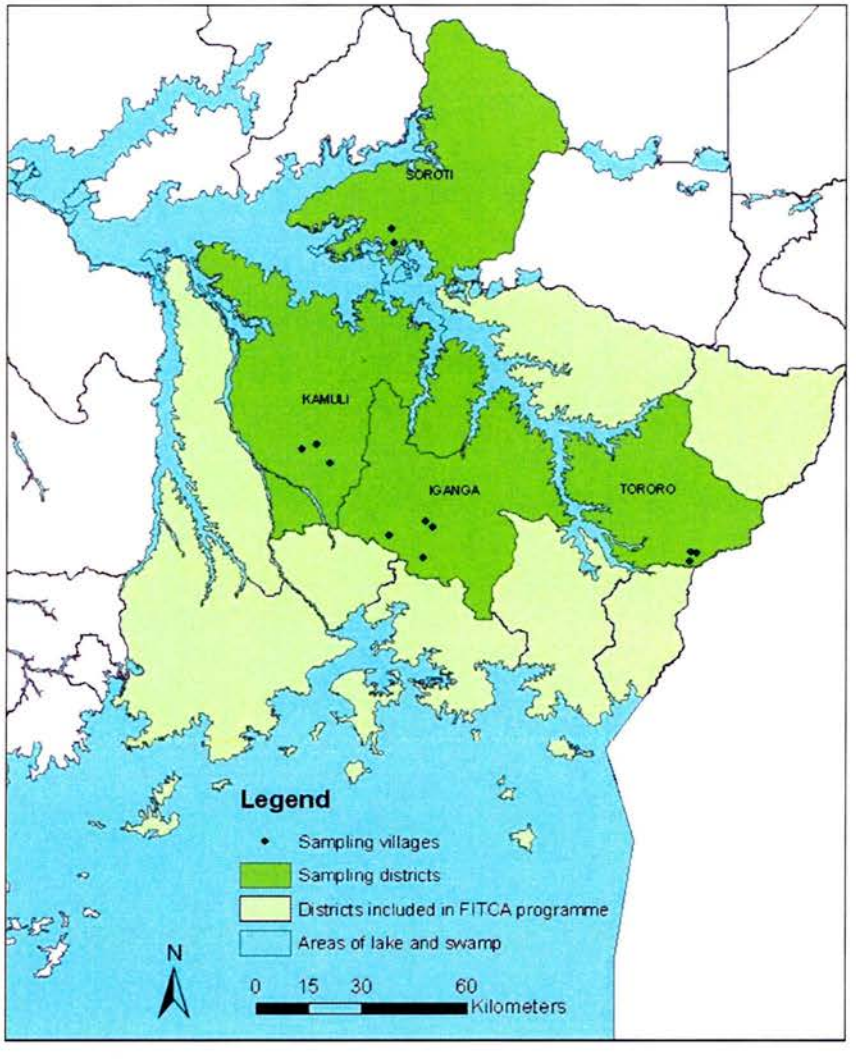


Figure 3.1 – Map of FITCA districts showing the sampling sites for this study

In all villages within the FITCA intervention areas farmers were asked to present their animals to a central point for treatment. In villages selected for this study, a blood sample was taken from the ear vein of each animal before treatment. On

subsequent visits to these villages, the incentive of a deworming treatment (albendazole) was offered for those farmers who brought their animals for sampling. Animals were sampled on a convenience basis in each study village at each time point.

3.2.2.2 Interventions

The predominant treatment was isometamidium chloride, administered intramuscularly and at the manufacturers recommended dose rate, 0.25-0.5mg/kg. District veterinarians carried out this treatment as part of the FITCA intervention programme. This drug is known to have a prophylactic effect and can remain active in the animal blood stream for three months (Holmes *et al.*, 2004). Diminazene aceturate was also used as a treatment but largely for clinical cases, this was administered at the manufacturers recommended dose rate, 3.5mg/kg.

3.2.2.3 Controls

No control villages were included in this study because of the nature of the intervention programme which targeted areas subject to the highest risk for human sleeping sickness. Consequently, it would have been highly unethical to withhold treatment in order to 'ring fence' a village within these high-risk areas to act as a scientific control. Areas outside of these high-risk zones could not act as suitable controls because by definition they had low prevalence of trypanosomes and no cases of sleeping sickness.

3.2.2.4 Sample size

Sample sizes within sub-counties were calculated with the Winepiscope programme (Thrusfield *et al.*, 2001), using the following equation:

$n = (t*SD/L)^2$

where,

n = sample size,

t = students t-value,

SD = $\sqrt{P*(1-P)}$, where P = prevalence,

L = the accepted absolute error or precision.

The values used in these calculations were, t = 1.96 (for the desired confidence level of 95%), P = 4% (Waiswa *et al.*, 2003; Magona *et al.*, 2005), L = 5%. This formula is valid when n is less than 10% of the total population size (winepiscope help) (Table 3.1). The total population was taken as the number of head of cattle reported in the FITCA livestock census, in the sub-county sampled.

District	**Sub-county (s/c)**	**No. head cattle per s/c**	**Minimum sample no.**	**Average no. sampled/ time point**	**Proportion of population sampled**
Iganga	Bulamagi	5529	60	352	6.4%
Kamuli	Kitayunjwa	4425	60	340	7.7%
Soroti	Pingire*	7589	60	262	3.5%
Tororo	Osukuru	8070	60	366	4.2%

Table 3.1 – Table showing the numbers of samples taken in each district. *No FITCA livestock census was completed therefore value is an estimation of cattle number from 2002 census, (s/c denotes sub-county).

In Kamuli, three additional villages were sampled, two have been removed from the analysis as they were located in a different sub-county and the other was removed as it could not be sampled at each of the time points. In Soroti, one additional village was sampled but was not included in the analysis as it was located in a different sub-county. The sampling errors for each of the districts were calculated using Winepiscope, and are shown in Table 3.2.

District	No. samples (1)	Exact error (1)	No. samples (2)	Exact error (2)	No. samples (3)	Exact error (3)
Iganga	360	1.96	336	2.03	321	2.08
Kamuli	409	1.81	291	2.18	319	2.07
Soroti	290	2.21	256	2.36	240	2.44
Tororo	360	1.98	378	1.93	360	1.98

Table 3.2 – Table showing the sampling error for each sampling point in each district – assuming a 4% prevalence. (1) is the baseline (pre-intervention) sample point, (2) is three month post-intervention sample point and (3) is one year post-intervention sample point.

Table 3.3 shows the number of samples collected at each site and the date of collection over the duration of the study.

		Baseline		First sampling point		Final sampling point	
District	Village	Date	Sample no.	Date	Sample no.	Date	Sample no.
Iganga	Budondo	12/11/2002	100	05/03/2003	120	10/11/2003	108
	Bulowoza	16/11/2002	60	08/03/2003	75	12/11/2003	52
	Bunyiro	14/11/2002	100	07/03/2003	116	11/11/2003	124
	Nawanyingi	15/02/2002	100	06/03/2003	25	11/11/2003	37
Kamuli	Bulagala	28/11/2002	120	22/03/2003	63	06/11/2003	108
	Buwaiswa	27/11/2002	143	21/03/2003	126	05/11/2003	101
	Nabigoagerya	27/11/2002	146	22/03/2003	100	05/11/2003	110
Soroti	Amuria	18/11/2002	146	13/03/2003	120	31/12/2003	120
	Okidi	19/11/2002	144	12/03/2003	136	20/12/2003	120
Tororo	Kasipodo	02/12/2002	112	17/03/2003	86	13/11/2003	132
	Kayoro	03/12/2002	108	18/03/2003	164	14/11/2003	100
	Manikori	04/12/2002	140	19/03/2003	128	15/11/2003	128
Totals			**1419**		**1261**		**1240**

Table 3.3– Date and number of samples collected in all four study districts

3.2.2.5 Assessment of treatment coverage

During both the post-intervention sampling points in all study sites, cattle owners who presented their cattle for sampling were asked whether the animal had been previously included in the FITCA mass treatment intervention. This data has allowed estimates to be made on the coverage of the mass treatment programme in the study areas used in this Chapter, the impact of which has been investigated.

3.2.2.6 Sample collection

The blood samples were collected onto Whatman FTA filter papers (Figure 3.2). Whatman FTA® is the cellulose matrix pre-treated to allow fixation and long term storage of genetic material. When samples are applied to FTA®-treated paper, cell lysis occurs and high molecular weight DNA is immobilised within the matrix. The system is designed to kill pathogens and prevent future colonisation by bacteria or fungi thus protecting DNA from microbial and environmental degradation. As bacteria and viruses are inactivated, the samples are rendered non-hazardous meaning that they can be collected, transported and stored at room temperature. PCR amplifications can be performed on biological samples stored on cards at room temperature with a desiccant for over ten years (Whatman, 2004).

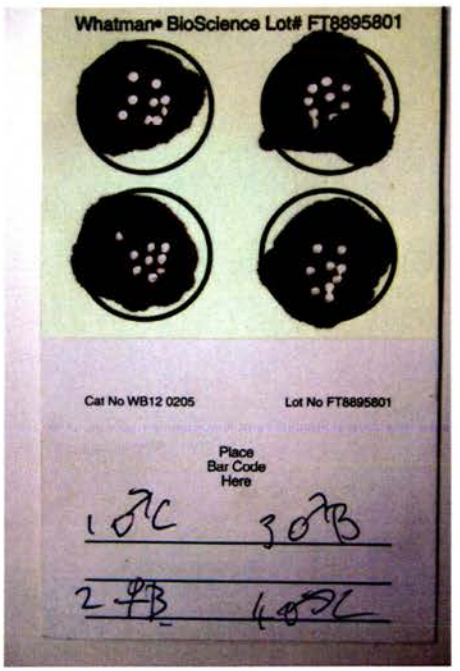


Figure 3.2– Picture of an FTA card, showing where the discs have been removed for analysis.

Blood samples were obtained by qualified veterinarians from the ear vein of cattle using a sterile lancet. Blood was collected into heparinised capillary tubes before being spotted directly onto FTA cards. Whole blood was used to keep the sampling process as simple as possible. After samples were collected, the cards were allowed to dry completely before storage. Post drying, cards were stored in FTA supplied multi barrier pouches with a desiccant. Five cards were stored in each pouch and after the addition of desiccant, pouches were sealed and stored at room temperature for transportation to the UK. All laboratory analyses were carried out by this author at the University of Edinburgh in the Centre for Tropical Veterinary Medicine (CTVM), Easter Bush.

The sex and age of each animal was recorded at the time of sampling. Cattle included in the study were separated into 3 age groups: animals of less than 1 year, animals of 1 to 2 years, and animals over 2 years. Where possible the baseline samples were taken immediately prior to the administration of isometamidium

chloride or diminazene aceturate, however, this was not possible in Tororo, in which the animals were treated a week later.

3.2.2.7 Preparation of sample from FTA card for PCR

To prepare an FTA sample for PCR amplification, discs were first cut from the card using a micro Harris 2.0 mm punch (Figure 3.2). Discs were transferred from the micro-punch to individual microtubes for washing. When more than one disc was cut from an individual sample, discs were washed together in the same microtube. To ensure no cross contamination occurred between samples an equivalent number of discs were taken from a blank filter card after each sample was cut and treated in the same way as the sample punches (detailed below).

Discs from each sample were then washed by the addition of 200 µl of FTA purification reagent per disc. Tubes were subsequently incubated at room temperature for 15 minutes while being continually agitated. The FTA reagent was removed and discarded using a fine tipped pastette. Pastettes were reserved for use throughout the washing procedure taking care to ensure the same pastette was only used for one sample to avoid cross contamination. After 2 washes with FTA reagent, the samples were rinsed with 200µl of TE (1xTris-EDTA (10mM Tris HCl, pH 8; 1mM EDTA)) per disc. Tubes were incubated for 15 minutes as before, after which the TE was removed using a pastette. This was repeated such that each sample was washed twice with FTA reagent and twice with TE. After the final wash, the disc was carefully removed using a pipette tip and placed into a PCR tube. The disc was then allowed to air dry at room temperature before amplification. A schematic illustrating the washing procedure is shown in Figure 3.3. A blank disc was included in the washing and subsequently in the PCR to act as a negative control.

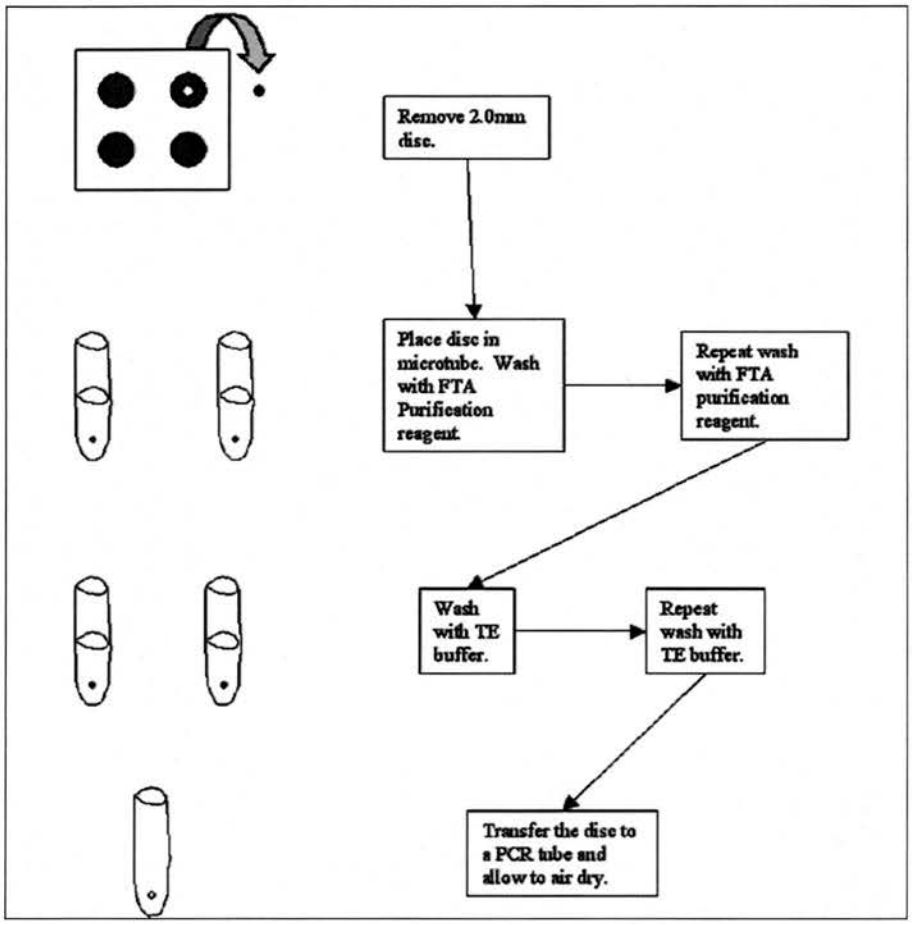


Figure 3.3 – Figure showing the washing procedure for samples stored on FTA cards

3.2.2.8 Detection of prevalence by PCR

Post washing and drying, discs were ready for PCR amplification. As the DNA template remained bound to the FTA disc and PCR amplification was carried out by adding the reaction mix to each sample directly without the need to isolate the DNA first. PCR amplifications were carried out in 25 µl reaction volumes, each containing a final concentration of 10 mM TrisHCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each of the 4 deoxynucleoside triphosphates (dNTPs) 1 Unit of *REDTaq* DNA polymerase (Sigma), with the required concentration of primer. Each sample was analysed for the presence of *T. brucei s.l.*, *T. vivax* and *T. congolense savannah* using species-specific primers at concentrations of 0.8, 2 and 2 µM

respectively (Picozzi *et al.*, 2002). Primer sequences and PCR amplification conditions are shown in Table 3.4; each reaction was heated for an initial denaturation step of 3 minutes at 94°C and a final extension step of 5 minutes at 72°C. Reactions were carried out in a DNA Engine DYAD™ Peltier Thermal Cycler.

Species	**Code**	**Sequence**	**Size**	**Reference**
T. brucei s.l.	TBR1 TBR2	CGAATGAATATTAAACAATGCGCAGT AGAACCATTTATTAGCTTTGTTGC	177bp	Artama *et al.* 1992
Amplification conditions : [(30 cycles) 94°C for 60s, 55°C for 60s, 72°C for 30s]				
T. vivax	TVW1 TVW2	GTGCTCCATGTGCCACGTTG CATATGGTCTGGGAGCGGGT	175bp	Masiga *et al.* 1996
Amplification conditions : [(30 cycles) 94°C for 30s, 60°C for 60s, 72°C for 30s].				
T. congolense savannah	TCS1 TCS2	CGAGAACGGGCACTTTGCGA GGACAAACAAATCCCGCACA	316bp	Masiga *et al. 1992*
Amplification conditions : [(30 cycles) 94°C for 60s, 55°C for 120s, 72°C for 120s]				

Table 3.4 – Table showing the primer sequences and conditions of the PCRs, (Artama *et al.*, 1992; Masiga *et al.*, 1996),(Masiga *et al.*, 1992).

3.2.2.9 Agarose gel electrophoresis

Species identification was conducted based on different sized amplified DNA fragments visualised using agarose gel electrophoresis with reference to size standards. Examples of the gels produced using three separate sets of primers to identify *T. brucei* s.l., *T. vivax* and *T. congolense savannah*, during this screening are shown in Figure 3.4, Figure 3.5 and Figure 3.6. The double bands seen in Figure 3.4 (*T. brucei* s.l.) both the positive control and positive samples is consistent with published results; this is explained by Artama and colleagues as resulting from the

primers being extended through multiple tandem copies during each PCR cycle (Artama *et al.*, 1992). Gels were prepared at a concentration of 1.2% agarose in TBE (Tris Borate EDTA, a composite buffer containing Tris base 89mM, Boric acid 289mM and EDTA 2mM at pH8.0) containing 5µM ethidium bromide and were run for 30 mins at 100 V. 25µl of sample PCR product was added to each well with one well containing 5µl of a 100 base pair ladder (Bioline) for band size comparison. Gels were visualised under ultraviolet light using a Biorad Gel Doc 2000.

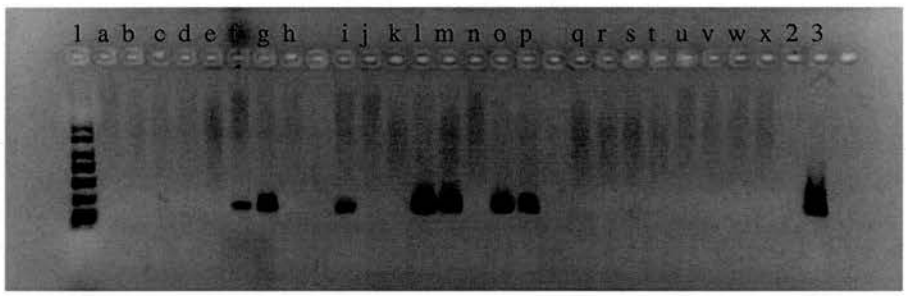


Figure 3.4 - Agarose gel showing the PCR products from *T. brucei s.l.* Lane 1 – ladder, lanes a to x - samples; f, g, i, l, m, o, p are positive for *T. brucei s.l.*, lane 2 - negative control and lane 3 - positive control.

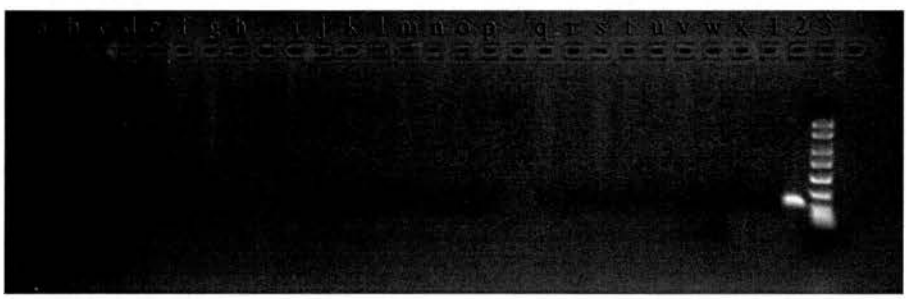


Figure 3.5 - Agarose gel showing the PCR products from *T. vivax*, lanes a to x - samples; there are no positives shown, lane 1 - negative control, lane 2 - positive control and lane 3 – ladder.

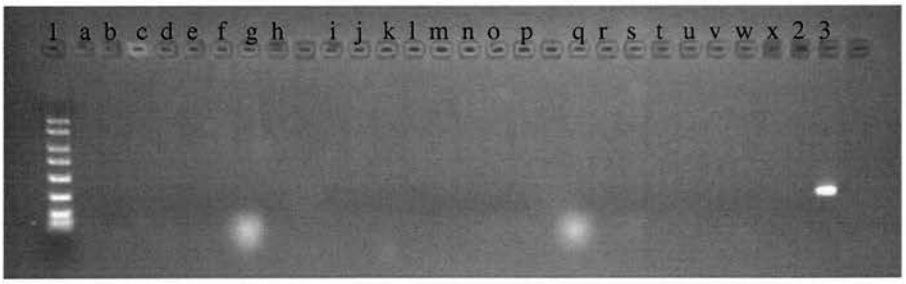


Figure 3.6 - Agarose gel showing the PCR products from *T. congolense savannah*, lane 1- ladder, lanes a to x – samples; there are no positive samples shown in this gel, lane 2 – negative control and lane 3 – positive control.

3.2.3 Calculation of costs relating to the FITCA programme in selected districts

3.2.3.1 Data Sources

All costs used in this analysis were taken from either internal FITCA reports or personal communication from the relevant component managers. During the FITCA programme each district had to submit quarterly progress reports to the main FITCA office in Entebbe, Uganda. These reports were complied into quarterly progress reports covering the whole of the FITCA project area. These reports were available on request in Entebbe.

The cost analysis was done only for the mass treatment of cattle with trypanocides within the four study districts. This treatment plan constituted only a part of the overall FITCA programme in Uganda. Therefore the cost analysis completed for this chapter is only a partial examination of the FITCA budget which relates only to the mass treatment in Kamuli, Iganga, Soroti and Tororo.

3.2.3.2 Included costs

The costs were divided into two groups - item costs and running costs - which referred to the cost of the items used in the program and the ongoing cost of implementing these items, respectively.

Item costs included the total cost of the trypanocides, distilled water, needles, syringes and vehicles, a motorbike and 4-wheel drive for each district. Running costs included the per diems paid to the veterinary staff to implement the programme, the cost of supervision visits, office expenses, vehicle insurance and fuel, for both implementation and supervision.

3.2.3.3 Excluded costs

Only the costs relating to the mass treatment programme in each of the study districts were included in this analysis, any costs incurred in different parts of the FITCA programme were excluded. This analysis is not intended to be an audit of FITCA Uganda; it was done purely to give an indication of how much a large scale mass treatment intervention would cost. The costs of distributing the supplies to the district and general administration are not included.

3.2.4 Statistical analysis

3.2.4.1 Confidence intervals

All confidence intervals were calculated as binomial confidence intervals in R version 2.4.0 (R project)(Figure 3.7).

```
round(100*(binconf(baseline_tbrucei_by_district$tbrucei,baseline_tbrucei_by_district$number)),1)
```

Figure 3.7 – Example of code used for calculating binomial confidence intervals in R, this example was used to calculate the CI for *T. brucei* s.l. at the district level.

3.2.4.2 Comparison of baseline data

To investigate any differences between the prevalence of *T. brucei* s.l., *T. vivax* and *T. c. savannah* in the different study areas χ^2 tests were conducted in Minitab version 14 (Minitab, Inc.). Differences were considered to be significant at $p<0.05$.

3.2.4.3 Effect of the mass treatment campaign

To investigate if there were any significant differences within districts included in the study pre- and post-intervention, paired t-tests were carried out using Minitab version 14 (Minitab, Inc.). Differences were considered to be significant at $p<0.05$.

To investigate if there were any significant differences within individual sample sites fisher exact tests were completed in R (Figure 3.8). Differences were considered to be significant at $p<0.05$.

```
for(k in 1:3){for(j in 1:2){for(i in
1:12){cat("\n",names(jenna)[k+5],names(jenna)[j+9],names(table(jenna$village))[i])print(table(j
enna[,j+9][jenna$village==names(table(jenna$village))[i]],jenna[,k+5][jenna$village==names(ta
ble(jenna$village))[i]]))print(fisher.test(table(jenna[,j+9][jenna$village==names(table(jenna$vill
age))[i]],jenna[,k+5][jenna$village==names(table(jenna$village))[i]])))}}}
```

Figure 3.8 – Code used for analysis of differences between individual sample sites, the Fishers exact test.

For analysis purposes, certain assumptions were made regarding normality and independence of data. The rationale and limitations of these assumptions will be examined in the discussion.

3.3 Results

3.3.1 Description of samples

There were 3,920 cattle blood samples collected from four districts at three time points over the whole study period. Of these, 1,419 were collected at the baseline pre-intervention sampling (November 2002), 1,261 of these at the first post-intervention sampling (March 2003) and lastly 1,240 at the second post-intervention sampling (November 2003). During sampling some basic epidemiological information was collected about each sampled animal, age in three categories (less than one year, one to two years and more than two years) and sex was recorded. The proportion of sampled animals in each age and sex category is shown in Table 3.5. This shows that there were differences in the make up of the samples from the different districts.

In Iganga and Kamuli district the majority of the animals sampled were female, ranging from 73.2% to 79.3%. In Soroti district the sampled animals were made up of almost equal numbers of males (44-50%) and females (50-56%)and in Tororo district there were slightly more females (63-68%than males (32-37%). Overall in each of the sampled areas the predominant age of the sampled cattle was over two years. The exact proportions of each age category are shown in the Table 3.5. Over time there is no significant difference in the proportions of cattle in each age group in Iganga ($\chi^2 = 8.120$, df = 4, p = 0.087) or Kamuli ($\chi^2 = 4.036$, df = 4, p = 0.401). However there are differences in the proportions sampled in Soroti and Tororo districts, in both situations there are variations in the proportions of animals less than two years old sampled at one of the time points. In Soroti at the final sampling point there are more animals in the less than one year old category and in Tororo at the baseline sample point there are fewer animals in the under one year old category.

District	Time point	Age < 1 year	Age 1 – 2 years	Age > 2 years	Female	Male
Iganga	Nov 2002	16.9%	22.2%	60.8%	74.4%	25.6%
	March 2003	12.5%	29.8%	57.7%	76.8%	23.2%
	Nov 2003	15.9%	29.3%	54.8%	73.2%	26.8%
Kamuli	Nov 2002	14.1%	20.5%	65.5%	79.3%	20.7%
	March 2003	12.3%	23.9%	63.7%	78.9%	21.1%
	Nov 2003	16.4%	27.1%	56.5%	76.8%	23.2%
Soroti	Nov 2002	6.1%	22.1%	71.8%	49.7%	50.3%
	March 2003	6.5%	21.1%	72.5%	55.3%	44.7%
	Nov 2003	11.9%	13.6%	74.4%	56.1%	43.9%
Tororo	Nov 2002	6.4%	30.3%	63.3%	62.8%	37.2%
	March 2003	11.4%	21.7%	66.9%	64.3%	35.7%
	Nov 2003	10.6%	20.3%	69.2%	68.6%	31.4%

Table 3.5 – Proportion of sampled animals at each age and sex category

3.3.2 Baseline pre-intervention results

Overall there were no statistically significant differences between age groups when total prevalence of all trypanosome species were compared (χ^2=2.221, df=2, p=0.329). When trypanosome species were examined individually, significant differences were observed between age groups for *T. vivax* (χ^2=21.320, df=2, p<0.001) but this was not the case for *T. brucei* s.l. (χ^2=1.714, df=2, p=0.425). *T. vivax* prevalence was significantly lower in animals under one year of age (χ^2=12.671, df=1, p<0.001) and those between one and two years (χ^2=17.084, df=1, p<0.001). The prevalence of *T. c. savannah* at baseline was very low and as such it was impossible to make any inferences regarding infection prevalence across any age or sex groups.

When the three trypanosome species combined were considered, there was a significant difference in infection prevalence between the sexes (x^2=7.70, df=1, p=0.006) with significantly more male cattle infected. When trypanosome species were considered separately, *T. vivax* continued to display the same trend (x^2=6.059, df=1, p=0.014) while *T. brucei* s.l. was approaching significance (x^2=3.283, df=1, p=0.07).

3.3.2.1 Prevalence at sample site level

3.3.2.1.1 T. brucei s.l.

The baseline prevalence of *T. brucei* s.l. in each sample site is shown in Figure 3.9. The prevalence by PCR detected in each of the study sites ranged from 0% to 33%, the highest prevalence was found in Okidi in Soroti district where 33% of the samples taken were positive for *T. brucei* s.l., the lowest prevalence was found in Kasipodo and Kayoro in Tororo district where no *T. brucei* s.l. was detected.

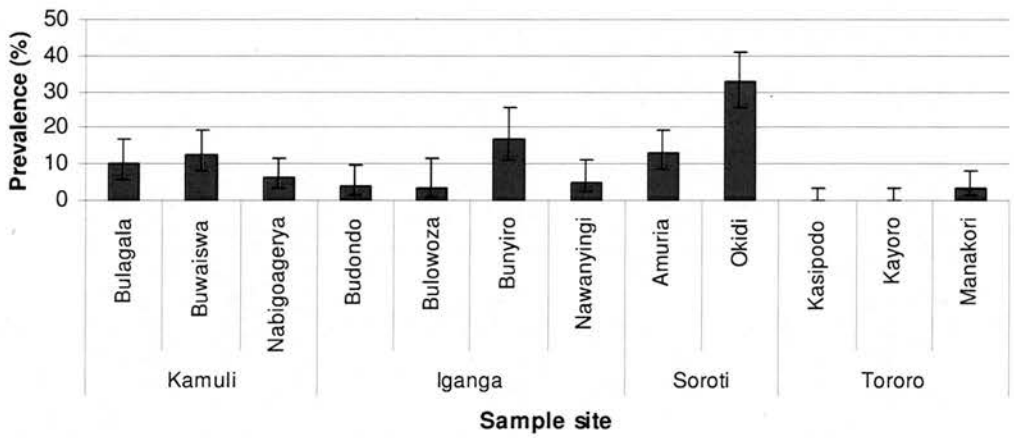


Figure 3.9 – Baseline prevalence of *T. brucei* s.l. at each sample site. Error bars shown on the graph represent the binomial confidence intervals for each sample site.

3.3.2.1.2 T. vivax

The baseline prevalence of *T. vivax* at each sample site is shown in Figure 3.10. The detected prevalence ranged from 0% to 10%. The highest prevalence was seen in Bunyiro in Iganga district where 10% of the samples tested were positive for *T. vivax,* the lowest prevalence was found in Kasipodo in Tororo district where no *T. vivax* was detected.

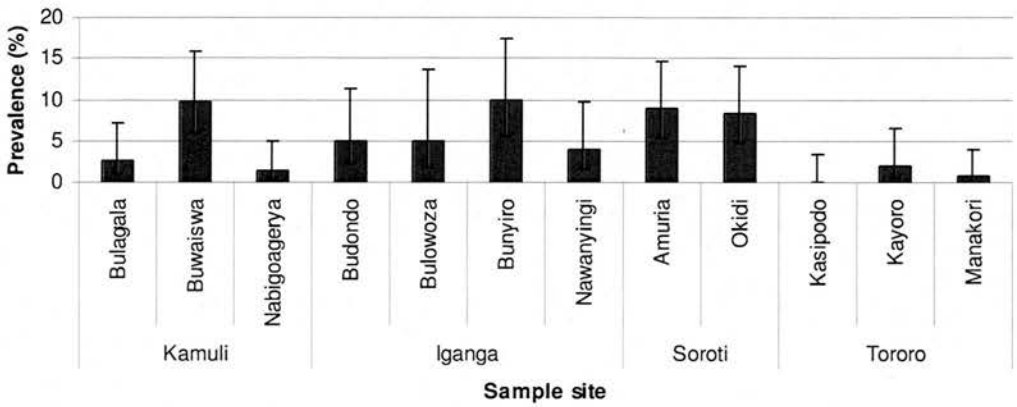


Figure 3.10 – Baseline prevalence of *T. vivax* at each sample site. Error bars shown on the graph represent the binomial confidence intervals for each sample site.

3.3.2.1.3 T. congolense savannah

As previously mentioned, very low levels of *T. c. savannah* were detected across all sample sites (Figure 3.11). There was no *T. c. savannah* detected in eight of the sample sites at the baseline while in the remaining four sites the prevalence varied between 1 and 4.5%. The highest prevalence was detected in Kasipodo in Tororo district.

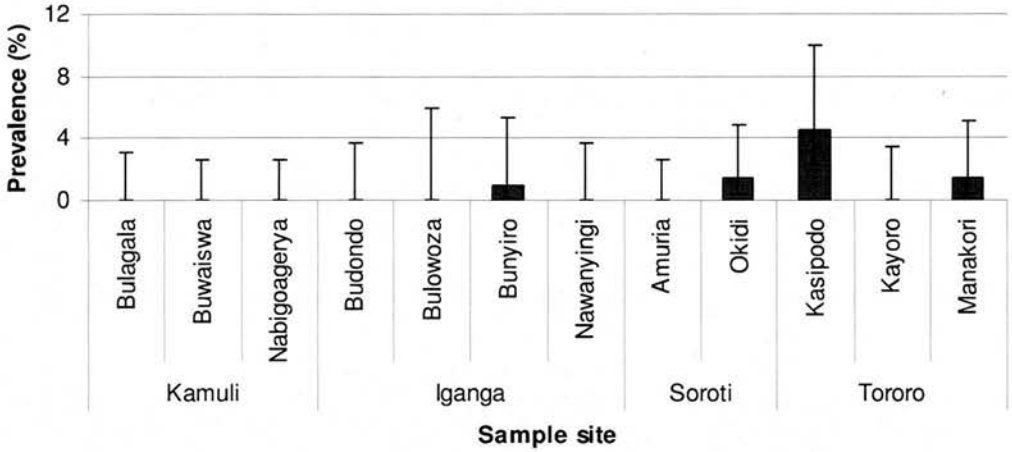


Figure 3.11 – Baseline prevalence of *T. congolense savannah* at each sample site. Error bars shown on the graph represent the binomial confidence intervals for each sample site.

3.3.2.2 Prevalence at district level

3.3.2.2.1 T. brucei s.l.

The prevalence of *T. brucei* s.l. across the four districts is shown in Figure 3.12. Significant differences in prevalence were observed across the districts (x^2=82.204, df=3, p<0.001) with the highest prevalence in Soroti (22.8%) and the lowest in Tororo (1.4%). On closer examination, Soroti and Tororo were both shown to be significantly different (p<0.001) from all other districts, as well as each other, while no difference was observed between Kamuli and Iganga.

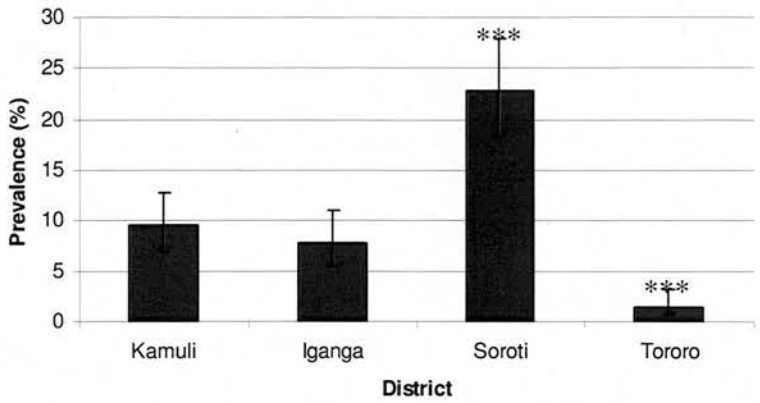


Figure 3.12 – Graph showing the prevalence of *T. brucei* s.l. pre-intervention, * denotes significance at p<0.001.**

3.3.2.2.2 T. vivax

The prevalence of *T. vivax* in the four study areas was lower than that of *T. brucei* s.l. however the patterns of infection across the districts remained similar with the highest prevalence seen in Soroti (8.6%) and the lowest in Tororo (0.8%). The results from all four districts are shown in Figure 3.13. Statistically there was a difference across the districts (x^2=22.742, df=3, p<0.001) which on closer examination was due to the prevalence of infection in Tororo being significantly lower than the other three districts. There was no difference between the prevalence of *T. vivax* in Kamuli, Iganga and Soroti (x^2=4.602, df=2, p = 0.100).

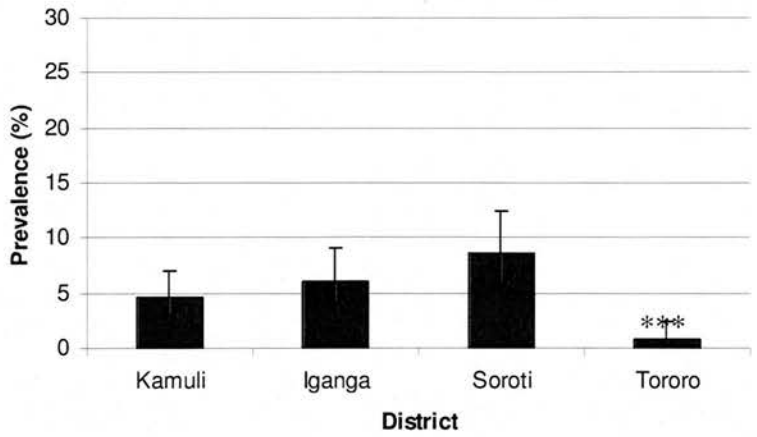


Figure 3.13 – Graph showing the prevalence of *T. vivax* pre-intervention, denotes significance at p<0.001.

3.3.2.2.3 T. congolense savannah

Levels of *T. c. savannah* detected throughout all districts were very low (Figure 3.14). No *T. c. savannah* was found in Kamuli district while the prevalence varied between 0.3% in Iganga district to 1.9% in Tororo district. No further analysis was carried out on this data due to the very low levels of prevalence detected.

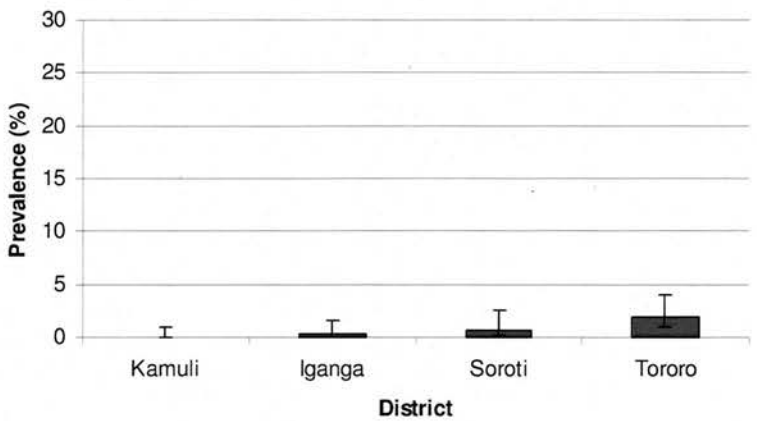


Figure 3.14 – Graph showing the prevalence of *T. congolense savannah* pre-intervention

3.3.2.2.4 Trypanosome species combined

When the trypanosome species were combined and compared across the four districts (Figure 3.15), a significant difference in prevalence levels was observed (x^2=93.827, df=3, p-value<0.0001). Soroti had a significantly higher prevalence of infection than any other district (p<0.001) while Tororo had a significantly lower prevalence of infection than any other district (p<0.001). The prevalence of infection in Iganga and Kamuli were not significantly different.

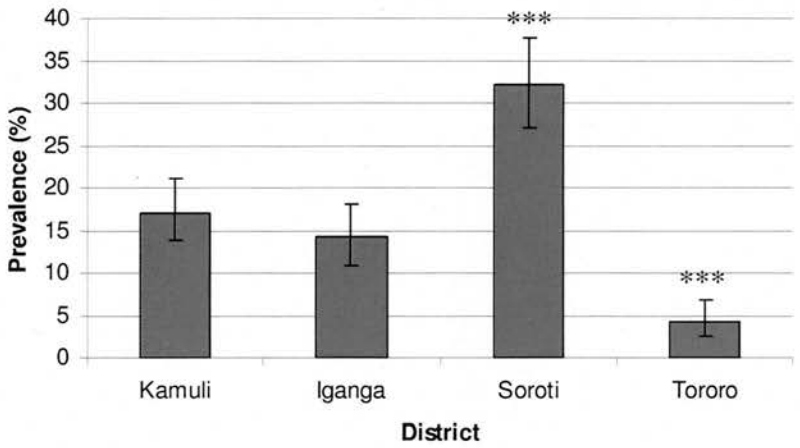


Figure 3.15 – Graph showing the prevalence of all tested trypanosome *spp.* pre-intervention, * denotes significance at p<0.001.**

3.3.2.3 Comparison of pre-intervention prevalence: PCR and microscopy

PCR prevalence data were compared to FITCA microscopy prevalence data to assess the correlation between the two diagnostic techniques. Table 3:6 shows a comparison between the baseline prevalence taken by FITCA using microscopy and the prevalence by PCR in the four study districts. Unfortunately the FITCA trypanosomiasis survey was not completed in Soroti district so there are no values available for microscopy. Prevalence data are shown for trypanosome species individually as well as combined.

District	Diagnostic tool	No. of samples	*T. brucei*	*T. vivax*	*T. c. savannah*	Total prevalence	P value
Iganga	Microscopy	1795	0.1%	2.0%	7.4%	**10.4%**	**0.038**
	PCR	360	7.8%	6.1%	0.3%	**14.2%**	
Kamuli	Microscopy	1756	1.1%	0.1%	0.8%	**2.8%**	**<0.001**
	PCR	409	12.5%	4.7%	0	**17.2%**	
Soroti	Microscopy	n/a	n/a	n/a	n/a	**n/a**	n/a
	PCR	290	22.8%	8.6%	0.7%	**32.1%**	
Tororo	Microscopy	1400	1.6%	2.6%	3.4%	**8.2%**	**0.009**
	PCR	360	1.4%	0.8%	1.9%	**4.2%**	

Table 3.6 – Baseline prevalence by PCR and microscopy. Microscopy and PCR total prevalence compared by x^2.

The total prevalence of all tested trypanosome species were compared rather than individual species as it is possible for misdiagnosis to occur when using microscopy (section 1.6). In Kamuli and Iganga, trypanosome prevalence was significantly higher by PCR than microscopy with the largest difference seen in Kamuli (14.4%). Conversely, prevalence was significantly higher by microscopy in Tororo compared to PCR.

3.3.3 Impact of FITCA mass treatment intervention on animal trypanosomiasis

3.3.3.1 Coverage of intervention

At the post-intervention sampling, cattle owners were asked if their animal had been included in the FITCA mass treatment (see section 3.2.2.5). Figure 3.16 shows the proportions of sampled animals at each post-intervention sampling which had been treated under the FITCA programme. This data allows an estimation of the coverage of cattle in treatment areas in each district, shown in Table 3.7. The district with the highest coverage was Kamuli where 81.1% of animals sampled three months post-

intervention had received treatment by FITCA. The district with the lowest coverage was Iganga where 45.2% of animals sampled three months post-intervention had received treatment by FITCA. In all circumstances, with the exception of Budondo and Bulowoza villages in Iganga district and Amuria in Soroti district, fewer of the sampled animals at the final post-intervention sampling had been included in the original FITCA treatment intervention. This is not surprising given the fluid nature of cattle keeping in these areas; animals are bought and sold regularly (pers. comm. C. Waiswa, Makerere University, Kampala, Uganda) and the fact that some farmers may not return for sampling.

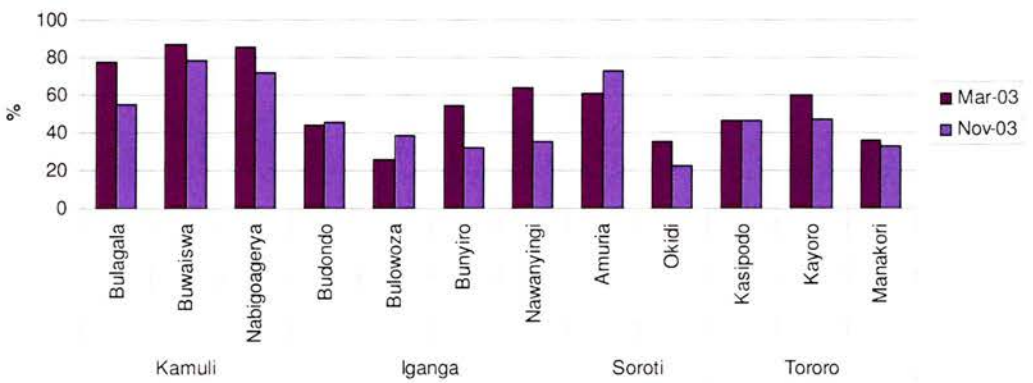


Figure 3.16 – Percentage of sampled animals which had been covered by the FITCA mass treatment at the first and second post-intervention sampling

District	Proportion of sampled animals which had been treated by FITCA	
	March 2003	**Nov 2003**
Kamuli	81.1%	67.2%
Iganga	45.2%	38.0%
Soroti	47.5%	47.3%
Tororo	48.4%	41.7%

Table 3.7 – The proportions of animals sampled in this study which were treated by the FITCA mass intervention

3.3.3.2 Impact of intervention across the study area

Figure 3.17 shows the combined prevalence of all trypanosome species in the entire study area. Pre-intervention the prevalence was 16.1%, three months post-intervention there was a significant reduction in the prevalence of all tested trypanosome species to 9.1% (T=3.02, p=0.011). This significant reduction was maintained over time and at one year post-intervention, the prevalence was 9.4% (T=3.94, p=0.002).

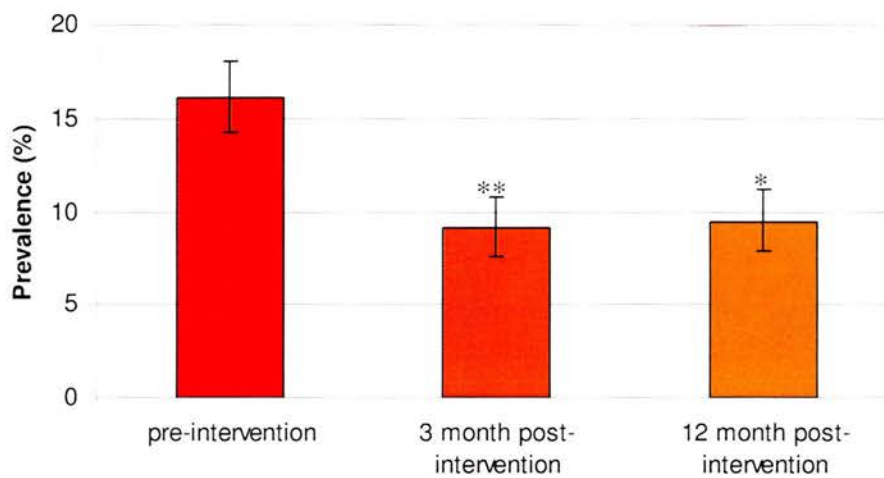


Figure 3.17 – Graph showing the prevalence of all tested trypanosome spp. in all 12 study sites, ** denotes significance at p<0.01, * denotes significance at p<0.05.

The prevalence of the individual tested species; *T. brucei* s.l., *T. vivax* and *T. c. savannah* are shown below in Table 3.8.

	Prevalence		
Species	Pre-intervention	3 month post-intervention	12 month post-intervention
T. brucei s.l.	9.7% (8.3-11.4)	6.1% (4.9-7.6)	5.8% (4.6-7.2)
T. vivax	4.9% (3.9-6.1)	2.9% (2.1-4.0)	3.4% (2.5-4.5)
T. c. savannah	0.7% (0.4-1.3)	0 (0-0.3)	0.2% (0.1-0.7)

Table 3.8 – Table showing the prevalence of *T. brucei* s.l., *T. vivax* and *T. c. savannah* for all 12 sample sites pre- and post-intervention. Binomial confidence intervals are shown in brackets for each value.

The prevalence of *T. brucei* s.l. reduced from 9.7% to 6.1% at three months post-intervention (T = 1.86, p = 0.090). One year post-intervention, prevalence continued to drop to 5.8% which was a significant reduction from the pre-intervention prevalence (T = 2.67, p = 0.022).

Pre-intervention the prevalence of *T. vivax* (Table 3.8) was 4.9%, this reduced to 2.9% three months post-intervention (T = 1.56, p = 0.147). One year post-intervention, there was a slight increase in prevalence to 3.4% but this was not a significant increase compared to three months post-intervention (T = 1.17, p = 0.267).

Pre-intervention the prevalence of *T. c. savannah* (Table 3.8) was 0.7%, three month post intervention there were no cases of *T. c. savannah* detected (T = 1.81, p = 0.098). One year post intervention, cases were again detected although the prevalence remained very low at 0.2% (T = 1.29, p = 0.222). As the detected prevalence of *T. c. savannah* across the study area was very low it will not be included in any future analysis.

3.3.3.3 Impact of intervention at the district level

3.3.3.3.1 Combined trypanosome species

The prevalence of all tested trypanosome species over time in each of the study districts show a similar trend with a reduction post-intervention in every district. Figure 3.18 shows the pre- and post-intervention combined trypanosome prevalence in each district. There is a similarity to the overall pattern in each of the districts with the largest reduction in prevalence occurring three month post-intervention. However this was only statistically significant in Kamuli, where the reduction three month post-intervention was significant ($T=8.56$, $p<0.05$) and remained so a year later ($p<0.05$). In Kamuli, Soroti and Tororo, a slight increase in prevalence was observed between three months and one year post intervention, however, this increase was not significant.

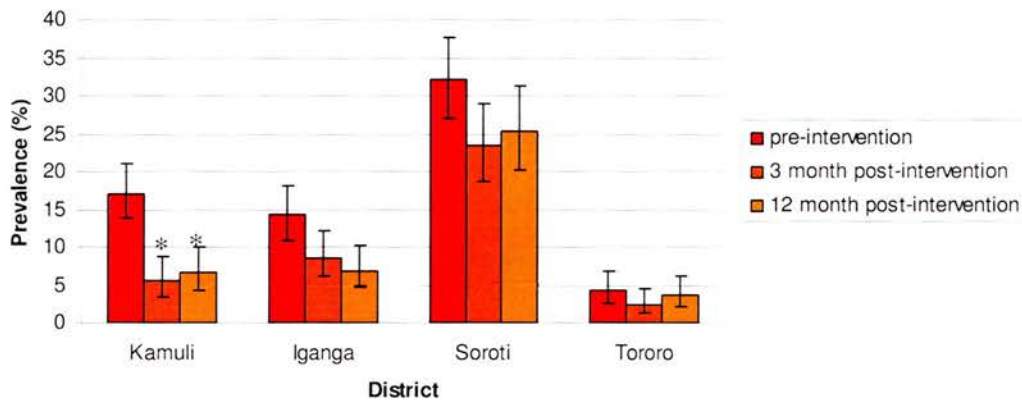


Figure 3.18 – Graph showing the prevalence of all tested trypanosome spp. in each district both pre- and post-intervention, * denotes statistical significance at $p<0.05$.

3.3.3.3.2 T. brucei s.l.

Figure 3.19 shows the pre- and post-intervention prevalence of *T. brucei* s.l. in each of the sampled districts. In Kamuli, Iganga and Soroti, a reduction in prevalence is

observed three months post-intervention, however, this was only significant in Kamuli (T=4.38, p<0.05). In Soroti and Iganga there was a further reduction in *T. brucei* s.l. one year post-intervention, however, these reductions were not statistically significant.

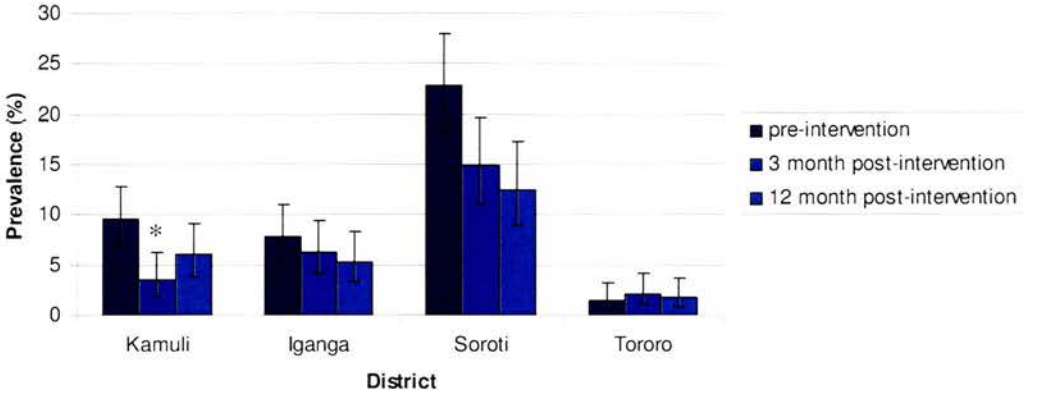


Figure 3.19 – Graph showing the prevalence of *T. brucei* s.l. in each district both pre- and post-intervention, * denotes significance at p<0.05.

3.3.3.3.3 T. vivax

Figure 3.20 shows the pre- and post-intervention prevalence of *T. vivax* in each of the sampled districts. In Kamuli, Iganga and Tororo, reductions in prevalence can be observed three months post-intervention, however, these were not statistically significant. In Soroti, there was no difference in prevalence three months post-intervention, however, the prevalence increased 12 months post-intervention (p>0.05). Tororo district also showed a non-significant increase in prevalence 12 months post-intervention. In contrast to the other districts, both Iganga and Kamuli continued to show further reductions 12 months post-intervention. Prevalence at 12 months post-intervention in Iganga was significantly reduced from prevalence pre-intervention (T=8.37, p<0.05).

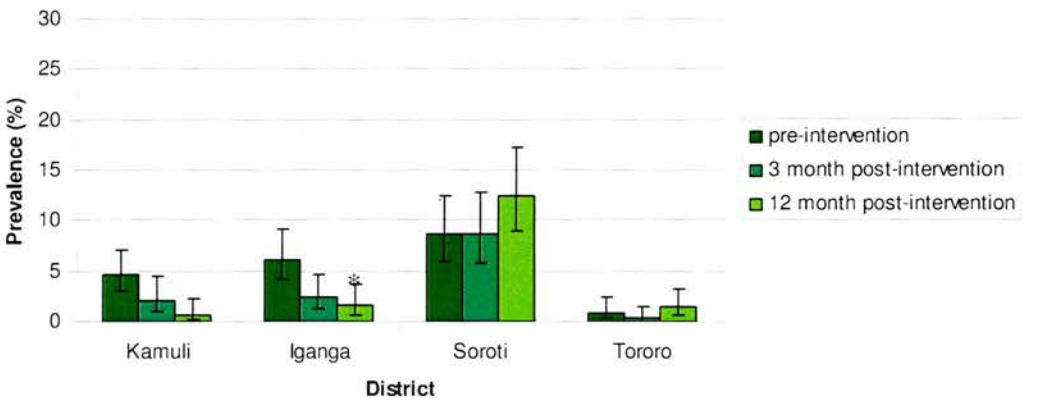
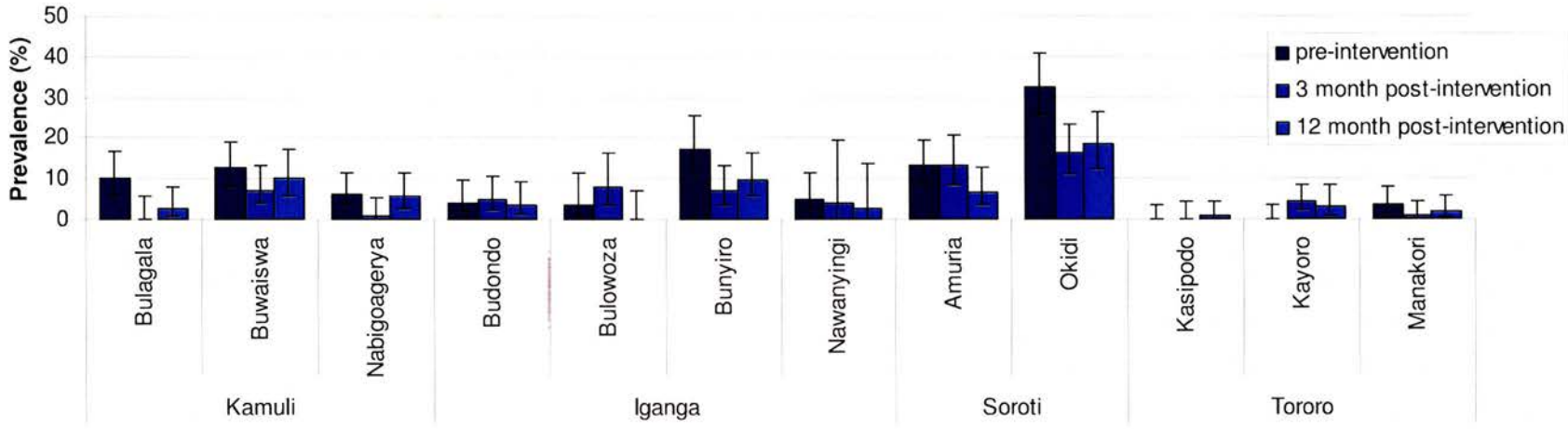


Figure 3.20 – Graph showing the prevalence of *T. vivax* in each district both pre- and post-intervention, * denotes statistical significance at p<0.05.

3.3.3.4 Impact of intervention at sample site level

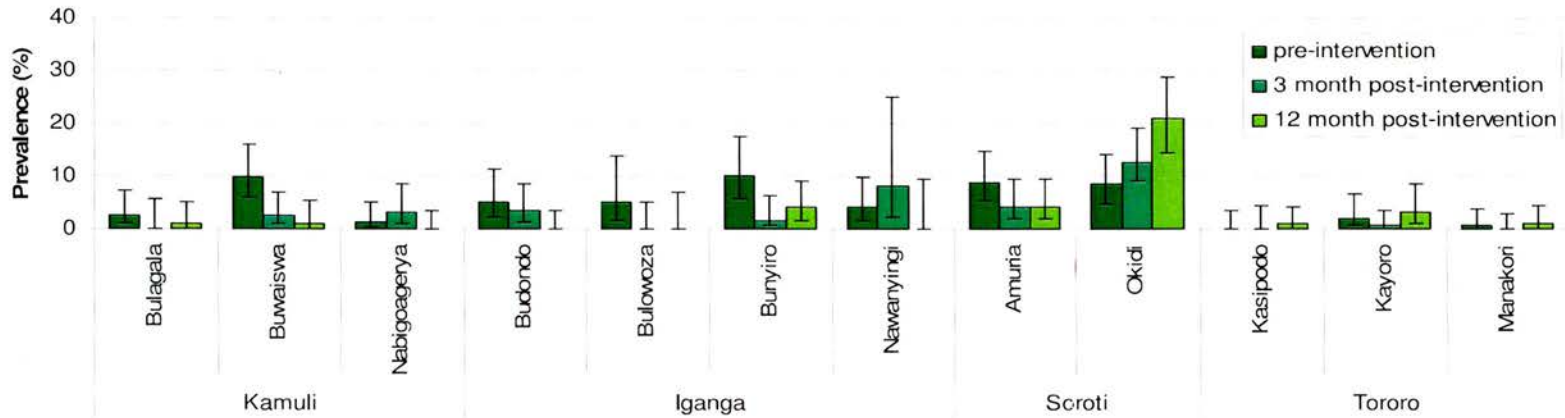
Figure 3.21 shows the prevalence of *T. brucei* s.l. at each of the 12 sample sites pre-intervention and post-intervention. For each sample site the pre-intervention prevalence was compared with both the three month and one year post-intervention prevalence using a fisher exact test. The p-values generated for each of these comparisons are shown in the table below, significant results are highlighted. Seven sites show a reduction in prevalence three months post-intervention and this was significant in three cases; Bulagala, Numyiro and Okidi. There are four sites which show an increase in prevalence; of which Kayoro is significant.

Figure 3.22 shows the prevalence of *T. vivax* at each of the 12 sample sites pre- and post-intervention. Overall there has been no significant reduction in the prevalence of *T. vivax* across any study sites. The only significant change is seen in Okidi where there was a significant increase in the prevalence of *T. vivax* one year post-intervention.



Village	Bulagala	Buwaiswa	Nabigoa.	Budondo	Bulowoza	Bunyiro	Nawan.	Amuria	Okidi	Kasipodo	Kayoro	Manakori
Significant change after 3 months	>0.001 ↓ Yes	0.158 ↓ No	0.052 ↓ No	0.759 ↑ No	0.299 ↑ No	0.031 ↓ Yes	1 ↓ No	1 ↑ No	0.001 ↓ Yes	1 - No	0.044 ↑ Yes	0.216 ↓ No
Significant change after 1 year	>0.001 ↓ Yes	0.549 ↓ No	1 ↓ No	1 ↓ No	0.498 ↓ No	0.114 ↓ No	1 ↓ No	0.104 ↓ No	0.011 ↓ Yes	1 ↑ No	0.109 ↑ No	0.450 ↓ No

Figure 3.21 –Graph showing the change in prevalence of *T. brucei* s.l. at each sample village at three time points, pre-intervention (Nov 02), three months post-intervention (Mar 2003) and one year post-intervention (Nov 03). The table shows the value from the fishers exact test and states the significance of that value, the coloured areas indicate sites where there was a significant change in the prevalence post-intervention.



Village	Bulagala	Buwaiswa	Nabigoa.	Budondo	Bulowoza	Bunyiro	Nawan.	Amuria	Okidi	Kasipodo	Kayoro	Manakori
Significant change after 3 months	1 ↓ No	1 ↓ No	0.242 ↑ No	1 ↓ No	0.244 ↓ No	0.222 ↓ No	0.077 ↑ No	1 ↓ No	0.266 ↑ No	1 - No	0.534 ↓ No	1 ↓ No
Significant change after 1 year	0.559 ↓ No	0.219 ↓ No	1 ↓ No	0.159 ↓ No	0.549 ↓ No	0.265 ↓ No	1 ↓ No	0.472 ↓ No	>0.001 ↑ Yes	0.388 ↑ No	0.332 ↑ No	1 ↓ No

Figure 3.22 - Graph showing the change in prevalence of *T. vivax* at each sample village at three time points, pre-intervention (Nov 02), three months post-intervention (Mar 2003) and one year post-intervention (Nov 03). The table shows the value from the fishers exact test and states the significance of that value, the coloured area indicates the site where there was a significant change in the prevalence post-intervention.

3.3.3.5 Coverage at individual sample site

While it may be hypothesised that sample sites receiving the highest levels of trypanocide treatment coverage would lead to the greatest reduction in trypanosome prevalence, this was not borne out statistically (Pearsons correlation=2.72, p=0.393).

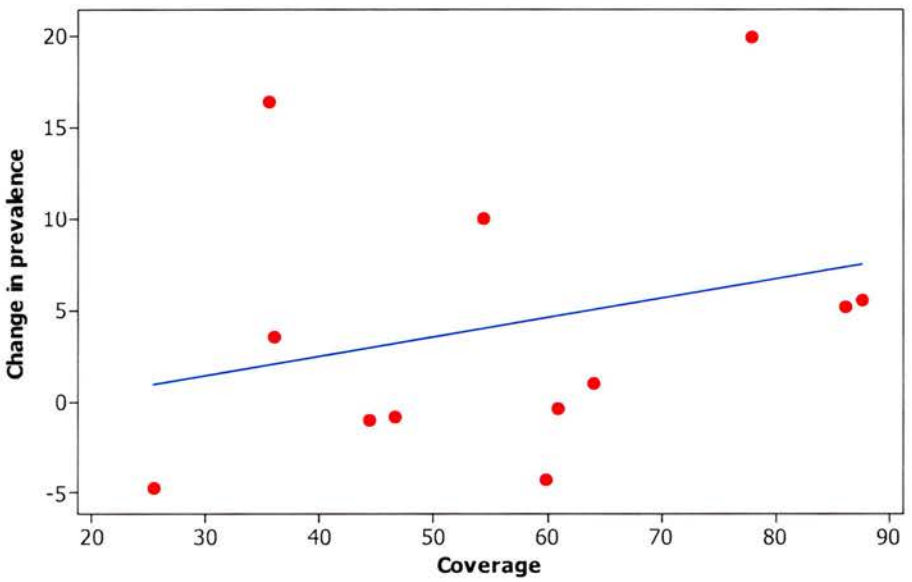


Figure 3.23 – Graph showing the change in prevalence against the % coverage of treatment at each study site. Sites which are shown to have a negative 'change in prevalence' in the Figure are those sites where the overall prevalence of trypanosomes increased post-intervention

3.3.4 Cost data

3.3.4.1 Item costs

All item costs were originally recorded in Ugandan shillings (UGS) and have been converted in USD as shown in Table 3.9. These costs include: the cost of the two trypanocides used, the equipment necessary to administer the drugs (distilled water, needles and syringes) and the vehicles needed to transport the drugs to the intervention sites.

Item	Unit	Unit cost (USD)
Isometamidium chloride	Packet (doses for 120 cattle)	$62.02
Diminazene aceturate	Packet (doses for 20 cattle)	$3.95
Distilled water	Litre	$0.85
Needles	Packet (12 needles)	$1.69
Metal syringe	1 syringe	$19.73
Motorbike		$2,649.98
4-wheel drive		$24,639.15

Table 3.9 – FITCA item costs for the mass treatment of cattle in Uganda conversion rates from Ugandan shillings (UGS) to USD were carried out using the average exchange rate over 2002 taken from the UN operational rates of exchange. The exchange rate was taken as 1773.60 UGS to 1 USD

3.3.4.2 Running costs

All running costs which were considered in cost calculations are shown in Table 3.10. These costs include: the allowances to staff, vehicle insurance and maintenance and fuel allowances.

Running cost	Cost (USD)
Day rate	$2.82
Night allowance (standard)	$27.06
Night allowance (driver)	$14.10
Motorbike insurance and maintenance	$563.82
4WD insurance and maintenance	$2,819.12
Fuel per day	$2.82

Table 3.10 – Running costs for FITCA interventions. The exchange rate was taken as 1773.60 UGS to 1 USD

3.3.4.3 Specific costs

The item and running costs for each of the four study districts, Kamuli, Iganga, Soroti and Tororo varied slightly depending on the numbers of doses of drugs distributed to each district and the number of staff involved in the intervention (Table 3.11). In all of the study districts the time period allowed for the intervention was 45 days, within that time five officers worked in Kamuli and Soroti, six in Tororo and eight in Iganga. Night allowances were claimed by officials from each district travelling to Entebbe for a workshop on the implementation of this intervention, these allowances were paid for four individuals from Iganga, Soroti and Tororo and for three individuals from Kamuli.

Item	Kamuli	Iganga	Soroti	Tororo
Doses of isometamidium chloride distributed	16000 doses (equivalent of 133 packs)	8000 doses (equivalent to 67 packs)	10400 doses (equivalent to 87 packs)	20000 doses (equivalent to 167 packs)
Doses of diminazene acteurate distributed	3000 doses (equivalent of 150 packs)	3500 doses (equivalent to 175 packs)	1500 doses (equivalent to 75 packs)	3000 doses (equivalent to 150 packs)
No. litres of water distributed	100	70	70	110
No. syringes distributed	6	6	6	6
No. of field days	45	45	45	45
No. of officers claiming day rate	5	8	5	6
No. of officers claiming night allowance	3	4	4	4

Table 3.11 – Quantities for item and running costs per study district

The numbers of doses shown in Table 3.11 can be compared to the number of cattle in each high risk sub-county in each of the study districts. The 19,000 doses of trypanocides distributed to Kamuli is slightly less than the 19,758 heads of cattle counted in the FITCA survey. Tororo district also received slightly less than the

census dictated 23,000 doses for 23,659 heads of cattle. Iganga on the other hand received 11,500 doses of trypanocide for 9,780 heads of cattle counted in the survey. Unfortunately the FITCA livestock survey was not completed in Soroti.

3.3.4.4 Cost of the intervention

The cost of the intervention for each district was calculated using both item and running costs values. As the vehicles were purchased for use in the whole of the FITCA project, the values of the vehicles for use in this specific part of the FITCA programme were considered on a cost per day basis. These values were calculated separately for the 4-wheel drive and motorbike assuming a 10 year working life and then calculating the cost of the 45 day intervention. These values were added to the values for the numbers of doses of drugs used in each district, the cost of equipment in each district and the running costs in terms of salaries and daily fuel use. The final costs for each of the four study districts and the average cost over the study area according to these calculations are shown in Table 3.12 in USD. Iganga district had the lowest cost for the intervention at $11,589 and Tororo district had the highest at $17,219. The average cost of the intervention across the four study districts was $13,816.

District	**Total cost of mass treatment**
Kamuli	$14,781
Iganga	$11,589
Soroti	$11,674
Tororo	$17,219
Average cost over study area	**$13,816**

Table 3.12 – Costs of the cattle treatment intervention in the study areas in US dollars. All currency exchange rates were taken as the average exchange rate over 2002 from the UN operational rates of exchange. The exchange rate was taken as 1773.60 UGS to 1 USD

In an attempt to put these costs into context, Table 3.13 shows the cost per person, this is also based on the population of the whole district not just those people residing in areas which were covered by the intervention. This averages out across the four districts as $0.02 per person. As 56.1% of the Ugandan population are children (under 18 years of age) (Ugandan Bureau of Statistics, 2002), the cost has also been calculated in terms of household. The mass treatment of cattle cost per household between $0.08 in Iganga district and $0.16 in Soroti district, which averaged out at $0.12 per household over the study area. The cost was also calculated in terms of cost per livestock owning household, this averaged out across the study area as $0.50 per livestock owning household. It should be pointed out at this time that these calculations have been based on the whole district not limited to the areas which benefited from the FITCA mass treatment. Values for the entire district have been used as the aims of the FITCA intervention were to impact on the whole district by targeting their interventions on the most heavily affected areas, therefore the benefits should be appreciated by all.

District	Cost per person	Cost per household	Cost per livestock keeping household
Kamuli	$0.02	$0.12	$0.45
Iganga	$0.02	$0.08	$0.49
Soroti	$0.03	$0.16	$0.56
Tororo	$0.03	$0.15	$0.51
Average cost over study area	$0.02	$0.12	$0.50

Table 3.13 – Cost of cattle treatment interventions by animal and human population and by administrative unit. Population data collected from the 2002 census. All currency exchange rates were taken as the average exchange rate over 2002 from the UN operational rates of exchange. The exchange rate was taken as 1773.60 UGS to 1 USD.

The cost of the intervention has also been analysed in terms of cost per 1% reduction of all tested species of trypanosomes (*T. brucei* s.l., *T. vivax* and *T. c. savannah*). Table 3.14 shows a summary of the prevalence of the tested species of trypanosome at the three sampling time points in each of the four districts and the difference in

prevalence between November 2002 and November 2003 (the first and last sampling points). This difference in prevalence has been used to calculate the cost at the district level per 1% reduction of *T. brucei, T. vivax* and *T. c. savannah.* It should be noted at this point that these prevalence figures are from a small number of sample sites within each of the districts and that these figures are being used to represent the whole district. The largest reduction in prevalence occurred in Kamuli where there was a 10.5% reduction in the prevalence of the tested trypanosome species between the samples taken in November 2002 pre-intervention and the samples taken in November 2003. This meant that in Kamuli each 1% reduction cost $1,408. On the other hand in Tororo where one year post-intervention the difference in prevalence was 0.5% the cost per 1% reduction of prevalence of the tested trypanosome species was $34,338. Overall the average reduction in prevalence across the four study sites was 6.3% which resulted in a $2,193 cost per 1% reduction of *T. brucei, T. vivax* and *T. c. savannah.*

	Prevalence (all *tryp. spp.*)			**Difference in prevalence (all *tryp. spp.*) Nov 02-Nov 03**	**Cost per 1% reduction (all tryp. species) in USD.**
	Nov 2002	**March 2003**	**Nov 2003**		
Kamuli	17.1%	6.9%	6.6%	10.5%	$1,408
Iganga	14.2%	7.7%	6.9%	7.3%	$1,588
Soroti	32.1%	23.4%	25.4%	6.7%	$1,742
Tororo	4.2%	2.4%	3.7%	0.5%	$34,338
Average over study site	16.9%	10.1%	10.6%	6.3%	$2,193

Table 3.14 – Cost per percentage reduction of all trypanosome species in each of the study districts. The exchange rate was taken as 1773.60 UGS to 1 USD.

Analysis was repeated to show the cost per 1% reduction of *T. brucei s.l.* and is shown in Table 3.15. The cost per 1% reduction of *T. brucei s.l.* varied between $1,133 in Soroti district to $4,636 in Iganga district with an average cost over the study area of $2,872. No value was calculated for Tororo district as there was an 0.3% increase in *T. brucei s.l.* over the study period.

	Prevalence (*T. brucei s.l.*)			Difference in *T. brucei* s.l. prevalence 02-Nov 03	Cost per 1% reduction (*T. brucei* s.l.) in USD.
	Nov 2002	**March 2003**	**Nov 2003**		
Kamuli	12.5%	3.4%	6.0%	6.5%	$2,274
Iganga	7.8%	5.4%	5.3%	2.5%	$4,636
Soroti	22.8%	14.8%	12.5%	10.3%	$1,133
Tororo	1.4%	2.1%	1.7%	-0.3%	n/a
Average over study site	10.6%	5.9%	5.8%	4.8%	$2,872

Table 3.15 – Cost per percentage reduction of *T. brucei s.l.* in each of the study districts. The exchange rate was taken as 1773.60 UGS to 1 USD.

3.4 Discussion

The main aim of the study presented in this chapter was to monitor, by PCR, the effect of a large scale treatment programme against trypanosomiasis by determining the prevalence of *T. brucei* s.l., *T. vivax* and *T. congolense savannah* over the course of the programme in the cattle population. Overall the FITCA Uganda programme appears to have been effective, the prevalence of all tested trypanosome species was 16.1% pre-intervention which was reduced significantly to 9.1% three months post intervention and remained significantly reduced one year post-intervention. The main control method used in this programme was a single dose of trypanocide, either isometamidium chloride or diminazene aceturate to all animals in areas of south-east Uganda which were deemed to be high-risk for human sleeping sickness. This study is a unique investigation into the impact of a large scale real-life control programme, using PCR to determine the effect on the prevalence of specific trypanosome species within the cattle population in south eastern Uganda. The effect of this programme on the zoonotic human infective *T. b. rhodesiense* will be covered in Chapter 4.

The results presented in this chapter look at the overall impact of this programme on the prevalence of *T. brucei* s.l., *T. vivax* and *T. c. savannah* separately. As stated, when these species are considered together there was a significant reduction in post-intervention prevalence, however, this was not true for each individual species. Three month post-intervention, the prevalence of each species showed a reduction, however, none were statistically significant. The reduction in the prevalence of *T. brucei* s.l. was nearing significance and one year post-intervention had showed a significant reduction from the pre-intervention prevalence. There should be caution in interpreting this reduction a year later as it cannot be proved that this was a direct result of the intervention programme and could be a natural reduction over time. As this study was monitoring a real-life control programme it would have been unethical to withhold treatment from an area to allow for a control to monitor the natural variations in the area (see section 3.2.2.3).

3.4.1 District level

The results were broken down at a district level to allow for observation of any differences between the districts included in the study. For all the tested species of trypanosomes there was a statistically significant reduction in prevalence in Kamuli both three month and one year post-intervention both Iganga and Soroti showed reductions in prevalence but these were not significant. Finally in Tororo there was an initial reduction three months post-intervention but this was negligible one year post-intervention.

When the results were considered by district and species the only significant reductions in prevalence were observed in *T. brucei* s.l. in Kamuli and Soroti. The prevalence of *T. vivax* showed no significant changes post-intervention and in fact there was a paradoxical increase in Soroti one year post-intervention although this was not statistically significant. Overall, the prevalence of *T. c. savannah* was very low across all sample areas which made it difficult to assess any impact of the intervention. This low level of *T. congolense spp.* was mirrored in a study published in 1998 on a naturally infected dairy herd in Mukono district in Uganda, where no *T. congolense spp.* were identified by PCR (Clausen *et al.*, 1998). In contrast, a study in Nguruman in neighbouring Kenya, showed that *T. congolense spp.* was most prevalent at 16.5%, followed by *T. vivax* at 4.95% and lastly *T. brucei* at 0.2% (Tarimo-Nesbit *et al.*, 1999). Interestingly the highest prevalence of *T. c. savannah* in this study was detected in Tororo district (2%) which is geographically the closest district to Kenya. The low prevalence of all tested trypanosome species in Tororo may be a sampling artefact rather than a true result as all the sample sites chosen were very close to Tororo town and the Livestock Research Institute (LIRI). Until additional PCR prevalence studies become available for this area, this data can only be compared to prevalence data determined by microscopy. In a recent study by Waiswa *et al*, prevalence in cattle was assessed by microscopy in two of the districts reported here, Kamuli and Tororo (Waiswa *et al.*, 2003). These authors reported *T. brucei* s.l. prevalence in Kamuli at 2.7%, compared with 12% (CI 9.1-14.9) in this study. However, the prevalence of *T. brucei* in Tororo reported by Waiswa *et al* was

6.7%, which is considerably higher than the prevalence detected by PCR in this study 1.4% (CI 0.2-2.6). This anomaly in prevalence levels suggests that selection of different sampling sites may have yielded different results.

The lack of statistically significant changes witnessed in the prevalence of *T. vivax* could be due to variations in the lifecycle of this species. The development of *T. vivax* within the tsetse fly is restricted to only the proboscis (Stevens & Brisse, 2004) which results in a shorter development period within the vector. This means that *T. vivax* transmission will resume more rapidly after any intervention. It is therefore possible that first post-intervention time point at three months was not early enough to observe any reduction in the prevalence of *T. vivax.*

3.4.1.1 Treatment coverage

The differences in impact of this intervention at district level could be linked to the estimates of coverage shown in the results. It is interesting to note that the most significant reductions in overall trypanosome prevalence are observed in Kamuli where coverage was estimated to be over 80%. This corresponds well to a recent theoretical paper by Coleman and Welburn which calculated that a mass treatment intervention would need to have a minimum coverage level of 80% to reduce the parasite load in the animal reservoir (Coleman & Welburn, 2004). However, this theory was not borne out when the change in prevalence at individual sample sites were compared to the level of treatment coverage. There was no significant correlation between reduction in prevalence and level of treatment coverage estimated in this study.

The differences in coverage between the districts were most likely due to local factors; Kamuli and Iganga districts were both part of the original Busoga sleeping sickness focus and as such have had many years of experience implementing trypanosomiasis control programmes. The differences in coverage between these two districts may be due to differing management practices. The mass treatment

campaigns were administered and implemented through the office of the DVO and differences between the approaches of these stakeholders may have affected the execution of the programme. From personal observation it was clear that Kamuli veterinary department was headed by a DVO who placed great importance on the implementation of the FITCA programme. However in Iganga, where sleeping sickness is not such a public health problem (FITCA Quarterly report 2002) , the veterinary department had some problems of motivation amongst its staff and the unfortunate ill health of the DVO may have had an adverse effect on the management of the FITCA programme within the district. Soroti was included in the FITCA programme late and therefore had not been included in the initial planning and organisation stages of the project. Consequently, the original cattle number and prevalence surveys were not completed in Soroti which may have had a knock on effect on the volume of drugs that were supplied. In addition the late inclusion of the district meant there was a delay in receiving the necessary resources to implement the project.

3.4.2 Sample site level

When the results from the monitoring of the FITCA Uganda programme were analysed at a higher level of resolution, i.e. at the individual sample site level, the general patterns seen at a district level became indistinct. Within each district there is very little consistency in the pattern of effect at the individual sample sites, with the exception of *T. brucei* s.l. in Kamuli which showed a reduction in prevalence at each site three months post-intervention, and then an increase at each site from three months to one year post-intervention. These changes were not all statistically significant due to the limitation in sample size when looking at the individual sample sites (n= 25 to164). It is important to observe these changes in prevalence at the individual site to investigate if a single site has a disproportional impact on the results of the district as a whole. In Soroti the effects observed at the district level are driven by the Okidi sample site which experienced dramatic changes in both *T. brucei* s.l. and *T. vivax* over the study period. The prevalence of *T. brucei* s.l. showed a significant reduction from 32.6% pre-intervention to 16.2% three months

post-intervention. However, it is difficult to be entirely certain that this significant reduction can be due to the effect of the intervention alone. The level of treatment coverage in the samples taken three months post-intervention was only 35.5%, which was one of the lowest levels of coverage recorded in this study. Therefore it is possible that the reduction was due to some other unmeasured effect, for example a high turn over of animals at this particular site as animals are officially treated at the point of sale (Fèvre, 2001). Alternatively owners may have treated their animals independently and been unwilling to reveal that for fear of being excluded from further treatments.

3.4.3 Microscopy and PCR

Prior to commencing this intervention, FITCA Uganda carried out a comprehensive cattle survey to determine where to target trypanocidal treatments. These data were collected by microscopy and compared with the results from the PCR survey detailed in this Chapter. As might be expected, there were a number of significant differences between the prevalence determined by microscopy and the prevalence determined by PCR (Picozzi *et al.*, 2002). In particular, the prevalence of *T. brucei* s.l. by microscopy was significantly lower than by PCR. For example, *T. brucei* s.l. prevalence in Kamuli was 12.5% by PCR compared with only 1.1% by microscopy. While PCR diagnosis of trypanosomes is known to be considerably more sensitive than microscopy, the level of difference in prevalence seen in Kamuli by the two methods is considerable. By comparison, studies show PCR trypanosome diagnosis to be 2-3 times more sensitive than microscopical diagnosis (Clausen *et al.*, 1998; Solano *et al.*, 1999). In addition, Duvallet *et al,* reviewed the use of PCR in West Africa and noted that 50% of parasitologically negative cattle with a haematocrit value of under 25% were shown to be positive for trypanosomes by PCR (Duvallet *et al.*, 1999).

Interestingly, the prevalence of trypanosome infection in Tororo was considerably higher by microscopy than PCR. This anomaly is likely due to the different sample sites used for each survey. The integrity of the sample sites used in this study appear

to have been compromised by their proximity to LIRI, which as a research institute has implemented trial control measures in this area. This confounding factor was unfortunately not identified at the study outset.

3.4.4 Study limitations

Field-based research has inherent problems associated with it, not least the inability to control all experimental parameters. As discussed previously, problems were identified with the location of some sample sites, most specifically in Tororo. In addition, the lack of control sites for ethical reasons meant that natural variation in disease prevalence due to any confounding factors could not be accounted for. Possible factors affecting the study outcomes may include: ongoing socio-economic activities associated with subsistence farming such as the sale and slaughter of livestock, unrecorded treatment of cattle, general use of insecticides and environmental changes.

Analysis of the dataset proved complicated for a number of reasons, not least of which was that the animals sampled in this study were not individually identified by tagging. As a consequence, there was no way to account for the potential of repeatedly sampled animals which meant the level of dependence between sample times could not be determined. The animals could not be tagged as logistically and financially there were no resources available to closely follow this number of animals (n=3,920). Additionally there is an ethical issue when dealing with a potentially lethal zoonotic pathogen as there is a moral obligation to give additional treatment to any animal found to be harbouring human infective parasites. For these reasons it was decided that the objective of the study would be to monitor the effect of the intervention programme at the herd level at each study site. Unfortunately it was very difficult to observe an effect at this level due to the sample sizes at individual sites; therefore it becomes more meaningful to cluster the sites at the district level. However, the sample number for analysis at the district level can only be the number of sites (Kamuli n=3, Iganga n=4, Soroti n=2 and Tororo n=3) and these low n values restrict the type of statistical tests which can be used thus reducing

the power of any test. Consequently, certain assumptions regarding the normality and independence of the prevalence data were made. Paired T tests were used to compare prevalence within districts over the intervention time points; these tests allow dependence between time points but do assume normality of the data. Fisher exact tests were used to compare each sample site individually over time. Small sample numbers necessitated the use of Fisher exact tests rather than conventional chi-squared tests, however, these tests do assume independence of data.

3.4.5 Cost effectiveness of FITCA Uganda treatment intervention

The second aim of this Chapter was to examine the cost of implementing a mass treatment programme in the four study districts. The main costs for each area were the trypanocides, fuel, vehicles and wages. The first cost which varied by district was the volume of drugs provided which was calculated by the number of areas to be treated and the estimated number of cattle in those areas. The second was the number of staff employed to deliver the intervention, although fuel and vehicle costs remained constant some districts chose to employ more staff from the DVO to implement treatment. The calculated costs were displayed per head of population, per household and per cattle keeping household for each district, these were calculated for the whole district not just the areas covered under FITCA as it was accepted that any reduction in human or animal trypanosomiasis would benefit the whole district. It was estimated that the FITCA treatment programme cost $0.50 per livestock keeping household. This can be favourably compared with the cost of treating an animal for trypanosomiasis, which costs approximately $1.00. However, it is important to stress that the calculated costs for the FITCA programme did not include administration or salary costs so the actual cost would be much greater. The cost data were also analysed, in terms of cost per 1% reduction in the level of animal trypanosomiasis in sampled cattle from each district. The most successful district in terms of cost for result was Kamuli, where the cost was $1,408 per 1% reduction of prevalence after one year. The least successful in terms of cost for benefit was Tororo where the cost for a 1% reduction in animal trypanosomiasis was $34,338. This figure is so high because one year post-intervention there was only a 0.5%

reduction in prevalence. These reductions are based on the PCR results from the sites sampled in this study which may not be representative of the district as a whole. This is especially important in the analysis of Tororo as prevalence levels detected in this study are considerably lower than other published results.

The cost assessment carried out in this Chapter was not a full cost-benefit analysis as during the period of this research there was not the time nor financial support to collect data on the general costs of livestock both healthy and those with trypanosomiasis. Thus, no comment can be made on the relative health and economic benefits of treating animals against trypanosomiasis. This meant that the cost of the programme could not be offset against the benefits to small holder farmers to establish whether the intervention could be deemed cost effective. It is important to emphasise at this time that this was not a full audit of the FITCA Uganda programme and the costs listed only referred to the mass treatment of cattle with trypanocides. The final results shown in this chapter stated the cost per 1% reduction in overall trypanosomiasis in the sampled cattle in each of the four districts. This result should be interpreted with caution as without control sites; it cannot be categorically shown that the reduction in prevalence was due to the intervention and not any other confounding factors.

3.4.6 Conclusions

As with all control programmes in the developing world there is the issue of sustainability. In most circumstances when the initial control operations are funded, controlled or assisted by foreign donors the cessation of funding invariably results in the collapse of the intervention effort. Local communities are either not motivated to maintain control because they consider it a public health service or more commonly are too poor to continue the work. Another pertinent issue is that for many communities tsetse fly control and elimination of sleeping sickness are not the main health concerns for the people living there. Malaria and HIV/AIDS have a far greater impact on many of these communities (World Health Organization, 2004).

The FITCA intervention assessed in this Chapter is an example of a top-down approach; however, the treatment of cattle as a control method is recognised as a strategy that can be implemented effectively by individual farmers. Unfortunately the majority of subsistence farmers treat their animals only when they are sick and not prophylactically. Consequently, local cattle breeds may harbour infection but not exhibit clinical pathology. This scenario is most common when cattle are infected with the potentially human-infective trypanosomes, *T. brucei* s.l.

Concerns do remain regarding the large-scale use of trypanocides leading to the occurrence of drug resistance. There have been reports of drug resistance against both diminazene aceturate and isometamidium chloride in Ethiopia (Codjia *et al.*, 1993; Leak *et al.*, 1993; Rowlands *et al.*, 1993) but so far there have been no studies showing any significant levels of drug resistance in Uganda. However, concern may be warranted as there were claims that in Ethiopia the emergence of drug resistance in *T. congolense spp.* parasites was associated with the wide-scale use of trypanocides (Codjia *et al.*, 1993; Rowlands *et al.*, 1993; Itty *et al.*, 1995).

Analysing a working intervention programme is not a simple matter as it is not possible to design or control many aspects of the study such as control sites or the timing or choice of intervention sites. But the merits of this should not be underestimated as it is an advantage to look at a true control programme with its many real-life pitfalls rather than a study under a completely scientifically controlled environment. It is important to look at the limitations of how and why things are implemented as they are in the field and try to assess what impact a theoretically 'good' intervention programme will have when faced with the realities of implementation in the developing world.

4 Chapter 4:

Impact of the FITCA intervention programme on the prevalence of *T. b. rhodesiense* in cattle in eastern Uganda

4.1 Introduction

In Chapter 3, the impact of the FITCA Uganda intervention on the prevalence of animal trypanosome species (*T. brucei* s.l., *T. vivax* and *T. congolense*) was investigated. This Chapter carries on this assessment but focuses solely on the prevalence of *T. brucei* s.l. and in particular *T. b. rhodesiense*. *T. b. rhodesiense* is a serious human pathogen responsible for considerable morbitiy and mortality sub-Saharan Africa (Fèvre *et al.*, 2006a). In Uganda, cattle represent a significant disease reservoir and indeed results in Chapter 3 demonstrated that *T. brucei* s.l. represented the most abundant trypanosome species. Without further investigation, however, it is impossible to know what proportion of identified *T. brucei* s.l. are the human-infective sub-species *T. b. rhodesiense*. Using PCR techniques this Chapter aims to identify the proportion of *T. brucei* s.l. infected cattle which are harbouring human infective *T. b. rhodesiense*.

4.1.1 *T. brucei* s.l.

There are three sub-species of *T. brucei* s.l.: *T. brucei brucei, T. brucei rhodesiense* and *T. brucei gambiense*. *T. b. brucei* is found across sub-Saharan Africa, *T. b. rhodesiense* is found in east and southern Africa, while *T. b. gambiense* is found in west and central Africa (Pepin & Meda, 2001). Uganda is unique in that both species of human-infective trypanosomes are present although their foci are currently distinct with *T. b. gambiense* in the northwest and *T. b. rhodesiense* in the southeast of the country (Welburn *et al.*, 2001a). In the areas under investigation in this thesis only *T. b. rhodesiense* is present. The three sub-species are morphologically identical but they differ in host range, *T. b. rhodesiense* and *T. b. gambiense* infect and cause disease in humans, while *T. b. brucei* cannot survive in human serum (Buscher & Lejon, 2004). *T. b. rhodesiense* and *T. b. brucei* can both infect cattle but in native African breeds the clinical symptoms are less pronounced and often the infection goes undetected (Welburn *et al.*, 2006).

Humans are unusual amongst mammals in being resistant to *T. b. brucei*, this resistance is due the presence of a trypanolytic factor. This trypanolytic factor was recently established to be Apolipoprotein L-1 (Vanhamme *et al.*, 2003), which causes lysis through the formation of pores in the trypanosomes lysosomal membrane (Perez-Morga *et al.*, 2005). The human infective trypanosomes, *T. b. rhodesiense* and *T. b. gambiense* have acquired resistance to this lytic factor, enabling them to establish patent infections causing disease, although the mechanisms to resistance differ. In *T. b. rhodesiense*, resistance to human serum is linked to antigenic variation which appears to be found at one expression site (R-ES) systematically selected for activity in human serum (Xong *et al.*, 1998). This expression site contains an ESAG termed SRA, which upon transfection into *T. b. brucei* parasites conferred resistance to human serum (Xong *et al.*, 1998). This identified *SRA* as the gene responsible for *T. b. rhodesiense* resistance to human serum. SRA is a truncated variant surface glycoprotein (VSG) with the region encoding the surface-exposed epitopes missing (Vanhamme & Pays, 2004) and was probably formed during DNA recombination of VSGs (Pays *et al.*, 2006). *SRA* is consistently found in human resistant stocks from across East Africa (Welburn *et al.*, 2001b; Gibson *et al.*, 2002).

4.1.2 Fitness cost of *T. b. rhodesiense*

Where *T. b. brucei* and *T. b. rhodesiense* co-exist, epidemiological theory predicts that the prevalence of *T. b. rhodesiense* would be higher than that of *T. b. brucei* due to the fact that *T. b. rhodesiense* can survive in a wider range of hosts (Coleman & Welburn, 2004). Based on Rogers general model for African trypanosomes (Rogers, 1988), assuming that the sub-species *T. b. brucei* and *T. b. rhodesiense* differ only in human infectivity, the model would predict that the basic reproductive number (Ro) of *T. b. rhodesiense* will be greater than that of *T. b. brucei*. Thus the prevalence of *T. b. rhodesiense* in non-human hosts (e.g. a domestic cattle reservoir) should always exceed that of *T. b. brucei*. This assumption has been investigated (Coleman & Welburn, 2004) using data from southeast Uganda (Hide *et al.*, 1996), which predicted that there would be a three times higher prevalence of *T. b. rhodesiense*

than *T. b. brucei* in the cattle reservoir and a 3.5 times higher prevalence within the vector population (Coleman & Welburn, 2004). However field observations have shown that this is not the case (Gibson & Wellde, 1985; Enyaru *et al.*, 1993; Hide *et al.*, 1996; Welburn *et al.*, 2001b; Waiswa *et al.*, 2003) and in fact the opposite is true, *T. b. brucei* always seems to predominate with a higher prevalence than *T. b. rhodesiense*. This led Coleman and Welburn to hypothesise that there are fitness costs associated with *T. b. rhodesiense* infections when outside the human host (Coleman & Welburn, 2004). In Uganda, cattle are the main reservoir host of *T. b. rhodesiense* and it is consequently of great epidemiological importance to able to distinguish the two species within the cattle reservoir population.

4.1.3 Methods of detection

Historically, the first tests to establish whether *T. brucei* s.l. parasites were human infective was to inoculate parasites found in an animal host into a human 'volunteer'. Heisch and colleagues took parasites from a bushbuck and inoculated them into a human volunteer, resulting in an infection (Heisch *et al.*, 1958). In a similar experiment, Onyango and colleagues took parasites isolated from infected cattle and showed that an infection could be caused in humans (Onyango *et al.*, 1966).

In 1970, the blood infectivity incubation test (BIIT) was developed. This test demonstrated the parasites ability to survive challenge with human serum in a mouse model thereby eliminating the need for testing on humans (Rickman & Robson, 1970). The test was subsequently validated by Geigy and colleagues on wildlife material. They tested blood from a variety of species and showed that blood taken from a *T. brucei* s.l. infected Coke's hartebeest was positive using the BIIT and infectious to human volunteers (Geigy *et al.*, 1973). The BIIT technique is still in use today and has shown the existence of *T. b. rhodesiense* in a variety of species; reedbuck, waterbuck, spotted hyena and lion (Gibson & Wellde, 1985) as well as domestic pigs (Waiswa *et al.*, 2003).

Since the advent of molecular methods of parasite characterisation, new ways have been developed to detect *T. b. brucei* and *T. b. rhodesiense*: restriction fragment length polymorphisms (RFLP) (Hide *et al.*, 1994), analysis of variability in mobile genetic elements by PCR (MGE-PCR) (Tilley *et al.*, 2003) and minisatellite marker analysis (MacLeod *et al.*, 2000). All of these methods require significant amounts of parasite DNA which means that the parasite material from a host animal must be amplified in mice prior to application of the technique. However, not all *T. brucei* s.l. collected in the field will amplify in mice; up to 50% of *T. brucei* s.l. field samples collected in southeast Uganda are lost during mouse passage (Welburn, pers com.). This has meant that estimates of the prevalence of both *T. b. brucei* and *T. b. rhodesiense* using BIIT, RFLP, MGE-PCR and minisatellite analysis are underestimates (Welburn *et al.*, 2005).

The *SRA* (serum-resistance-associated) gene is a single copy gene that can be used as a marker to distinguish *T. b. brucei* from *T. b. rhodesiense* (Xong *et al.*, 1998). It is conserved in all known *T. b. rhodesiense* isolates (Gibson *et al.*, 2002) as well as within human and domestic animal reservoirs (Welburn *et al.*, 2001b; Gibson *et al.*, 2002; Radwanska *et al.*, 2002a) in East Africa. Consequently, the *SRA* gene has been developed for use as a diagnostic marker for the identification of *T. b. rhodesiense* infections (Welburn *et al.*, 2001b). There are, however, problems with using this gene as a PCR marker as *SRA* is a single copy gene and infections in the animal reservoir can be chronic with low levels of parasitemia (Katunguka-Rwakishaya, 1996). A negative result therefore, may be due to a lack of parasite DNA rather than a true absence of *T. b. rhodesiense* infection. On the basis of this, Picozzi and colleagues have developed a multiplex PCR which contains two different sets of primers (Picozzi *et al.*, *In press*). One set, targets the *SRA* gene as a diagnostic for *T. b. rhodesiense* and one targets *PLC*, a phosopholipase C (*GPI-PLC*) which is a single copy gene found in *T. brucei* s.l. (Hereld *et al.*, 1988; Carrington *et al.*, 1989). The positive amplification of the *PLC* sequence indicates that there is enough genetic material present in the sample to detect the presence or absence of the *T. b. rhodesiense SRA* gene (Picozzi *et al.*, *In press*).

4.1.4 Chapter aims

The aim of this Chapter is to investigate the impact of the FITCA Uganda intervention on the prevalence of human-infective *T. b. rhodesiense* in the cattle reservoir. Samples which were identified in Chapter 3 as positive for *T. brucei* s.l. were re-analysed using the *PLC-SRA* multiplex PCR at both pre- and post-intervention time points. The levels of *T. b. rhodesiense* identified will be presented as a percentage of *T. brucei* s.l. infections rather than a percentage of total samples collected. However, the relative contribution of *T. b. rhodesiense* to the total number of trypanosome infections identified within cattle in each district will be discussed. In addition, this Chapter will present data collected from Soroti at a third time point, 18 months post-intervention, to investigate whether any lasting impact of the FITCA intervention could be detected.

4.2 Materials and Methods

4.2.1 Impact of the FITCA intervention on prevalence of *T. b. rhodesiense*

4.2.1.1 Sample collection

Blood samples were collected from cattle in four districts (Kamuli, Iganga, Soroti and Tororo) and stored on FTA cards as described in Chapter 3. The samples were collected at three time points to assess the effectiveness of the FITCA intervention programme. Baseline samples were collected pre-intervention and then samples were collected at 3 and 12 months post-intervention. The specific details of the study sites and sample collection are detailed in Chapter 3, section 3.3.2. Any samples which were found to be positive for *T. brucei* s.l. in Chapter 3 were selected for inclusion in this study. These samples were then tested for *T. b. rhodesiense* using a multiplex PCR for both *T. brucei* s.l. and *T. b. rhodesiense*.

4.2.1.2 Sample analysis

A multiplex PCR was used to detect the presence of the *T. b. rhodesiense SRA* gene (Picozzi *et al.*, *In press*). As mentioned, *T. brucei* s.l. *PLC* was amplified as a positive control to ensure sufficient parasite DNA was present in the samples. PCR amplifications were carried out in 25µl reaction volumes, each containing 1.5 Units of HotstarTaq® DNA polymerase (Qiagen), 1.25 µl of rediload dye (Invitrogen), with a final concentration of 3 mM $MgCl_2$, 200µM of each of the 4 deoxynucleoside triphospahtes and 0.2 µM of each primer. PCR products were separated by electrophoresis using a 1.7% agarose gel electrophoresis (see section 3.2.2.9). Primer sequences and cycling steps are shown in Table 4.1.

Trypanosome species	Code	Sequence	Product size	Reference
T. b. rhodesiense	SRA – B651	5'-GAA GAG CCC GTC AAG AAG GTT TG-3'	669bp	Picozzi *et al. in press*
	SRA – B652	5'-TTT TGA GCC YYC CAC AAG CTT GGG-3'		
	PLC – B657	5'-CGC TTT GTT GAG GAG CTG CAA GCA-3'	325bp	
	PLC – B658	5'-TGC CAC CGC AAA GTC GTT ATT TCG-3'		
Amplification conditions: 94°C for 15mins [(42 cycles) 94°C for 30s, 63°C for 90s, 72°C for 70s] 72°C for 10mins, storage at 4°C				

Table 4.1 – Primer sequences, amplification conditions and product size of *T. brucei s.l.* and the multiplex PCR for *T. b. rhodesiense*

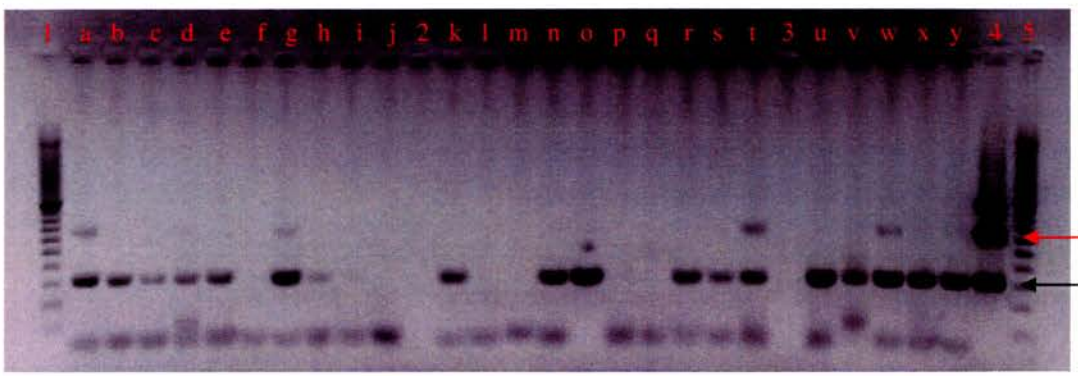


Figure 4.1- Agarose gel picture illustrating the PCR products from the *PLC-SRA* multiplex, the black arrow shows the *PLC* and the red arrow shows *SRA*. Lanes 1 and 5 are molecular ladder, lanes 2 and 3 are negative controls, lane 4 is the positive control and lanes a – y are samples. Lanes a-e, g-i, k, n, o, r-t and u-y are positive for *PLC*, lanes a, g, t and w are positive for *SRA*.

4.2.1.3 Blotting

Southern blotting was performed on each of the samples to increase the detection sensitivity of *T. b. rhodesiense* and to ensure that the correct products were identified. Southern blotting is a technique where DNA is transferred from a gel on to a positively charged membrane. A labelled probe of the desired sequence is then introduced to the membrane under strict conditions. The presence of the desired

PCR sequence on the membrane is visualised by activation of the labelled probe that has bound to the membrane.

4.2.1.3.1 Probe preparation

To obtain the probe, genomic DNA of *T. b. rhodesiense* (LIRI024) was amplified by PCR as described in section 3.2.1.2, this amplification generated both *SRA* and *PLC* material. The products were separated by gel electrophoresis (see section 3.3.10) and the bands of *SRA* and *PLC* were excised. The DNA was then extracted using a MiniElute Gel extraction kit (Qiagen) according to the manufacturers instructions, following the centrifugation extraction protocol. The extracted *SRA* and *PLC* DNA were then denatured at 100°C for 10 minutes and immediately cooled on ice. The DIG-High Prime (Roche) labelling mix was then added in a 1:5 ratio and incubated at 37°C for a minimum of 1 hour. This reaction was stopped by heating the DNA and label to 65°C.

4.2.1.3.2 Probe yield estimation

A 10x dilution series was prepared from DIG-labelled control DNA provided by the manufacturer, ranging from 1ng μl^{-1} to 0.1pg μl^{-1}. A similar dilution series was prepared using the newly labelled *SRA* and *PLC* probe. 1µl of each dilution, both control and freshly labelled *SRA* and *PLC*, DNA fragments were spotted onto a positively charged nylon membrane and allowed to air dry. The probes were then fixed to the membrane by a 5 minute exposure to UV light. The membrane was washed twice with 1x Washing buffer (0.1M maleic acid, 0.15M NaCl: pH 7.5, 0.3% (v/v) Tween®20), then incubated for 30 minutes at room temperature in 1x Blocking solution (1g blocking reagent in 100cm^3 washing buffer without the Tween®20). This was followed by 30 minute incubation at room temperature with Anti-DIG-alkaline phosphatase, diluted 1:5000 in blocking solution. The membrane was then washed twice in 1x Washing buffer for 2x15 minutes and then incubated with detection buffer (0.1M Tris-HCl at pH 9.5; 0.1M NaCl) for 2 minutes. The colour

substrate solution (80µl NBT/BCIP in $10cm^3$ detection buffer) was then introduced to the membrane; colour development was carried out in the dark, without shaking. The reaction was stopped by a 5 minute wash in H_20. Probe yield was estimated by a visual comparison of colour development of the control and test probe dilutions.

4.2.1.3.3 Transfer

After agarose gel electrophoresis of the PCR products, the gel was soaked in denaturing solution (0.5M NaOH, 1.5M NaCl) for 20 minutes at room temperature, to ensure denaturation of the DNA within the gel to single strands. The gel was then soaked in neutralising solution (0.5M Tris-HCl at pH 7.5; 3M NaCl) for a further 20 minutes also at room temperature. Transfer of DNA from the gel to the nitrocellulose membrane was performed on a vacuum blotter (QBiogene). For the transfer, the membrane was soaked in 20x SSC (3M NaCl, 0.3M sodium citrate, pH 7) which inhibits the re-naturation of the DNA. The membrance was placed on top of a carrier filter paper (Whatman 3mm), which was also soaked in SSC, and place between the vacuum blotter and the gel (Figure 4.2). Negative pressure of 65mbar was applied on the vacuum blotter over 1 hour. Throughout the transfer procedure, it was ensured that the gel was covered with 20x SSC to prevent air bubbles blocking the DNA transfer. After the transfer the position of the wells was marked on the membrane by punching through the wells with a pipette tip. The DNA was permanently bound to the membrane by a 5 minute exposure to UV light; this cross-links the DNA to the membrane. During exposure to UV light the membrane was placed on a filter paper (Whatman 3mm) soaked with 10x SSC, to avoid drying. After UV cross-linking, the membrane was washed in double distilled water and allowed to air dry, at this point the membrane can be stored at 2-8°C.

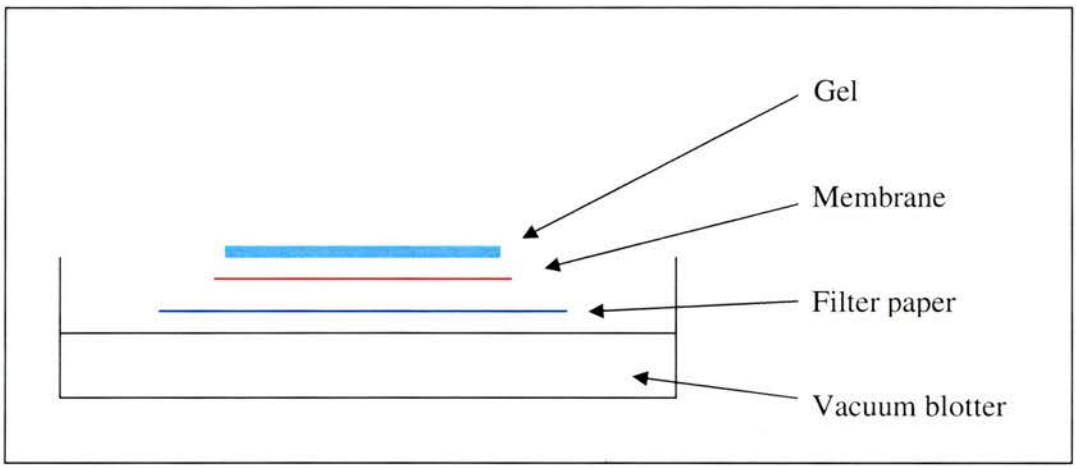


Figure 4.2 – Diagram showing the arrangement for southern blotting

4.2.1.3.4 Hybridisation

The nitrocellulose membrane was pre-hybridised in a standard hybridisation buffer (Roche) at 42°C for 1 hour. This prepared the membrane by blocking any non-specific nucleic acid binding sites.

The DNA probes was denatured, by heating at 100°C for 10 minutes, then directly transferred on to ice. The probes were then diluted to 25ng cm-3 in preheated hybridisation buffer and introduced to the membrane. Hybridisation with the probes took place overnight at 42°C. The membrane was then washed twice, 2 x 5 minutes, in 2x SSC (0.3M NaCl, 30mM sodium citrate, pH 7, 0.1% (w/v) SDS) at room temperature, which removed any unbound probe. The membrane was then washed twice, 2 x 15 minutes, in 0.5 x SSC (75mM NaCl, 7.5mM sodium citrate, pH7, 0.1 (w/v) SDS) at 68°C. Regions of hybridisation were detected as described in section 4.2.1.3.2 estimation of probe yield.

4.2.1.4 Screening using 5 repeats of each sample

On the FTA card, parasite material will not be uniformly distributed over the surface of the card, so five 2mm diameter circular punches were taken from each sample on the card to maximise the chance of amplifying parasite material. If after five punches no amplification of *PLC* was observed it was assumed that the parasitemia was too low to correctly distinguish between *T. b. brucei* and *T. b. rhodesiense*. Only those samples that were *PLC* positive were included in this analysis.

4.2.2 Soroti district follow up study [1]

A follow up study was carried out in Soroti district in April 2004, 18 months after the mass administration of isometamidium chloride and diminazene aceturate by FITCA Uganda. Blood samples were taken from cattle in areas that had been included in the FITCA intervention and also from areas which had not.

4.2.2.1 Sampling sites

Sampling sites were selected in Soroti to assess the continued effectiveness of the FITCA intervention programme. Three sites were selected from an area which had been included in the FITCA intervention programme; each of these three sites had reported cases of sleeping sickness pre-intervention. An additional three sites were selected that had not been included in the FITCA intervention; these sites were selected on the basis of a recent reported case of sleeping sickness (January-April 2004). In addition samples were collected from a livestock market within the district, Brookes Corner, which had been at the centre of the original reported outbreak starting in 1998 (Fèvre *et al.*, 2001).

([1] Work presented in this section was published in (Fèvre *et al.*, 2005))

4.2.2.2 Sample collection

Farmers were asked to present their animals to a central point for sampling at which point blood samples were taken from ear veins and blood was spotted directly onto FTA cards (see section 3.2.2.). 51-56 animals were sampled at each site (Table 4.2). The incentive of a de-worming treatment was offered for participating farmers and animals were sampled on a convenience basis.

Site description	Name of site	Sample number
Intervention sites	Obur	56
	Obar	55
	Akoroi	56
	Total	167
Non-intervention site	Omagoro	56
	Odunguru	56
	Ayepe	56
	Total	168
Market	Brookes Corner	51

Table 4.2 – Number of samples collected in the Soroti follow-up study

Exact details regarding the sampling methodology and analysis can be found in Fèvre *et al*, 2005 (shown in appendix).

4.2.3 Statistical analysis

4.2.3.1 Confidence intervals

All confidence intervals were calculated as binomial confidence intervals in R version 2.4.0 (R project)(Figure 3.7).

```
round(100*(binconf(baseline_tbrucei_by_district$tbrucei,baseline_tbrucei_by_district$number)),1)
```

Figure 4.3 – Example of code used for calculating binomial confidence intervals in R, this example was used to calculate the CI for *T. brucei* s.l. at the district level.

4.2.3.2 Comparison of baseline data from all districts

To investigate any differences between the prevalence of *T. brucei* s.l. and *T. b. rhodesiense* in the different study areas χ^2 tests were conducted in Minitab version 14 (Minitab, Inc.). Differences were considered to be significant at $p<0.05$.

4.2.3.3 Comparison of pre- and post-intervention

To investigate if there were any significant differences within districts included in the study pre- and post-intervention, paired t-tests were carried out using Minitab version 14 (Minitab, Inc.). Differences were considered to be significant at $p<0.05$.

4.3 Results

4.3.1 Description of the samples

T. brucei s.l. positive samples were tested for *SRA* to determine the prevalence of *T. b. rhodesiense*. Table 4.3 shows the number of *T. brucei* s.l. samples successfully tested by the multiplex PCR and the number from which *PLC* was amplified. As discussed, only those samples positive for *PLC* were confirmed as containing enough DNA to detect the single copy *SRA* gene.

District	**No. tested by multiplex**	**No. positive for *PLC* *T. brucei* s.l. marker (%)**
Kamuli	60	51 (85%)
Iganga	47	38 (81%)
Soroti	124	113 (91%)
Tororo	13	13 (100%)

Table 4.3 – Detection rate of single copy marker (*PLC*) for *T. brucei* s.l.

4.3.2 Baseline pre-intervention results

Figure 4.4 shows the prevalence of *T. b. rhodesiense* as a percentage of total *T. brucei* s.l. positive samples. Kamuli and Soroti were found to have the highest proportion of *T. b. rhodesiense* - 56% and 28% respectively. Prevalence was 20% in Tororo but this represents only one positive sample out of five *T. brucei* s.l. samples. Iganga as a district had a considerable portion of *T. brucei* s.l. samples (see Chapter 3) but unfortunately many of these did not amplify well in the multiplex PCR. As a result, out of the nine successful amplifications, the prevalence of *T. b. rhodesiense* in Iganga was shown to be 11%.

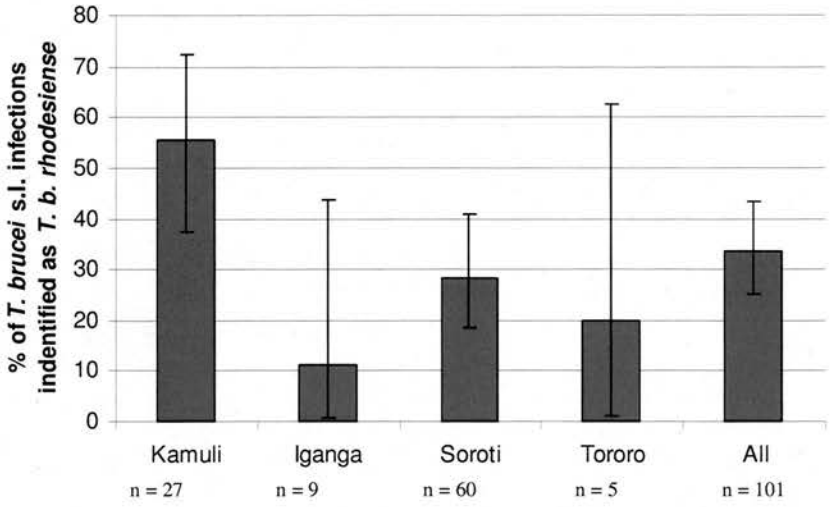


Figure 4.4 – Graph showing the minimum baseline prevalence of *T. b. rhodesiense*, n represents the total number of samples positive for *T. brucei* s.l. marker. Error bars represent 95% binomial confidence intervals.

It is clear from Figure 4.4 that *T. b. rhodesiense* makes up a considerable portion of *T. brucei* s.l. infections at baseline in each of the studied districts. Figure 4.5 illustrates the overall minimum prevalence of *T. b. rhodesiense* within the total sampled population at baseline. The highest prevalence of *T. b. rhodesiense* is in Soroti - 6%, followed by Kamuli where the overall prevalence is just under 4%. In both Tororo and Iganga the prevalence of *T. b. rhodesiense* detected in the collected samples was significantly lower than the prevalence found in Kamuli (for Iganga $\chi^2 = 10.09$, df = 1, $p<0.001$) and Soroti.

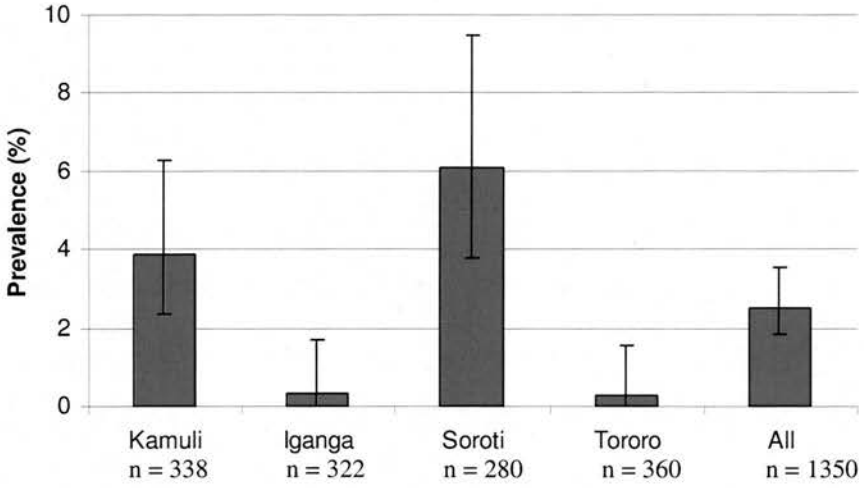


Figure 4.5 – Minimum prevalence of *T. b. rhodesiense* across the districts at baseline, n represents the total number of samples in each district. Error bars represent 95% binomial confidence intervals.

4.3.2.1 Impact of the intervention

4.3.2.1.1 All districts

Figure 4.6a shows the combined prevalence of *T. brucei* s.l. in all districts at each sampled time point. Figure 4.6b shows the prevalence of *T. b. rhodesiense* detected from those samples positive for *T. brucei* s.l. at each sampled time point. The prevalence of *T. brucei* s.l., as determined by the presence of *PLC,* reduces step-wise at each consecutive time point. The pre-intervention prevalence of 7.5% reduces to 4% one year post-intervention ($p<0.05$) (Figure 4.6a). Pre-intervention, 33% of the of *T. brucei* s.l. was found to be *T. b. rhodesiense*, this reduced significantly three months post-intervention to under 10% ($p<0.001$)(Figure 4.6b). However, despite a continued decrease in the overall prevalence of *T. brucei* s.l. one year post-intervention, there was an increase in the relative percentage of those infections which were *T. b. rhodesiense* (Figure 4.6b).

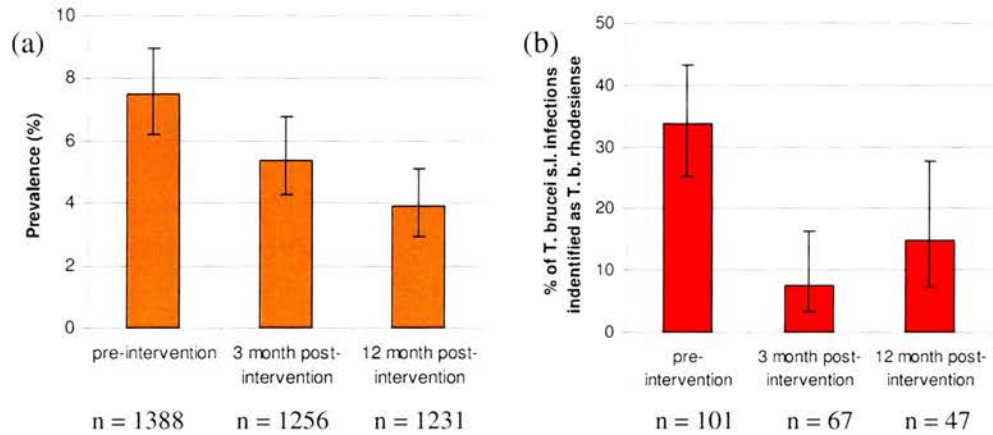


Figure 4.6 – Graphs showing the prevalence of (a) *T. brucei* s.l. (yellow) confirmed by amplification of the *PLC* marker as a percentage of the total number of samples collected in all sites and (b) *T. b. rhodesiense* (red) as a percentage of the total number of *T. brucei* s.l. positive samples in all sample sites. n represents the total number of samples positive for the specific trypanosome species displayed. Error bars represent 95% binomial confidence intervals.

4.3.2.1.2 Kamuli

If we look more closely at the results, some variations in the prevalence of *T. b. rhodesiense* at the district level can be observed. Figure 4.7a shows the prevalence of *T. brucei* s.l. in Kamuli district at each sampled time point. The pre-intervention prevalence of *T. brucei* s.l., confirmed by the amplification of *PLC*, reduced three months post-intervention and then increased slightly one year post-intervention. Figure 4.7b shows the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples in Kamuli. Pre-intervention, 55% of the 27 tested *T. brucei* s.l. samples were shown to be *T. b. rhodesiense*. However, at both three and twelve months post intervention no *T. b. rhodesiense* infections were detected.

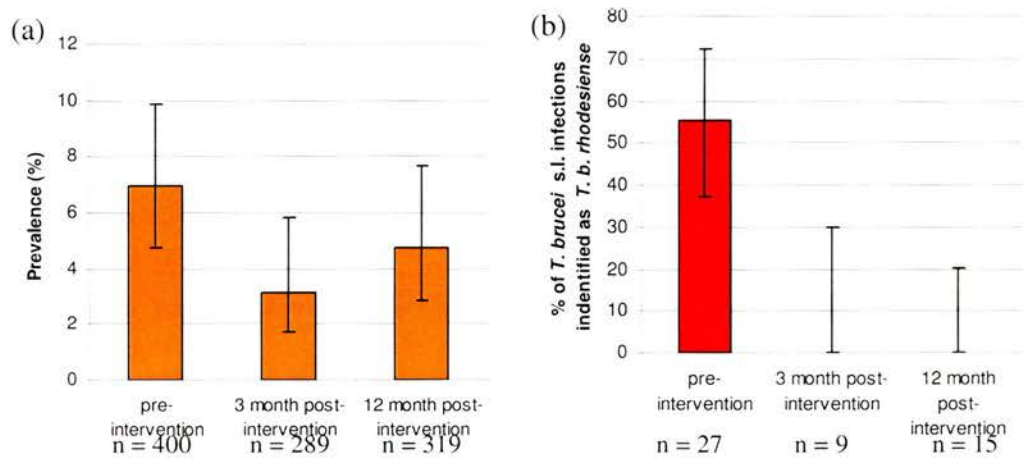


Figure 4.7 – Graphs showing the prevalence of (a) *T. brucei* s.l. (yellow) confirmed by amplification of the *PLC* marker as a percentage of the total number of samples collected in Kamuli and (b) *T. b. rhodesiense* (red) as a percentage of the total number of *T. brucei* s.l. positive samples in Kamuli at each time point. n represents the total number of samples positive for the specific trypanosome species displayed. Error bars represent 95% binomial confidence intervals.

4.3.2.1.3 Iganga

Figure 4.8a shows the prevalence of *T. brucei* s.l. in Iganga at each sampled time point. Pre-intervention, the prevalence of *T. brucei* s.l., confirmed by the amplification of *PLC* was very low compared to other districts – under 3%. Post-intervention, the prevalence of *T. brucei* s.l. increased in the initial three months before decreasing one year post-intervention; however, neither change was significant. Figure 4.8b shows the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples. Very low numbers of *T. b. rhodesiense* were detected both pre- and three month post-intervention, one and three respectively, while no infections were detected one year post-intervention.

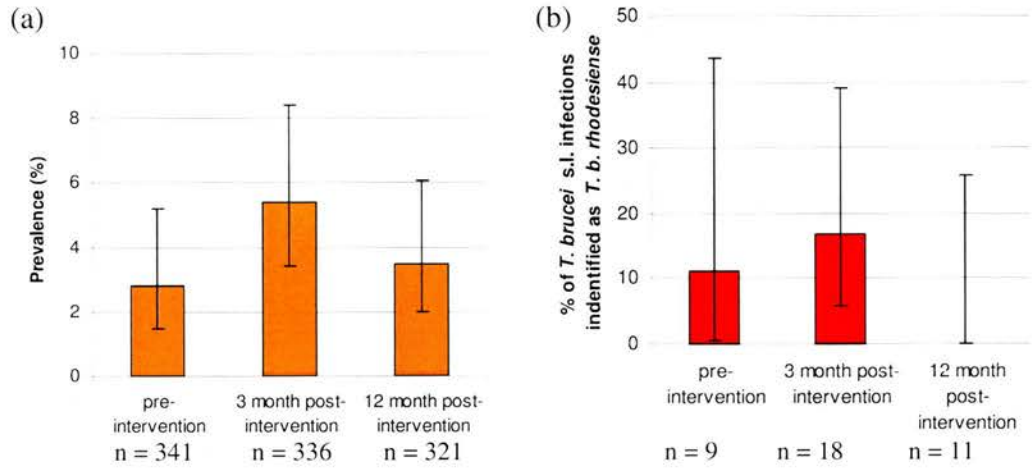


Figure 4.8 – Graph showing the prevalence of (a) *T. brucei* s.l. (yellow) confirmed by amplification of the *PLC* marker, as a percentage of the total number of samples collected in Iganga at each time point and (b) *T. b. rhodesiense* (red) as a percentage of the total number of *T. brucei* s.l. positive samples in Iganga at each time point. n represents the total number of samples positive for the specific trypanosome species displayed. Error bars represent 95% binomial confidence intervals.

4.3.2.1.4 Soroti district

Figure 4.9a shows the prevalence of *T. brucei* s.l. within sampled cattle in Soroti. The pre-intervention prevalence of *T. brucei* s.l., confirmed by the amplification of *PLC*, was 21%. This reduced at both three months and one year post-intervention ($p<0.01$). Figure 4.9b shows the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples. Pre-intervention, 28% of *T. brucei* s.l. samples were confirmed as *T. b. rhodesiense*. This reduced dramatically three months post-intervention to less than 10% ($p<0.05$). One year post-intervention, despite a continued decrease in the prevalence of *T. brucei* s.l. (Figure 4.9a), there was a dramatic increase in the percentage (32%) ($p<0.01$) of those samples which were *T. b. rhodesiense* (Figure 4.9b).

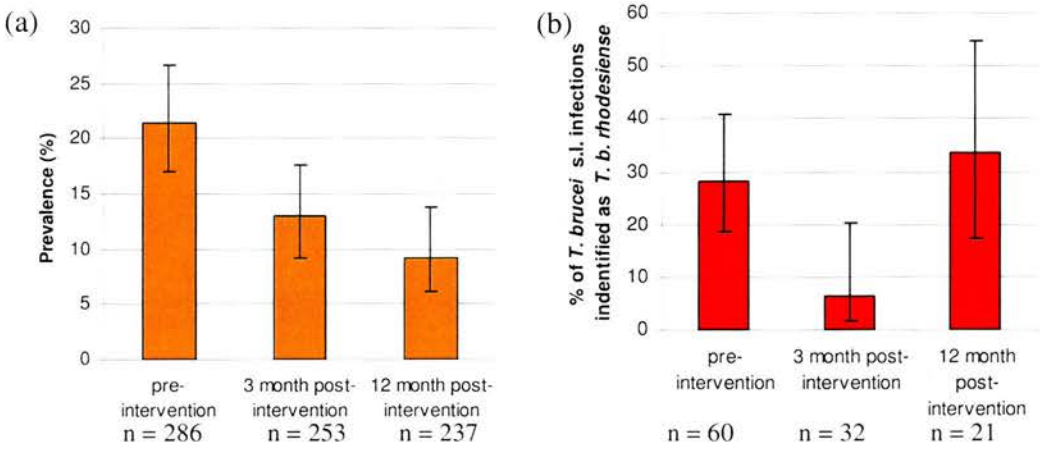


Figure 4.9 Graph showing the prevalence of (a) *T. brucei* s.l. (yellow) confirmed by amplification of the *PLC* marker, as a percentage of the total number of samples collected in Soroti at each time point and (b) *T. b. rhodesiense* (red) as a percentage of the total number of *T. brucei* s.l. positive samples in Soroti at each time point. n represents the total number of samples positive for the specific trypanosome species displayed. Error bars represent 95% binomial confidence intervals.

4.3.3 Soroti follow up study

Figures 4.10a and 4.10b show the prevalence of *T. brucei s.l.* and *T. b. rhodesiense* 18 month post-intervention in Soroti. Sites where the FITCA interventions took place are grouped together as intervention sites and sites where no intervention took place are grouped as non-intervention sites. The results from the samples collected at Brookes Corner market are shown separately.

18 months post-intervention there was no significant difference between sites which had been included in the FITCA intervention and those that had not (χ^2=0.056, DF = 1, p>0.05). The prevalence of *T. brucei s.l.* in both intervention and non-intervention villages was then compared to that found at Brookes Corner market shown in Figure 4.10a. Overall the prevalence at the market was significantly higher than the intervention villages (χ^2=24.269, df=1, p<0.0001) and non-intervention villages (χ^2=21.401, df=1, p<0.0001) (Fèvre *et al.*, 2005).

Similarly there were differences in the prevalence of *T. b. rhodesiense* found in the market samples (48%) compared to the intervention villages (30%) and non-intervention villages (36%). However there was no difference in the prevalence of *T. b. rhodesiense* between the intervention and non-intervention villages (χ^2=0.145, df=1, p>0.05).

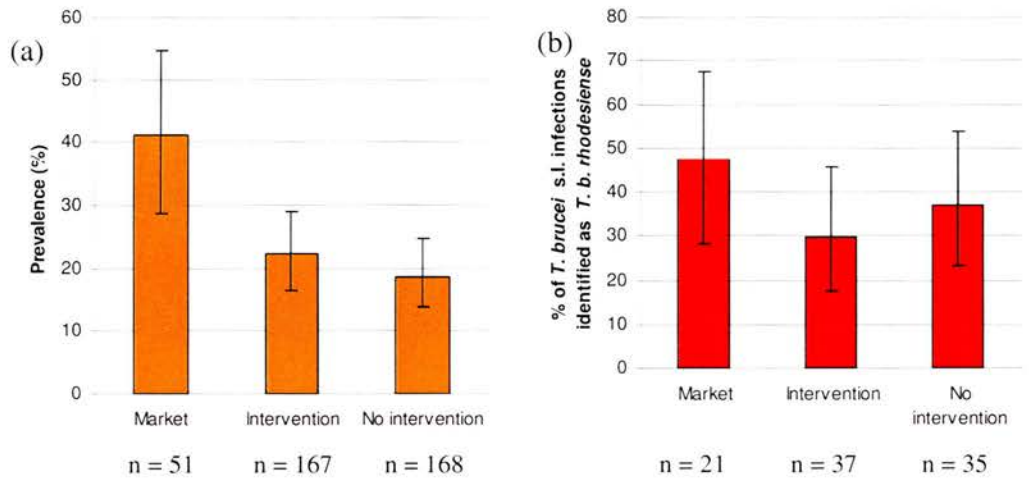


Figure 4.10 – (a) Graph showing the prevalence of *T. brucei* s.l. (yellow) in Brookes Corner market, intervention and non-intervention sites as a percentage of the total number of samples collected. (b) The prevalence of *T. b. rhodesiense* (red) in Brookes Corner market, intervention and non-intervention sites as a percentage of positive *T. brucei* s.l. samples.

The percentage of *T. brucei* s.l. observed 18 months post-intervention in both intervention (22%) and non-intervention (19%) sites was similar to the prevalence seen pre-intervention in the original study in Soroti (21%). However, one year post-intervention the prevalence was 8% which was lower than that observed 18 months after infection. Interestingly the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples was similar pre-intervention (28%), one year post-intervention (32%) in the original study and 18 months post-intervention in both intervention (30%) and non-intervention (36%) sites.

4.4 Discussion

The aim of this chapter was to investigate the impact of the FITCA Uganda intervention programme on the prevalence of *T. b. rhodesiense* in cattle in the study area. This was completed by determining the prevalence of *T. b. rhodesiense* within the positive *T. brucei* s.l. samples. All the *T. brucei* s.l. prevalence described in this chapter are from the samples in which the single copy gene *PLC* was amplified. The single copy *PLC* was amplified in over 80% of *T. brucei* s.l. positive samples. This is a fairly successful amplification rate when compared to the amplification of other single copy gene PCRs in trypanosomes, e.g. study by Macleod *et al* showed that single copy genes can be hard to find, only 56% of samples containing a single trypanosome were successfully amplified for the single copy gene TIM (triosephosphate isomerase) found in *T. brucei* s.l.(MacLeod *et al.*, 1997).

Overall there was a significant reduction in the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. samples three months post-intervention, from 33% pre-intervention to less than 10% three months post-intervention. Interestingly, although the prevalence of *T. brucei* s.l. within the total sample population continued to decrease one year post-intervention, the prevalence of *T. b. rhodesiense* within these *T. brucei* s.l. samples increased to 15%. The results presented in this Chapter firstly, investigated the pre-intervention prevalence of *T. b. rhodesiense* both within *T. brucei* s.l. samples and the prevalence within the total sample populations. These results were then compared with the prevalence both three months and one year post-intervention within each sampled district.

4.4.1 Baseline prevalence of *T. b. rhodesiense*

Pre-intervention there were significant differences in the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples. The highest levels of *T. b. rhodesiense* were detected in Kamuli and Soroti. In Kamuli 55% of *T. brucei* s.l. samples were confirmed as *T. b. rhodesiense*, this was by far the highest prevalence within *T. brucei* s.l. samples across any district. Overall, 33% of all *T. brucei* s.l.

infections, confirmed by the amplification of *PLC*, were determined to be human-infective *T. b. rhodesiense*. The prevalence of *T. b. rhodesiense* across the study corresponds well to the ratio of 1:3 (*T. b. rhodesiense*: *T. brucei* s.l.) presented in the review by Coleman and Welburn (Coleman & Welburn, 2004). Overall there was a 2.5% population prevalence of *T. b. rhodesiense* in all sampled cattle across the study site. This compares to a prevalence of 4.9% *T. vivax* and 0.7% *T. c. savannah*. There are few other published studies on the prevalence of *T. b. rhodesiense* within the cattle reservoir. The first field based study on the prevalence of *T. b. rhodesiense* in cattle in Uganda was carried out in 2001. In Soroti district 45% of the sampled cattle (n = 200) tested positive for *T. brucei* s.l. and 40% of these were characterised as *T. b. rhodesiense* (Welburn *et al.*, 2001b). Another more recent study in 2006 study in Uganda showed that using *SRA* as a diagnostic marker (Gibson *et al.*, 2002; Radwanska *et al.*, 2002a) 11.5% (n = 87) of parasitologically positive cattle were infected with *T. b. rhodesiense* in Kaberamaido district (Enyaru *et al.*, 2006).

4.4.2 Effect of FITCA intervention on prevalence of *T. b. rhodesiense*

As previously mentioned there was a significant reduction in the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples three months post-intervention however, there were variations at the district level. The most dramatic differences post-intervention were in Kamuli, pre-intervention 55% of *T. brucei* s.l. samples were found to be *T. b. rhodesiense*. However, post-intervention no further *T. b. rhodesiense* were identified in *T. brucei* s.l. positive samples (three month post-intervention, n = 9 and one year post-intervention n = 15). The lasting *T. b. rhodesiense* reductions observed in Kamuli were not detected in Soroti. In Soroti, 28% of *T. brucei* s.l. samples were identified as *T. b. rhodesiense* pre-intervention this reduced to lower than 10% three months post intervention but increased to 33% one year post-intervention. It is worth noting that the 1:3 (*T. b. rhodesiense*: *T. b. brucei*) ratio is present pre-intervention in Soroti and although this decreases dramatically three months post-intervention to 1:10, ultimately one year on the 1:3 ratio has recovered corroborating the views of Coleman and Welburn (Coleman & Welburn, 2004). The increase in the percentage of *T. b. rhodesiense* one year post-

intervention occurred despite an overall reduction in the prevalence of *T. brucei* s.l. Interestingly the recovery of the proportion of *T. b. rhodesiense* within *T. brucei* s.l. samples one year post-intervention is only observed in Soroti where there is currently an epidemic of human sleeping sickness (Fèvre *et al.*, 2005).

4.4.2.1 Soroti follow-up study

It was particularly important to complete a follow up study in Soroti due to the continuing epidemic of sleeping sickness in that area. By this time it was becoming clear that human sleeping sickness had continued to spread in Soroti (this is detailed in Chapter 5) and had in fact spread from Soroti to the neighbouring district of Kaberamaido leading to further concerns about the overlap of *T. b. rhodesiense* and *T. b. gambiense* (Picozzi *et al.*, 2005). As it seemed likely that the continued northward spread of *T. b. rhodesiense* originated from Soroti district it was important to complete a follow up study to determine the long term impacts of the FITCA programme in reducing *T. brucei* s.l. and specifically *T. b. rhodesiense*. Overall when the intervention and non-intervention sites were compared there was no significant difference in the prevalence of either *T. brucei* s.l. or *T. b. rhodesiense*. There was also no difference in the proportion of *T. brucei* s.l. samples which were *T. b. rhodesiense*, this was consistent at approximately 30% in each group. This follow-up study suggests that there was no lasting impact of the FITCA intervention in this district.

The prevalence in these villages was then compared to the prevalence at a local livestock market within the district called Brookes Corner. This market was considered to be the introduction point of *T. b. rhodesiense* into this district (Fèvre *et al.*, 2001). The prevalence of *T. brucei* and *T. b. rhodesiense* were shown to be significantly higher in the samples collected in the market than either of the groups of villages. Additionally approximately 50% of *T. brucei* s.l. cases were determined to be *T. b. rhodesiense*; this was considerably higher than the other sample groups. This result highlights that livestock markets continue to be a risk factor in the spread

of disease to new areas and the maintenance of the disease in the local area. It is government policy to treat all cattle which are sold in a markets, the animal should be given a dose of trypanocide at the point of sale (Fèvre, 2001). However in practice this treatment is rarely given (C. Waiswa, pers. com.) which means these animals can be moved into uninfected areas and have the potential to spread sleeping sickness into a new area. Overall there was no lasting impact of the FITCA intervention in the sampled areas in Soroti district after 18 months and there was still a higher prevalence of *T. brucei* s.l. and *T. b. rhodesiense* at the market highlighting the continued risk of disease spread.

4.4.3 Study limitations

There are similar limitations to the study presented in this Chapter as discussed in section 3.4.4 (Chapter 3), in addition there are implications of sample size. These limitations are due to the fact that when a species complex such as *T. brucei* s.l. is broken down there are very low infection levels. To try to illustrate the changes observed in the prevalence of *T. b. rhodesiense*, infection levels have been shown as percentage of the total positive *T. brucei* s.l. samples. Additionally the PCR for a single copy gene was relying on moderate parasite parasitemia in these cattle to allow detection of *T. b. rhodesiense* (Picozzi *et al.*, *In press*). It is therefore possible that false negatives occurred due to a low parasitemia or the uneven spread of DNA on the cards. In an attempt to compensate for these problems multiple punches were taken from each sample however, this does reduce the amount of sample available for repeats. The multiplex PCR also attempted to account for potentially low parasitemia by ensuring the amplification of a single copy marker for *T. brucei* s.l. (*PLC*). However, this could not inform about the presence of mixed infections of *T. b. brucei* and *T. b. rhodesiense*, meaning that the level of *T. b. rhodesiense* could still be very low. Despite these limitations it is clear that over course of the FITCA intervention, there was a significant decrease in the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples.

4.4.4 Final comments

The results showed that three months post-intervention there was a significant impact on the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples. However, one year post-intervention the prevalence of *T. b. rhodesiense* within the *T. brucei* s.l. positive samples had shown an increase despite the continued decline in the prevalence of *T. brucei* s.l. This implies that although single dose of trypanocide may have an initial impact on the prevalence of *T. b. rhodesiense*, this is not maintained over time. This is corroborated by the 18 month follow up study in Soroti district which showed no significant differences in the prevalence of *T. brucei* s.l. and *T. b. rhodesiense* in sites where the FITCA intervention had taken place and those that had not. This follow up study also highlighted the continued importance of livestock markets as a route for spreading *T. b. rhodesiense*, almost 50% of *T. brucei* s.l. infected cattle carried human infective *T. b. rhodesiense*. This emphasises the need for the stringent upkeep of the policy to treat animals sold at markets with a trypanocide, which is a key strategy in reducing the potential transmission of *T. b. rhodesiense* to new areas. Although there were regional differences in the effectiveness of this intervention it is clear that overall a one off trypanocide treatment of cattle will not achieve a lasting effect in areas where there is an ongoing epidemic.

5 Chapter 5:

Effect of mass trypanocidal treatment of cattle on the epidemiology of sleeping sickness

5.1 Introduction

5.1.1 Introduction

It is well established that in Uganda cattle form an important reservoir for human infective *Trypanosome brucei rhodesiense* (Welburn *et al.*, 2001b). Furthermore, movement of infected cattle has been implicated in the recent outbreak of sleeping sickness in Uganda (Fèvre *et al.*, 2001). Based on mathematical modelling it has been proposed that mass trypanocidal treatment of cattle, such as that undertaken as part of the FITCA programme, may form the most effective way to control sleeping sickness caused by *T. b. rhodesiense* (Welburn *et al.*, 2001a). The effect of the FITCA Uganda mass treatment programme on the prevalence of the trypanosomiases in cattle has been examined in the previous two chapters. Observations in Chapters 3 demonstrated that the overall prevalence of *T. brucei* s.l., *T. vivax* and *T. c. savannah* was reduced after the intervention by FITCA and Chapter 4 showed a reduction of *T. b. rhodesiense* in the cattle population sampled post-intervention. In this chapter the number and distribution of the reported human sleeping sickness cases in two of the study districts (Soroti and Kamuli) covered by the intervention programme are examined to determine if the programme had any impact on the incidence of human trypanosomiasis.

5.1.2 Soroti and Kamuli districts

Soroti and Kamuli districts are situated on the northern and southern shores of Lake Kyoga respectively. Although separated by Lake Kyoga, these two districts share similar demographics; both are comprised predominantly of rural populations with 89% of people in Soroti and 98% of people in Kamuli involved in subsistence agriculture (Ugandan Bureau of Statistics, 2002). Due to the increased contact with tsetse flies associated with this employment, it is the working population that is at greatest risk of contracting sleeping sickness. Consequently the potential socio-economic impact of HAT on the collective community can be dramatic (Fèvre, 2002).

5.1.3 History of sleeping sickness in Soroti

Prior to 1998 there was only one reported case of sleeping sickness in Soroti, which was diagnosed and treated in the Ugandan Trypanosomiasis Research Organisation Hospital in Tororo in 1967. The female patient claimed to have lived in Soroti on the shores of Lake Kyoga all her life, but had recently travelled to and from Tanzania, a journey that would have required her to pass through endemic sleeping sickness areas. (Onyango, 1967). The case prompted an extensive survey of trypanosome infection of people (n = 650), tsetse flies (n = 1800) and cattle (n = 2444) in the area (Persoons, 1967; Mwambu, 1969b; Mwambu, 1969a). No *T. brucei* s.l. infections in either humans or cattle was identified but 2 tsetse flies (0.1%) were found to be infected with parasites that displayed characteristics considered by the authors to be indicative of *T. brucei* s.l. (Persoons, 1967).

In the current epidemic of sleeping sickness the first case was identified in Soroti in December 1998 at Serere hospital (Fèvre *et al.*, 2001) in the south of the district. Since that time, there have been over 500 reported cases of sleeping sickness in Soroti district (Fèvre *et al.*, 2005) with the source of the outbreak linked to the movement of cattle into the area, and in particular to a specific cattle market at Brookes Corner (Fèvre *et al.*, 2001). Extensive cattle movement into the district at this time was due to a major cattle restocking programme to replace those lost in cattle raids (Fèvre, 2002). Since 1998, the disease has spread from the point of introduction throughout a larger part of the district.

5.1.4 History of sleeping sickness in Kamuli

At independence, Kamuli district was part of Busoga district and as such is part of the established Busoga focus of sleeping sickness (Langlands, 1967). The first cases of sleeping sickness were identified at the turn of the 19th century on the shores of Lake Victoria before spreading across the Busoga district (Christy, 1903). This epidemic was not brought under control until the late 1920's by which time it had claimed more than a quarter of a million lives (Mbulamberi, 1990). A further epidemic occurred in

this area between 1939 and 1945 followed by an outbreak in neighbouring Iganga district in 1976 which expanded to include some areas of Kamuli through the 1980's (Berrang-Ford *et al.*, 2006). In 1990, Mbulamberi *et al.* produced a detailed account of the numbers of cases reported in affected sub-counties within Kamuli (Mbulamberi, 1990). Throughout the 1990's small number of sleeping sickness cases continued to be reported in Kamuli, but in 2001 there was a substantial increase with >100 cases reported (Gould, 2003).

5.1.5 Spatial analysis

Examining data sets in the context of their spatial distribution is a method that has been used extensively in epidemiology and can refer to anything from simple maps to complicated mathematical analysis. In one of the most famous early epidemiological studies, John Snow identified the source of a cholera outbreak in the Soho district of London in 1854 by mapping the location of confirmed cases. His simple but detailed spatial analysis revealed clustering of the cholera cases around a specific water pump and earned Snow a place amongst the most famous scientists of that era (Snow, 2002). The development of geographical information systems (GIS) since the 1980's as a method of storing and displaying spatial data has meant that analysis of spatial data is now quicker, easier and more sophisticated (Longley *et al.*, 2001). The concurrent development of readily available and portable global positioning system (GPS) devices has permitted the rapid and accurate acquisition of reliable data from the field.

5.1.5.1 Spatial clustering analysis

A simple definition of disease clustering is *"the occurrence of a disease within a particular area at a frequency greater than that expected by chance"* (Robinson, 2000). Many statistical tests aim to characterise spatial clustering of disease cases and a detailed description of the mathematical principals underlying these different clustering algorithms are beyond the scope of this thesis (comprehensive discussions are given in (Wakefield *et al.*, 2000)).

Besag and Newell (1991) identified 3 types of tests for clustering (Besag & Newell, 1991). (i) *Tests for clustering* - which describe the existence of spatial clustering within a dataset but do not define the location or size of clusters. The use of such tests is restricted to studies in which the location of clusters is not of primary concern, for example in investigating whether or not a disease is infectious e.g. (Moran, 1948). (ii) *Tests for the detection of clustering 'focused'* - which are designed to test whether there is clustering around a pre-specified putative health-hazard e.g. incidence of cancer around a nuclear power station. Examples of the application of such tests are given in (Schulman *et al.*, 1988; Stone, 1988; Waller *et al.*, 1992). (iii) *Tests for the detection of clustering 'generalised'* - which are capable of specifying the size and location of clusters over a large study region in which there are no preconceived notions of the location of clusters.

The geographical analysis machine (GAM) developed by Openshaw and colleagues was the first major attempt to describe clusters of a rare disease over a large region using a 'generalised' test for the detection of clustering. The GAM utilises multiple overlapping circles of a fixed radius laid out on a fine regular lattice as quadrats. Observations on the case counts in overlapping quadrats and separate significance tests for each quadrat facilitate identification of potential clusters (Openshaw *et al.*, 1988). Although the GAM represented a significant advance in tests for the detection of clustering under 'generalised' conditions, it doesn't satisfactorily quantify the significance of identified clusters (Turnbull *et al.*, 1990; Besag & Newell, 1991). Based on the Openshaw method, Turnbull and colleagues developed the cluster evaluation permutation procedure (CEPP). The CEPP also uses overlapping circles as quadrats, but with the radius of each circle fixed for population size rather than geographical distance. The incorporation of a Monte-Carlo simulation in the CEPP also permitted the statistical significance of apparent clusters to be quantitatively assessed (Turnbull *et al.*, 1990).

5.1.5.2 Spatial scan statistic

Modifications of the CEPP by Kulldorff and Nagarwalla led to the creation of the spatial scan statistic (Kulldorff & Nagarwalla, 1995). The spatial scan statistic works by centring a circular quadrat over each point in a dataset and allowing it to vary in size during each iteration of the analysis, thereby evaluating potential clustering around each point within the area defined by numerous radii. The maximum size of the circle is normally defined so as to include no more than 50% of the points in the data set (Kulldorff *et al.*, 1998). For each circle of each size at each location the observed number of cases is determined and compared to the number that would be expected under the null hypothesis (i.e. that there are no clusters). The applicability of the null and alternative (i.e. that there is clustering) hypotheses for each circle are compared by a likelihood ratio calculation. The spatial scan statistic identifies clusters as quadrats expressing the highest likelihood ratio for the alternative hypothesis and permits the identification of secondary as well as primary clusters within a data set, which are ranked according to their likelihood ratio (Kulldorff, 1997). A large number of replications using a Monte-Carlo simulation are used to determine the probability that a detected cluster arose by chance and gives a corresponding p-value. Only clusters demonstrating p-values <0.05 are considered to be significant. Hence the final output from the spatial scan statistic defines the location, size and an indication of the significance of detected clusters. Although the location of the cluster is given as a fixed point, this should not be interpreted too literally – the true central point of the cluster will lie within the radius of the cluster (Kulldorff & Nagarwalla, 1995). The adoption of the likelihood ratio test permits the spatial scan statistic to be applied to study areas in which the population density is heterogenous (Kulldorff & Nagarwalla, 1995). The spatial scan statistic can be applied to data conforming to either a Poisson-based model (e.g. where the number of cases in a geographical area is Poisson-distributed, according to the known underlying population at risk) or a Bernoulli model (e.g. with the binary data generated in case-control studies) as discussed in (Kulldorff, 1997).

5.1.5.3 Applications of the spatial scan statistic

Since its inception the spatial scan statistic has been used in numerous human and veterinary epidemiological studies (http://www.satscan.org/references). It has been widely used in infectious disease investigations to assess the importance of potential aetiological factors (Odoi *et al.*, 2004), evaluate risk factors associated with disease (e.g. (Bakker *et al.*, 2004; Brooker *et al.*, 2004), monitor disease outbreaks (e.g. (Norstrom *et al.*, 2000; Pearl *et al.*, 2006)) and evaluate the effect of intervention programmes (e.g. Washington *et al* 2004). As envisaged by (Kulldorff & Nagarwalla, 1995), an important application of the spatial scan statistic has been the identification of statistically significant clustering of disease cases as an approach to rationally allocating resources for disease control. The spatial scan statistic has been adopted in disease surveillance programmes (e.g. (Mostashari *et al.*, 2003) and a variety of studies employing this method have provided results that can inform the most efficient use of healthcare and disease control provision (e.g. (Sankoh *et al.*, 2001; Jennings *et al.*, 2005; Tiwari *et al.*, 2006). The spatial scan statistic has been used in two studies into the epidemiology of sleeping sickness in Uganda. Berrang-Ford et al (2006) used the spatial scan statistic to define clusters of sleeping sickness cases in south east Uganda from 1970 to 2003 (Berrang-Ford *et al.*, 2006) and demonstrated the expansion of the sleeping sickness focus during the 1970's and the subsequent infiltration into new areas in the 1980's and 1990's. Fevre et al (2001) used the method to illustrate the association between cattle movements and the origins of the 1998 outbreak of the disease in Soroti (Fèvre *et al.*, 2001).

5.1.6 Chapter aims

As described previously, the main component of the FITCA Uganda programme was the mass treatment of cattle in east Uganda with a single dose of trypanocide completed in November and December 2002. The aim of this Chapter is to assess if this intervention had any impact on the number and distribution of sleeping sickness cases in the programme area.

To achieve this aim a combination of descriptive statistical analysis and the spatial scan statistic was used to analyse the sleeping sickness cases reported over a five and a half year period (January 2000 - July 2005) in two of the districts (Soroti and Kamuli) within the FITCA programme area. In the first half of the chapter the data was analysed on an annual basis and in the second half of the chapter comparisons are made between the data for the pre- and post-intervention periods. The results of this analysis suggest that the impact of the FITCA intervention on the number and geographical distribution of sleeping sickness cases was negligible. Possible explanations for the level of impact are discussed in section 5.4 (Chapter discussion).

5.2 Materials and Methods

5.2.1 Study area

Prior to instigation of the programme, FITCA Uganda completed a sleeping sickness and animal trypanosomiasis survey throughout all districts in the FITCA programme area. The results indicated that Soroti and Kamuli districts had the highest reported numbers of sleeping sickness cases, and to maximise the potential for statistically significant observations to be made, these two districts were selected for this analysis. Both districts had three sub-counties categorised as high-risk; in Soroti these were Pingire, Kateta and Kyere sub-counties and in Kamuli these were Bumanya, Kitayunjwa and Namwendwa sub-counties (Figure 5.1). FITCA interventions in Soroti and Kamuli were preferentially targeted within these sub-counties, with the location of intervention sites selected by the respective DVOs.

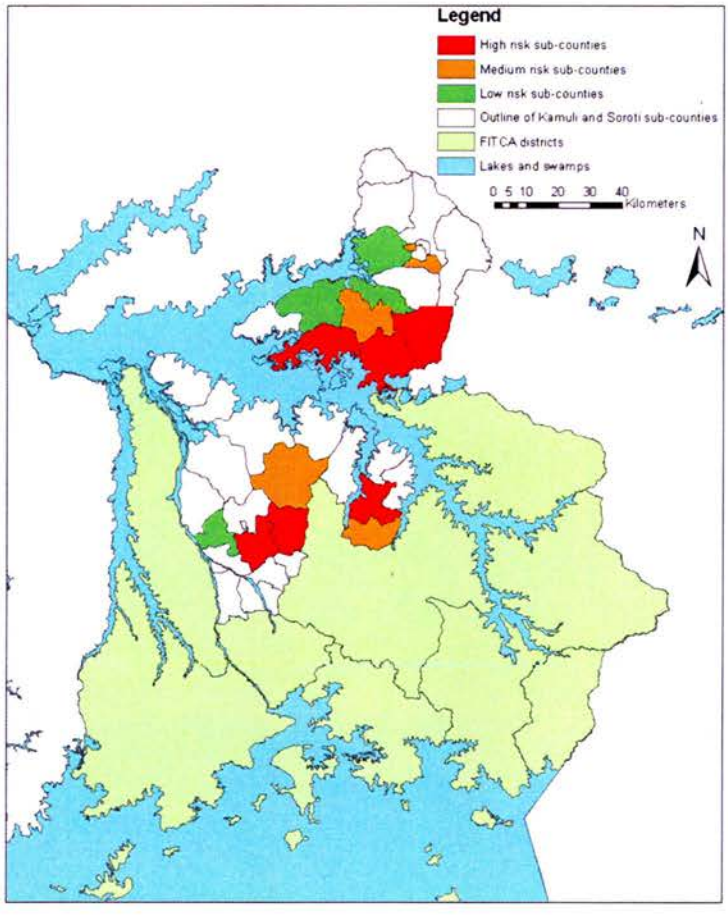


Figure 5.1- Map showing FITCA high, medium and low risk sub-counties within Soroti and Kamuli districts, other FITCA districts shown in pale green.

There were 25 intervention sites selected by the DVO in Soroti district. These sites were situated across seven sub-counties: Bugondo, Serere, Pingire, Kateta, Kyere, Asuret and Atiira (Figure 5.2). In Kamuli 22 intervention sites were selected by the DVO. The majority of these sites were situated in Namwendwa sub-county with the addition of one site in Kiatyunjwa sub-county very close to the border with Namwendwa (Figure 5.2). A single dose trypanocide treatment of cattle, either isometamidium chloride or diminazene aceturate (Chapter 3, section 3.2.2.2) was carried out at these sites from November 2002 to December 2002. However, to permit analysis it was assumed that the time of intervention was at the end of December 2002.

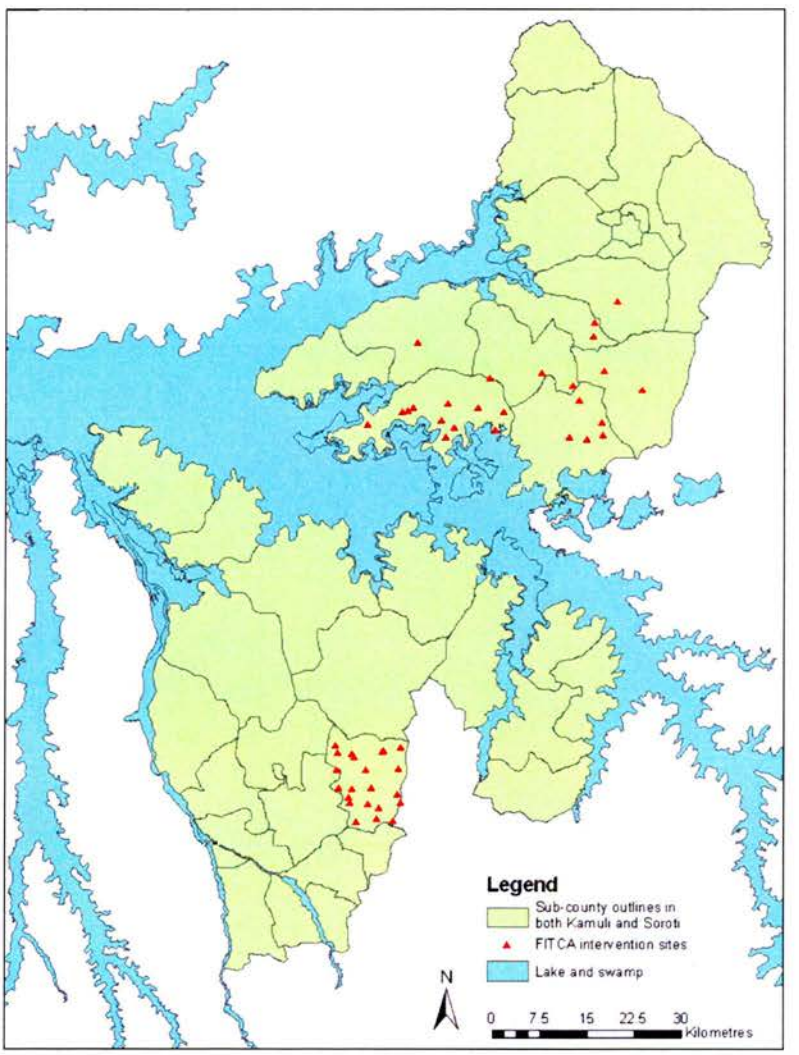


Figure 5.2 – Map showing the location of the intervention sites in Soroti and Kamuli districts.

5.2.2 Data collection

In Soroti, all sleeping sickness cases are recorded by Serere Hospital, the only sleeping sickness diagnostic centre in the district. In Kamuli, there are three active sleeping sickness diagnostic centres located in Bulopa, Nankandulo and Namwendwa. Data for all cases of sleeping sickness reported in Soroti and Kamuli districts from January 2000 through to July 2005 were collected retrospectively during visits to the diagnostic centres in August 2005. Sleeping sickness diagnosis was based on the WHO criteria of detection of trypanosomes in the blood or cerebrospinal fluid (World Health Organization, 1998a). Details including (i) village, parish and sub-county of residence, (ii) the admission date and (iii) stage of disease were obtained, where possible, for every individual case of sleeping sickness reported. All cases were considered for inclusion in the study and data were digitized and stored in Excel. However, all records are hand written (Figure 5.3) and a small number of cases (n=5) had to be excluded from spatial scan statistic analysis due to missing/illegible data regarding the patients village of residence.

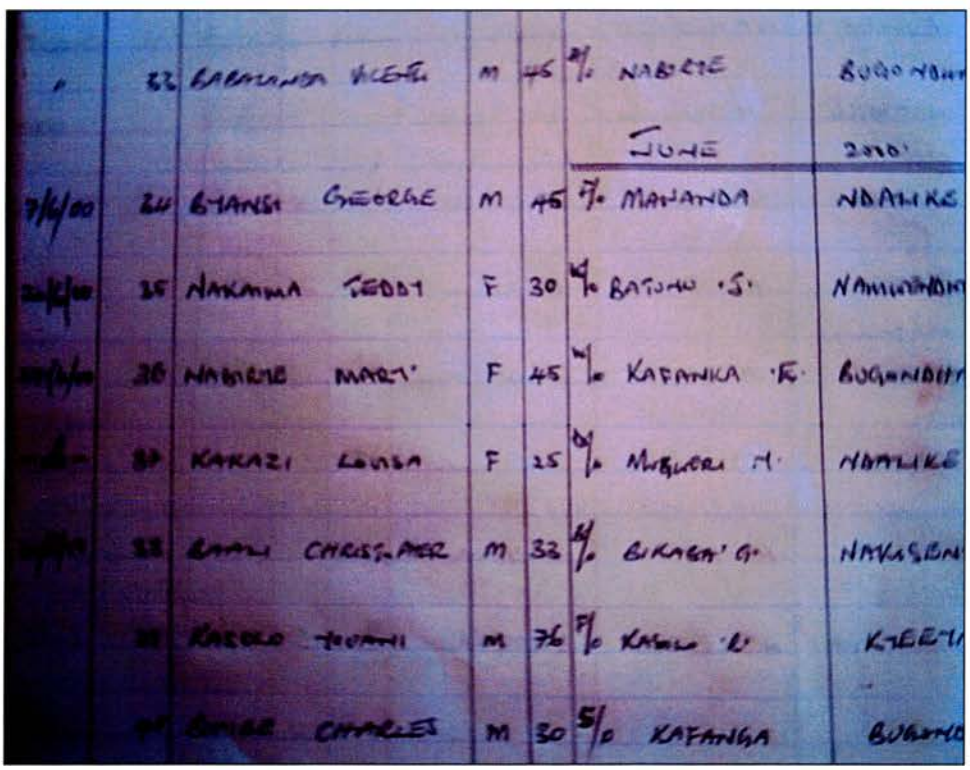


Figure 5.3 – Picture showing sleeping sickness records

5.2.3 Data geo-referencing

5.2.3.1 Geo-referencing maps (image warping)

Paper maps covering the study areas were scanned and then geo-referenced using the image warp version 2.0 for ArcView version 3.1 Geographical Information System software (ERSI Systems, Redlands, CA, USA). Image warp version 2.0 is an extension to ArcView which was used to geo-reference the 1:50,000 maps. This process takes a digital image and accurately positions it in geographical space. To ensure accurate geo-referencing from each of the original maps the latitude and longitude co-ordinates of 8 reference points were established. During an image warping session the error in the location of these reference points on the digitised maps generated were calculated and the map image accordingly warped using the nearest neighbour method to rectify the error. Final warped maps had an error within 30 metres.

The following 1:50,000 maps were digitised and geo-referenced (i) for Soroti; Bugondo (series Y732, sheet 42/4, edition 1-U.S.D.1963), Kelle (series Y732, sheet 42/3, edition 1-U.S.D. 1964), Kumi (series Y732, sheet 53/2, edition 1-U.S.D. 1963), Kyere (series Y732, sheet 53/1, edition 1-U.S.D. 1964), Sambwa (series Y732, sheet 52/2, edition 1-U.S.D. 1969) and Soroti (series Y732, sheet 43/3, edition 1-U.S.D. 1968) and (ii) for Kamuli; Balawoli (series Y732, sheet 52/3, edition 1-U.S.D. 1963), Kamuli (series Y732, sheet 62/1, edition 3-U.S.D. 1998), Namwendwa (series Y732, sheet 62/2, edition 3-U.S.D. 1998) and Nawaikoke (series Y732, sheet 52/4, edition 1-U.S.D. 1963).

5.2.3.2 Village location

The locations of all villages in the study areas were taken from the 1:50,000 maps detailed above (section 5.2.3.1). For each village the latitude and longitude co-ordinates were established on the digitally manipulated maps. Using the ESRI XY

coordinates extension function of ArcView version 3.1 (ESRI systems®, Redlands, CA, USA), the co-ordinate location for each village was transformed for the input projection detailed in Table 5.1 and the co-ordinate data stored in a Excel spreadsheet (Microsoft Office XP, Redmond, USA).

Projection	**Transverse Mercator**
Spheroid	Clark 1880
Central meridian	33
Reference latitude	0
Scale factor	0.9996
False easting	500000
False northing	0

Table 5.1 – Projection details for the Ugandan maps

5.2.3.3 Intervention site location

The locations of the sites at which the FITCA interventions were completed in both districts were provided by the relevant DVOs. The geographic co-ordinates of these sites had been collected by veterinary officers attending the interventions using a hand held Garmin GPSII+ device (Garmin Europe Ltd. Romsey, Hants., UK) and reflected the position of the animal crush pens used. The co-ordinate data was entered into an Excel spreadsheet (Microsoft Office XP, Redmond, USA) and any required geographical manipulations of this data were carried out in ArcView version 3.1 (ESRI systems®, Redlands, CA, USA).

5.2.4 Analysis

5.2.4.1 Descriptive statistics

Mann Whitney *U* tests, conducted in Minitab version 14 (Minitab, Ltd. Coventry, UK) were completed to permit comparisons of the median number of monthly cases reported in different years. A p-value <0.05 was considered significant. Median values were used for analysis to limit any skewing effect that may have been introduced by outlying values.

The incidence of reported sleeping sickness was calculated using the 2002 census data for sub-county and parish populations (Ugandan Bureau of Statistics, 2002). In absence of more recent census data it was assumed that there were no significant changes in populations within the study districts in the years subsequent to 2002.

5.2.4.2 Cluster detection

The spatial scan statistic (Kulldorff & Nagarwalla, 1995; Kulldorff, 1997) implemented by SaTScan version 5.1.3 (http://www.satscan.org) was used to investigate spatial clustering of sleeping sickness in Soroti and Kamuli during the study period. Analysis was performed assuming a Bernoulli distribution. Villages with residents that reported as sleeping sickness cases were categorised as 'case villages', with due regard given to villages in which multiple residents reported with sleeping sickness. All other villages in the districts (which did not have any reported cases of sleeping sickness within the time-frame of the study) were considered to represent 'control villages'. The maximum cluster size was set at 50% of the dataset points as is convention (Kulldorff & Nagarwalla, 1995) and the analysis was run to identify areas of clustering ('high rates') rather than areas with no clustering (Kulldorff *et al.*, 1998). The analysis was run with 9999 Monte-Carlo iterations.

5.3 Results

5.3.1 Descriptive statistical analysis of the numbers of sleeping sickness cases reported

Between January 2000 and July 2005 there were 403 cases of sleeping sickness reported in Soroti district and 283 cases reported in Kamuli district. The number of sleeping sickness cases reported each month in Soroti and Kamuli during the study period is shown in Figures 5.4a and b respectively.

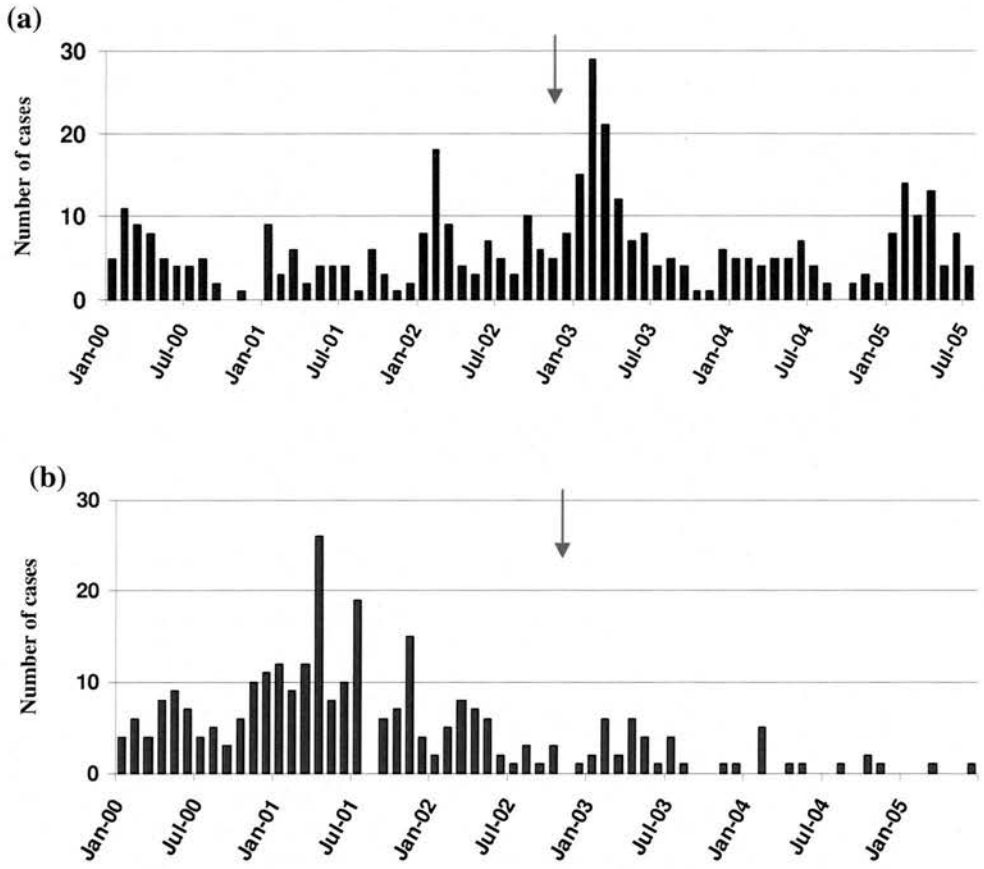


Figure 5.4 – Graph showing the number of reported sleeping sickness cases per month during the study period from January 2000 to July 2005 in (a) Soroti and (b) Kamuli districts. Red arrows indicate the time point of the intervention

Visual observation suggests that the number of cases recorded each month in Soroti is subject to seasonal variation, with a peak in February/March (particularly notable in 2002 and 2003, Figure 5.4a). However, this variation is not evident in all years e.g.

2004. Disregarding these peak months in 2002 and 2003, the number of cases reported in Soroti appears relatively consistent throughout the study period. Seasonal variation appears to be absent from the data collected for Kamuli district (Figure 5.4b). Except for the high numbers of cases reported in April, July and October 2001, in the months from January 2000 until the middle of 2002 the number of cases reported is comparatively stable. In the subsequent months there is a general trend of declining number of cases reported and from the middle of 2003 until the end of the study cases are only reported intermittently.

Using the Mann-Whitney *U* test, the median monthly number of cases reported in each year was used to statistically describe the year-by-year changes in the number of cases reported in the two districts (Figure 5.5 and Table 5.2). As there was not data for the full year in 2005 it was excluded from this part of the analysis. In Soroti, the median number of monthly cases remained fairly stable throughout the five-year period (Figure 5.5a). Only the increase seen between 2001 and 2002 was statistically significant ($p = 0.017$), although the decrease from 2003 to 2004 was nearing significance ($p = 0.057$). In Kamuli, there was an increase in the median monthly number of cases from 2000 to 2001, which was followed by a continual decrease in the subsequent years (Figure 5.5b). Application of Mann-Whitney *U* test (Table 5.2b) indicated that only the decrease seen between 2001 and 2002 was statistically significant ($p = 0.003$), although the decrease between 2003 and 2004 was approaching significance ($p = 0.061$).

Comparison of the median monthly number of cases reported in the first year (2000) and the last complete year (2004) of the study indicates that there were no statistically significant differences in Soroti ($p = 0.686$), but that in Kamuli a statistically significant decrease was seen over the course of the study period ($p = 0.0001$).

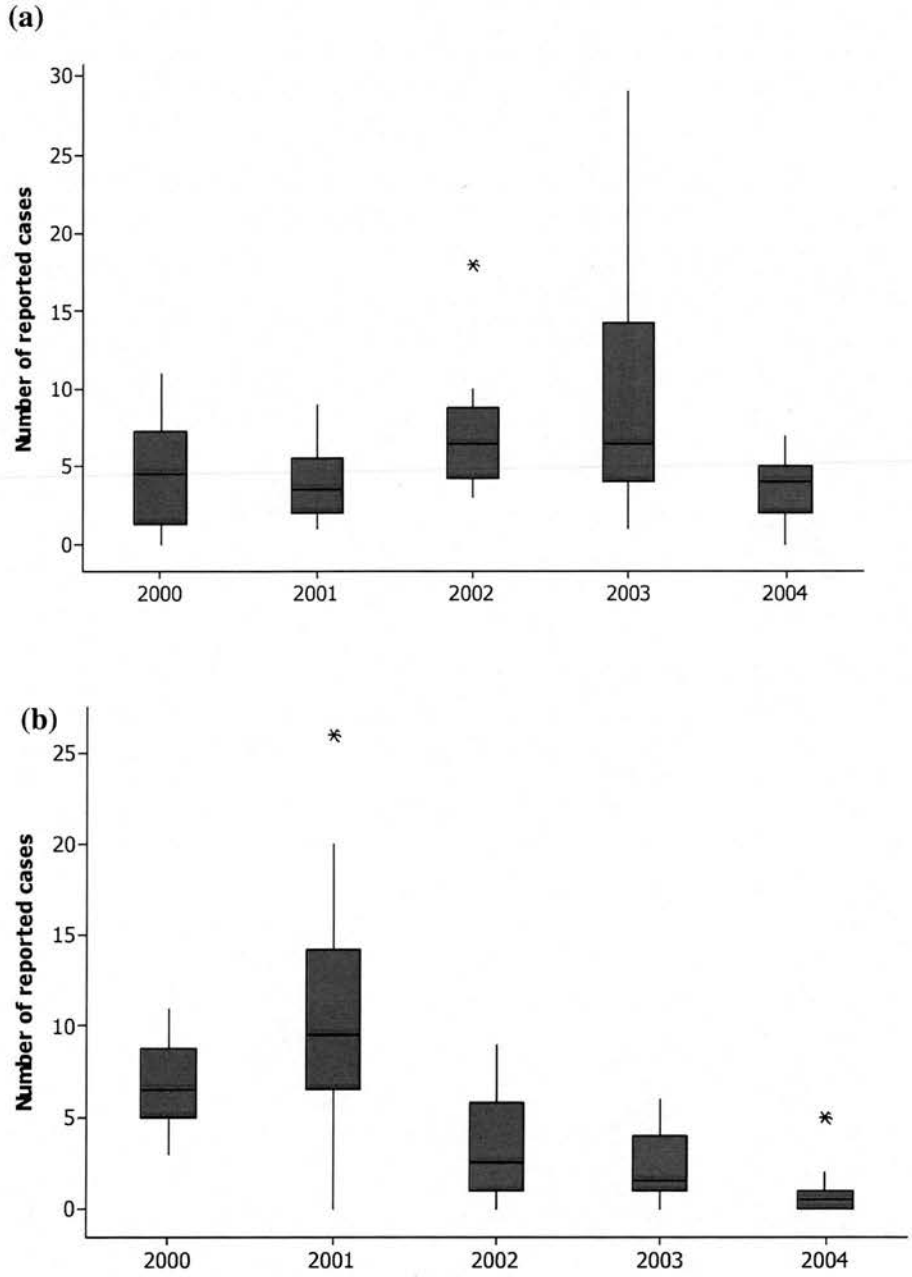


Figure 5.5 – Box plots showing the number of sleeping sickness cases reported each month in 2000 – 2004 in (a) Soroti and (b) Kamuli. Horizontal black lines denote the median monthly number of cases reported for each year. Data for 2005 has not been shown as the complete dataset for the year was not collected.* denotes outlying data points.

(a)

Year	Median monthly number of sleeping sickness cases reported	
	Soroti	**Kamuli**
2000	**4.5**	**6.5**
2001	**3.5**	**9.5**
2002	**6.5**	**2.5**
2003	**6.5**	**1.5**
2004	**4.0**	**0.5**

(b)

Comparison	p-value (Mann Whitney *U*)	Significant change
2000 to 2001	0.6442	None
2001 to 2002	0.0166	Significant increase
2002 to 2003	0.8852	None
2003 to 2004	0.0567	Nearing significance
2000 to 2004	0.6861	None
Pre- to post-intervention	0.9671	None

(c)

Comparison	p-value (Mann Whitney *U*)	Significant change
2000 to 2001	0.0783	None
2001 to 2002	0.0029	Significant decrease
2002 to 2003	0.4025	None
2003 to 2004	0.0606	Nearing significance
2000 to 2004	0.0001	Significant decrease
Pre- to post-intervention	0.0002	Significant decrease

Table 5.2 – Statisitical analysis of numbers of sleeping sickness cases reported in Soroti and Kamuli in 2000 - 2004. (a) Annual median monthly number of sleeping cases reported. Year-by-year comparison of the annual median monthly number of sleeping sickness cases reported in (b) Soroti and (c) Kamuli districts. Pre-intervention incorporates the years 2001 and 2002 combined and post-intervention the years 2003 and 2004 combined.

5.3.2 Geographical distribution of sleeping sickness cases

5.3.2.1 Soroti District

In Soroti, the 403 sleeping sickness cases reported during the study period came from 77 villages which are distributed over six of the seventeen sub-counties in the district (Asuret, Atiira, Kateta, Kyere, Serere/Olio and Pingirie). These sub-counties form a contiguous group in the south of the district (Figure 5.6).

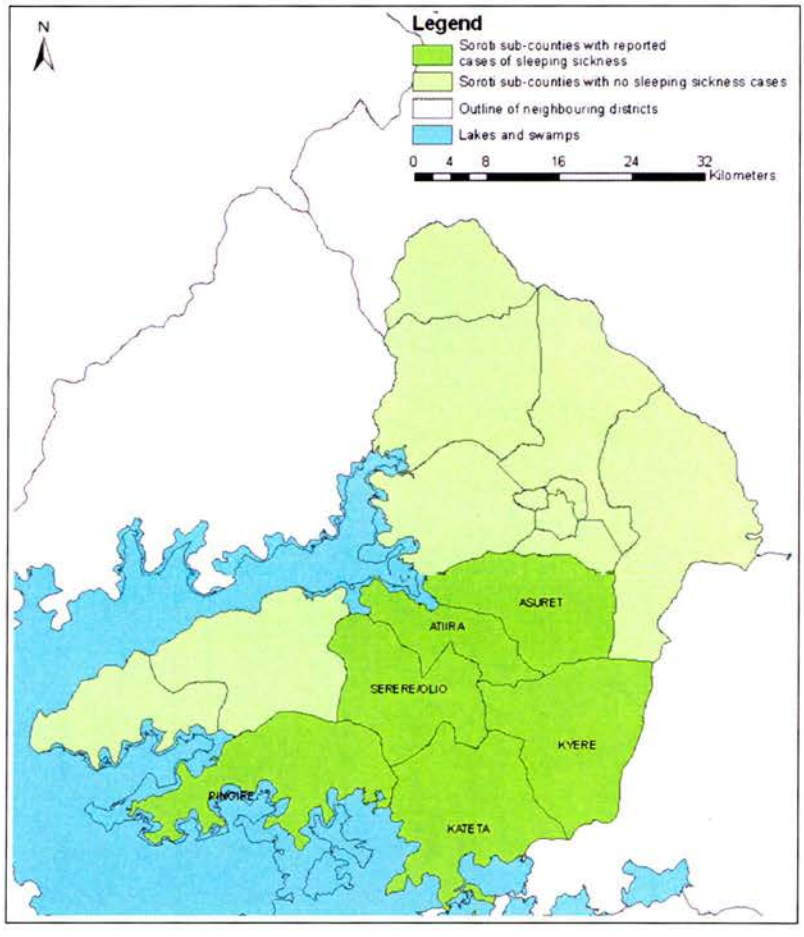


Figure 5.6 – Map of Soroti showing which sub-counties have been affected by sleeping sickness during the study period January 2000 to July 2005.

In different years within the period of study there was a degree of variation in the distribution of cases reported at both the sub-county (Figure 5.7) and village level

(Figure 5.8). Cases were reported from Asuret, Kateta, Kyere and Pingirie in each year of the study and in Serere/Olio in every year except 2004, whilst in Atiira cases were only reported in 2001 and 2004. There is no identifiable trend across the different sub-counties for changes in number of cases reported annually (Figure 5.7). The peak number of cases reported in 2003 appears to be attributable to the continued high number of cases from Pingire and an increase in cases reported from Asuret for that year (Figure 5.7).

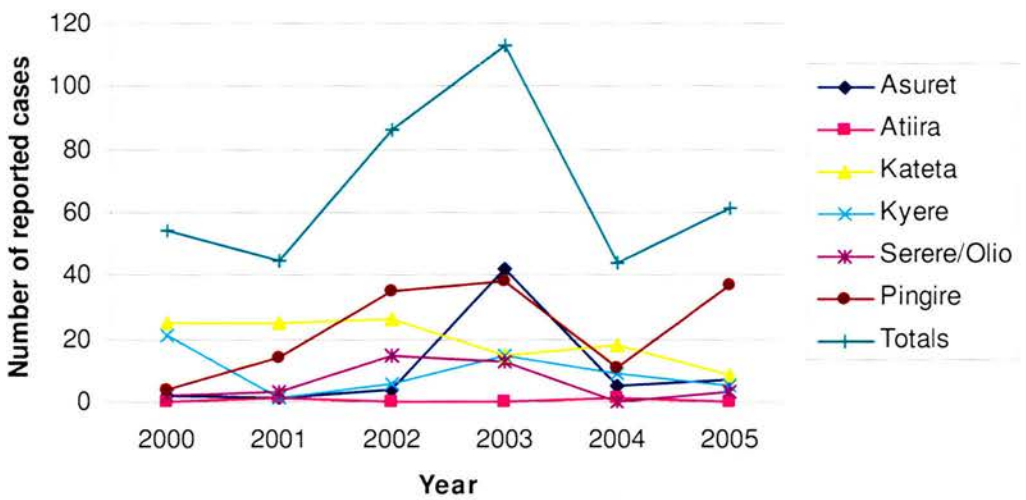


Figure 5.7 – Graph showing the number of sleeping sickness cases reported in the six affected sub-counties and the total number of cases in Soroti district from January 2000 to July 2005.

Analysis with the spatial scan statistic identified significant clustering of sleeping sickness cases in each year of the study (Figure 5.8 and Table 5.3). In 2000 a single primary cluster of large radius (17.52km) covered most of Kateta and Kyere sub-counties. In the following two years (2001 and 2002) a significant cluster was located further west, being centred on the border between Kateta and Pingire sub-counties. In 2003 a secondary cluster was located in the same area, but the primary cluster was situated to the north-east, in Asuret sub-county. In 2004 the primary cluster returned to Kateta and Kyere sub-counties before relocating once again westwards to the Kateta/Pingirie border in 2005.

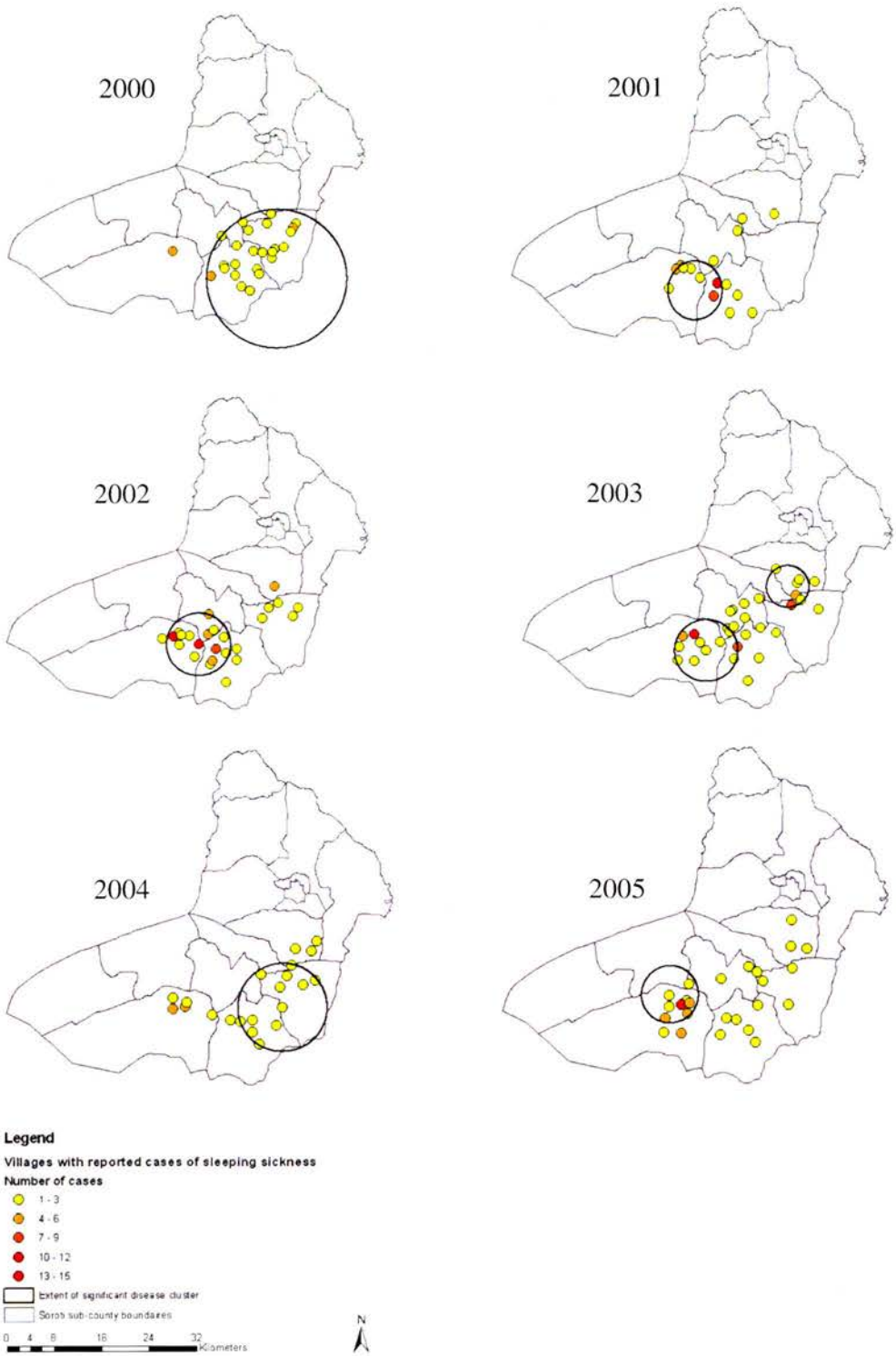


Figure 5.8 – Geographical distribution of sleeping sickness cases and significant clusters in Soroti district in the years 2000 – 2005. Villages reporting sleeping sickness cases are shown according to the legend above, control village are not shown. Significant clusters of cases as determined by application of the spatial scan statistic are shown by the circles. Detailed outputs of the spatial scan statistic are given in Table 5.3. Note that the data for 2005 is incomplete as the study period ended in July 2005.

Year	Type of cluster	No. of expected cases	Actual number of cases	Latitude	Longitude	Radius (km)	Log likelihood ratio	Overall relative risk	p-value	Majority of cluster located in (sub-county)
2000	Primary	17	50	1.41693	33.61796	17.52	54.47	2.91	0.0001	Kyere/Kateta
2001	Primary	7	36	1.43409	33.42795	6.78	61.66	5.23	0.0001	Pingire
2002	Primary	21	65	1.45978	33.43994	7.89	71.90	3.04	0.0001	Pingire/Kateta
2003	Primary	20	51	1.58486	33.58711	5.16	41.30	2.59	0.0001	Pingire
2003	Secondary	17	40	1.44117	33.41028	7.66	24.01	2.32	0.0001	Asuret
2004	Primary	10	27	1.47618	33.58633	10.32	19.16	2.77	0.0002	Kyere/Kateta
2005	primary	9	38	1.43722	33.36991	6.63	54.30	4.36	0.0001	Pingire

Table 5.3 – Table showing the results of the annual cluster detection of reported cases of sleeping sickness in Soroti district by application of the spatial scan statistic. The number of expected cases refers to the number of cases expected in the cluster if distribution was random (i.e. the null hypothesis of no clustering).

5.3.2.2 Kamuli district

In Kamuli, the 283 sleeping sickness cases reported between January 2000 and July 2005 came from eleven out of the twenty-three sub-counties in the district. These included the four sub-counties in the far east of the district (Namugongo, Bumanya, Gadumire and Namwiwa), a group of six centrally located sub-counties (Bugaya, Bugulambya, Balawoli, Kitayunjwa, Nabwigulu and Namwendwa) and Kisozi sub-county in the south-west of the district (Figure 5.9).

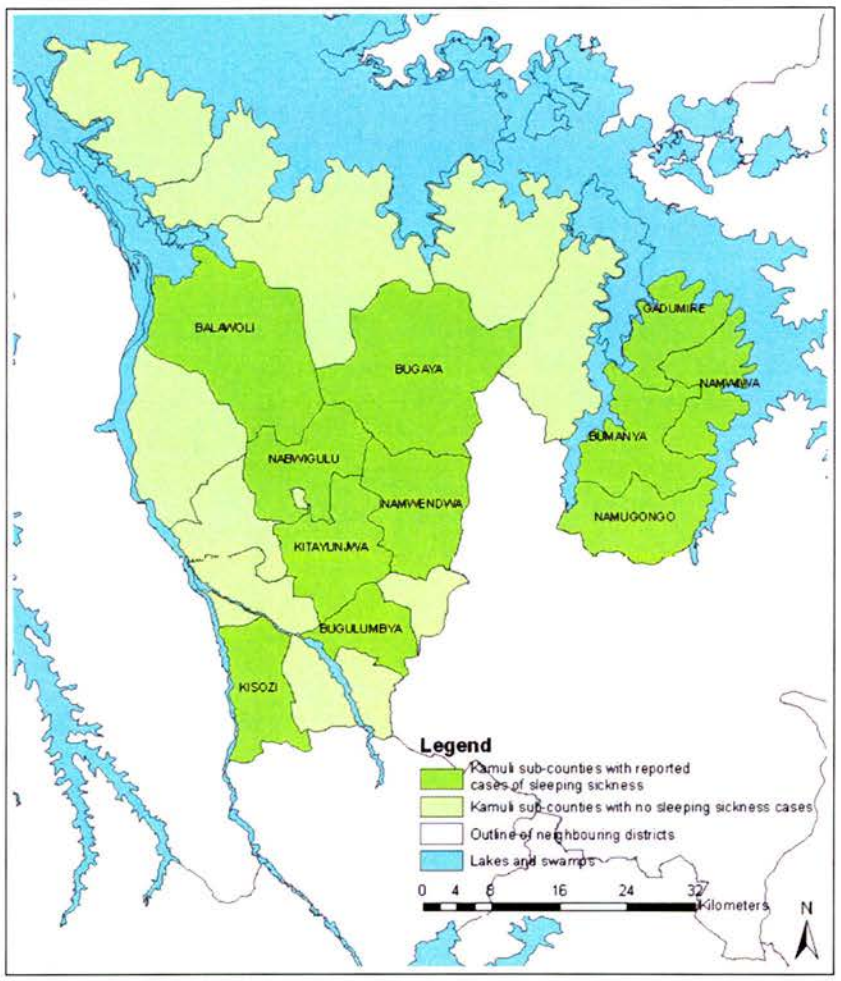


Figure 5.9– Map showing the sub-counties in Kamuli which have been affected by sleeping sickness from 2000 – 2005.

Notably only three sub-counties consistently reported sleeping sickness cases throughout the study period. Namwendwa had cases every year of the study and Bugaya and Kitayunjwa had cases in every year except 2005. In the other sub-counties, sleeping sickness was only reported in one or two years of the study (Figure 5.10). Of particular note is that 79% of all sleeping sickness cases reported in Kamuli district were from Namwendwa sub-county and that a subset of villages (Kyeeya, Kinu, Ndalike and Isingo) consistently reported sleeping sickness cases in each year from 2000 – 2003 (Figure 5.11). The dominance of Namwendwa sub-county in Kamuli is demonstrated by the close approximation between the number of cases reported in this sub-county and the district as a whole throughout the study period (Figure 5.10)

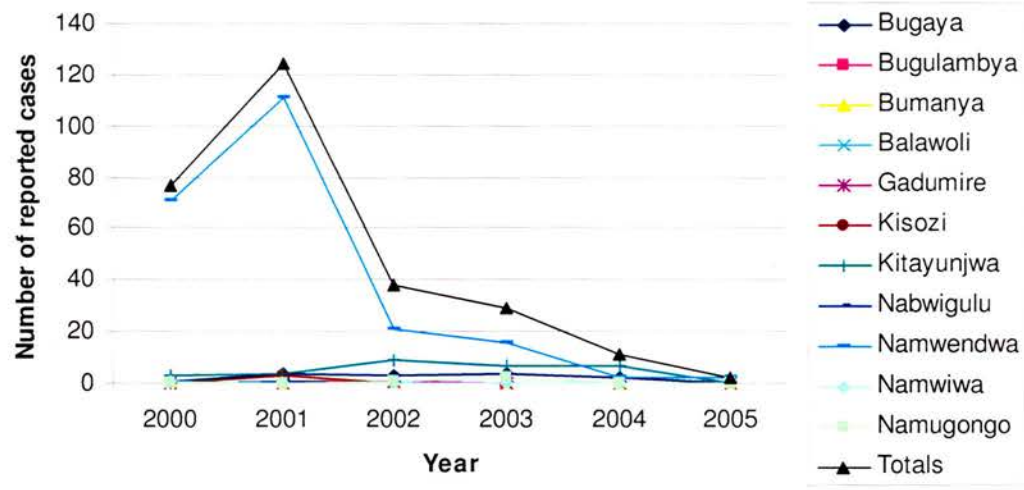


Figure 5.10 – Graph showing the number of sleeping sickness cases reported in each affected sub-county in Kamuli district from January 2000 to July 2005.

Spatial scan statistic analysis demonstrated a statistically significant primary cluster of sleeping sickness cases in all years of the study except 2005 (Table 5.4). From 2000 – 2003 a cluster was detected with a radius of 5.76 – 8.14km which was centred in the north-west of Namwendwa sub-county (Figure 5.11). In 2004 the cluster was of a larger radius (13.85km) and the gradual northward migration of the cluster that was evident during the course of the study had moved its centre into southern Bugaya (Figure 5.11).

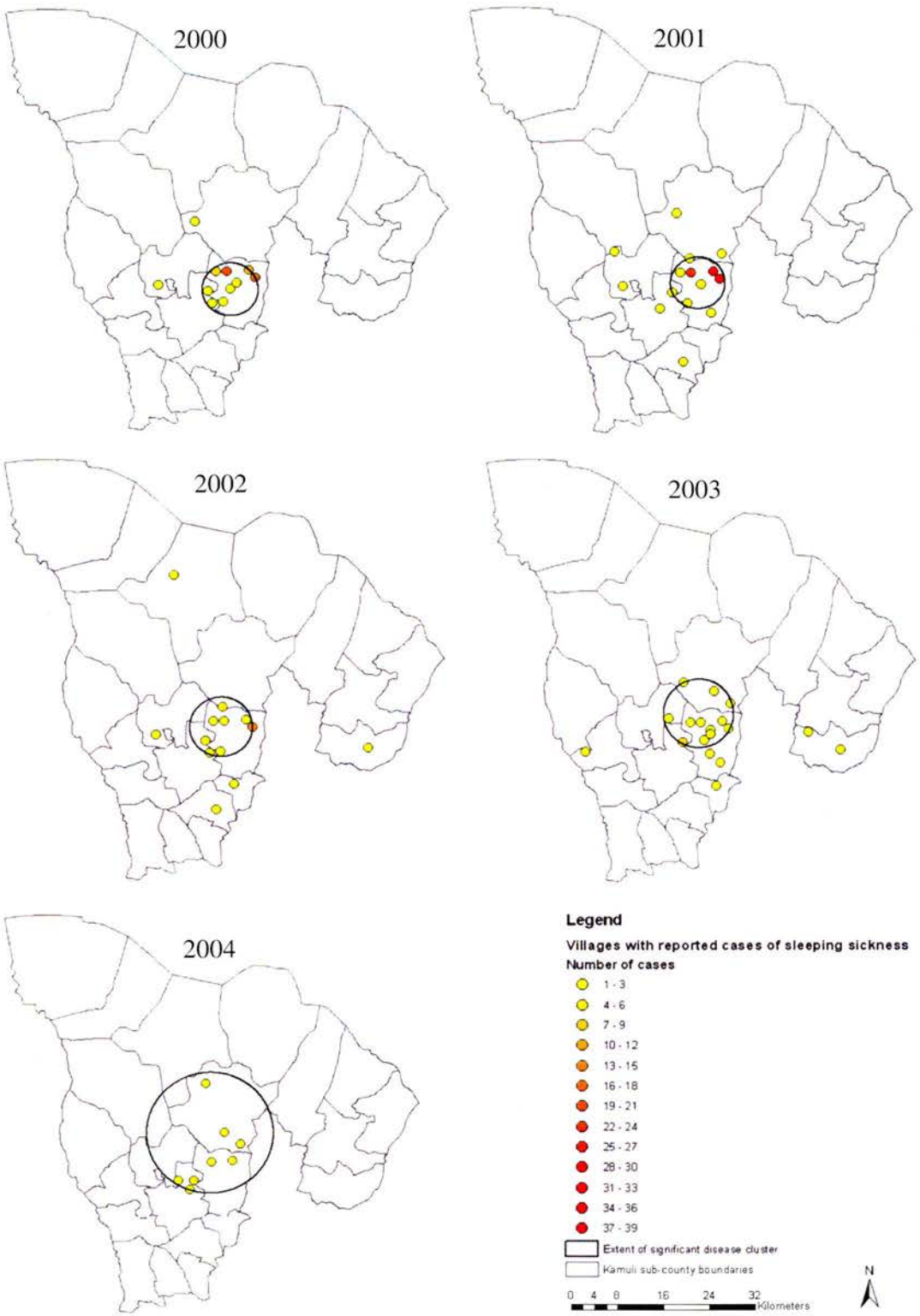


Figure 5.11 – Geographical distribution of sleeping sickness cases and significant clusters in Kamuli district in the years 2000 – 2004. Villages reporting sleeping sickness cases are shown according to the legend above, control villages are not shown. Significant clusters of cases as determined by application of the spatial scan statistic are shown by the circles. Detailed outputs of the spatial scan statistic are given in Table 5.4. The data for 2005 has not been presented.

Year	Type of cluster	No. of expected cases	Actual number of cases	Latitude	Longitude	Radius (km)	Log likelihood ratio	Overall relative risk	p-value	Majority of cluster located in (sub-county)
2000	Primary	13	71	0.93440	33.23451	5.76	135.76	5.43	0.0001	Namwendwa
2001	Primary	29	116	0.94977	33.24039	5.60	190.24	4.01	0.0001	Namwendwa
2002	Primary	5	32	0.95838	33.22258	6.17	59.33	6.70	0.0001	Namwendwa
2003	Primary	4	24	0.99740	33.22494	8.14	39.58	6.41	0.0001	Namwendwa
2004	Primary	2	11	1.03022	33.22514	13.85	18.15	5.00	0.0001	Namwendwa/Bugaya
2005	primary	0	2	1.03211	33.18958	11.22	4.11	7.74	0.3475*	None

Table 5.4– Table showing the results of the annual cluster detection of reported cases of sleeping sickness in Kamuli district by application of the spatial scan statistic. The number of expected cases refers to the number of cases expected in the cluster if distribution was random (i.e. the null hypothesis of no clustering). *This is not a significant result and so has not been shown in Figure 5.11.

5.3.3 Comparison of incidence and distribution of sleeping sickness cases pre- and post-intervention

5.3.3.1 Soroti

Comparison of the average annual incidence of sleeping sickness cases in the pre-intervention (January 2000 – December 2002) and post-intervention (January 2003 – July 2005) periods of the study show there was an increase from 3.82 to 4.50 cases reported per 10,000 people in the six sub-counties. Similarly, the median monthly number of cases reported in the two years after the intervention (2003 and 2004) was higher than that reported in the two years immediately prior to the intervention (2001 and 2002); however the results of a Mann-Whitney *U* test (Table 5.2b) indicates that this difference was not statistically significant ($p = 0.9671$).

Examination of the incidence of reported sleeping sickness cases at the parish level (the smallest administrative unit in Uganda) permitted a higher resolution comparison of the pre- and post-intervention periods and revealed a complex pattern. Whilst some parishes demonstrated decreased annual incidence of reported sleeping sickness cases in the post-intervention period compared to the pre-intervention period, others demonstrated either an increase or no change (Figures 5.12). This inconsistent pattern was replicated in the fourteen parishes in which the intervention sites were located, with five showing no change, four showing an increase and five a decrease in incidence in the post-intervention period compared to the pre-intervention period (Table 5.5).

In the parishes which hosted an intervention site the average incidence of reported sleeping sickness cases increased from 1.27 cases per 1000 head of population in the pre-intervention period (January 2000 - December 2002) to 1.59 cases per 1000 head of population in the post-intervention period (January 2003 – July 2005), despite the latter period being 6 months shorter. By comparison, the average incidence of sleeping sickness in parishes which reported cases but did not host intervention sites showed a smaller increase - from 1.55 cases per 1000 head of population in the pre-intervention period to 1.58 cases per 1000 head of population in the post-intervention

period. Therefore, it would appear that intervention sites didn't exert a local effect that reduced the incidence of reported cases.

Description of reported case clustering by application of the spatial scan statistic implies that the intervention had minimal impact on the geographical distribution of sleeping sickness cases in Soroti district (Table 5.5). In both the pre- and post-intervention periods a primary cluster of cases was approximately centered on the Kateta/Pingirie border whilst a secondary cluster was located to the north-east in either Kyere (pre-intervention) or neighboring Asuret (post-intervention) sub-counties (Figures 5.12).

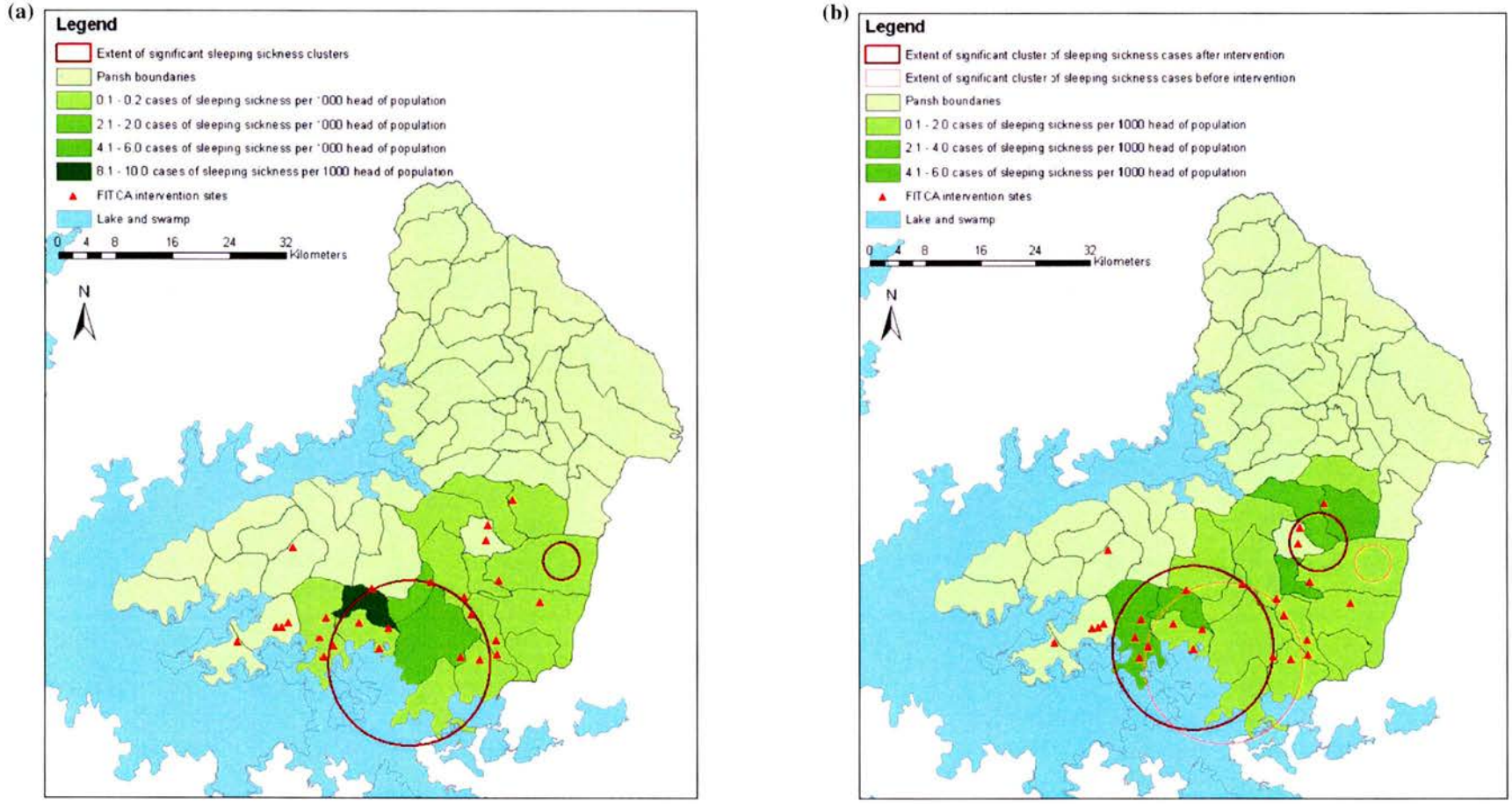


Figure 5.12 – Maps showing the incidence of reported sleeping sickness cases in Soroti during (a) the pre-intervention period (January 2000 – December 2002) and (b) the post-intervention period (January 2003 – July 2005). Annual incidence of sleeping sickness cases in each parish are shown according to the legend above.

Sub-county	Parish	Number of intervention sites	No. of sleeping sickness cases pre-intervention (Jan 00 to Dec 02)	No. of sleeping sickness cases post-intervention (Jan 03-July 05)	Change
Bugondo	Kangeta	1	0	0	-
Serere	Okulonyo	1	0	1	↑
	Osuguro	1	14	13	↓
	Oburin	1	0	2	↑
Pingire	Aaropoo	1	0	0	-
	Labori	3	0	0	-
	Pingire	4	1	43	↑
	Kadapakol	3	1	1	-
Kateta	Kateta	1	51	22	↓
	Ojetenyang	1	4	4	-
	Kamusala	3	7	4	↓
Kyere	Kelim	1	9	6	↓
	Kyere	1	8	4	↓
Asuret	Mukura	1	5	25	↑
Atiira	Atiira	2	0	0	-
Total		25	100	124	↑

Table 5.5 – Location and number of FITCA intervention sites in Soroti district by sub-county and parish. The numbers of reported sleeping sickness cases from parishes containing an intervention site is also shown. The relative change in numbers of reported cases in these areas is shown as ↓ for a decrease and ↑ for an increase in the number of reported cases.

5.3.3.2 Kamuli

The average annual incidence of reported sleeping sickness cases in the eleven sub-counties in Kamuli which reporting sleeping sickness cases during the study decreased from 2.03 to 0.36 cases per 10,000 people from the pre- (January 2000-December 2002) to the post- intervention period (January 2003 – July 2005). This is reflected in the statistically significant decrease identified in the median monthly number of cases seen in the two years immediately after the intervention compared to the two years immediately prior to it using the Mann-Whitney *U* test ($p = 0.0002$ – Table 5.2c).

Analysis of the incidence of sleeping sickness cases reported at the parish level emphasizes that in Kamuli a disproportionate number of cases were reported from a geographically small area. In the pre-intervention period two parishes in the north-east of Namwendwa sub-county (Keeya and in particular Ndalike) had markedly high incidence rates and accounted for a high proportion of the reported cases. Ndalike parish alone reported 109 cases in the pre-intervention period (54% of the total cases reported in Kamuli during this period) and had an incidence of 15.9 cases per 1000 head of population. Notably in the post-intervention period no parishes in Kamuli exhibited such dramatically high incidence rates (Figure 5.13b).

In the nine parishes that hosted intervention sites in Kamuli there was a substantial decrease in the incidence of reported sleeping sickness cases from 5.54 cases per 1000 head of population in the pre-intervention period to 0.76 cases per 1000 head of population. A decrease in numbers of cases reported was evident in the majority of the parishes in which an intervention site had been located, with only Nawansaso parish demonstrating an increase in numbers of cases reported in the post-intervention period (Table 5.6). A decrease in the incidence of reported cases was also seen in the parishes which had reported cases of sleeping sickness within the study period but did not host an intervention site with a drop from 0.37 cases per 1000 head of population pre-intervention to 0.01 cases per 1000 head of population post-intervention.

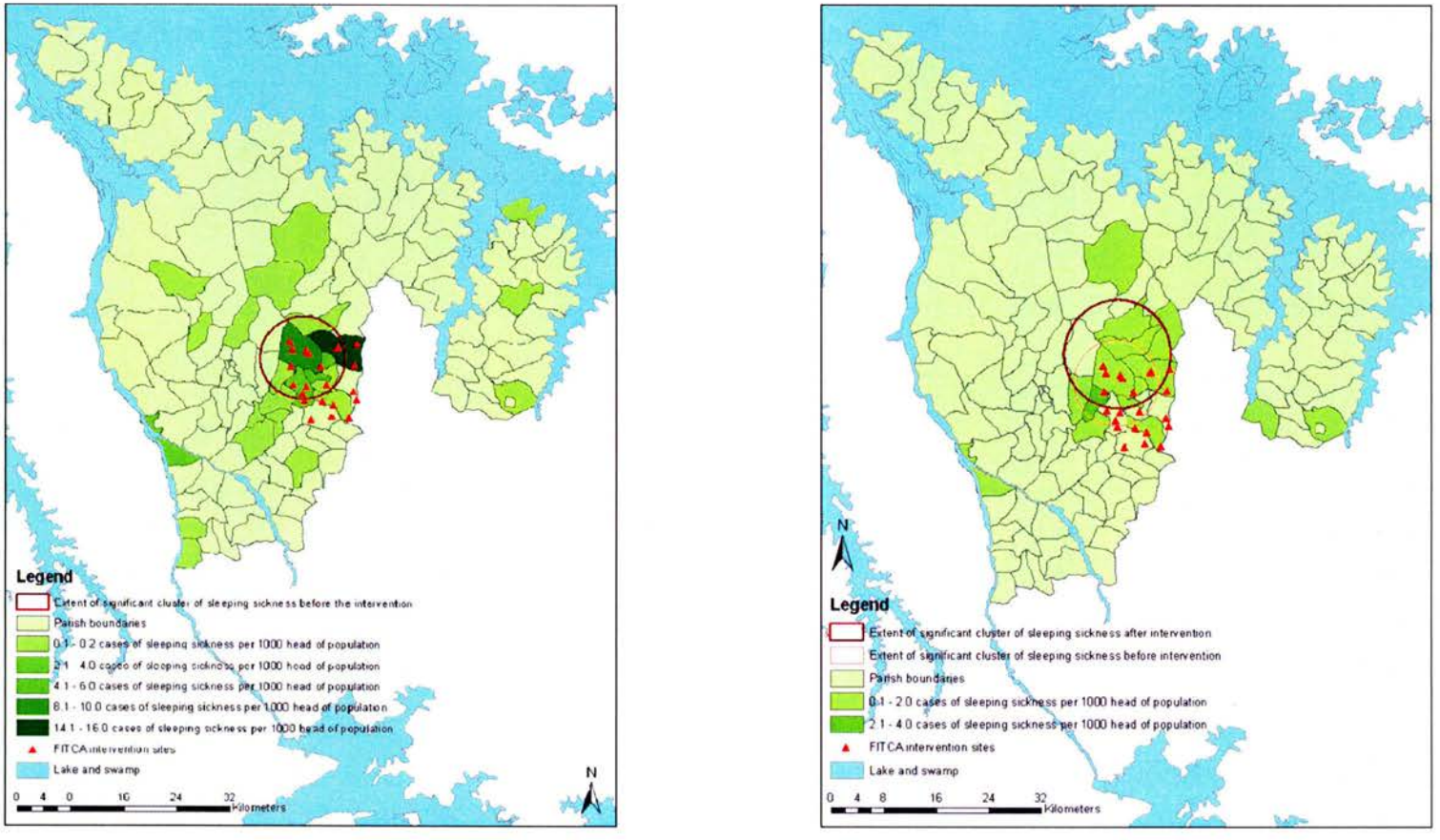


Figure 5.13 – Maps showing the incidence of reported sleeping sickness cases in Kamuli during (a) the pre-intervention period (January 2000 – December 2002) and (b) the post-intervention period (January 2003 – July 2005). Annual incidence of sleeping sickness cases in each parish are shown according to the legend above.

Sub-county	Parish	Number of intervention sites	No. of sleeping sickness cases pre-intervention (Jan 00 to Dec 02)	No. of sleeping sickness cases post-intervention (Jan 03-July 05)	Change
Namwendwa	Kyeeya	4	44	4	↓
	Ndalike	4	109	5	↓
	Isingo	1	15	4	↓
	Namwendwa	1	6	0	↓
	Kidiki	2	0	0	-
	Bulange	3	0	2	↑
	Makoka	2	0	0	-
	Bulogo	4	14	0	↓
Kitayunjwa	Nawansaso	1	9	12	↑
Total		22	197	27	↓

Table 5.6 – List of FITCA the location and number of intervention sites in Kamuli district by sub-county and parish. The numbers of reported sleeping sickness cases from parishes containing an intervention site is also shown. The relative change in numbers of reported cases in these areas is shown as ↓ for a decrease and ↑ for an increase in the number of reported cases.

5.4 Discussion

Cattle are recognised as the most important reservoir host for the human infective *T. b. rhodesiense* in Uganda (Welburn *et al.*, 2001b). Based on mathematical modelling it has been proposed that chemoprophylactic treatment of the animal reservoir may form an effective method in control of *T. b. rhodesiense* sleeping sickness in humans (Welburn *et al.*, 2001a). Under the FITCA Uganda programme a mass treatment on cattle was completed at the end of 2002. By examining the number and distribution of sleeping sickness cases reported in two of the districts (Soroti and Kamuli) included within the FITCA programme area over a five and a half year period (January 2000 – July 2005) this chapter aimed to assess the impact this intervention had on the incidence of human disease.

5.4.1 Sleeping sickness in Soroti and Kamuli districts during the study period

The analysis completed in this chapter has provided descriptions of very different scenarios in Soroti and Kamuli districts. In Soroti the reported sleeping sickness cases all came from a block of six sub-counties in the south-east of the district; Asuret, Atiira, Kateta, Kyere, Pingire and Serere/Olio. Although the annual number of sleeping sickness cases reported in the district remained relative consistent from 2000 through to 2004, the number of cases reported from individual sub-counties showed marked annual variation (Figure 5.7). This was reflected in the substantial movement of significant clusters of reported cases identified in different years of the study period by application of the spatial scan statistic (Figure 5.8).

In contrast to Soroti, the number of cases reported in Kamuli varied substantially over the study period; after an initial increase from 2000 to 2001, the number of cases reported progressively decreased in the subsequent years (Figure 5.5b). Cases were reported in geographically distant and discrete parts of the district ranging from

Gadumire sub-county in the far north-east to Kisozi sub-county in the extreme south-west of the district. However, in most of the sub-counties cases were only reported intermittently. The exceptions to this were the adjoining sub-counties of Bugaya, Kitayunjwa and Namwendwa located in the centre of the district, which reported cases in most/all years of the study period. The most notable feature in Kamuli district was the dominance of Namwendwa sub-county, where 78% (222 out of 287) of all cases in Kamuli during the study period were reported. This dominance is demonstrated by the similar trends in annual numbers of cases reported in Kamuli district and Namwendwa sub-county (Figure 5.10). When examined at a parish level it was evident that within Namwendwa a large proportion of the cases originated from just two parishes (Keeya and Ndalike) in the north-east of the sub-county. As a consequence of this, reported cases in Kamuli were significantly clustered around the north-east of Namwendwa from 2000 – 2003. To understand the highly localized nature of this outbreak detailed village level analysis would need to be completed (T. Zoller, in prep).

The different scenarios described in Soroti and Kamuli most likely represent the differences in the contemporary history of sleeping sickness in these two districts. Sleeping sickness was only introduced into Soroti four years prior to the beginning of this study when an epidemic was initiated following movement of infected cattle (Fèvre *et al.*, 2001), whereas it has been endemic in Kamuli for at least a century (Mbulamberi, 1990; Koerner *et al.*, 1995). The continuous reporting of a high/moderate number of cases over a geographically defined area in Soroti, with a high degree of motility in the focus of clustered disease is consistent with an ongoing unstable epidemic. In contrast, the reporting of a large number of cases from a small area, complemented by the sporadic reporting of low numbers of cases from other locations, as seen in Kamuli, is suggestive of a significant recrudescence (and then decline) of disease from one of numerous endemic loci. The reasons for these localized outbreaks remain unclear but may be triggered by changes in tsetse numbers or changes in vector-human exposure (Berrang-Ford *et al.*, 2006).

In Soroti an element of seasonal variation was evident in the number of cases reported, an observation that has been made previously in this district (Fèvre, 2002) and is currently under further investigation (E. Fèvre, pers. comm.). Intriguingly evidence of seasonal variation in the number of cases reported appears to be absent in Kamuli despite the geographical proximity and similarities in demographics and ecology of the two districts.

5.4.2 Assessment of the impact of the FITCA Intervention

The results from the comparison of the pre- and post-intervention periods in Soroti indicate that the intervention had minimal impact on the numbers of cases reported, with the annual incidence of reported sleeping sickness cases actually higher in the post-intervention period (n = 218) than in the pre-intervention period (n = 185). This reasons for this are unclear, however, it could be due to an increasing awareness about sleeping sickness promoted by the FITCA interventions. Analysis with the spatial scan statistic suggests the intervention also had negligible impact on the geographical distribution of cases, with the significant clusters of reported disease being similarly located in the pre- and post-intervention periods (Figure 5.12). This lack of effect was also evident at the local level, with parishes hosting intervention sites showing a greater increase in incidence of reported cases post-intervention (n = 127) than parishes in which no intervention sites were located (n = 86).

In Kamuli comparison of the pre- and post-intervention period indicates that the intervention was associated with a significant decrease in the numbers of sleeping sickness cases reported. However, it is evident from Figure 5.4b and 5.5b that the number of cases being reported in Kamuli had already begun to decline prior to the intervention. Indeed, it was between 2001/2002 (i.e. a year before the intervention) that a significant decrease in the annual median monthly number of cases (Mann Whitney U Test $p > 0.01$) was recorded. Hence, it would appear that the intervention was co-incidental with, rather than the cause of, the reduction in the number of reported cases in Kamuli district. Similarly the dramatic decrease in reported case incidence seen in parishes hosting intervention sites (from 197 to 27) is probably an

artifact and attributable to the location of most of the intervention sites in Namwendwa.

5.4.3 Study limitations

Accurately assessing the impact of large scale interventions implemented in the 'field' is subject to numerous difficulties. Prominent amongst these is the unquantifiable effect of the many uncontrolled factors that may be concurrently affecting the epidemiology of a disease. In the case of human sleeping sickness caused by *T. b. rhodesiense* ecological factors associated with the tsetse fly (e.g. weather conditions and changes in land usage causing alteration in the vegetation habitat of the fly), and changes in reservoir host populations (e.g. the continuing re-introduction of potential infected cattle into study areas (Fèvre *et al.*, 2006b)) will obviously have the potential to influence the epidemiology of HAT. Furthermore, socio-political disturbances which cause large scale movement of people and their livestock, such as that following the presence of the Lord's Resistance Army (LRA) in Soroti during 2003, are also known to promote cases of sleeping sickness (Maudlin, 2006; Welburn *et al.*, 2006). Another factor to be considered is that the study used reported rather than actual number of sleeping sickness cases to evaluate the impact of the intervention and it is known that in Uganda there is substantial under-reporting of the disease (Odiit *et al.*, 2005).

The ability to control or quantify the impact of these factors or instigate an active case finding programme were beyond the scope of this study. However, it is important that the effects of intervention programmes are assessed under 'real-life' conditions rather the under strictly controlled conditions that can not be realistically replicated in the 'field'. Although the results of the study indicate the intervention had negligible impact on HAT it is important to appreciate that it isn't possible to determine how the pattern of disease would have been different in the absence of the intervention. Establishment of control sites in which no intervention occurred was not an option within the FITCA programme and the district-specific scenarios of

HAT incidence described would have made comparison between control and intervention areas difficult to interpret.

5.4.4 Conclusions

Reducing the size of the animal reservoir of zoonotic pathogens is a control strategy employed for various diseases including leishmaniasis (Gramiccia & Gradoni, 2005), rabies (Cleaveland, 1998) and schistosomiasis (Guo *et al.*, 2006). It has been suggested that reducing the prevalence of *T. b. rhodesiense* in the cattle reservoir may be an effective method of controlling human East African sleeping sickness (Welburn *et al.*, 2001b; Welburn *et al.*, 2006).

The work completed in this Chapter provides the first analysis on the impact that mass trypanocidal chemoprophylaxis of cattle has on the incidence of human sleeping sickness. The results indicate that in both study areas, the impact of this intervention was negligible. However, the intervention in Soroti was implemented during the course of an epidemic outbreak and in Kamuli at a time when the human disease was already remitting. Thus, the intervention may have had an impact that was masked by these events. However, it is certain that the FITCA intervention did not prevent the spread of *T. b. rhodesiense* into previously unaffected districts beyond Soroti. In 2004 the first cases of sleeping sickness were reported in Kaberamaido and Lira districts to the west and north of Soroti (Picozzi *et al.*, 2005).

Modifying the way in which mass treatment of cattle interventions were implemented may provide more satisfactory results. In Soroti the percentage of the cattle population treated was approximately 40%, (Chapter 3) which was substantially below the 80% which the mathematical model suggests would be effective (Coleman & Welburn, 2004), and use of serial rather than a single dose regime may have more substantive effects. Various other HAT control strategies have been employed in Uganda, (see Chapter 2), but few have assessed the impacts

in terms of the sleeping sickness incidence in the human population. In a recent review, Welburn and colleagues present numbers of reported sleeping sickness cases during the 1980's and 90's pinpointing when aerial spraying occurred in the region, the numbers of human cases decrease dramatically but it appears that the peak of the epidemic occurred before the spraying was implemented (Welburn *et al.*, 2006). It is therefore difficult to make comparisons between the effectiveness of the FITCA programme and alternative control policies. In future, integration of control programmes that concurrently target the animal reservoir, tsetse vector and human population will probably offer the best means of controlling sleeping sickness in eastern Uganda.

6 Chapter 6:

General discussion

6.1 Discussion

Farming in Tsetse Controlled Areas (FITCA) is a regional EU funded programme designed to promote sustainable agriculture in areas where trypanosomiases is prevalent. In Uganda the programme predominantly took the form of a mass treatment of cattle with trypanocidal drugs, administered in November/December 2002. The aim of this thesis was to evaluate the impact of this intervention on the prevalence of bovine and human trypanosomiases in the treated districts and provide comparative data on real-world, large scale interventions. The main conclusions obtained from the results of this thesis are:

1. Mass treatment of cattle with trypanocidal drugs effected a significant reduction in the prevalence of pathogenic trypanosome species in the sampled cattle population. The prevalence of *T. brucei* s.l, *T. c. savannah* and *T. vivax* all decreased at both three months and one year post-intervention. Although decreases in prevalence were seen in all districts, the effects varied across districts with Kamuli the only district demonstrating a significant decrease.

2. There was a significant reduction in the proportion of human infective *T. brucei rhodesiense* detected in the cattle population after mass treatment. In Soroti where epidemic conditions exist, this effect appeared to be temporary with a reduction in prevalence evident three months post-intervention before returning to pre-intervention levels one year post-intervention. In contrast, this effect was more persistent in Kamuli, with no *T. b. rhodesiense* detected at either three months or one year post-intervention.

3. Despite the reduction observed in *T. b. rhodesiense* within the cattle reservoir there appeared to be no impact on the incidence and distribution of reported cases of human sleeping sickness following the FITCA intervention. This was studied in two districts: Soroti which is experiencing an epidemic of sleeping

sickness and Kamuli which is within an area endemic for sleeping sickness. Although the incidence of human disease and distribution of disease varied considerably between the districts, the intervention appeared to have negligible effect in either.

In summary, although the FITCA intervention reduced the prevalence of tested trypanosome species within the sampled cattle population, and specifically, the levels of *T. b. rhodesiense* within the cattle reservoir, there was no associated reduction in the incidence of reported human sleeping sickness cases. The intervention therefore did not have a public health benefit.

6.1.1 The FITCA Uganda programme

As the name implies, FITCA, was initially intended as a programme to promote sustainable agriculture practices of which tsetse related issues were a part. However, faced with the reality of the situation in Uganda, these priorities changed under pressure to address specifically, the problem of sleeping sickness within eastern Uganda. Thus, measures to control trypanosomiasis were accepted as part of the programme and consequently, the methods used to implement the control strategies may not have been optimal.

One of the first problems with the FITCA study design was the decision to restrict control measures to designated high-risk areas. These high-risk areas were determined prior to treatment by compiling a risk map based on the location of reported human sleeping sickness cases coupled with a microscopy survey of cattle throughout the area (Figure 6.1). In theory the decision was shrewd as limited resources were available for the intervention, meaning that cattle in all areas could not be treated, but unfortunately a number of problems arose. The first of these was the time delay between the completion of the human and cattle trypanosomiasis surveys (carried out in 2000) and the launch of the intervention (2002). Unfortunately, in the intervening years the range of the disease increased, meaning

that the FITCA interventions were not always targeted to the correct areas – this was most apparent in Soroti which was latterly added to the programme. Considerable under reporting of sleeping sickness cases across eastern Uganda (Odiit *et al.*, 2005) may also have affected the accurate identification of high risk areas. Additionally, the construction of the risk map based on administrative structure, using the sub-county, meant that in some circumstances high-risk areas were situated adjacent to low-risk areas (Figure 6.1) in which no interventions occurred. Obviously, in reality the spread of sleeping sickness is not limited by administrative boundaries therefore sub-counties adjacent to high-risk areas must have had a higher level of risk.

While under reporting of human sleeping sickness cases no doubt affected the accuracy of the risk map, so too the decision to use microscopy to screen cattle for disease as it is considerably less sensitive than PCR for trypanosome detection (as demonstrated in Chapter 3). This survey was also not carried out in all sub-counties within the FITCA area, resulting in areas of unknown risk (shown as white in Figure 6.1).

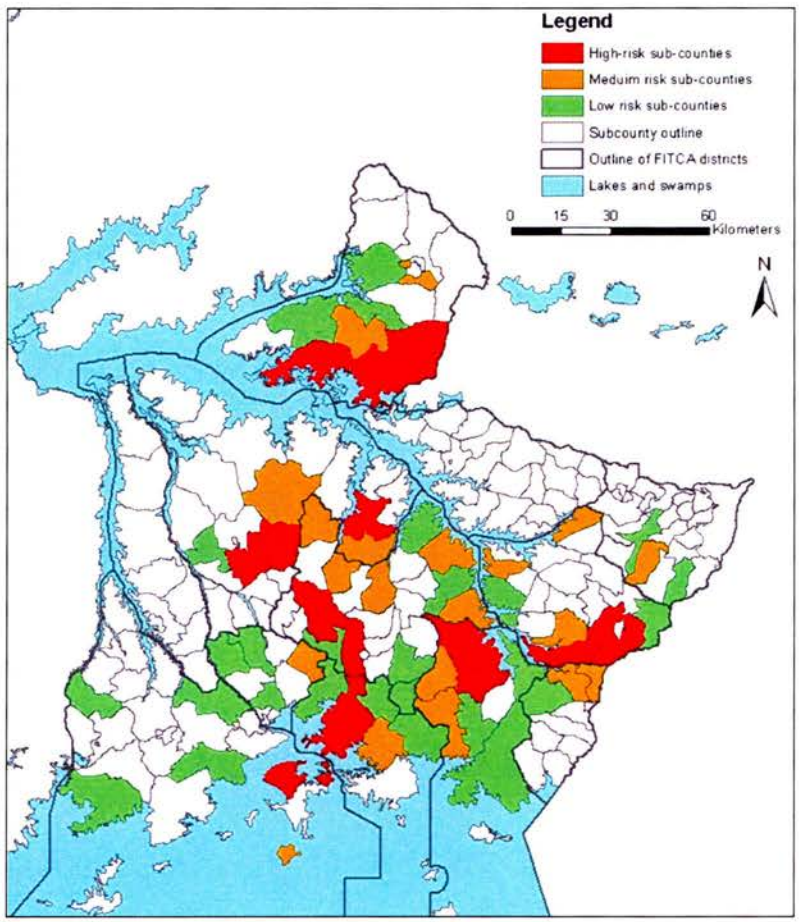


Figure 6.1 - Map showing high, medium and low risk sub-counties within the FITCA area

The FITCA Uganda interventions were implemented in a top-down strategy through the administration structures of the individual districts within the FITCA areas. The ongoing decentralisation of Ugandan veterinary services resulted in the FITCA project money being used to support these under-resourced decentralised structures rather than the intervention activities themselves. Ceding responsibility for the administration of FITCA to the veterinary services in each district led to numerous district to district differences in the way that interventions were implemented. This was clearly illustrated in Kamuli district where the decision was made to target almost all interventions to a single high-risk sub-county, Namwendwa, while in Soroti the intervention sites were distributed across all the high-risk sub-counties (shown in Chapter 5). Differences in implementation between districts may also

account for the difference in treatment coverage – results in Chapter 3 showed that Kamuli achieved a coverage rate of over 80% in each of the sample sites, almost double that found in the other districts.

Another factor hampering the implementation of the FITCA project in Uganda was administrative delays. It is clear from FITCA Uganda quarterly reports, that the programme suffered from administrative delays at every level, for example, there were delays in the arrival of money from the EU to the country programme, which resulted in planning delays in-country, which in turn resulted in delaying the flow of resources to the districts. Despite these problems with the design and implementation of the FITCA project in Uganda, the mass treatment of cattle with trypanocidal drugs was shown in Chapter 3 and 4 to have a significant impact on the prevalence of trypanosomes within the sampled cattle population. This success highlights how effective the mass treatment of cattle could be as a trypanosomiasis control strategy under a different implementation regime.

Beyond the study period covered in this thesis, FITCA continued working in eastern Uganda. After the implementation of the mass treatment programme, the focus of FITCA returned to the original aims of promoting sustainable farming practices, including encouraging farmers to use animal traction for ploughing, encouraging the use of zero grazing units and the forming of insecticide spraying cooperatives. The spraying cooperatives were based in the designated high-risk areas; livestock keepers were encouraged to form farmer groups and construct spraying crushes. Each group was then given one litre of Decatix® and hand pump sprayers were provided to be shared between a number of groups. The groups were informed they should charge an amount for each animal sprayed to allow the purchase of the next batch of insecticide. It has been observed by this author that in the majority of cases spraying was discontinued when the supplied insecticide was finished. In one situation in Kamuli, where farmers groups had the dedicated support of the local animal husbandry officer (AHO), some of the groups managed to acquire enough funds to purchase more insecticide. However, in many cases this had required the direct

intervention of the AHO, for example, in situations when money had gone missing from within the group or when problems had arisen in the sharing of the spray unit (Dr Waako, DVO Kamuli, pers. com.). At the time of writing FITCA was continuing to operate in eastern Uganda, in a partnership with a British NGO, Send a Cow, providing netting for the zero grazing units necessary for the housing of the exotic cross breed animals provided by Send a Cow (S. Gould, technical advisor for FITCA Uganda, pers. com).

6.1.2 Control options for trypanosomiasis in Uganda

Over the past 100 years many strategies have been employed to control both human and animal trypanosomiasis, of which some have been detailed in the historic review in Chapter 2. In the past, just as today, methods of trypanosomiasis control have been influenced not only by scientific advances but also by economics and politics. The most controversial, complicated and expensive solution currently available is the sterile insect technique which despite many concerns among tsetse and trypanosomiasis researchers (Molyneux, 2001a; Torr *et al.*, 2005) is currently supported by PATTEC as the best solution for tsetse control across Africa (Kabayo, 2002; Kabayo, 2005). However, the prohibitive cost and concerns regarding its practicality, cost, safety, and public acceptance make it increasingly likely that in the twenty first century we will continue to rely on low tech solutions and in particular, the use of insecticides and trypanocides.

One of the current methods for trypanosomiasis control in Uganda is the use of insecticide treated traps which are required at a density of ten traps per square kilometre to be effective at reducing fly densities sufficiently to interrupt the transmission of sleeping sickness (Lancien, 1991). Changing attitudes of donor organisations in the 1990's shifted the emphasis of tsetse control to the local community level (Torr *et al.*, 2005). Subsequently, limited success has been demonstrated with community-based trapping in Tororo using locally produced materials (Okoth *et al.*, 1991) however, these are unlikely to achieve the coverage required. The success of such community based approaches are governed by the

ability of farmers groups to cooperate and these types of community-based approaches are often dependant on the use of farmers private resources for a 'public' good (Torr *et al.*, 2005). Consequently, the constraints faced by poor rural communities in terms of financial wealth and a lack of technical advice have resulted in traps not being optimally utilised (Dransfield & Brightwell, 2004).

An alternative to the use of traps is insecticide treated cattle which act as live bait for the tsetse. However, this can only be effective in areas where there is a high concentration of cattle and can result in patchy coverage (Hargrove *et al.*, 2000). As shown in this thesis the mass trypanocidal treatment of cattle can be an effective strategy to reduce the prevalence of trypanosomes within cattle population and combined with the simultaneous use of some form of tsetse control, for example insecticide spraying of cattle, the effect could be even greater. However, farmers will probably only adopt regular insecticide spraying of cattle if it is made affordable. This has become a reality with the use of restricted application of insecticides to tsetse predilection sites, namely the legs and belly (Torr & Mangwiro, 2000), reducing the cost of treatment to less than US$1 per animal (Vale & Torr, 2005). Coordinated activities resulting in the implementation of integrated control, targeting cattle, humans and tsetse requires co-ordination between veterinary and medical authorities. Such cross-sectorial collaborations could also assist in targeting emerging sleeping sickness problem areas, where rapid communication between the health and veterinary sectors could result in the implementation of cattle treatments and spraying to prevent further spread. As shown in this thesis, treatment of the cattle reservoir must be an important part of any sleeping sickness control programme in Uganda as cattle are a significant reservoir of sleeping sickness caused by *T. b. rhodesiense* and have been implicated in the spread of disease (Fèvre *et al.*, 2001).

6.1.3 Immediate future

The FITCA mass treatment of cattle failed to stem the northward spread of sleeping sickness, however, this mass treatment was shown to reduce the cattle reservoir of *T.*

b. rhodesiense. There is currently great concern in Uganda concerning the overlap of the *T. b. rhodesiense* focus with the *T. b. gambiense* focus of sleeping sickness in the north west of the country (Picozzi *et al.*, 2005). This situation is becoming more acute given the potential resolution of the ongoing civil disturbances in the north of the country. There have been *T. b. rhodesiense* sleeping sickness cases reported from an internally displaced peoples (IDP) camp in Aloi in northern Lira (A. Mpairwe, Medical officer, Lwala hospital, Uganda) and these people are now being encouraged to return to their homes in northern Uganda leading to a potential spread of *T. b. rhodesiense* sleeping sickness to areas further north. Although, a greater potential risk factor is the movement of cattle with the returning IDPs, as recent surveys have shown the presence of *T. b. rhodesiense* within the cattle population in Lira (B. Wissmann, unpublished). This risk will undoubtedly increase with the peaceful resolution to the current conflict as the Ugandan government/ World Bank aid administered through the Northern Uganda Social Action Fund (NUSAF) is already seeing cattle restocking as a major part of re-building the northern economy (C. Laker, pers. com). It is therefore essential for the cattle sold at markets as well as those belonging to returning IDPs to be treated.

In areas newly affected by sleeping sickness there is a lack of knowledge about the disease, which can be confused with HIV/AIDS. In one situation a local official described how an elderly widow had become ostracised from her community as it was suspected that she was suffering from HIV/AIDS and only after the intervention of a distant family member was it was revealed to be sleeping sickness. This confusion leads to a reluctance to seek treatment resulting in unnecessary suffering and without treatment certain death. It is therefore, essential that education about the symptoms of sleeping sickness is part of any control programme, this is especially true in the areas in which the disease is new and there is little knowledge of the disease and its symptoms.

Currently in Uganda there is an ongoing control programme established by a public-private partnership to stop the spread of *T. b. rhodesiense* further north. The Stamp

Out Sleeping sickness (SOS) campaign is targeting the cattle reservoir using similar methods to those described in this thesis. Mass treatment using diminazene aceturate was completed in three districts – Kaberamaido, Lira and Apac – treating all cattle in these areas, involving approximately 220,000 animals. As discussed above, it is difficult to accurately target high-risk areas, thus, the best way to ensure maximum effect is to treat all cattle and include all areas at risk from the spread of sleeping sickness, not just areas where cases have already occurred. The SOS programme is multi-pronged with cattle treatment accompanied by the simultaneous spraying of insecticide to target the tsetse population. The combined use of trypanocides to clear the cattle reservoir and insecticides to reduce the tsetse population is anticipated to prevent any further spread of *T. b. rhodesiense,* and hopefully prevent the merging of the *T. b. rhodesiense* focus with the north western *T. b. gambiense* focus. A public health communication project aimed at disseminating information about sleeping sickness through local media and local leaders has also been implemented to help educate and change perceptions about the disease.

Trypanosomiasis remains a neglected disease but with human disease burdens a mere shadow of its infamous compatriots such as malaria, HIV, and tuberculosis, the lack of investment in its control is understandable. However, trypanosomiasis places more of a toll on human welfare than is immediately apparent from looking at morbidity and mortality figures alone. Nagana is a devastating disease of cattle placing immeasurable constraints on agricultural production and consequently socio-economic development across vast swathes of sub-Saharan Africa. With limited resources available for disease control it is therefore, imperative that the best strategies are employed in order to gain maximum benefit.

7 References

Abaru, D.E. (1985). Sleeping sickness in Busoga, Uganda 1976 - 1983. *Tropical Medical Parasitology,* **36**, pp. 72-6.

Afewerk, Y., Clausen, P.H., Abebe, G., Tilahun, G., & Mehlitz, D. (2000). Multiple-drug resistant *Trypanosoma congolense* populations in village cattle of Metekel district, north-west Ethiopia. *Acta Tropica,* **76**, pp. 231 - 238.

Allsopp, R. (1984). Control of tsetse flies (Diptera: Glossinidae) using insecticides: a review and future prospects. *Bulletin of Entomological Research,* **74**, pp. 1-23.

Allsopp, R. (2001). Options for vector control against trypanosomiasis in Africa. *Trends in Parasitology,* **17**, pp. 11-19.

Allsopp, R. & Hursey, B.H. (2004). Insecticidal control of tsetse. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases*: CAB International.

Anene, B.M., Onah, D.N., & Nawa, Y. (2001). Drug resistance in pathogenic African trypanosomes: what hopes for the future? *Veterinary Parasitology,* **96**, pp. 83-100.

Annual Medical Report. (1913). *Annual report of the Medical and Sanitary department*. Entebbe: Protectorate of Uganda.

Annual Medical Report. (1925). *Annual report of the Medical and Sanitary department*. Entebbe: Protectorate of Uganda.

Annual Research Report. (1958). *The East African council for medical research annual report*. East African High Commission.

Annual Tsetse Control Report. (1952). *Annual report of the Tsetse Control department*. Entebbe: Protectorate of Uganda.

Annual Tsetse Control Report. (1953). *Annual report of the Tsetse Control department*. Entebbe: Protectorate of Uganda.

Annual Tsetse Control Report. (1955). *Annual report of the Tsetse Contol department*. Entebbe: Protectorate of Uganda.

Annual Tsetse Control Report. (1959). *Annual report of the Tsetse Control department*. Entebbe: Ugandan Protectorate.

Annual Veterinary Report. (1932). *Annual report of the Veterinary department.* Entebbe: Protectorate of Uganda.

Annual Veterinary Report. (1938). *Annual report of the Veterinary department.* Entebbe: Protectorate of Uganda.

Annual Veterinary report. (1945). *Annual report of the Veterinary department.* British Protectorate of Uganda.

Annual Veterinary Report. (1947). *Annual report of the Veterinary department.* British Protectorate of Uganda.

Annual Veterinary Report. (1953). *Annual report of the Veterinary department.* British Protectorate of Uganda.

Annual Veterinary Report. (1954). *Annual report of the Veterinary department.* British Protectorate of Uganda.

Annual Veterinary Report. (1959). *Annual report of the Veterinary department.* British Protectorate of Uganda.

Anon. (1911). *Sleeping sickness clearing scheme, revised November 8th 1911.* Entebbe, Uganda.

Artama, W.T., Agey, M.W., & Donelson, J.E. (1992). DNA comparisons of Trypanosoma evansi (Indonesia) and Trypanosoma brucei spp. *Parasitology,* **104 Pt 1**, pp. 67-74.

Awan, M.A.Q. (1971). The use of human plasma in the blood incubation infectivity test to differentiate *Trypanosoma brucei* and *Trypanosoma rhodesiense. Tropical Animal Health and Production,* **3**, pp. 183-186.

Awan, M.A.Q. (1979). Identification by the blood incubation infectivity test to of *Trypanosoma brucei* subspecies isolated from game animals in Zambia. *Acta Tropica,* **36**, pp. 343-347.

Bacchi, C.J. (1993). Resistance to clinical drugs in African trypanosomes. *Parasitology Today,* **9**, pp. 190-193.

Bailey, C.P. (1998). Tsetse fly eliminated on Zanzibar. *Nuclear News, January*, 56-61.

Bakker, M.I., Hatta, M., Kwenang, A., Faber, W.R., van Beers, S.M., Klatser, P.R., & Oskam, L. (2004). Population survey to determine risk factors for *Mycobacterium leprae* transmission and infection. *International Journal of Epidemiology,* **33**, pp. 1329-1336.

Barrett, M.P. (1999). The fall and rise of sleeping sickness. *The Lancet,* **353**, pp. 1113-1114.

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.

Bauer, B., Amsler-Delafosse, S., Clausen, P.H., Kabore, I., & Petrich-Bauer, J. (1995). Successful application of deltamethrin pour on to cattle in a campaign against tsetse flies (*Glossina* spp.) in the pastoral zone of Samorogouan, Burkina Faso. *Tropical Medical Parasitology,* **46**, pp. 183-189.

Becker, S., Franco, J.R., Simarro, P.P., Stich, A., Abel, P.M., & Steverding, D. (2004). Real-time PCR for detection of *Trypanosoma brucei* in human blood samples. *Diagnostic Microbiology and Infectious Disease,* **50**, pp. 193-199.

Belete, H., Tikubet, G., Petros, B., Oyibo, W.A., & Otigbuo, I.N. (2004). Control of human African trypanosomiasis: trap and odour preference of tsetse flies (Glossina morsitans submorsitans) in the upper Didessa river valley of Ethiopia. *Tropical Medicine & International Health,* **9**, pp. 710-714.

Berberof, M., Perez-Morga, D., & Pays, E. (2001). A receptor-like flagellar pocket glycoprotein specific to *Trypanosoma brucei gambiense*. *Molecular and Biochemical Parasitology,* **113**, pp. 127-138.

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

Berriman, M., Ghedin, E., Hertz-Fowler, C., Blandin, G., Renauld, H., Bartholomeu, D.C. & Lennard, N.J. et al. (2005). The genome of the african trypanosome *Trypanosoma brucei*. *Science,* **309**, pp. 416-422.

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

Boseley, S. (2001). Drug firm wakes up to sleeping sickness. *The Guardian,* 7/5/2001.

Bouteille, B., Oukem, O., Bisser, S., & Dumas, D. (2003). Treatment perspectives for human African trypanosomiasis. *Fundamental and Clinical Pharmacology,* **17**, pp. 171-181.

Braakman, H.M.H., van de Molengraft, F.J.J.M., Hubert, W.W.A., & Boerman, D.H. (2006). Lethal African trypanosomiasis in a traveller: MRI and neuropathology. *Neurology,* **66**, pp. 1094-1096.

Brenzel, L. & Claquin, P. (1994). Immunization programs and their costs. *Social Science & Medicine,* **39**, pp. 527-536.

Bromidge, T., Gibson, W., Hudson, K., & Dukes, P. (1993). Identification of *Trypanosoma brucei gambiense* by PCR amplification of variant surface glycoprotein genes. *Acta Tropica,* **53**, pp. 107-119.

Brooker, S., Clarke, S., Njagi, J.K., Polack, S., 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 & International Health,* **9**, pp. 757-766.

Brownlow, A. (2007). *Novel approaches for the use of trypanocidal agents in south east Uganda.* University of Edinburgh, Edinburgh.

Bruce, D. (1895). *Preliminary Report on the Tsetse fly Disease or Nagana in Zululand.* Durban, South Africa: Bennet & Davis.

Bruce, D., Hamerton, A.E., Bateman, H.R., & Mackie, F.P. (1910). Experiments to ascertain if cattle may act as a reservoir of the virus of sleeping sickness (Trypanosoma gambiense). *Proceedings of the Royal Society of London. Series B.,* **82**, pp. 480-484.

Bruce, D. & Nabarro, D. (1903). Progress report on sleeping sickness in Uganda. *Proceedings of the Royal Society of London.,* **1**, pp. 11-88.

Burchmore, R.J.S., Ogbunude, P.O.J., Enanga, B., & Barrett, M.P. (2002). Chemotherapy of Human African Trypanosomiasis. *Current Pharmaceutical Design,* **8**, pp. 257-267.

Burri, C. & Brun, R. (2003). Eflornithine for the treatment of human African trypanosomiasis. *Parasitological Research,* **90**, pp. s49-52.

Buscher, P. & Lejon, V. (2004). Diagnosis of Human African Trypanosomiasis. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases*: CAB International.

Buscher, P., Lejon, V., Magnus, E., & Van Meirvenne, N. (1999). Improved latex agglutination test for detection of antibodies in serum and cerebrospinal fluid of *Trypanosoma brucei gambiense* infected patients. *Acta Tropica,* **73**, pp. 11-20.

Buxton, P.A. (1947). *Trypanosomiasis in Eastern Africa*. London: HMSO.

Carrington, M., Bulow, R., Reinke, H., & Overath, P. (1989). Sequence and expression of glycosyl-phosphatidylinositol-specific phospholipase C of *Trypanosoma brucei. Molecular and Biochemical Parasitology,* **33**, pp. 289-296.

Castellani, A. (1903). *Presence of Trypanosoma in sleeping sickness*. London: Royal Society.

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

Challier, A., Eyraud, M., Lafaye, A., & Laveissiere, C. (1977). Amelioration du rendement du piege biconique pour glossines (*Diptera, Glossinidae)* par l'emploi d'un cone inferieur bleu. *Cahiers d'ORSTOM, Sere Entomologie Medicale et Parasitologie,* **15**, pp. 283-286.

Chappuis, F., Loutan, L., Simarro, P., Lejon, V., & Buscher, P. (2005). Options for field diagnosis of human African trypanosomiasis. *Clinical Microbiological Review,* **18**, pp. 133-146.

Christy, C. (1903). *The epidemiology and etiology of sleeping sickness in equatorial East Africa with clinical observations.* London: Royal Society.

Claes, F., Verloo, D., De Waal, D.T., Urakawa, T., Majiwa, P., Goddeeris, B., & Buscher, P. (2002). Expression of RoTat 1.2 cross reactive variable antigen type in *Trypanosoma evansi* and *T. equiperdium. Annals of the New York Academy of Science,* **969**, pp. 174-179.

Clausen, P.H., Wiemann, A., Patzell, R., Kakaire, D., Poetzsch, C., Peregrine, A.S.,

& Mehlitz, D. (1998). Use of a PCR assay for the specific and sensitive detection of *Trypanosoma spp.* in naturally infected dairy cattle in peri-urban Kampala, Uganda. *Annals New York Academy of Sciences,* **849**, pp. 21-31.

Cleaveland, S. (1998). Epidemiology and control of rabies: The growing problem of rabies in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **92**, pp. 131-134.

Codjia, V., Mulatu, W., Majiwa, P.A.O., Leak, S.G.A., Rowlands, G.J., Authie, E., d'Ieteren, G.D.M., & Peregrine, A.S. (1993). Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia.

3. Occurrence of populations of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium. *Acta Tropica,* **53**, pp. 151-163.

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

Contamin, H., Fandeur, T., Bonnefoy, S., Skouri, S., Ntoumi, F., & Mercereau-Puijalon, O. (1995). PCR typing of field isolates of *Plasmodium falciparium. Journal of Clinical Microbiology,* **33**, pp. 944-951.

Cook, G.A. & Zumla, A.I. (2002). *Manson's Tropical Diseases.* (21 Edition ed.): Saunders Ltd.

Cook, J.H. (1901). Notes on cases of "sleeping sickness" occurring in the Ugandan Protectorate. *Journal of Tropical Medicine,* **4**, pp. 236-239.

Cox, F.E.G. (1993). *Modern Parasitology.* (Second edition ed.): Blackwell science.

Curtis, C.F. & Lines, J.D. (2000). Should DDT be banned by international treaty. *Parasitology Today,* **16**, pp. 119 - 121.

Dale, C., Welburn, S.C., Maudlin, I., & Milligan, P.J.M. (1995). The kinetics of maturation of trypanosomes in tsetse. *Parasitology,* **111**, pp. 187-191.

Davies, J.N.P. (1968). Informed speculation on the cause of sleeping sickness 1898 - 1903. *Medical History,* **12**, pp. 200 - 204.

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

Desquesnes, M. & Davila, A.M.R. (2002). Applications of PCR-based tools for detection and identification of animal trypanosomes: a review and perspectives. *Veterinary Parasitology,* **109**, pp. 213 - 231.

Director of Medical and Sanitary services. (1928). *Memorandum on Prevention of Sleeping sickness*. Entebbe, Uganda.

Docampo, R. & Moreno, S.N.J. (2003). Current chemotherapy of human African trypanosomiasis. *Parasitology Research,* **90**, pp. s10-13.

Dransfield, R.D. & Brightwell, R. (2004). Community participation in tsetse control: the principles, potential and practice. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases*: CAB International.

Dutton, J.E. (1902). Note on a *Trypanosoma* occurring in the blood of man. *British Medical Journal,* **2**, pp. 881-884.

Duvallet, G., de la Rocque, S., Reifenberg, J.M., Solano, P., Lefrancois, T., Michel, J.F., Bengaly, Z., Sidibe, I., Cuisance, D., & Cuny, G. (1999). Review on the molecular tools for the understanding of the epidemiology of animal trypanosomiasis in West Africa. *Mem Inst Oswaldo Cruz,* **94**, pp. 245-248.

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* (pp. 253-267): CABI.

Eisler, M.C., Magona, J.W., Jonsson, N.N., & Revie, C.W. (2007). A low cost decision support tool for the diagnosis of endemic infectious diseases in the mixed crop-livestock production system of sub-Saharan Africa. *Epidemiology and Infection,* **135**, pp. 67-75.

Ekwanzala, M., Pepin, J., Khonde, N., Molisho, S., Bruneel, H., & De Wals, P. (1996). In the heart of darkness: sleeping sickness in Zaire. *The Lancet,* **348**, pp. 1427-1430.

Engstler, M., Thilo, L., Weise, F., Grunfelder, C.G., Schwarz, H., & Boshart, M. (2004). Kinetics of endocytosis and recycling of the GPI-anchored variant surface glycoprotein in *Trypanosoma brucei*. *Journal of Cell Science,* **117**, pp. 1105-1115.

Enyaru, J.C.K., Matovu, E., Akol, M., Sebrikali, C.G., Kyambadde, J.W., Schmidt, C., Brun, R., Kaminsky, R., Ogwal, L.M., & Kansiime, F. (1998). Parasitological detection of *Trypanosoma brucei gambiense* in serlogically negative sleeping-

sickness suspects from north-western Uganda. *Annals of Tropical Medicine and Parasitology,* **92**, pp. 845-850.

Enyaru, J.C.K., Matovu, E., Nerima, B., Akol, M., & Sebikali, C. (2006). Detection of *T. b. rhodesiense* Trypanosomes in humans and domestic animals in South East Uganda by amplification of serum resistance-associated gene. *Annals of the New York academy of science,* **1081**, pp. 311-319.

Enyaru, J.C.K., Odiit, M., Gashumba, J.K., Carasco, J.F., & Rwenderie, A.J.J. (1992). Charaterization by isoenzyme electrophoresis of *Trypanozoon* stocks from sleeping sickness endemic areas of south-east Uganda. *Bulletin of the World Health Organization,* **70**, pp. 631-636.

Enyaru, J.C.K., Oditt, M., Winyi-Kaboyo, R., Sebrikali, C.G., Matovu, E., Okitoi, D., & Olaho-Mukani, W. (1999). Evidence for the occurence of *Trypanosoma brucei rhodesiense* sleeping sickness outside the traditional focus in south-eastern Uganda. *Annals of Tropical Medicine and Parasitology,* **93**, pp. 817-822.

Enyaru, J.C.K., Stevens, J.R., Odiit, M., Okuna, N.M., & Carasco, J.F. (1993). Isoenzyme comparison of *Trypanosoma* isolates from two sleeping sickness areas of south-eastern Uganda. *Acta Tropica,* **55**, pp. 97 - 115.

EU Newsletter Uganda. (2004). *A quarterly news letter of the delegation of the European commission in Uganda*. Kampala: EU commission Uganda.

Fairbairn, H. (1948). Sleeping sickness in Tanganyika Territory, 1922-1946. *Tropical Medical Bulletin,* **45**, pp. 1-17.

Fairlamb, A.H. (1990). Future prospects for the chemotherapy of human trypanosomiasis : 1. Novel approaches to the chemotherapy of trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **84**, pp. 613-617.

Fèvre, E. (2001). More thoughts on the control of trypanosomes in cattle. *Trends in Parasitology,* **17**, pp. 412-413.

Fèvre, E.M. (2002). *The epidemiology of trypanosomiasis, a re-emerging zoonosis in Uganda.* University of Edinburgh, Edinburgh.

Fèvre, E.M., Coleman, P.G., Oditt, M., J.W., M., 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 1990-1920 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., Picozzi, K., Jannin, J., Welburn, S.C., & Maudlin, I. (2006a). Human African Trypanosomiasis: Epidemiology and Control. *Advances in Parasitology*, pp. 167-221.

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

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

Fuller, J. (2000, 18.12.2000). Bill and Melinda Gates Foundation awards $15.1 million to treat African sleeping sickness and Leishmaniasis. *Bill and Melinda Gates Foundation.*

Geake, M. (2001). Send a cow. *New Scientist,* **172**, pp. 52.

Geerts, S., Diarra, B., Eisler, M.C., Brandt, J., Lemmouchi, Y., Kageruka, P., De Deken, R., Ndao, M., Diall, O., Schacht, E., Berkvens, D., Speybroeck, N., & Holmes, P.H. (1999). Extension of the prophylactic effect of isometamidium against trypanosome infections in cattle using a biodegradable copolymer. *Acta Tropica,* **73**, pp. 49-58.

Geerts, S. & Holmes, P.H. (1997, September 20 - October 4). *Drug management and parasite resistance in animal trypanosomiasis in Africa.* Paper presented at the International Scientific Council for Trypanosomaisis Research and Control, Maputo, Mozambique.

Geerts, S. & Holmes, P.H. (1998). Drug management and parasite resistance in bovine trypanosomiasis in Africa. *PAAT Technical Sciences Series, FAO, Rome,* **1**, pp.

Geerts, S., Holmes, P.H., Diall, O., & Eisler, M.C. (2001). African bovine trypanosomiasis: the problem of drug resistance. *Trends in Parasitology,* **17**, pp. 25 - 28.

Geerts, S., Kageruka, P., De Deken, R., Brandt, J.R.A., Kazadi, J.M., Diarra, B., Eisler, M.C., Lemmouchi, Y., Schacht, E., & Holmes, P.H. (1997). Prophylactic effects of isometamidium- and ethidium-sustained release devices against *Trypanosoma congolense* in cattle. *Acta Tropica,* **65**, pp. 23-31.

Geigy, R., Jenni, L., Kauffmann, M., Onyango, R.J., & Weiss, N. (1975). Identification of *T. brucei*-subgroup strains isolated from game. *Acta Tropica,* **32**, pp. 190-205.

Geigy, R., Kauffmann, M., & Jenni, L. (1973). Wild animals as reservoirs for Rhodesian sleeping sickness in the Serengeti, 1970-71. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **67**, pp. 284-286.

Geigy, R., Mwambu, P.M., & Kauffmann, M. (1971). Sleeping sickness survey in Musoma district Tanzania. IV. Examination of wild animals as a potential reservoir for *Trypanosoma rhodesiense. Acta Tropica,* **28**, pp. 211-220.

Gibson, W. (2002). Will the real *Trypanosoma brucei rhodesiense* please step forward? *Trends in Parasitology,* **18**, pp. 486 - 490.

Gibson, W., Backhouse, T., & Griffiths, A. (2002). The human serum resistance associated gene is ubiquitous and conserved in *Trypanosoma brucei rhodesiense* throughout East Africa. *Infection, Genetics and Evolution,* **1**, pp. 207 - 214.

Gibson, W.C. & Gashumba, J.K. (1983). Isoenzyme characterisation of some Trypanozoon stocks from a recent trypanosomiasis epidemic in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **77**, pp. 114-118.

Gibson, W.C. & Wellde, B.T. (1985). Characterization of *Trypanozoon* stocks from south Nyanza sleeping sickness focus in western Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **79**, pp. 671-676.

Gould, S. (2003). *Present status of Uganda component of Farming In Tsetse Controlled Areas project.*

Gramiccia, M. & Gradoni, L. (2005). The current status of zoonotic leishmaniases

and approaches to disease control. *International Journal for Parasitology,* **35**, pp. 1169-1180.

Green, C.H. (1988). The effect of colour on trap and screen orientated responses in *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae). *Bulletiin of entomological research,* **78**, pp. 591-604.

Green, C.H. (1994). Bait methods for tsetse fly control. *Advances in Parasitology,* **34**, pp. 229-291.

Guo, J., LI, Y., GRAY, D., NING, A., HU, G., CHEN, H., DAVIS, G.M., SLEIGH, A.C., FENG, Z., McMANUS, D.P., & WILLIAMS, G.M. (2006). A drug based intervention study on the importance of buffaloes for human *Schistosoma japonicum* infectionaround Poyang Lake, People's Republic of China. *American Journal of Tropical Medicine and Hygiene,* **74**, pp. 335-341.

Gutierrez, C., Juste, M.C., Corbera, J.A., Magnus, E., Verloo, D., & Montoya, J.A. (2000). Camel trypanosomosis in the Canary Islands: assessment of seroprevalence and infection rates using the card agglutination test (CATT/*T. evansi*) and parasite detection tests. *Veterinary Parasitology,* **90**, pp. 155-159.

Hall, D.R., Beevor, P.S., Cork, A., Nesbit, B.F., & Vale, G.A. (1984). 1-Octen-3-ol: a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Science and its Application,* **5**, pp. 335-339.

Hargrove, J.W. (2003). *Tsetse eradication: sufficiency, necessity and desirability.* Edinburgh, UK: Centre for Tropical Veterinary Medicine.

Hargrove, J.W. (2004). Tsetse population dynamics. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases*: CABI.

Hargrove, J.W., Holloway, M.T.P., Vale, G.A., Gough, A.J.E., & Hall, D.R. (1995). Catches of tsetse (*Glossina* spp.) (Diptera: Glossinidae) from traps and targets baited with large doses of natural and synthetic host odour. *Bulletin of Entomological Research,* **85**, pp. 215-227.

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.

Hargrove, J.W. & Williams, B.G. (1998). Optimised simulation as an aid to modelling, with an application to the study of a population of tsetse flies, *Glossina*

morsitans morsitans Westwood (Diptera: Glossinidae). *Bulletin of Entomological Research,* **88**, pp. 425-435.

Haynes, D.M. (2000). Framing Tropical Disease in London: Patrick Manson, *Filaria perstans*, and the Uganda sleeping sickness epidemic 1891 - 1902. *Social History of Medicine,* **13**, pp. 467 - 493.

Heisch, R.B., McMahon, J.P., & Manson-Bahr, P.E.C. (1958). The isolation of *Trypanosoma rhodesiense* from a bushbuck. *British Medical Journal,* **2**, pp. 1203-1204.

Hendrickx, G., La Rocque de, S., Reid, R., & Wint, W. (2001). Spatial trypanosomiosis management: from data - layers to decision making. *Trends in Parasitology,* **17**, pp. 35 - 41.

Herold, D., Hart, G., & Englund, P.T. (1988). cDNA encoding the glycosyl-phosphatidylinositol-specific phospholipase C of *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences of the United States of America,* **85**, pp. 14-18.

Hide, G., Buchanan, N., Welburn, S., Maudlin, I., Barry, J.D., & Tait, A. (1991). *Trypanosoma brucei rhodesiense*: Characterisation of stocks from Zambia, Kenya, and Uganda using repetitive DNA probes. *Experimental Parasitology,* **72**, pp. 430-439.

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. (1964). Morphological and taxonomic studies on mammalian trypanosomes. X. Revision of the systematics. *Journal of Protozoology,* **11**, pp. 200-207.

Hodges, A. (1905). *Report on sleeping sickness in Unyoro and the Nile valley.* London: Royal Society.

Hodges, A. (1910a). Progress report on the Uganda sleeping sickness camps from December, 1906, to November, 1909. *Sleeping sickness bureau bulletins,* **19**, pp. 260 - 271.

Hodges, A. (1910b). Sleeping sickness in the Uganda Protectorate. *Sleeping sickness bureau bulletins,* **2**, pp. 218 - 223.

Hodges, A. & Will, J. (1906). *Report on sleeping sickness in Uganda from January 1st to June 30th, 1906*. London: Royal Society.

Holmes, P.H. (1997). New approaches to the integrated control of trypanosomiasis. *Veterinary Parasitology,* **71**, pp. 121-135.

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*: CAB International.

Hunt, A.R. & Bloss, J.F.E. (1945). Tsetse fly control and sleeping sickness in the Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **39**, pp. 43-58.

Hutubessy, R.C.W., Bendib, L.M., & Evans, D.B. (2001). Critical issues in the economic evaluation of interventions against communicable diseases. *Acta Tropica,* **78**, pp. 191-206.

Itty, P., Swallow, B.M., Rowlands, G.J., Mulatu, W., & d'Ieteren, G.D.M. (1995). The economics of village cattle production in a tsetse-infested area of southwest Ethiopia. *Preventative Veterinary Medicine,* **22**, pp. 183-196.

Jennings, J.M., Currriero, F.C., Celentano, D., & Ellen, J.M. (2005). Geographic identification of high gonorrhea transmission areas in Baltimore, Maryland. *American Journal of Epidemiology,* **161**, pp. 73-80.

Jeppson, A. (2001). Financial priorities under decentralization in Uganda. *Health Policy Plan.,* **16**, pp. 187-192.

Johannesson, M. & Jonsson, B. (1991). Economic evaluation in health care: is there a role for cost benefit analysis? *Health Policy,* **17**, pp. 1-23.

Jordan, A.M. (1978). Principles of eradication or control of tsetse flies. *Nature,* **273**, pp. 607-609.

Kabayo, J.P. (2002). Aiming to eliminate tsetse from Africa. *Trends in Parasitology,* **18**, pp. 473-475.

Kabayo, J.P. (2005). Africa will be free when its tsetse free, *Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC)* (pp. 16). Addis Ababa.

Kanmogne, G.D., Asonganyi, T., & Gibson, W. (1996). Detection of *Trypanosoma brucei gambiense*, in serologically positive but aparasitaemic sleeping-sickness suspects in Cameroon, by PCR. *Annals of Tropical Medicine and Parasitology,* **90**, pp. 475-483.

Kapiriri, L., Norheim, O., F., & Heggenhougen, K. (2003). Using the burden of disease information for health planning in developing countries: the experience from Uganda. *Social Science & Medicine,* **56**, pp. 2433-2441.

Kappmeier, K. (2000). A newly developed odour-baited "H trap" for the collection of *Glossina brevipalpis* and *Glossina austeni* (Diptera: Glossinidae) in South Africa. *Onderstepoort Journal of Veterinary Research,* **67**, pp. 15-26.

Katunguka-Rwakishaya, E. (1996). The prevalence of trypanosomiasis in small ruminants and pigs in a sleeping sickness endemic area of Buikwe county, Mukono district, Uganda. *Revue d'Elevage et de Medecine Veterinaire des Pay Tropicaux,* **49**, pp. 56-58.

Kennedy, P.G.E. (2004). Human African trypanosomiasis of the CNS: current issues and challenges. *Journal of Clinical Investigation,* **113**, pp. 496-504.

Kinghorn, A. (1925). Human trypanosomiasis in the Luangwa valley, Northern Rhodesia. *Annals of Tropical Medicine and Parasitology,* **57**, pp. 281-300.

Knight, C.G. (1971). The Ecology of African Sleeping Sickness. *Annals of the Association of American Geographers,* **61**, pp. 23-44.

Koerner, T., de Raadt, P., & Maudlin, I. (1995). The 1901 Ugandan 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 cost of animal trypanosomiasis, 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., Feuer, E.J., Miller, B.A., & Freedman, L.S. (1998). Breast cancer

clusters in the northeast United States: A geographic analysis. *American Journal of Epidemiology,* **146**, pp. 161-170.

Kulldorff, M. & Nagarwalla, N. (1995). Spatial disease clusters: detection and inference. *Statistics in Medicine,* **14**, pp. 799-810.

Kumar, N., Orenstein, R., Uslan, D.Z., Berbari, E.F., Klein, C.J., & Windebank, A.J. (2006). Melarsoprol-associated multifocal inflammatory CNS illness in African trypanosomiasis. *Neurology,* **66**, pp. 1120-1121.

Kuzoe, F.A. (1993). Current situation of African trypanosomiasis. *Acta Tropica,* **54**, pp. 153-162.

Lancien, J. (1991). Campaign against sleeping sickness in South-East Uganda by trapping tsetse flies. *Annals de la Societe Belge de Medecine Tropicale,* **71**, pp. 35-46.

Langlands, B.W. (1967). *The sleeping sickness epidemic of Uganda 1900 - 1920 - A study in historical geography.* Makerere University College, Makerere.

Leak, S.G.A. (1999). *Tsetse Biology and Ecology: Their Role in the Epidemiology and Control of Trypanosomiasis*: CAB International.

Leak, S.G.A., Woudyalew, M., Authie, E., d'Ieteren, G.D.M., Peregrine, A.S., Rowlands, G.J., & Trail, J.C.M. (1993). Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 1. Tsetse challenge and its relationship to trypanosome prevalence in cattle. *Acta Tropica,* **53**, pp. 107-120.

Legros, D., Evans, S., Maiso, F., Enyaru, J.C.K., & Mbulamberi, D. (1999). Risk factors for the treatment failure after melarsoprol for *Trypanosoma brucei gambeinse* trypanosomiasis in Uganda. *Transactions of the Royal Society of Tropical Medicine and Parasitology,* **93**, pp. 439-442.

Longley, P.A., Goodchild, M.F., Maguire, J.D., & Rhind, D.W. (2001). *Geographic information systems and science*: John Willey & Sons, LTD.

Lutumba, P., Robays, J., Miaka mia Bilenge, C., Mesu, V.K., Molisho, D., Declercq, J., Van der Veken, W., Meheus, F., Jannin, J., & Boelaert, M. (2005). Trypanosomiasis control, Democratic Republic of Congo, 1993-2003. *Emerging Infectious Diseases,* **11**, pp. 1382-1388.

Lyons, M. (1992). *The colonial disease: A social history of sleeping sickness in Northern Zaire, 1900-1940*: Cambridge University Press.

MacKichan, I.W. (1944). Rhodesian sleeping sickness in Eastern Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **38**, pp. 49 - 61.

MacLean, L., Chisi, J.E., Odiit, M., Gibson, W.C., Ferris, V., Picozzi, K., & Sternberg, J.M. (2004). Severity of human African Trypanosomiasis in East Africa is associated with geographic location, parasite genotype, and host inflammatory cytokine response profile. *Infection and Immunity,* **72**, pp. 7040-7044.

MacLeod, A., Turner, C.M.R., & Tait, A. (1997). Detection of single copy gene sequences from single trypanosomes. *Molecular and Biochemical Parasitology,* **84**, pp. 267-270.

MacLeod, A., Tweedie, A., C., W.S., I., M., Turner, C.M.R., & Tait, A. (2000). Minisatellite marker analysis of *Trypanosoma brucei*: reconciliation of clonal, panmictic and epidemic population structures. *Proceedings of the National Academy of Sciences of the United States of America,* **97**, pp. 13442-13447.

Magnus, E., Vervoot, T., & Van Meirvenne, N. (1978). A card-agglutination test with stained trypanosomes (C.A.T.T.) for the serological diagnosis of *T. b. gambiense* trypanosomiasis. *Annals de la Societe Belge de Medecine Tropicale,* **58**, pp. 169-76.

Magona, J.W., Walubengo, J., Odiit, M., Okedi, L.A., Abila, P., Katabazi, B.K., Gidudu, A.M., & Olaho-Mukani, W. (2005). Implications of the re-invasion of Southeast Uganda by Glossina pallidipes on the epidemiology of bovine trypanosomosis. *Veterinary Parasitology,* **128**, pp. 1-9.

Mandavilli, A. (2006). Health agency backs use of DDT against malaria. *Nature,* **443**, pp. 250-251.

Masake, R.A., Njuguna, J.T., Brown, C.C., & Majiwa, P.A.O. (2002). The application of PCR-ELISA to the detection of *Trypanosoma brucei* and *T. vivax* infections in livestock. *Veterinary Parasitology,* **105**, pp. 179-189.

Masiga, D.K., McNamara, J.J., Laveissiere, C., Truc, P., & Gibson, W. (1996). A high prevalence of mixed trypanosome infections in tsetse flies in Sinfra, Cote d'Ivorie, detected by DNA amplification. *Parasitology,* **112**, pp. 75 - 80.

Masiga, D.K., Smyth, A.J., Hayes, P., Bromidge, T.J., & Gibson, W.C. (1992). Sensitive detection of trypanosomes in tsetse flies by DNA amplification. *International Journal for Parasitology,* **22**, pp. 909-918.

Matovu, E., Seebeck, T., Enyaru, J.C.K., & Kaminsky, R. (2001). Drug resistance in *Trypanosoma brucei* spp., the causative agents of sleeping sickness in man and nagana in cattle. *Microbes and Infection,* **3**, pp. 763-770.

Maudlin, I. (2006). African trypanosomiasis. *Annals of Tropical Medicine and Parasitology,* **100**, pp. 1-23.

Maudlin, I. & Welburn, S.C. (1989). A single trypanosome is sufficent to infect a tsetse fly. *Annals of Tropical Medicine and Parasitology,* **83**, pp. 431-433.

May, E. & Allolio, B. (1991). Fatal toxic epidermal necrolysis during suramin therapy. *European Journal of Cancer,* **27**, pp. 1338.

Mbulamberi, D. (1990). Current epidemiological situation of human African trypanosomiasis in Uganda: patterns of distribution of the disease and its control. *Uganda Medical Journal,* **7**, pp. 2-17.

Meslin. (1997). Global aspects of emerging and potential zoonoses: a WHO perspective. *Emerging Infectious Diseases,* **3**, pp. 223-228.

Molyneux, D.H. (1993). Vectors. In F.E.G. Cox (Ed.), *Modern Parasitology* (Second edition ed.): Blackwell science.

Molyneux, D.H. (2001a). Sterile insect release and trypanosomiasis control: a plea for realism. *Trends in Parasitology,* **17**, pp. 413-414.

Molyneux, D.H. (2001b). Vector-borne infections in the tropics and health policy issues in the twenty-first century. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **95**, pp. 223-238.

Moore, D.A.J., Edwards, M., Escombe, R., Agranoff, D., Bailey, J.W., Squire, S.B., & Chiodini, P. (2002). African trypanosomiasis in travellers returning to the United Kingdom. *Emerging Infectious Diseases,* **8**, pp. 74-76.

Moran, P.A.P. (1948). The interpretation of statistical maps. *Journal of the Royal Statistical Society, Series B,* **10**, pp. 243-251.

Morrison, L.J., Majiwa, P., Read, A.F., & Barry, J.D. (2005). Probabilistic order in antigenic variation of *Trypanosoma brucei*. *International Journal for Parasitology,* **35**, pp. 961-972.

Mostashari, F., Kulldorff, M., Hartman, J.J., Millar, J.R., & Kulasekera, V. (2003).

Dead bird clustering: a potential early warning system for West Nile virus activity. *Emerging Infectious Diseases,* **9**, pp. 641-646.

Mudo, S. (2003). Tsetse control operations in Botswana. *Newsletter on Integrated Control of Pathogenic Trypanosomes and their Vectors,* **7**, pp. 8 - 9.

Mulla, A.F. & Rickman, L.R. (1988). The isolation of human serum-resistant *Trypanosoma* (Trypanozoon) species from Zebra and Impala in Luangwa valley, Zambia. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **82**, pp. 718.

Murray, C.J.L. (1994). Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bulletin of the World Health Organisation,* **72**, pp. 429-445.

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

Mwambu, P.M. (1969a). Cattle trypanosomiasis in Teso District, Eastern Uganda. *EATRO Annual report*, pp. 114-116.

Mwambu, P.M. (1969b). Prevalence of *Trypanosoma vivax* infection in cattle in Teso district, Eastern Uganda. *Annals of Epizootic Diseases in Africa,* **17**, pp. 395 - 402.

Ngaira, J.M., Bett, B., Karanja, S.M., & Njagi, E.N.M. (2003). Evaluation of antigen and antibody rapid detection tests for *Trypanosoma evansi* infection in camels in Kenya. *Veterinary Parasitology,* **114**, pp. 131-141.

Ngaira, J.M., Njagi, E.N.M., Ngeranwa, J.J.N., & Olembo, N.K. (2004). PCR amplification of RoTat 1.2 VSG gene in *Trypanosoma evansi* isolates in Kenya. *Veterinary Parasitology,* **120**, pp. 23-33.

Njiokou, F., Laveissiere, C., Simo, G., Nkinin, S., Grebaut, P., Cuny, G., & Herder, S. (2006). Wild fauna as a probable animal reservoir for *Trypanosoma brucei gambiense* in Cameroon. *Infection, Genetics and Evolution,* **6**, pp. 147-153.

Norstrom, M., Pfeiffer, D.U., & Jarp, J. (2000). A space-time cluster investigation of an outbreak of acute respiratory disease in Norwegian cattle herds. *Preventative Veterinary Medicine,* **47**, pp. 107-119.

Nugent, P. (2004). *Africa since Independance*: Palgrave MacMillian.

Odiit, M., Amulen, D., Kansiime, F., Enyaru, J.C.K., & Okitoi, D. (1997). *Comparison of the epidemiology of sleeping sickness and the enviroment profile in Tororo district.* Paper presented at the International scientific council for Trypanosomiasis research and council (ISCTRC), Maputo, Mozambique.

Odiit, M., Coleman, P.G., Liu, W.-C., McDermott, J.J., Fevre, 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.

Odoi, A., Martin, S.W., Michel, P., Middleton, D., Holt, J., & Wilson, J. (2004). Investigation of clusters of giardiasis using GIS and a spatial scan ststistic. *International Journal of Health Geographics,* **3**, pp.

Okiria, R. (1985). The prevalence of human trypanosomiasis in Uganda, 1970 to 1983. *East African Medical Journal,* **62**, pp. 813 - 816.

Okoth, J.O. (1999). Tsetse and trypanosomiasis control problems in South-East Uganda: past, present and alternative strategies. *Schweiz med wochenschr,* **129**, pp. 1091 - 1098.

Okoth, J.O., Kirumira, E.K., & Kapaata, R. (1991). A new approach to community participation in tsetse control in the Busoga sleeping sickness focus, Uganda. A preliminary report. *Annals of Tropical Medicine and Parasitology,* **85**, pp. 315-322.

Onyango, R.J. (1967). *Human trypanosomiasis discovered in Teso District, Uganda.* EATRO.

Onyango, R.J., Geigy, R., Kauffmann, M., Jenni, L., & Steiger, R. (1973). New animal reservoirs of *T. rhodesiense* sleeping sickness. *Acta Tropica,* **30**, pp. 275.

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., Charlton, M., Craft, A., & Birch, J. (1988). Investigation of leukemia clusters by use of a geographical analysis machine. *Lancet*, pp. 272-3.

Pays, E. (2005). Regulation of antigen gene expression in Trypanosoma brucei. *Trends in Parasitology*

21 years following trends in parasitology, **21**, pp. 517-520.

Pays, E. (2006). The variant surface glycoprotein as a tool for adaptation in African trypanosomes. *Microbes and Infection,* **8**, pp. 930-937.

Pays, E., Lips, S., Nolan, D.P., Vanhamme, L., & Perez-Morga, D. (2001). The VSG expression sites of *Trypanosoma brucei*: multipurpose tools for the adaptation of the parasite to mammalian hosts. *Molecular and Biochemical Parasitology,* **114**, pp. 1-16.

Pays, E., Vanhollebeke, B., Vanhamme, L., Paturiaux-Hanocq, F., Nolan, D.P., & Perez-Morga, D. (2006). The trypanolytic factor of human serum. *Nature Reviews,* **4**, pp. 477-486.

Pearl, D.L., Louie, M., Chui, L., Dore, K., Grimsrud, K.M., Leedell, D., Martin, S.W., Michel, P., Svenson, L.W., & McEwen, S.A. (2006). The use of outbreak information in the interpretation of clustering of reported cases of *Escherichia coli* O157 in space and time in Alberta, Camada, 2000-2002. *Epidemiology and Infection,* **134**, pp. 699-711.

Pepin, J. & Meda, H.A. (2001). The epidemiology and control of human African trypanosomiasis. *Advances in Parasitology,* **49**, pp. 71-132.

Pepin, J. & Milord, F. (1994). The treatment of human African trypanosomiasis. *Advances in Parasitology,* **33**, pp. 1-47.

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

Perez-Morga, D., Vanhollebeke, B., Paturiaux-Hanocq, F., Nolan, D.P., Lins, L., Homble, F., Vanhamme, L., Tebabi, P., Pays, A., Poelvoorde, P., Jacquet, A., Brasseur, R., & Pays, E. (2005). Apolipoprotein L-I Promotes Trypanosome Lysis by Forming Pores in Lysosomal Membranes. *Science,* **309**, pp. 469-472.

Persoons, C.J. (1967). A tsetse survey in Serere County, Teso District, Uganda. *EATRO Annual report*, pp. 47.

Picozzi, K., Carrington, M., & Welburn, S.C. (*In press*). A multiplex that discriminates between *Trypanosoma brucei brucei* and *T. b. rhodesiense*. *Experimental Parasitology*, pp.

Picozzi, K., Fevre, 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.

Picozzi, K., Tilley, A., Fevre, E.M., Coleman, P.G., Magona, J.W., Oditt, M., Eisler, M.C., & Welburn, S.C. (2002). The diagnosisof trypanosome infections: applications of novel technology for reducing disease risk. *African Journal of Biotechnology,* **1**, pp. 39-45.

Pugh, J. (1910). Letter to the Chief Medical Officer, dated 11/7/1910. Sleeping Sickness camp, Kaniamka, Ugandan protectorate.

Radwanska, M., Chamekh, M., Vanhamme, L., Claes, F., Magez, S., Magnus, E., de Baetselier, P., Buscher, P., & Pays, E. (2002a). The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma brucei rhodesiense. American Journal of Tropical Medicine and Hygiene,* **67**, pp. 684-690.

Radwanska, M., Claes, F., Magez, S., Magnus, E., Perez-Morga, D., Pays, E., & Buscher, P. (2002b). Novel primer sequences for polymerase chain reaction-based detection of *Trypanosoma brucei gambiense. American Journal of Tropical Medicine and Hygiene,* **67**, pp. 289-295.

Reader, J. (1997). *Africa: a Biography of the Continent.* London.

Reid, H.W., Burridge, M.J., Pullan, N.B., Sutherst, R.W., & Wain, E.B. (1970). Survey for trypanosome infections in domestic cattle and wild animals in areas of East Africa. I. Introduction. *British Veterinary Journal,* **126**, pp. 622-6.

Remme, J.H.F., Blas, E., Chitsulo, L., Desjeux, P.M.P., Engers, H.D., Kanyok, T.P., Kayondo, J.F.K., Kioy, D.W., Kumaraswami, V., & Lazdins, J.K. (2002). Strategic emphases for tropical diseases research: a TDR perspective. *Trends in Microbiology,* **10**, pp. 435-440.

Rickman, L.R. & Robson, J. (1970). The testing of Proven *Trypanosoma brucei* and *T. rhodesiense* strains by the Blood Incubation Infectivity test. *Bull World Health Organization,* **42**, pp. 911 - 916.

Robays, J., Bilengue, M.M.C., Stuyft Van der, P., & Boelaert, M. (2004). The effectiveness of active population screening and treatment for sleeping sickness control in the Democratic Republic of Congo. *Tropical Medical and Internatinal Health,* **9**, pp. 542-550.

Robinson, T.P. (2000). Spatial statistics and geographical information systems in epidemiology and public health. *Advances in Parasitology,* **47**, pp. 81-128.

Rogers, D.J. (1988). A general model for the African trypanosomiasis. *Parasitology,* **97**, pp. 193 - 212.

Rowlands, G.J., Leak, S.G.A., Mulatu, W., Nagda, S.M., Wilson, A., & d'Ieteren, G.D.M. (2001). Use of deltamethrin 'pour-on' insecticide for the control of cattle trypanosomosis in the presence of high tsetse invasion. *Medical and Veterinary Entomology,* **15**, pp. 87-96.

Rowlands, G.J., Woudyalew, M., Leak, S.G.A., Authie, E., d'Ieteren, G.D.M., 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., Ye, Y., Sauerborn, R., Muller, O., & Becher, H. (2001). Clustering of childhood mortality in rural Burkina Faso. *International Journal of Epidemiology,* **30**, pp. 485-492.

Saunderson, M. (1911). Notes on *Glossina fusca*, Walk in North Nyanza. *Bulletin of Entomological Research,* **1**, pp. 299-302.

Saunderson, P.R. (1995). An economic evaluation of alternative programme designs for tuberculosis control in rural Uganda. *Social Science & Medicine,* **40**, pp. 1203-1212.

Schofield, C.J. & Maudlin, I. (2001). Trypanosomiasis control. *International Journal for Parasitology,* **31**, pp. 614-619.

Schulman, J., Selvin, S., & Merrill, D.W. (1988). Density equalized map projections: a method for analysing clustering around a fixed point. *Statistics in Medicine,* **7**, pp. 491-506.

Shaw, A. (2004). Economics of African Trypanosomiasis. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases*: CABI.

Sheader, K., Berberof, M., Isobe, T., Borst, P., & Rudenko, G. (2003). Delineation of the regulated Variant Surface Glycoprotein gene expression site domain of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology,* **128**, pp. 147-156.

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. 858-861.

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.

Simpson, J.J. (1911). Entomological research in British West Africa. I. Gambia. *Bulletin of Entomological Research,* **2**, pp. 187-239.

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., Pepin, J., & Stich, A. (1998). Human African trypanosomiasis: an emerging public health crisis. *British Medical Bulletin,* **54**, pp. 341-355.

Snow, S.J. (2002). Commentary: Sutherland, Snow and water: the transmission of cholera in the nineteenth century. *International Journal of Epidemiology,* **31**, pp. 908-911.

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 Burkino Faso. *Veterinary Parasitology,* **86**, pp. 95-103.

Stephens & Fantham. (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*). *Proceedings of the Royal Society of London, B.,* **83**, pp. 28.

Sternberg, J.M. (2004). Human African trypanosomiasis: clinical presentation and immune response. *Parasite Immunology,* **26**, pp. 469-476.

Stevens, J.R. & Brisse, S. (2004). Systematics of Trypanosomes of Medical and Veterinary Importance. In I. Maudlin, P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases* (pp. 1-24).

Stich, A., Abel, P.M., & Krishna, S. (2002). Human African trypanosomiasis. *British Medical Journal,* **325**, pp. 203-206.

Stone, R.A. (1988). Investigations of excess enviromental risk around putative sources: statistical problems and a proposed test. *Statistics in Medicine,* **7**, pp. 649-660.

Swallow, B.M. (2000). *Impacts of trypanosomiasis on African agriculture*. Food and Agriculture Organization.

Swynerton, C.F.M. (1933). Some traps for tsetse flies. *Bulletin of Entomological Research,* **24**, pp. 69-102.

Swynerton, C.F.M. (1936). The tsetse flies of East Africa: a first study of their ecology, with a view to their control. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **84**, pp. 1-579.

Tarimo-Nesbit, R.A., Golder, T.K., Dransfield, R.D., Chaudhury, M.F., & Brightwell, R. (1999). Trypanosome infection rate in cattle at Nguruman, Kenya. *Veterinary Parasitology,* **81**, pp. 107-117.

Taylor, K.A. & Authie, E. (2004). Pathogenesis of Animal Trypanosomiasis. In M. I., P.H. Holmes, & M.A. Miles (Eds.), *The Trypanosomiases* (pp. 331-353): CABI Publishing.

Taylor, L.H., Latham, S.M., & Woolhouse, M.E.J. (2001). Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences,* **356**, pp. 983-989.

Thompson, J.W., Mitchell, M., Rees, R.B., Shereni, W., Schoenfeld, A.H., & Wilson, A. (1991). Studies on the efficacy of deltamethrin applied to cattle for the control of tsetse flies (*Glossina* spp.) in southern Africa. *Tropical Animal Health and Production,* **23**, pp. 221-226.

Thompson, M.C. (1987). The effect on tsetse flies (Diptera: Glossinidae) of deltamethrin applied to cattle. *Tropical Pest Management,* **33**, pp. 329-335.

Thrusfield, M. (2005). *Veterinary Epidemiology*. (3rd ed.).

Thrusfield, M., Ortega, C., de Blas, I., Noordhuizen, J., & Frankena, K. (2001). WIN EPISCOPE 2.0: improved epidemiological software for veterinary medicine. *Vet Rec.,* **148**, pp. 567-572.

Tilley, A., Welburn, S.C., Fevre, E.M., Feil, E.J., & Hide, G. (2003). *Trypanosoma brucei*: Trypanosome strain typing using PCR analysis of mobile genetic elements (MGE-PCR). *Experimental Parasitology,* **104**, pp. 26-32.

Tiwari, N., Adhikari, C., Tewari, A., & Kandpal, V. (2006). Investigation of geo-spatial hotspots for the occurrence of tuberculosis in Almora district, India, using

GIS and spatial scan statistic. *International Journal of Health Geographics,* **5**, pp. 33.

Torr, S.J., Hargrove, J.W., & Vale, G.A. (2005). Towards a rational policy for dealing with tsetse. *Trends in Parasitology,* **21**, pp. 537-541.

Torr, S.J. & Mangwiro, T.N.C. (2000). Interactions between cattle and biting flies: effects on the feeding rate of tsetse. *Med Vet Entomol,* **14**, pp. 400-409.

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

Turnbull, B.W., 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. S136-S143.

Turusov, V., Rakitsky, V., & Tomatis, L. (2002). Dichlorodiphenyltrichloroethane (DDT): Ubiquity, Persistance and Risks. *Enviromental Health Perspectives,* **110**, pp. 125-128.

Ugandan Bureau of Statistics. (2002). *Ugandan Population and Housing Census.* Entebbe: Uganda Bureau of Statistics.

Ugandan Bureau of Statistics. (2005). *Uganda districts infrormation handbook.* (Expanded edition ed.). Kampala: Fountain publishers.

Uilenberg, G. (1998). A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis. *Food and Agriculture Organization of the United Nation*, pp.

Vale, G.A. (1974). The reponses of tsetse flies (Diptera: Glossinidae) to stationary baits. *Bulletin of entomological research,* **64**, pp. 545-588.

Vale, G.A. (1980). Field studies of the responses of tsetse flies (*Glossinidae*) and other Diptera to carbon dioxide, acetone and other chemicals. *Bulletin of Entomological Research,* **70**, pp. 563 570.

Vale, G.A. (1982a). The improvement of traps for tsetse flies (Diptera: Glossinidae). *Bulletin of Entomological Research,* **72**, pp. 95-106.

Vale, G.A. (1982b). The trap-orientated behavior of tsetse flies (Glossinidae). *Bulletin of Entomological Research,* **72**, pp. 71-93.

Vale, G.A., Flint, S., & Hall, D.R. (1986). The field repsonses of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to odours of host residues. *Bulletin of Entomological Research,* **76**, pp. 685-693.

Vale, G.A., Lovemore, D.F., Flint, S., & Cockbill, G.F. (1988). Odour-baited targets to control tsetse flies *Glossina* spp. in Zimbabwe. *Bulletin of Entomological Research,* **78**, pp. 31-49.

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 Trypanosomiases*: CAB International.

Vale, G.A. & Torr, S.J. (2005). User-friendly methods of the costs and efficacy of tsetse control: application to sterilizing and insecticidal techniques. *Medical and Veterinary Entomology,* **19**, pp. 293-305.

Van den Bossche, P., Ky-Zerbo, A., Brandt, J., Marcotty, T., Geerts, S., & De Deken, R. (2005). Transmissibility of *Trypanosoma brucei* during its development in cattle. *Tropical Medicine & International Health,* **10**, pp. 833-839.

Van der Ploeg, L.H.T., Cornelissen, A.W.C.A., Michels, P.A.M., & Borst, P. (1984). Chromosome rearrangements in *Trypanosoma brucei. Cell,* **39**, pp. 213-221.

Van Nieuwenhove, S., Betu-Ku-Mesu, V.K., Diabakana, P.M., Declercq, J., & Bilenge, C.M.M. (2001). Sleeping sickness resurgence in the DRC: the past decade. *Tropical Medicine and International Health,* **6**, pp. 335-341.

Vanhamme, L., Paturiaux-Hanocq, F., Poelvoorde, P., Nolan, D.P., Lins, L., Van Den Abbeele, J., Pays, A., Tebabi, P., Van Xong, H., Jacquet, A., Moguilevsky, N., Dieu, M., Kane, J.P., De Baetselier, P., Brasseur, R., & Pays, E. (2003). Apolipoprotein L-I is the trypanosome lytic factor of human serum. **422**, pp. 83-87.

Vanhamme, L. & Pays, E. (2004). The trypanosome lytic factor of human serum and the molecular basis of sleeping sickness. *International Journal for Parasitology,* **34**, pp. 887-898.

Vanhamme, L., Poelvoorde, P., Pays, A., Tebabi, P., Van Xong, H., & Pays, E. (2000). Differential RNA elongation controls the variant surface glycoprotein gene expression sites of *Trypanosoma brucei. Molecular Microbiology,* **36**, pp. 328-340.

Verloo, D., Magnus, E., & Buscher, P. (2001). General expression of RoTat 1.2

variable antigen type in *Trypanosoma evansi* isolates from different origins. *Veterinary Parasitology,* **97**, pp. 183-189.

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.

Wakefield, J.C., Kelsall, J.E., & Morris, S.E. (2000). Clustering, cluster detection and spatial variation in risk. In P. Elliott, J.C. Wakefield, N.G. Best, & D.J. Briggs (Eds.), *Spatial Epidemiology: Methods and Applications*. New York: Oxford University Press.

Walker, D. & Fox-Rushby, J. (2000). Economic evaluation of parasitic diseases: A critique of the internal and external validity of published studies. *Tropical Medicine and International Health,* **5**, pp. 237-249.

Waller, L.A., Turnbull, B.W., Clark, L.C., & Nasca, P. (1992). Chronic disease surveillance and testing of clustering of disease and exposure: application to leukemia incidence and tce-contaminated dumpsites in upstate New York. *Environmetrics,* **3**, pp. 281-300.

Welburn, S.C., Coleman, P.G., Maudlin, I., Fevre, 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., Fevre, 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. (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., Picozzi, K., Fevre, E.M., Coleman, P.G., Odiit, M., Carrington, M., & Maudlin, I. (2001b). Identification of human-infective trypanosomes in the animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (*SRA*) gene. *The Lancet,* **358**, pp. 2017-2019.

Welburn, S.C., Picozzi, K., Kaare, M., Fevre, E.M., Coleman, P.G., & Mlengeya, T. (2005). Chapter 8 - Control options for human sleeping sickness in relation to the

animal reservoir of disease. In S.A. Osofsky (Ed.), *The occasional paper of the IUCN species survival commision No. 30.*

Wellde, B.T., Reardon, M.J., Chumo, D.A., Kovatch, R.M., Waema, D., Wykoff, D.E., Mwangi, J., Boyce, W.L., & Williams, J.S. (1989). Cerebral trypanosomiasis in naturally-infected cattle in the Lambwe Valley, South Nyanza, Kenya. *Annals of Tropical Medicine and Parasitology,* **83**, pp. 151 - 160.

Wendo, C. (2002). Global Fund money won't increase health spending, says Uganda. *The Lancet,* **360**, pp. 1312.

West, T.F. & Campbell, G.A. (1950). *DDT and Newer Persistant Insecticides.* London: Chapman & Hall.

Whatman. (2004). FTA Technology (Vol. 2006, pp. http://www.whatman.com/products/?pageID=7.31.31).

WHO. (1959). *Zoonoses.* Geneva: World Health Organization.

WHO. (2006). Weekly epidemiological record. *World Health Organization,* **81**, pp. 69-80.

Wilkinson, D., Floyd, K., & Gilks, C.F. (1998). Antiretroviral drugs as a public health intervention for pregnant HIV-infected women in rural South Africa: an issue of cost-effectiveness and capacity. *AIDS,* **12**, pp. 1675-1682.

Wireless, C. (2003). CSI Wireless' Space-Age GPS products aiding in eradication of centuries-old African scourge - the deadly "sleeping sickness". In C. Wireless (Ed.), *CSI Wireless* (Vol. 2006, pp. Website article).

World Health Organization. (1998a). Control and Surveillance of African Trypanosomiasis. *WHO Technical Report Series 881. Geneva: WHO*, pp.

World Health Organization. (1998b). *Control and Surveillance of African Trypanosomiasis.* Geneva: WHO.

World Health Organization. (2002). *WHO Programme to eliminate sleeping sickness: Building a global alliance.* WHO/CDC/CSR/EPH/2002.13. Geneva: World Health Organization.

World Health Organization. (2004). *The World Health Report 2004 - Changing History.* Geneva: WHO.

Xong, H.V., Vanhamme, L., Chamekh, M., Chimfwembe, C.E., Abbeele, J.V.D., Pays, A., Meirvenne van, N., Hamers, R., Baetselier de, P., & Pays, E. (1998). A *VSG* expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell,* **95**, pp. 839-846.

Yorke, W. (1920). Research into the trypanosomiasis problem: a critical consideration of suggested measures. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* **14**, pp. 31 - 47.

8 Appendix:
Publications arising to date

Printed copies of this thesis contain the following published papers. Papers in preparation are not included:

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

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

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

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Central point sampling from cattle in livestock markets in areas of human sleeping sickness

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Abstract

We present the results of a study to determine the value of central point sampling in cattle markets as a means of estimating the trypanosomiasis (*T. brucei s.l.*) prevalence in the surrounding landscape in Uganda. We find that in the epidemic area studied, central point sampling is a good predictor of prevalence in surrounding villages, but not in endemic areas. We also find that animals infected with trypanosomiasis are more likely to be brought for sale in livestock markets in endemic areas; we discuss these results in relation to the prevention of the spread of sleeping sickness.

Keywords: Sleeping sickness; Trypanosomiasis; Cattle; Market; Sampling

1. Introduction

Sleeping sickness, caused by the zoonotic trypanosome *Trypanosoma brucei rhodesiense,* affects large areas of eastern Africa. In southeast and eastern Uganda, the disease occurs in discrete foci, although in recent years there has been an expansion into previously unaffected districts. In the virtual absence of wildlife in these areas of Uganda, domestic livestock—in particular cattle—have become an important reservoir for the parasite, and a significant source of bloodmeals for the tsetse host (Clausen et al., 1998; Okiria et al., 2002). This has been recognized both in the persistence of sleeping sickness during endemic periods (Waiswa et al., 2003), and in the epidemic zones of south-eastern (Hide et al., 1996) and more recently eastern Uganda (Fèvre et al., 2001), where 18% of cattle were found to be harbouring human-infective parasites (Welburn et al., 2001). The current epidemic in eastern Uganda is spreading northwards, with cattle movements a likely driving factor (Fèvre et al., 2005).

In areas affected by *T.b. rhodesiense* sleeping sickness, particularly during epidemics, a rapid response to reducing the prevalence in the reservoir is essential to prevent widespread transmission. However, it is often only the passive surveillance of sleeping sickness at treatment centres that provides the first indication of disease resurgence or spread, and reporting to health centres is beset with problems (Odiit et al., 2004). Outbreaks may occur in areas covering many hundreds of square kilometres, so that assessment of the extent of infection in the animal reservoir can be problematic. Strategies designed to assess the degree of the problem without

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requiring sampling from many disparate geographical locations would therefore greatly improve efficiency and reduce costs. In contrast to other infectious diseases of livestock such as foot- and -mouth disease, animal movement restrictions are not put in place to prevent sleeping sickness and livestock are brought to market regularly from across risk areas.

Using PCR based methods for identification of animals infected with *T. brucei s.l.*, we investigated the value of central point sampling strategies; we tested the hypothesis that livestock markets could be used as central point sampling locations representative of the surrounding area. This study was carried out prior to a large-scale sleeping sickness control programme which targeted livestock in this region.

2. Methods

2.1. Study design

Surveys were conducted in three districts in Uganda: Soroti district, where sleeping sickness is currently epidemic, and Tororo and Kamuli districts, both endemic areas. In Uganda, each district operates one or two major livestock markets in which animals from across the district are traded, and a number of smaller ones dealing mainly with inter-village trade. We sampled each of the major markets in the districts studied. Cattle were also sampled in four to six typical and arbitrarily chosen villages from the surrounding area in each district. This was a cross-sectional study, conducted in the first quarter of 2003; in both the markets and villages, we sampled all the cattle present on the sampling day, with a minimum of 50 animals in each location, sufficient to detect an effect in a village/market with an estimated maximum cattle population of 100 animals and an expected prevalence of up to 4% for *T. brucei s.l.* (Waiswa et al., 2003; Magona et al., 2005), with a 95% confidence level.

2.2. Sample analysis

Bovine ear vein blood was collected into heparinised capillary tubes and applied directly onto Whatman FTA cards (Whatman Inc., NJ, USA). These were analysed for the presence of *T. brucei s.l.* using species specific primers, as described by Picozzi et al. (2002). Differences in the number of *T. brucei s.l.* positive and negative animals among markets and villages in the epidemic and endemic districts were assessed using generalised linear models with binomial errors in S+ (Insightful, Seattle, USA).

3. Results

Fig. 1 shows the PCR-based prevalence for *T. brucei s.l.* in cattle from markets and villages within the three districts studied. The epidemic area of Soroti had a significantly higher prevalence of *T. brucei s.l.* than the endemic areas of Kamuli and Tororo

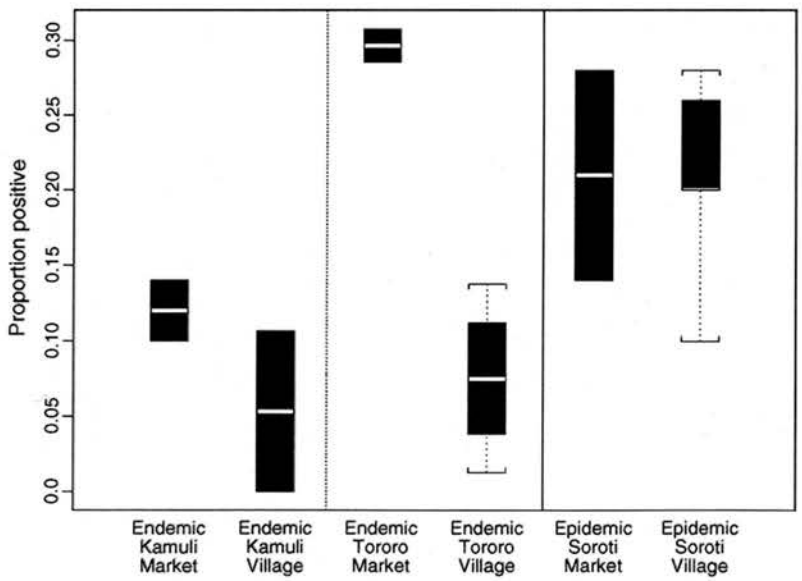

Fig. 1. Box plots of the prevalence of *T. brucei s.l.* in markets and villages in Kamuli (two markets; four villages), Tororo (two markets; four villages) and Soroti districts (two markets; five villages) of Uganda. Soroti is denoted as an epidemic zone, Kamuli and Tororo as endemic. Whiskers represent range of data, while bars and boxes the 25–75% percentile.

($\chi^2_1 = 18.37$; $p < 0.001$); however, there was an interaction between epidemic/endemic status and sampling location ($\chi^2_1 = 10.78$; $p = 0.001$). There was no significant difference between *T. brucei s.l.* prevalence in markets and villages within Soroti district ($\chi^2_1 = 0.001$; $p = 0.967$); this study was undertaken prior to the implementation of control activities in that region. Within this epidemic area, prevalence determined for markets provided reasonable estimates of prevalence in surrounding villages. In contrast, in the endemic areas, markets did not accurately reflect village *T. brucei s.l.* prevalence; markets had a significantly higher proportion of infected animals ($\chi^2_1 = 29.20$; $p < 0.001$).

4. Discussion

The data presented suggest that prior to the implementation of sleeping sickness control in the epidemic area studied (Soroti district), *T. brucei s.l.* prevalences in cattle at markets could be used to estimate the prevalence of the parasite in the surrounding area; Soroti was the only *T.b. rhodesiense* sleeping sickness epidemic area in Uganda at the time of this study. In the endemic zones studied, market prevalences were not representative of those in the surrounding area; however, for assessment of the extent of *T. brucei s.l.* prevalence in livestock during sleeping sickness epidemics, markets may be used as a convenient central point. This has benefits in terms of reducing costs and improving the efficiency of screening programmes, and might be applied in emerging epidemic areas to which sleeping sickness has recently spread (Fèvre et al., 2005). Sampling of cattle would not, however, replace the need to screen humans in an effort to identify and treat patients infected with *T.b. rhodesiense*. The treatment of cattle at markets in epidemic areas would also prevent the spread of *T.b. rhodesiense* in the animal reservoir, though this should be accompanied by monitoring for the possible development of drug resistance (Barrett, 2001). Our results also show that while the market as a central point approach is inappropriate in endemic areas, there is a need for researching and testing cost-effective strategies for efficient sampling where cattle and human trypanosomiasis are well established. Improving our understanding of the levels of infection in reservoir populations where sleeping sickness persists should have important impacts on the efficiency of reservoir-targeted interventions.

At present, microscopy is the best diagnostic tool available for the identification of trypanosomes in the field, and the detection of the *SRA* gene—the marker for *T.b. rhodesiense* (Welburn et al., 2001; Gibson et al., 2002) is not field-adapted. While *T. brucei s.l.* prevalence continues to be used as a proxy for *T.b. rhodesiense*, Coleman and Welburn (2004) have shown that the ratio of *T. b. brucei*: *T.b. rhodesiense* in a given population is fairly constant, at 3:1. The level of *T. brucei s.l.* prevalence in a reservoir population in a sleeping sickness affected area is thus indicative of the prevalence of *T.b. rhodesiense* in that same reservoir population.

T. brucei s.l. does not result in severe clinical disease in local (primarily zebu) breeds of cattle. However, it is likely, that animals carrying *T. brucei s.l.* infections are also infected with other, more pathogenic species of trypanosome such as *T. vivax* and *T. congolense* (Magona et al., 2003), and the overt clinical signs associated with these infections may encourage farmers to attempt to sell animals, accounting for infection rates at markets in endemic areas higher than those in the surrounding villages. In addition, if animals infected with pathogenic trypanosomes are brought to market on the basis of poor performance (e.g. loss of body condition), such animals may not be rapidly sold to traders; farmers and traders have a tendency of returning to the market with the unsold animals on subsequent market days, leading to a build up of infected animals in the market—which may greatly contribute to the high prevalence recorded. The preferential sale of potentially infected animals in areas serving as a source of livestock for country-wide restocking programmes has implications for disease control policy (Fèvre et al., 2001), and emphasises the need for a better understanding of the livestock trade and for control measures designed specifically for preventing the long-range spread of zoonotic diseases.

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References

Barrett, M.P., 2001. Veterinary link to drug resistance in human African trypanosomiasis? Lancet 358, 603–604.

Clausen, P.H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F., Staak, C., 1998. Host preferences of tsetse (Diptera: Glossinidae) based on bloodmeal identifications. Med. Vet. Entomol. 12, 169–180.

Coleman, P.G., Welburn, S.C., 2004. Are fitness costs associated with resistance to human serum in Trypanosoma brucei rhodesiense? Trends Parasitol. 20, 311–315.

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. Lancet 358, 625–628.

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. Lancet 366, 745–747.

Gibson, W., Backhouse, T., Griffiths, A., 2002. The human serum resistance associated gene is ubiquitous and consereved in *Trypanosoma brucei rhodesiense* throughout East Africa. Infect. Genet. Evol. 25, 1–8.

Hide, G., Tait, A., Maudlin, I., Welburn, S.C., 1996. The origins, dynamics and generation of *Trypanosoma brucei rhodesiense* epidemics in East Africa. Parasitol. Today 12, 50–55.

Magona, J.W., Mayende, J.S.P., Olaho-Mukani, W., Coleman, P.G., Jonsson, N.N., Welburn, S.C., Eisler, M.C., 2003. A comparative study on the clinical, parasitological and molecular diagnosis of bovine trypanosomosis in Uganda. Onderstepoort J. Vet. Res. 70, 213–218.

Magona, J.W., Walubengo, J., Odiit, M., Okedi, L.A., Abila, P., Katabazi, B.K., Gidudu, A.M., Olaho-Mukani, W., 2005. Implications of the re-invasion of Southeast Uganda by *Glossina pallidipes* on the epidemiology of bovine trypanosomosis. Vet. Parasitol. 128, 1–9.

Odiit, M., Shaw, A., Welburn, S.C., Fèvre, E.M., Coleman, P.G., McDermott, J.J., 2004. Assessing the patterns of health-seeking behaviour and awareness among sleeping-sickness patients in eastern Uganda. Ann. Trop. Med. Parasitol. 98, 339–348.

Okiria, R., Okuna, N.M., Magona, J.W., Mayende, J.S.P., 2002. Sustainability of tsetse control by subsequent treatment of 10% of a previously treated Ugandan cattle population with 1%, w/v deltamethrin. Trop. Anim. Health Prod. 34, 105–114.

Picozzi, K., Tilley, A., Fèvre, E.M., Coleman, P.G., Magona, J.W., Odiit, M., Eisler, M.C., Welburn, S.C., 2002. The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk. Afr. J. Biotechnol. 1, 39–45.

Waiswa, C., Olaho-Mukani, W., Katunguka-Rwakishaya, E., 2003. Domestic animals as reservoirs for sleeping sickness in three endemic foci in south–eastern Uganda. Ann. Trop. Med. Parasitol. 97, 149–155.

Welburn, S.C., Picozzi, K., Fèvre, E.M., Coleman, P.G., Odiit, M., Carrington, M., Maudlin, I., 2001. Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. Lancet 358, 2017–2019.

A burgeoning epidemic of sleeping sickness in Uganda

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The epidemic of *Trypanosoma brucei rhodesiense* sleeping sickness in eastern Uganda, which began in 1998 as a result of movements of the livestock reservoir of the parasite, has continued to spread. An additional 133 000 people have been put at risk of infection in Kaberamaido, another newly affected district. The few resources committed to control interventions in Soroti district have failed to contain the epidemic. The high prevalence of the parasite in cattle presents a significant risk for transmission to human beings and further spread of this neglected zoonotic disease. Targeted interventions are urgently needed to control epidemics and reduce the high mortality resulting from sleeping sickness.

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There are two areas in Uganda affected by sleeping sickness; *Trypanosoma brucei rhodesiense* occurs in the east of the country and *T b gambiense* in the northwest. The geographic distribution of these parasites is separate, although there are concerns that an overlap might occur.[1] In 2001, we reported on the origins and causes of a newly described outbreak of *T b rhodesiense* sleeping sickness in Serere county, Soroti district, lying to the north of historical endemic foci.[2] The greatest risk factor for infection at the start of the outbreak was proximity to a livestock market; cattle are the principal reservoir of the zoonotic *T b rhodesiense* parasite in this ecosystem, and we have shown that livestock movements into Soroti resulted in the spread of the parasite to the area.[2]

Here, we report on the current extent of the sleeping sickness situation in Soroti district. Before control activities were undertaken across the affected area, up to 18% of cattle in some parts of Soroti were shown to be carrying human infective parasites.[3] The primary response to outbreaks such as this in Uganda is to treat human patients (treatment in the early stage of the disease with suramin, while melarsoprol is used in the late stage). Second, to prevent further transmission to human beings, both vector control (tsetse flies of the genus *Glossina*) and mass treatment of the livestock reservoir with anti-trypanocidal drugs is undertaken. Such control interventions have taken place in a few areas of Soroti; however, this region has also been plagued by violence and instability since 2003, which has had a substantial negative effect on the general health of the population.[4]

Interventions were put in place between January, 2000, and December, 2003, and consisted of mass treatment of livestock with long-acting trypanocides and some vector control. In April, 2004, domestic cattle in six villages (51–56 animals per village; total sample size=335) in the areas of Soroti affected by sleeping sickness were screened for trypanosome infections. Three villages in the non-intervention area were selected based on a history of recent (<3 months) sleeping sickness reporting, and three villages known to have reported sleeping sickness before the interventions were selected in the intervention area. Additionally, 51 animals were screened at the livestock market that had been at the centre of the originally reported outbreak.[2] All animals were screened by PCR for both *T brucei* sensu lato and *T b rhodesiense* (table 1).[3] Cattle at the local livestock market were also sampled on one market day. We calculated CI using binomial regression, taking account of the clustering of cattle at the village level, producing robust estimates of the standard errors; χ^2 values are uncorrected. The mean prevalence of human infective *T b rhodesiense* in the cattle population in areas with interventions is not significantly different from in those areas where there had been no intervention (χ^2=0·167, df=1, p=0·6829). To be effective, mass treatment interventions need to cover enough of the livestock population to prevent the transmission of parasites to tsetse. Similarly, effective tsetse control must reduce vector challenge sufficiently to effect transmission to human beings. The prevalence of *T b rhodesiense* in cattle at the livestock market was higher than in villages in both the intervention (χ^2=7·609, df=1, p=0·0058) and non-intervention (χ^2=5·864, df=1, p=0·0155) zones. The very high prevalence of *T b rhodesiense* in cattle at the market, which are imported mainly from endemic sleeping sickness areas,[2] indicates that the trade and resultant movement of animals infected with *T b rhodesiense* continues. While *T brucei* sl is not highly pathogenic to local cattle breeds, co-infections with other more pathogenic livestock-infective trypanosomes are common, and farmers might be choosing to sell their sick or less productive animals. The risk of spreading sleeping sickness via this reservoir host in the area

	T brucei sensu lato	*T b rhodesiense* alone
Intervention area		
Village 1 (Obur)	8/56 (14%)	0/56 (0%)
Village 2 (Obar)	13/55 (24%)	5/55 (9%)
Village 3 (Akoroi)	16/56 (29%)	6/56 (11%)
Total	37/167 (22%; 95% CI 15·0–31·5)	11/167 (7%; 95% CI 2·4–17·0)
Livestock market		
Brookes Corner	21/51 (41%; 95% CI 28·6–55·0)	10/51 (20%; 95% CI 10·9–32·7%)
Non-intervention area		
Village 4 (Omagoro)	22/56 (39%)	8/56 (14%)
Village 5 (Odunguru)	9/56 (16%)	3/56 (5%)
Village 6 (Ayepe)	4/56 (7%)	2/56 (4%)
Total	35/168 (21%; 95% CI 7·8–45·1%)	13/168 (8%; 95% CI 3·3–17·2%)

***Table 1*: Prevalence of *T brucei* sensu lato and *T b rhodesiense* in livestock in Soroti district in 2004, diagnosed by PCR**

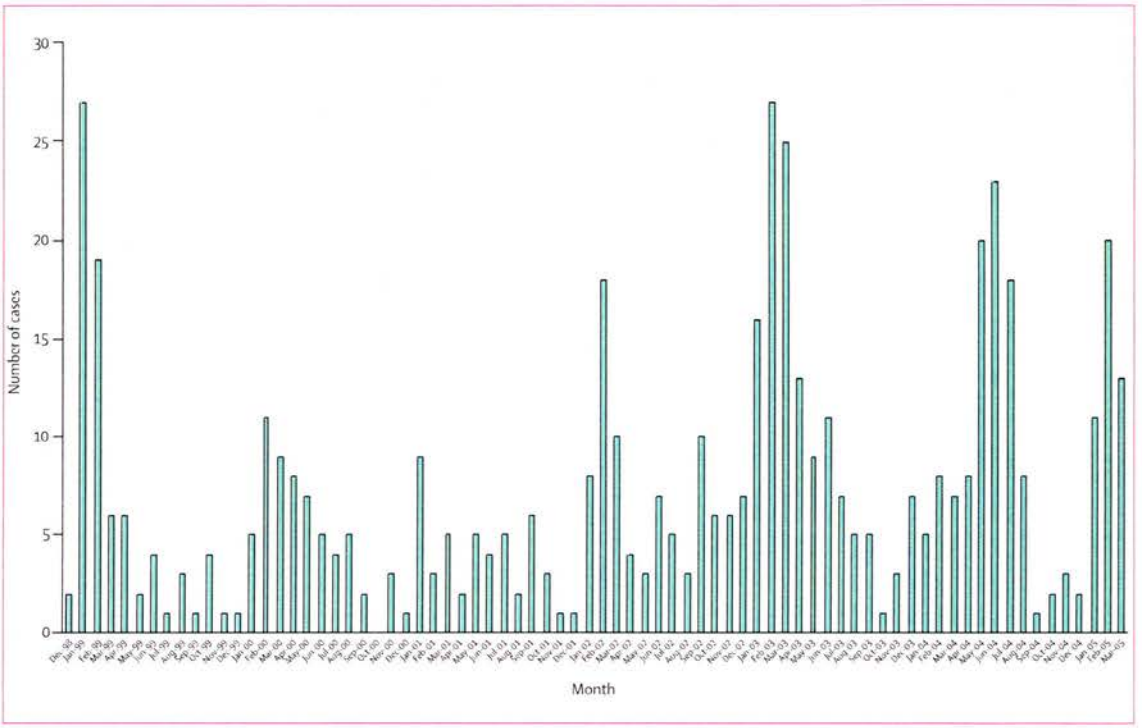

Figure 1: Number of cases of sleeping sickness during each month of the epidemic in Serere, from the time the disease was first recognised (December, 1998) to March, 2005

continues to be a public-health challenge. Livestock for sale are required to be treated at their point of origin or before sale; this requirement forms part of the well designed national policy for trypanosomiasis control, but has proved difficult to implement at a local level with increasing decentralisation of public services.

From the first reported case in 1998, and up to April, 2004, 428 cases of sleeping sickness presented at the only health facility equipped to diagnose and treat the disease in Soroti district (Serere Health Centre; figure 1). There was a marked seasonal peak in case reporting; this could indicate increased parasite transmission by tsetse during the preceding wet season, which occurs between September and November (the late stage of infection typically develops after 3–6 months). Of the reported cases, 103 were early stage cases, 287 were late stage, and 38 were not staged. The mortality rate in the hospital was 4% (18/428 deaths). 67% of staged cases were in the late stage of sleeping sickness, indicative of poor early detection of the disease by both the health system and communities. A deterministic model has been developed to estimate the proportion of undetected *T b rhodesiense* cases in a given population on the basis of knowledge of the early to late ratio.[3] Application of the model to the Soroti data suggests that an additional 299 unreported cases (95% CI 170–438), or 0·7 (0·4–1·02) unreported cases for each reported case, might have occurred in the communities in the Serere Health Centre catchment area up to April, 2004. Untreated cases have almost 100% fatality rate. Sleeping sickness tends to affect the poorest and most disenfranchised rural communities with the least access to health care. Public-health messaging and extension

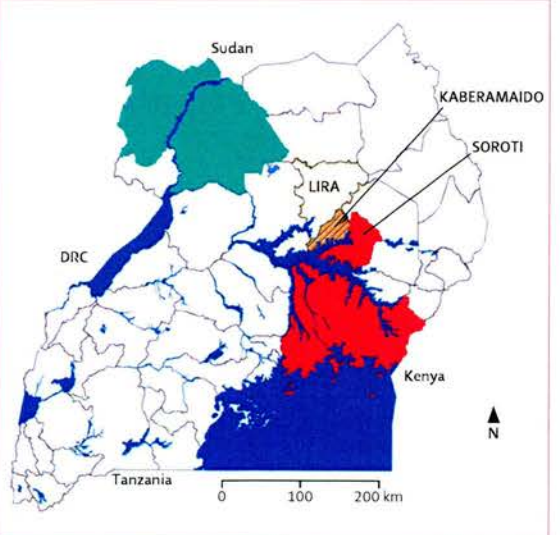

Figure 2: Map of Uganda showing established *Trypanosome brucei rhodesiense*-affected areas in the south and east (red), and *T b gambiense*-affected areas in the northwest (green)

health services are needed to improve knowledge and reporting of this disease.

From April, 2004, onwards, patients with *T b rhodesiense* sleeping sickness have also been reporting from areas outside the catchment area of Serere hospital, and a new sleeping sickness focus has become established in Kaberamaido District, to the north-west of Soroti across a major branch of the Lake Kyoga drainage system (figure 2). Disease control has not been effective in Soroti and, as a result, the parasite has spread to Kaberamaido district; there are also reports of *T b rhodesiense* transmission in the southern part of Lira district. Between February, 2004, and January, 2005, 144 cases presented from this area, which is more remote and less well equipped to handle the situation than Soroti, and has also suffered from civil unrest. The direction of spread of *T b rhodesiense* sleeping sickness in Uganda over the past 5 years, towards the *T b gambiense* foci in northwestern Uganda and southern Sudan, increases the risk of a geographic overlap of the two causative organisms. Since treatments and diagnostic methods for the two diseases differ, any convergence in their range will have important implications for patient care and national control policy. As livestock movements result in the spread of *T b rhodesiense*, controlling such movements or treating livestock before movement is a matter of urgency. Where livestock migration can be monitored, such as at livestock markets, control activities should be targeted there. Additionally, given the human population movements consequent upon civil instability,[4] and their possible role in *T b gambiense* spread, it would be prudent to monitor for the presence of *T b gambiense* in those areas where only *T b rhodesiense* occurs at present. Outbreaks of *T b rhodesiense* in new areas are conceptually straightforward to prevent with effective veterinary interventions but, once established, very difficult to control. *T b rhodesiense* will probably become endemic in Soroti with long-term consequences for the rural population. Like other zoonoses, this disease stretches the capacity of veterinary and medical services affecting those sections of society least able to deal with the problem.

Contributors

E M Fèvre, K Picozzi, S C Welburn, and P G Coleman conceived of the study, which was comanaged by E M Fèvre, M Odiit, and S C Welburn. E M Fèvre, K Picozzi, J Fyfe, and C Waiswa conducted the field components. Laboratory analyses were undertaken by K Picozzi and J Fyfe. E M Fèvre, M Odiit, and P G Coleman did the data analyses, and all authors contributed to the data interpretation and manuscript writing.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

1 Hutchinson OC, Fèvre EM, Carrington M, Welburn SC. Lessons learned from the emergence of a new *Trypanosoma brucei rhodesiense* sleeping sickness focus in Uganda. *Lancet Infect Dis* 2003; **3**: 42–45.

2 Fèvre EM, Coleman PG, Odiit M, Magona JW, Welburn SC, Woolhouse MEJ. The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in eastern Uganda. *Lancet* 2001; **358**: 625–28.

3 Welburn SC, Picozzi K, Fèvre EM, et al. Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (*SRA*) gene. *Lancet* 2001; **358**: 2017–19.

4 Nathan N, Tatay M, Piola P, Lake S, Brown V. High mortality in displaced populations of northern Uganda. *Lancet* 2004; **363**: 1402.

5 Odiit M, Coleman PG, Liu W-C, et al. Quantifying the level of under-direction of *Trypanosoma brucei rhodesiense* sleeping sickness cases. *Trop Med Int Health* (in press).

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A PCR based assay for detection and differentiation of African trypanosome species in blood

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Abstract

Direct PCR analysis of trypanosome infected blood samples in the quantities required for large scale epidemiological study has always been problematic. Current methods for identifying and differentiating trypanosomes typically require several species-specific reactions, many of which rely on mouse passaged samples to obtain quality concentrated genomic DNA. As a consequence important epidemiological information may be lost during the sample preparation stage. Here, we report a PCR methodology that reduces processing and improves on the sensitivity of present screening methods. The PCR technique targets the gene encoding the small ribosomal subunit in order to identify and differentiate all clinically important African trypanosome species and some subspecies. The method is more economical, simple, and sensitive than current screening methods, and yields more detailed information, thereby making it a viable tool for large-scale epidemiological studies.

Index Description and Abbreviations: Trypanosomiasis; Epidemiology; PCR; Diagnosis; Human African sleeping sickness; Ribosomal DNA; Whatman FTA; DNA, deoxyribonucleic acid; ITS, internal transcribed spacer; PCR, polymerase chain reaction; RNA, ribonucleic acid

Keywords: Trypanosomiasis; Epidemiology; PCR; Diagnosis; Human African sleeping sickness; Ribosomal DNA; Whatman FTA

1. Introduction

The African trypanosomes comprise a group of important and complex pathogens, affecting animal and human health in much of sub Saharan Africa. The causative organisms are represented by a variety of species and subspecies of a heteroxenous parasite of the genus *Trypanosoma*, some of which are zoonotic, causing disease in man and animals (domestic and wild). Animal trypanosomiasis, or nagana, costs livestock producers and consumers an estimated $1340 million annually, this figure excludes indirect livestock benefits such as manure and traction (Kristjanson et al., 1999). Human African trypanosomiasis occurs in endemic foci across East and West Africa. The human infective *Trypansoma brucei rhodesiense* subspecies is maintained in wild animals (van Hoeve et al., 1967) and domestic livestock (Hide et al., 1996; Onyango et al., 1966) from where it may play a significant role in the generation of acute sleeping sickness epidemics in east Africa (Fevre, 2001; Hide et al., 1996; Welburn et al., 2001). Effective disease control and management depends heavily upon knowledge of the epidemiology of the disease, which in turn relies upon

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methods that incorporate screening of both animal and human populations (Hutchinson et al., 2003).

Methods of epidemiological screening include direct parasite examination using traditional dark ground microscopy, examination of buffy coat and more recently molecular methodologies based on the polymerase chain reaction (PCR). Microscopy is labour intensive and can lack sensitivity under field conditions due to routinely low peripheral parasitaemia in infected livestock (Picozzi et al., 2002). PCR based diagnostic methods have largely overcome difficulties associated with sensitivity and specificity. A number of methods have been developed for the following species and subspecies of *Trypansoma–Trypanozoon* (Artama et al., 1992; Kabiri et al., 1999), *Trypanosoma congolense* (Riverine/Forest) (Masiga et al., 1992), *T. congolense* (Kilifi) (Masiga et al., 1992), *T. congolense* (Savannah) (Masiga et al., 1992), *Trypanosoma vivax* (Masake et al., 1994, 1997), *Trypanosoma simiae* (Masiga et al., 1992), *Trypanosoma evansi* (Artama et al., 1992), *T. congolense* (Kenya Coast) (Masiga et al., 1992), and *Trypanosoma theileri* (Rodrigues et al., 2003). Using these approaches accurate species/subspecies differentiation requires up to eight different PCRs per sample, which increases the costs and impacts on the practical application of the technique for large-scale epidemiological studies. Furthermore, many of the PCR techniques developed in recent years are based on complex protocols requiring samples to be mouse passaged, and therefore mouse adapted, a process which some trypanosome isolates do not survive (Hoare, 1972; Masiga et al., 1992) resulting in loss of species or strains and selection and sampling bias (Coleman and Welburn, 2004).

Recent developments in matrices for sample collection and archive, which permit direct PCR identification from tissue/fluids may overcome such bias. Simplified protocols incorporating these improved sample collection techniques, together with rapid PCR-based screening methodologies for the direct analysis of field samples are therefore required. The internal transcribed spacers (ITS) located within the ribosomal RNA genes have been used to establish relationships and differentiate species in an extremely wide range of organisms (Mai and Coleman, 1997; Samuel, 1998; Schlotterer et al., 1994; Wesson et al., 1992). A high copy number combined with inter-species length variation makes the ITS region a useful marker for species differentiation in trypanosomes, as has been recently demonstrated (Desquesnes et al., 2001; McLaughlin et al., 1996; Njiru et al., 2004). However, this technique was shown to be relatively insensitive and in some cases was problematic for detection of *T. vivax* (the principal pathogenic species in cattle) in either concentrated genomic DNA or DNA extracted from field samples. Here, we report the development of a simple nested PCR method, which detects the inter-specific length variation of the ITS regions of ribosomal genes and thereby produces a unique size of PCR product for each species of trypanosome. The technique is able to detect the following African trypanosome species. (*Trypanozoon*, *T. congolense* (River/Forest), *T. congolense* (Kilifi), *T. congolense* (Savannah), *T. vivax* , *T. simiae*, *T. evansi*, *T. congolense* (Kenya Coast), and *T. theileri*). It is able to detect a single trypanosome and has been optimised for PCR amplification of blood applied to filter paper (Whatman FTA) permitting direct PCR analysis of field material.

2. Materials and methods

2.1. Samples

Field samples consisted of 245 samples of bovine blood taken from two villages in the Soroti and Tororo districts of Uganda and collected on Whatman FTA cards. Genomic DNA stocks are as detailed in Table 1.

2.2. Primer design

Sixteen trypanosome ribosomal DNA sequences were selected from the NCBI database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide). *T. brucei* AF306771, AF306772, AF306773, AF306774, AF306775, AF306776, AF306777, and X05862; *T. congolense* U22315; *T. congolense* (Kilifi) U22316; *T. congolense* (River/Forest) U22317; *T. congolense* (Tsavo) U22318; *T. vivax* U22319, *T. simiae* U22320. Two additional sequences (*Trypanosoma cruzi* AY362826 and *Trypanosoma rangeli* AY230240) were selected for comparison as out-groups to ensure optimal specificity of the primers. Sequences were aligned with CLUSTALX software (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX) (Thompson et al., 1997) and viewed using the Bioedit programme (Hall, 1999). A set of nested primers targeting the ribosomal gene locus was selected using PRIMER3 web

Table 1
Details and origin of trypanosome genomic DNA used in the development of the ITS-PCR protocol

Species	Stock code	Origin
T. brucei brucei	BUTEBA135	Tororo, SE Uganda, Cow, 1990
T. brucei rhodesiense	BUG H2	Kamuli, Uganda, Human, 2000
T. brucei rhodesiense	DO	Katerema, Uganda Human, 1990
T. congolense (Savannah)	IL1180 (ILNat3.1)	Serengeti, Tanzania
T. congolense (Forest)	IL3900	Burkina Faso
T. congolense (Kalifi)	IL45.1	Kilifi, Kenya
T. vivax	ILDatt1.2	Kenya
T. brucei	OBUR C19	Soroti, Uganda, Cow, 2000
T. congolense (Forest)	TSW103	Liberia, Pig
T. simiae	TV008	Unknown

Table 2
Expected and obtained band sizes for amplification using nested ITS primers as calculated from the sequences present in bioinformatic databases

Species	Expected band size from NCBI database (bp)	Band sizes obtained (bp)
T. congolense (Forest)	1513	1501
T. congolense (Kilifi)	1422	1430
T. congolense (Savannah)	1413	1408
T. congolense (Tsavo)	954	951
T. brucei	1207–1224	1215
T. simiae	850	847
T. vivax	611	620
T. theileri	988	998

primer selection software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi). Primers were evaluated using NETPRIMER software available at (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html). The specificity of the primers was evaluated using a BLAST search against human and mouse genomes (http://www.ncbi.nlm.nih.gov/BLAST). The outer primer sequences were ITS1 (5′-GAT TAC GTC CCT GCC ATT TG-3′), and ITS2 (5′-TTG TTC GCT ATC GGT CTT CC-3′) (MWG Biotech), and inner primer sequences ITS3 (5′-GGA AGC AAA AGT CGT AAC AAG G-3′), and ITS4 (5′-TGT TTT CTT TTC CTC CGC TG-3′) (MWG Biotech). All PCR conditions were optimised using modified 'Taguchi' methods (Cobb and Clarkson, 1994). Expected band sizes were calculated from the distance between the primer locations as determined from the sequences for each trypanosome species present in bioinformatic databases. The expected band sizes are shown in Table 2.

2.3. Amplification of DNA

Blood and genomic DNA samples were applied to Whatman FTA cards and allowed to dry for a minimum of 24 h at room temperature. A 2 mm diameter punch was cut from the cards and was washed according to the following protocol; three washes of 10 min in Whatman FTA reagent followed by two washes of 5 min in 1 mM TE buffer. The punches were dried at room temperature for a minimum of 90 min then placed directly in the PCR tubes for the first round of PCR. The reaction volume of 25 μl contained the following components. Super-Taq PCR buffer from HT Biotechnologies, Cambridge (final concentrations of 10 mM Tris–HCl, pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, and 0.01%(w/v) stabilizer), 2 μM of each outer primer ITS1 and ITS2, 1 mM total dNTP's and 1.25 U of Biotaq (Bioline, London). The reaction conditions were as follows: 1 cycle of 95 °C for 7 min followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, the thermal cycling was carried out on a Stratagene Robocycler. For the second round reaction 1 μl of the PCR product from the first round reaction was placed in a fresh tube and 24 μl of the reaction mixture was added as detailed for the outer primers, with the exception of the substitution of the outer primers (ITS1 and 2) with the inner primers. (ITS3 and 4) The reaction conditions were as detailed previously. Ten microlitres of the PCR product was run on a 30 cm × 20 cm 1.5% agarose gel run at 100 V. The gel was stained with ethidium bromide and visualised using a Flowgen Alpha 1220 gel imaging system.

3. Results

To differentiate important species (and some subspecies) of African trypanosome a nested PCR was developed which amplified the variable ITS region of the ribosomal gene locus, using primers designed to the conserved flanking sequences (Fig. 1).

3.1. Specificity

Amplification of genomic DNA from trypanosome stocks (Table 1) resulted in a specific size band for each species, which was within the bounds of measurement error and was in complete agreement with the expected band sizes (Table 2). Control DNA samples were not available for some trypanosome species (e.g., *T. theileri*), therefore when unexpected band sizes appeared in field samples the bands were cut out, sequenced, and compared with database sequences to confirm species identity (data not shown). The specificity of the primers was further tested by PCR amplification with host DNA (human, cow, and mouse), which produced no visible bands.

3.2. Sensitivity

To investigate the sensitivity of the nested PCR, the technique was tested on a dilution series of whole try-

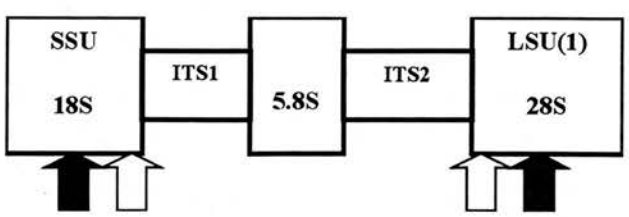

Fig. 1. The structure of part of the ribosomal RNA gene locus. Ribosomal genes are present in tandem arrays of around 100–200 copies per trypanosome. Each gene consists of a number of conserved coding regions and non-coding spacer regions. Large boxes represent conserved coding regions (SSU, small sub-unit; LSU, large subunit) and small boxes represent spacer regions. The two spacers, internal transcribed spacers (ITS) 1 and 2 are known to vary in size between species and occasionally subspecies. A set of nested primers designed to the conserved regions are represented by black arrows (outer primers) ITS1 and ITS2 and white arrows (inner primers) ITS3 and ITS4.

panosomes (diluted in phosphate-buffered saline) on Whatman FTA cards and a dilution series of genomic DNA (diluted in water) in liquid form and also applied to Whatman FTA cards. In the two dilutions of genomic DNA positive amplification was detected at a DNA concentration of 49 pg ml^{-1} (or less than a single trypanosome equivalent). To investigate the efficacy of the technique on samples containing host material, trypanosomes were diluted in bovine blood (UK origin) and applied to Whatman FTA cards to mimic field samples. Positive amplification was detected at DNA a concentration of 55 pg ml^{-1}, which is again equivalent to less than a single trypanosome.

3.3. Application to field samples

Application of the nested ITS primers to 245 samples of bovine blood taken from the Tororo and Soroti Districts of Uganda and collected on Whatman FTA cards resulted in successful amplification of the target ITS region as shown by species specific band sizes (Fig. 2). This technique was also able to show samples infected with multiple species (e.g., Fig. 2; lanes 2, 16, and 19), as shown by the presence of multiple bands.

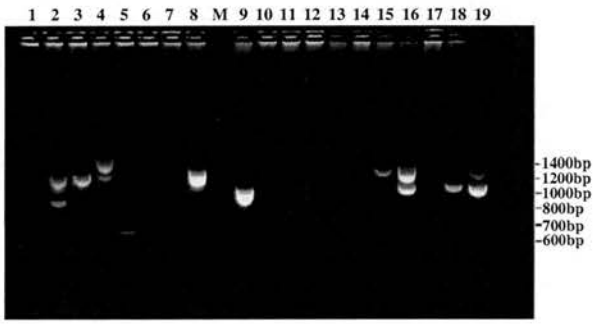

Fig. 2. Representative gel showing bands obtained from PCR amplification (using nested ITS primers) of 19 blood samples (on Whatman FTA cards) taken from cattle in the Tororo district of Uganda. Samples 2, 3, 8, 16, and 19 are all positive for *T. brucei*, Samples 2, 9, 16, 18, and 19 are positive for *T. theileri*. Sample 17 is positive for *T. simiae*, sample 5 is positive for *T. vivax*, and samples 1, 6, 7, 10, 11, 12, 13, and 14 are negative. Lane M represents a marker graduated in 100 bp intervals (band sizes illustrated). Mixed species infections were found in lanes 2, 3, 4, 5, 16, and 17.

3.4. Technique evaluation

The efficacy of the technique was tested against the most widely used screening method; individual species specific PCR's (Artama et al., 1992; Clausen et al., 1998; Majiwa et al., 1994; Masake et al., 1997; Masiga et al., 1992), using samples collected from two different villages in Uganda (Cow blood applied to Whatman FTA cards). Analysis of the 245 samples using the individual species-specific PCR screening method demonstrated a low prevalence of trypanosomes in cows from the first village and a high prevalence of trypanosomes cows from the second village. The new nested PCR analysis method showed that a comparable prevalence and greater number of species were detected in each case (Table 3).

4. Discussion

Existing methods for screening samples for detection and differentiation of trypanosomes are not suited to large-scale epidemiological analysis. This study addressed the requirement for improved techniques that simplify the sample analysis process but maintain the sensitivity and specificity required for directly analysing field samples.

We developed, a new nested PCR targeted to include both internal transcribed spacers of the ribosomal RNA genes (ITS PCR), that was capable of detecting trypanosomes in the presence of host DNA and the PCR inhibitors present in blood (Heme, Lactoferrin IgG and non-target DNA). This nested technique was found to be sensitive enough for detection of a single parasite in blood samples and has been shown to be able to differentiate all important African trypanosome species and some subspecies. To simplify the sample collection and processing methodology, we investigated the storage of samples on treated filter paper cards, which make possible the direct

Table 3
Evaluation of detection of trypanosome DNA from Whatman FTA cards containing blood from low and high prevalence villages in the Tororo and Soroti districts of Uganda, using a nested PCR amplification, compared with utilisation of individual species specific primers (Artama et al., 1992; Clausen et al., 1998; Majiwa et al., 1994; Masake et al., 1997; Masiga et al., 1992)

Species	Low prevalence village prevalence (%)		High prevalence village prevalence (%)	
	Species specific PCR method	ITS-PCR	Species specific PCR method	ITS-PCR
T. brucei	5	7	32	33
T. theileri	ND	3	ND	47
T. congolense	0	1	1	5
T. vivax	1	1	8	5
T. simiae	ND	0	ND	2
		($N = 101$)		($N = 144$)

ND, not done.

analysis of biological samples, in addition to circumventing the requirement for mouse passage. When the nested technique was evaluated against the current single PCR per species screening method, using a complete sample set containing positive and negative samples, it was found to have a similar level of detection, but was capable of detecting a greater number of species in both high and low prevalence sample sets.

The epidemiology of African trypanosomiasis is complex and poorly understood and requires large-scale field based investigation. This technique has greatly simplified epidemiological studies involving sample screening. As a result the costs and time involved in screening samples for the eight major species/subspecies of trypanosome have been reduced by a factor of four (conservative estimate). This nested PCR technique can be used to screen large numbers of biological samples directly, quickly, and accurately, making it a simple, cost effective, robust, and reliable tool for investigating the complex epidemiology of African Trypanosomiasis.

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References

Artama, W., Agey, M., Donelson, J., 1992. DNA comparisons of *Trypanosoma evansi* (Indonesia) and *Trypanosoma brucei* spp. Parasitology 104, 67–74.

Clausen, P.H., Wiemann, A., Patzelt, R., Kakaire, D., Poetzsch, C., Peregrine, A., Mehlitz, D., 1998. Use of a PCR assay for the specific and sensitive detection of *Trypanosoma* spp. In naturally infected dairy cattle populations in Peri-Urban Kampala, Uganda. In: Jongejan, F., Goff, W., Camus, E. (Eds.), Tropical Veterinary Medicine: Molecular Epidemiology, Hemoparasites and Their Vectors, and General Topics, vol. 849. Eurospan Ltd, pp. 21–31.

Cobb, D.C., Clarkson, J., 1994. A Simple Procedure for Optimising the Polymerase Chain Reaction (PCR) Using Modified Taguchi Methods. Nucleic Acids Research 22, 3801–3805.

Coleman, P., Welburn, S., 2004. Are fitness costs associated with resistance to human serum in *Trypanosoma brucei rhodesiense*?. Trends in Parasitology 20, 311–315.

Desquesnes, M., McLaughlin, G., Zoungrana, A., Davila, A.M., 2001. Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. International Journal for Parasitology 31, 609–613.

Fevre, E.M., 2001. The origins of a new *Trypanosoma brucei rhodesiense* sleeping sickness outbreak in Eastern Uganda. Lancet, 625–628.

Hall, T.A., 1999. Bioedit: a user-friendly biological sequence alignment editor and analysis. Nucleic Acids Symposium Series 41, 95–98.

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, 50–54.

Hoare, C.A., 1972. The Trypanosomes of Mammals. A Zoological Monograph, vol. 46. Blackwell Scientific Publications, pp. 427–434.

Hutchinson, O.C., Fevre, E.M., Carrington, M., Welburn, S.C., 2003. Lessons learned from the emergence of a new *Trypanosoma brucei rhodesiense* sleeping sickness focus in Uganda. Lancet Infectious Diseases 3, 42–45.

Kabiri, M., Franco, J.R., Simarro, P.P., Ruiz, J.A., Sarsa, M., Steverding, D., 1999. Detection of *Trypanosoma brucei gambiense* in sleeping sickness suspects by PCR amplification of expression-site-associated genes 6 and 7. Tropical Medicine and International Health 4, 658–661.

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, 79–98.

Mai, J.C., Coleman, A.W., 1997. The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. Journal of Molecular Evolution 44, 258–271.

Majiwa, P.A.O., Thatthi, R., Moloo, S.K., Nyeko, J.H.P., 1994. Detection of trypanosome infections in the saliva of tsetse flies and buffy-coat samples from Antigenaemic but Aparasitaemic cattle. Parasitology 108, 313.

Masake, R.A., Majiwa, P.A.O., Moloo, S.K., Makau, J.M., Njuguna, J.T., Maina, M., Kabata, J., Ole MoiYoi, O.K., Nantulya, V.M., 1997. Sensitive and specific detection of *Trypanosoma vivax* using the polymerase chain reaction. Experimental Parasitology 85, 193–205.

Masake, R.A., Nantulya, V.M., Pelle, R., Makau, J.M., 1994. A species-specific antigen of *Trypanosoma* (Duttonella) *vivax* detectable in the course of infection is encoded by a differentially expressed tandemly reiterated gene. Molecular and Biochemical Parasitology 64, 207.

Masiga, D.K., Smyth, A.J., Hayes, P., Bromidge, T.J., 1992. Sensitive detection of trypanosomes in Tsetse flies by DNA amplification. International Journal for Parasitology 22, 909.

McLaughlin, G., Ssenyonga, S., Nanteza, E., Rubaire, A., Wafula, O., Hansen, R.D, Vodkin, M., Novak, R.J., Gordon, V.R., Montenegro-James, S., James, M., Aviles, H., Armijos, R., Santrich, C., Weigle, K., Saravia, N., Wozniak, E., Gaye, O., Mdachi, R., Shapiro, S., CHang, K.P., Kakoma, I., 1996. PCR based detection and typing of parasites. In: Azcel, M., Alkan, M. (Eds.), Parasitology for the 21st Century CAB International, Wallingford, Oxon, pp. 261–287.

Njiru, Z.K., Constantine, C.C., Guya, S., Crowther, J., Kiragu, J.M., Thompson, R.C., Davila, A.M., 2004. The use of ITS1 rDNA PCR in detecting pathogenic african trypanosomes. Parasitology Research [E published ahead of print].

Onyango, R., van Hoeve, K., De Raadt, P., 1966. The epidemiology of *Trypanosoma rhodesiense* sleeping sickness in alego location, central Nyanza, Kenya. Evidence that cattle may act as a reservoir host of trypanosomes infective to man. Transactions of the Royal Society for Tropical Medicine and Hygiene 60, 175–182.

Picozzi, K., Tilley, A., Févre, E.M., Coleman, P.G., Magona, J.W., Odiit, M., Eisler, M.C., Welburn, S.C., 2002. The diagnosis of trypanosome infections: applications of novel technology for reducing disease risk. African Journal of Biotechnology 1, 39–45.

Rodrigues, A.C., Campaner, M., Takata, C.S., Dell' Porto, A., Milder, R.V., Takeda, G.F., Teixeira, M.M., 2003. Brazilian isolates of *Trypanosoma* (Megatrypanum) *theileri*: diagnosis and differentiation of isolates from cattle and water buffalo based on biological charac-

teristics and randomly amplified DNA sequences. Veterinary Parasitology 116, 185–207.

Samuel, R.E.A., 1998. Its sequences from nuclear rRNA suggest unexpected phylogenetic relationships between euro-mediterranean, East Asiatic and North Atlantic taxa of *Quercus* (Fagaceae). Plant Systematics and Evolution 211, 129–139.

Schlotterer, C., Hauser, M.T., von Haeseler, A., Tautz, D., 1994. Comparative evolutionary analysis of rDNA ITS regions in *Drosophila*. Molecular Biology and Evolution 11, 513–522.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25, 4876–4882.

van Hoeve, K., Onyango, R., Harley, J., 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 for Tropical Medicine and Hygiene 61, 684–687.

Welburn, S.C., Fevre, E.M., Coleman, P.G., Odiit, M., Maudlin, I., 2001. Sleeping sickness: a tale of two diseases. Trends in Parasitology 17, 19–24.

Wesson, D.M., Porter, C.H., Collins, F.H., 1992. Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). Molecular Phylogenetics and Evolution 1, 253–269