THE UNIVERSITY
of EDINBURGH

The molecular epidemiology of trypanosomiasis in Ugandan cattle during the Stamping Out Sleeping Sickness control programme, 2006 – 2008

Louise Claire Hamill

Submitted in fulfilment of the requirement of the degree of Doctor of Philosophy

The University of Edinburgh

2012

Ph.D – The University of Edinburgh – 2012

Declaration

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

Louise Claire Hamill

Edinburgh 2012

Ph.D – The University of Edinburgh – 2012

Abstract

Over the past two decades movement of cattle towards the north of Uganda has enabled the *Trypanosoma brucei rhodesiense* focus in south-eastern Uganda to spread into previously unaffected districts. This thesis brings together important epidemiological data regarding the impact of mass cattle drug treatment on the point prevalence of several different species of trypanosome in a newly endemic area of human sleeping sickness. Crucially the findings illustrate mass drug treatment is effective in reducing the prevalence of *T. b. rhodesiense* in cattle, thus minimising the reservoir potential of these animals in the epidemiology of human disease.

During 2006 a control programme was launched to halt the northward spread of this zoonotic parasite. This programme, entitled 'Stamping Out Sleeping Sickness' (SOS) proposed to reduce the prevalence of Human African Trypanosomiasis (HAT) in the newly affected districts by reducing the prevalence of this parasite in the main animal reservoir of infection – domestic cattle. Cattle were mass treated using trypanocides to clear infections.

Previous work demonstrated the prevalence of *T. brucei* s. l. and *T. b. rhodesiense* in cattle was higher in the districts of Dokolo and Kaberamaido than in the other SOS intervention districts (Selby 2011). To determine whether animals in these areas were also exposed to pathogenic cattle trypanosomes samples were screened for the presence of *T. vivax* and *T. congolense* savannah using PCR. Chapter three of this thesis determined the prevalence of these trypanosomes in cattle in these districts. Before treatment had taken place the prevalence of *T. vivax* was 2% (4/200, 95% CI 3.57 – 0.12%) in Dokolo and 7.3% (21/310, 95% CI 10.17 - 4.24 %) in Kaberamaido. The prevalence of *T. congolense* savannah at baseline was 3.5% (7/200, 95% CI 7.08–1.42 %) in Dokolo and 9.1% (21/230, 95% CI13.6–5.7 %) in Kaberamaido. Monitoring was conducted three, nine and 18 months post treatment and both pathogens were detected at all time points. The impact the treatment had on point prevalence varied by trypanosome species and between the two districts.

Several clusters of villages in Dokolo and Kaberamaido continued to report cases of HAT after the initial SOS intervention due in part to their proximity to livestock markets (Batchelor *et al.,* 2009). In 2008 re-treatment of these 'high risk' areas was undertaken. Monitoring was performed before and six months after treatment. Cattle blood samples were collected at 20 village sites from ten 'case-positive villages' (from which human sleeping sickness cases had been reported six months prior to June 2007) and from ten 'case-negative villages' (no reported human sleeping sickness cases six months prior to June 2007). These samples were screened for all of the aforementioned trypanosomes using species specific PCR protocols.

Chapter five details the results of this screening, and assessed whether re-treatment in Dokolo and Kaberamaido was effective in reducing the prevalence of trypanosomiasis. The re-treatment had a dramatic effect, significantly reducing the point prevalence of overall trypanosomiasis in the 20 villages screened from 38.1% (95% CI = 40.5 – 35.79%) at baseline to 26.9% (95% CI 28.96 – 24.97, $p < 0.0001$) at six months. Looking at each species separately, point prevalence of three out of four detected species of trypanosome fell significantly, including *T. b. rhodesiense*, which was reduced to 25% of its baseline prevalence.

Finally the two SOS treatment cycles were compared both statistically and spatially with emphasis on trends at village level and the occurrence of mixed infections.

Contents

List of Figures

List of Tables

List of Abbreviations

AAT – African animal trypanosomiasis

BCT – Buffy coat technique

CAR – Central African Republic

CATT - Card Agglutination Trypanosomiasis Test

CEVA - Ceva Santé Animale

CID – Centre for infectious diseases

COCTU – Coordinating office for control of trypanosomiasis in Uganda

CSF – Cerebrospinal fluid

DFID – Department for international development

DNA – Deoxyribonucleic acid

DRC – Democratic Republic of Congo

DVO – District veterinary officer

FITCA – Farming in tsetse control areas

FTA – Flinders technology associated

EG – Equatorial Guinea

G-B – Guinea – Bissau

H_o – Null hypothesis

HAT – Human African Trypanosomiasis

HCT – Heamatocrit centrifugation technique

IK – Industria Kaptial

LAMP – Loop - mediated isothermal amplification

mAEC – Miniature anion exchanger centrifugation

MH – Mantel - Haenszel

MINTRACS – Makerere In-training Community Services

NGO – Non-government organisation

OIE – World Organisation for Animal Health

PCR – Polymerase chain reaction

QBC – quantitative buffy coat

RAP – Restricted application of pesticide

S-L – Sierra Leone

SOS – Stamping out sleeping sickness

SRA – Serum resistance associated (*T. b. rhodesiense*)

TBSL – *T. brucei* s. l.

TC – *T. congolense* savannah

TV – *T. vivax*

UBOS – Ugandan Bureau of Statistics

UV – Ultraviolet

V_c – intercluster variance

WHO – World Health Organization

Acknowledgements

My great thanks go to my two supervisors, Dr Kim Picozzi and Professor Sue Welburn. Sue also deserves credit for first inspiring my love of parasitology and introducing me to the fascinating world of trypanosomiasis as an undergraduate. Throughout my postgraduate studies Kim gave much needed encouragement and support, read countless drafts of this document and exhibited patience and kindness which were much needed and gratefully received. I must also express my sincere appreciation and gratitude to everyone in the group, Kim and Sue included, for their contribution towards a thoroughly pleasant working environment.

Next I extend my thanks to Dr Jenna Fyfe, a valuable guardian of my sanity at times, and someone I could always rely on for an honest opinion. Dr Ewan Macleod was a great source of advice, and one of the fastest readers and walkers I have ever met. Dr Beatrix von Wissmann must receive a special mention for bestowing upon me some of her considerable statistical talents, which required no small amount of patience. Pauline McManus has been a great source of administrative support, moral support and good company. My thanks to Pauline.

I would like to thank Dr Richard Selby, who has made an excellent friend and mentor for the past four years. Thanks also to Dr Nicola Wardrop, who was brilliant at providing technical support for GIS, going out of her way to help me time and again. I have enjoyed the company of all my colleagues and fellow PhD students, and each has in their own way, helped me on this journey, so thank you Cristina Santiriso, Vincenzo Lorusso, Christine Amongi Acup, Ayo Majekodunmi, Sally Best, Phillip Auckland, Maire Ducrotoy and Dennis Muhanguzi. Anna Walker Okello gets a special mention for her many hilarious insights over the past four years. Hayley Mableson has proved herself an invaluable friend and confidant, and was always ready to assist in the lab when needed.

There are many wonderful people in Uganda without whom this work would not have been possible, my special thanks go to Professor Charles Waiswa and Professor John David Kabasa. The DVOs of Dokolo and Kaberamaido also deserve great thanks for ensuring the smooth running of field operations in their respective districts. Recognition must also go to the other members of my field team, Benard Agwai, Dr Bea von Wissmann, Dr Sally Best and Joesph Ssempijja, all of whom were great company and hard workers.

Lauren Sandford gets a huge thank you, for pulling me through the nightmares of writing, doing truly magical things with spreadsheets and generally being a constant source of support and encouragement. Jane Appleby is the most understanding and accommodating flatmate anyone could hope to live with, and I thank her for that and many other things besides. I would like to thank Bex Hamill, my sister and best friend, who stood by me unfailingly for the duration of my studies, as she always does. My parents have provided me with unconditional love and support, and from an early age encouraged my learning and my ambition. Without them this would not have been possible.

Chapter 1 – Introduction

African trypanosomiasis occurs in sub-Saharan Africa in countries that lie between the latitudes of 14°N and 29°S. It is caused by protozoan parasites from the genus *Trypanosoma*, and is transmitted by members of the genus *Glossina*, known as tsetse flies. African trypanosomiasis affects a number of mammalian species, including domestic livestock and humans, causing considerable morbidity, mortality, and economic loss. The members of the genus *Trypanosoma* are diverse and varied, as are the types of host they can infect. The main species causing African trypanosomiasis are shown in Figure 1.1.

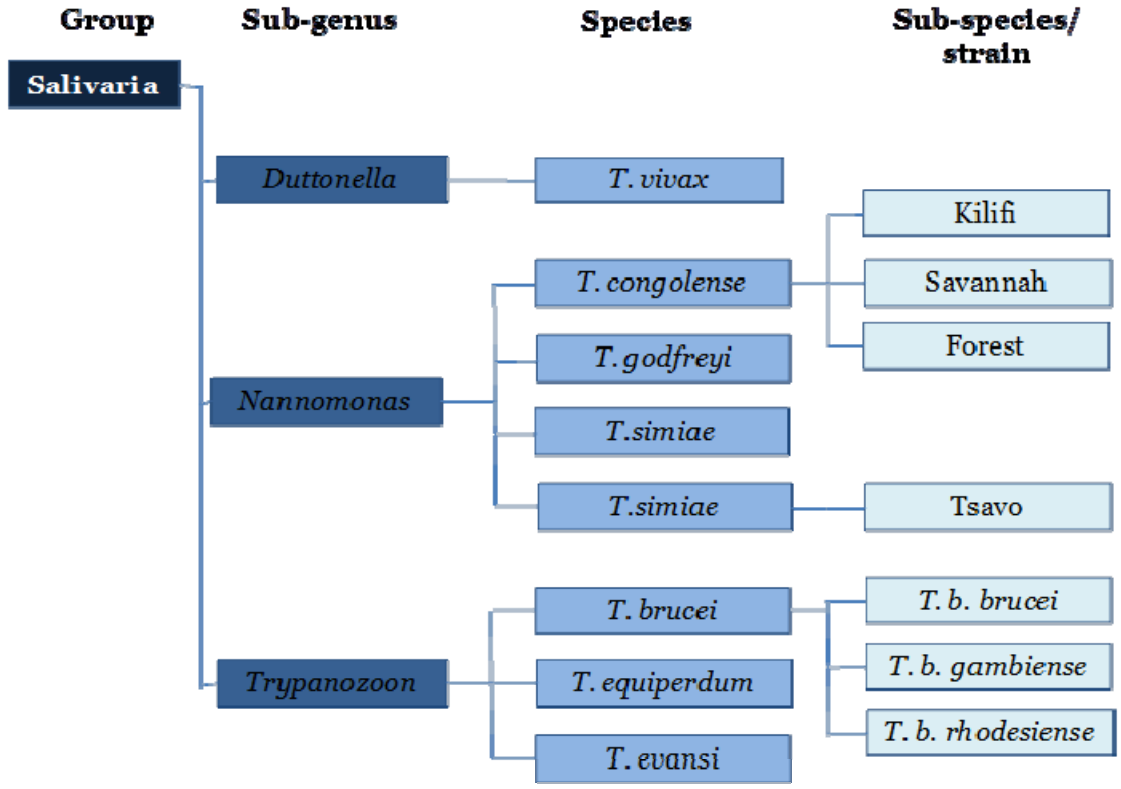

Figure 1.1 – classification of the Salivarian group of Trypanosoma (summarised from (Stevens and Brisse, 2004)).

These species and subspecies of trypanosome are infective to various different animal and human hosts. Of economic importance are those with the capability to cause disease in man and his livestock. In man, the causative agents of Human African Trypanosomiasis (HAT) are two subspecies of *Trypanosoma brucei*: *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. Of the many species of trypanosome capable of infecting animals most notable are those that cause "Nagana" or African Animal Trypanosomiasis (AAT): *Trypanosoma congolense* and

Trypanosoma vivax. The causative agents and epidemiology of AAT and HAT are discussed separately in sections 1.1 and 1.2.

1.1 African Animal Trypanosomiasis (AAT)

African Animal Trypanosomiasis (AAT) inflicts a serious burden upon animal health in sub-Saharan Africa, decreasing the health and productivity of millions of livestock per year and placing serious economic constraints on the communities that depend upon them for their livelihood. It is the most economically important livestock disease of Africa, especially of cattle (OIE, 2009). At least 48 million cattle are at risk of contracting AAT in the 10 million square kilometres of Africa infested with tsetse flies (Ilemobade, 2009), costing at least US$35 million per annum in treatment alone (Kristjanson et al., 1999). Annual production losses in cattle amount to US$1 – 1.2 billion per annum, as a result of direct losses such as mortality and poor growth, or indirectly, through reduced off-take of milk and meat, lower reproductive rates, and loss of traction and manure to general agricultural activities (Ilemobade, 2009). The combined treatment and production costs total an estimated US$1.55 billion every year. AAT renders large areas of sub-Saharan Africa unsuitable for farming as profitable cattle keeping is impossible, thus limiting agricultural output across the region (Murray and Trail, 1987). In affected rural areas AAT influences where people decide to live, how they manage their livestock, and the intensity and mix of crop agriculture (Ilemobade, 2009).

The main species recognised as causing AAT in cattle are *T. brucei* s. l., *T. congolense* and *T. vivax* (O'Gorman *et al.,* 2009). However it is important to note that while animal trypanosomiasis is regarded under one umbrella term — Nagana, or AAT — the infective parasites differ from each other in pathogenicity and reaction to drugs, so in actuality these umbrella terms represent a complex spectrum of diseases (Richardson, 1928). Two species, *T. congolense* and *T. vivax*, cause by far the greatest losses; *T. congolense* produces the more often virulent form of animal trypanosomiasis in East Africa, while in West Africa *T. vivax* is considered more pathogenic and prevalent (Losos and Ikede, 1972). Despite being more likely to invade other tissues, like the

central nervous system (CNS), *T. brucei* s. l. is generally less pathogenic than *T. vivax* or *T. congolense* (Taylor and Authie, 2004). Brief summaries of the main points in the development and transmission of each of the three species forming the AAT complex of diseases in cattle are outlined in the corresponding sections that follow.

1.1.1 *T. brucei* s. l.

The *T. brucei* s. l. group consists of three morphologically identical subspecies: *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense*. As two of the three subspecies are the causative agents of HAT, *T. brucei* s. l. is undoubtedly the most frequently studied of all trypanosome species. After inoculation into a tsetse fly via blood meal, it takes between 17 – 45 days for *T. brucei* s. l. to mature into a transmissible infection in the salivary glands of the fly (Hoare, 1970). As well as cattle and man, *T. brucei* s. l. is able to infect a wide variety of other domestic and wild game animals (Stevens and Brisse, 2004).

It was initially thought *T. brucei* s. l. was the causative agent of pathogenic AAT in cattle (Bruce, 1895, Bruce *et al.*, 1910c). However, many researchers have since proven otherwise, and it has been hypothesised the seemingly pathogenic nature of this species in bovids in early studies was due to its presence in mixed infections with either *T. congolense* or *T. vivax* (Killick-Kendrick, 1971, Losos and Ikede, 1972, Richardson, 1928). Experimental infection of cattle with *T. b. rhodesiense* indicated just under half developed CNS disease comparable to that found in man, 85 – 1613 days post infection (Wellde *et al.*, 1989). However it is still held *T. brucei* s. l. infection in cattle usually produces chronic low grade infections (Morrision *et al.*, 1981); such infections in cattle are frequently observed but clinical signs are seldom seen (Gray, 1970), suggesting cattle can tolerate *T. brucei* s. l. for many years without any appreciable ill effect. This has huge ramifications when considering the control of HAT; in the absence of treatment, cattle could harbour *T. b. rhodesiense* indefinitely without any outward signs of infection.

1.1.2 *T. congolense*

There are three distinct subtypes of *T. congolense*: savannah type *T. congolense*, riverine/forest type *T. congolense,* and Kenya coast type (Kilifi) *T. congolense* (Stevens and Brisse, 2004). A fourth group named *T. congolense* Tsavo was reclassified as a strain of *T. simiae* (Gibson *et al.,* 2001). The development of *T. congolense* in the tsetse fly takes between 19 – 53 days, and the transmissible form resides in the proboscis (Hoare, 1970).

A broad range of domestic and wild mammals can be infected by *T. congolense*, including cows, horses, sheep, goats, camels, pigs and dogs (Mulligan, 1970). Infections in cattle do not invariably cause disease; animals have been found to be infected and yet have no visible ill effects (Losos and Ikede, 1972). While this tolerance is possible, strains that cause disease are far more widely reported. Intermittent fever and lethargy, weakness, confusion, weight loss and progressive anaemia are all signs of a virulent *T. congolense* infection (Wellde *et al.,* 1974).

1.1.3 *T. vivax*

Trypanosoma vivax is infective to wild and domestic mammals in Africa, being pathogenic to domestic ungulates such as cattle, sheep, goats, horses and camels (Hoare, 1970). It is transmitted cyclically by the tsetse fly and mechanically by other species of biting fly (Stevens and Brisse, 2004). The development of *T. vivax* in the tsetse vector is limited to the proboscis, as first observed by Sir David Bruce (Bruce *et al.,* 1910a). This suggests *T. vivax* is better adapted to development within the tsetse fly, being able to produce a mature infection in as little as four days from the infective feed (Hoare, 1972). The fact that *T. vivax* is able to be transmitted mechanically under certain conditions has enabled it to spread from West Africa into South America. Cattle were imported along this route to support the European colonisation of South America, and there they encountered biting flies, which transmitted the parasite mechanically and allowed it to become established outside the tsetse belt (Jones and Davila, 2001).

Particular stocks of *T. vivax* found predominantly in East Africa can cause a haemorrhagic disease unlike that of typical *T. vivax* infections, characterised by high parasitaemia, severe anaemia, enlargement of the spleen and extensive haemorrhage into viscera and at external mucosal surfaces (Assoku and Gardiner, 1989). Haemorrhagic *T. vivax* was first reported in Kenyan cattle in 1944 (Hudson, 1944). Since that time, haemorrhagic *T. vivax* has been described in Central, Rift Valley and Coastal Provinces of Kenya (Gardiner *et al.,* 1989, Wellde *et al.,* 1983) and South Eastern Uganda (Magona *et al.,* 2008). The exact mechanism by which *T. vi*vax causes this haemorrhagic syndrome is unclear. Symptoms of non-haemorrhagic *T. vivax* in cattle include fever, loss of appetite, weight loss, anaemia and loss of condition, with greater severity of disease, higher mortality rates and a more rapid progression associated with West African strains (Stephen, 1970b).

1.1.4 Diagnosis, treatment and control of AAT

African animal trypanosomiasis is frequently cited as one of the most important constraints limiting agricultural and economic growth in sub-Saharan Africa (Eisler *et al.*, 2004, Machila *et al.*, 2003, O'Gorman *et al.*, 2009). To fully understand the debilitating impact of AAT on affected communities the diagnosis and distribution of AAT must be considered. The sensitivity of the diagnostic techniques routinely used in the field setting directly affects the accuracy of the reported distribution.

There are several serological methods developed to detect trypanosomiasis, however they are not routinely used for field diagnosis. With regards to AAT, results of serological test methods may be difficult to interpret since cattle can be infected simultaneously with several different species of trypanosome and the persistence of antibodies in treated recovered animals varies among individuals (Luckins and Mehlitz, 1978). A battery of molecular diagnostic techniques specific for the causative agents of AAT have been developed since the advent of PCR, but they remain useful only in the remit of epidemiological studies and scientific settings, as the costs and logistical problems associated with such techniques strictly limit their use to the laboratory.

Currently, routine diagnosis and treatment of bovine trypanosomiasis is almost exclusively reliant on the recognition of the clinical signs and symptoms in the animals themselves (Eisler *et al.,* 2004). Although a wide range of diagnostic tools have been developed to detect the various causative parasites, few are applicable to routine field use. While the clinical signs of bovine trypanosomiasis are widely regarded as non-specific, they will remain the mainstay of detection for the foreseeable future (Eisler *et al.,* 2004).

1.1.4.1 Field diagnosis of AAT

Examination of the blood by light microscopy is the most readily applied method for diagnosis of trypanosomiasis and, more importantly, is a technique which can be easily applied in the field (Luckins, 1992). Examination of wet blood films is the simplest and fastest method of diagnosis. It is also the least sensitive, and of limited value for routine diagnosis of apparently healthy animals; however, where an animal has clinical signs it can prove useful as a preliminary screening test, as in symptomatic animals the infection is being poorly controlled by the immune system and parasitaemia is likely to be high (Robson and Ashkar, 1972). Thick and thin blood smears, whereby the sample is dried (thick) or stained (thin) onto a slide for examination, are marginally more sensitive than wet blood film examination (Uilenberg, 1998). There are numerous concentration techniques described for trypanosome detection in blood, many of which have been subject to modification and amendment several times over. The most commonly used methods are described here.

In 1970, Woo described one of the first concentration methods readily adaptable to field use. His heamatocrit centrifugation technique (HCT) involved taking blood into an anticoagulant coated capillary tube (haematocrit tube) and centrifuging at high speed so that the blood separates into its constituent parts (Woo, 1970). Trypanosomes are concentrated at the buffy coat/plasma junction and can be observed by placing the tube directly under a microscope or by making a smear of this area (Uilenberg, 1998). When applied to diagnosis of bovine AAT in the field HCT was much more sensitive than thick, thin or wet blood films (Toro *et al.*, 1981). It was subsequently discovered expression of the buffy coat/plasma junction onto a slide and examination using dark

ground illumination (buffy coat technique, BCT) offered improved sensitivity over standard HCT, as well as being markedly more sensitive than wet blood films or thin smears (Murray *et al.*, 1977). A comparison between parasitological concentration techniques concluded BCT was the most sensitive, as well as having the added advantages of being able to provide an estimation of parasitaemia and anaemia (Paris *et al.*, 1982). Additional diagnostic sensitivity was added via modified capillary tubes containing plastic inserts which were originally designed for quantitative buffy coat analysis (QBC) (Levine *et al.*, 1989). This plastic cap reduces the length of capillary tube available for the blood sample to separate into, effectively making each fraction shorter and more concentrated, "squashing" the trypanosomes up against the sides of the capillary tube, making them clearly visible (Levine *et al.*, 1989).

The miniature anion exchanger centrifugation technique (mAEC) separates trypanosomes from blood cells by utilizing the differing ionic charge on the surface of these cells when suspended in a buffer solution of optimal pH and ionic strength (Lumsden *et al.*, 1977). The buffered blood sample is passed through the mAEC column containing DEAD cellulose beads which the red blood cells adhere to, therefore by centrifugation and microscopic examination of the filtrate any trypanosomes present can be readily observed (Lumsden *et al.*, 1979). The downside of this technique is it takes several hours to complete, and some authors have called into question its reported sensitivity as they found it missed a significant number of sub-patent infections in goats (Kalu *et al.*, 1986).

Sub inoculation of blood from suspect animals into susceptible laboratory ones can be useful in certain situations, and is one of the most sensitive techniques as diagnosis of AAT can be made where no other technique had detected a single trypanosome (Molyneux, 1975). However there are several drawbacks, apart from the question of cost and ethical considerations necessary when transporting test animals to and from the field; as well as the logistical problems this poses, it is also not a suitable method for routine diagnosis because suitable rodents have to be maintained and monitored over long periods (Uilenberg, 1998). Furthermore the trypanosomes are not immediately apparent in the test animals, so diagnosis is not confirmed for more than a week in most cases (Molyneux, 1975). On such a time scale, and given the fluctuating

parasitaemias associated with AAT, re-testing the suspected animal a week later with a less sensitive technique may prove more practical than rodent inoculation as parasitaemia may have risen to detectable levels over this time. While generally very sensitive for other trypanosome species, rodent inoculation is not sufficient for the detection of *T. vivax*, as laboratory mice are refractory to the majority of *T. vivax* strains (Leeflang *et al.*, 1976).

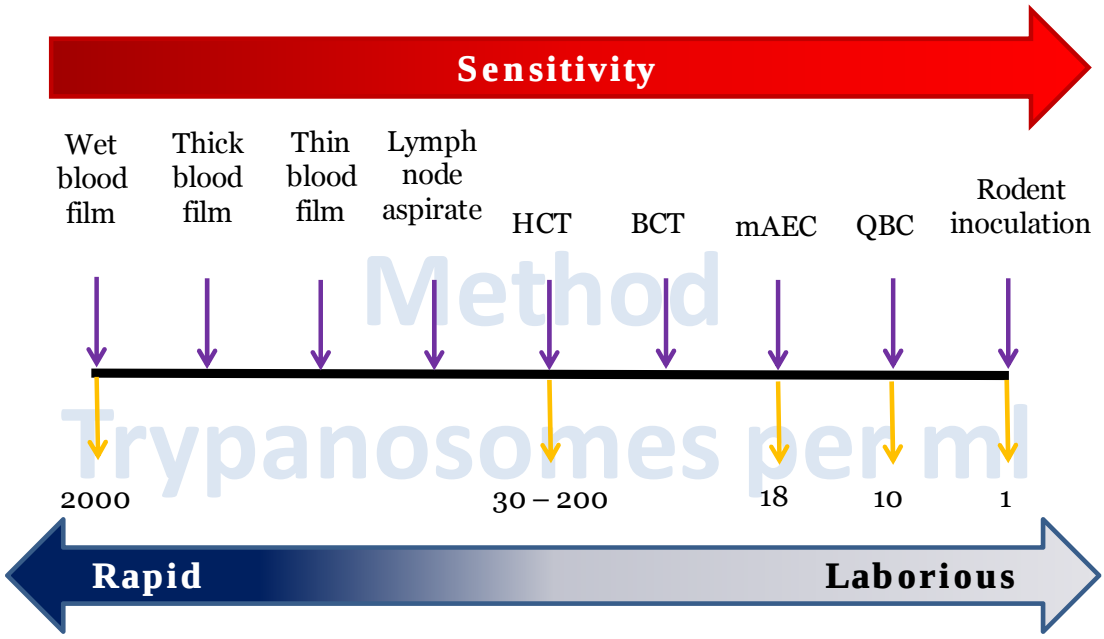

Figure 1.2 - relative sensitivities of commonly used parasitological diagnostic techniques which can be applied in the field setting. HCT = heamatocrit centrifugation technique, BCT = buffy coat technique, mAEC = miniature anion exchanger centrifugation technique, QBC = quantitative buffy coat technique. The lower limit of detection of trypanosomes per ml of blood is also shown for those techniques where an estimate was given.

The sensitivities of each diagnostic technique relative to the others are displayed in Figure 1.2. As is clearly shown, the more sensitive the diagnostic technique, the more laborious it is to execute. This highlights the lack of rapid, sensitive, field friendly diagnostics so urgently needed for the detection of AAT. Until such a technique is developed epidemiological information pertaining to AAT will continue to be gathered using techniques that underestimate the prevalence of AAT. Molecular diagnostic techniques, which have excellent sensitivity and specificity when compared to the methods above (between 1 and 0.001 trypanosomes per ml depending on the primer set

used), are not usually employed in the detection and control of AAT. Even in epidemiological surveys parasitological techniques tend to be favoured e.g. (Mamoudou *et al.*, 2006, Mihret and Mamo, 2007, Rowlands *et al.*, 2001, Van den Bossche *et al.*, 2000, Waiswa *et al.*, 2003).

1.1.4.2 Treatment and control of AAT

There are three drugs available for the treatment of AAT: Homidium bromide (Ethidium bromide), isometamidium chloride (Samorin) and diminazene (Berenil) (Stephen, 1970a). Of these three compounds, which have been in constant use for over 40 years, isometamidium provides prophylaxis for up to six months, homidium provides limited prophylaxis, and diminazene is therapeutically active only (Holmes *et al.*, 2004).

Current and historical methods for controlling bovine AAT include mass treatment of cattle with trypanocides, using livestock that are more resistant to disease, reducing the proximity of livestock to reservoir hosts, and controlling tsetse populations either by clearing bush to eliminate tsetse habitats, by spraying insecticides, or by trapping (Taylor, 1998). As the responsibility for control of AAT falls on the individual farmer in the largely decentralised veterinary systems present in sub-Saharan Africa today, bovine trypanosomiasis is controlled almost exclusively by trypanocides (Holmes *et al.*, 2004). Resistance to the available drug treatments has been reported in many different regions of Africa (Geerts *et al.*, 2001), raising concerns about long term treatment and control prospects for AAT.

1.1.5 Distribution and impact of AAT

AAT is widely reported to the World Health Organization's (WHO) World Organisation for Animal Health (OIE) from across sub-Saharan Africa, occurring almost everywhere that tsetse are found, as shown in Figure 1.3.

Figure 1.3 – distribution of AAT in Africa, January – June 2008 (WAHID-OIE, 2012).

A systematic review of all the available literature, conducted in 2007, found AAT to be one of the leading causes of cattle mortality on smallholder dairy farms in Eastern and Southern Africa, alongside tick borne diseases and diarrhoea (Phiri *et al.*, 2010). However, the precise nature of AAT differs in each country, as too does the amount of information available about each country. Many countries such as Burundi, Rwanda, and the Democratic Republic of Congo (D.R.C.) have recently experienced or are still experiencing civil war and violence, as a result veterinary services and disease-monitoring capabilities are severely compromised. With the possible exception of Mauritania all the countries in political West Africa are affected with tsetse-transmitted trypanosomiasis to some extent (Agyemang, 2005). In many of these countries, research and control programmes are geared towards chronic HAT.

Information on suitable habitat for each species of tsetse fly in each country was compiled most recently by Rodgers and Robinson, (2004), using satellite imagery to produce a series of detailed maps (Rogers and Robinson, 2004). The number of species

of tsetse fly in a given area gives some indication of the potential for AAT to impact upon agricultural activities. A summary of the number and species of tsetse that are thought to be found in each country in sub-Saharan Africa based on the presence of suitable habitat is given in Table 1.1.

Country	*G. brevipalis*	*G. fuscipleuris*	*G. fusca fusca*	*G. fusca congolensis*	*G. haningtoni*	*G. longipennis*	*G. medicorum*	*G. nashi*	*G. nigrofusca nigrofusca*	*G. nigrofusca hopkinsi*	*G. severini*	*G. schwetzi*	*G. tabaniformis*	*G. vanhoofi*	*G. caliginea*	*G. fuscipes fuscipes*	*G. fuscipes matinii*	*G. fuscipes quanzensis*	*G. palpalis palpalis*	*G. palpalis gambiensis*	*G. pallicera pallicera*	*G. pallicera newsteadi*	*G. tachinoides*	*G. austeni*	*G. longipalpis*	*G. morsitans morsitans*	*G. morsitans submorsitans*	*G. morsitans centralis*	*G. palidipes*	*G. swynnertoni*	Totals
Angola	X				X						X		X				X	X	X			X						X			9
Benin			X	X			X												X		X		X		X						7
Botswana	X																									X		X			3
Burkina							X												X	X			X								4
Burundi	X																X											X	X		4
Cameroon		X	X	X	X			X	X				X		X	X			X	X	X	X	X		X						15
C.A.R.		X		X	X			X	X				X			X			X	X		X	X		X		X				13
Chad		X																					X				X				3
D.R.C.	X	X		X	X			X	X	X	X	X	X	X		X	X	X				X			X		X	X	X		19
Congo				X	X			X				X	X			X		X	X			X						X			10
Cote d'Ivoire			X				X		X				X						X	X	X		X				X				9
Djibouti																															0
E.G.					X							X	X		X	X			X		X	X									8
Eritrea																															0
Ethiopia	X					X										X							X				X		X		6
Gabon				X	X			X				X	X		X			X			X	X									9
Gambia																				X							X				2
Ghana							X		X				X						X		X		X		X		X				8
Guinea			X						X				X						X		X				X						6
G-B			X																X						X						3
Kenya	X					X										X								X			X	X	X	7
Lesotho																															0
Liberia							X		X				X						X	X					X		X				7
Malawi																										X					1
Mali																											X				1
Mauritania																															0
Mozambique	X																							X		X			X		4
Namibia																															0
Niger																															0
Nigeria			X	X			X	X					X		X					X	X		X		X		X				11
Rwanda	X	X		X		X											X											X	X	X	8
Senegal																			X	X					X		X				4
S-L			X																X	X		X	X		X		X				7
Somalia	X																							X							2
South	X																							X					X		3
South						X										X											X				3
Sudan																							X				X				2
Swaziland	X																												X		2
Tanzania	X					X										X	X							X		X		X	X	X	9
Togo			X				X												X				X		X		X				6
Uganda	X	X		X		X					X					X											X	X	X		9
Zambia	X																X									X		X	X		5
Zimbabwe																										X		X	X		3

Table 1.1 - suitable habitat for each species/subspecies of tsetse fly in the countries of sub-Saharan Africa. Abbreviations: C.A.R. = Central African Republic, D.R.C. = Democratic Republic of Congo, E.G. = Equatorial Guinea, G-B = Guinea –Bissau, S-L = Sierra Leone. Information summarised from (Rodgers and Robinson, 2004).

As shown in Table 1.1, there is an abundance of suitable tsetse habitat in sub-Saharan Africa. Given the wide range of possible tsetse infestation and the comparatively miniscule amount of surveillance for AAT that is carried out, actively or passively, it is likely the impact of AAT on sub-Saharan Africa is severely underestimated. Of the epidemiological surveys that have been performed many of them employed only parasitological diagnostic techniques, compounding the problem of under-reporting for AAT.

1.1.5.1 AAT in East Africa

Of the countries that share a border with Uganda, South Sudan, the D.R.C., and Rwanda suffer from a severe lack of data regarding AAT. These countries are potential hosts to numerous species of tsetse (Table 1.1) and all are known to contain foci of HAT, indicating favourable conditions for the transmission of trypanosomes in general. It is likely AAT is also present in these countries; however, more information is not readily available at this time.

The costal countries of Kenya and Tanzania have comparatively peaceful histories and for decades, the economic and social situation has been one conductive to the investigation of AAT.

1.1.5.1.1 AAT in Kenya

Approximately 25% of Kenya is tsetse infested, including more than half of the rangelands with pasture suitable for raising cattle (Chemuliti *et al.*, 2005), placing major constraints on livestock production. A report in 1969 detailed the transmission of cattle trypanosomiasis on the shores of Lake Victoria in the far south of the country, but does not specify which species of trypanosome were involved (Bertram, 1969). In the Lambwe Valley in 1986 AAT was documented as causing livestock losses on a scale significant enough to seriously affect the local economy (Turner, 1986). At that time Lambwe Valley was shared between wild animals in a national park and human settlements with domestic livestock (Turner, 1986).

T. congolense was isolated from cattle and tsetse flies in the Nguruman area and two particular isolates were found to be resistant to isometamidium (Gray *et al.*, 1993), which could seriously hinder the treatment and management of this trypanosome in years to come. In Busia it should be noted that monitor lizards act as reservoirs for trypanosomiasis (Njagu *et al.*, 1999), although as *T. brucei* s. l. was the only species detected, monitor lizards likely play a more important epidemiological role as potential reservoirs for HAT than for AAT. In Nguruman an investigation into the trypanosome infection rate in cattle found 16.5% of animals were infected with *T. congolense*, 4.95% with *T. vivax* and 0.19% with *T. brucei* s. l. (Tarimo-Nesbitt *et al.*, 1999). In contrast to the majority of other areas studied, work in the coastal province of Kenya revealed AAT was not a major constraint to cattle production, with trypanosome prevalence less than 1% (Maloo *et al.*, 2001).

Sampling from tsetse at several different locations in southern Kenya revealed pathogenic trypanosome species present in flies at all sample sites, with *T. congolens*e being most common (Njiru *et al.*, 2004a). In Kwale district a study on the main causes of morbidity and mortality in calves found trypanosomiasis occurred at the highest incidence of all vector borne diseases, making it an important factor limiting cattle productivity on the smallholder farms studied (Muraguri *et al.*, 2005). A later study also conducted in Kwale district detected AAT at a prevalence of 18% in cattle, with *T. congolense* being the predominant species (Ohaga *et al.*, 2007). In the Nkineji and Nhuruman areas of Kenya, *T. vivax* and *T. congolense* were detected in tsetse and local cattle herds (Bett *et al.*, 2008, Bett *et al.*, 2010). Cattle in the Suba and Teso districts had trypanosome prevalence of 41% and 29% respectively, with *T. vivax* accounting for the majority of infections (Thumbi *et al.*, 2010). Busia district was visited in another study; this time AAT was detected in 11.9% of animals, with *T. brucei* s. l. the most common species (von Wissmann *et al.*, 2011).

1.1.5.1.1.1 Investigations into the epidemiology and control of AAT at Galana Ranch

Galana Ranch has long been used to study cattle trypanosomiasis, and many detailed experiments and field trials have been carried out investigating trypanosome prevalence, incidence, and predisposing risk factors. Evidence from one study suggests

circulating strains of *T. vivax* are less susceptible to prophylactic trypanocides than expected, with the period of protection lasting for a maximum of four weeks with isometamidium as opposed to the two to three months the manufacturer and previous studies indicate (Dolan *et al.*, 1992). A review which brought together much of the data collected on the ranch over a fifteen-year period concluded the incidence of *T. congolense* did not change significantly over the course of the studies but the incidence of *T. vivax* did, increasing markedly in the years when tsetse challenge was shown to be the highest (Dolan, 1998). According to one study, *T. congolense* and *T. vivax* are the only species responsible for AAT on the ranch (Bett *et al.*, 2004), although species determination was by microscopy alone which is much less accurate and sensitive than the molecular techniques used in other studies.

For decades, AAT has been documented as a significant constraint to agricultural development in Kenya. As is the case with HAT, AAT is not uniformly an issue across the entire country; rather, in certain areas it has a significant impact while in others it poses little threat to cattle production. In the areas where AAT is problematic the dominant species of trypanosome changes; some areas are most heavily afflicted with *T. vivax*, others with *T. congolense*. Few areas see *T. brucei* s. l. dominate. The heterogeneity of the AAT epidemiological situation in Kenya highlights the importance of the complex and in some cases poorly understood interaction between tsetse, environment, ecosystem, and wild and domestic animals.

1.1.5.1.2 AAT in Tanzania

Approximately 60% of Tanzania is infested with tsetse flies (Moloo, 1985). On beef farms in Morogoro 12.3% of animals were infected with trypanosomes, with 80% of those infections due to *T. congolense* and 20% due to *T. vivax* (Mugittu *et al.*, 2001). A cattle ranch in North-Eastern Tanzania reported considerable success in controlling bovine trypanosomiasis using deltamethrin dipping: the tsetse population decreased by 90% and disease mortality decreased by 66% (Fox *et al.*, 1993). Trypanosomiasis was eliminated from Zanzibar with the eradication of the tsetse vector using the sterile insect technique (Vreysen *et al.*, 2000), while the prevalence of AAT on nearby Mafia Island was found to be too low to merit a similar intervention (Goossens *et al.*, 2006). *Trypanosoma vivax*, *T. brucei* and *T. congolense* savannah were detected in cattle in

Northern Tanzania, in areas adjacent to Serengeti National Park where these trypanosome species were also detected in wild animals (Kaare *et al.*, 2007). In the cattle samples *T. brucei* s. l. was most commonly detected, while *T. congolense* was most commonly detected in the wildlife samples (Kaare *et al.*, 2007). A study on trypanosomiasis in horses in the north west of the country detected *T. b. brucei* and *T. congolense* savannah in an area adjacent to a game reserve heavily infested with tsetse flies (Auty *et al.*, 2008).

Considerable work has been carried out in Tanzania identifying trypanosome infections in wild caught tsetse flies. Although not a direct measure of AAT prevalence in livestock, the data provided in these studies gives an indication of the circulating strains livestock and wild animals may be challenged with in that area, and also indicates potential areas to avoid cattle grazing if tsetse numbers and infections rates are high. On the Ruvu flood plain in Eastern Tanzania tsetse were trapped and examined for trypanosomes: 4.9% of flies were positive for *T. congolense*, 3.3% were positive for *T. vivax* and 0.1% were positive for *T. brucei* s. l. as determined by microscopy (Msangi *et al.*, 1998). Generic trypanosomal PCR primers targeting ribosomal DNA (rDNA) have been used to identify trypanosomes in tsetse midgut samples taken from Msubugwe Forest Reserve and Serengeti National Park (Adams *et al.*, 2006). *T. congolense* savannah, *T. congolense* Kilifi, *T. brucei* s. l., *T. simiae*, *T. simiae* Tsavo, and *T. godfreyi* were all detected (Adams *et al.*, 2006). Sequencing and phylogenetic analysis recently revealed new strains of *T. vivax*, identified from tsetse fly proboscises from two distinct regions of Tanzania (Msubugwe Forest Reserve and Tarangire National Park) (Adams *et al.*, 2010). As both these sampling locations were areas with high concentrations of wild animals, and bloodmeal analysis was not conducted, the host range of these new strains is unknown as is their potential infectivity and pathogenicity to domestic livestock.

The majority of published papers investigating AAT in Tanzania are conducted in areas where wildlife and domestic animals co-exist, indicating the most badly affected areas are those where cattle keeping overlaps into habitats where wild animals are present. This mirrors the situation in Zambia, where AAT is said to be a interface disease, exacting a high toll on cattle health where they are kept in close proximity to wild

animals, and having a more endemic character where cattle constitute the main food source for tsetse (Van den Bossche, 2001).

1.1.5.1.3 AAT in Uganda

Approximately 70% of Uganda is infested with tsetse (Kauta *et al.*, 2010). *T. vivax* and *T. congolense* were found infecting cattle and causing considerable losses in the fifties and early sixties in Mbarara in Western Uganda, limiting the area available for grazing (Ford and Clifford, 1968). During the late sixties cattle trypanosomiasis was considered to be an enormous economic hindrance, again with reports that many areas of suitable grazing were not utilised due to extensive tsetse infestation (Wooff, 1969). On the northern shores of Lake Victoria a survey of porcine trypanosomiasis was conducted, and *T. brucei* s. l. was the only species detected (Okuna *et al.*, 1986). A survey of trypanosome infection rates in pigs, goats, and sheep in Mukono found 16.8% of animals were infected, with *T. congolense* and *T. brucei* s. l. detected in almost even amounts overall, and *T. vivax* being detected less frequently (Katunguka-Rwakishaya, 1996). The trypanosomes were detected and species determined via examination of buffy coat, and thick and thin smears (Katunguka-Rwakishaya, 1996). BCT and HCT were used to survey cattle in Mbale, Tororo, Busia and Bugiri districts for trypanosomiasis; *T. congolense* was most commonly detected, followed by *T. vivax* and *T. brucei* (Magona *et al.*, 2005). As part of a major control programme in East Africa (Farming in Tsetse Controlled Areas, FITCA) baseline samples were taken from cattle in four districts of Uganda: Kamuli, Iganga, Soroti, and Tororo, and analysed using species specific PCR protocols (Fyfe, 2007). The precise prevalence varied depending upon district, however across all districts *T. brucei* s. l. was the most commonly detected species, followed by *T. vivax*, and *T. congolense* savannah was the least commonly detected species, being totally absent from the district of Kamuli (Fyfe, 2007). In Tororo district in the south east of the country the prevalence of *T. congolense* and *T. vivax* in cattle were 3% and 13% respectively (Magona *et al.*, 2008). This area also suffers from sporadic outbreaks of haemorrhagic *T. vivax*, with the last reported outbreak having a fatality rate of 35% (Magona *et al.*, 2008). In Kasese and Jinja districts pigs were found to be infected with *T. brucei*, *T. vivax* and *T. congolense*, in villages where cattle and goats were also raised (Biryomumaisho *et al.*, 2009).

In the HAT endemic districts of Kamuli, Mukono and Tororo a testse survey was conducted, 1 – 1.82% of flies were infected by microscopy, PCR analysis of the most recent blood meal taken by each fly found 14% of feeds contained *T. brucei* s. l. (Waiswa *et al.*, 2006). On Buvuma Island in Lake Victoria *T. vivax* was the most common species of trypanosome detected in tsetse, with in infection rate of 4.32% compared with 1.15% for *T. congolense* (Ogwal *et al.*, 2007).

AAT in Uganda undoubtedly hampers agricultural activities, as well as livestock health and productivity (Magona *et al.*, 2005). As outlined in section 1.2, cattle movements in Uganda have allowed HAT to expand outwith its traditional boundaries into new, previously unaffected districts. There is also a danger AAT trypanosomes could be similarly exported to new areas, amplifying the economic constraints this disease currently places on livestock production in Uganda.

1.2 Human African Trypanosomiasis

Human African Trypanosomiasis (HAT), commonly known as sleeping sickness, is caused by two trypanosome subspecies, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*, transmitted to human hosts via an insect vector, the tsetse fly (Bruce, 1895). Dr David Bruce is credited with making the link between trypanosomes and sleeping sickness in 1895, but he himself admitted the disease had been known in the West Coast of Africa for "as long as any history of these parts had been documented" (Bruce, 1908). He was also the first to the recognise the importance of cattle as a reservoir for *T. b. rhodesiense*, at first by realising the tsetse fly remained infected with trypanosomes even after the human population had been evacuated from the tsetse infested shores of Lake Victoria (Bruce *et al.,* 1909), and then by proving cattle were able to harbour and transmit human infective trypanosomes between cattle and other susceptible hosts (Bruce *et al.,* 1910b).

Trypanosoma brucei gambiense and *T. b. rhodesiense* are morphologically identical but spatially separate, divided roughly along the line of the Great Rift Valley, with *T. b. gambiense* to the north and west and *T. b. rhodesiense* to the south and east (Rogers *et al.,* 2000). The two parasites are not uniformly distributed within these areas; they occur in discrete disease foci (Leak, 1999). Sleeping sickness caused by *T. b. rhodesiense* manifests as a predominantly acute disease, whereas *T. b. gambiense* causes a chronic form of sleeping sickness, with symptoms and disease pathology taking much longer to become apparent (Apted, 1970). Both forms of sleeping sickness exhibit distinct early and late stages of disease pathogenesis. Early stage symptoms including chancre, lymph node swelling (particularly for *T. b. gambiense*), fever, facial oedema, anaemia, and weight loss, and correspond to parasite multiplication within the haemo-lymphatic system (Pentreath and Kennedy, 2004). Symptoms of the late encephalitic stage correspond to parasites crossing the blood brain barrier, and as most regions of the central nervous system (CNS) are susceptible to malfunction, a wide range of symptoms may present including: anxiety, tremors, visual disturbance, disturbance of the normal pattern of sleep, seizures, coma, and ultimately death in the absence of treatment (Kennedy, 2006). HAT causes considerable morbidity and mortality, with an estimated 1.6 million disability-adjusted life years lost per year (WHO, 2000). It is estimated 30,000 people are infected with HAT (WHO, 2010). However, these figures are only estimates and may under represent the problem, due to under-reporting and a lack of surveillance in endemic areas (Welburn and Odiit, 2002). Historically *T. b. gambiense* has been the cause of the majority of HAT cases; between 2000 and 2009 it accounted for more than 95% of all recorded HAT cases (Simarro *et al.,* 2011).

1.2.1 Diagnosis, treatment and control of HAT

The presence of one or more of the symptoms of HAT may be considered as indirect evidence of trypanosome infection; however, due to the nonspecific and variable nature of these symptoms, suspects should be confirmed by parasite detection (Buscher and Lejon, 2004). Diagnosis of HAT is usually by means of parasitological examination of blood, lymph or cerebrospinal fluid (Wastling and Welburn, 2011). As the two subspecies of *T. brucei* s. l. that cause HAT are morphologically identical, this method is unable to differentiate between the two. With *T. b. rhodesiense,* parasitaemias are

usually high, and so comparatively easy to detect via microscopy; for *T. b gambiense*, where parasitaemias are generally lower, the Card Agglutination Trypanosomiasis Test, CATT, can be used to detect suspected infections (Magnus *et al.,* 1978). Once infection with either subspecies has been confirmed, stage determination based on examination of CSF is necessary to determine the appropriate course of treatment (Kennedy, 2008).

There are currently four drugs licensed for the treatment of HAT: for treatment of stage one haemo-lymphatic HAT there is pentamidine and suramin, and for the treatment of stage two CNS HAT there is melarsoprol and eflornithine, although *T. b. rhodesiense* is unfortunately not susceptible to the latter compound (Burri *et al.,* 2004). These drugs are regarded by many as unsatisfactory for various reasons, including unacceptable toxicity (melarsoprol), poor efficacy, undesirable route of administration, and drug resistance (Fairlamb, 2003). Furthermore, all drugs require hospital admission for administration, increasing the disease burden and cost to the affected individual.

When it comes to control of HAT the picture is complicated somewhat by the presence of human infective trypanosomes in animal hosts. Man is the only important reservoir of infection for *T. b. gambiense* (Scott, 1970), as the course of disease from infection to death can last as long as 10 years, with the individual being infectious to the tsetse fly for much of this time. Although infection in other species such as pigs (Penchenier *et al.,* 2005) and wild animals (Njiokou *et al.,* 2006) does occur, they are not thought to play a significant role in the spread of disease, and domestic pigs are able to spontaneously clear their infection within a year (Penchenier *et al.,* 2005). Furthermore, given that *T. b. gambiense* control has been effective in the past simply by case finding and treatment, the animal reservoir of *T. b. gambiense* does not appear important to the maintenance and transmission of the disease among humans (Welburn *et al.,* 2004).

For control of *T. b. gambiense* mathematical modeling identified active case detection as the most effective means of control (Welburn *et al.*, 2001a). The delayed onset of symptoms in Gambian HAT contraindicates passive control strategies or those that are not human-centric, as they allow ‘silent epidemics’ to build where the disease is not

detected until a large number of people are already infected. Moreover, as the clinical progression of *T. b. gambiense* HAT is little studied and poorly understood, the possibility remains there may well be some people who become infected with this parasite and yet never develop disease, or those who take much longer than expected to develop clinical disease (Checchi *et al.,* 2008). This ambiguity hampers diagnosis and the interpretation of diagnostic techniques; a recent study found CATT and PCR positive individuals who were apparently healthy in northern Uganda (Wastling *et al.*, 2010b). As positivity with CATT did not always equate to positivity by PCR and vice versa, and initially positive individuals were negative at follow up testing, the clinical significance of these results is unclear (Wastling *et al.*, 2010b). However they serve to highlight the lack of knowledge surrounding this important area (Wastling *et al.*, 2010b). Overall, while control of *T. b. gambiense* is simpler in comparison to that of *T. b. rhodesiense*, it is by no means clear-cut.

For *T. b. rhodesiense* the picture is very different as there are many species of animal capable of harbouring infection. It has been detected by a number of investigators in animals such as cattle, sheep, goats, pigs, dogs, bushbuck, reedbuck, hyena, warthogs, and lions: (Bruce, 1915, Corson, 1936, Heisch *et al.*, 1958, Kaare *et al.*, 2007, Moloo *et al.*, 1973, Njiru *et al.*, 2004b, Onyango *et al.*, 1966, Waiswa *et al.*, 2003) to cite but a small selection of the literature on this topic. The contribution of an animal reservoir to the epidemiology of HAT depends upon local climatic and ecological variables, which differ extensively on a small scale. The importance of cattle as a reservoir for *T. b. rhodesiense* in Uganda will be extensively reviewed in section 1.3.2.

For *T. b. rhodesiense* control, early colonial efforts focused on the destruction of wildlife and habitat and the evacuation of populations from affected areas. During the mid twentieth century aerial insecticide spraying against tsetse became the most popular choice, whereas modern day activities tend to be focused instead on control of the tsetse vector or elimination of the parasite from the local reservoir species (Welburn *et al.,* 2009). When it comes to tsetse control there are a number of methods available: bi-conical traps (Challier and Laveissière, 1973), screens or localised insecticidal spraying of the environment (Vale, 1987), and the utilization of cattle as live bait, using insecticidal footbaths (Bouyer *et al.,* 2007), pour-ons or sprays, particularly

in a restricted fashion targeting only the preferred feeding sites of the fly (Torr *et al.*, 2007). The sterile insect technique has not been widely used, mainly due to its prohibitively expensive nature as well as the problematic issues of reinvasion of cleared areas, international co-operation, and targeting multiple species of fly (Torr *et al.*, 2005). Despite the large number of control options available, particularly for the tsetse fly, there are concerns they are being applied on too small a scale and in an uncoordinated fashion, rendering them ineffectual (Torr *et al.*, 2005).

1.3 Uganda

Uganda is situated in East Africa, bordering Kenya to the East, Tanzania and Rwanda to the South, the Democratic Republic of Congo to the West, and South Sudan to the north, and including a large portion of Lake Victoria within its territory (NG, 2010). Uganda has a total surface area of 241,440 square kilometres, of which 41,743 square kilometres consist of swamps, lakes and waterways; the altitude above sea level ranges from 620 metres (Albert Nile) to 5,111 metres (Mt. Rwenzori peak) (UBOS, 2010). Uganda has an estimated population of 31.8 million (UBOS, 2010) and is split into 111 districts, as depicted in Figure 1.4.

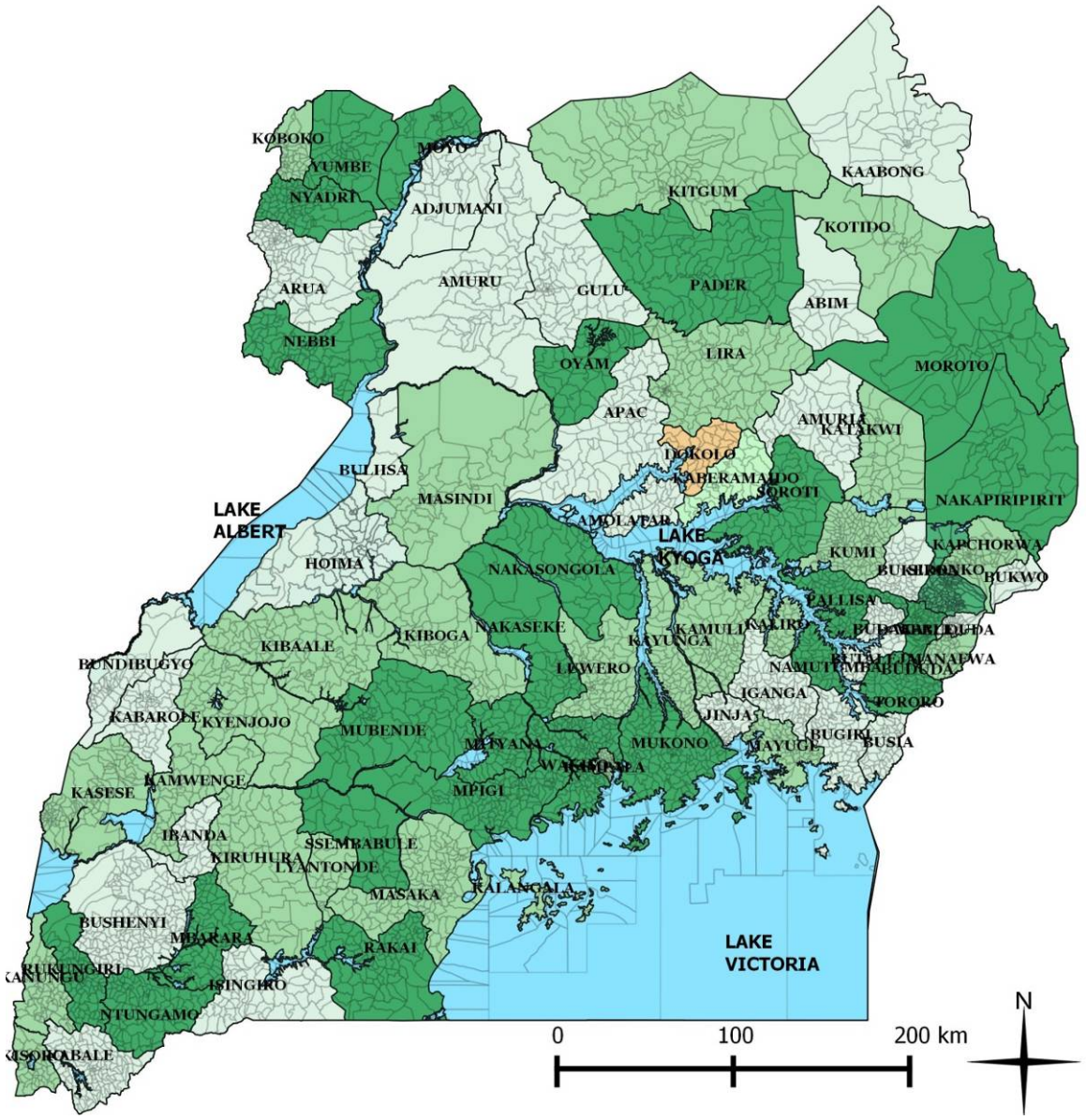

Figure 1.4 - map of Uganda delineating district boundaries as they stood in 2007.

1.3.1 The importance of cattle in rural Uganda

In Uganda cattle are an important livestock species, making a larger contribution to the country's economy than any other single livestock species (Behnke and Nakirya, 2012). It is estimated mixed farming smallholders and pastoralists own over 90% of cattle in the country (Mwebaze, 2006). Particularly in districts on the northern shores of Lake Kyoga, cattle play an essential role in everyday life. They provide a fifth of the total household income; as well as providing milk for family consumption and sale, and

traction for ploughing, the income generated from the sale of excess animals and animal products pays for school fees and essential healthcare (Butcher, 2009). In these areas cattle are also of cultural importance as they form a major part of many wedding dowries, and keeping cattle for the purpose of being able to marry well was frequently ranked in the top three reasons for cattle ownership (Butcher, 2009).

1.3.2 HAT in Uganda

Due to the fact Uganda straddles the Great Rift Valley, it has the unique misfortune of harbouring endemic foci of both chronic and acute human African trypanosomiasis within its borders; *T. b. gambiense* is present in the north and west of the country, and *T. b rhodesiense* is found in the south east (MacKichan, 1944). In the past, accurate differentiation between subspecies has not been necessary, as the two were separated by hundreds of kilometres and so clinical diagnosis was based solely upon patient location. However, the modern day expansion of *T. b. rhodesiense*, discussed in detail in the following paragraphs, brings these two parasites perilously close to one another.

The earliest recorded cases of sleeping sickness in Eastern Uganda can be traced back to 1901 when reports of a mysterious disease, similar to the description of sleeping sickness in West Africa, started to emerge (Low, 1929). This heralded the start of a massive epidemic in the Busoga region of Uganda. At first, this epidemic was attributed to *T. b. gambiense*. According to MacKichan (1944), *T. b. rhodesiense* originated in Zambia and spread northwards to arrive in South East Uganda in the forties, and was not present in Busoga at this time. Ormerod (1961) also supported this theory, chronologically tracing the epidemic spread of *T. b. rhodesiense* from its origin in Zambia, progressing northwards through time to Zimbabwe, Malawi, Tanzania, Uganda, and Kenya (Ormerod, 1961). However, this has been challenged in recent years for a number of reasons.

Firstly, comparative analysis of hospital archives shows the clinical progression of sleeping sickness during the 1901 epidemic to be consistent with the classical acute pathogenesis of *T. b. rhodesiense* as opposed to the chronic progression of *T. b.*

gambiense (Fèvre *et al.,* 2004). Secondly, molecular analysis revealed *T. b. rhodesiense* retains a stable genomic composition over time within a given focus (Gashumba *et al.,* 1994, Hide *et al.,* 1998). Thirdly, strains isolated from Uganda and Zambia are phylogenetically distinct from each other (Tilley *et al.,* 2003), an unexpected finding if Ugandan strains of *T. b. rhodesiense* originated in Zambia. Fourth, it has been postulated the ease with which investigators of the epidemic were able to achieve experimental cyclical transmission of the causative agent within tsetse flies indicate they were in fact dealing with *T. b. rhodesiense*, as *T. b. gambiense* infection in tsetse is much more difficult to reproduce (Koerner *et al.,* 1995). Fifth, the experimental cyclical transmission those early researchers reported was between cattle, tsetse flies and monkeys, and upon discovering transmission was possible they screened cattle living in tsetse infested areas of the lake shore and found some harbouring *T. b. gambiense* (Bruce *et al.,* 1910b). Since the development of molecular diagnostics, cattle are not routinely found naturally harbouring *T. b. gambiense* in areas endemic for Gambian sleeping sickness, but have frequently been reported harbouring *T. b. rhodesiense* in Uganda, including Busoga. Finally, in 1901 *T. b. rhodesiense* had not yet been discovered, making it likely *T. b. gambiense* was identified in Busoga as it was the only species linked with human infection at that time (Leak, 1999).

On top of all this, it must be highlighted ancient foci of HAT are not known to simply disappear without trace, therefore *T. b. gambiense* should still be present in Busoga today. It is not, but the isoenzyme profiles of endemic *T. b. rhodesiense* isolates in Busoga include isolates with rapid pathogenesis and those with a slower disease progression (Smith and Bailey, 1997). The clinical spectrum of infection associated with the isolates circulating in Busoga in 1997 could account for all of the previous epidemics (Smith and Bailey, 1997). Without any parasitic materials from the 1901 epidemic to scrutinise using molecular techniques, this debate will never be unequivocally resolved. Nevertheless, there is a large body of circumstantial evidence indicating *T. b. rhodesiense* was the likely causative agent of epidemic sleeping sickness in Busoga at the turn of the last century.

A further epidemic of sleeping sickness was reported in Busoga in 1940, and this time there was no doubt it was caused by *T. b. rhodesiense* (MacKichan, 1944). The

epidemic, which began in 1976, heralded the northerly spread of *T. b. rhodesiense* out-with its previous boundaries in Busoga. The disease was recorded for the first time in Tororo in 1984, and similar strains were found to be circulating in both human and bovine isolates (Enyaru *et al.,* 1992). Some of these strains were identical to strains isolated previously from Busoga, confirming *T. b. rhodesiense* had spread from there (Enyaru *et al.,* 1993). Hide *et al.,* (1994) went on to show 23% of cattle harboured human infective parasites in Tororo (Hide *et al.,* 1994). Furthermore, taking into account transmission parameters, it was calculated the cattle-fly-human transmission cycle was five times more probable than the human-fly human transmission cycle in this locality (Hide *et al.,* 1996), highlighting the importance of the cattle reservoir in this disease outbreak.

In December 1998 *T. b. rhodesiense* was again detected north of its previous limit, when a case was reported for the first time in Soroti district; in the next six months 119 cases were recorded in this previously disease free area (Fèvre *et al.*, 2001). Due in part to the incomplete implementation of early control measures, and despite the identification of cattle as the source of the outbreak (Welburn *et al.,* 2001b), it spread again into the neighbouring districts of Kaberamaido, Lira and Apac during the course of 2004 and 2005 (Fèvre *et al.,* 2005). At this point major concerns were raised regarding the possibility of an overlap of the two forms of sleeping sickness in Uganda, which would complicate and debilitate a diagnostic process which has no way to differentiate between *T. b. rhodesiense* and *T. b. gambiense* (Picozzi *et al.,* 2005). The authors were able to spatially track the spread of *T. b. rhodesiense* and estimate the distance separating the two forms of HAT as less than 150km, as seen in Figure 1.5 (Picozzi *et al.,* 2005).

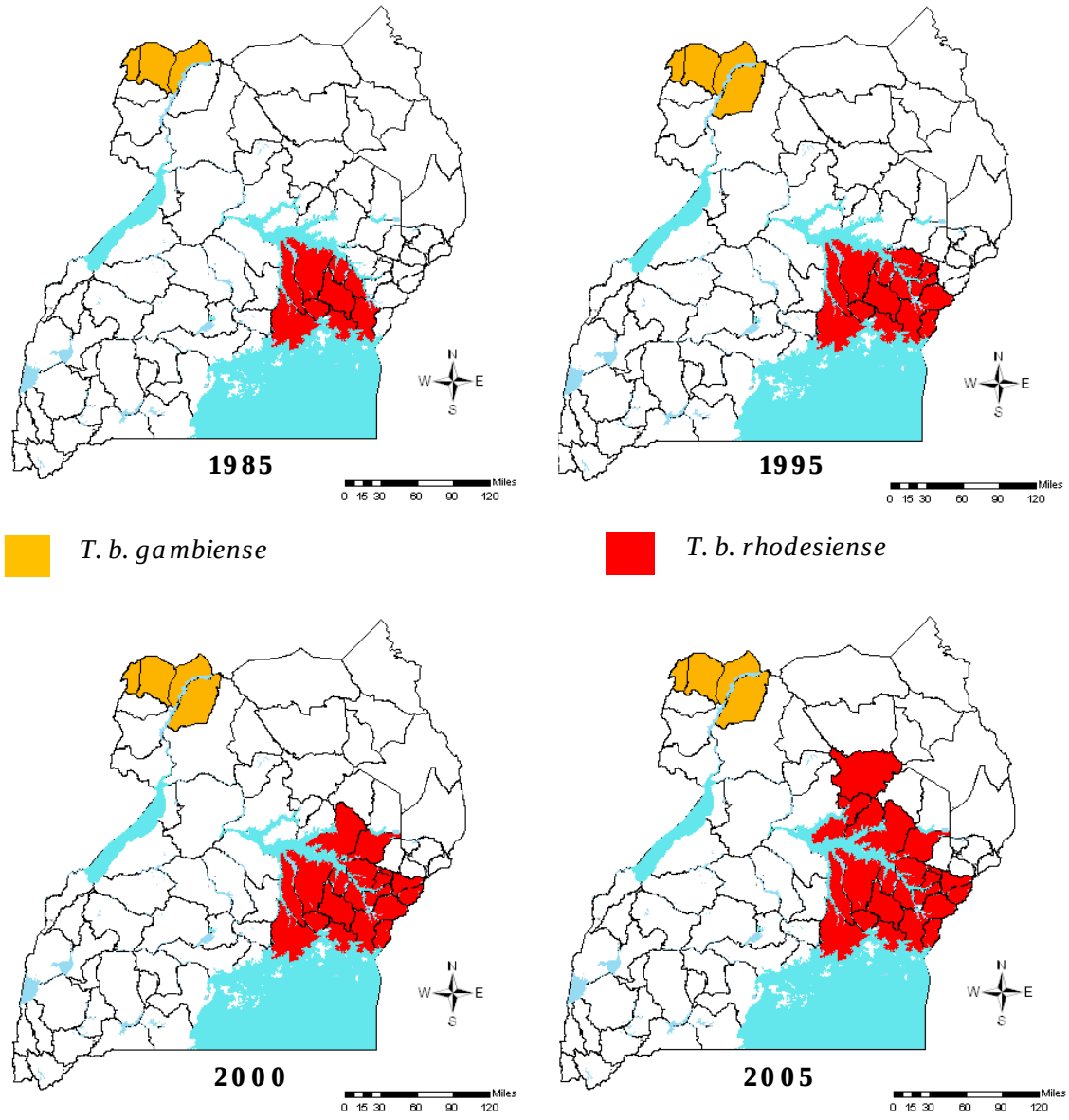

Figure 1.5 - the spread of *T. b. rhodesiense* in Uganda (after Picozzi *et al.*, 2005).

Further geo-statistical modelling revealed proximity to local cattle markets was a significant risk factor for the acquisition of HAT, strongly indicating the disease had been introduced into these new areas via the movement of infected cattle (Batchelor *et al.*, 2009).

During 2006, a control programme was launched to halt the northward spread of this zoonotic parasite. This programme, entitled 'Stamp Out Sleeping Sickness' (SOS),

proposed to reduce the prevalence of HAT in the newly affected districts by reducing the prevalence of *T. b. rhodesiense* in the main animal reservoir of infection – domestic cattle. Cattle were mass treated using trypanocides to clear infections. The programme was extremely successful in reducing the prevalence of this parasite in cattle. A second round of treatment took place in 2008 to quell the prevalence of *T. b. rhodesiense* in cattle in Dokolo and Kaberamaido, which had been identified as high risk areas during monitoring of the initial treatment. The design and implementation of the SOS programme is reviewed in chapter two.

1.4 Thesis aims

This thesis aims to evaluate the overarching impact of the SOS control programme on the epidemiology of trypanosomiasis in Dokolo and Kaberamaido districts, with particular emphasis on the second round of treatment. After reviewing the SOS programme in chapter two, chapter three sets out the materials and methods used in field and laboratory data collection and analysis.

With relation to *T. vivax* and *T. congolense* savannah, the most common species causing AAT in cattle, samples from SOS phase one districts taken before and after treatment were subsequently screened for these parasites. Chapter four sets out to establish if these species were present in Dokolo and Kaberamaido and if the treatment had any effect on their prevalence, working on the following null hypothesis:

H_0 – the SOS control programme had no effect on the prevalence of *T. vivax* or *T. congolense* savannah in these districts.

Chapter five is an investigation into the effect of the second round of SOS treatment, which focused particularly on Dokolo and Kaberamaido. In these districts, trypanosomiasis prevalence was significantly higher than anywhere else in the SOS region, alongside continued HAT cases, indicating a need for further control measures in afflicted localities. This chapter aims to investigate the prevalence of *T. brucei* s. l., *T.*

b. rhodesiense, *T. vivax* and *T. congolense* savannah before and after the second round of treatment, working on a similar null hypothesis to chapter four:

H_0 – the SOS re-treatment had no effect on the prevalence of *T. brucei* s. l., *T. b. rhodesiense*, *T. vivax* or *T. congolense* savannah.

Chapter six is an analysis of the epidemiology of trypanosomiasis in Dokolo and Kaberamaido on the micro-scale. Measurements of trypanosome prevalence taken at the village level over the duration of the SOS programme are collated and compared. This chapter draws and expands on data from the previous chapters, with a particular focus on mixed infections and particular cattle traits that may increase the likelihood of trypanosome infections. Mixed infections in all areas at all time points were investigated under the following null hypothesis:

H_0 – there is no association between species of trypanosome within the sample population, and mixed infections do not occur at a high rate than expected by chance.

Chapter seven is the general discussion, wherein the wider implications of the findings of this work are discussed and contextualized. This thesis brings together important epidemiological data regarding the impact of mass cattle drug treatment on the point prevalence of several different species of trypanosome in a newly endemic area of human sleeping sickness. Crucially, this large scale epidemiological study utilizes the superior sensitivity and specificity of molecular diagnostic techniques, providing invaluable information on an almost unprecedented scale.

Chapter 2 – Stamping out sleeping sickness in Uganda: The SOS campaign

In response to the threat of convergence of the two forms of HAT in Uganda, The Stamp Out Sleeping sickness (SOS) campaign was launched in October 2006 (Anon, 2010). This public-private partnership brought together the veterinary pharmaceutical group Ceva Santé Animale (CEVA), the Coordinating office for Control of Trypanosomiasis in Uganda (COCTU), The University of Edinburgh's Centre for Infectious Diseases (CID), IK investment partners, and Makerere University's Faculty of Veterinary Medicine. The aims of the SOS programme were:

- To maintain a barrier zone between the two forms of HAT in Uganda (Butcher, 2009).
- To reduce the prevalence of *T. b. rhodesiense* in cattle by treating more than 86% of the cattle population with trypanocides in a one-off mass treatment.
- To maintain a reduction in the prevalence of *T. b. rhodesiense* in cattle via repeated insecticidal spraying of 86% of cattle with deltamethrin.
- To reduce HAT prevalence in newly affected areas (Butcher, 2009).

The programme set out to achieve these aims by mass trypanocidal treatment of cattle in the areas of Uganda newly affected with *T. b. rhodesiense* HAT outbreaks: all of Dokolo, Kaberamaido and Amolatar, as well as large parts of Lira and Apac, as shown in Figure 2.1.

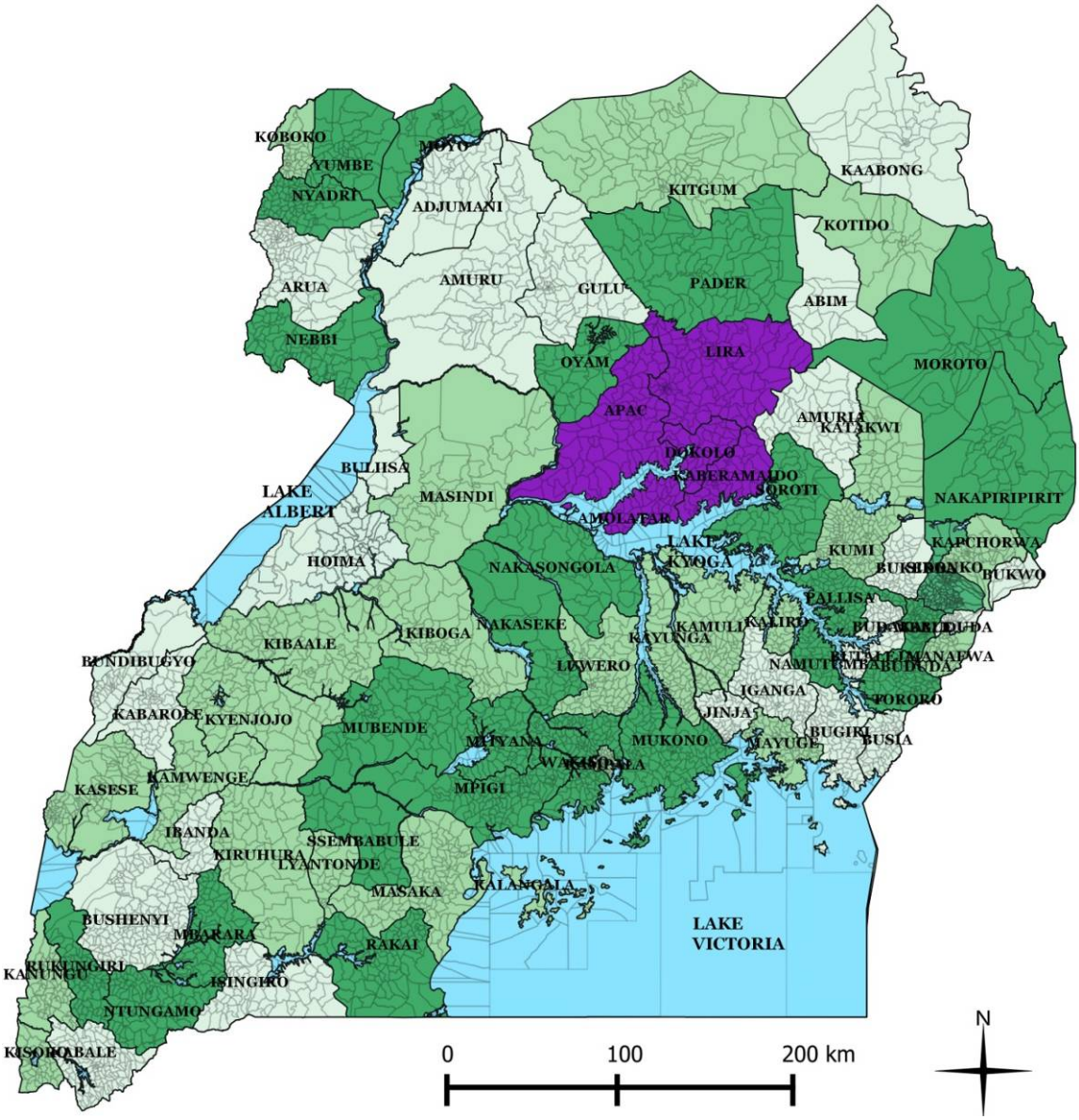

Figure 2.1 - map of Uganda indicating district boundaries as they stood in 2007, SOS phase one districts in purple

Lira and Apac were not included in their entirety due to the activity of insurgents in the north of Uganda at that time. To retard re-infection after this treatment, cattle were also sprayed with a deltamethrin-based insecticide using the restricted application of pesticide (RAP) technique (Torr *et al.*, 2007).

To monitor if these aims were successfully met, cattle within the intervention area were blood sampled at a number of time points. Twenty three villages within the SOS intervention area were selected using a two-stage cluster sample method; at each of these villages 100 cattle were randomly sampled at four time points: pre-treatment and three, nine, and eighteen months after treatment had occurred. Cattle herders and owners were questioned as to whether or not animals in their care had been treated, and each cow blood sample underwent molecular analysis to detect the presence of *T. brucei* s. l.

The approach SOS took to controlling trypanosomiasis in Uganda was unique in that the RAP technique had not, to this author's knowledge, been applied before on such a grand scale. Neither had mass trypanocidal drug treatment of cattle been used in response to a HAT epidemic. In combining veterinary and medical expertise alongside vector control this "One Health" intervention aimed to integrate the many and often remote facets of trypanosomiasis control to effectively combat the disease.

2.1 Chapter aims

This chapter aims to give a comprehensive overview of the SOS control programme, its implementation, and key findings by drawing on a number of resources. Background to SOS will be given by highlighting the key findings in trypanosomiasis research which influenced and informed those designing the programme. The discovery of continued HAT transmission, the decision to administer an additional treatment, and the selection of high-risk areas to re-treat is also reviewed.

2.2 Key findings influencing the design of the SOS campaign

The SOS programme drew knowledge from many important discoveries regarding trypanosomiasis control, including research particular to Uganda. Mathematical

modelling indicated control of *T. b. rhodesiense* in the animal reservoir was the most effective approach for acute HAT control (Welburn *et al.,* 2001a). The importance of cattle in the spread of *T. b. rhodesiense* in Uganda had already been established (Fèvre *et al.*, 2001, Fèvre *et al.*, 2006b, Welburn *et al.*, 2001b). Mass trypanocidal drug treatment of cattle had been shown to effectively reduce the prevalence of *T. b. rhodesiense* in those animals elsewhere in South Eastern Uganda; however, the reduction was not sustained over a long period of time in the absence of sustained control and in the face of continuing challenge (Fyfe, 2007). In order to maintain a reduction in bovine *T. b. rhodesiense* the SOS programme opted to use insecticidal spraying of cattle to prevent re-infection through tsetse control. Prior to the launch of the SOS campaign, RAP was gaining increasing recognition as a cost effective method of tsetse and tick control that has low environmental impact when compared to other insecticidal methods (Eisler *et al.,* 2003, Fox *et al.,* 1993, Torr *et al.,* 2005, Van den Bossche and Mudenge, 1999). The SOS programme intended spraying to be undertaken at regular intervals following the drug treatment, to maintain protection of cattle from trypanosome infection. The long term aim was to increase awareness and uptake of insecticidal cattle spraying within the community, particularly with deltamethrin, which is effective against ticks and tsetse. Initial research indicated cattle owners in the intervention area were already spraying their cattle against ticks, providing a knowledgebase within the community for SOS to build on (Butcher, 2009).

The incorporation of the above research findings into the design of SOS provided a firm scientific basis for the programme to proceed. Each partner in the initiative contributed expertise and resources to ensure the logistical practicalities were covered. Makerere University's Faculty of Veterinary Medicine played a central role in the project, providing final year veterinary medicine students to deliver treatment through innovative changes to the curriculum brought together in the MINTRACS programme (Makerere In-training Community Services, http://mintracs.org), as well as expert staff to plan and supervise treatment activities in liaison with COCTU. CEVA donated drugs for spraying and treatment, IK provided the financial resources for treatment delivery, and The University of Edinburgh provided input during the planning phase of the project and were responsible for monitoring the impact of the treatment and spraying by assessing the prevalence of *T. b. rhodesiense* in cattle in the intervention areas.

2.3 SOS phase one study design

The study design has been covered in detail by Selby (2011). An overview of the relevant detail pertaining to the selection of sample sites, sampling and treatment dates is provided here.

Cattle blood samples were taken at five different time points over the course of SOS phase one monitoring, as shown in Figure 2.2. The samples taken in July, October and November 2006 together form the pre-treatment baseline.

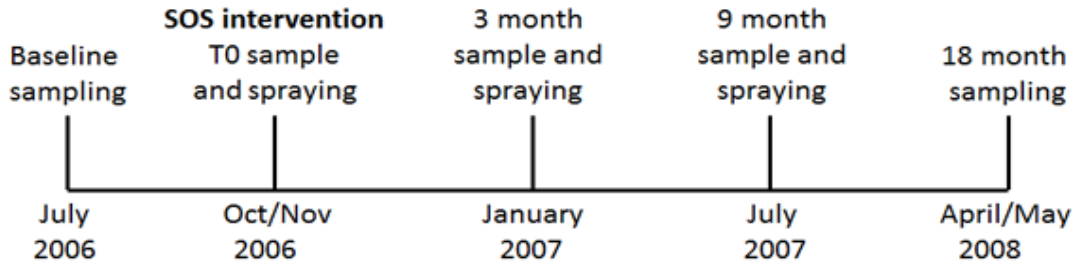

Figure 2.2 - dates of cattle blood samples taken for SOS phase one area monitoring as well as spraying and the trypanocidal drug treatment (from Selby (2011)).

2.3.1 Selection of sample villages

Repeated cross-sectional sampling was selected as the most suitable means of monitoring trypanosome prevalence within the cattle population. Sample size calculations were adjusted for cluster sampling (Thrusfield, 2005). Based on an initial exploratory sampling conducted in the intervention area in July 2006, the prevalence of *T. brucei* s. l. was estimated to be 15%, with an expected intercluster variance (V_c) of 0.013 between sample villages (Selby, 2011). From this, random sampling of 80 – 100 cattle in 23 sampling villages was calculated to achieve a desired absolute precision of below 5% (Selby, 2011).

These 23 sample villages were selected by two-stage cluster sampling. The first stage was the random selection of sub-county from within the SOS region; by allowing a maximum of one village per sub-county the villages were distributed as evenly as possible across the SOS treatment area. Stage two was the selection of the village. A village within each sub-county was chosen at random from a short list of suitable villages within the sub-county provided by each District Veterinary Officer (DVO). In order to be deemed suitable for sampling the short listed villages had to meet the following criteria: be home to a suitable number of livestock (~80 – 100), the owners of which would be likely to cooperate (distrust of outside personnel was in some locations deemed to be insurmountable in the wake of civil unrest), and with no current outbreak of infectious diseases of cattle, such as foot and mouth, that would limit access to the village.

2.4 Implementation of SOS phase one

At the inception of the SOS programme cases of HAT from the intervention region were almost exclusively from the districts of Dokolo and Kaberamaido. Therefore cattle resident in these two districts were treated with isometamidium, which has prophylactic activity of up to three months. It was deemed unnecessary to treat cattle in the remaining areas with isometamidium considering the absence of HAT; they were instead treated with diminazene, due to its lower cost and reduced risk of complications (Selby, 2011). The drugs used and the precise geographical boundaries included within the intervention area are shown in Figure 2.3.

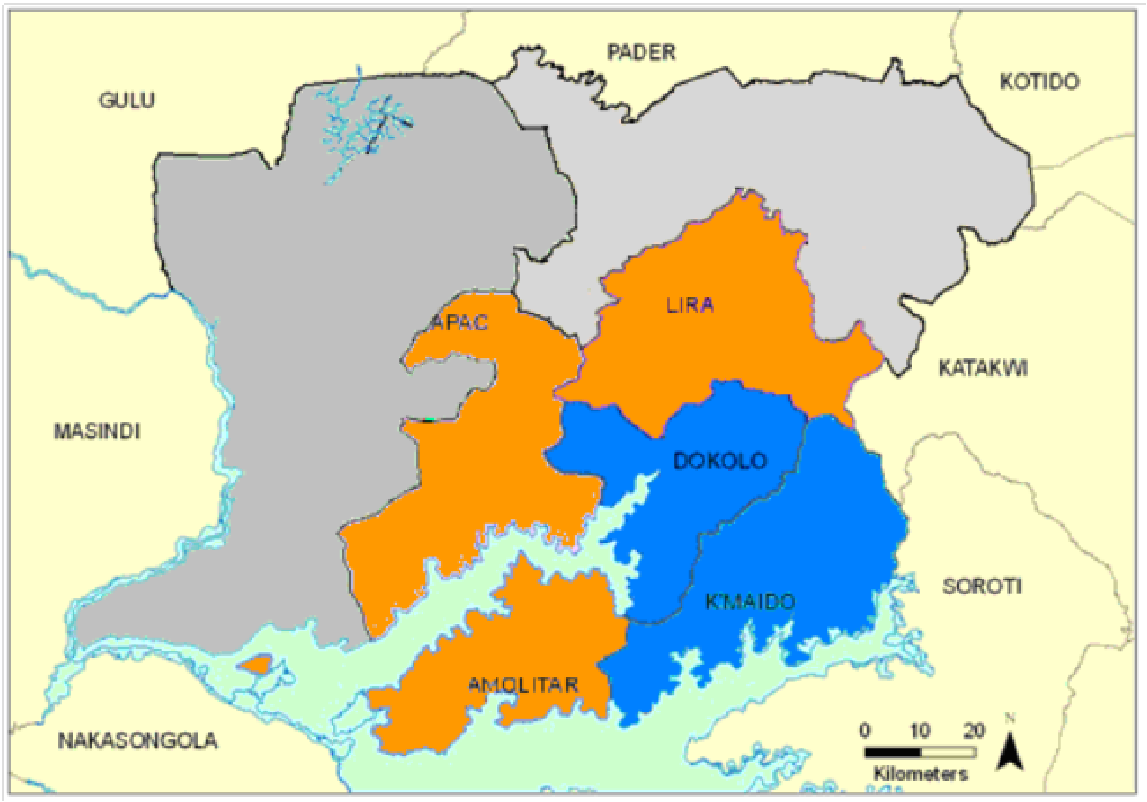

Figure 2.3 - areas included in SOS phase one mass treatment (Selby, 2011). Areas treated with diminazene are shown in orange, areas treated with isometamidium are shown in blue, grey areas depict the parts of Lira and Apac excluded from the intervention for security reasons.

Injection and spraying commenced late in October of 2006. Before any treatment took place residents in the SOS area were informed about the planned activities, using local language radio stations, seminars with local leaders, and posters in villages; immediately before the treatment commenced local people and students were deployed to further explain the aims and requirements of the treatment campaign to the target communities (Butcher, 2009). DVOs were also involved during SOS community sensitisation: each DVO was asked to inform their subordinate veterinary staff and local community animal health extension officers about the SOS programme's aims and objectives (Selby, 2011).

As treatment was carried out, Makerere staff recorded the number of cattle treated, creating an exceptionally accurate account of the number of cattle which took part in the programme. In an ideal situation, the number of animals treated would be compared to the total cattle population in the target area to determine the percentage

coverage of the treatment. However, estimating or even counting the number of animals present in Northern Uganda was difficult for a number of political and social reasons. Local people in the SOS region mistrust government interference in livestock rearing practices as a cattle tax was applicable in the recent past. This led people to underreport the number of animals they owned during census activities (Selby, 2011). During the war and civil unrest, the cattle population of the area was almost completely wiped out; in recent years there has been extensive restocking from a multitude of different organisations and locations, as well as by individual farmers. With so many different parties involved it is difficult to keep track of the overall population, or indeed find a point when the cattle were reliably counted to estimate from. Furthermore, during the two decades of war and civil unrest it was much too dangerous for government officials or Makerere staff to visit the area to collect data on the cattle population.

Despite these many difficulties, there was census data available at the time of the SOS treatment; however, it had been collected some years prior to 2006. The cattle population in Uganda is known to be expanding rapidly. With such a fast changing picture it was thought the census data, while as accurate as possible in relation to the cattle population in the past, would not be an accurate reflection of the cattle population in 2006. Therefore Makerere University's Faculty of Veterinary Medicine compiled estimates of the total number of cattle likely to be present, using their extensive knowledge of cattle trading and re-stocking initiatives in Uganda and of the importance of cattle in the SOS region. The cattle population estimates were compared to the number of animals treated to provide a rough idea of treatment coverage.

Figures provided by Makerere University estimated 174,851 cattle resided within the SOS intervention area. They recorded a total of 179,478 cattle treated, giving a coverage rate of 102.6%, much in excess of the 86% target for treatment coverage. As these figures are based on estimates, they should be interpreted with caution. Another estimation of treatment coverage is provided by the field derived data collected by the University of Edinburgh. Three months post intervention, 78.1% of sampled animals had received the initial treatment, suggesting that the target coverage had not been met (Selby, 2011). However, as there is no way to mark or differentiate treated from

untreated cattle without negatively impacting upon the value of the animal, the field data is reliant on the memory of individual cattle keepers and may be subject to error due to incorrect memory recall since they were questioned three months after the treatment took place. Furthermore, these figures do not account for turnover of the cattle population as a function of births, deaths, import, and export.

The first round of deltamethrin spraying was carried out alongside the trypanocidal treatment; however, a number of issues were reported regarding faulty backpack sprayers, which had a negative impact on the spraying coverage (Selby, 2011). Nevertheless, Makerere derived data indicates 110% of the cattle population were covered in the initial spray, down to 65% coverage for the second spray in January 2007, and then in June 2007 160% of cattle were reportedly sprayed (Selby, 2011). However, the field derived data from the University of Edinburgh does not reflect this: 33% of sampled cattle were reported to have been sprayed at the time of trypanocidal treatment in October 2006, with a further 32% having been sprayed at either the January or June 2007 sprayings; it was also discovered 17% of sampled animals were not present at the time of treatment, i.e., had been born or imported since (Selby, 2011). Allowing for this 17% proportion within the cattle population Selby (2011) suggested the actual percentage of cattle sprayed at the initial spraying could be considered to be 50%, which is considerably lower than the desired 86%.

2.5 Assessing the impact of SOS phase one upon the prevalence of *T. b. rhodesiense* in cattle

The SOS intervention aimed to reduce the prevalence of *T. b. rhodesiense* by mass trypanocidal and insecticidal treatment of cattle. To assess if these aims were achieved, cattle blood samples were subjected to molecular analysis. This analysis was carried out chiefly by Richard Selby in collaboration with other staff and students at the University of Edinburgh, and an in-depth breakdown of the results of this analysis is set out in his 2011 doctoral thesis (Selby, 2011). What follows in the subsections here is an overview of the data produced by (Selby, 2011), with a special focus on the districts of Dokolo and Kaberamaido, as they comprise the study area investigated in subsequent chapters of

this thesis. Section 2.5.1 sets out an overview of results of SOS phase one, section 2.5.2 deals with problems encountered during SOS phase one, and section 2.5.3 looks at Dokolo and Kaberamaido in detail and their subsequent designation as high transmission areas.

2.5.1 Overview of SOS phase one results

The point prevalence of *T. brucei* s. l. detected in cattle sampled from 23 villages within the SOS phase one intervention area was significantly ($p < 0.0001$) reduced from a baseline prevalence of 15.57% to 4.87% three months post treatment, but by nine months post treatment prevalence had rebounded to 16.11%, insignificantly different from the baseline prevalence ($p = 0.6422$) (Selby, 2011). At eighteen month sampling, the prevalence of *T. brucei* s. l. had significantly increased to 27.44%, as summarised in Figure 2.4.

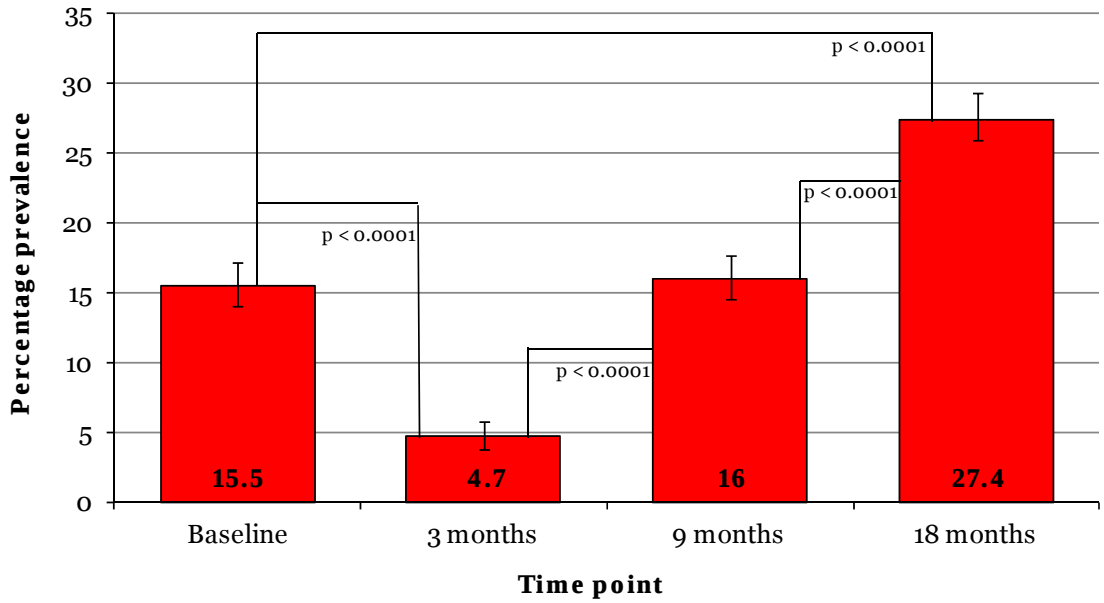

Figure 2.4 - percentage prevalence of *T. brucei* s. l. detected in sampled cattle in the SOS phase one region at each sampling time point (Selby, 2011). Vertical lines dissecting bars represent 95% confidence intervals. Lines linking bars represent a significant difference between the two as determined by the Chi squared test.

Similarly, the detected prevalence of *T. b. rhodesiense* in sampled cattle was significantly reduced (p = 0.0018) from a baseline prevalence of 0.81% to 0.11% at three month sampling (Selby, 2011). Nine and eighteen month sampling returned prevalences of 0.48% and 0.53% respectively, which although lower than baseline were not significantly different, as summarised in Figure 2.5.

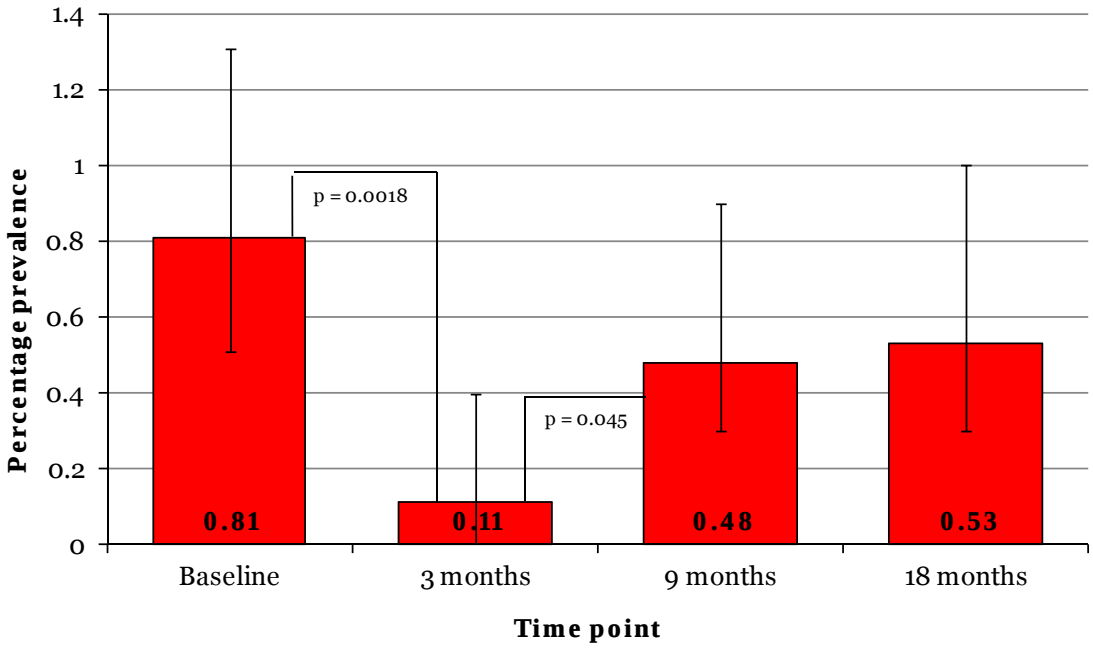

Figure 2.5 - percentage prevalence of *T. b. rhodesiense* in sampled cattle in the SOS phase one region at each sampling time point (Selby, 2011). Vertical lines dissecting bars represent 95% confidence intervals. Lines linking bars represent a significant difference between the two as determined by the Chi squared test.

Furthermore, not only did the prevalence of *T. b. rhodesiense* reduce significantly, the number of villages where *T. b. rhodesiense* was detected in the sampled cattle also reduced, from ten villages at baseline to two villages at three months, three villages at nine months and five villages at eighteen months (Selby, 2011). At three and nine month sampling these villages containing *T. b. rhodesiense* cattle were clustered around the Dokolo and Kaberamaido border, and were situated in or close to parishes which were continuing to report cases of HAT (Selby, 2011). This region, which was still presenting *T. b. rhodesiense* positive cattle and humans, was hence designated a high transmission area, and a mop-up re-treatment was proposed, incorporating all

parishes still affected with HAT, and all parishes bordering the affected areas (Selby, 2011). Examining the prevalence of *T. b. rhodesiense* in the high-risk area and comparing it to the rest of the SOS region, the case for re-treatment becomes clear, as highlighted in section 2.5.3.

2.5.2 Problems encountered in the implementation of SOS phase one

Although SOS phase one successfully reduced the prevalence and spatial distribution of *T. b. rhodesiense* within cattle, it can only be considered a partial success due to the persistence of a localised pocket of continued transmission centred around the border between Dokolo and Kaberamaido districts. The reasons for this continued persistence were unclear, as analysis had shown there was no significant difference in the prevalence of *T. b. rhodesiense* in areas treated with the prophylactic drug and those which were administered a purely curative treatment (Selby, 2011). To clarify the situation in these problematic areas, and in the SOS region as a whole, participants and organisers took part in structured interviews, the aim being to ascertain if problems were associated with delivering the mass treatment in any particular areas (Selby, 2011).

All respondents were sure cattle were not under dosed with trypanocides; therefore, it seems unlikely the persistent *T. b. rhodesiense* is due to trypanosomes surviving suboptimal drug doses or having developed drug resistance. The most frequently reported problems across the SOS phase one region as a whole were poor mobilisation, poor response to mobilisation, and treatment sites lacking an animal crush (Selby, 2011). Accordingly, the most frequently suggested improvements related to community education, community awareness of SOS, and mobilisation (Selby, 2011). Interviewees were also asked to recall any particular regions where they felt problems were most encountered; of those 14 who could recall a specific location the majority (eight) named Kaberamaido, with another two naming Dokolo (Selby, 2011), meaning 10/14 interviewees perceived the proposed re-treatment area as being the most problematic. This suggests the persistence of *T. b. rhodesiense* in the cattle in these districts and the

continued occurrence of HAT cases were at least partly due to the large number of problems encountered by treatment personnel in the implementation of SOS phase one.

To further evaluate the success and impact of the SOS campaign, Department For International Development (DFID) personnel travelled to the area in December 2008 to conduct focus group discussions and semi-structured interviews. These revealed Dokolo had the lowest community uptake of spraying with only 38% of cattle keepers reporting they had sprayed their animals in the last year, compared to 74% in Lira, 78% in Apac, 92% in Amolatar, and 92% in Kaberamaido (Butcher, 2009). Awareness of nagana in Dokolo was also the lowest out of all SOS districts at just 48% (Butcher, 2009), meaning farmers were unsure of the signs, cause, and prevention of AAT. It is therefore unsurprising they were not spraying their animals if they were not aware of the potential benefits to cattle health and productivity this practice could bring. This reinforces the results from the SOS participant interviews, which stated community education and community awareness of SOS were key problems encountered during implementation.

The information gathered in these two rounds of interviews consistently indicates Kaberamaido and Dokolo as problematic areas during the implementation of SOS phase one. Those tasked with delivering the treatment felt community awareness was subpar, particularly in Kaberamaido, and those dwelling in Dokolo had much less knowledge of the purpose of spraying and the diseases caused by AAT. The high number of problems encountered in the area of continued transmission greatly increases the likelihood that less than 86% of the cattle population were treated, rendering the programme ineffectual in the long term. The analysis to follow in section 2.5.3 shows the effect of this, by comparing the prevalence of *T. brucei* s. l. and *T. b. rhodesiense* in Dokolo and Kaberamaido to the prevalence in the rest of the SOS region.

2.5.3 Comparison of high transmission areas to the remainder of the SOS region

As already stated, post treatment monitoring of SOS phase one revealed continued transmission of *T. b. rhodesiense* in a discrete zone along the Dokolo and Kaberamaido border. Questionnaires and structured interviews revealed Dokolo and Kaberamaido had suffered problems which may have negatively impacted on the effect of the SOS programme overall. To ascertain if this was the case, these problematic districts were analysed separately to the rest of the SOS region.

As shown in Figure 2.6, the prevalence of *T. brucei* s. l. was higher in the high-risk areas at baseline, three and nine month sampling. At eighteen months this situation was reversed, but this could be an artifact of the inclusion of additional sampling sites in the remainder of the SOS region for this round of sampling (see (Selby, 2011) for a detailed explanation).

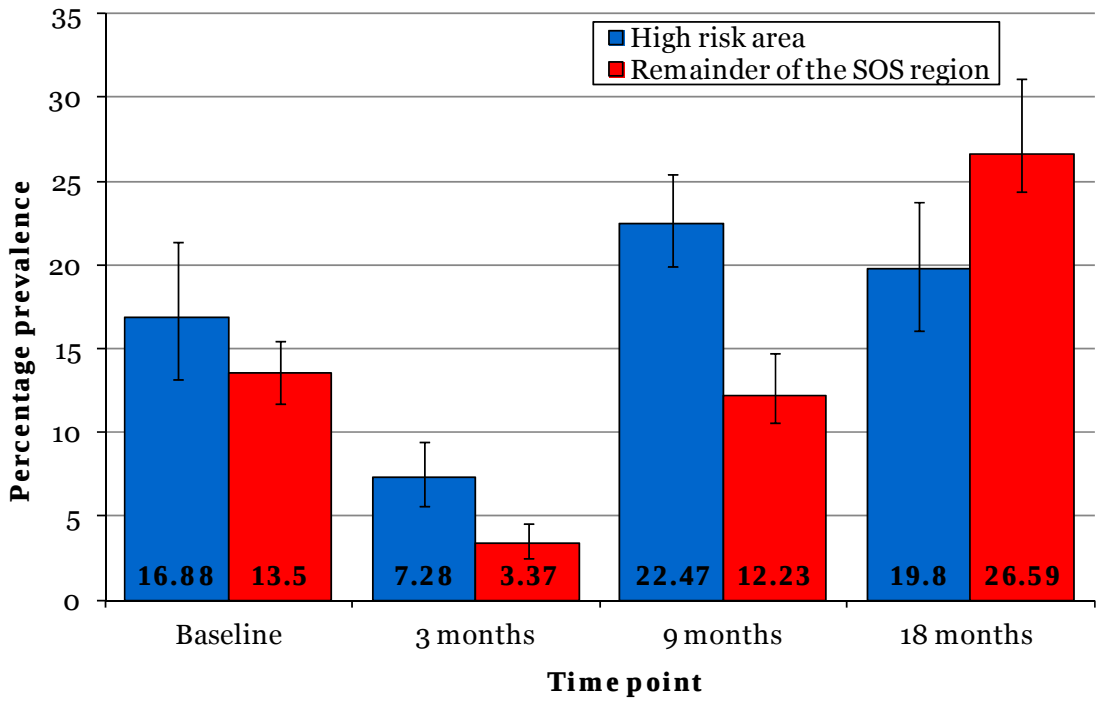

Figure 2.6 - prevalence of *T. brucei* s. l. in high-risk areas of Dokolo and Kaberamaido compared to the prevalence in the rest of the SOS region (Selby, 2011). Vertical lines dissecting bars represent 95% confidence intervals.

The trend is similar when comparing the prevalence of *T. b. rhodesiense* within the high-risk areas and in the rest of the SOS region: Dokolo and Kaberamaido record a

higher prevalence of *T. b. rhodesiense* at all sampling time points, as shown in Figure 2.7.

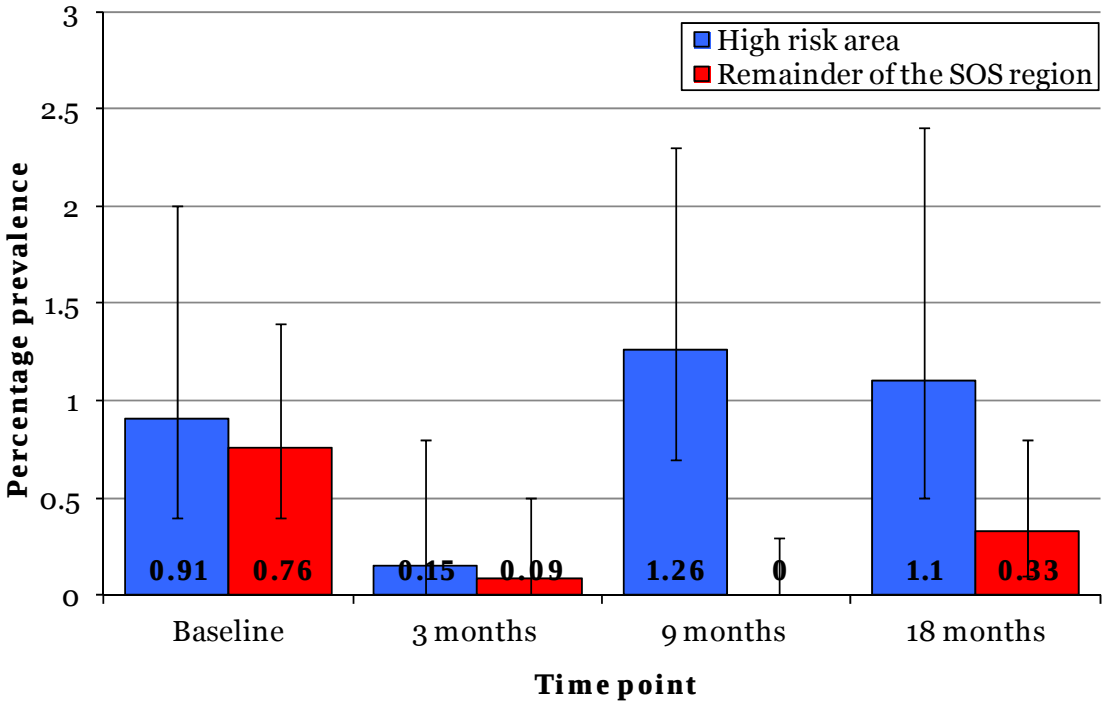

Figure 2.7 - prevalence of *T. b. rhodesiense* in high-risk areas of Dokolo and Kaberamaido compared to the prevalence in the rest of the SOS region (Selby, 2011). Vertical lines dissecting bars represent 95% confidence intervals.

Not only was the detection of *T. b. rhodesiense* in sampled cattle restricted to Dokolo and Kaberamaido sample sites in the nine months after treatment, these areas overlapped parishes that were continuing to suffer from HAT.

The problems reported from within Dokolo and Kaberamaido during treatment – the decreased community uptake of spraying, the low levels of nagana awareness, and the persistence of *T. b. rhodesiense* in cattle residing in parishes suffering from HAT – all strongly indicated a mop-up re-treatment was required to help the SOS programme fully achieve its aims.

While these factors go a long way towards explaining the continued persistence of *T. b. rhodesiense* in cattle in Dokolo and Kaberamaido, there still remains the possibility

that other unknown forces could be exerting their influence. In order to get a clearer picture of the epidemiology of both human and animal trypanosomiasis in Dokolo and Kaberamaido it is extremely relevant, timely, and informative to conduct further analysis on samples from SOS phase one, to assess whether other species of trypanosome are present, discover how the prevalence of these trypanosomes responded to the treatment and if any interaction between different species of trypanosomes occurs.

2.6 The SOS re-treatment programme

Following the successful reductions in the prevalence of *T. brucei* s. l. and *T. b. rhodesiense* in the majority of the SOS phase one area, a mop-up re-treatment was proposed along the Dokolo and Kaberamaido border. In 2008, re-treatment of these high-risk areas was carried out, aiming to:

- Reduce the prevalence of *T. b. rhodesiense* in the cattle population within the re-treatment area by treating more than 86% of the cattle population with trypanocides.
- Have a knock on reduction in the number of HAT cases originating within the re-treatment area.

By achieving these aims, the hope was to stop the parasite spreading back into cattle in neighbouring districts, in addition to the aims originally set out in the opening section of this chapter. To properly interrupt the transmission cycle of *T. b. rhodesiense* it was again estimated more than 86% of the total cattle population needed to be treated. Due to another arm of the SOS project specifically aiming to increase community uptake of spraying by supporting Makerere University veterinary graduates to set up small businesses in the area (3v) (Butcher, 2009), and the low coverage of the last mass spraying, re-spraying of the cattle was not undertaken. Isometamidium is expensive, and the benefit of its prophylactic activity was not evident during the previous round of treatment; therefore, the cheaper diminazene was chosen for this round of treatment.

2.6.1 SOS re-treatment study design

The area in need of re-treatment was defined as every parish in Dokolo and Kaberamaido that had HAT within its borders during the first nine months of monitoring and evaluation of the initial SOS treatment, plus all parishes that shared a boundary with these infected parishes (Selby, 2011).

Samples were taken from a maximum of 100 cattle at each location, both before and after re-treatment, as shown in Figure 2.8.

Figure 2.8 - sampling timeline for the SOS re-treatment.

2.6.2 Treatment coverage

Given the numerous problems encountered with insecticidal spraying in SOS phase one, and the short time frame in which the re-treatment was designed and implemented, RAP was not included as part of the re-treatment intervention. Instead, tsetse control was targeted by increasing the uptake of RAP at the individual farmer level, through support grants and business loans to three Makerere veterinary graduates to set up veterinary businesses in the area. By approaching vector control from the bottom up rather than top down, sustainability should be increased.

As baseline sampling happened directly before treatment, none of the animals sampled at that time had been treated. However, when the treatment was carried out Makerere staff recorded the total number of animals treated in each parish. Based on estimates of the total cattle population, the percentage coverage is shown in Figure 2.9.

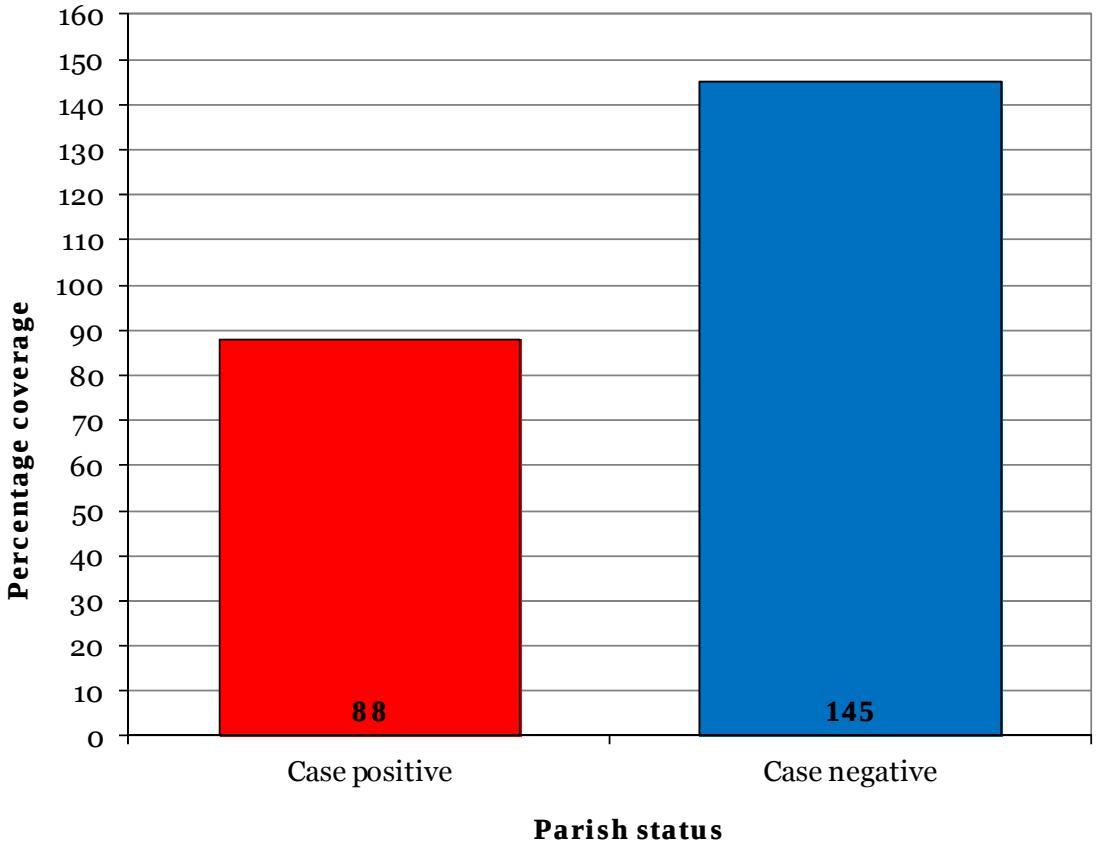

Figure 2.9 - initial treatment coverage in each of the treated parishes according to their sleeping sickness status, provided by Makerere University.

As can be seen from the value higher than 100% the total cattle population is not well known in this area as a whole. The difference between the proportion of animals treated in case-positive parishes is significantly lower than in case negative parishes (Chi squared = 544.712 with 1 degrees of freedom $p < 0.0001$). However, coverage levels in both sets of villages were above the threshold of 86% deemed necessary for the successful interruption of the *T. b. rhodesiense* transmission cycle.

These treatment coverage figures are prone to the same difficulties encountered when estimating the coverage rate of SOS phase one, as outlined in section 2.4 Implementation of SOS phase one

2.7 HAT cases in Dokolo and Kaberamaido, 2004 – 2010

The SOS control programme was implemented due to HAT in northern Uganda, and aimed to reduce the number of HAT cases occurring; therefore, the number of HAT cases originating from within the SOS region is a key measure of the success of the programme.

In addition to cattle blood samples, HAT case records from the local sleeping sickness hospitals in Serere and Lwala were collected. Since control efforts began, it has been standard protocol to record individual HAT patient details at the treatment centre they present to. The name, age, sex, and date of admission and discharge (or death) of each patient is recorded. As much information as possible about the geographic location in which they reside is also recorded, with a minimum of village name, but in most cases also the parish and sub-county. In digitalising these records for use within this thesis, no patient names were recorded in order to maintain patient confidentiality and to meet the standard set out in the international ethical guidelines for biomedical research involving human subjects.

The response of HAT to SOS phase one has already been detailed (Selby, 2011). The distribution of HAT in Dokolo and Kaberamaido is shown in relation to the re-treatment sample sites in Figure 2.10.

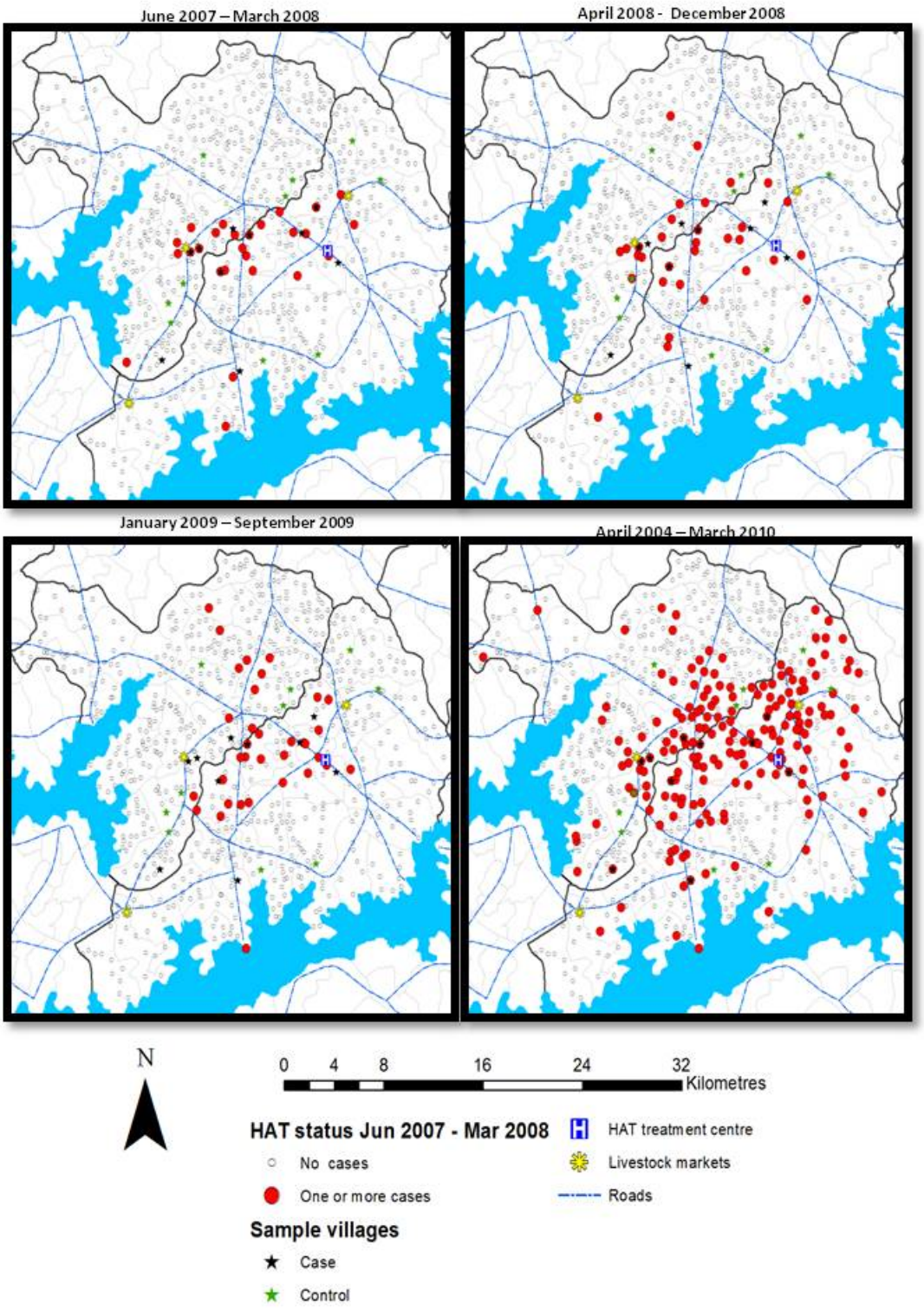

Figure 2.10 - distribution of HAT in Dokolo and Kaberamaido (Wardrop, 2011). Top left panel — before re-treatment, top right — six months after re-treatment, bottom left — Jan – September 2009, bottom right — had a case since records began.

The distribution of HAT in Dokolo and Kaberamaido is sporadic in nature and spread over a large number of villages. In any one time period, afflicted villages seem relatively close to one another; however looking at the number of villages that have reported a case since records began, a large number have been affected. Affected villages seem to aggregate along the Dokolo/Kaberamaido border in a wide band, with villages on the lake shore or at the extreme boundaries of the district less affected. The aggregation of villages reporting sleeping sickness along the border could occur for a number for reasons:

1. Local environmental conditions in the border area are more favourable for trypanosomiasis transmission when compared to outlying areas
2. The remote, inaccessible location of outlying villages and the long distance from health care facilities, particularly those villages along the lake shore, could accentuate under reporting.
3. Residents in villages at the extreme boundary of the district may travel to a health centre in a neighbouring district for convenience

The number of cases originating within the SOS re-treatment area, which excluded the periphery of Dokolo and Kaberamaido districts, is shown in Figure 2.11.

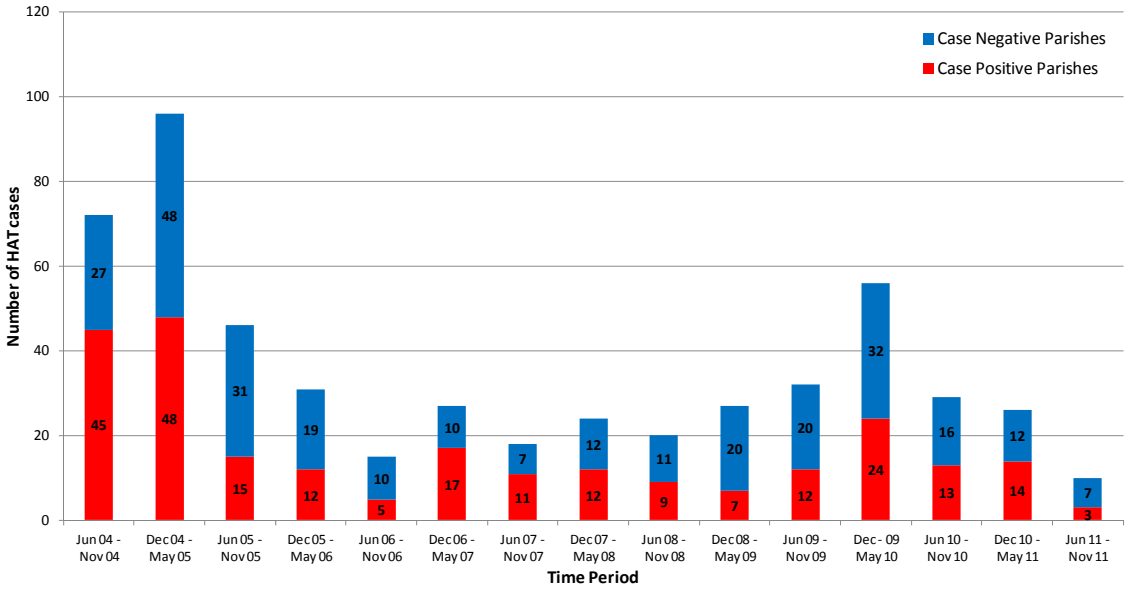

Figure 2.11 - HAT cases recorded from case-positive and case-negative parishes within the re-treatment area since June 2004.

As the graph clearly demonstrates, the initial peak of cases occurred at the start of the epidemic, with the highest numbers reported between December 2004 and May 2005. After this time period the number of cases began to decrease. The impact of SOS is not immediately obvious on this graph. The reduction in tsetse numbers brought about by insecticidal spraying is more likely to lead to a gradual reduction in HAT cases rather than an immediate large reduction. Furthermore, as education and sensitization about HAT symptoms and disease progression occurred alongside SOS treatment activities, it could be increased awareness of HAT lead to an increase in health care seeking behaviour in infected individuals, masking the effect the control programme was having. Similarly, it is difficult to assess if the re-treatment had an impact on HAT cases, as there is no obvious dip in case numbers in the months following the mass treatment. This could again be due to increased education and awareness facilitated by the 3v veterinary start up businesses, and ongoing government education and awareness drives. The number of HAT cases has been prevented from reaching the initial peak observed at the start of the epidemic.

For both SOS phase one and the re-treatment there is no way to tell how many cases may have occurred in the absence of any control measures; in the face of a rapidly fatal

disease epidemic leaving a section of the at risk population without any control measures to accurately assess treatment efficacy would have been ethically questionable. Previous HAT epidemics in Uganda have claimed thousands of lives. That the death toll for the current epidemic extends only into the hundreds can be at least partly attributed to SOS control efforts.

Chapter 3 – Materials and Methods

Contained within this chapter are the materials and methods commonly used throughout this thesis. Where materials and methods are particular to an individual piece of work they are explained within the relevant chapter.

3.1 Description of the study area

This thesis focuses on two districts of Uganda, Dokolo and Kaberamaido, shown in Figure 3.1.

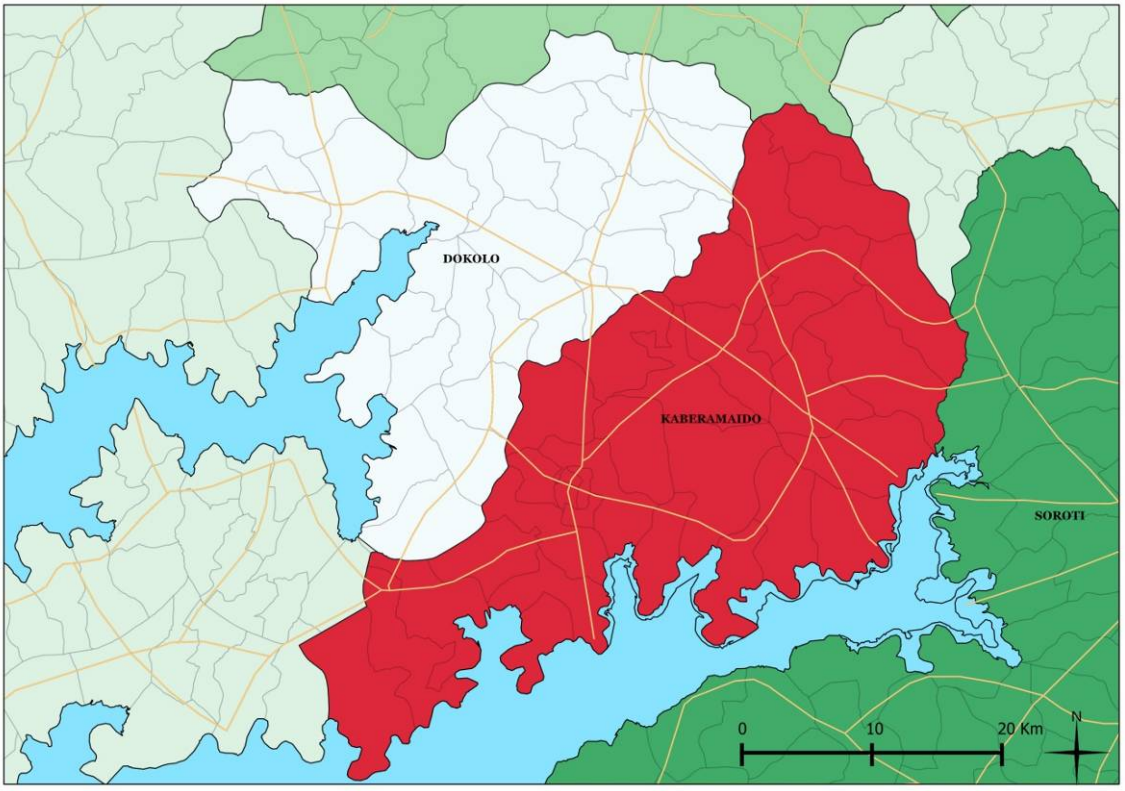

Figure 3.1 -map showing location of Dokolo and Kaberamaido. The water body shown is that of Lake Kyoga. The main road networks are shown in yellow.

Kaberamaido was formerly part of Soroti until the year 2000, when it became a district in its own right. Similarly, until 1st of July 2006 Dokolo was a sub county of Lira. These districts share a large border with one another. Both districts are situated on the shores of Lake Kyoga, bordering the district of Amolatar to the South West and Lira to the North. To the West Dokolo borders the district of Apac, while Kaberamaido boarders

Amuria and Soroti districts to the north and east respectively. There are two rainy seasons each year, the first between April – June and the second between August – November. Neither district is connected to the national electricity grid.

Kaberamaido covers a total area of 1,627.9 sq. km. It lies at an approximate altitude of 1036 – 1,127m above sea level, with rainfall totaling between 1000 mm and 1500 mm. The town of Kaberamaido serves as the administrative head quarters for the district. The main economic activities undertaken in the area are fishing and agriculture. The most common food crops are millet, potatoes, soya beans, sesame seeds, groundnuts and sunflowers. Cotton is also grown as a cash crop. In 2006 the population was estimated to be 152,900. According to the 2008 livestock census (first available online in 2010) Kaberamaido is home to 76,109 cattle (UBOS, 2010). Cultivation by oxen is the main agricultural technology (Mwebaze, 2006).

Dokolo covers a total area of 1049.3 sq. km. It lies at a similar altitude to Kaberamaido, and has similar average annual rainfall estimates. The town of Dokolo forms the administrative head quarters of the district. Subsistence agriculture, animal husbandry and commercial fishing from Lake Kyoga form the main economic activities in the district. The main crops are millet, potatoes, soya beans, sesame seeds, groundnuts and sunflowers. In 2006 the population of the district was estimated at 147,500. According to the 2008 livestock census Dokolo is home to 58,902 cattle.

3.2 Mobilisation

To enable a good turnout at each sampling, and to ensure the community were informed of the activities of the SOS treatment and sampling teams, the sample villages were visited prior to the planned sampling date. Typically each location was visited two to three days before sampling was due to occur, and a minimum of 3 local people were informed, where possible one of these people was to be the village chairperson. These individuals were informed of the sample location, number of cattle required, and of the de-wormer incentive. They were also assured the sampling would not cause any injury

to their animals, and that the blood would be taken from the ear, meaning the hide would not be significantly damaged.

Due to the remote, resource poor area the samples were taken from, it was not possible to carry out analysis of samples as they were taken. Blood was applied to Flinders Technology Associated (FTA) cards and shipped to the UK for lab analysis. FTA cards are well suited to this application in many ways; they contain chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, as well as oxidative and UV damage (www.whatman.com). DNA collected on FTA cards can be stored for years at room temperature. The chemicals impregnated within the filter paper matrix are designed to prevent bacterial and fungal growth (Smith and Burgoyne, 2004).

3.3 Collection of cattle blood samples

Cattle were restrained and cast to the ground by local herdsmen (Figure 3.2 left panel). Blood was obtained by veterinary personnel from an ear vein using a lancet (Figure 3.2 centre panel). Two 50µl capillary tubes were filled with the blood, which was then applied to an FTA card in accordance with the manufacturer's guidelines (Figure 3.2 right panel). Each animal sampled was given oral drench de-wormer (10% albendazole) as an incentive to take part in the sampling. The youngest calves (under 4 weeks of age) were not sampled, just given de-wormer. Detailed information was gathered for each cow sampled. An age estimate was provided for each sample, where animals were grouped into 3 classes: A <18 months, B 18 – 36 months, C >36 months. Body condition score was assessed using the guidelines set out by Nicholson and Butterworth, 1986; using this standard there are 9 possible body conditions, where the 3 main conditions of fat (F), medium (M) and lean (L) are further subdivided into L -/L /L+ categories (Nicholson and Butterworth, 1986). Sex, breed and treatment status were also recorded.

Figure 3.2 - collection and storage of cattle blood samples.

3.4 Extracting DNA from FTA cards

To free the DNA from the FTA cards, five 3mm diameter discs were cut from each sample using a Harris unicore 300 hole punch. Five dummy punches were preformed on clean filter paper between each sample to avoid cross contamination.

The discs from each sample were placed together in an empty 1.5ml Eppendorf tube (Alpha laboratories, Eastleigh, UK). 1 ml of Whatman FTA purification regent (Scientific Laboratory Supplies LTD, Nottingham, UK) was added to each Eppendorf, using a new 3 ml graduated pasteur pipette (Scientific Laboratory Supplies LTD, Nottingham, UK). The Eppendorfs were placed on a rocker and gentle agitated for 15mins. The liquid was removed from each Eppendorf with a fine tip pastette, taking care not to remove the discs also. This was repeated three times, once more with FTA purification regent, and twice with 1ml of 1.0 M Tris – HCl, pH 8, containing 0.1M Ethylenediamine Tetracetic Acid (Tris - EDTA buffer, Sigma-Aldrich LTD, Gillingham, UK)

The discs were then dried by transferring the discs to PCR tubes (Alpha laboratories, Eastleigh, UK) using a 1-200 µl pipette tip (Starlab LTD, UK), and placing them in an incubator set for 37^{o}C, for 30 – 45mins.

100 µl of 5% (w/v) chelex solution (Chelex 100 sodium form, 50-100 dry mesh, Sigma-Aldrich LTD, Gillingham, UK) was then added to each PCR tube. They were then heated at 90°C for 30 mins in a peltier thermal cycler, in order to free the DNA from the surface of the card in preparation for PCR reactions.

3.5 PCR reactions

Samples were analysed using the following published PCR protocols. As large numbers of samples were screened at once mastermix was made in batches of 50 reactions, with *Taq* DNA Polymerase always added at the last minute to avoid non-specific amplification. Once *Taq* had been added to the mastermix it was pipetted onto the samples on ice, again to prevent non-specific amplification since *Taq* is active at low levels at room temperature. Each set of amplifications includes both positive and negative controls; the positive control is DNA derived from known trypanosomal stocks, while the negative controls are PCR mix seeded with water alone (a on Figure 3.3), and the eluate from a blank FTA card (b on Figure 3.3).

3.5.1 PCR for *T. brucei* s. l. (Moser *et al.*, 1989).

The reaction was carried out in 25µl mixtures containing 16mM $(NH_4)_2SO_4$, 67mM Tris-HCl (pH 8.8 at 25 °C) 0.01% tween-20, (collectively 10x NH_4 Reaction Buffer, Bioline, UK), 1.5mM $MgCl_2$ (Bioline, London, UK), 5 µl of eluted DNA, 0.7 units of RED*Taq* (Bioline, London, UK), 0.2mM of each of the four dNTPs (Bioline, London, UK), and the primers TBR1 and TBR2 (MWG Biotech, London, UK, sequences detailed in Table 3.1) at a final concentration of 0.8 µM each. Thermal cycling was carried out in a peltier thermal cycler and the conditions were 94°C for 3mins followed by 35 cycles of 94°C for 1min, 55°C for 1 min and 72°C for 30 seconds, with a final extension step of 72°C for 5 mins.

3.5.2 PCR for *T. vivax* (Masake *et al.*, 1997).

The reaction mix consisted of 5µl of eluted DNA in NH_4 Reaction Buffer, 1.5mM $MgCl_2$, 0.2mM of each of the four dNTPs, the primers ILO1264 and ILO1265 (MWG Biotech, London, UK, sequences detailed in Table 3.1) at a final concentration of 1µM each and 1 unit of RED*Taq*, diluted to 25µl. After an initial step of 94 °C for 3 min, there were 30 cycles of 94 °C for 1 min, 55 °C for2 min, and 72 °C for 2 mins, and a final extension step of 72 °C for 5 mins, all of which were carried out on a peltier thermal cycler.

3.5.3 PCR for *T. congolense* savannah (Masiga *et al.*, 1992).

The reaction mix consisted of 5µl of eluted DNA in NH_4 Reaction Buffer, 1.5mM $MgCl_2$, 0.2mM of each of the four dNTPs, the primers TCS1 and TCS2 (MWG Biotech, London, UK, sequences detailed in Table 3.1) at a final concentration of 1µM each and 1 unit of RED*Taq*, diluted to 25µl. After an initial step of 94 °C for 3 min, there were 30 cycles of 94 °C for 1 min, 55 °C for2 min, and 72 °C for 2 mins, and a final extension step of 72 °C for 5 mins, all of which were carried out on a peltier thermal cycler.

3.5.4 Multiplex PCR (Picozzi *et al.*, 2008).

All samples identified as *T. brucei s.l.* positive by the above *T. brucei* PCR in 3.5.1 were subjected to the multiplex PCR for discrimination between *T. b. brucei* and *T. b. rhodesiense*. Reactions were carried out in 25 µl reaction mixtures containing 5 µl of DNA template, HotStart Buffer (Tris-CL, KCl, $(NH_4)_2SO_4$ 1.5mM $MgCl_2$, Qiagen, UK), Rediload red dye (Invitrogen), 1mM $MgCl_2$, 0.2mM of each of the four dNTPs, primers SRA-F, SRA-R, PLC-F and PLC-R (MWG Biotech, London, UK, sequences detailed in Table 3.1) at a final concentration of 0.2µM each and 1.5 units of Hot Star*Taq* (Qiagen, UK). PCR cycling was carried out on a peltier thermal cycler and consisted of an initial denaturing step of 95°C for 15 mins followed by amplification cycles of 94°C for 30 secs, 63°C for 1 min 30 secs, and 72°C for 1 min 10 sec, with a final extension step of 72°C for 10 min. The PCR was carried out in duplicate at 45 and 50 cycles.

PCR	Primer	Sequence (5'-3')	Band size	Reference
T. brucei s. l. Stock: Buteba135	TBR1	CGA ATG AAT ATT AAA CAA TGC GCA GT	173 bp	Moser *et al.*, 1989
	TBR2	AGA ACC ATT TAT TAG CTT TGT TGC		
T. vivax Stock: ILDatt1.2	ILO1264	CAG CTC GGC CAC TTC GCT GGG GTG	400bp	Masake *et al.*, 1997
	IL01265	TCG CTA CCA CAG TCG CAA TCG TCG TCT CAA GG		
T. congolense savannah Stock: IL1180	TCS1	CGA GAA CGG GCA CTT TGC GA	316bp	Masiga *et al.*, 1992
	TCS2	GAA CAA ACA AAT CCC GCA CA		
Multiplex PCR Stock: DO	SRA -F	GAA GAG CCC GTC AAG AAG GTT TG	669bp	Picozzi *et al.*, 2008
	SRA-R	TTT TGA GCC TTC CAC AAG CTT GGG		
	PLC - F	CGC TTT GTT GAG GAG CTG CAA GCA	325bp	
	PLC - R	TGC CAC CGC AAA GTC GTT ATT TCG		

Table 3.1 - PCR primer sequences and expected band sizes. The parasitic stocks used as positive controls in these reactions are also provided.

3.6 Visualisation of PCR products

A minimum of 12µl of PCR product were separated at 120 volts for a minimum of 45 minutes on a 1.5% (w/v) agarose gel containing a 1x concentration of GelRed nucleic acid gel stain (Cambridge Bioscience Ltd), with a supperladder-mid 100bp ladder (ABgene, UK) for sizing of bands (see Figure 3.3). Gel pictures were taken using a BioRad GelDock™ imaging system. Depending on band intensity and ladder separation gels were on occasion run for longer in order to clearly visualise the exact location and size of any bands present. In such cases, gels were returned to the gel tank and checked at 5 minute intervals until band size and location was clearly visible.

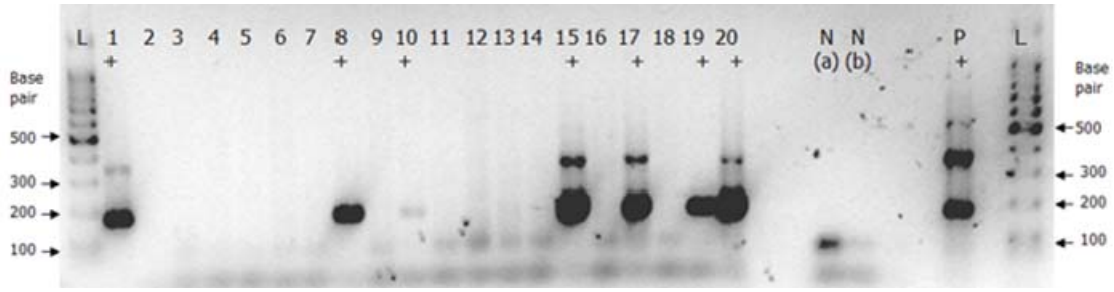

Figure 3.3- a typical gel picture for *T. brucei* s. l. PCR. L = 100bp DNA ladder, lanes 1 – 20 = screened cattle blood samples with positive amplification of parasitic DNA indicted by a 173bp fragment, this is denoted by +; the negative (N) and positive (P) controls are also identified.

3.7 Statistical analysis

From the results obtained the prevalence of *T. brucei* s. l., *T. b. rhodesiense, T. vivax* and *T. congolense* savannah was calculated as the proportion of samples which were PCR positive. Using graphpadinstat software, the 95% confidence interval was computed based on the exact binomial distribution and displayed as error bars on the respective graphs (http://www.graphpad.com/quickcalcs/index.cfm). Using the same software package the difference between prevalence at baseline and six months follow up was compared using Fisher's Exact Test, the differences were assigned statistical significance at p values less than 0.05. Fisher's Exact test was used in the place of the chi squared test due to the small frequencies in some of the categories tested.

3.7.1 Statistical analysis particular to chapter six

The detailed comparisons conducted in chapter six required additional statistical tests, outlined in the following sections.

3.7.1.1 Mantel – Haenszel test

The Mantel-Haenszel (MH) test was computed using R statistical software. Age and sex where chosen as the only two factors to analyse as they were the only cattle factors that had a significant impact on trypanosome prevalence, determined using Fisher's exact test.

The MH procedure calculates an estimate of a common effect of the exposure across the confounder strata using a weighted mean of an appropriate measure of association (Stefano and Ezio, 2007). In this case the strata are M, male, or F, female for sex, or the A, B and C age groups. Under the null hypothesis of no association between the exposure and the outcome, the MH estimator of relative risk will tend to one while measures of absolute effect will tend to zero (Stefano and Ezio, 2007). When the MH approaches its expected value under the null hypothesis the test statistic will tend to zero (Stefano and Ezio, 2007).

When using R to calculate MH, the odds ratio is computed for each stratification level, and plotted on a graph. A homogeneity test p value is also computed, this is a test of how similar the odds ratios (OR) are between the two groups, if there is a significant difference this value will be less than 0.05. If the homogeneity p value is significant this indicates an interaction between factors, and possible confounding bias. The MH estimator of relative risk is given as MH-OR. An example of the typical output given by R for the MH test is illustrated below in figure Figure 3.4.

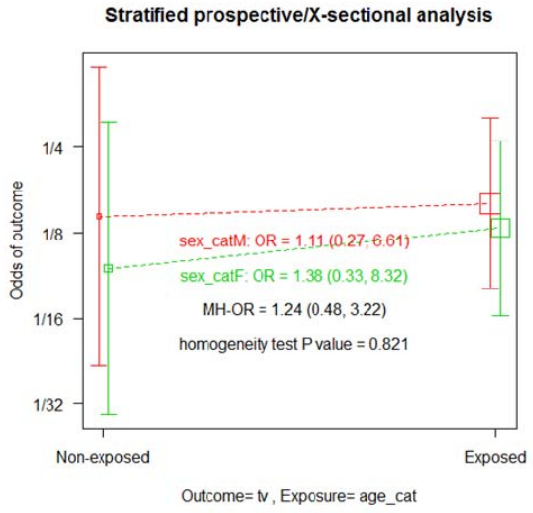

Figure 3.4 - typical R output for the MH test. Exposed = A age group, non-exposed = B age group. In the above example, where the combined effect of age and sex on the prevalence of *T. vivax* is assessed, it is clear there is no significant interaction, as OR confidence intervals of the strata overlap (the green and red vertical lines), MH-OR value is close to 1 with the 95% confidence interval including 1, and homogeneity test value > 0.05.

Comparisons were conducted and interpreted in this way for Dokolo and Kaberamaido separately at each time point for SOS phase one, and separately for case-positive and case-negative villages at the re-treatment. As age has three categories three comparisons were run at each time point, comparing A to B, A to C, and B to C.

3.7.1.2 Exact binomial test

The exact binomial test is a goodness of fit test which directly computes the probability of obtaining the observed data under the null hypothesis (McDonald, 2009). It is used when the data is such that a predicted probability can be computed and compared to the observed probability. The exact binomial test is used in this study to analysis the proportion of mixed trypanosome infections occurring at each time point. The methods for computing the predicted probability are given in section 3.7.1.3.

Exact binomial probabilities are calculated through repeated applications of the formula –

$$Y = \frac{p^{k}\,(1-p)^{(n-k)} n\,!}{k\,!\,(n-k)!}$$

Where -
n= sample size
k = event of interest
p = expected probability if null hypothesis is true
Y = the probability of observing k events
! = factorial value

(Adapted from (McDonald, 2009)).

The formula is computed for every possible outcome, including the observed value, values more than the observed value, and less than the observed value. The large number of calculations necessitates the use of a software package, and again graphpadinstat was used. P values are given for the chance of getting exactly the observed value, more than the observed value and less than the observed value, three values per comparison. Statistical significance was set at the 5% level, so values more than 0.975 and less than 0.025 were considered significant. Probability had to be above significance threshold across all three values for a given comparison to determine overall significant difference between the observed and predicted probabilities.

3.7.1.3 Formulae for predicted probability

Ho – there is no link between the distribution of trypanosome species within the sample population, therefore mixed infections should not occur at a significantly higher rate than expected by chance.

As described by (Willett, 1972), if infections with the three different species of trypanosome, in this case b (*T. brucei* s. l.), v (*T. vivax*), and c (T. *congolense* savannah), are randomly distributed in a sample population, eight mutually exclusive outcomes are possible when considering the infection status of an individual cow:

1. Triple infection with b, v, and c
2. Double infection with b and v
3. Double infection with b and c
4. Double infection with c and v
5. Single infection with b only
6. Single infection with v only
7. Single infection with c only
8. No infection with b, v, or c

The sum of all classes will naturally be equal to the total number of animals sampled. The relationship between the different species of trypanosome can be visualized by the following diagram:

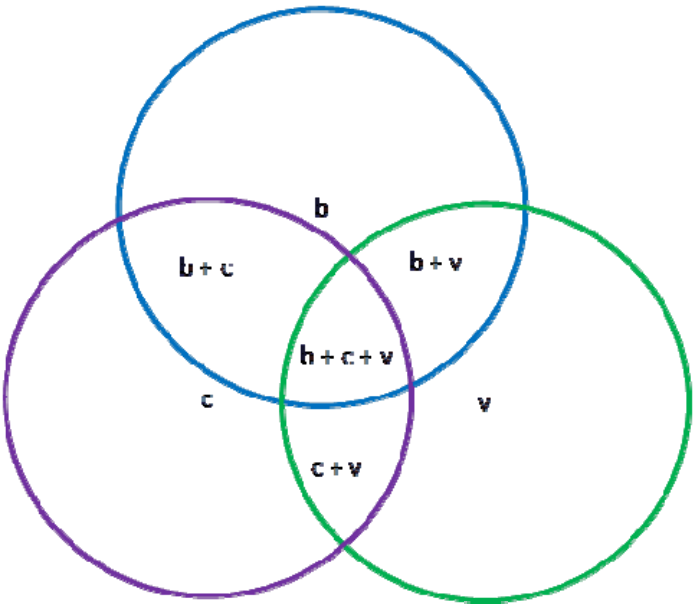

Figure 3.5 - graphical representation of the occurrence of mixed trypanosome infections within the sample population. b = *T. brucei* s. l., v =*T. vivax*, c= *T. congolense* savannah.

The probable frequency for each of the eight classes can be calculated in the following way, assuming the hull hypothesis to be correct and no associations between trypanosome species exist –

- p_b = the prevalence of *T. brucei* s. l.
- p_v = the prevalence of *T. vivax*
- p_c = the prevalence of *T. congolense* savannah

x = *T. brucei* s.l. only

y = *T. vivax* only

z = *T. congolense* savannah only

So the eight outcomes become -

1. x
2. y
3. z
4. xy
5. xz
6. yz
7. xyz
8. negative

The formula for the predicted probability of each class is given as –

1. $p_x = p_b - (p_{xy} + p_{xz} + p_{xyz})$
2. $p_y = p_v - (p_{xy} + p_{yz} + p_{xyz})$
3. $p_z = p_c - (p_{xz} + p_{yz} + p_{xyz})$
4. $p_{xy} = p_b\, p_v - p_{xyz}$
5. $p_{xz} = p_b p_c - p_{xyz}$
6. $p_{yz} = p_v p_c - p_{xyz}$
7. $p_{xyz} = p_b p_v p_c$
8. 1 – (the sum of all of the above)

Chapter 4 – African animal trypanosomiasis in Dokolo and Kaberamaido during SOS phase one

This chapter details the results of an investigation into the prevalence of *T. vivax* and *T. congolense* savannah during a mass cattle treatment programme that aimed to reduce the prevalence of *T. brucei* s. l. and *T. b. rhodesiense*. While these AAT agents were not the primary target of the treatment programme, the trypanocidal drugs are active against all species of trypanosome. High levels of *T. brucei* s. l. were detected during initial monitoring and evaluation, particularly in the districts of Dokolo and Kaberamaido, suggesting these areas were particularly well suited for the transmission of trypanosomiasis.

The fall in prevalence of *T. brucei* s. l. following the SOS intervention was not as marked in the districts of Dokolo and Kaberamaido as compared to other areas; the literature suggests that the presence of concurrent challenge from other trypanosome species may affect the efficacy of trypanocidal control programmes. If present these infections may contribute to the maintenance of *T. brucei* s. l., and may also have had a detrimental effect on cattle health. Cattle in poor health are more prone to adverse drug reactions, increasing the likelihood of treatment failure and negatively affecting treatment success.

This chapter presents the research aimed at discovering whether these trypanosome species are present, at what prevalence and to what extent they may be interacting with *T. brucei* s. l. in mixed infections. There is little data from Dokolo and Kaberamaido pertaining to AAT; however there are reports from neighboring districts and other locations in Uganda, East Africa and Sub-Saharan Africa from which parallels may be drawn, as reviewed in Chapter 1, section 1.1.5.1.

4.1 Chapter aims

Dokolo and Kaberamaido had already been identified as high transmission areas with regards to *T. brucei* s. l. Therefore the decision was taken, given limited time and resources, to screen only samples from Dokolo and Kaberamaido for the presence of *T. vivax* and *T. congolense* savannah. The high prevalence of *T. brucei* s. l. in these two districts indicates favorable conditions for trypanosomiasis transmission. Therefore this chapter particularly sets out to –

- To determine the presence and possible prevalence of *T. vivax* and *T. congolense* savannah in Dokolo and Kaberamaido districts of Uganda.
- To compare the prevalence of *T. congolense* savannah and *T. vivax* in Dokolo and Kaberamaido with that of *T. brucei* s. l. to assess the overall level of trypanosomiasis.
- To determine if cattle age, sex, breed or body condition score influence trypanosome infection in Dokolo and Kaberamaido.

By exploring these aims the central null hypothesis of this chapter is tested –

H_0 – the SOS control programme had no effect on the prevalence of *T. vivax* or *T. congolense* savannah in Dokolo and Kaberamaido.

4.2 Study design

Of the 23 villages selected across the entire SOS region using the criteria detailed in chapter two, 14 were located in Dokolo or Kaberamaido, as shown in Figure 4.1. These villages were sampled at baseline, and three, nine and eighteen months post treatment.

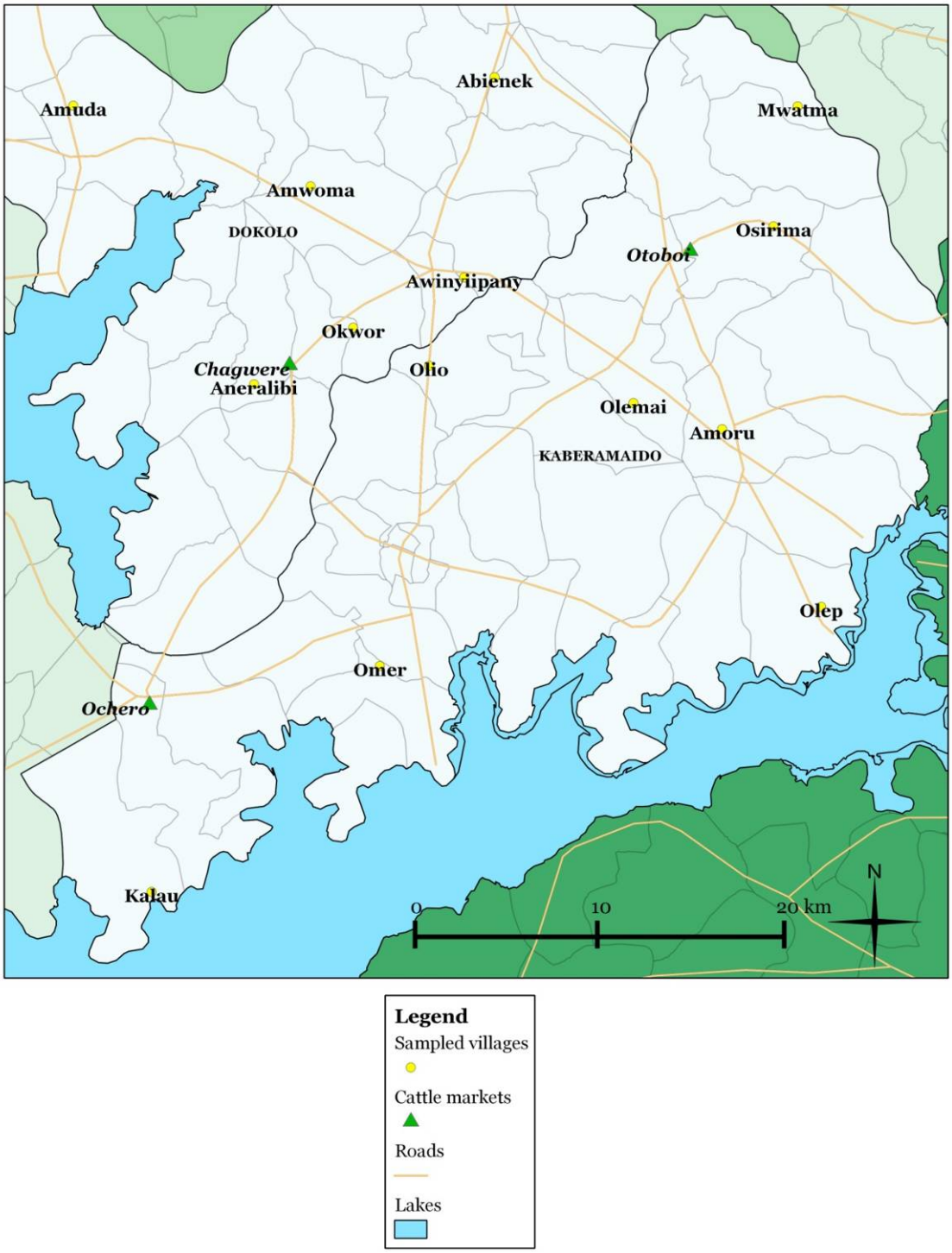

Figure 4.1 - location of village sampling sites in Dokolo and Kaberamaido visited during the monitoring of SOS phase one. The water mass shown is that of Lake Kyoga.

4.3 Summary of key characteristics of sampled cattle

For each cow sampled information the age, sex, location, body condition score and breed were made note of, as detailed in chapter two. In order to contextualize the information on trypanosome prevalence detailed in the results section, a summary of the general characteristics of the sampled cattle at each time point is found below.

4.3.1 Number of cattle sampled

The number of samples collected in each district can be seen in

Table 4.1.

	Dokolo	Kaberamaido
Baseline	200	310
3 months	486	717
9 months	600	714
18 months	343	637

Table 4.1 - total number of cattle sampled in Dokolo and Kaberamaido at each time point.

4.3.2 Age of cattle sampled

The field team estimated the age of each cow sampled, and accordingly classified it as A (<18 months), B (18-36 months), or C (36< months).

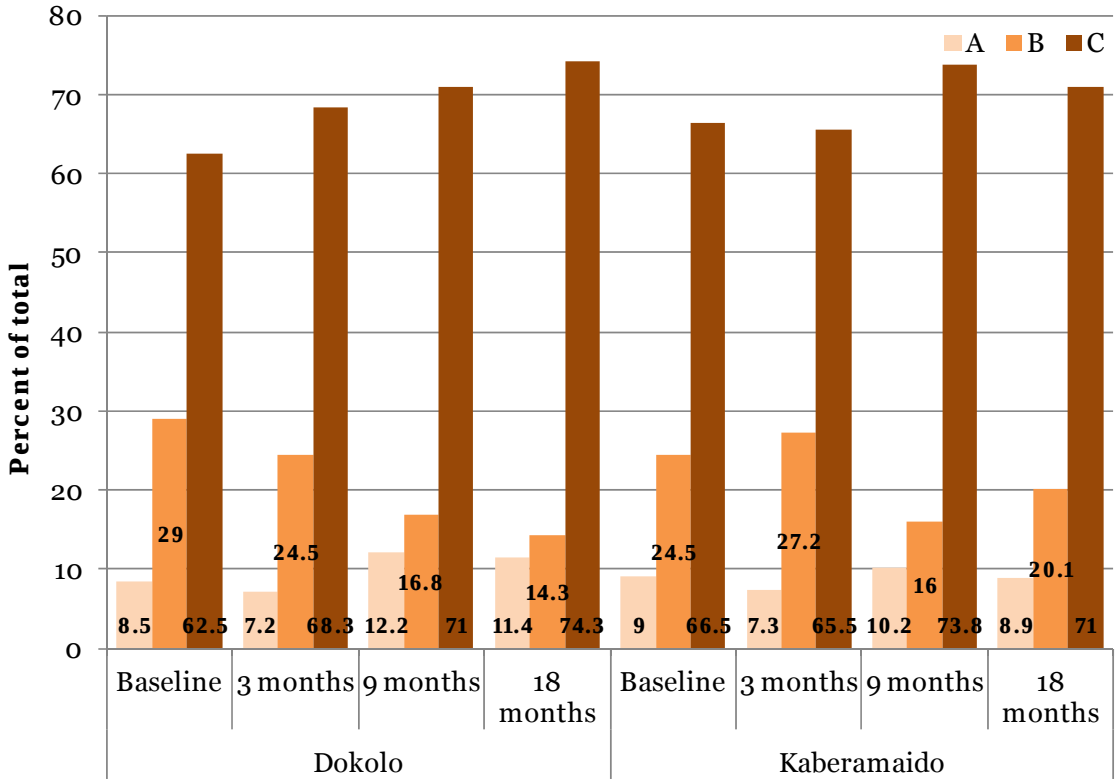

Figure 4.2 - percentage of the total sampled made up from animals in the A (<18 months), B (18-36 months), or C (>36 months) age groups at each time point in Dokolo and Kaberamaido.

As can be seen in Figure 4.2, the C age group was by far the most commonly sampled at each time point in both districts. The A age category was the least common at all time points, followed by the B category. Cattle in the A category become less likely to have been included in the mass treatment of October 2006, depending on the timing of their birth.

4.3.3 Breed

During sampling, the breed of each cow was recorded; previous studies have demonstrated that some breeds of cow are more susceptible to trypanosomiasis than others, e.g. - (Mwangi *et al.*, 1998). The number of breeds present at each time point and their relative abundance in the sample can be seen in Table 4.2.

		Breed (%)				
District	Time point	Ankole	Boran	Crossbreed	Zebu	Not given
Dokolo	Baseline	9.0	14.5	5.5	71.0	0.0
	3 months	8.8	0.0	3.3	87.9	0.0
	9 months	5.7	0.0	4.5	89.6	0.2
	18 months	6.4	0.0	1.5	91.8	0.3
Kaberamaido	Baseline	18.1	0.0	25.2	56.7	0.0
	3 months	13.9	0.0	4.4	81.7	0.0
	9 months	14.6	0.1	5.6	79.7	0.0
	18 months	18.7	0.2	3.3	77.8	0.0

Table 4.2 - breed of sampled animals in Dokolo and Kaberamaido at each of the sampling time points, shown as a percentage of the total.

The most common breed of cow in both Dokolo and Kaberamaido at all time points is the Zebu. After the Zebu, the Ankole breed is the second most common apart from at baseline, where in Dokolo a greater number of Boran cattle are recorded, and in Kaberamaido a high number of cross breeds are recorded. The presence of these breeds not usually found in the area at other time points could be due to the work of an NGO, of which several are active within the two districts. There were very few cattle for which breed could not be determined and was not given by the cattle owner.

The figures on the breed of cattle sampled at each time point illustrate time points are comparable to each other, as the proportion of each breed at each time point is in line with the other time points.

Zebu are the most abundant breed sampled across the intervention area, therefore statistical comparisons of the prevalence of trypanosome species between the different breeds becomes problematic. Due to the small number of cattle sampled in the other breeds there is not enough statistical power for meaningful analysis to be carried out. Further comparison of the trypanosome prevalence in each breed of cattle is not included due to small sample sizes in the majority of breed categories.

4.3.4 Sex

The sex of cattle sampled in Dokolo and Kaberamaido can been seen in Figure 4.3.

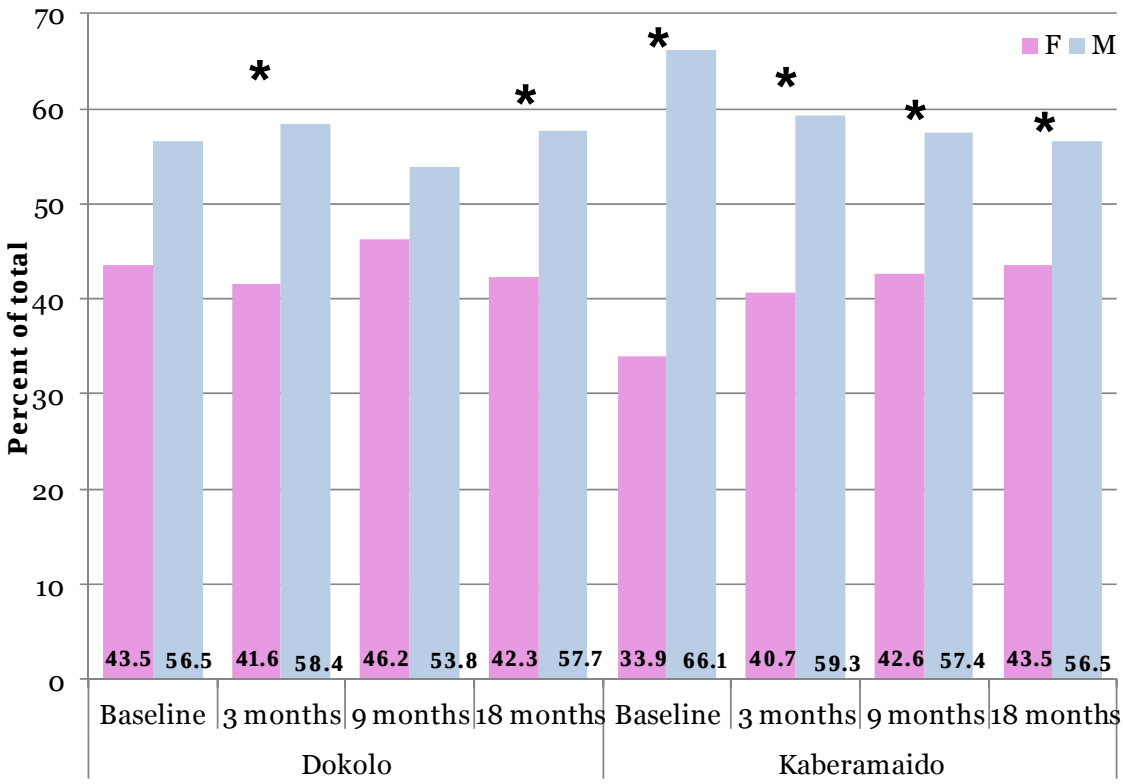

Figure 4.3 - sex of cattle sampled in Dokolo and Kaberamaido at each time point during SOS phase one monitoring. A star above a time point indicates a significant difference between the number of male (M) and female (F) animals sampled as determined by Fisher's Exact test ($p < 0.05$).

In each district at all time points males are more common than females. The number of males is significantly higher than the number of females in Dokolo at three months ($p = 0.0026$) and 18 months ($p = 0.02$). In Kaberamaido the number of males sampled are significantly greater than the number of females sampled at baseline ($p < 0.0001$), three months ($p < 0.0001$), nine months ($p = 0.0013$) and 18 months ($p = 0.0076$).

The only two time points were this is not significant, namely Dokolo at baseline ($p = 0.1412$) and nine months ($p = 0.1335$), where the ratio of males to females does not deviate significantly from an even split.

Although males made up a significantly greater proportion of the sample, this pattern remains consistent across all sample time points. Therefore comparisons across time points are valid and the data collected provides sufficient representation to assess the impact of the intervention. Reasons for the observed male to female ratio are examined in the discussion.

4.3.5 Body condition score

Body condition score is a useful indicator of overall herd health; in addition weight loss is a sign often associated with AAT, therefore body condition may correlate with trypanosome infection.

Although body condition scores across nine categories were originally collected, these nine categories have been collapsed into three for the following reasons.

Firstly, the body condition score can be said to be a subjective measurement in this context, as no physiological measurements such as weight, height or circumference were recorded. Guidelines for standardization of body condition score were used to make these observational measurements less subjective, but are still open to differing interpretations. By widening the definition of “lean” to include a greater range of physiological attributes more accuracy is introduced at the expense of sensitivity.

Secondly these guidelines were interpreted by a number of different people at each sampling time point; only one member of the field team responsible for assigning body condition scores was present throughout. Therefore the possibility of disagreement between assessors when rating an animal’s body condition score might be anticipated on a detailed subjective scale such as the one used here.

Thirdly, with nine variables very few animals fell into the categories at the extremes of the scale, and so numbers sampled in those categories were too small for meaningful comparisons or statistical analysis to be made.

Therefore, in order to maximize the usability of this information, it was decided to reduce the resolution and consolidate the modifiers onto the central category. The L - , L, and L + descriptors were collapsed to form a larger, general lean category, as were the medium and fat categories. The body condition score of cattle in Dokolo and Kaberamaido is shown in Figure 4.4

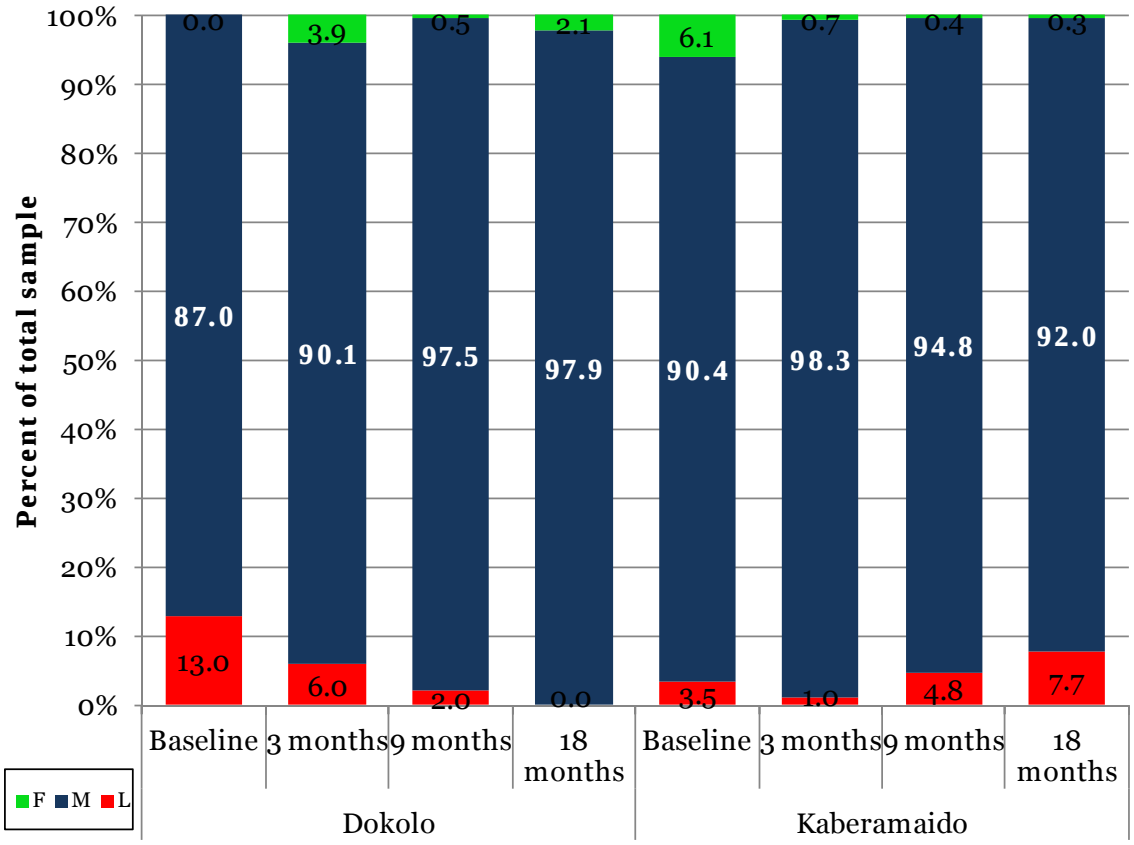

Figure 4.4 - consolidated body condition scores for cattle sampled in Dokolo and Kaberamaido at each of the time points. Cattle categorised as fat (F) are shown in green, medium(M) as blue and lean (L) as red.

As shown in Figure 4.4 at any given time point comparatively few cattle fall into the lean or fat categories.

In Dokolo the number of cattle classed as lean steadily declines from a baseline of 13% to 6% at three months 2% at nine months and 0% at eighteen months. The highest number of fat cattle is recorded at the 3 month time point (3.9%).

In Kaberamaido the number of lean cattle falls slightly from baseline to three months, but at nine and eighteen months slight increases were observed. Very few cattle were classified as fat beyond the baseline time point. None of these small changes were statistically significant, as such the body condition score of the sample remains constant across time points, illustrating these time points are comparable to one another.

For body condition score too few cattle fall into the lean or fat categories to allow meaningful statistical analysis. Further analysis of trypanosome prevalence in the different body condition categories is excluded due to the small sample size in the majority of categories.

4.4 Overview of previously collected *T. brucei* s. l. prevalence data

A comprehensive analysis of the prevalence of *T. brucei* s. l. and *T. b. rhodesiense* has already been conducted and included in the thesis of Selby 2011. Data particularly relevant to the work done in this thesis, i.e. pertaining to Dokolo and Kaberamaido, as opposed to the SOS region as a whole, is included in this section so comparisons may easily be drawn. The PCR protocol and sample preparation methods are exactly as described in chapter two, and were carried out by R. Selby during 2007 – 2009.

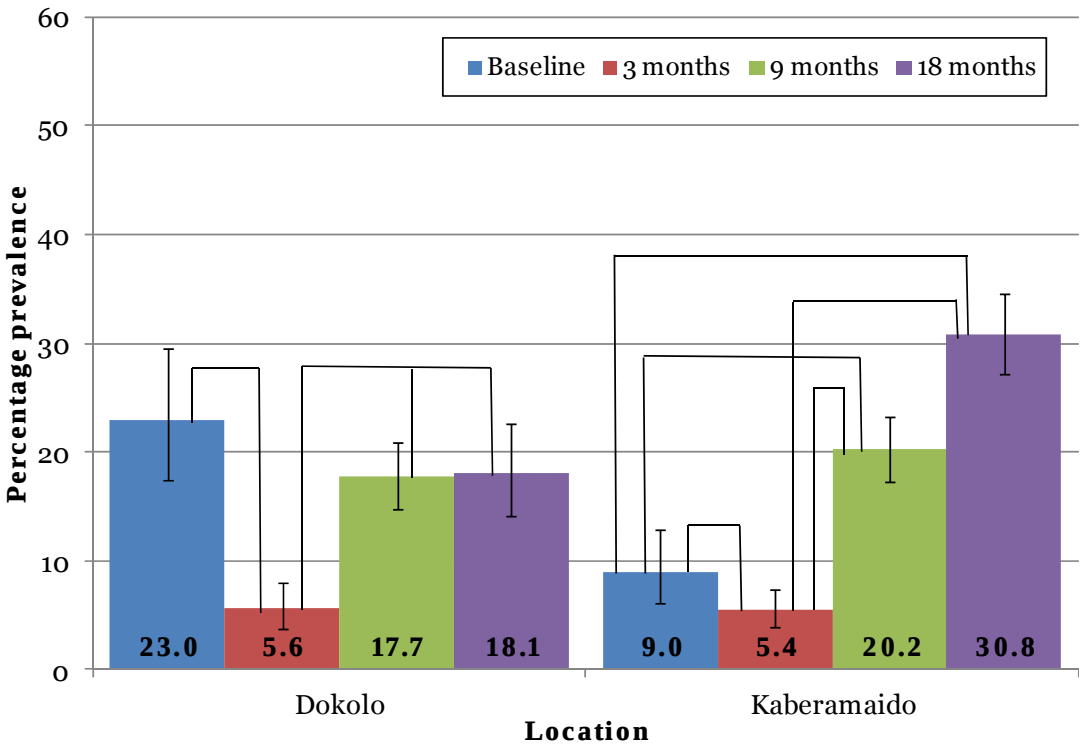

Figure 4.5 - prevalence of *T. brucei* s. l. in Dokolo and Kaberamaido at each of the sampling time points; graph produced using data from (Selby, 2011). The vertical lines transecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two as determined by Fisher's Exact test.

As shown in Figure 4.5, at baseline the prevalence of *T. brucei* s. l. was 23.0% (46/200, 95% CI 22.5 – 43.9%) in Dokolo and 9.0 % (28/310, 95% CI 6.1 – 12.8%) in Kaberamaido.

At the three month sampling a significant drop in the prevalence of *T. brucei* s. l. had occurred, to 5.6 %(27/486, 95% CI 3.69 – 7.98%, $p < 0.0001$) in Dokolo and 5.4% (39/717, 95% CI3.9 – 7.4%, $p = 0.03$) in Kaberamaido.

At the nine month sampling there had been a significant rise in the prevalence of *T. brucei* s. l. prevalence, to 17.7% (106/600, 95% CI 14.7 – 21%, $p < 0.0001$) in Dokolo and 20.2 %(144/714, 95% CI 17.3 -23.3%, $p < 0.0001$) in Kaberamaido. The nine month prevalence of 17.7 % in Dokolo was insignificantly different from the 23.0%

detected at baseline (p = 0.09). In Kaberamaido the nine month prevalence of 20.2% represented a significant increase from the baseline prevalence of 9% (p < 0.0001).

At the 18 month sampling the prevalence of *T. brucei* s. l. had risen slightly in Dokolo to 18.1% (62/343, 95% CI 14.2 – 22.6%, p = 0.8601). Prevalence had risen considerably in Kaberamaido to 30.8% (196/637, 95% CI 27.2 – 34.5%, p < 0.0001). In Dokolo the 18 month prevalence of 18.1% was insignificantly different from the baseline prevalence of 23.0% (p = 0.1815), but significantly greater than the 5.6% detected at the three month sampling (p < 0.0001). In Kaberamaido the 18 month prevalence of 30.8% was significantly greater than the prevalence detected at baseline (p < 0.0001) or three month (p < 0.0001) sampling, having more than tripled since monitoring began.

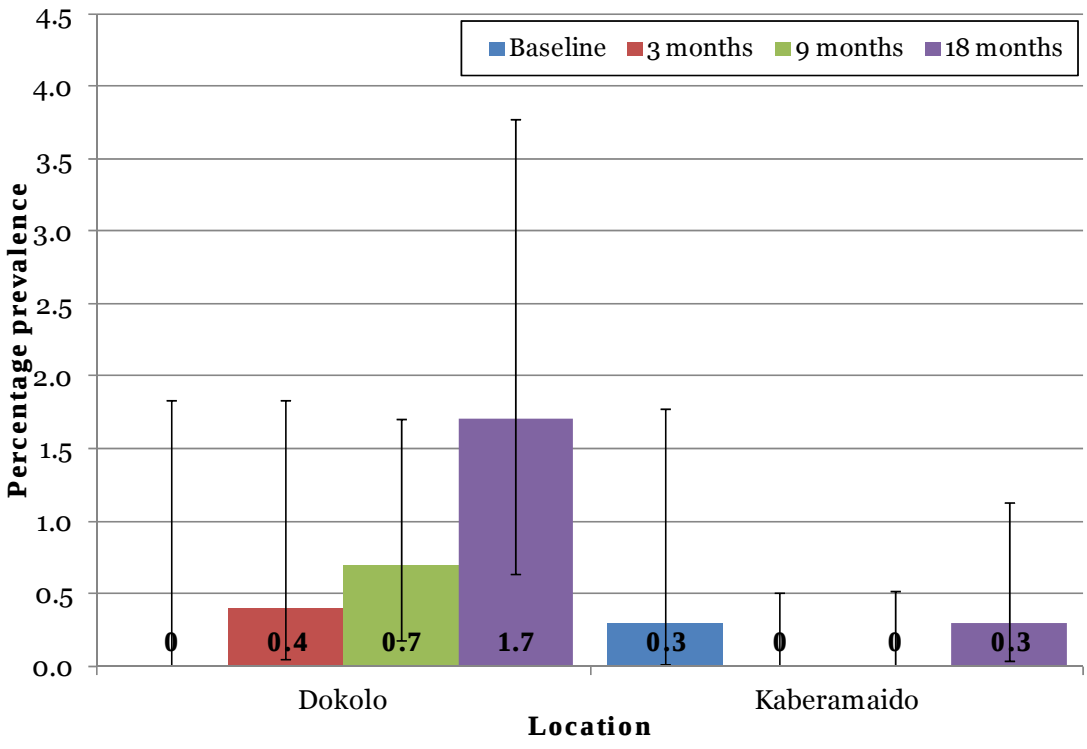

Figure 4.6 - percentage prevalence of *T. b. rhodesiense* in Dokolo and Kaberamaido at each of the sampling time points; graph produced using data from (Selby, 2011). The vertical lines transecting each bar represent the 95% confidence intervals.

As depicted in Figure 4.6, at baseline no *T. b. rhodesiense* was detected in Dokolo (0%, 0/200, 95% CI 0 - 1.83), while in Kaberamaido 0.3% (1/310, 95% CI 0.01 – 1.8%) of cattle were found to be infected.

At three month sampling the prevalence of *T. b. rhodesiense* had risen insignificantly to 0.4% (2/486, 95% CI 0.05 – 1.48%, p = 1) in Dokolo, and fallen insignificantly to 0% in Kaberamaido (0/717, 95% CI 0 – 0.51% p = 0.3019).

At nine month sampling prevalence of *T. b. rhodesiense* has risen insignificantly in Dokolo to 0.7% (4/600, 95% CI 0.18 – 1.7%, p = 0.7), while in Kaberamaido it remained at 0% (0/714, 95% CI 0 – 0.52, p = 1).

At the 18 month sampling the prevalence had again risen insignificantly in Dokolo to 1.7% (6/343, 95% CI 0.64 – 3.8%, p =0.09), and also risen insignificantly in Kaberamaido to 0.3% (2/637, 95% CI 0.04 – 1.1%, p = 1). While there is no statistical significance in any of the rises in *T. b. rhodesiense* prevalence recorded here, it is worrying to note the steady incremental rise in the prevalence of this zoonosis in cattle in Dokolo, and hypothesize whether or not these increases could be of biological significance given the continued occurrence of HAT in the region.

4.5 Results of AAT retrospective screening

A large number of samples were analysed generating a wealth of data to be explored in the following sections. The first section will combine the results from all PCRs conducted to give the prevalence of animals found to be positive for one or more of the trypanosome species under investigation, *T. vivax* and *T. congolense* savannah and *T. brucei* s. l. After this overview the prevalence of *T. vivax* and *T. congolense* savannah will be looked at individually. Following that, the effect of cattle age and sex on the prevalence of *T. vivax* and *T. congolense* savannah will be examined.

4.5.1 Overall trypanosomiasis

The results from the retrospective screening and original *T. brucei* s. l. screening were combined to give the overall level of infection with any species of trypanosome. Infection with a single species of trypanosome made up the majority of parasitic events, however there were numerous instances of individual cows infected with more than one species of trypanosome. These multiple infections have been taken to account, as evidenced by the fact prevalence of overall trypanosomiasis is less than the sum of all *T. brucei* s. l., *T. vivax* and *T. congolense* savannah infections. The species of trypanosome involved in mixed infections is covered in detail in chapter six. This section deals with the prevalence of trypanosomiasis within the cattle in terms of positive individual cows, regardless of how many species of trypanosome each cow may be infected with.

At baseline sampling in Dokolo the prevalence of animals testing positive for one or more species of trypanosome was 27.5% (55/200, 95% CI 21.44 – 34.24%). In Kaberamaido the prevalence was 18.4% (57/310, 95% CI 14.45 – 23.09%). There was significantly more trypanosomiasis in Dokolo than in Kaberamaido ($p = 0.01$)

In Dokolo three months post treatment a significant drop in prevalence of trypanosomiasis occurred, to 10.3% (50/487, 95% CI 7.72 – 13.31%, $p < 0.0001$). In Kaberamaido the prevalence of trypanosomiasis fell slightly, but not by a significant amount to 17.6% (126/717, 95% CI 14.86 – 20.56%, $p = 0.79$). At this time point overall trypanosomiasis was significantly higher in Kaberamaido than in Dokolo ($p = 0.0005$).

In Dokolo at nine months the prevalence had risen to 29.5% (177/600, 95% CI 25.88 – 33.33%), making it insignificantly ($p = 0.65$) different from the 27.5% detected at baseline, but extremely significantly different ($p < 0.0001$) from the 10.3% detected at three months. Similarly in Kaberamaido the prevalence had risen significantly to 28.2% (201/714, 95% CI 24.88 – 31.61%), significantly exceeding the prevalence detected at baseline ($p < 0.0001$) and three months ($p < 0.0001$). At the nine months time point there was no significant ($p = 0.6246$) difference between trypanosomiasis prevalence in Dokolo and Kaberamaido.

Eighteen months post treatment in Dokolo prevalence had fallen slightly to 25.7% (85/331, 95% CI 21.26– 30.65%). This prevalence was significantly higher than that detected at three months ($p < 0.0001$), but not significantly different from that detected at baseline ($p = 0.68$) or nine months (0.2239) . In Kaberamaido the prevalence had risen to 43.8% (270/617, 95% CI 39.8 – 47.78%). This rise made the prevalence detected at eighteen months significantly higher than baseline ($p < 0.0001$), three months ($p < 0.0001$) or nine months ($p < 0.0001$). At the eighteen month time point the prevalence of overall trypanosomiasis was significantly ($p< 0.0001$) higher in Kaberamaido than in Dokolo.

The information in the preceding four paragraphs is summarised in Figure 4.7 for clarity and ease of comparison.

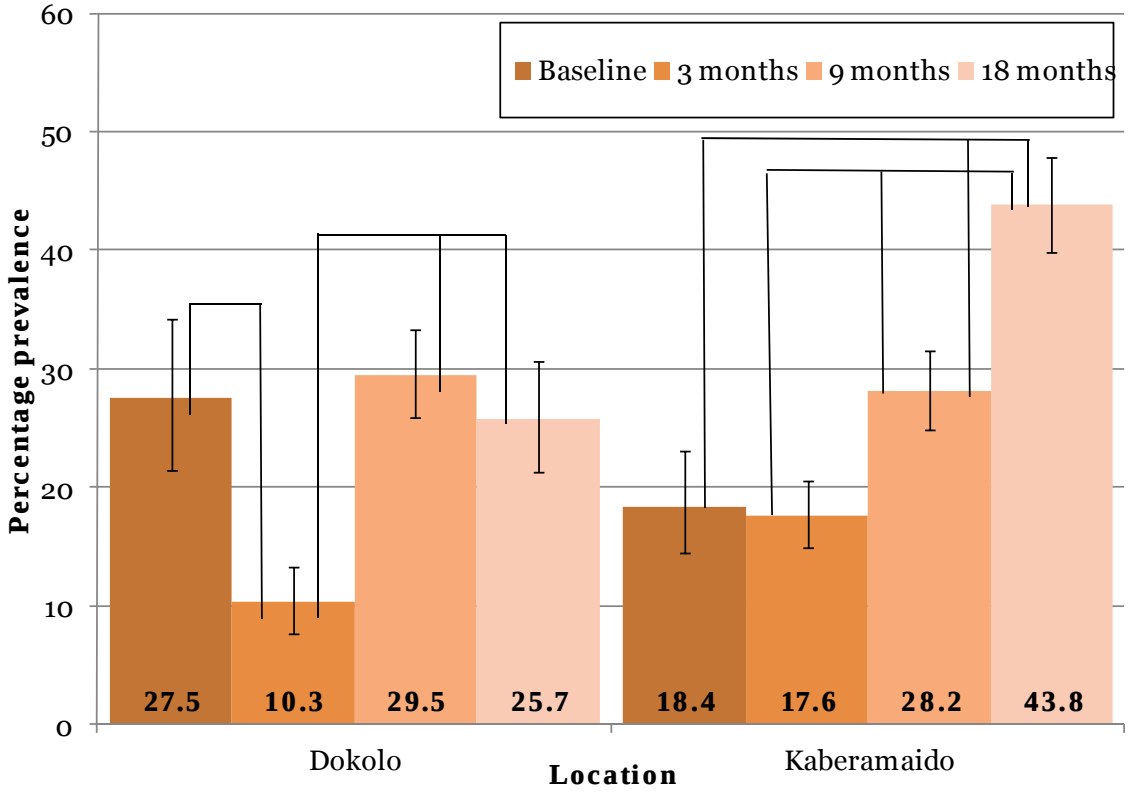

Figure 4.7 - percentage prevalence of overall trypanosomiasis detected at each of the four sampling time points in Dokolo and Kaberamaido. Vertical lines transecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two as determined by Fisher's Exact test ($p < 0.05$).

4.5.1.1 Age and overall trypanosomiasis

The prevalence of trypanosomiasis in each age group varied by time point and by district. There was no evidence of a distinct pattern; the A, B and C age groups have the highest prevalence at different time points. This could be due to different species of trypanosome having different patterns of age and infection. This is summarised in Figure 4.8.

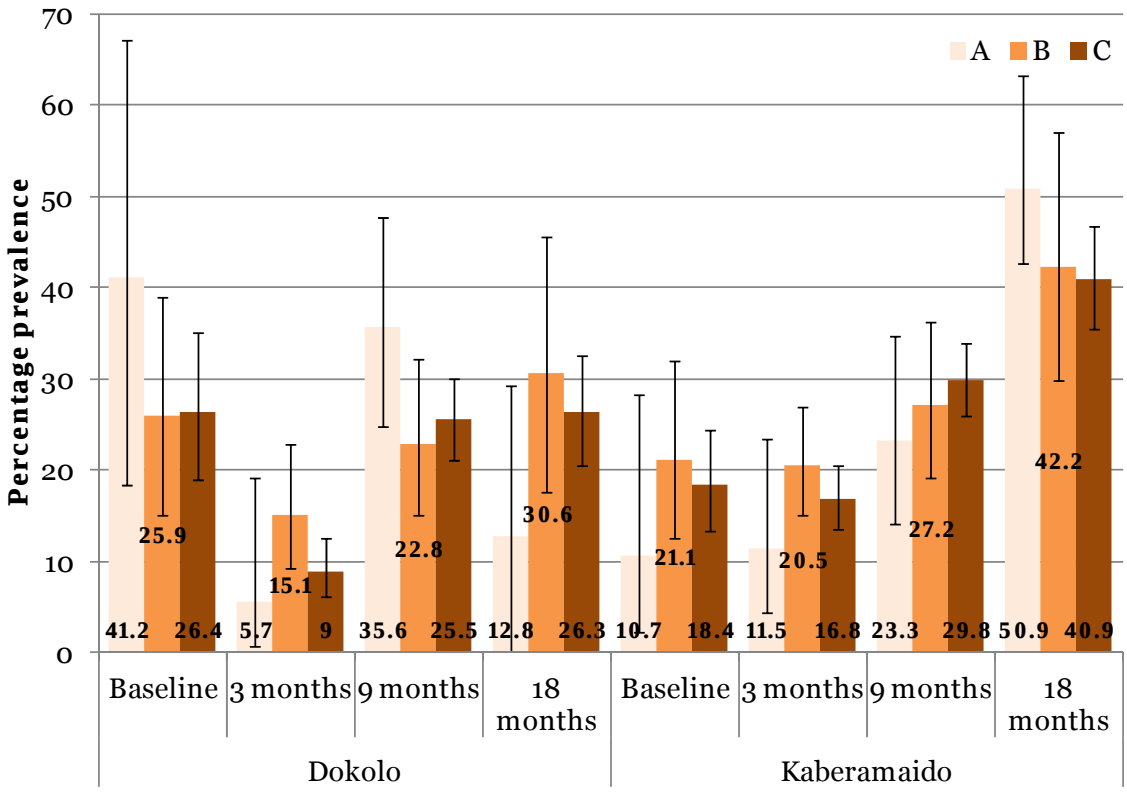

Figure 4.8 - prevalence of trypanosomiasis in cattle in each of the A (<18 months), B (18-36 months), C (>36 months). Vertical lines dissecting the bars show the 95% confidence intervals.

An analysis of age in relation to individual species of trypanosome is conducted in section 4.5.2 for *T. vivax* and section 4.5.3 for *T. congolense* savannah.

4.5.1.2 Sex and overall trypanosomiasis

The prevalence of trypanosome infection in male and female cattle fluctuates slightly at each time point, but a significant difference in prevalence between the two sexes was

not observed at any point. The level of trypanosomal infection in male and female cattle is plotted on Figure 4.9.

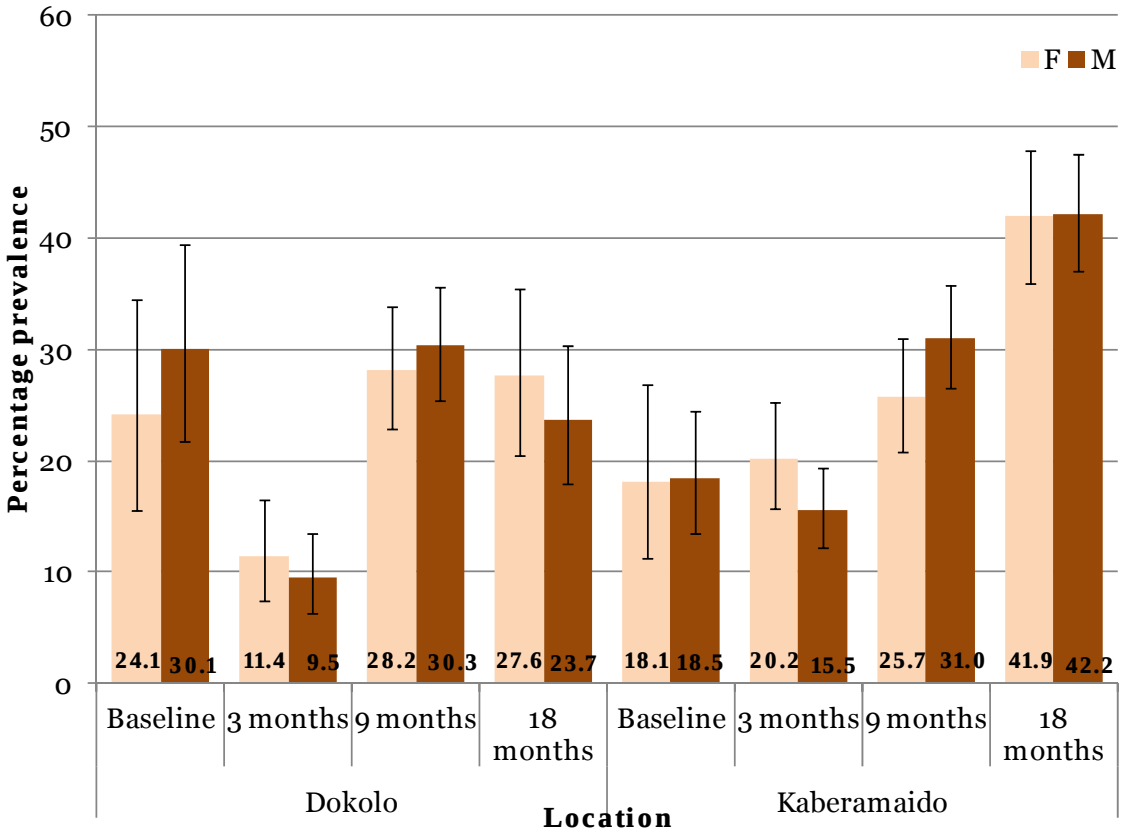

Figure 4.9 - prevalence of overall trypanosomiasis in male (M) and female (F) cattle in Dokolo and Kaberamaido at each of the sampling time points. Vertical lines transecting each bar represent the 95% confidence intervals.

4.5.1.3 Comparison of the prevalence of each species of trypanosome

As previously mentioned, overall trypanosomiasis figures consist of the total number of cattle infected with one or more of *T. brucei* s. l., *T. b. rhodesiense*, *T. vivax* or *T. congolense* savannah. Having already illustrated the combined figures, the prevalence of each individual species of trypanosome at each time point is considered in this section, plotted alongside each other to aid comparison. *T. brucei* s. l. and *T. b. rhodesiense* will not be compared to each other statistically, as the *T. brucei* s. l. prevalence encompasses all of the *T. b. rhodesiense* positives.

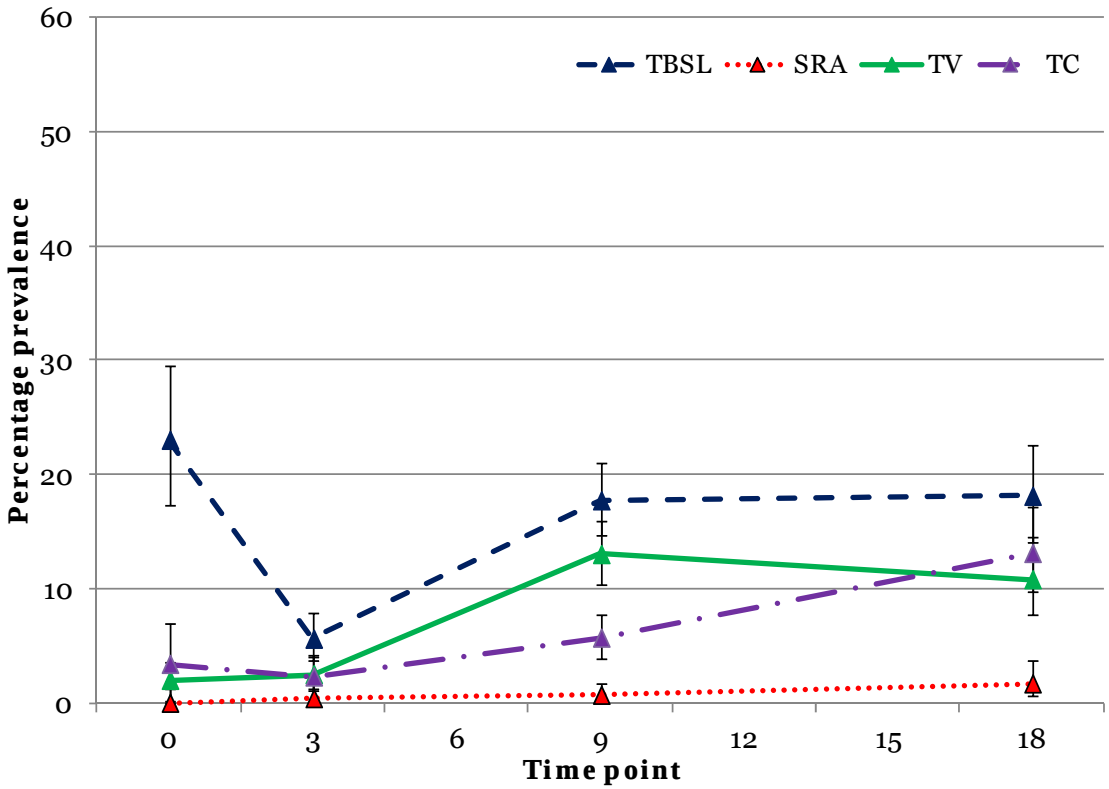

Figure 4.10 - prevalence of each species of trypanosome detected at each time point in Dokolo. Vertical lines dissecting each point represent the 95% confidence intervals. TBSL = *T. brucei* s. l., SRA = *T. b. rhodesiense*, TV = *T. vivax*, TC = *T. congolense* savannah.

In Dokolo *T. brucei* s. l. was the most commonly detected trypanosome at baseline, as shown in Figure 4.10. 23.0% (46/200, 95% CI 22.5 – 43.9%) of animals were positive for this species, significantly more than the percentage of animals positive for *T. vivax* (2%, 4/200, 95% CI 0.12 – 3.57%, p < 0.0001) or *T. congolense* savannah (3.5 % 7/200, 95% CI 1.42 – 7.08%, p < 0.0001). There was no significant difference in the prevalence of *T. vivax* and *T. congolense* savannah (p = 0.54). The prevalence of *T. congolense* savannah is significantly (p = 0.02) higher than that of *T. b. rhodesiense*, the prevalence of *T. vivax* is not (p = 0.12).

At the three month time point *T. brucei* s. l. is still the most commonly detected species (Figure 4.10). 5.6 %(27/486, 95% CI 3.69 – 7.98%) of cattle tested positive for *T. brucei* s. l., significantly more than tested positive for *T. vivax* (2.5%, 12/474, 95% CI 1.28 - 4.27% p = 0.02) or *T. congolense* savannah (3.5 % 7/200, 95% CI 1.42 – 7.08% p

= 0.01). There was no significant difference between the prevalence of *T. vivax* and *T. congolense* savannah (p = 0.84). The prevalence of *T. vivax* was significantly (p = 0.01) higher than that of *T. b. rhodesiense*, (0.4% 2/486, 95% CI 0.05 – 1.48%), as was the prevalence of *T. congolense* savannah.

At the nine month time point 17.7% (106/600, 95% CI 14.7 – 21.0%) of cattle tested positive for *T. brucei* s. l., making it the most commonly detected species of trypanosome again. The prevalence of *T. brucei* s. l. was significantly higher than that of *T. vivax* (13%, 78/600, 95% CI 10.41 – 15.96%, p = 0.03) or *T. congolense* savannah (5.7%, 34/600, 95% CI 3.96 – 7.83%, p < 0.0001). The prevalence of *T. vivax* was significantly higher than the prevalence of *T. congolense* savannah (p < 0.0001). The prevalence of both *T. vivax* and *T. congolense* savannah was significantly higher than that of *T. b. rhodesiense* (0.7%, 4/600, 95% CI 0.18 – 1.7%, p < 0.0001).

At the eighteen month sampling *T. brucei* s. l. was again the most common species of trypanosome detected, with 18.1% (62/343, 95% CI 14.2 – 22.6%) of cattle positive. The prevalence of *T. brucei* s. l. is significantly higher than that of *T. vivax* (10.8%, 37/343, 95% CI 7.71 - 14.56%, p = 0.009), but not significantly different to the prevalence of *T. congolense* savannah (13.1%, 45/343, 95% CI 9.73 – 17.16%, p = 0.09). The prevalence of both *T. vivax* and *T. congolense* were both significantly higher than the prevalence of *T. b rhodesiense* (1.7%, 6/343, 95% CI 0.64 – 3.8%, p < 0.0001).

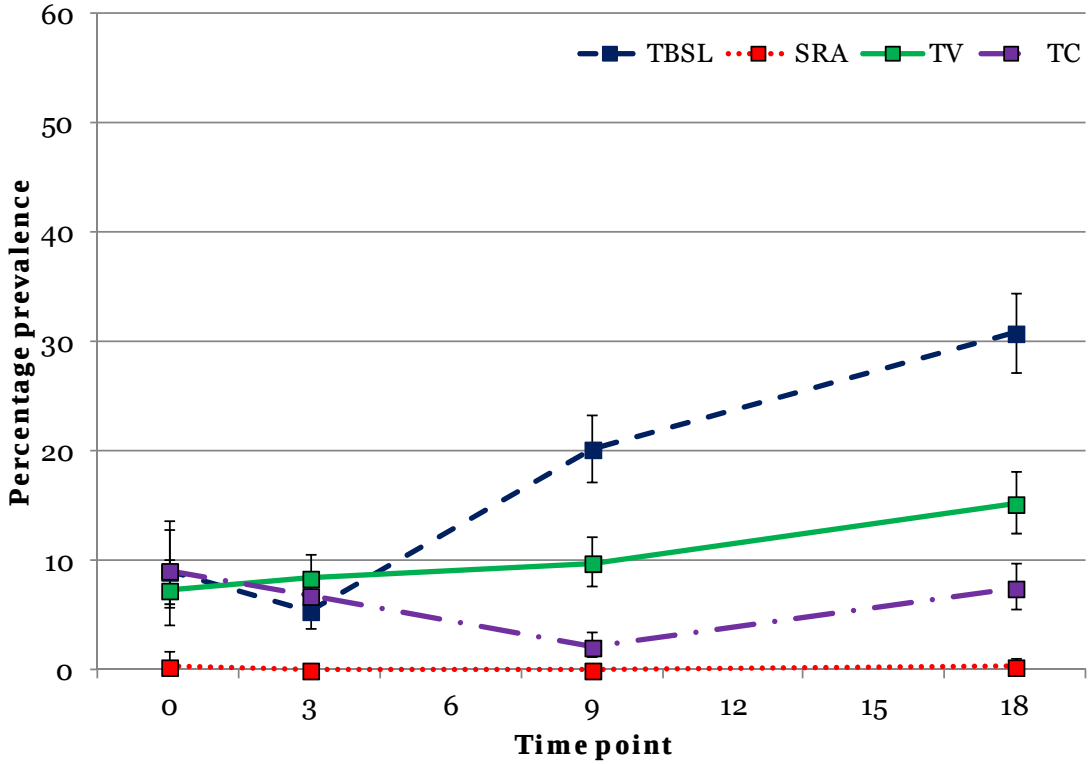

Figure 4.11 - prevalence of each species of trypanosome detected at each time point in Kaberamaido. Vertical lines dissecting each point represent the 95% confidence intervals. TBSL = *T. brucei* s. l., SRA = *T. b. rhodesiense*, TV = *T. vivax*, TC = *T. congolense* savannah.

As shown in Figure 4.11, at the baseline in Kaberamaido the prevalence of *T. brucei* s. l. and *T. congolense* savannah were near identical, with 9.0 % (28/310, 95% CI 6.1 – 12.8%) and 9.1% (21/230, 95% CI 5.7 – 13.6%) detected respectively, p = 1. There was no significant difference between the prevalence of *T. brucei* s. l. and that of *T. vivax* (7.3%, 21/310, 95% CI 4.24 – 10.17%, p = 0.37). There was no significant difference between the prevalence of *T. vivax* and *T. congolense* savannah, and both were significantly greater than *T. b. rhodesiense* (0.3%, 1/310, 95% CI 0.01 – 1.8%, p < 0.0001).

At three month sampling *T. vivax* was the most commonly detected species of trypanosome, with a prevalence of 8.4% (60/657, 95% CI 6.45 – 10.64%). There was no significant difference in the prevalence of *T. vivax* when compared to *T. congolense* savannah (6.8% 49/717, 95% CI 5.1 – 8.93%, p = 0.32). There was no significant

difference in the prevalence of *T. brucei* s. l. (5.4%, 39/717, 95% CI 3.9 – 7.4%) when compared to *T. congolense* savannah (p = 0.32). However the prevalence of *T. vivax* was significantly higher than that of *T. brucei* s. l. (p = 0.03). The prevalence of both *T. vivax* and *T. congolense* savannah was significantly higher than that of *T. b. rhodesiense* (0%, 0/717, 95% CI 0 – 0.51%, p < 0.0001).

At nine month sampling 17.7% (106/600, 95% CI 14.7 – 21%) of cattle were *T. brucei* s. l. positive, making it the most commonly detected species of trypanosome. The prevalence of *T. brucei* s. l. was significantly higher than that of *T. vivax (*9.8% 70/714, 95% CI 7.72 – 12.22%, p < 0.0001) or *T. congolense* savannah (2.1%, 15/714, 95% CI 1.18 – 3.44%, p < 0.0001). *T. vivax* prevalence was significantly higher than the prevalence of *T. congolense* savannah (p< 0.0001). Both *T. congolense* savannah and *T. vivax* had significantly higher prevalence than *T. b. rhodesiense* (0% 0/714, 95% CI 0 – 0.52 p < 0.0001).

At eighteen month sampling *T. brucei* s. l. was the most common species of trypanosome, with a prevalence of 30.8% (196/637, 95% CI 27.2 – 34.5%). This was significantly greater than that of either *T. vivax* (15.2%, 97/637, 95% CI 12.53 – 18.26%, p < 0.0001) or *T. congolense* savannah (7.5% 48/637, 95% 5.61 – 9.87%, p < 0.0001). The prevalence of *T. vivax* was significantly higher than that of *T. congolense* savannah (p < 0.0001), and both *T. congolense* savannah and *T. vivax* prevalence was significantly higher than that of *T. b. rhodesiense* (0.3%, 2/637, 95% CI 0.04 – 1.1%, p < 0.0001).

4.5.2 *T. vivax*

At baseline sampling 2% (4/200, 95% CI 0.12 – 3.57%) of animals in Dokolo tested positive for *T. vivax*, while in Kaberamaido 7.3% (21/310, 95% CI 4.24 – 10.17%) were infected. The prevalence of *T. vivax* was significantly higher in Kaberamaido than in Dokolo (p = 0.0191).

At three month sampling the prevalence in Dokolo remained relatively static at 2.5% (12/474, 95% CI 1.28 - 4.27%), which was not significantly (p = 1) different from the baseline prevalence. In Kaberamaido the prevalence of *T. vivax* rose slightly to 8.4% (60/657, 95% CI 6.45 – 10.64%), a rise which was not statistically significant (p = 0.4497). Again the prevalence *of T. vivax* was significantly higher in Kaberamaido than in Dokolo (p < 0.0001).

At the nine month time point in Dokolo the percentage of infected cattle had risen to 13% (78/600, 95% CI 10.41 – 15.96%). This rise was significantly higher than baseline (p < 0.0001) or three months (p < 0.0001). In Kaberamaido the *T. vivax* prevalence was 9.8% (70/714, 95% CI 7.72 – 12.22%). This was not significantly different from the prevalence detected in this district either at baseline (p = 0.1221) or three months (p = 0.3588). At nine months the difference in *T. vivax* prevalence between Dokolo and Kaberamaido is no longer statistically significant (p = 0.0796).

In Dokolo at the 18 month sampling prevalence had dropped a little to 10.8% (37/343, 95% CI 7.71 - 14.56%). This decrease meant the prevalence of *T. vivax* was still significantly higher than that detected at baseline (p < 0.0001) and three months (p < 0.0001), but that it was not significantly (p = 0.3525) different from the nine month prevalence. The Kaberamaido *T. vivax* prevalence had increased to 15.2% (97/637, 95% CI 12.53 – 18.26%), which is significantly higher than at baseline (p = 0.0001), three months (p < 0.0001) or nine months (p = 0.0028). At eighteen months the difference between the two districts is not statistically significant (p = 0.0637).

The information in the preceding four paragraphs is summarised in Figure 4.12 to aid clarity and comparison.

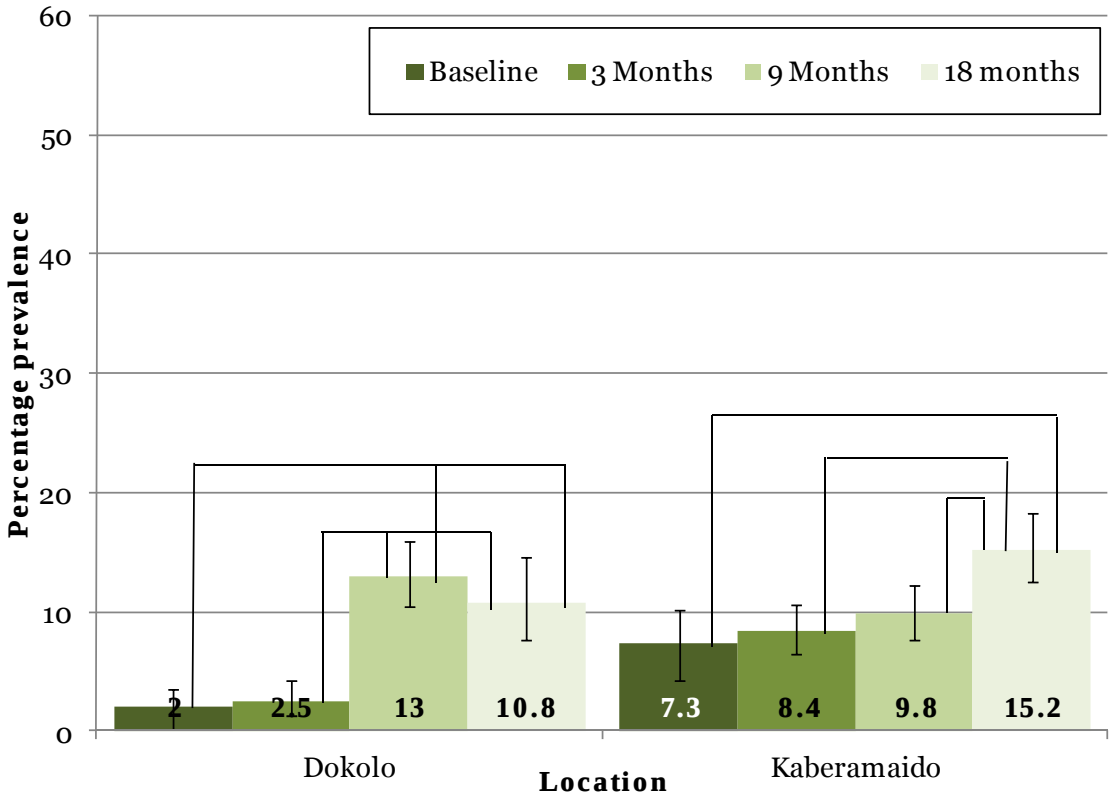

Figure 4.12 - prevalence of *T. vivax* detected in Dokolo and Kaberamaido at each of the sampling time points. Vertical lines transecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two as determined by Fisher's exact test ($p < 0.05$).

4.5.2.1 Age and *T. vivax* infection

The percentage prevalence of *T. vivax* found in each of the three age groups is plotted on Figure 4.13.

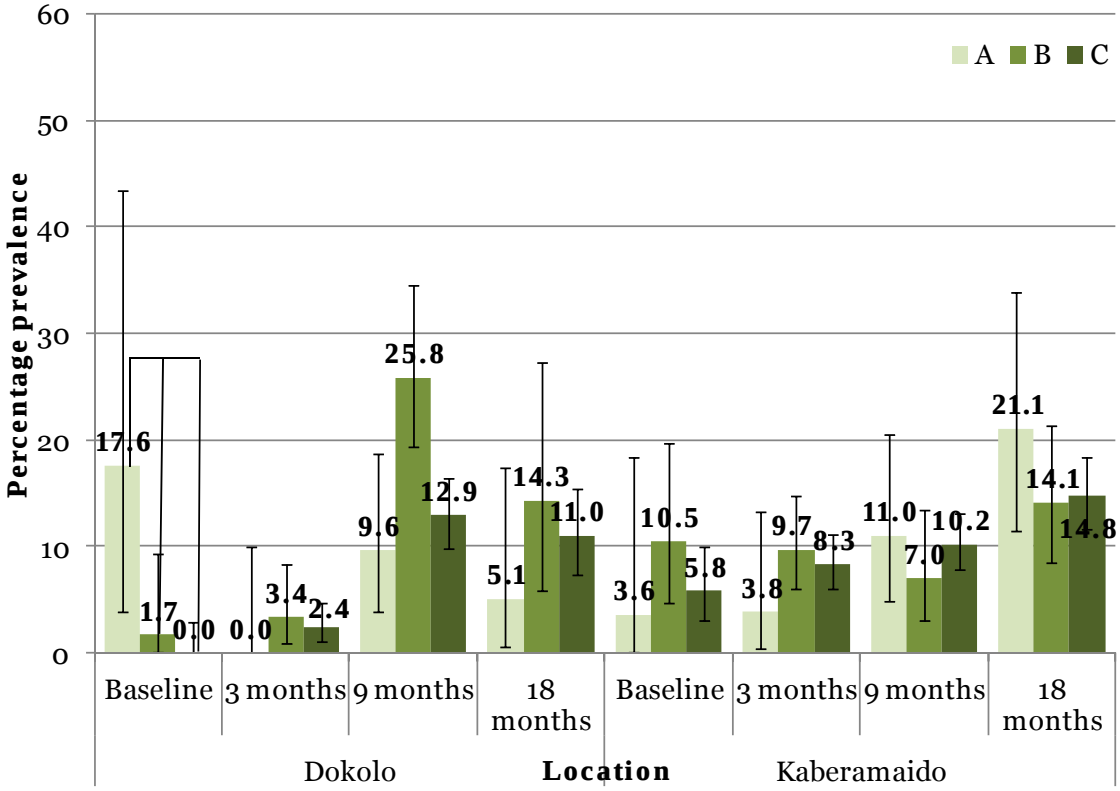

Figure 4.13 - prevalence of *T. vivax* in each of the three age categories in Dokolo and Kaberamaido at each sampling time point; A (<18 months), B (18-36 months) and C (36> months). Vertical lines transecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two as determined by Fisher's Exact test ($p < 0.05$).

As can be observed in Figure 4.13, in Dokolo at baseline a statistically significant pattern exists in the distribution of *T. vivax* in cattle of different ages. At this time point cattle in the A age group (<18 months) have a significantly higher prevalence of *T. vivax* than those in the B or C age groups. After the treatment this pattern is lost, and was not demonstrated again during the period of monitoring. In Kaberamaido there were no statistically significant differences in the prevalence of *T. vivax* in each age group. While the differences in prevalence are not statistically significant there is a perhaps important trend which remains constant across all districts at all time points; that is, the highest prevalence of *T. vivax* never occurs in the adult (C) age group. While this is not statistically significant it may be clinically relevant.

4.5.2.2 Sex and *T. vivax* infection

The percentage of male and female cattle infected with *T. vivax* is shown in Figure 4.14.

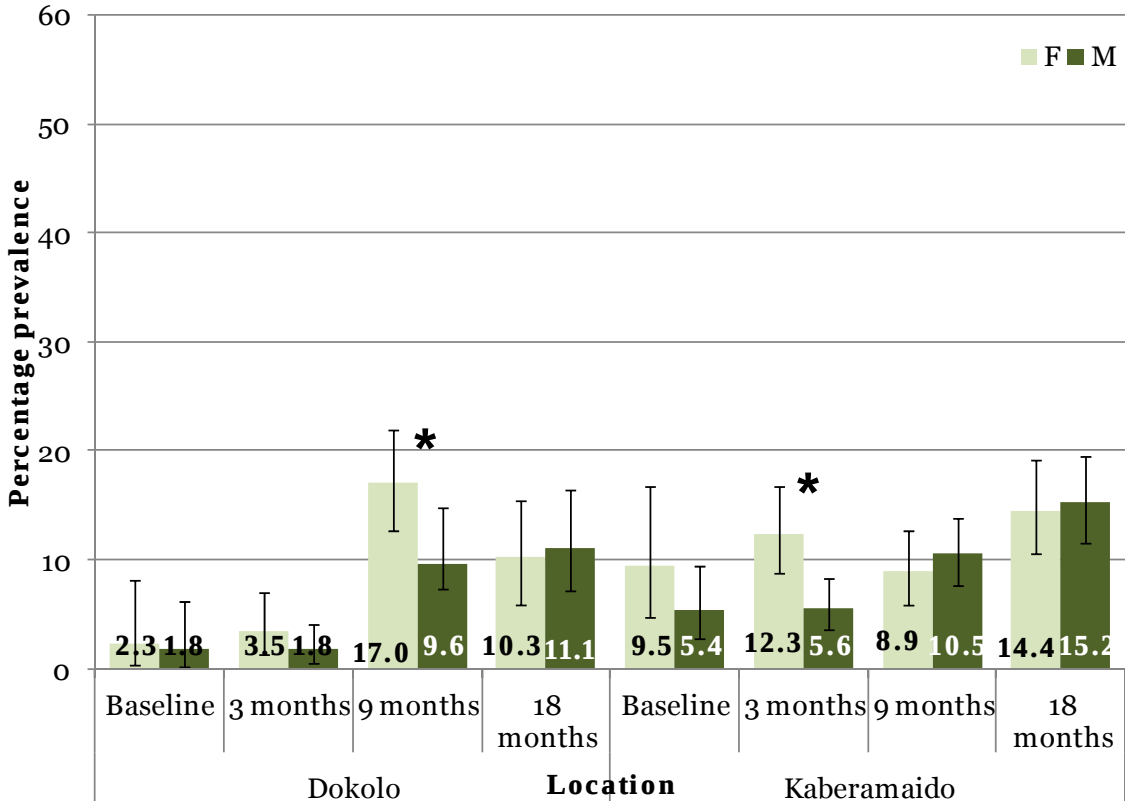

Figure 4.14 - prevalence of *T. vivax* in male (M) and female (F) cattle in Dokolo and Kaberamaido at each of the sampling time points. Vertical lines transecting each bar represent the 95% confidence intervals. Stars above a time point indicate a significant difference between male and female cattle as determined by Fisher's Exact test ($p < 0.05$).

As shown in Figure 4.14 the prevalence of *T. vivax* in male and female cattle varies across all the time points, but only at one time point per district is this difference actually significant. In both instances females are the more infected sex.

4.5.3 *T. congolense* savannah

At baseline sampling 3.5 % (7/200, 95% CI 1.42 – 7.08%) of animals in Dokolo were positive for *T. congolense* savannah, while in Kaberamaido 23.5% (73/310, 95% CI 18.94 – 28.67%) of cattle were infected. However in Kaberamaido the overwhelming

majority of these positive cattle (52/73) came from the same village, Omor, where the village prevalence of *T. congolense* savannah was 53.5%. This figure is significantly higher than the prevalence detected in any village in either district at any time point, and as such can be considered a localized epidemic outbreak of disease rather than an indicator of the overall *T. congolense* savannah prevalence. Having deemed the prevalence in this village to be unusually high it was excluded from the final analysis. The combined prevalence for the remaining villages in Kaberamaido was 9.1% (21/230, 95% CI 5.7 – 13.6%), and this is the figure depicted in graph 3.10, as it is a better representation of the prevalence in district as a whole. The prevalence of *T. congolense* savannah was significantly higher in Kaberamaido than in Dokolo at baseline ($p = 0.0007$).

At the three month time point 2.3% (11/486, 95% CI 1.14 – 4.01%) of animals in Dokolo tested positive for *T. congolense* savannah, a small fall that was not statistically significant ($p = 0.4301$). In Kaberamaido the prevalence was 6.8% (49/717, 95% CI 5.1 – 8.93%), a decrease that was insignificant when compared with baseline prevalence ($p = 0.25$). There were no villages experiencing outbreaks at this time point, and all villages sampled were included in the final prevalence tally. Again, the prevalence of *T. congolense* savannah was significantly higher in Kaberamaido than in Dokolo.

At nine months in Dokolo prevalence had risen to 5.7% (34/600, 95% CI 3.96 – 7.83%), which was insignificantly different from the prevalence detected at baseline ($p = 0.1937$), but was significantly higher than the prevalence detected at three months ($p =0.0055$). In Kaberamaido the prevalence of *T. congolense* savannah had taken another fall to 2.1% (15/714, 95% CI 1.18 – 3.44%). This was significantly lower than either the baseline ($p < 0.0001$) or three month ($p < 0.0001$) prevalence detected in Kaberamaido. At nine months the prevalence of *T. congolense* savannah was higher in Dokolo than in Kaberamaido, and this difference was statistically significant ($p = 0.0007$).

In Dokolo at 18 month sampling the prevalence was 13.1% (45/343, 95% CI 9.73 – 17.16%). However again upon closer inspection this was due to a localized epidemic in

one village, this time the village of Aneralibi. 29/45 positive cows came from this village, giving a village prevalence of 51.8%. This village was excluded from the final analysis. The prevalence of *T. congolense* savannah in the remainder of villages in Dokolo was 5.6 % (16/287, 95% CI 3.22 – 8.9%). This was not significantly different from the prevalence detected at baseline (p = 0.38) or nine months (p = 1), and significantly higher than that detected at three months (p = 0.02). In Kaberamaido prevalence had risen to 7.5% (48/637, 95% 5.61 – 9.87). This was insignificantly lower than the prevalence detected at baseline (p = 0.48), not significantly different to the prevalence at three months (p = 0.599), and significantly higher than the nine month prevalence (p < 0.0001). At eighteen month sampling there was no significant difference in the prevalence of *T. congolense* savannah between Dokolo and Kaberamaido (p = 0.3278).

The information regarding *T. congolense* savannah prevalence at differing locations and time points is summarised in Figure 4.15 to aid clarity and comparison.

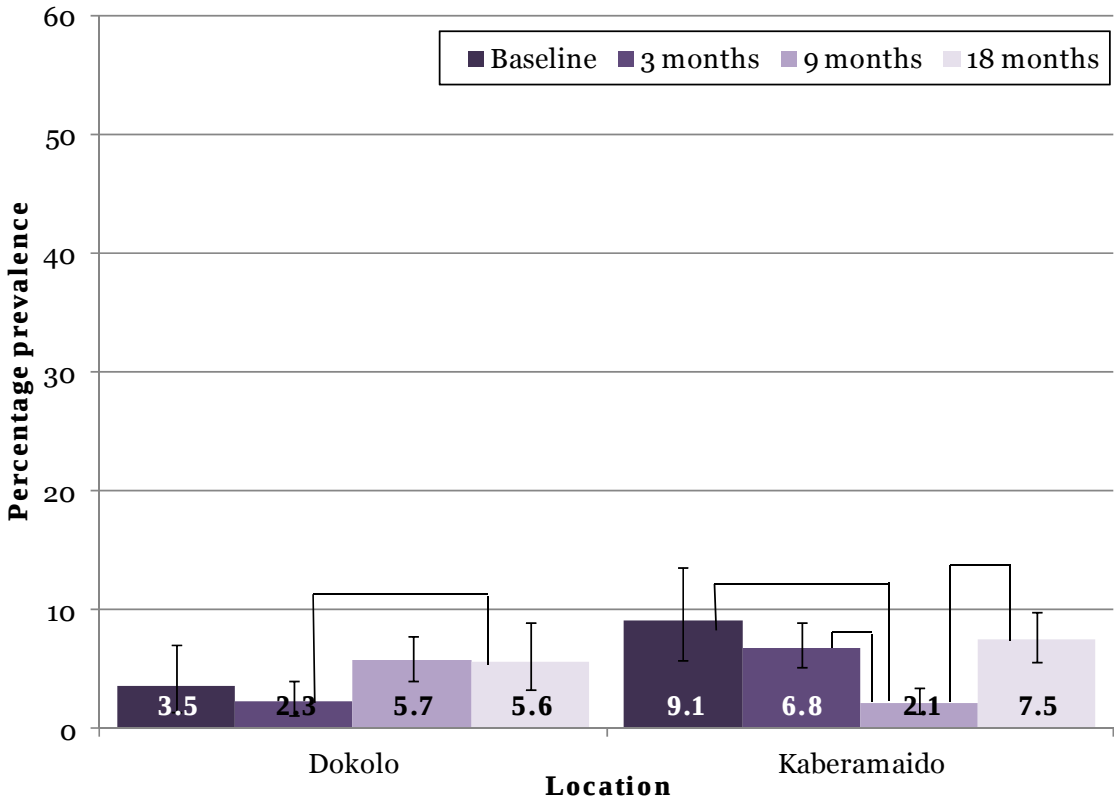

Figure 4.15 - prevalence of *T. congolense* savannah in Dokolo and Kaberamaido at each of the sampling time points. Vertical lines transecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two as determined by Fisher's Exact test ($p < 0.05$).

4.5.3.1 Age and *T. congolense* savannah infection

In Dokolo there is no significant difference in the prevalence of *T. congolense* savannah in the three different age categories at baseline, three or nine months. At each of these time points highest prevalence is found in the A age group. At eighteen months there was no *T. congolense* savannah detected in the A age group, and the prevalence in the B age group was significantly higher than that in the C age group ($p = 0.04$).

In Kaberamaido the prevalence of *T. congolense* savannah was significantly higher in the B age group when compared with the C age group at baseline ($p = 0.03$) and three months ($p = 0.04$). At nine month sampling there were no significant differences in prevalence between age groups. At eighteen months the prevalence of *T. congolense*

savannah was significantly higher in the A age group when compared with the C age group (p = 0.03).

This is summarised in Figure 4.16 for clarity and ease of comparison.

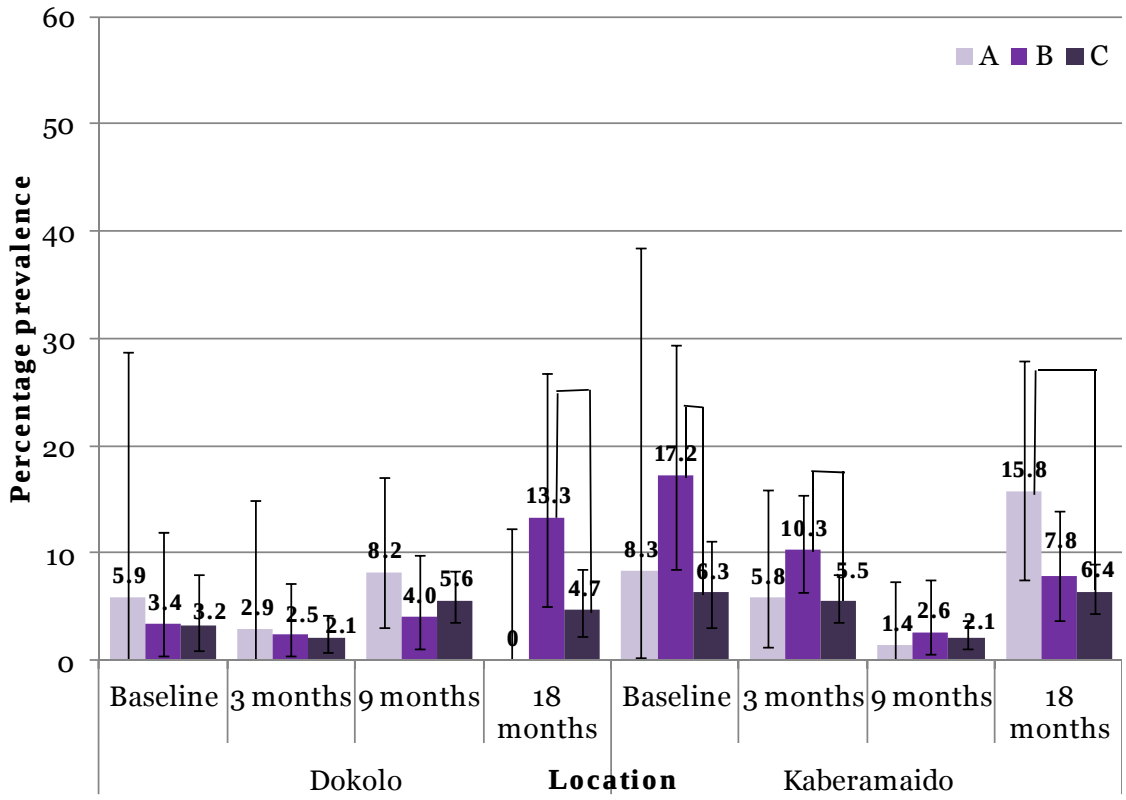

Figure 4.16 - percentage prevalence of *T. congolense* savannah in each of the three age categories in Dokolo and Kaberamaido at each sampling time point; A (<18 months), B (18-36 months) and C (36+ months). Vertical lines transecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two as determined by Fisher's Exact test (p < 0.05).

4.5.3.2 Sex and *T. congolense* savannah infection

The prevalence of *T. congolense* savannah in the two sexes varies across the time points sampled, but the difference between the two is not significant in either district at any time point, as summarised in Figure 4.17.

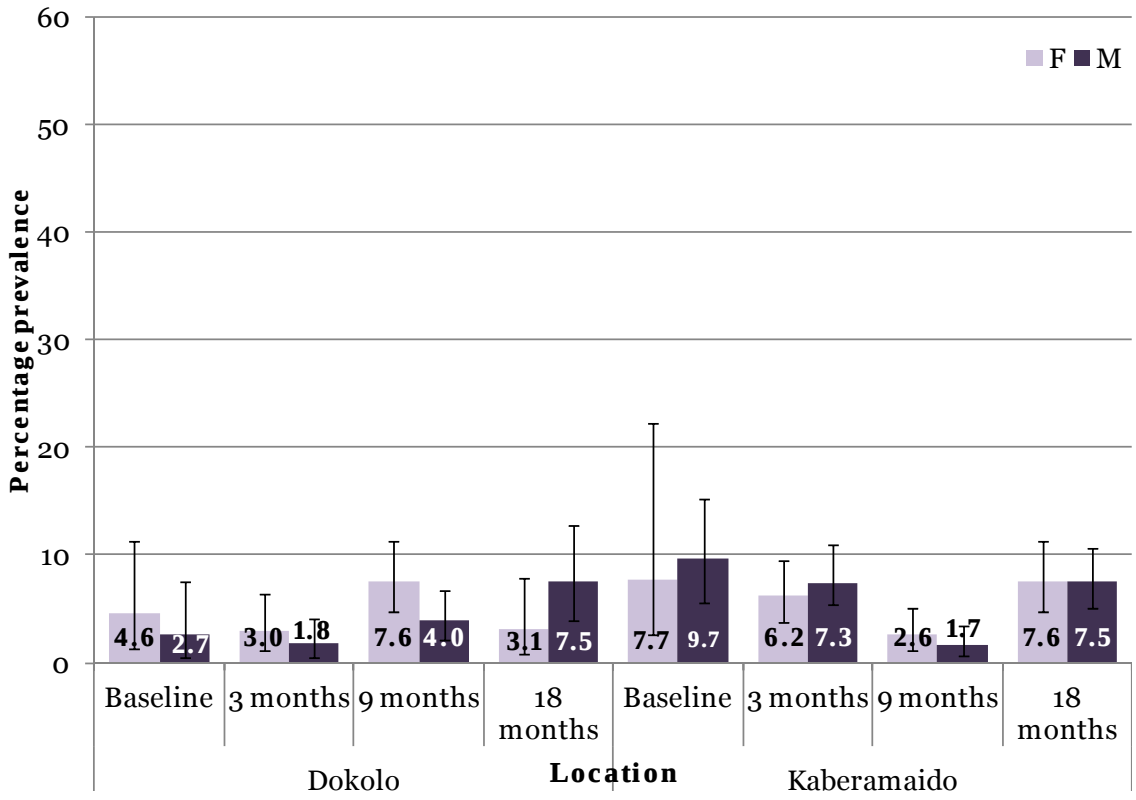

Figure 4.17 - prevalence of *T. congolense* savannah in male (M) and female (F) cattle in Dokolo and Kaberamaido at each of the sampling time points. Vertical lines transecting each bar represent the 95% confidence intervals. Stars above a time point indicate a significant difference between male and female cattle as determined by Fisher's Exact test ($p < 0.05$).

4.6 Discussion

Throughout the SOS programme the epidemiological situation of *T. b. rhodesiense* in animals and man has received much attention; however there is a need to clarify the prevalence and distribution of the causative agents of AAT. This clarity is essential to fully assess the impact of the mass treatment programme and ensure decisions on future control programmes can be made on a sound evidence base. Currently, accurate evidence on the epidemiology of AAT in Sub-Saharan Africa is lacking, due to lack of application of sensitive diagnostic techniques in the field. Similarly there is a great paucity of evidence regarding the impact of mass trypanocidal drug treatment on the prevalence of *T. vivax* and *T. congolense* savannah.

The general aim of SOS phase one was to control the spread of *T. b. rhodesiense* in Uganda and reduce its prevalence in cattle at the northern limit of *T. b. rhodesiense* distribution. This was motivated by the continual encroachment of *T. b. rhodesiense* into new districts of Uganda, bringing it perilously close to overlapping with *T. b. gambiense* (Picozzi *et al.*, 2005). These aims were successfully achieved through the mass application of a single trypanocidal drug treatment, augmented by deltamethrin spraying. Taking the intervention region as a whole the point prevalence of *T. brucei* s. l. was significantly reduced from 15.5% at baseline to 4.7% ($p < 0.0001$) three months post treatment (Selby, 2011). The point prevalence of *T. b. rhodesiense* was similarly reduced across the region as a whole, from a baseline prevalence of 0.81% to 0.11% three months post treatment (Selby, 2011).

Taking a more detailed look at individual districts and areas covered by SOS phase one, in some districts the treatment had significantly more impact than others. Two districts, Dokolo and Kaberamaido, had significantly higher prevalence of *T. brucei* s. l. than the remainder of the intervention area (Selby, 2011). The prevalence of *T. b. rhodesiense* detected within Dokolo and Kaberamaido was continually higher than that detected in the remainder of the SOS region, and HAT cases were also concentrated here (Selby, 2011). These findings triggered a re-treatment of the high risk areas within Dokolo and Kaberamaido. The implementation and success of this second mass trypanocidal treatment is detailed in Chapter five.

This chapter aimed to determine if other species of trypanosome were circulating in the high risk areas of Dokolo and Kaberamaido. *T. vivax* and *T. congolense* savannah were both detected by molecular means at all time points. The baseline data, collected before control measures were implemented, serve as a snapshot of the natural composition of bovine trypanosomiasis. This is one of a very small number of studies where molecular diagnostics have been used in a large scale epidemiological survey of bovine trypanosomiasis. The detection of *T. vivax* and *T. congolense* savannah highlight the presence of suitable ecological and environmental conditions for the transmission of trypanosomiasis. The detected prevalence of *T. vivax* and *T. congolense* savannah was combined with the previously detected prevalence of *T. brucei* s. l. from the same

samples, to produce data concerning the overall level of infection with any species of trypanosome within the sampled cattle population.

The treatment impacted the overall prevalence of trypanosomiasis in the two districts differently. The prevalence of overall trypanosomiasis was initially significantly reduced ($p < 0.0001$) in Dokolo, falling from 27.5% at baseline to 10.3% at three months, a reduction of 62.5% in the sample. In Kaberamaido there was no significant change in prevalence from baseline to three months, going from 18.4% at baseline to 17.6% at three months. After the three month sampling prevalence of overall cattle trypanosomiasis subsequently rose, at eighteen month sampling in Kaberamaido the recorded prevalence of 43.8% was significantly higher than that detected at baseline ($p < 0.0001$). In Dokolo there was no statistically significant increase in the prevalence of trypanosomiasis between baseline and nine or eighteen months. This suggests the impact of the treatment did not last longer than three months in this district, as this is the only time point where a significant reduction in trypanosomiasis is detected. It must also be noted that while trypanocidal drug treatment coverage approached optimal levels, the reported level of spraying coverage within the sampled population was substantially lower. The failure of the control programme to sustain a reduction in overall trypanosomiasis past the three month time point could be attributed to suboptimal spraying coverage, which was not sufficient to impact the tsetse population and prevent treated cattle becoming re-infected (Selby, 2011).

Optimal trypanocide coverage could be rendered less effective by the continual import of untreated cattle on an unknown scale. A closer look at the male to female cattle ratio in Dokolo and Kaberamaido gives some indication of the scale of cattle imports. Male cattle are favored over females for the purpose of ploughing and draft power, due to their increased size, weight and muscle power. The high demand for male cattle cannot be sustained by natural herd productivity as this would see a male to female ratio of close to one. Therefore the significantly skewed ratio of male to female cattle is a very strong indicator of a high level of cattle movements, with male cattle constantly being brought in from out width Dokolo and Kaberamaido as part of restocking by government, NGOs and individual farmers. NGO and government organizations most frequently report they are providing cattle for the purpose for providing draught power,

and eleven out of the 18 organizations questioned stated they only provided male cattle for restocking activities (Selby, 2011). The discrepancy between the number of male and female cattle is greatest in Kaberamaido and significant at all time points, suggesting this district has a higher importation rate of untreated, trypanosome infected cattle than Dokolo. This, alongside poor spraying coverage, could explain the discrepancy between the effect of treatment in Dokolo and Kaberamaido.

The short time in which the prevalence of overall trypanosomiasis was able to recover suggests high tsetse challenge, particularly in Kaberamaido. It could also be indicative of better cattle management practices in Dokolo as compared with Kaberamaido; however this cannot be investigated further as no records of trypanocidal drug treatments administered by cattle owners themselves were kept. Additionally uptake of and participation in SOS spraying activities may have been more prevalent in Dokolo than Kaberamaido. Again this theory is difficult to substantiate as records of farmer administered deltamethrin are also not kept. While it is difficult to assess the root cause of the high trypanosomiasis prevalence in Kaberamaido, this district is severely affected by both HAT and AAT, highlighting the need for further education and control measures.

4.6.1 *T. vivax*

The treatment had no discernible impact on the prevalence of *T. vivax*. At baseline in Dokolo the prevalence was 2.0%, at three months it was 2.5%, an insignificant increase of 25%. In Kaberamaido baseline prevalence of *T. vivax* was 7.3%, at three months it was 8.4%, an insignificant increase of 15%. *T. vivax* has a short developmental cycle within the tsetse vector, with flies becoming infectious to subsequent hosts in as little as five days after the initial infective feed (Lloyd and Johnson, 1924). *T. vivax* can also be transmitted mechanically, both by tsetse flies and other biting flies (Gardiner and Wilson, 1987). *T. vivax* hence has the capacity to quickly recover from any reduction in prevalence, much more so than *T. brucei* s. l. or *T. congolense savannah*, both of which take considerably longer to reach maturity within tsetse. The treatment may have had an effect on the prevalence of *T. vivax* immediately after it was administered, but monitoring was not performed at this time. Either the treatment with isometamidium

had no impact on *T. vivax* prevalence, suggesting possible resistance to this trypanocide, or the impact was so short lived monitoring was not frequent enough to detect it. Strains of drug resistant *T. vivax* have been reported in several studies in both East and West Africa (Gray and Roberts, 1971, Mwambu and Mayende, 1971, Sow *et al.*, 2012). However trypanocides are not thought to have been widely used in Dokolo and Kaberamaido in the past due to low numbers of cattle in the area during decades of conflict and poor availability of veterinary services and drugs post conflict. Therefore drug resistance seems an unlikely consequence of a single mass application of trypanocide to an apparently naive population of trypanosomes. Further investigation is needed to determine the possible presence of drug resistant *T. vivax* in Dokolo and Kaberamaido.

Assuming *T. vivax* was drug sensitive, importation of *T. vivax* infected animals into the SOS region would allow the prevalence of this trypanosome to recover much more quickly than that of the other species detected, given its comparatively shorter maturation rate in tsetse. Therefore it is possible *T. vivax* prevalence was reduced, but due to the import of infected cattle was able to rebound to baseline prevalence before the first monitoring sampling took place.

Over the course of monitoring prevalence of *T vivax* continued to increase in both Dokolo and Kaberamaido. At eighteen months the prevalence in Kaberamaido is more than double that recorded at baseline, while in Dokolo a five-fold increase in the prevalence of *T. vivax* is recorded over the same time. This reiterates earlier observations made by Selby, (2011) that vector control was insufficient to interrupt the transmission cycle of trypanosomiasis in the area. It also suggests use of trypanocidal drugs by cattle owners is low; as *T. vivax* is ordinarily pathogenic infected cattle should display signs such as weight loss, prompting treatment with trypanocides if they were being widely used. Alternatively the strain or strains of *T. vivax* circulating in Dokolo and Kaberamaido could be of low virulence, negating the need for livestock owners to treat their cattle in the absence of clinical trypanosomiasis. The steady and significant increase of *T. vivax* prevalence over time suggests a high level of tsetse challenge. Alternatively mechanical transmission by biting flies could also be facilitating this increase in prevalence.

4.6.2 *T. congolense* savannah

T. congolense savannah was similarly unaffected by the treatment, however there was no significant increase in prevalence between baseline and eighteen months. *T. congolense* savannah seems to have an epidemic nature in this area. Most villages have low prevalence of *T. congolense* savannah, but twice in different locations village prevalence in excess of 50% was detected. This illustrates the fact that although the district prevalence of *T. congolense* savannah was low this parasite is still likely to cause considerable morbidity and mortality. The reason for the fall in prevalence in Kaberamaido between three and nine months is unclear. It is possible this is a seasonal fluctuation. It is also possible that a particularly severe epidemic outbreak of this trypanosome occurred, whereby death of infected animals immediately before sampling significantly reduced prevalence. It could also be that in response to an outbreak large numbers of cattle owners treated their cattle which were showing signs of trypanosomiasis, leading to the reduction in prevalence.

There was some association between *T. congolense* savannah prevalence and age, particularly in Kaberamaido. Since baseline samples were taken before any treatment had been administered, these samples are representative of the natural distribution of trypanosomiasis within the sample population. In Kaberamaido at baseline and three months the prevalence of *T. congolense* savannah is highest in animals aged between eighteen and thirty six months, the B age group. At eighteen months animals in the A age group, <18 months, have significantly higher *T. congolense* prevalence than the other age groups. Most notable across all time points is the fact adults in the C age category never have the highest prevalence of *T. congolense* savannah. This is surprising for a number of reasons. These cattle are fully grown, and so larger than those in the younger age categories, meaning they give off more odor and present a larger visual target for tsetse. Hypothetically then, adults should attract more tsetse and have a higher prevalence of trypanosomiasis, yet the opposite is observed here. Diminishing levels of infection in older age groups is associated with the development of immunity for other pathogens.

This study also acts as a cautionary tale against the generalization of epidemiological information onto too large a scale. For example, if previous authors had not studied the

precise distribution of *T. b. rhodesiense* at a localized level the existence of the high transmission area would not have been discovered. Here too it has been demonstrated that ignoring the nuances of parasite prevalence at a local level can skew the results for a whole district, giving an inaccurate picture of the extent of the disease. In Kaberamaido at baseline the majority of positive samples were all from the one village, Omor. This village was excluded from the district total in the final analysis as the village prevalence of 53.5% can be considered unusually high for this area. It seems likely the village of Omor was suffering from a localized epidemic of *T. congolense* savannah at baseline sampling. A similar phenomenon occurred at eighteen months in the Dokolo village of Aneralibi. Further circumstantial evidence for this localized epidemic in Omor can be gained via analysis of the age of cattle sampled at each time point. At baseline and three months there are significantly ($p < 0.0001$) fewer adult animals sampled than at nine months. This suggests increased mortality within the village herd, and purchase of younger animals to replace the animals that died. This highlights that while the overall district prevalence of *T. congolense* savannah is not unduly high this trypanosome may still cause considerable cattle morbidity and mortality alongside the associated economic losses to the farmer.

4.7 Conclusion

The treatment did not appear to have a sustained impact on the overall prevalence of trypanosomiasis in Dokolo and Kaberamaido. However the importation of cattle into Dokolo and Kaberamaido is extremely high. Imported cattle are unlikely to have received a trypanocidal drug treatment, as the enforcement of government policy of treating cattle at the point of sale is reported to be low. Movement of cattle into Dokolo and Kaberamaido as part of ongoing restocking activities has mostly likely been a key factor reducing the efficacy of the SOS phase one treatment in these districts.

The treatment differently impacted upon the prevalence of each species of trypanosome. *T. brucei* s. l. prevalence was initially significantly reduced, *T. vivax* and *T. congolense* savannah prevalence were seemingly unaffected. Given the result presented in this chapter mass trypanocidal drug treatment is not an effective measure

for reducing or controlling the prevalence of these two agents of AAT in the face of high levels of cattle importation. Although it is worth reiterating that the original objectives of the SOS intervention programme, namely to reduce the burden and spread of *T. b. rhodesiense* have been met.

The results presented in this chapter mean it is not possible to reject the null hypothesis that SOS phase one treatment had no effect on the prevalence of *T. vivax* or *T. congolense* savannah.

Chapter 5 – The effectiveness of a targeted re-treatment programme in reducing the prevalence of trypanosomiasis in cattle in Uganda

The SOS control programme began in October 2006; over a quarter of a million cattle were mass treated using trypanocides to reduce the burden and spread of the zoonotic parasite *T. b. rhodesiense*. The programme was extremely successful in reducing the prevalence of this parasite in cattle.

Within the five districts treated, the residual prevalence of *T. brucei* s. l. was notably higher in two districts in particular, Dokolo and Kaberamaido, both before and after the treatment. Furthermore, within these problem districts there were several villages where the prevalence of *T. b. rhodesiense* remained stubbornly high within the cattle sampled, see Figure 5.1. These villages were located close to one another and within parishes that continued to report human sleeping sickness cases, indicating a further trypanocidal drug treatment of the cattle population may be needed.

In 2008 re-treatment of these high risk areas was carried out, aiming to reduce the prevalence of *T. b. rhodesiense* in the cattle population, thereby preventing this parasite spreading back into previously cleared neighbouring districts and reducing the onward transmission to the human population. To properly interrupt the transmission cycle of *T. b. rhodesiense* it was estimated more than 86% of the total cattle population needed to be treated (Welburn *et al.,* 2006). The trypanosome prevalence within the re-treatment area was monitored by PCR surveillance of cattle blood from 20 selected villages Figure 5.2. As the SOS programme focused on the reduction of *T. b. rhodesiense* in cattle and in humans, the detection of *T. brucei* s. l. and *T. b. rhodesiense* was considered a priority.

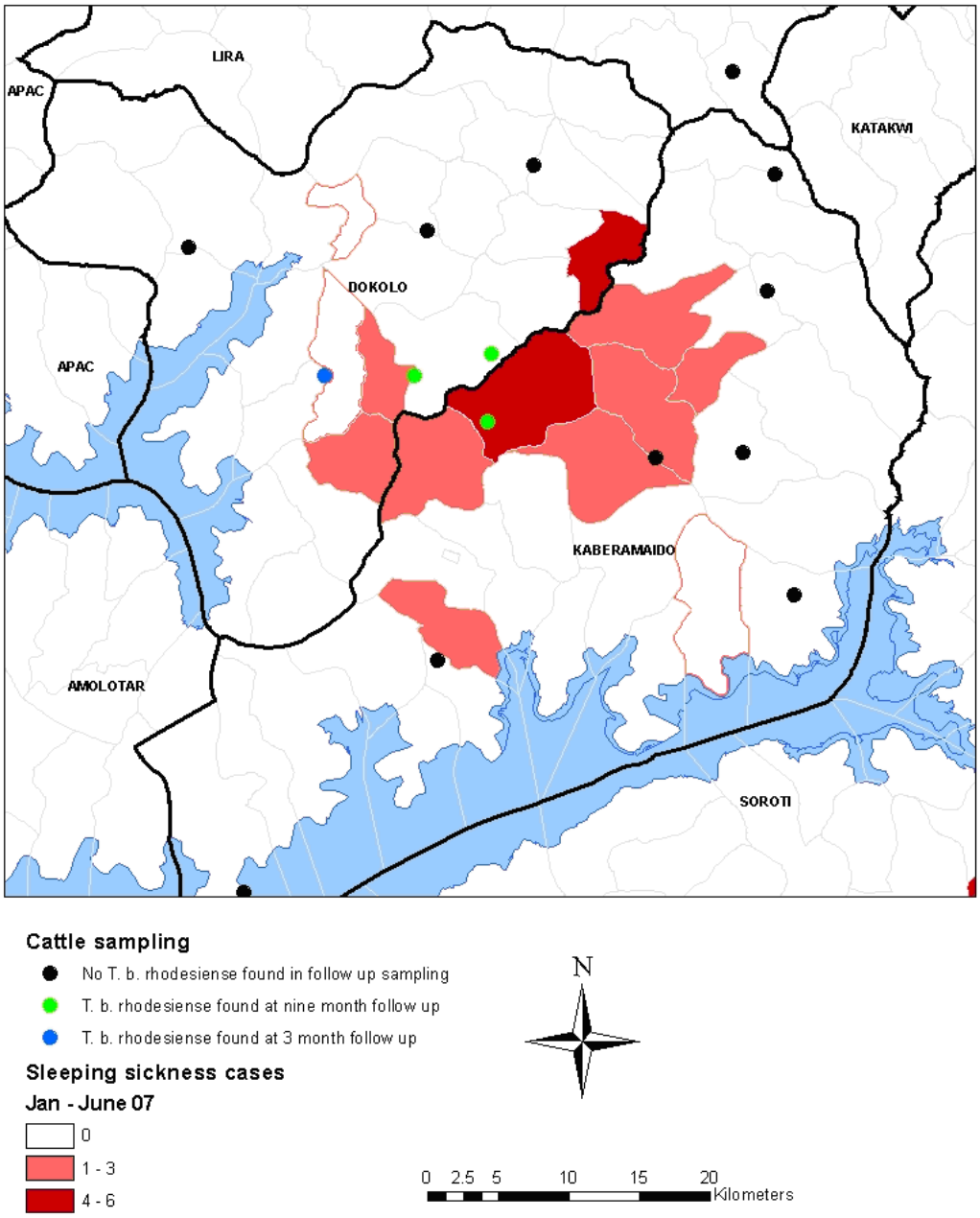

Figure 5.1 – parishes where cases of HAT originated and the location of *T. b. rhodesiense* cattle villages (Batchelor *et al.*, 2009).

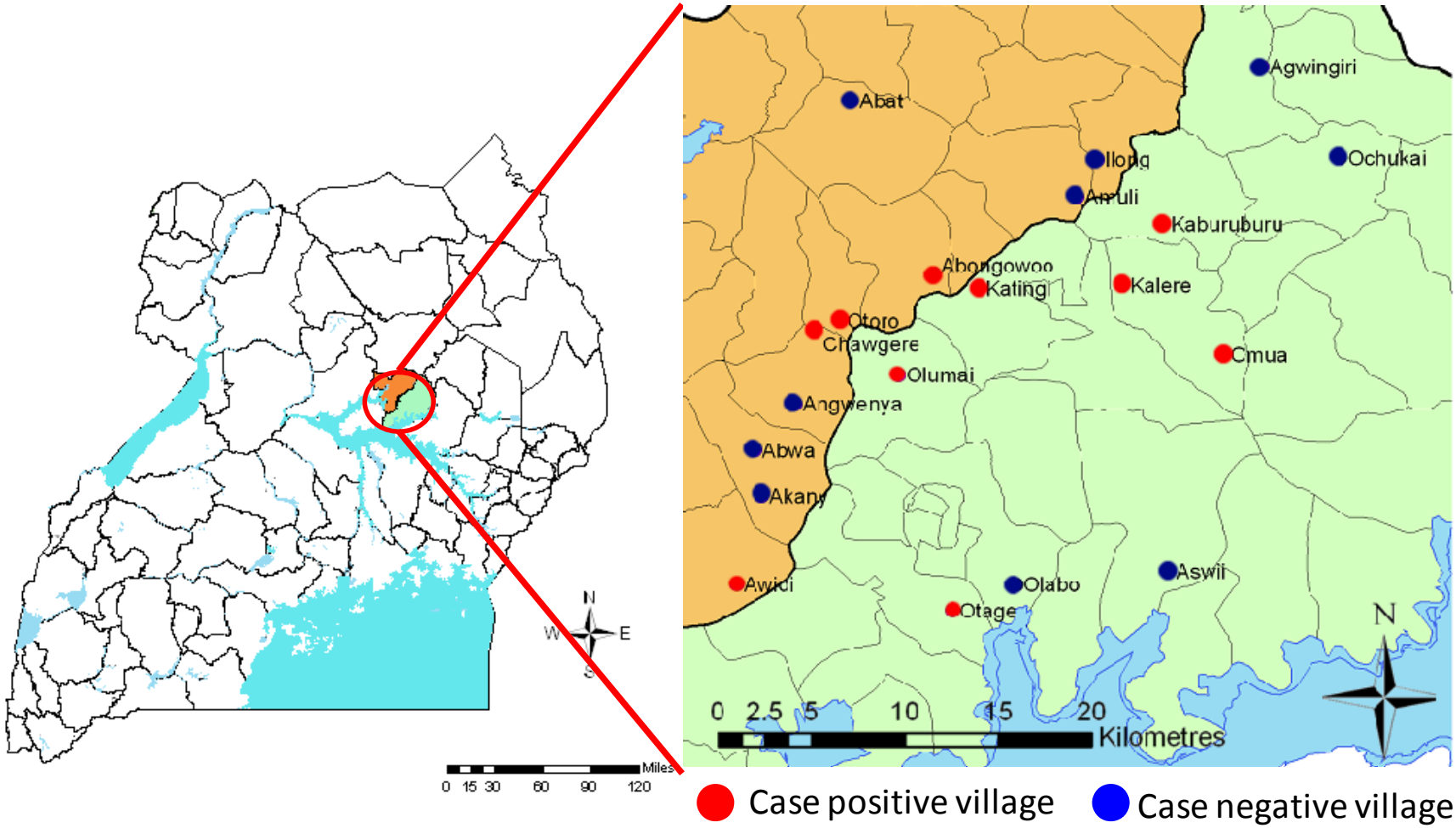

Figure 5.2 - location of case-positive and case-negative sample sites within the re-treatment area.

Given the favourable conditions for the maintenance of trypanosomal infections, the economically important cattle trypanosomes, *T. vivax* and *T. congolense* savannah, were also investigated. Both parasites can cause significant clinical disease in cattle, and while not the primary target for this intervention they are susceptible to the trypanocidal treatment used.

As well as helping to assess the overall tsetse challenge in the retreatment area, the prevalence of *T. congolense* savannah is also important for other reasons. Work by Van den Bossche *et al.* 2004 suggests that cattle chronically infected with *T. brucei* s. l. at low level, undetectable parasitaemias, will experience a dramatic rise in the circulating parasitaemia of *T. brucei* s. l. when challenged with *T. congolense* (Van den Bossche *et al.,* 2004). Earlier studies (Maudlin and Welburn, 1989) suggest it is probable that cattle chronically infected with *T. brucei* s. l. have parasitaemias so low as to be below the threshold required to infect tsetse flies. Under these conditions, transmission will be minimal.

Any factor that increases the *T. brucei* s. l. parasitaemia to a level above the minimum infectious threshold is likely to have a significant effect on the infection rate of tsetse and thus the epidemiology of *T. brucei* s. l. (Van den Bossche *et al.*, 2004). Therefore, in the context of the SOS retreatment programme, it was essential to investigate any link between the prevalence of these two parasites, to ascertain if previously documented interactions between these two species are occurring within the re-treatment area and impacting upon the effectiveness of the re-treatment or the epidemiology of HAT in the area.

5.1 Chapter Objectives

This chapter sets out to assess if the SOS re-treatment was effective in reducing the prevalence of trypanosomiasis, working against the null hypothesis-

H_0 – the SOS re-treatment had no effect on the prevalence of *T. brucei* s. l., *T. b. rhodesiense*, *T. vivax* or *T. congolense* savannah.

To determine whether or not to reject the null hypothesis, the prevalence of each species was ascertained through PCR analysis of cattle blood samples collected before and after the treatment took place.

5.2 Summary of key characteristics of sampled cattle

All villages within the re-treatment parishes were designated as case-positive or case-negative. Case-positive villages had at least one case of HAT in the 6 months prior to June 2007; case-negative villages had no reported HAT cases during this time. Ten villages were chosen from each category at random for inclusion into this study. The location of these villages and their sleeping sickness status can be seen in Figure 5.2.

For each cow sampled information about sex, breed, treatment status, body condition and an age estimate were recorded for each animal sampled.

5.2.1 Sample size

The number of animals sampled in each of the case-positive and case-negative villages at baseline and six months is shown in

Table 5.1.

Status	Village	Baseline	Six Months
Case-positive	Kaburuburu	100	100
	Kalere	100	100
	Katingi	56	100
	Olumai	100	100
	Omua	100	101
	Otage	100	100
	Abongowoo	100	100
	Awidi	100	102
	Chawgere	46	100
	Otoro	52	53
	Total	854	956
Case-negative	Agwingiri	100	100
	Aswii	90	100
	Ochukai	88	100
	Olabo	100	100
	Abat	50	73
	Abwa	80	100
	Akany	80	62
	Amuli	65	100
	Angwenya	51	100
	Ilong	100	100
	Total	804	935
Grand Total		1658	1891

Table 5.1 - number of cattle sampled at baseline and six months.

The sampling target was reached in the majority of villages, particularly at the six month sampling. The villages of Abat, Akany and Otoro did not reach the sampling target of 100 animals at either time point, which may indicate fewer cattle or underlying issues in these areas.

5.2.2 Age

The field team estimated the age of each cow sampled, and accordingly classified it as A <18 months, B 18 - 36 months, C >36 months.

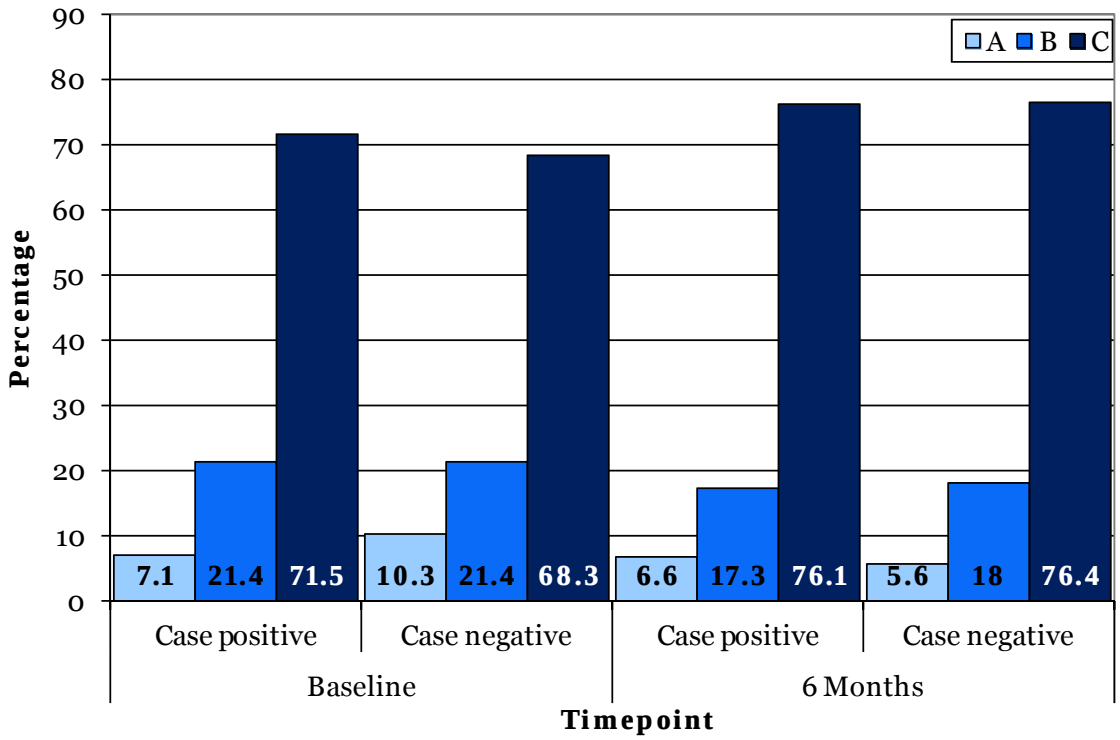

Figure 5.3 - proportion of animals from each age group (A <18 months, B 18 - 36 months, C >36 months) sampled in case-positive and case-negative villages at baseline and six month sampling.

As can be seen above in Figure 5.3, the majority of cattle sampled were in the adult age category. The number of individuals in the calf age category (A) may help contribute to the reduced numbers of treated animals present at 6 month sampling, as any animals in this category younger than six months old are too young to have been treated.

5.2.3 Breed

Where ever possible the breed of each cow sampled was recorded. Below in the breeds present at baseline and six month sampling are displayed in Figure 5.4.

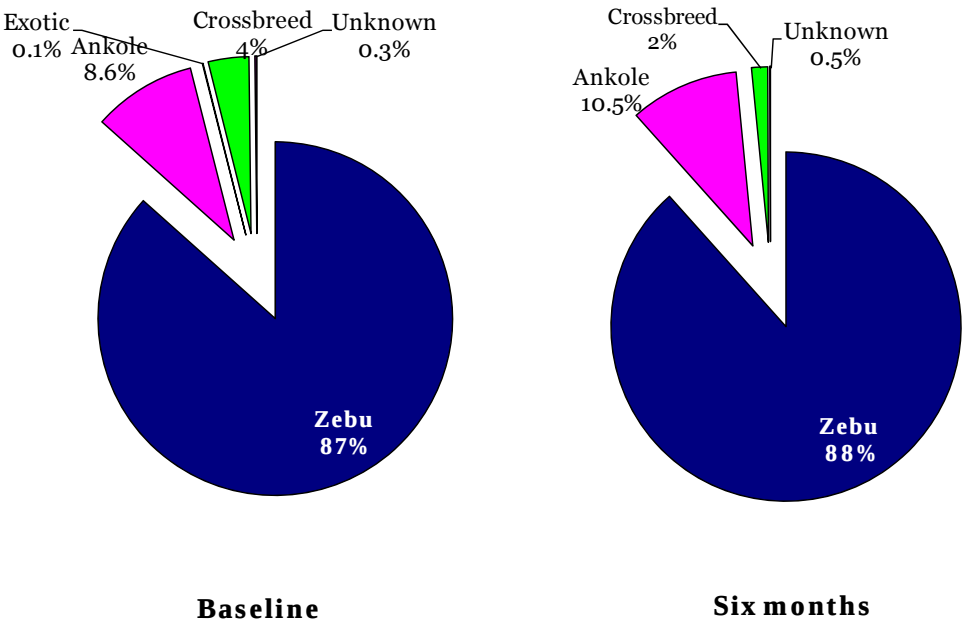

Figure 5.4 - breed of cattle sampled in the re-treatment area at baseline and six months.

By far the most common breed of cow in the re-treatment area was Zebu, followed by Ankole. There were a small number of cross bred animals at both time points, these usually were Ankole - Zebu crosses. At baseline exotic breeds were very rare, with just two Friesian type animals being recorded, and at 6 months there were no exotic breeds sampled.

5.2.4 Sex

As some studies report a difference in the prevalence of trypanosomiasis between male and female cattle, the ratio of male to female cattle sampled could impact upon trypanosome prevalence. The proportion of male and female cattle sampled at each time point is shown below in Figure 5.5.

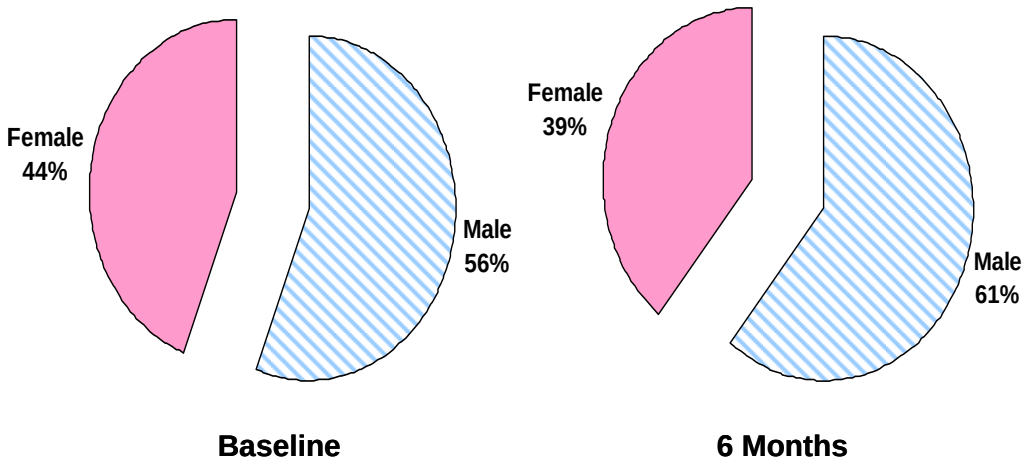

Figure 5.5 - percentage of male and female cattle sampled at baseline and six months.

The male to female ratio did not deviate significantly from an even split at both time points, with males being slightly more common than females, more so at six month sampling.

5.2.5 Body condition score

Weight loss is a common sign of trypanosomiasis, therefore the body condition score of each cow could prove useful in predicting infections, but also gives an important snap shot of overall herd health. The body condition scores allocated to cattle at baseline and six months are shown in Figure 5.6 and Figure 5.7.

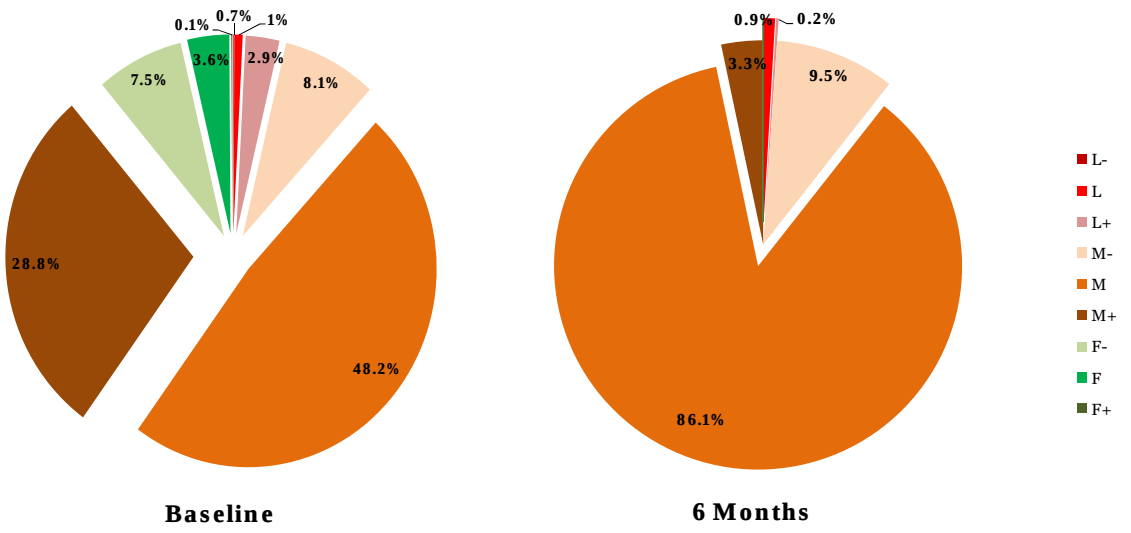

Figure 5.6 - body condition score of cattle sampled in case-positive villages at baseline and six months.

Between baseline and six month sampling there was a considerable change in the body condition scores assigned to cattle sampled in case-positive villages. At baseline 0.1% of cattle were classed as L-, 0.7% of animals were classed as L, and 2.9% of animals were classified L+. So at baseline 3.7% of animals received one of the three lean scores. At 6 months no animals were classified as L – ($p = 0.47$), 0.9% of cattle were classified as L ($p = 0.61$) and 0.2 % of cattle were classed as L + ($p<0.0001$). In total 1.1% of cattle were assigned one of the three lean categories at six month sampling.

At baseline sampling in case-positive villages 8.1% of cattle were classified M - , 48.2% of cattle were classified M and 28.8% of cattle were classified M +. Roughly 85.1% of cattle received a medium classification at baseline. By six months significant changes had occurred in these categories; 9.1% of cattle received an M – classification ($p = 0.32$), 86.1% of cattle received a classification of M ($p < 0.0001$), and 3.3% of cattle received a classification of M+ ($p <0.0001$). Roughly 98.5% of cattle received a medium classification at six months.

At baseline sampling in case-positive villages 7.5% of cattle were classified F -, 3.6% of cattle were classified F and 0.1% of cattle were classified F+. In total 10.2% of cattle

received one of the fat classifications. By six month sampling no cattle were assigned to any of the fat categories, a drop which was statistically significant for the F- ($p < 0.0001$) and F ($p < 0.0001$) categories.

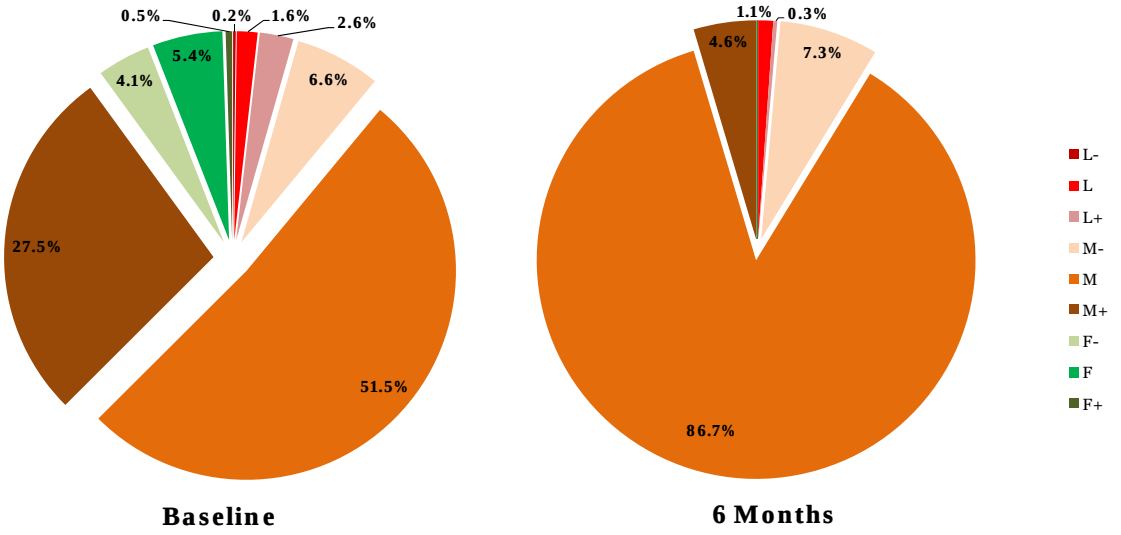

Figure 5.7 - body condition score of cattle sampled in case-negative villages at baseline and six months.

A similar picture emerges from case-negative villages, as shown in Figure 5.7. At baseline 0.2% of cattle were scored L -, 1.6% were scored L and 2.6% scored L +, making a total of 3.4% receiving one of the three lean body condition scores. At six months no cow received an L - score, a significant ($p < 0.0001$) drop from baseline, 1.1% of cattle received a score of L ($p = 0.4$) and 0.3% of acattle received a score of L + ($p < 0.0001$). Overall 1.4% of cattle received one of the three L body condition scores, a reduction from baseline.

At baseline in case-negative villages 6.6% of cattle received an M – score, 51.5% of animals received a score of M, and 27.5% of cattle received a score of M +, a total of 85.6% scoring in one of the three medium categories at baseline. At six months 7.3% of cattle were scored M-, an insignificant ($p = 0.57$) increase from baseline , 86.7% M and 4.6% M +, a total of 98.6% of cattle scored in one of the three medium categories at six

month sampling. The number of cattle scoring M increased significantly ($p < 0.0001$), while the number scoring M + fell significantly when compared to baseline.

In case-negative villages at baseline 4.1% of cattle were scored F - , 5.4% of cattle F and 0.5% of cattle F +, a total of 10% of cattle receiving one of the three fat body condition scores. At six months no cattle were classified as any of the three fat body condition scores, a reduction which was significant ($p< 0.0001$) across all three categories.

In general small but significant reductions in the number of cattle classified as lean can be observed in both case-positive and case-negative villages between baseline and six months. A much greater increase in the number of cattle classified as medium can be observed, in particular a decrease in those classified as M+ and an increase in those classified as the leaner M. There were no fat cattle observed at six month sampling. This suggests herd health, at least in relation to body condition and fat retention, has significantly reduced. In general the herds sampled have lost significant condition.

While the body condition scores have given a useful overview of herd health, it must be noted comparatively few cattle fall into any of the lean or fat categories at either baseline or six months. These low numbers rule out meaningful statistical analysis of trypanosome prevalence within groups due to the low statistical power of such small samples. Further analysis of trypanosome prevalence in the different body condition categories is excluded due to the small sample size in the majority of categories.

5.2.6 Treatment coverage at six months

At six months each cattle owner bringing a cow to be sampled was questioned as to whether their animal/s had been treated. The percentage of treated animals sampled at six months is shown in Figure 5.8.

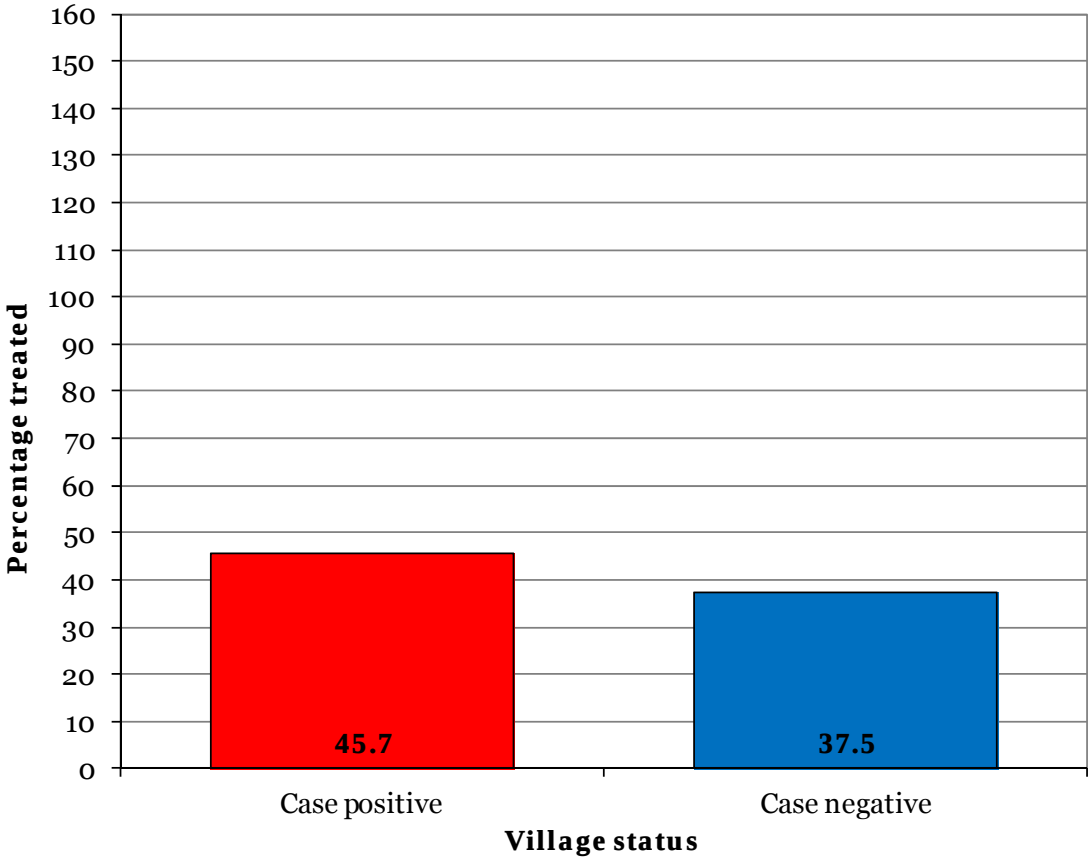

Figure 5.8 - percentage of treated cattle sampled at six months.

At six month sampling in both case-positive and case-negative parishes less than half of cattle owners reported their animals had been treated. There was no significant difference in the coverage in case-positive or case-negative villages.

5.3 Results

Initially the conversion of case-negative to case-positive villages and the overall trypanosomiasis prevalence as identified by PCR is presented. This figure gives the percentage of cattle with any trypanosome infection, be that single, double or triple species. The exact composition and distribution of mixed infections is given in Chapter six. Subsequently there is a detailed breakdown for each species and subspecies of

trypanosome and analyses of the effect that the re-treatment has had on each species in terms of district, case-positive and case-negative villages and overall.

5.3.1 HAT cases in sample villages

As sample villages were selected on the basis of whether or not they had recently presented a case of HAT it is relevant to this study to assess if case-positive villages continued to produce HAT cases and if case-negative villages continued to remain disease free.

Since the retreatment took place in April 2008, 4 case-positive villages have had a HAT case; one originating in Chawgere, two from Kaburuburu, one from Olumai and one from Otage. Angwenya was the only case-negative village to have had any HAT, presenting a single case in April 2008.

5.3.2 Overall trypanosomiasis

In case-positive villages at baseline overall trypanosomiasis prevalence was 42.4% (362/854, 95% CI = 39.1 – 45.8%). By six months this had fallen significantly to 27.8% (266/956, 95% CI = 25.0 – 30.8%, $p < 0.0001$), as shown in Figure 5.9.

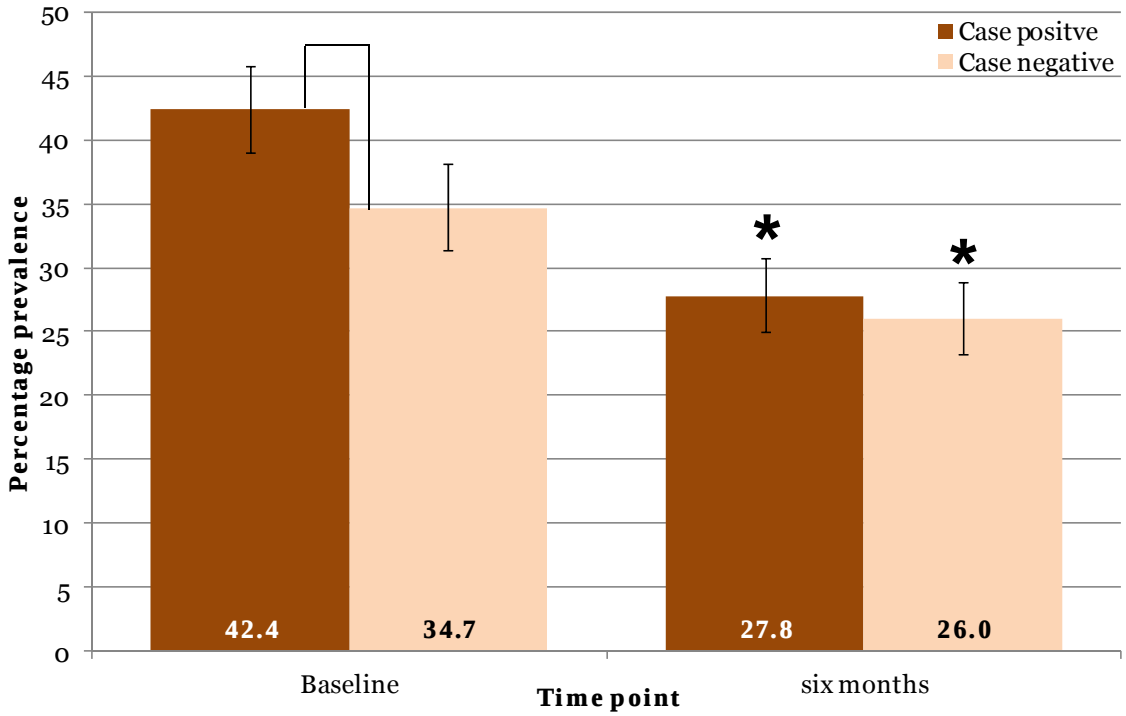

Figure 5.9 - percentage of sampled animals positive for one or more species of trypanosome at case-positive and case-negative villages, at baseline and six month sampling. Vertical lines indicate the 95% confidence intervals. Linking lines indicate a significant difference between groups at that time point as determined by Fisher's Exact test ($p < 0.05$). Stars indicate a significant drop in trypanosome prevalence between baseline and six months ($p < 0.05$).

There were more cattle positive for one or more species of trypanosome in case-positive villages at baseline, with 34.7% (279/804, 95% CI = 31.4 – 38.1%) of cattle testing positive. At six month sampling there had been a significant ($p < 0.0001$) reduction in the number of animals testing positive in case-negative villages; just 26.0% (243/935, 95% CI =23.2 - 28.9%).

The species tested for and detected were *T. brucei* s. l., *T. b. rhodesiense*, *T. vivax* and *T. congolense* savannah as detailed in the following sections.

5.3.3 *T. brucei* s. l.

The prevalence of *T. brucei* s. l. was significantly higher in case-positive villages than in case-negative villages at both baseline (p = 0.002) and six months (p = 0.008). The prevalence of T. *brucei* s. l. in case-positive and case-negative villages before and after treatment can be seen in Figure 5.10.

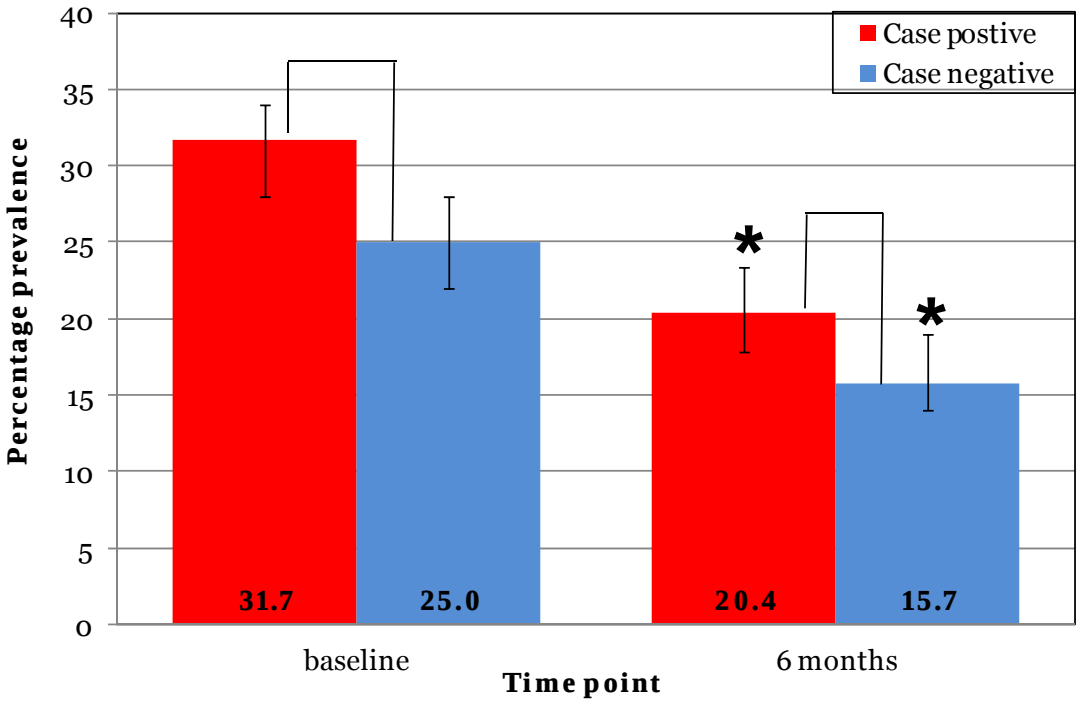

Figure 5.10 - percentage prevalence of *T. brucei* s. l. in case-positive and case-negative villages at baseline and six months. Vertical lines indicate the 95% confidence intervals. Linking lines indicate a significant difference between groups at the same time point as indicated by Fisher's exact test ($p < 0.05$); stars indicate a significant change in that group between baseline and six months ($p < 0.05$).

In case-positive villages at baseline *T. brucei* s. l. prevalence was 31.7% (271/854, 95% CI = 34.97 – 28.62%). At six months this had fallen significantly to 20.4% (195/956, 95% CI = 23.09 – 17.89%, p < 0.0001).

In case-negative villages at baseline the prevalence of *T. brucei* s. l. was 25% (201/804, 95% CI = 28.14 – 22.04). At six months this had fallen significantly to 15.7% (147/935 95% CI = 18.22 – 13.45%, p < 0.0001).

5.3.3.1 *T. b. rhodesiense*

T. b. rhodesiense was more prevalent in case-positive compared with case-negative villages, although there was no statistically significant difference between the two at either time point. Prevalence dropped significantly between baseline and six months in both sets of villages, as displayed in Figure 5.11.

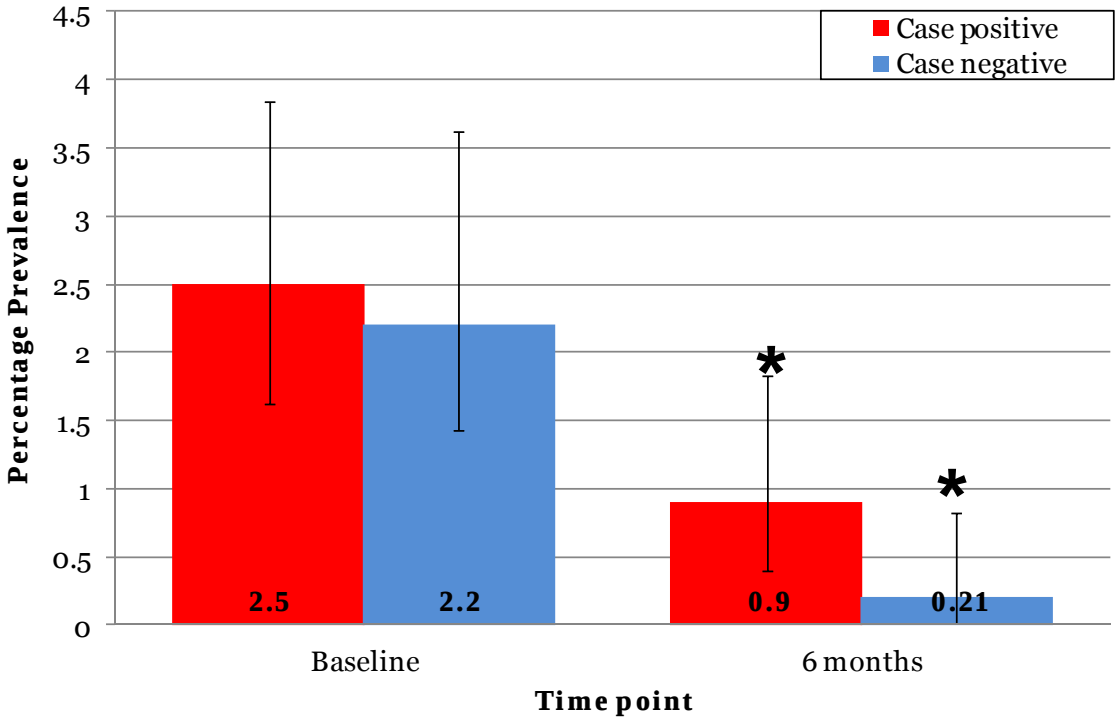

Figure 5.11 - percentage prevalence of *T. b. rhodesiense* in case-positive villages and case-negative villages at baseline and 6 months. Stars indicate a significant change in that group between baseline and 6 months (p < 0.05). Vertical lines indicate the 95% confidence intervals.

At baseline in case-positive villages *T. b. rhodesiense* prevalence was 2.5% (21/854, 95% CI = 3.73 – 1.53%). At six months the prevalence in case-positive villages had fallen significantly (p = 0.01) to 0.9% (9/956, 95% CI = 1.78 – 0.43%).

At baseline in case-negative villages *T. b. rhodesiense* prevalence was 2.2% (18/804, 95% CI = 3.52 – 1.33%). In case-negative villages at six months prevalence had fallen significantly to 0.21% (2/935, 95% CI = 0.77 – 0.03%, p < 0.0001)

5.3.3.2 Percentage of *T. brucei* s. l. made up of *T. b. rhodesiense*

At baseline sampling the percentage of *T. brucei* s. l. that was *T. b. rhodesiense* was slightly higher in case-negative villages as opposed to case-positive villages, but there is no significant difference between the groups at either time points. At six month sampling there was a significant reduction in the amount of *T. brucei* s. l. that is *T. b. rhodesiense* in case-negative villages only (p = 0.004). Although levels had also fallen in case-positive villages, they have not fallen enough to represent a statistically significant change, as shown in Figure 5.12.

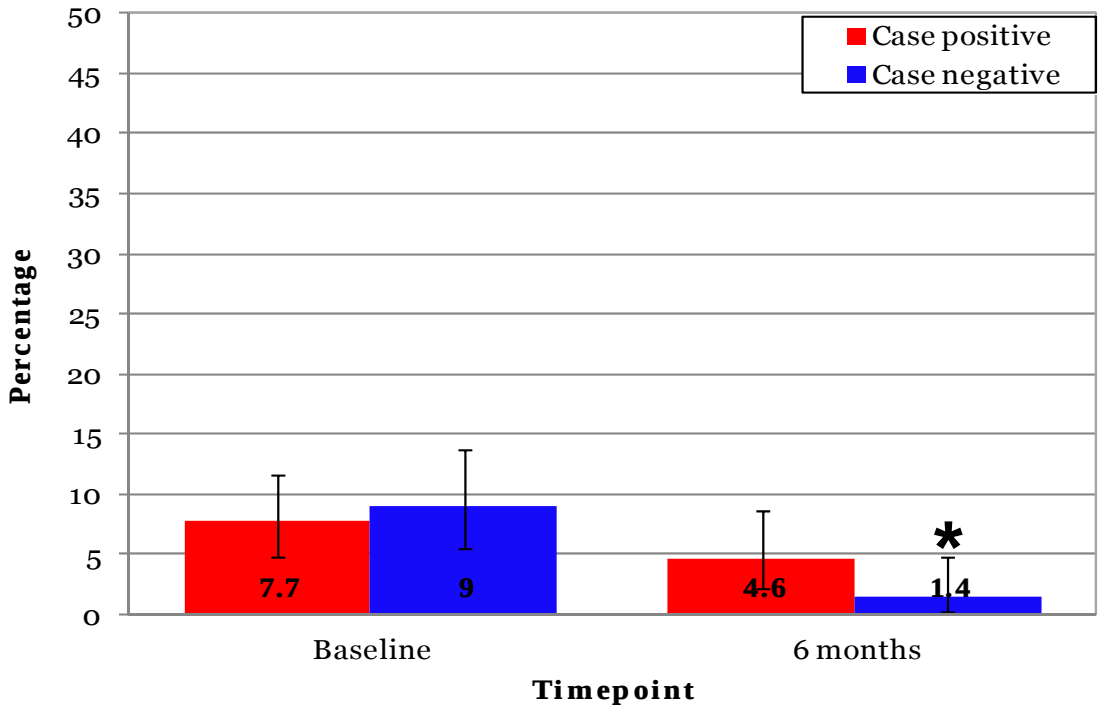

Figure 5.12 - percentage of *T. brucei* s. l. that was *T. b. rhodesiense* in case-positive and case-negative villages at baseline and six months. Vertical lines represent the 95% confidence intervals; stars indicate a significant change in that group between baseline and six months ($p < 0.05$).

5.3.4 *T. vivax*

The prevalence of *T. vivax* was slightly higher in case-negative as opposed to case-positive villages, both at baseline and six months, this difference was not significant at either time point.

At baseline 5.7% (49/854, 95% CI = 7.51 – 4.27%) of case-positive cattle tested positive for *T. vivax*. At six months, the prevalence in case-positive villages had dropped significantly to 0% (0/956, 95% CI = 0.39 – 0%, $p < 0.0001$).

At baseline 6.1% (49/804, 95% CI = 7.98 – 4.54%) of case-negative animals tested positive for *T. vivax*. Prevalence of *T. vivax* dropped significantly at six months to 0.54% (1/935, 95% CI = 0.59 – 0.01%, $p < 0.0001$). This is summarised in Figure 5.13.

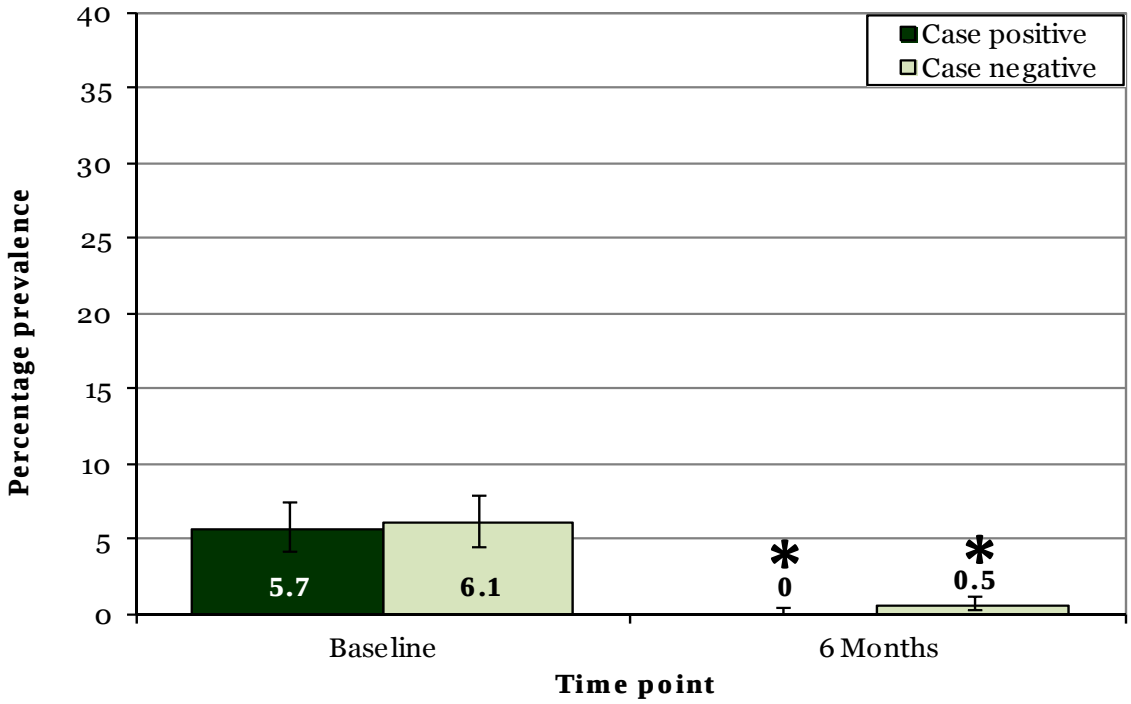

Figure 5.13 - percentage prevalence of *T. vivax* in case-positive and case-negative villages at baseline and six months. Vertical lines indicate the 95% confidence intervals. Stars indicate a significant change in that group between baseline and six months ($p < 0.05$).

5.3.5 *T. congolense* savannah

In case-positive villages, the prevalence of *T. congolense* savannah rises between baseline and six months, from 9.7% (83/854, 95% CI = 11.91 - 7.82%) to 11.3% (108/956, 95% CI = 13.48 - 9.36%). This change was not statistically significant ($p = 0.2842$).

In case-negative villages the baseline prevalence of T. *congolense* savannah was 6.7% (54/804, 95% CI = 8.67 - 5.09%). This was significantly lower than in case-positive villages ($p = 0.039$). However at six months this situation was reversed, and the case-negative prevalence of 14.4% (135/935, 95% CI = 16.86 - 12.25%) was significantly higher than the case-positive prevalence ($p = 0.0461$). The considerable rise in prevalence of *T. congolense* savannah in case-negative villages is statistically significant ($p < 0.0001$). This information is summarised in Figure 5.14.

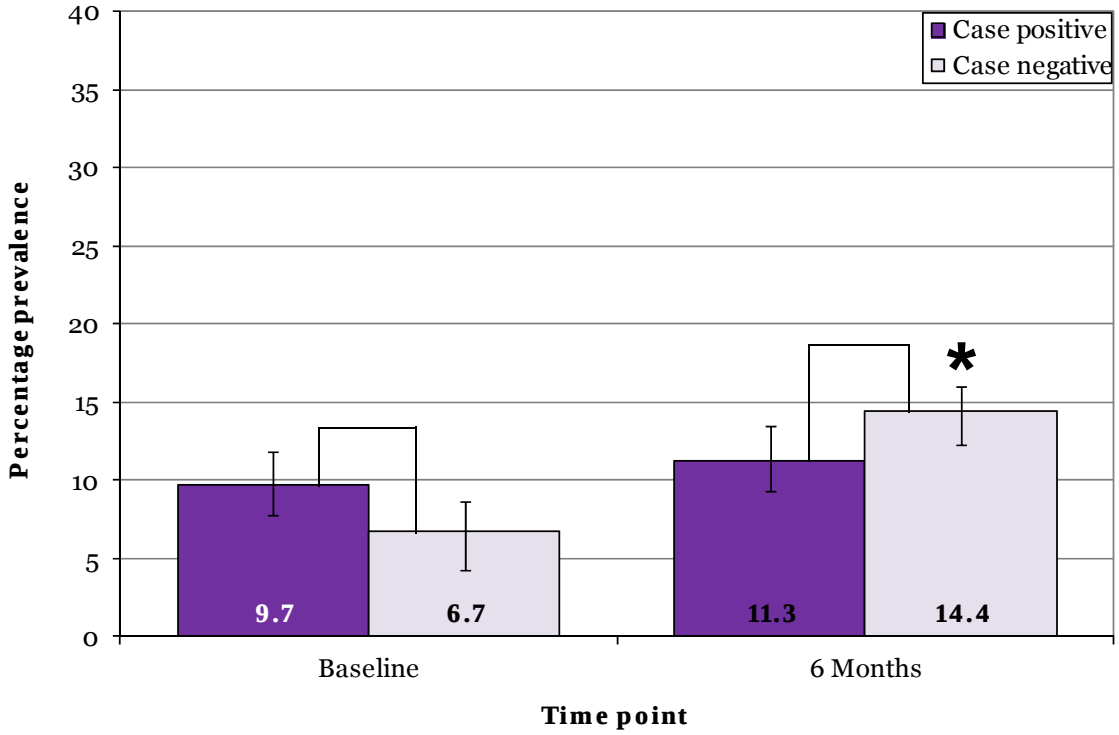

Figure 5.14 - percentage prevalence of *T. congolense* savannah in case-positive villages and case-negative villages at baseline and six months. Vertical lines indicate the 95% confidence intervals. Linking lines indicate a significant difference between groups at the same time point; stars indicate a significant change in that group between baseline and six months ($p < 0.05$).

5.3.6 Other trypanosome associations

The possibility exists that cattle demographic factors could have had an effect on the prevalence on trypanosomiasis. In this section the relationship between cattle age and trypanosome infection is examined, then the relationship between sex of cattle and trypanosome infection.

5.3.6.1 Age and infection

Below in Figure 5.15 the prevalence of each trypanosome species detected in each of the age groups at baseline and six months is shown.

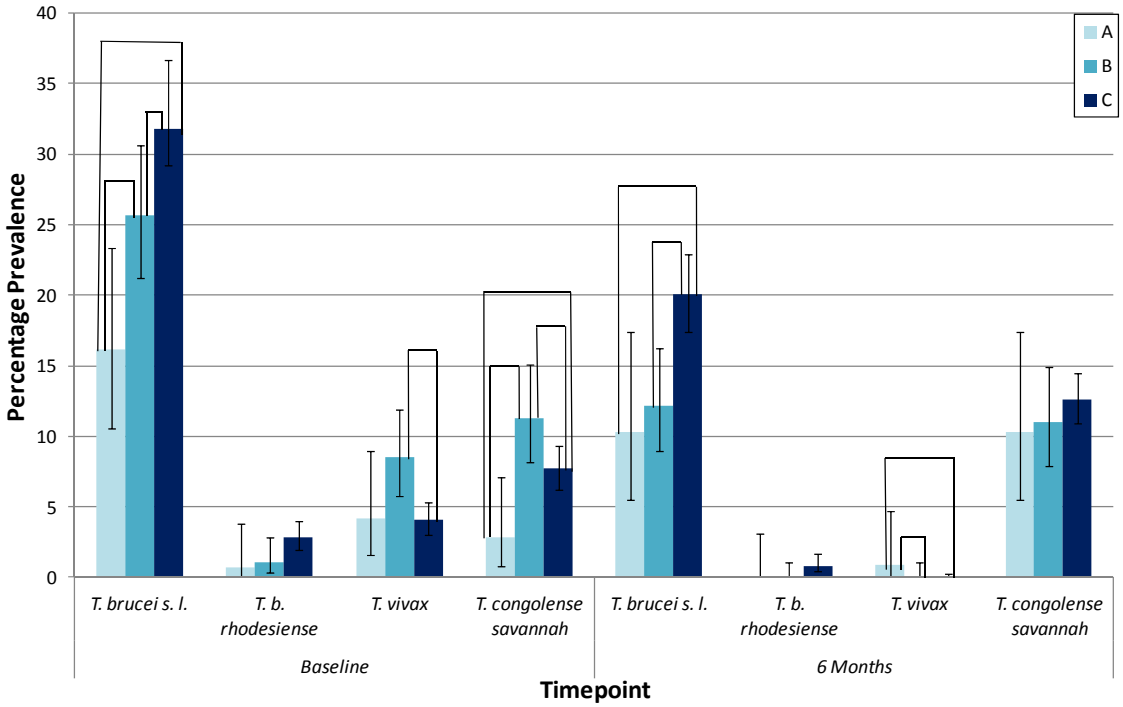

Figure 5.15 - prevalence of trypanosomiasis by cattle age group at baseline and six months; A (<18 months), B (18–36 months) and C (>36 months). Vertical lines dissecting each bar represent the 95% confidence intervals. Lines linking bars indicate a significant difference between the two ($p < 0.05$).

The prevalence of *T. brucei* s. l. is significantly different across age groups at baseline sampling. The prevalence in A aged cattle is significantly less than in B ($p = 0.02$) and C ($p < 0.0001$) aged cattle. The prevalence in B aged cattle is significantly less than in C ($p = 0.02$) aged cattle. The pattern of infection is similar at six month sampling; however there is no significant difference between cattle in A and B age groups. C aged cattle have significantly more *T. brucei* s. l. than any other age group, both at baseline and six month sampling, suggesting as age increases so too does *T. brucei* s. l. prevalence.

For *T. b. rhodesiense* the patterns in the age groups are similar to that of *T. brucei* s. l., but at much lower levels and without significant differences. For this subspecies there is no firm correlation between age and infection.

The highest prevalence of *T. vivax* is in B aged cattle at baseline sampling. The prevalence in this age group is significantly higher ($p = 0.0021$) than in the C aged adult cattle. At six month sampling *T. vivax* is only detected in the A age group, at significantly higher levels than in B ($p < 0.0001$) and C ($p < 0.0001$) age groups. Overall, it seems as if the trend is for *T. vivax* to infect younger animals, however due to the extremely low levels of *T. vivax* detected at 6 month sampling these results should be interpreted with caution.

T. congolense savannah shows a similar pattern to that of *T. vivax* at baseline, with a spike in prevalence in the B aged animals. The prevalence in this aged group is significantly higher than in A ($p = 0.0016$) or C ($p = 0.0385$) aged cattle. Furthermore, the prevalence of *T. congolense* savannah in A aged cattle is significantly ($p = 0.0365$) lower than in C aged cattle. So at baseline, the youngest cattle carry the least *T. congolense* savannah, and the juveniles carry the most. Interestingly, at 6 month sampling there is no significant difference between infection levels in age groups.

While this gives an insightful overview of the relative burden of trypanosomiasis in each age group, they do not show how this relates to the total prevalence of each individual trypanosome species in the sample population overall. If there is no link between age and trypanosome infection then the percentage of each trypanosome found in any age category should be similar to the percentage of that age group within the sample population.

5.3.6.1.1 Age contribution in case-positive villages

The percentage of the total sample each age group makes up can be used as a rough predictor of the contribution that age group should make towards the prevalence of each species. Below in Table 5.2 the percentage of age group in the sample is shown alongside their contribution to the prevalence of each species.

Case positive			Percentage of each trypanosomes prevalence accounted for by each age group			
	Age	Percentage of sample (predicted contribution to prevalence)	*T. brucei* s. l.	*T. b. rhodesiense*	*T. vivax*	*T. congolense* savannah
Baseline	A	7.1%	4.0%	4.8%	12.2%	3.6%
	B	21.4%	19.5%	9.5%	46.9%	26.5%
	C	71.5%	76.5%	85.7%	40.8%	69.9%
6 months	A	6.6%	4.0%	0.0%	NA	6.5%
	B	17.3%	14.1%	0.0%	NA	16.7%
	C	76.1%	81.8%	100.0%	NA	76.9%

Table 5.2 - percentage of animals in each of the age categories sampled at baseline and six months, and percentage of the total prevalence of each of the four trypanosome species found in each age group: A (<18 months), B (18–36 months) and C (>36 months).

At baseline in case-positive villages, cattle in the A age group account for 7.1% (60/854) of all animals sampled. 31.9% (272/854) of all cattle were positive for *T. brucei* s. l. 1.3% (11/854) of cattle were both in the A age category and infected with *T. brucei* s. l. Therefore 4% (11/272) of all *T. brucei* s. l. in case-positive villages were found in the A age category. If there was no link between trypanosome infection and age the amount of *T. brucei* s. l. detected in the A age category should be closer to 7.1%. This logic was followed to make comparisons between all age groups and species at both time points. The main differences between predicted and actual contribution to prevalence are outlined below.

At baseline in case-positive villages the predicted contribution of the A age group to the prevalence of each trypanosome species is 7.1%. As shown in Table 5.2 cattle in the A age group contribute a little less than would be expected to the prevalence of T. *brucei* s. l., *T. b. rhodesiense* and *T. congolense* savannah. The A age group contributed more than predicted towards the prevalence of *T. vivax*. At six months a similar pattern was evident, with the A age group contributing less than the predicted 6.6% to the prevalence of *T. brucei* s. l., and *T. b. rhodesiense*. The proportion of *T. congolense* savannah accounted for by infections in the A age category correlates roughly with the predicted percentage. No *T. vivax* was detected.

The predicted contribution of cattle in the B age group to trypanosome prevalence at baseline is 21.4%. As Table 5.2 shows, cattle in the B age group contribute less than predicted to the prevalence of *T. brucei* s. l and *T. b. rhodesiense*, and much more than predicted to the prevalence of *T. vivax* and *T. congolense* savannah. At the 6 month time point the B age group is predicted to contribute 17.3% towards trypanosome prevalence. Therefore this group is contributing less than expected to the prevalence of *T. brucei* s. l. and *T. b. rhodesiense*, and roughly the expected amount to *T. congolense* savannah. No *T. vivax* was detected at six months.

The predicted contribution of cattle in the C age group to trypanosome prevalence at baseline is 71.5%. According to Table 5.2, adult cattle account for a lower proportion of *T. vivax* prevalence than would be expected, close to the expected value for *T. brucei* s. l. and *T. congolense* savannah, and make a larger contribution to the prevalence of *T. b. rhodesiense* than would be expected given the proportion of the sample they make up. At six months the predicted contribution of the C age group to trypanosome prevalence is 76.1%. Therefore they contribute more than predicted to the prevalence of *T. brucei* s. l. and *T. b. rhodesiense*, and close to the predicted contribution towards the prevalence of *T. congolense* savannah.

5.3.6.1.2 Age contribution in case-negative villages

In Table 5.3 the percentage of each age group in the sample is shown alongside their contribution to the prevalence of each species case-negative villages, in order to again assess how much each age group contributes towards the overall prevalence of each species.

Case negative			Percentage of each trypanosomes prevalence found in each age group			
	Age	Percentage of sample (predicted contribution to prevalence)	*T. brucei* s. l.	*T. b. rhodesiense*	*T. vivax*	*T. congolense* savannah
Baseline	A	10.3%	5.7%	0.0%	2.0%	2.0%
	B	21.4%	17.9%	11.8%	32.7%	36.0%
	C	68.3%	76.4%	88.2%	61.2%	62.0%
6 months	A	5.6%	2.7%	0.0%	100.0%	3.2%
	B	18.0%	9.5%	0.0%	0.0%	15.3%
	C	76.4%	87.8%	100.0%	0.0%	80.6%

Table 5.3 - percentage of animals in each of the age categories sampled at baseline and six months and the percentage of the total prevalence of each of the four trypanosome species found in each age group; A (<18 months), B (18–36 months) and C (>36 months).

At baseline the predicted contribution of cattle in the A age group to the prevalence of each trypanosome species is 10.3%. According to Table 5.3, cattle in this age group seem to be contributing less than predicted to the prevalence of all four species of trypanosome. At six months the predicted contribution of cattle in the A age group is 5.6%. Again they contribute less than predicted to the prevalence of *T. brucei* s. l. *T. b. rhodesiense* and *T. congolense* savannah. The only case of *T. vivax* detected at six months was found in the A age group.

At baseline 21.4% of sampled animals were from the B age category. As shown in table 4.2, cattle in this age group contribute less than predicted to the prevalence of *T. brucei* s. l. and *T. b. rhodesiense*, and more to prevalence of *T. vivax* and *T. congolense* savannah. At six months cattle in the B age category contribute less than the predicted 18.0% to the prevalence of all four species of trypanosome.

At baseline 68.3% of animals belonged to the C age group. Therefore they are contributing more than predicted to the prevalence of *T. brucei* s. l. and T. *b. rhodesiense*, and less to the prevalence of *T. vivax* and *T. congolense* savannah. At six months the predicted contribution of cattle in the C age group to the prevalence of each species of trypanosome is 76.4%. According to Table 5.3, cattle in this age category are

contributing more than predicted to the prevalence of *T. brucei* s. l., *T. b. rhodesiense* and *T. congolense* savannah, and less to the prevalence of *T. vivax*.

Across all age groups and time points a common theme emerges in relation to age and infection. The level of trypanosome infection in each age category rarely correlates with the proportion of the sample which that age group makes up. Therefore the assumption that age has no effect on trypanosome prevalence does not seem to hold true.

5.3.6.2 Sex and infection

The sex of each cow sampled was recorded, and the results for each trypanosome species were analysed to see if there was any association between sex and trypanosome infection. This is represented in Figure 5.16.

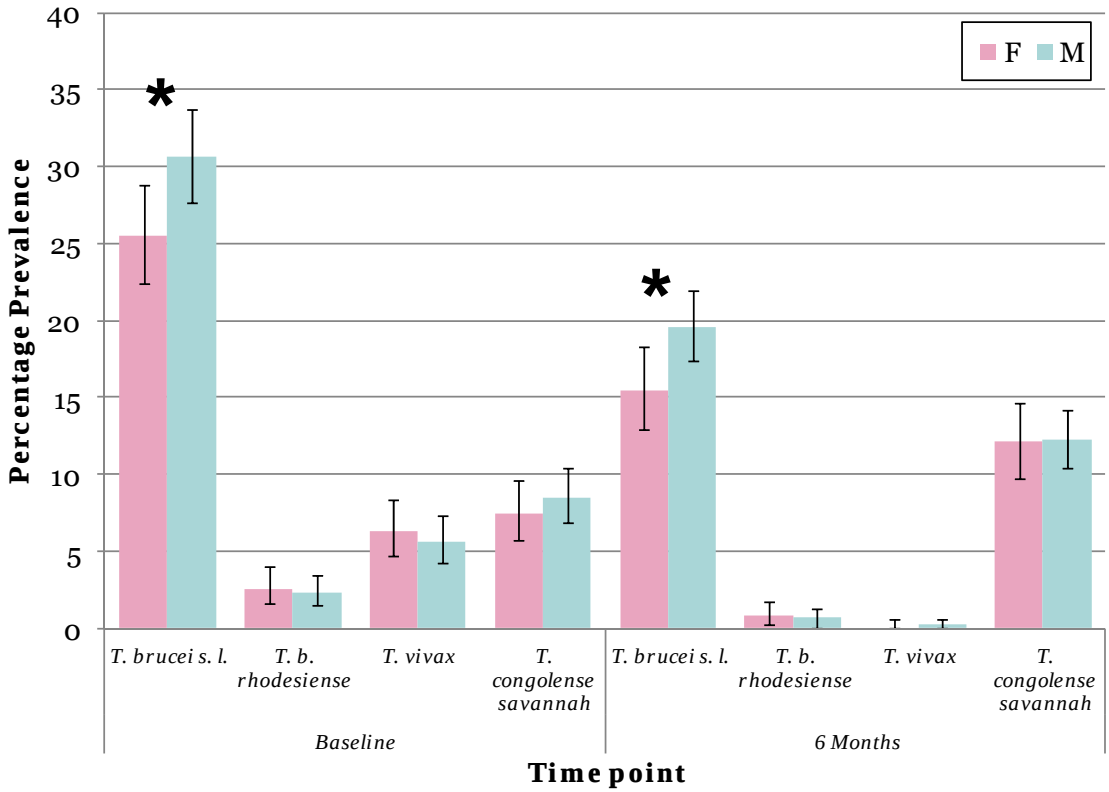

Figure 5.16 - prevalence of trypanosomiasis by sex of animal. Vertical lines dissecting each bar represent the 95% confidence intervals. Stars represent a significant difference between the sexes for that parasite, as determined by Fisher's Exact test ($p < 0.05$).

Male cows carry significantly higher levels of *T. brucei* s. l. than females do, both at baseline (p = 0.02) and 6 months (p = 0.03). This pattern is not repeated for any of the other species of trypanosome detected, where the levels are roughly equal in both male and female animals.

5.4 Discussion

The primary aim of the SOS re-treatment programme was to reduce the prevalence of *T. b. rhodesiense,* an aim which was successfully achieved. The re-treatment had a dramatic effect, significantly reducing the point prevalence of overall trypanosomiasis from 38.1% (95% CI = 40.5 – 35.79%) at baseline to 26.9% (95% CI 28.96 – 24.97, p < 0.0001) at 6 months. Looking at each species separately, point prevalence of 3 out of 4 detected species of trypanosome fell significantly, including *T. b. rhodesiense*.

5.4.1 Treatment coverage

The Makerere figures, outlined in chapter two of this thesis, show higher treatment coverage in case-negative parishes than in case-positive. Because these figures are based on cattle population estimates, they should be interpreted with caution.

The accuracy of the original cattle population estimates introduces a level of uncertainty in the treatment coverage calculations. Reports of the number of treated cattle reveal there was roughly half the number of cattle treated in case-positive parishes (6, 671) than in case-negative parishes (12, 930) indicating that the positive parish herd may be half that of negative parishes if the level of coverage was comparable. The highest discrepancy between the estimated population and the number of animals treated occurs in the case-positive parish of Opilitok, with 1050% treatment coverage, more than 10 times the expected number of animals.

There are a number of unknowns that should be addressed when considering these calculations; the cattle population in this area may be highly mobile and while the numbers treated are accurate, the size of the residential village herd cannot be assumed. Equally the sustainable cattle capacity of these retreatment areas is unknown, although the environmental conditions between the positive and negative parishes were noted to be very similar. There may also be an underlying cause that has influenced cattle productivity, thus diminishing the herd within the positive parishes.

The number of cattle reported to have been treated was well below 50% for both case-positive (45.7%) and case-negative (37.5%) villages when the owners were asked to recall the event at the 6 month sampling. This can be attributed to the import of untreated cattle, export of treated cattle, poor memory recall of cattle owners, birth of cattle, and the presence of cattle which were missed by the original treatment. The relative contribution of each of these factors to the high number of reported untreated animals is unknown.

The fact treatment coverage in case-positive parishes is reported to be higher at 6 months, despite these areas having more trypanosomiasis, suggests the link between treatment coverage and trypanosomiasis is weak at best. In addition, where transmission of trypanosomes has become established it is very difficult to disrupt this transmission cycle.

As was observed during SOS phase one, male cattle are more common in the re-treatment sample than females, both at baseline and six months. Although these figures are not statistically significant, they are again indicative of the high level of cattle movement within Dokolo and Kaberamaido. These imported cattle will not have been present while the re-treatment was being carried out, therefore they impact negatively on treatment coverage and the overall efficacy of the programme.

5.4.2 Impact of treatment on trypanosome prevalence

The prevalence of overall trypanosomiasis was reduced from 38.1% (95% CI = 40.5 – 35.79%) at baseline to 26.9% (95% CI 28.96 – 24.97, p < 0.0001) at 6 months. Of these infections, *T. brucei* s. l. makes up the majority of infections, followed by *T. congolense* savannah, *T. vivax* and *T. b rhodesiense*, with the prevalence of *T. vivax* falling lower than that of *T. b. rhodesiense* at 6 months. Each species is considered individually in the sections below.

5.4.2.1 *T. brucei* s. l.

The prevalence of *T. brucei* s. l. was significantly reduced, from 28.5% (95% CI 26-30%) at baseline to 18.1% (95% CI 16 - 20%, p < 0.0001), a reduction of 31.3%. At both time points there is a significantly higher prevalence of *T. brucei* s. l. in case-positive villages. This indicates a continued correlation between human and bovine trypanosomiasis. In case-negative villages the reduction in prevalence of *T. brucei* s. l. is 38.4% (p < 0.0001). The treatment is slightly less effective in the case-positive villages, where *T. brucei* s. l. prevalence falls by 35.6%, which is still a significant drop (p < 0.0001). *T. brucei* s. l. is the most common trypanosome detected within the re-treatment area, both at baseline and 6 months.

These findings concur with the previous work within the re-treatment area, and the SOS area as a whole. Before the re-treatment treatment took place monitoring of the SOS project had been carried out for baseline, 3 and 9 months post sampling. At each of these time points the prevalence of *T. brucei* s. l. was higher in the high risk re-treatment area, significantly so at 3 and 9 months, becoming more pronounced at the 9 month sampling where the prevalence within the high risk area is 22.5% compared to 12.2% in the rest of the SOS region (Selby, 2011). Although this is significantly less than 38.1% detected here, during the monitoring of SOS phase 1 it could be seen at 9 months the prevalence of *T. brucei* s. l. had already exceeded that at baseline (Selby, 2011), a reasonable assumption is that the prevalence kept rising in the absence of further intervention from either cattle owners or another mass intervention.

The prevalence of *T. brucei* s. l. is in line with the findings of other studies conducted in neighbouring areas in Uganda and other Sub-Saharan African countries. Looking broadly across East Africa, point prevalence of 34.3% of cattle in Tororo, Uganda, (Cox *et al.*, 2010), 45% of cattle in Soroti, Uganda (Welburn *et al.*, 2001b) and 21.2% of cattle in Kenya (Onyango *et al.*, 1966) illustrate the fact prevalence is very variable depending upon the conditions in each locality. Focusing on a smaller area within central Uganda, a 2003 study found baseline prevalence of *T. brucei* s. l. ranged between 1.4% and 22.8% across four districts (Fyfe, 2007). Similarly, when considering each individual sample site in this study point at village level, prevalence ranges between 8.8 – 53%.

The prevalence of zoonotic *T. b. rhodesiense* was significantly reduced in the cattle reservoir; this was a key aim of the re-treatment intervention. Prevalence fell from 2.35% (95% CI 3.21 – 1.72%) to 0.6% (95% CI 1.06 – 0.31%, P<0.0001), a reduction of 74.5% in the sample. The majority of *T. b. rhodesiense* at 6 months is detected in case-positive villages, again suggesting a continued correlation between bovine and human trypanosomiasis. As these villages are known to have had cases of HAT in the recent past, it could be that humans are the source of cattle re-infection. However as *T. b. rhodesiense* persists in case-negative villages as well, it is likely some *T. b. rhodesiense* survived the treatment or was imported into the region after treatment took place.

The findings here are also in line with those recorded during SOS phase 1. As was observed for *T. brucei* s. l., the prevalence of *T. b. rhodesiense* in the high risk area is consistently higher than in the rest of the SOS phase one areas, at baseline, 3 and 9 month post sampling - again being most pronounced at the 9 month time point, where *T. b. rhodesiense* was only detected in the high risk area, and not in the remainder of the SOS region (Selby, 2011). Therefore it is not surprising *T. b. rhodesiense* was detected by this study both at baseline and 6 month sampling; during SOS phase 1 monitoring even at the 3 month time point *T. b. rhodesiense* is still present in the cattle reservoir, albeit at a reduced prevalence (Selby, 2011).

Balyeidhusa and colleagues report a point prevalence of *T. b. brucei* of 14.5% in Western Uganda, using the same TBR PCR used here yet considerably lower than

reported here (Balyeidhusa *et al.*, 2012). However the areas in which Balyeidhusa and colleagues collected samples from is not a *T. b. rhodesiense* endemic area, unlike the high transmission area studied here, although it is one of the areas deemed to be at risk of an overlap. Neither human forms were detected in any of the animal species they tested, suggesting *T. b. rhodesiense* has not spread this far north, and, so far, overlap between the chronic and acute forms of sleeping sickness has not occurred.

5.4.2.2 *T. vivax*

The treatment had a marked impact on the prevalence of *T. vivax*, nearly eliminating this species from the re-treatment area. At baseline *T. vivax* prevalence was 5.4% (95% CI = 6.63 – 4.33%), at 6 months it was just 0.26% (95% CI = 0.03 – 0.01%), a statistically significant drop of 95.4% ($p < 0.0001$). No other species of trypanosome was so profoundly affected by the treatment. Out of all species detected at baseline, *T. vivax* was the least prevalent, so it stands to reason it would also be least prevalent at 6 months. A study conducted by Duffy *et al.,* 2009 in Gambia found *T. vivax* had a clonal population structure, with no evidence of mating (Duffy *et al.,* 2009). If the *T. vivax* detected here had a similar clonal population structure, this could account for its extreme sensitivity to the drug treatment, as with little genetic diversity there would be little chance for a resistant phenotype to exist or emerge. It seems more likely that one or two drug sensitive clones were the dominant circulating strains, and so treatment was very effective.

However a later study by Adams *et al.* (2010) isolated apparently new *T. vivax* genotypes from tsetse flies in Tanzania (Adams *et al.,* 2010). It had not previously been thought *T. vivax* was as genetically diverse as this, and the study casts doubts on previous assertions that *T. vivax* is unable to undergo sexual reproduction. The Tanzanian study was conducted in an area with many different species of wild life and domestic livestock, where as the Gambian study utilized samples only from cattle. Tsetse can be thought of as sampling the trypanosomes in many different species of mammalian and reptilian hosts depending on where they take a feed from, and so a reasonable expectation would be that these samples contained more diversity than trypanosomes isolated from one species of mammalian host. The studies performed by

both Adams and Duffy have limitations, including the fact they analysed a relatively small number of samples, from a small geographical area, over a short period of time. Valuable as these studies are, they provide mere snapshots in time of very small sections of the *T. vivax* population, especially when considering this is a parasite found across sub Saharan Africa. Direct extrapolation from either of those studies to make assumptions about the data presented here would be premature. Based on the extreme sensitivity of the Ugandan *T. vivax* to drug treatment, a clonal population structure is a sensible suggestion, which microsatellite analysis would be able to confirm.

5.4.2.3 *T. congolense* savannah

After the treatment the prevalence of *T. congolense* savannah rose significantly. This is in contrast to all other species of trypanosome detected, where the prevalence fell. *T. congolense* savannah was the second most prevalent species of trypanosome detected, both at baseline and 6 months. This parasite has not been detected by many other studies in Uganda, so finding it here at all constitutes something of an anomaly. A study in neighbouring south-east Uganda found the overall prevalence of *T. congolense* savannah in cattle to be 2.06%, compared to 5.44% for *T. vivax* and 4.99% for *T. brucei* s. l., although as microscopy was the primary detection method the prevalences of all three were probably underestimated (Waiswa *et al.,* 2003). Another recent study in central Uganda detected a maximum prevalence of just 0.7% of *T. congolense* savannah in cattle (Fyfe, 2007). This leads to the speculation that the strain or strains of *T. congolense* savannah circulating in this area are only mildly pathogenic, or else recently introduced, to account for the fact *T. congolense* savannah is seldom detected at these levels in Uganda. Variation in pathogenicity between strains of *T. congolense* savannah has been demonstrated previously (Masumu *et al.,* 2006).

If *T. congolense* savannah was newly introduced into the area, then it could be whatever allowed it to move into a new area allowed it to re-establish more quickly after the treatment, and lead to the continuing rise in the prevalence of this species. There could also be another animal species in the area, such as pigs, goats, sheep or dogs, which is more tolerant to *T. congolense* savannah infection than cattle are, and so is serving as a reservoir of infection, both before and after the treatment took place. As

cattle were the only species included in this intervention the treatment will therefore not have had such a marked effect on the prevalence trypanosomiasis in any potential reservoir species that inhabits the re-treatment area.

Some samples from the SOS phase one monitoring were also screened for *T. congolense* savannah as part of a study assessing the best molecular detection methods for trypanosomiasis, and the prevalence was found to be extremely low; 0.2% (Ahmed, 2009). Although the PCR primers and reaction conditions were identical to those used here, and samples were also stored on FTA cards, they were prepared for PCR following a different protocol. This protocol involved screening ten 1.2mm discs (Ahmed, 2009), as opposed to five 3mm discs screened here meaning the total area of blood on FTA card screened previously was 11.3mm^2 as opposed to 35.3mm^2 screened here. This increase in the surface area of blood saturated FTA card matrix from which DNA was eluted may have improved the detection of *T. congolense* savannah in this study.

While the findings in this study are in contrast to those reported previously, it is known from that work and work preceding and following it the introduction of trypanosomiasis into an area can happen quickly and unexpectedly, as evidenced by the introduction of sleeping sickness into previously unaffected areas which led to the conception of the SOS intervention in the first place. Judging by the high levels of *T. brucei* s. l. detected throughout the SOS campaign in this high risk area it seems it is an area well suited to the transmission of trypanosomes.

There are several factors which could explain the post treatment rise in *T. congolense* savannah prevalence. As six months had passed between treatment re-sampling, it could be *T. congolense* savannah was able to rebound to normal levels more quickly than the other species which were detected. A quick recovery in prevalence after treatment would also enable this species to take advantage of reduced competition from other trypanosome species, and so expand the ecological niche it occupies within the vector host trypanosome system. Furthermore, as the other cattle pathogenic species of trypanosome *T. vivax* almost disappeared after treatment this could have left a gap in the ecosystem which the similarly pathogenic, but more genetically diverse *T. congolense* savannah was able to exploit much more successfully. Tsetse fly have been

proven to have a higher feeding success rate on *T. congolense* infected cattle than uninfected controls (Baylis and Nambiro, 1993, Moloo *et al.*, 2000). This in turn could lead to increased transmission of *T. congolense* savannah and the rise in prevalence between baseline and six months. The fact this was not observed earlier, during SOS phase one monitoring, could indicate *T. congolense* savannah has been introduced to the area relatively recently, and is gradually becoming more established.

It could be that *T. congolense* savannah circulating in the area contained drug resistant genotypes, rendering the drug treatment less effective for this species of trypanosome. The discovery of population structure consistent with frequent mating in *T. congolense* savannah (Morrison *et al.,* 2009) means if a drug resistant trait was present in the area, frequent mating could have distributed it widely throughout the population. Drug resistant strains of *T. congolense* savannah have been reported across Sub –Saharan Africa, in areas of Ethiopia (Assefa and Abebe, 2001, Codjia *et al.,* 1993, Mulugeta *et al.,* 1997), Burkina Faso (Clausen et al., 1992, McDermott et al., 2003, Gall et al., 2004), Zambia (Delespaux *et al.*, 2008) and many other countries and areas. *As T. congolense* savannah is not frequently reported in Uganda in the literature, there are similarly few reports on the susceptibility or resistance of this parasite to trypanocides in this country. Further investigation would be required to determine if drug resistance was a factor influencing the epidemiology of this trypanosome in the re-treatment area.

5.4.3 Cattle characteristics that affect trypanosome prevalence

The pattern of contact between blood sucking insects, such as tsetse flies, and their hosts, is varied and non-random (Kelly, 2001). Therefore some hosts are challenged significantly more than others and may contribute more to the transmission of disease, including vector borne parasites (Woolhouse *et al.,* 1997), such as trypanosomes. As well as contact with tsetse, other factors may affect the level of trypanosomiasis in different demographic groups of cattle. The prevalence of trypanosomiasis has been found to vary according to age and sex in a number of studies, which in part has been attributed to the effect of these variables on the attractiveness of the individual host to the tsetse vector. A selection of these studies are discussed below, in relation to the correlation between age, sex and trypanosomiasis in the re-treatment area.

5.4.3.1 Age and infection

The results presented in section 5.3.8.1 show that not only do some age groups have a significantly higher prevalence of trypanosomiasis than others, and that the most affected age group varies according to species. Further evidence of the link between animal age and trypanosome infection comes from the analysis of the percentage of trypanosomal infection detected in each age group, where for many species each age group contributes more or less than would be expected according to the percentage of that sample that age group makes up. Taken together these findings strongly suggest the distribution of trypanosomiasis within the sample population is not random and may be affected by cattle age.

While it is well known age effects trypanosome prevalence, the literature on this topic is conflicting as to the precise nature of this effect. For example; studies on the feeding preferences of tsetse flies have found mature animals are more attractive to tsetse flies than their younger counterparts, as they provide stronger visual and olfactory cues to attract the flies, due in part to their larger size (Torr, 1994). Young animals have also been observed to exhibit more defensive behaviour than adults (Torr, 1994, Torr and Mangwiro, 2000), and so are hypothesized to be bitten less. This would indicate younger animals should be at less risk of contracting trypanosomiasis than adults.

When considering *T. brucei* s. l. this seems to be the case, as shown in graph 5.14 the youngest animals are the least infected, followed by animals falling into the juvenile age group and then adults harbouring the highest prevalence. However this pattern is not repeated when looking at the prevalence of *T. congolense* savannah or *T. vivax*. The results here split the pattern of age and infection into two categories, the pattern observed in cattle pathogenic species, *T. vivax* and *T. congolense* savannah, and the pattern observed in the non-pathogenic *T. brucei* s. l. A search of the literature reveals this may be considered something of an atypical distribution of pathogenic

trypanosome infection in different cattle age categories, as discussed in more detail below.

In Ethiopia prevalence of *T. congolense* savannah was highest in cattle older than 48 months, which would fall into the adult age category here (Rowlands *et al.,* 2001), in contrast to the drop in prevalence detected in that age category here. The same investigators also analysed *T. vivax*, and found prevalence steadily increased from the calf age group, was highest between 18 and 42 months of age, and declined slightly after this (Rowlands *et al.,* 2001). The shape of the curve is similar to that here, but much flatter and consequently without significant differences in levels of infection in animals of different ages. A more recent study in a different area of Ethiopia found prevalence of either *T. congolense* savannah or *T. vivax* did not vary according to age (Mihret and Mamo, 2007).

However the parameters defining each age category are not uniform, and so the age of an animal classified as a calf, juvenile or adult differs depending upon the paper being considered. As a further caveat it should be noted many studies determining the differing attractiveness of animals to tsetse usually consider individual animals alone, when cattle herding and management practices mean each individual is part of a large group of animals, and that group will exert a much larger attractive stimulus then any individual of a given age, weight or sex. Therefore, the apparent link or lack of link between trypanosomiasis prevalence and age reported in the above studies may not be relevant where epidemiological conditions or study parameters differ even slightly.

Despite these difficulties comparing and interpreting age and infection patterns within and between studies, there is a growing body of evidence to support the findings here. In Uganda a recent study found a similar pattern of age and infection in Tororo district, with *T. vivax* prevalence being highest in animals that would fall into the juvenile age group of this study (Magona *et al.,* 2008). Another study in the SOS area also found a similar pattern of age and infection with *T. vivax*, with adults being the least infected, followed by juveniles, and calves harbouring the highest prevalence of this parasite (Ahmed, 2009). Investigators in the Democratic Republic of Congo found N'Dama

calves there harbour significantly less *T. vivax* and *T. congolense* savannah than their mothers, with the highest prevalence of both occurring in juvenile animals, before tailing off in the adult age category slightly for *T. congolense* savannah and a lot for *T. vivax* (Trail *et al.,* 1994). Based on the aforementioned information, lower prevalence of pathogenic trypanosomiasis in adult cows is a feasible situation in some areas.

This pattern of infection becomes even more feasible when considering the relevant epidemiological theory. The term peak shift refers to a pattern where peak levels of infection are higher and occur at a younger age in populations subject to high levels of transmission, but are lower and occur at an older age when transmission is lower (Woolhouse, 1998). The main assumption of this theory is exposure to the pathogen in question leads to a degree of acquired immunity, a controversial topic in the field of trypanosomiasis research.

A study on the pathogenesis of *T. congolense* in cattle found animals under the age of 1 were able to clear infection without treatment, whereas animals aged between 2 and 5 years needed treatment to clear the infection or else they died (Wellde *et al.,* 1981). Self-cure has also been reported in calves infected with *T. vivax* (Uzoigwe, 1986). Wellde and colleagues also reported a certain degree of immunity/tolerance, when cattle were challenged with exactly the same strain of *T. congolense*, but that this tolerance was not transferred to other strains of *T. congolense* from different geographical locations (Wellde *et al.,* 1981). Theoretically then it could be possible for cattle to build up tolerance to the circulating strain or strains of trypanosome in a given area, meaning severe outbreaks of clinical disease in adults occur only when a new strain is introduced into the area. Crucially, *T. brucei* s. l. would not be affected by peak shift, as it is not pathogenic causing at worst mild disease; therefore immunity to this species is not required or advantageous within the cattle population.

It is also critical to consider the effect treatment had on the pattern of age and infection. *T. brucei* s. l. prevalence remains highest in adult cattle at both time points, significantly so, while at six months there is not enough *T. vivax* detected to attribute any particular pattern to the age distribution. However the pattern for *T. congolense*

savannah has changed between baseline and six months, and a pattern no longer exists at six months, as there is no significant difference in the prevalence of this trypanosome in any of the age groups. This is interesting considering the significant differences between all age groups at baseline. These observations lend weight to the theory that some sort of immunity develops to *T. congolense* savannah, at least in a section of the population. By administering mass treatment to the entire population young animals were effectively cured of this infection, removing the chance for immunity to develop. Therefore, as adults, these animals are more susceptible to *T. congolense* savannah than they would otherwise have been, facilitating the rise in prevalence observed for this species in the sample population overall. The mass treatment has in effect increased the burden of *T. congolense* savannah in the C age group. Tables 5.2 and 5.3 show that at six month sampling the adult age group contributed more than the predicted percentage towards the overall prevalence of *T. congolense* savannah.

While some of the data here may be suggestive of an underlying acquisition of herd immunity over time, it is important not to discount other possible explanations. It could be that young animals contract and die from *T. congolense* savannah and *T. vivax*, and so fewer infected animals survive into adulthood. This theory assumes challenge is equal across age groups and young animals suffer higher mortality from pathogenic trypanosome infections.

5.4.3.2 Sex and infection

T. brucei s. l. was the only species of trypanosome where a link between sex and infection was evident. Male cattle were significantly more infected with *T. brucei* s. l. than females, both at baseline and 6 month sampling. Several previous studies have found male cattle, and in particular oxen, to be at a higher risk of trypanosome infection (Rowlands *et al.*, 2001), due to their large size and so increased odour excretion and therefore attractiveness to tsetse (Torr and Mangwiro, 2000). Perhaps this effect is only statistically significant for *T. brucei* s. l. due to the power of the statistical testing methods requiring a large number of samples to detect a small difference. Perhaps it is an artifact of *T. brucei* s. l. low virulence, males are more at risk from infection with any trypanosome, however males infected with pathogenic strains

would be more likely to be treated than females, as they usually provide valuable traction. Thus infection rates between sexes are the same due to treatment of trypanosomiasis signs, apart from *T. brucei* s. l. because of its symptom free pathogenesis.

5.5 Conclusion

The re-treatment programme was effective in reducing the overall prevalence of trypanosomiasis in Dokolo and Kaberamaido; it also significantly reduced the prevalence of *T. b. rhodesiense*, the target pathogen of this control programme, and so can be considered successful in this aspect.

The results presented here lead to a rejection of the null hypothesis, that the treatment had no effect on the prevalence of *T. brucei* s. l., *T. b. rhodesiense*, *T. vivax* or *T. congolense* savannah.

The movement of cattle into the re-treatment area from locations that were not covered by the intervention continues to be a significant, yet difficult to quantify, factor in the epidemiology of trypanosomiasis in these areas.

While this re-treatment programme has undoubtedly saved lives and improved animal health it has also raised important questions that ultimately cannot be answered with this data alone.

Strong circumstantial evidence has been provided to support the theory that immunity to pathogenic trypanosomes does develop in areas of high challenge. However serological tests to demonstrate the basis of this hypothesized immunity are needed, and analysis of the pathogenicity of the strains of trypanosome circulating in the area.

Questions raised around the possibility of drug resistant strains of *T. congolense* savannah need to be addressed by genetic analysis of existing specimens and collection of live trypanosomes in order to determine their susceptibly to trypanocides. This in itself could form the basis of an entire PhD thesis.

Ultimately the main conclusion of this chapter is that mass treatment of cattle is an effective way to reduce the prevalence of trypanosomiasis in these cattle; the question remains as to the best way to sustain this reduction over time.

Chapter 6 – A comparative analysis of SOS phase one and the re-treatment in Dokolo and Kaberamaido

Chapters four and five of this thesis document the impact of the Stamp Out Sleeping sickness programme following two rounds of mass trypanocidal treatment of cattle. Monitoring was based on the molecular analysis of cattle blood, in order to assess the impact in terms of trypanosome prevalence within the treated herds. In this chapter information from both these treatments will be compared, as a meta-analysis of the general epidemiology of trypanosomiasis in Dokolo and Kaberamaido between 2006 and 2008. The central tenet of this chapter, and moreover this thesis, is examining the overarching impact of SOS for the duration of its running in Dokolo and Kaberamaido.

Both rounds of treatment shared a similar aim: to reduce the prevalence of the zoonotic parasite *T. b. rhodesiense* within the cattle reservoir; for both rounds of treatment this aim was successfully achieved. The data in chapter four illustrated the first mass treatment did not impact equally on the prevalence of all trypanosome species. While *T. brucei* s. l. prevalence was significantly reduced, the prevalence of *T. vivax* and *T. congolense* savannah were seemingly unaffected. The re-treatment also did not impact equally upon the prevalence of all trypanosome species, this time the prevalence of both *T. brucei* s. l. and *T. vivax* were significantly reduced, while a significant increase occurred in the prevalence of *T. congolense* savannah.

Despite this shared aim SOS phase one and the re-treatment programme were implemented differently; SOS phase one took in a much larger area, including parts of Lira and Apac and all of Amolatar, Dokolo and Kaberamaido. The re-treatment targeted only the highest risk areas within Dokolo and Kaberamaido. As such the inclusion of the areas surrounding Dokolo and Kaberamaido in SOS phase one will have had an impact on the re-infection of these districts. Furthermore, SOS phase one ran deltamethrin spraying alongside the trypanocidal treatment; the re-treatment was reliant on single administration of trypanocide only.

The reason or reasons for the differing outcomes of the two treatments is unclear, but by looking in more detail at trypanosomiasis on the micro-scale possible explanations

will be explored. It could be the differing implementation of the two treatments lies at the heart of the difference, but other epidemiological factors may also play a role.

6.1 Background

Within this chapter potential correlates of trypanosome infection will be investigated; links between trypanosomiasis and age, sex, treatment coverage, type of drug treatment, and geographical location will be explored. The presence and determining factors of mixed infections will also be investigated. The rationale for investigating these particular factors is given in the following sections.

6.1.1 Drug treatment and trypanosomiasis

As stated in Chapter two, the first mass treatment delivered in Dokolo and Kaberamaido was isometamidium, a curative drug that also has prophylactic activity. For SOS phase one Dokolo and Kaberamaido were treated with isometamidium, while the remaining areas were treated with diminazene. Dokolo and Kaberamaido were singled out for this different drug treatment due to the much higher burden of HAT when compared to the other SOS districts (Selby, 2011). However comparison of the areas under the two different treatment regimes showed no significant difference in prevalence of *T. brucei* s. l. despite the reported prophylactic effect of isometamidium (Selby, 2011). Hence when the re-treatment was initiated in 2008 the decision was taken to treat with the cheaper diminazene.

While the analysis of Selby (2011) showed no significant difference in the prevalence *of T. brucei* s. l. in isometamidium and diminazene treated areas, it is important to remember the areas compared did not have equal levels of trypanosome prevalence. The re-treatment area was shown to have significantly higher prevalence of *T. brucei* s. l. compared to the rest of the SOS area. If challenge and transmission of trypanosomiasis was higher in Dokolo and Kaberamaido than the rest of the SOS region, the prophylactic effect of isometamidium could have been masked. Therefore it is possible the prophylactic effect of isometamidium did retard the re-infection rate in cattle. Under this hypothesis trypanosomiasis in isometamidium treated areas should

have been higher than in diminazene areas, but the prophylactic effect of isometamidium lowered the prevalence of trypanosomiasis to the extent there was no significant difference in trypanosomiasis prevalence between the isometamidium and diminazene treated areas.

Isometamidium has been reported to have prophylactic activity lasting between one and three months in some situations (Eisler *et al.*, 1994). If this is true for the epidemiological situation in Dokolo and Kaberamaido, cattle falling into the treated category should have less trypanosomiasis than those in the untreated or unknown categories, as they were sampled within the period when isometamidium could still be prophylatically active. Even if the period of prophylaxis had passed, prevalence should still be reduced due to the complete removal of trypanosomiasis from this group three months prior to sampling (assuming a 100% cure rate).

6.1.2 Cattle age, sex and trypanosomiasis

Data presented in chapters four and five of this thesis showed age and sex of cattle had some effect on the prevalence of trypanosomiasis. During SOS phase one, few significant differences were detected in the prevalence of trypanosomiasis in the different sexes. The re-treatment results showed *T. brucei* s. l. infection is higher in males than in females, and other studies have shown males in general are more susceptible to trypanosomiasis (Torr and Mangwiro, 2000).

With regards to age, patterns of age and infection were observed during SOS phase one and the re-treatment. During SOS phase one the prevalence of *T. congolense* savannah and *T. vivax* was highest in the A or B age groups consistently, but the difference was not significant at all time points. For the re-treatment at baseline the B age group had significantly higher prevalence of both *T. vivax* and *T. congolense* savannah. The prevalence of *T. brucei* s. l. increased in a stepwise fashion, with lowest prevalence in the youngest animals and highest prevalence in adults. By six months, the pattern of age and trypanosome prevalence had disappeared for *T. congolense* savannah and *T. vivax* but was maintained in *T. brucei* s. l. Other studies have similarly shown age

having an effect on trypanosome prevalence (Magona *et al.*, 2008, Rowlands *et al.*, 2001, Trail *et al.*, 1994, Welburn *et al.*, 2008).

Breed and body condition score had no effect on trypanosomiasis prevalence (see section 4.3.3). Furthermore, the uneven distribution of cattle between the descriptors in these categories made statistical analysis problematic, so these categories were excluded from further analysis. To ascertain whether sex and age interacted with one another to give an amplified effect on trypanosome prevalence, or obscure an underlying pattern, the Mantel – Haneszel test was applied (section 3.7). This method was used to test for interaction between the effect of age and sex on trypanosome prevalence. Failure to control for any possible interaction between two factors could lead to confounding bias.

Time since treatment and location (Dokolo/Kaberamaido, case-positive/case-negative) were shown to have a significant impact upon trypanosome prevalence. Since each area at each time point has been shown to be significantly different from the others, and the same animals were not sampled at each time point, they have been kept separate for this analysis. Combining areas known to be measurably different from one another with regards to trypanosomiasis will not further understanding of the epidemiology of the disease in these areas.

Therefore the Mantel-Haneszel test was utilized to test for a possibly confounding between sex and age only. The results of this analysis are given in section 6.3.1

6.1.3 Mixed species trypanosome infections

Epidemiological studies usually focus on single parasite systems, even though most hosts are capable of supporting multiple parasite species, meaning the potential impacts of co-infection on disease dynamics are only beginning to be recognized (Jolles *et al.*, 2008). Section 6.3.3 examines the prevalence and distribution of mixed infections during SOS phase one and the re-treatment.

If there is no association between species of trypanosome then one cow should be just as likely to be infected with a particular trypanosome as another, and the occurrence of mixed double and triple infections should be comparatively rare. According to the law of basic probability, if there is no association between trypanosome distributions within the sample population, the probability of getting a mixed infection with any two species should be the probability of getting a species A positive animal multiplied by the probability of getting a species B positive animal, minus the number positive for A, B and C (Willett, 1972). *T. brucei* s. l., *T. vivax* and *T. congolense* savannah have been detected within the sample population and using the known values for the prevalence of each of the three species of trypanosome it is possible to work out the expected probabilities for combinations of these trypanosomes. Using the exact binomial test (as described in 3.7) these predicted probabilities are compared to the actual frequency of mixed infections and p values are given assessing the likelihood of observing exactly this number of mixed infections, less than this number, or more than this number. In this way, it is possible to tell not only if the result is unexpected, but in which direction the result is skewed.

6.1.4 Village level trypanosomiasis

Thus far, this thesis has examined trypanosomiasis prevalence at the district level. Examining the prevalence in each of the villages sampled with reference to the spatial distribution of these villages may reveal areas of high trypanosome prevalence. As such, HAT is known to be a focal disease, the distribution and impact of which varies over relatively small distance. Therefore continual extrapolation to the district level risks overlooking important spatial patterns and indicators of prevalence on a village level.

6.2 Chapter aims

Given the background information outlined above, the main aims of this chapter are:

- To identify if any group or subgroup of cattle are consistently more infected than others, in order in identify parts of the “district herd” which should be given special attention should future control measures be considered in these areas. This information should also be considered by those designing and implementing trypanosomiasis control programmes elsewhere in sub-Saharan Africa.
- To compare the efficacy of isometamidium and diminazene in reducing trypanosome prevalence in Dokolo and Kaberamaido
- To investigate the occurrence of mixed infections and assess whether trypanosome species are evenly distributed in the sample population, or if association between different species occurs.
- To identify spatial patterns in the distribution of trypanosomiasis by comparing prevalence data at the village level.

6.3 Results

This result section is a split into four sections, corresponding to each of the chapter aims. The first section gives an overview of the results of Mantel-Haneszel test for association between age and infection. Next the coverage of SOS phase one and the re-treatment are outlined, as too is the prevalence of trypanosomiasis in treated and untreated animals at each round of treatment. The third section of results deals with the prevalence and distribution of mixed infections within the sample population. The spatial distribution of mixed infections is also mapped. Finally, the last results section analyses the epidemiology of trypanosomiasis at the village level, with a particular focus on villages or groups of villages that could be considered most at risk.

6.3.1 Results of Mantel-Haenszel test for association between trypanosomiasis prevalence and the age and sex of cattle.

At each time point the prevalence of each species of trypanosome was compared in males and females of each age group. Dokolo and Kaberamaido were examined separately at baseline, three, nine and eighteen months. Case-positive and case-negative areas were tested separately at baseline and six months. Section 3.7.1.1

provides a detailed explanation on the calculation involved in the Mantel-Haenszel (MH) test. The test was run using R statistical software, and the results for the effect of age and sex on *T. vivax* prevalence in Kaberamaido at SOS phase one baseline sampling are given below in Figure 6.1.

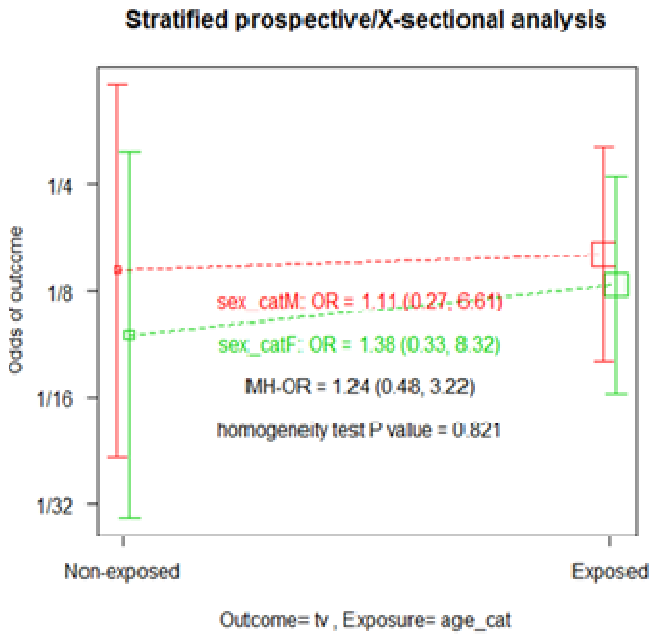

Figure 6.1 - results for *T. vivax* infection in Kaberamaido during SOS phase one at baseline. Red = male, green = female, exposed = A age group, non-exposed = B age group.

In the above example, where the combined effect of age and sex on the prevalence of *T. vivax* is assessed, it is clear there is no significant interaction, as OR confidence intervals of the strata overlap (the green and red vertical lines), the MH-OR value is close to 1 with the 95% confidence interval including 1, and homogeneity test value > 0.05.

Comparisons were conducted and interpreted in this way for Dokolo and Kaberamaido separately at each time point for SOS phase one, and separately for case-positive and case-negative villages at the re-treatment. As age has three categories three comparisons were run at each time point, comparing A to B, A to C, and B to C. Across all of these time points no significant interaction between age and sex was detected (remaining data not shown).

6.3.2 Comparison of isometamidium and diminazene treatment

Parts of Dokolo and Kaberamaido were included in both the SOS phase one mass treatment and the re-treatment. SOS phase one treatment was with isometamidium. The re-treatment used diminazene. Therefore the opportunity now arises to compare the effect of diminazene and isometamidium treatments in the same area.

6.3.2.1 Isometamidium coverage and trypanosome prevalence: SOS phase one

At every post treatment sampling cattle owners were asked if their cattle had been treated. They responded that their cattle were either treated, untreated or can't remember/don't know. For SOS phase one the information gathered at three months is likely to be most reliable, as this data is based on the memory of each farmer, the less time elapsed since treatment the better memory recall is likely to be.

Three months post treatment the majority of farmers recalled their animals being treated (in Dokolo 83.3%, 405/486, 95% CI 79.7 – 86.5% and in Kaberamaido 76.3%, 547/717, 95% CI 73.0 – 79.4). Some farmers recalled their animals were present at the time of treatment but did not receive a dose (in Dokolo 11.9%, 58/486, 95% CI9.2 – 15.2%, and in Kaberamaido 18.8%, 135/717, 95% CI 16.0 – 21.9%). Few farmers were unable to recall the treatment status of their cattle (in Dokolo 4.7%, 23/486, 95% CI 2.1 – 3.1%, and in Kaberamaido 4.9%, 35/717, 95% CI 3.4 – 6.7%). The difference between the three categories in each district was statistically significant ($p < 0.0001$ for all). This is summarised in Figure 6.2.

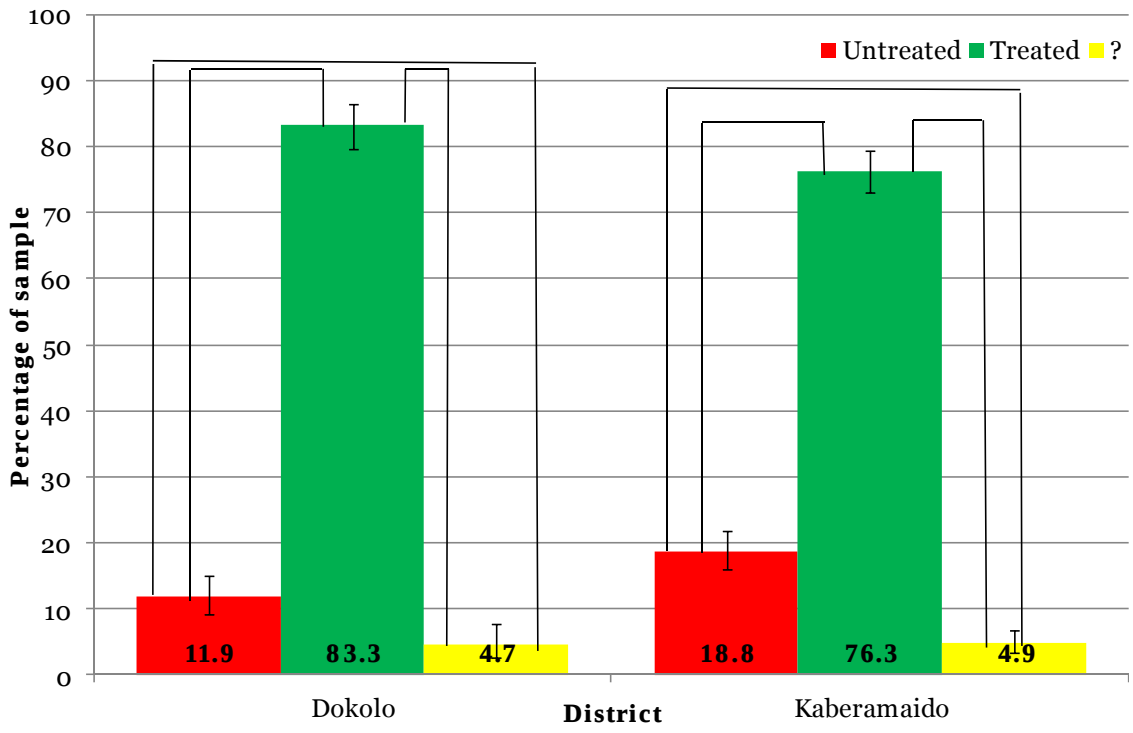

Figure 6.2 - percentage of treated, untreated, and unknown treatment status cattle present in Dokolo and Kaberamaido at three month sampling (? = unknown treatment status). Vertical lines dissecting bars represent the 95% confidence intervals. Lines linking bars represent a significant difference between the two as determined by Fisher's Exact Test ($p < 0.05$).

6.3.2.1.1 Isometamidium coverage by age

At three months many of the cattle in the A age group may not have been born when the treatment took place, and therefore they might unduly affect the estimated treatment coverage overall. This is clearly the case in Dokolo, where there is no significant difference in the percentage of treated and untreated animals in the A age group. Furthermore, coverage in the C age group in this district has exceeded the target of 86%. In Kaberamaido the effect is not as pronounced, but still coverage in the C age group coverage is more than 3% higher than the combined total across all age groups. This is summarised in Figure 6.3.

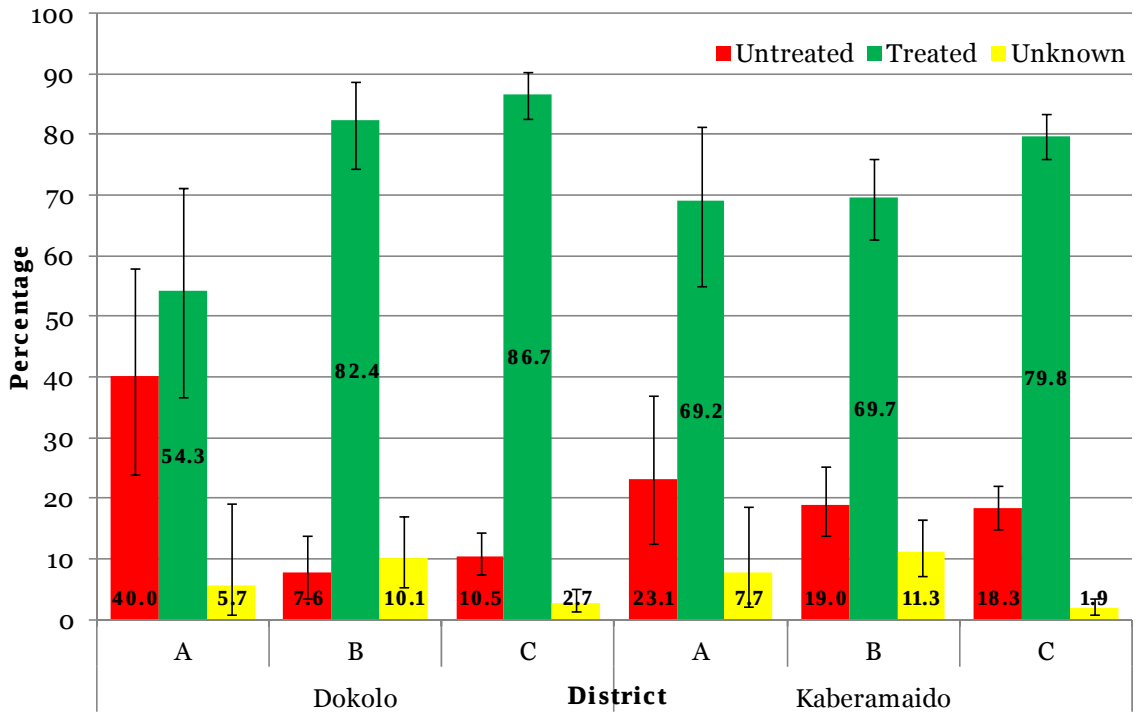

Figure 6.3 - percentage of untreated, treated and unknown cattle in each of the three age group: A (<18 months), B (18 - 36 months) and C (>36 months) at SOS phase one three month sampling.

6.3.2.1.2 Trypanosome prevalence and isometamidium coverage

In Dokolo the prevalence of overall trypanosomiasis, caused by one or more species of trypanosome, and labeled any in Figure 6.4, is significantly ($p = 0.02$) higher in cattle where the treatment status is unknown, when compared to treated cattle. Similarly prevalence of *T. brucei* s. l. is significantly higher in cattle of unknown treatment status than treated cattle ($p = 0.03$). There were no other significant differences detected between trypanosome prevalence in treated, untreated and unknown cattle. This is summarised in Figure 6.4.

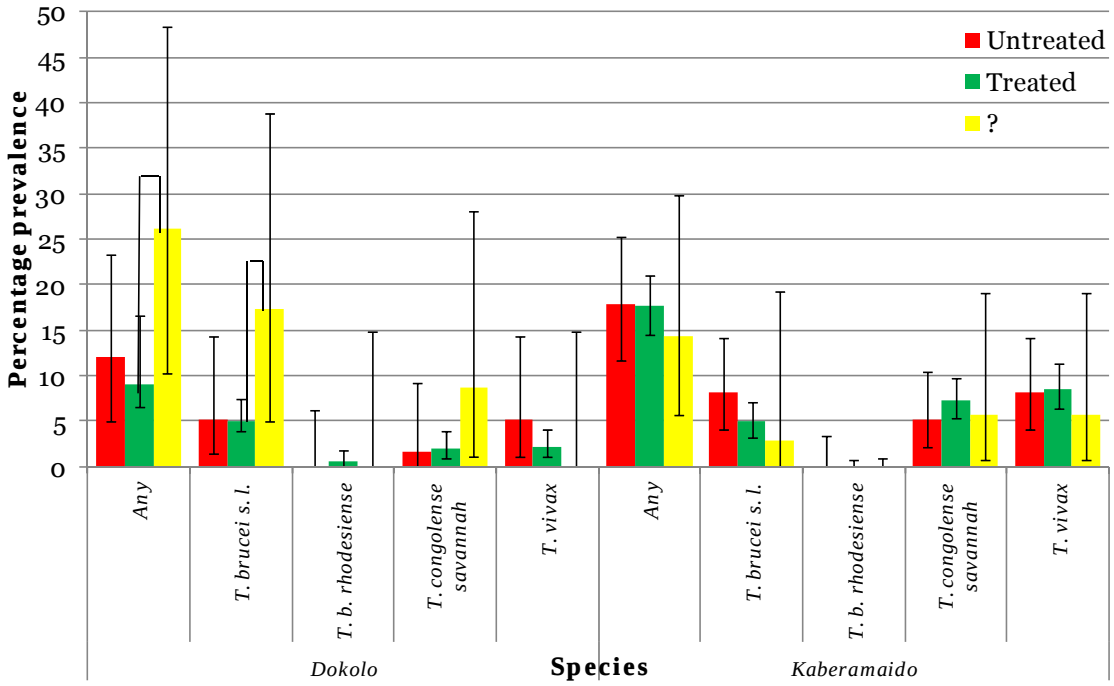

Figure 6.4 - prevalence of trypanosomiasis in each of the untreated, treated and unknown groups of cattle (? = unknown) at SOS phase one three months sampling. Vertical lines dissecting bars represent the 95% confidence intervals. Lines linking bars represent a significant difference between the two as determined by Fisher's Exact Test ($p < 0.05$).

6.3.2.2 Diminazene coverage: SOS re-treatment

At six month post treatment sampling farmers were questioned as to whether their animals had been treated or not. They responded that their cattle were either treated, untreated or can't remember/don't know.

The majority of cattle keepers reported their animals had not been treated at six month sampling in the re-treatment area (Figure 6.5). In case-positive areas 50.8% (486/956, 95% CI 47.6 – 54.1 %) of cattle were reported untreated, significantly more than were reported treated ($p = 0.03$), or unknown ($p < 0.0001$). In case-negative areas 59.7% (558/935, 95% CI 56.5 – 62.8%) were reportedly untreated, significantly more than were reported treated ($p < 0.0001$) or unknown ($p > 0.0001$). This is summarised in Figure 6.5.

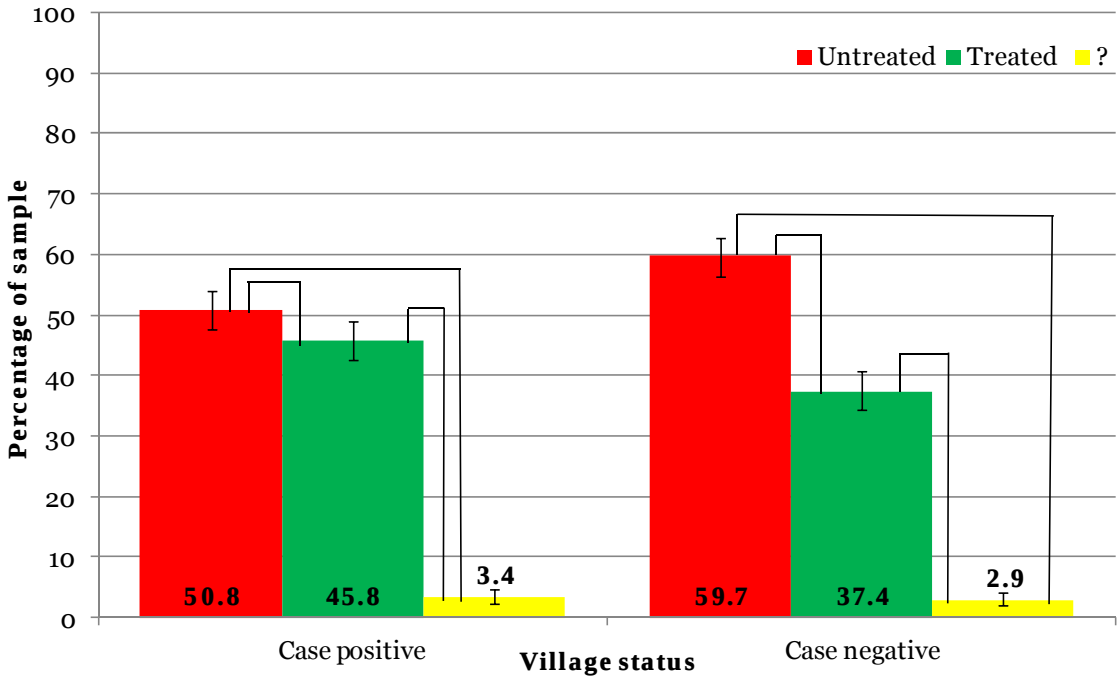

Figure 6.5 - percentage of cattle falling into the untreated, treated and unknown categories at six month sampling in the re-treatment area (? = unknown). Vertical lines dissecting bars represent the 95% confidence intervals. Lines linking bars represent a significant difference between the two as determined by Fisher's Exact Test ($p < 0.05$).

6.3.2.2.1 Diminazene coverage by age

Many of the cattle in the A age group may not have been born when the treatment took place, and therefore they might unduly affect the estimated treatment coverage overall. In case-positive villages at six months 72.9% of cattle in the A age group had not been treated, significantly higher than the percentage of untreated animals in the B or C age groups ($p > 0.0001$). The age group with the highest reported diminazene coverage was the B age group. Similarly in case-negative villages 94.2% of cattle in the A age group had not been treated at the six month sampling, significantly more than the percentage of untreated animals in the B or C age groups ($p < 0.0001$). In case-negative villages the highest reported diminazene coverage was in the C age group. This is summarised in Figure 6.6.

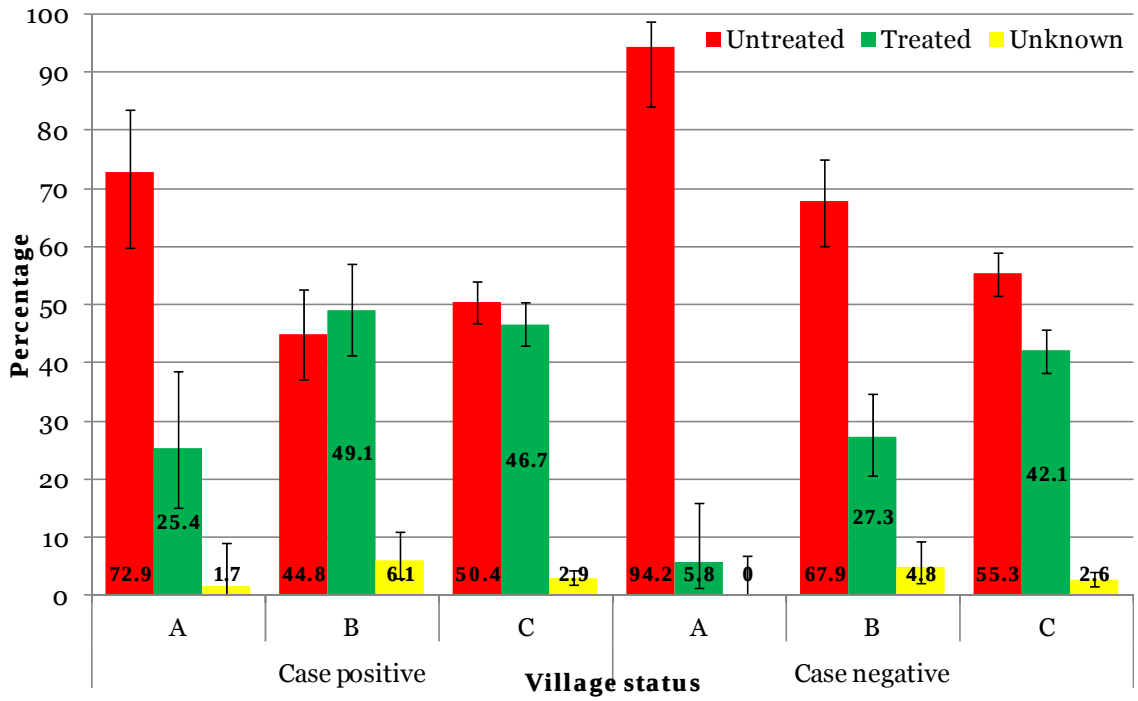

Figure 6.6 - the percentage of untreated, treated and unknown cattle in each of the three age groups: A (<18 months), B (18 - 36 months) and C (>36 months).

6.3.2.2.2. Trypanosome prevalence and diminazene coverage

There is no difference in the prevalence of trypanosomiasis in treated and untreated cattle in case-positive or case-negative villages six months post treatment. In case-negative villages *T. brucei* s. l. was not detected in cattle in the unknown group, and so the prevalence in untreated and treated cattle was significantly higher, $p = 0.02$ and $p = 0.01$ respectively. This is summarised in Figure 6.7.

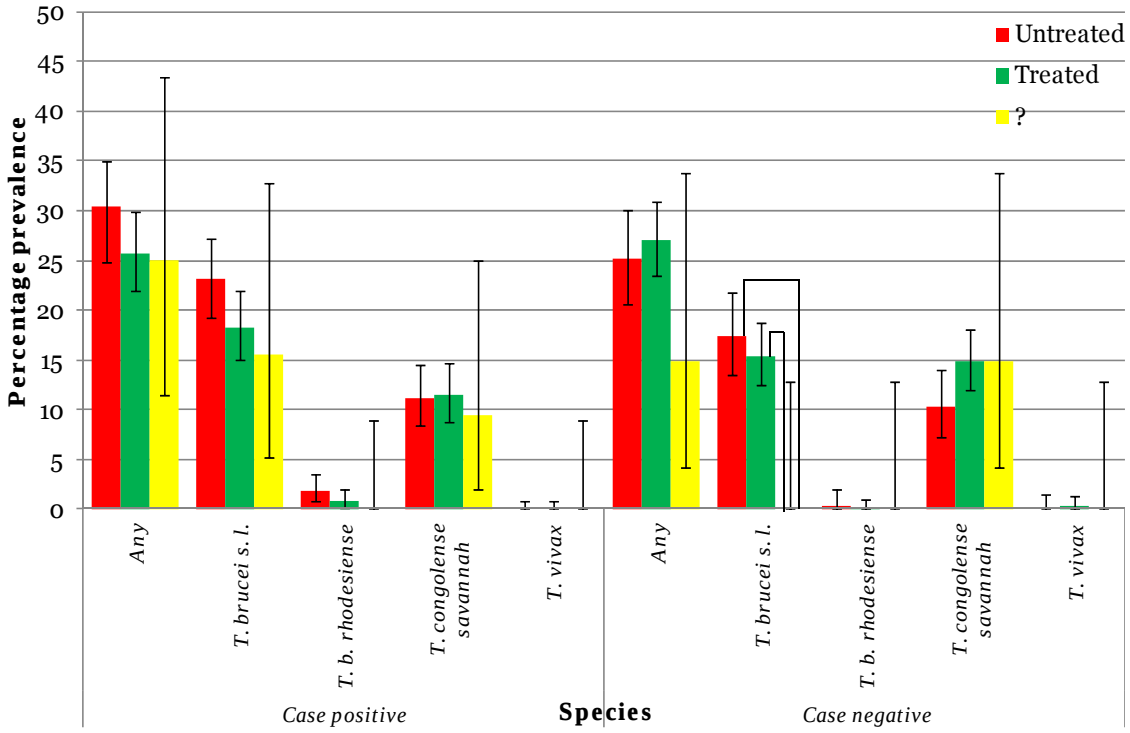

Figure 6.7 - the percentage prevalence of trypanosomiasis in each of the untreated, treated and unknown groups of cattle six months post SOS re-treatment (? = unknown). Vertical lines dissecting bars represent the 95% confidence intervals. Lines linking bars represent a significant difference between the two as determined by Fisher's Exact Test ($P < 0.05$).

6.3.3 Mixed infections

The present study has detected several species of trypanosomes, and thus far considered them only in isolation. In this section the presence and distribution of mixed trypanosome infections is considered, and associations between different species of trypanosome within the sample population considered.

6.3.3.1 Mixed infections during SOS phase one

Mixed infections with more than one species of trypanosome were detected at all time points during SOS phase one. Mixed infections are considered in each district separately in sections 6.3.3.1.1 and 6.3.3.1.2.

6.3.3.1.1 Occurrence of mixed infections in Dokolo

Over the course of SOS phase one monitoring in Dokolo the percentage of each trypanosome's prevalence attributable to single, double or triple infections varied considerably. The prevalence of double infections can be seen to increase from a baseline of 1% to 7% at eighteen months. Immediately after the treatment there were no mixed infections detected. The number of single, double and triple infections detected in Dokolo at each sampling time point is shown in Table 6.1.

Dokolo		Number			Percentage		
Time point	Sampled	Single	Double	Triple	Single	Double	Triple
Baseline	200	53	2	0	26.5	1	0
Three months	486	50	0	0	10.3	0	0
Nine months	600	137	36	3	22.8	6	0.5
Eighteen months	343	90	24	2	26.2	7	0.6

Table 6.1 - number and percentage of mixed infections detected in Dokolo during SOS phase one.

At baseline sampling the vast majority of trypanosome infections consisted of a single species, with only two double infections occurring. One of these was mixed *T. brucei* s. l. and *T. vivax*, the other was *T. brucei* s. l. and *T. congolense* savannah. There were no triple infections. *T. brucei* s. l. was detected in 23.0% (46/200, 95% CI 22.5 – 43.9%) of cattle at baseline. Of these 46 infections, 44 (95.7%) were single infections and two (4.3%) were double. At baseline sampling 2% (4/200, 95% CI 0.12 – 3.57%) of animals in Dokolo were positive for *T. vivax*. Of these four infections three (75%) were single infections and 1 (25%) was a double infection. *T. congolense* savannah was detected in 3.5 % (7/200, 95% CI 1.42 – 7.08%) of cattle at baseline. Of these seven infections, six (85.7%) were single and one (14.3%) was a double.

At three month sampling all trypanosome infections were single species.

At nine month sampling in Dokolo the majority of trypanosome infections still consisted of one species alone, however the number of double infections had increased and triple infections were detected in this district for the first time. *T. brucei* s. l. was detected in 17.7% (106/600, 95% CI 14.7 – 21%) of cattle. Of these 106 infections, 90 (84.9%) were single, 13 were double (12.3%, 11 *T. vivax* and 2 *T. congolense* savannah), and three (2.8%) were triple. *T. vivax* was detected in 13% (78/600, 95% CI 10.41 – 15.96%) of cattle. Of these 78 infections 41 were single infections (52.6%), 34 were double infections (43.6%, 11 with *T. brucei* s. l. and 23 with *T. congolense* savannah) and three were triple (3.8%). 5.7% (34/600, 95% CI 3.96 – 7.83%) of cattle were positive for *T. congolense* savannah. Of these 34 infections, six were single infection (17.6%,) 25 were double infections (73.5%, two with *T. brucei* s. l. and 23 with *T. vivax*) and three were triple (8.8%).

At eighteen month sampling the majority of infections were still due to single species, double and triple infections were also present. *T. brucei* s. l. was detected in 18.1% (62/343, 95% CI 14.2 – 22.6%) of cattle. Of these 62 infections, 43 were single infections (69.4%), 17 were double infections (27.4%, seven *with T. congolense* savannah and ten with *T. vivax*) and two were triple (3.2%). *T. vivax* was detected in 10.8% (37/343, 95% CI 7.71 - 14.56%) of cattle. Of those 37 infections 18 were single infections (48.6%) 17 were double infections (45.9%, ten with *T. brucei* s. l. and seven with *T. congolense* savannah) and two were triple (5.4%). *T. congolense* savannah was detected in 13.1% (45/343, 95% CI 9.73 – 17.16%) of cattle. Of these 45 infections 29 were single infections (64.4%) 14 were double infections (31.1% seven with *T. brucei* s. l. and seven with *T. congolense* savannah) and two were triple (4.4%).

6.3.3.1.2 Distribution of mixed infections in Dokolo

The results of the exact binomial test run for all time points are outlined in this section. In Dokolo at baseline, three, and eighteen months no significant deviations from the expected frequency of mixed infections were detected (data not shown). At these time points mixed infections with combinations of two or three species did not occur any more frequently than would be expected by chance.

There is considerable association evident in the distribution of certain species of trypanosome within the sample population at nine months. *T. vivax* is occurring more frequently than expected by chance in mixed infections, both with *T. brucei* s. l. and *T. congolense* savannah. All species of trypanosome occur in single infections less often than expected by chance as shown in Table 6.2. What caused this increase in the occurrence of mixed infections at just one time point is unknown. Possible explanations are examined in the discussion.

Species	Observed	Predicted	Exactly this	This or more	This or less
triple	3	1	0.03629	0.0446	0.99169
TBSL + TV	2	6	0.00017*	0.9997*	0.0002*
TBSL + TCS	2	5	0.07289	0.96705	0.10584
TV + TCS	23	4	< 0.000001*	< 0.000001*	1*
TBSL only	43	48	< 0.000001*	1*	< 0.000001*
TV only	41	68	0.00005*	0.99993*	0.000012*
TCS only	6	24	0.000001*	1*	0.000001*

Table 6.2 - results of the exact binomial test for significant differences between observed and expected frequencies in Dokolo at nine months. TBSL = *T. brucei* s. l., TV = *T. vivax*, TCS = T. *congolense* savannah. The first column shows the probability of getting exactly the observed number of "successes", if the population the sample is drawn from has a normal distribution. The second column shows the probability of getting this number, or more than the observed number of "successes". The third column shows the probability of getting this number, or less than the observed number of "successes". p values must reach significance threshold in all three columns (<0.025 or >0.975) before a significant difference between observed and expected frequencies can be observed.

6.3.3.1.3 Occurrence of mixed infections in Kaberamaido

At all time points sampled in Kaberamaido infections with one species of trypanosome account for the majority of parasitic events. The prevalence of double infections mixed infections is unchanged between baseline, three and nine months. However at eighteen months the prevalence of double infections is more than twice the previously observed level. The number and percentage of single, double, and triple infections is shown in Table 6.3.

Kaberamaido		Number			Percentage		
Time point	Sampled	Single	Double	Triple	Single	Double	Triple
Baseline	310	98	9	2	31.6	2.9	0.6
Three months	717	103	21	1	14.4	2.9	0.1
Nine months	714	182	21	1	25.5	2.9	0.1
Eighteen months	637	207	49	12	32.5	7.7	1.9

Table 6.3 - number and percentage of mixed infections detected in Kaberamaido during SOS phase one.

At baseline sampling in Kaberamaido the vast majority of trypanosome infections are due to one species alone, however double and triple infections do occur. *T. brucei* s. l. was detected in 9.0 % (28/310, 95% CI 6.1 – 12.8%) of cattle. Of these 28 infections 25 were single infections (89.3%), one was a double infection with *T. vivax* (3.6%), and two were triple infections (7.1%). *T. vivax* was detected at a prevalence of 7.3% (21/310, 95% CI 4.24 – 10.17%). Of these 21 infections, ten were single infections (47.6%), nine were double infections (42.9% one with *T. brucei* s. l. and eight with *T. congolense* savannah), and two were triple infections (9.5%). *T. congolense* savannah was detected in 23.5% (73/310, 95% CI 18.94 – 28.67%) of cattle. Of these 73 infections, 63 were single infections (86.3%), eight were double infections (11%, all with *T. vivax*), and two were triple (2.7%).

At three month sampling in Kaberamaido single infections still make up the majority of infections. However, while the prevalence of single infections has fallen as a result of the treatment, the prevalence of double infections, 2.9%, remains unchanged. *T. brucei* s. l. was detected in 5.4% (39/717, 95% CI3.9 – 7.4%) of cattle. Of these 39 infections 37 were single infections (95.9%) one was a double infection (2.6%, with *T. congolense* savannah) and one was a triple infection (2.6%). *T. vivax* was detected in 8.4% (60/657, 95% CI 6.45 – 10.64%) of cattle. Of those 60 infections, 39 were single infections (65%), 20 were double infections (33.3%, all with *T. congolense* savannah), and one was a triple infection (1.7%). *T. congolense* savannah was infecting 6.8%

(49/717, 95% CI 5.1 – 8.93%) of cattle. Of these 49 infections 27 were single infections (55.1%), 21 were double infections (42.9%, one with *T. brucei* s. l. and 20 with *T. congolense* savannah), and one was a triple infection (2%).

At nine month sampling in Kaberamaido the prevalence of single infections rose, again they account for the majority of trypanosome infections. The prevalence of mixed infections remains relatively unchanged from three months. *T. brucei* s. l. was detected in 20.2 %(144/714, 95% CI 17.3 -23.3%) of cattle. Of these 144 infections, 126 were single infections (87.5%) 17 were double (11.8% all with *T. vivax*) and 1 was triple (0.7%). *T. vivax* was detected in 9.8% (70/714, 95% CI 7.72 – 12.22%) of cattle. Of these 70 infections, 47 were single infections, 22 were double infections (31.4%, 17 with *T. brucei* and five with *T. congolense* savannah) and one was a triple infection (1.4%). *T. congolense* savannah was detected in 2.1% (15/714, 95% CI 1.18 – 3.44%) of cattle. Of these 15 infections nine were single infections (60%) five were double infections (33.3%, all with *T. vivax*) and one was a triple infection.

At eighteen month sampling single infections again constituted the majority of trypanosome infections. The prevalence of single, double and triple infections had risen since nine months. *T. brucei* s. l. was detected in 30.8% (196/637, 95% CI 27.2 – 34.5%) of cattle. Of these 196 infections, 156 were single infections (79.6%), 28 were double infections (14.3%, five with *T. congolense* savannah and 23 with *T. vivax*), and 12 were triple infections (6.1%). *T. vivax* was detected in 15.2% (97/637, 95% CI 12.53 – 18.26%) of cattle. Of these 97 infections 41 were single infections (42.3%), 44 were double infections (45.4%, 23 with *T. brucei* s. l. and 21 with *T. congolense* savannah), and 12 were triple infections (12.4%). *T. congolense* savannah was detected in 7.5% (48/637, 95% 5.61 – 9.87) of cattle. Of these 48 infections ten were single infections (20.8%), 26 were double infections (54.2%, 5 with *T. brucei* s. l. and 21 with *T. vivax*) and 12 were triple infections (25%).

Over the course of SOS phase one monitoring in Kaberamaido the percentage of each trypanosome's prevalence attributable to single, double or triple infections varied considerably. The significance of the difference in the composition of mixed infections

between time points was tested with the exact binomial test, and results are given in section 6.3.3.1.4.

6.3.3.1.4 Mixed infection distribution in Kaberamaido

The results of the exact binomial test run for all time points in Kaberamaido are given here. There were significant differences between the observed and expected frequency of mixed infections at all time points.

At baseline in Kaberamaido there was only one deviation away from the expected frequency of infections (full data set not shown). There were less than expected mixed infections with *T. brucei* s. l. and *T. congolense* savannah (exactly this, p = 0.0002, this or more p = 1, this or less p = 0.0002).

At three month sampling there were several significant deviations between the observed and expected frequencies of trypanosome infection. The results demonstrate a clear association between *T. vivax* and *T. congolense* savannah at the three month time point. The occurrence of these two trypanosomes together is higher than predicted by chance, and the occurrence of each individual species alone is less than predicted by chance, as summarised below in Table 6.4.

Species	Observed	Predicted	Exactly this	This or more	This or less
triple	1	0	0.1785	0.19994	0.97856
TBSL + TV	0	3	0.0475	1	0.0475
TBSL + TCS	1	2	0.21223	0.9134	0.29884
TV + TCS	20	4	< 0.000001*	< 0.000001*	1*
TBSL only	37	33	0.05452	0.27858	0.77594
TV only	39	53	0.00647*	0.98642*	0.02005*
TCS only	27	42	0.00257*	0.9963*	0.00628*

Table 6.4 - results of the exact binomial test for significant differences between observed and expected frequencies in Kaberamaido at three months. TBSL = *T. brucei* s. l., TV = *T. vivax*, TCS = *T. congolense* savannah. The first column shows the probability of getting exactly the observed number of "successes", if the population the sample is drawn from has a normal distribution. The second column shows the probability of getting this number, or more than the observed number of "successes". The third column shows the probability of getting this number, or less than the observed number of "successes". p values must reach significance threshold in all three columns (<0.025 or >0.975) before a significant difference between observed and expected frequencies can be observed.

At nine month sampling there are fewer single *T. vivax* infections than predicted (exactly this p = 0.002, this or more p = 0.995, this or less p = 0.007). There is no significant increase in the occurrence of *T. vivax* with any other particular species (full data not shown).

Several species of trypanosome do not appear to be randomly distributed within the sample population at eighteen months. Significantly more triple infections occurred than predicted. *T. congolense* savannah was occurring more frequently in mixed infections than predicted, both with *T. brucei* s. l. and *T. vivax*. Significantly less *T. vivax* and *T. congolense* savannah than predicted occurred as single infections. At the eighteen month time point there is a clear aggregation of AAT causing trypanosomes occurring together in the sample population, as summarised in Table 6.5.

Species	Observed	Predicted	Exactly this	This or more	This or less
triple	12	2	< 0.000001*	< 0.000001*	1*
TBSL + TV	23	28	0.05528	0.8396	0.21568
TBSL + TCS	5	13	0.00899*	0.99504*	0.01395*
TV + TCS	21	5	< 0.000001*	< 0.000001*	1*
TBSL only	156	154	0.03587	0.42835	0.60752
TV only	41	77	< 0.000001*	< 0.000001*	1*
TCS only	10	28	0.00004*	0.99998*	0.00006*

Table 6.5 - results of the exact binomial test for significant differences between observed and expected frequencies in Kaberamaido at eighteen months. TBSL = *T. brucei* s. l., TV = *T. vivax*, TCS = T. *congolense* savannah. The first column shows the probability of getting exactly the observed number of "successes", if the population the sample is drawn from has a normal distribution. The second column shows the probability of getting this number, or more than the observed number of "successes". The third column shows the probability of getting this number, or less than the observed number of "successes". p values must reach significance threshold in all three columns (<0.025 or >0.975) before a significant difference between observed and expected frequencies can be observed.

6.3.3.2 Mixed infections in the re-treatment area

Mixed infections were detected in both case-positive and case-negative villages at baseline and six months, as detailed in the following sections.

6.2.3.2.1 Mixed infections in case-positive villages

The number and percentage of single, double and triple infections detected in case-positive villages is shown in Table 6.6.

Case-positive		Number			Percentage		
Time point	Sampled	Single	Double	Triple	Single	Double	Triple
Baseline	854	323	37	2	37.8	4.3	0.2
Six months	956	229	37	0	24	3.9	0

Table 6.6 - number and percentage of single, double and triple species trypanosome infections detected in case-positive villages at baseline and six months.

At baseline *T. brucei* s. l. was detected in 31.7% (271/854, 95% CI = 34.97 – 28.62%) of cattle. Of these 271 infections 232 were single infections (85.6%), 37 were double infections (13.7%, 23 with *T. congolense* savannah and 14 with *T. vivax*) and 2 were triple infections (0.7%). *T. vivax* was detected in cattle at a prevalence of 5.7% (49/854, 95% CI = 7.51 – 4.27%). Of these 49 infections 33 were single infections (67.3%), 14 were double infections (28.6%, all with *T. brucei* s. l.) and two were triple infections (4.1%). *T. congolense* savannah was detected in 9.7% (83/854, 95% CI = 11.91 - 7.82%) of cattle. Of these 83 infections, 58 were single infections (69.9%), 23 were double infections (27.7%, all with *T. brucei* s. l.) and two were triple infections (2.4%).

At six months *T. brucei* s. l. was detected in 20.4% (195/956, 95% CI = 23.09 – 17.89%) of cattle. Of these 195 infections 158 were single infections (81%), and 37 were double infections with *T. congolense* savannah (19%). No *T. vivax* was detected. *T. congolense* savannah was detected in 11.3% (108/956, 95% CI = 13.48 - 9.36%) of cattle. Of these 108 infections, 71 were single infections (65.7%) and 37 were double infections with *T. brucei* s. l. (34.3%).

6.3.3.2.2 Distribution of mixed infections in the case-positive sample

At baseline in case-positive villages mixed infections did not occur any more frequently than predicted by chance. In terms of single infections there were less single *T. vivax* infections than predicted. This is summarised in Table 6.7.

Species	Observed	Predicted	Exactly this	This or more	This or less
triple	2	2	0.25642	0.46532	0.79211
TBSL + TV	14	14	0.10638	0.57422	0.53216
TBSL + TCS	27	25	0.07284	0.38407	0.68877
TV + TCS	0	3	0.0348	1	0.0348
TBSL only	232	231	0.03057	0.47883	0.55174
TV only	33	20	0.000175	0.000395	0.9978
TCS only	58	54	0.04603	0.30063	0.74539

Table 6.7 - results of the exact binomial test for significant differences between observed and predicted frequencies in case-positive villages at baseline. TBSL = *T. brucei* s. l., TV = *T. vivax*, TCS = T. *congolense* savannah. The first column shows the probability of getting exactly the observed number of "successes", if the population the sample is drawn from has a normal distribution. The second column shows the probability of getting this number, or more than the observed number of "successes". The third column shows the probability of getting this number, or less than the observed number of "successes". p values must reach significance threshold in all three columns (<0.025 or >0.975) before a significant difference between observed and expected frequencies can be observed.

At six months in case-positive villages an association between *T. brucei* s. l. and *T. congolense* savannah was detected. There were significantly more mixed infections with *T. brucei* s. l. and *T. congolense* savannah than predicted by chance. Although there were fewer than predicted single infections with *T. congolense* savannah, the difference between observed and predicted only borders significance. The number of single infections with *T. brucei* s. l. did not differ significantly from the number predicted. This is summarised in Table 6.8.

Species	Observed	Predicted	Exactly this	This or more	This or less
triple	0	0	N/A	N/A	N/A
TBSL + TV	0	0	N/A	N/A	N/A
TBSL + TCS	37	22	0.00087	0.000196	0.99892
TV + TCS	0	0	N/A	N/A	N/A
TBSL only	158	173	0.01545	0.90428	0.11117
TV only	0	0	N/A	N/A	N/A
TCS only	71	86	0.01081	0.96281	0.048

Table 6.8 - results of the exact binomial test for significant differences between observed and expected frequencies in case-positive villages in the re-treatment area. TBSL = *T. brucei* s. l., TV = *T. vivax*, TCS = T. *congolense* savannah. The first column shows the probability of getting exactly the observed number of "successes", if the population the sample is drawn from has a normal distribution. The second column shows the probability of getting this number, or more than the observed number of "successes". The third column shows the probability of getting this number, or less than the observed number of "successes". p values must reach significance threshold in all three columns (<0.025 or >0.975) before a significant difference between observed and expected frequencies can be observed.

6.3.3.2.3 Mixed infections in case-negative villages

The number and percentage of single, double and triple infections in case-negative villages at baseline and six months is shown in Table 6.9.

Case-negative		Number			Percentage		
Time point	Sampled	Single	Double	Triple	Single	Double	Triple
Baseline	804	261	17	2	32.5	2.1	0.2
Six months	935	214	29	0	22.9	3.1	0

Table 6.9 – number and percentage of single, double and triple infections in case-negative villages.

At baseline in case-negative villages 25% (201/804, 95% CI = 28.14 – 22.04) of cattle were infected with *T. brucei* s. l. Of these 201 infections 187 were single infections (93%), 12 were double infections (6%, six with *T. congolense* savannah and six with *T.*

vivax), and two were triple infections (1%). *T. vivax* was detected in 6.1% (49/804, 95% CI = 7.98 – 4.54%) of cattle. Of these 49 infections 36 were single infections (73.5%), eleven were double infections (22.4%, six with *T. brucei* s. l. and five with *T. congolense* savannah), and two were triple infections (4.1%). *T. congolense* savannah was detected in 6.7% (54/804, 95% CI = 8.67 - 5.09%) of cattle.

At six months in case negative villages 15.7% (147/935 95% CI = 18.22 – 13.45%) of cattle were infected with *T. brucei* s. l. Of these 147 infections 118 were single infections (80.3%), 29 were double infections with *T. congolense* savannah (19.7%). No triple infections were detected. *T. vivax* was detected in 0.54% (1/935, 95% CI = 0.59 – 0.01%) of cattle. 100% of these infections were single infections. *T. congolense* savannah was detected in 14.4% (135/935, 95% CI = 16.86 - 12.25%) of cattle. Of these 135 infections, 94 were single infections (76.4%) and 29 were double infections with *T. brucei* s. l. (23.6%)

6.3.3.2.4 Distribution of mixed infections in the case-negative sample

In case-negative villages at baseline *T. brucei* s. l. *T. vivax* and *T. congolense* savannah were evenly distributed in the sample population. The frequency of single, double and triple infections observed was not significantly different to the frequency predicted by chance (data not shown).

At six month sampling in case-negative villages an association between *T. brucei* s. l. and *T. congolense* savannah was observed. These two parasites occurred together significantly more frequently than expected by chance, as shown in Table 6.10.

Species	Observed	Predicted	Exactly this	This or more	This or less
triple	0	0	N/A	N/A	N/A
TBSL + TV	0	0	N/A	N/A	N/A
TBSL + TCS	29	19	0.00155	0.00327	0.99828
TV + TCS	0	0	N/A	N/A	N/A
TBSL only	118	127	0.02774	0.20542	0.82202
TV only	2	0	0.27096	0.59428	0.67668
TCS only	94	103	0.03733	0.30064	0.73668

Table 6.10 - results of the exact binomial test for detecting significant differences between observed and predicted frequencies in case-negative villages at six months. TBSL = *T. brucei* s. l., TV = *T. vivax*, TCS = T. *congolense* savannah. The first column shows the probability of getting exactly the observed number of "successes", if the population the sample is drawn from has a normal distribution. The second column shows the probability of getting this number, or more than the observed number of "successes". The third column shows the probability of getting this number, or less than the observed number of "successes". p values must reach significance threshold in all three columns (<0.025 or >0.975) before a significant difference between observed and expected frequencies can be observed.

6.3.4 Village level trypanosomiasis

Examining trypanosomiasis at the village level enables a greater understanding of the nuances in variation of the epidemiology of trypanosomiasis across small and seemingly similar geographic locations. Thus far in this thesis aggregated trypanosomiasis data has been overviewed and analyzed. In this section trypanosomiasis is considered on a village by village basis for the first time.

6.3.4.1 Change in trypanosome prevalence in SOS phase one villages

Between baseline and three months three villages experienced a significant drop in the prevalence of *T. brucei* s. l.: Amoru ($p = 0.01$), Okwor ($p < 0.0001$) and Aneralibi ($p = 0.01$). Omor village experienced a significant rise in the prevalence of *T. brucei* s. l. ($p = 0.007$), while at the same time experiencing a significant reduction in the prevalence of both *T. vivax* ($p = 0.04$) and *T. congolense* savannah ($p < 0.0001$). Mwatma village had a significant drop in the prevalence of both *T. vivax* ($p = 0.002$) and *T. congolense*

savannah ($p < 0.0001$). There were no significant changes in the prevalence of *T. b. rhodesiense*; however it was only detected in one village at baseline and one village at three months. This is summarised in

Figure 6.8 .

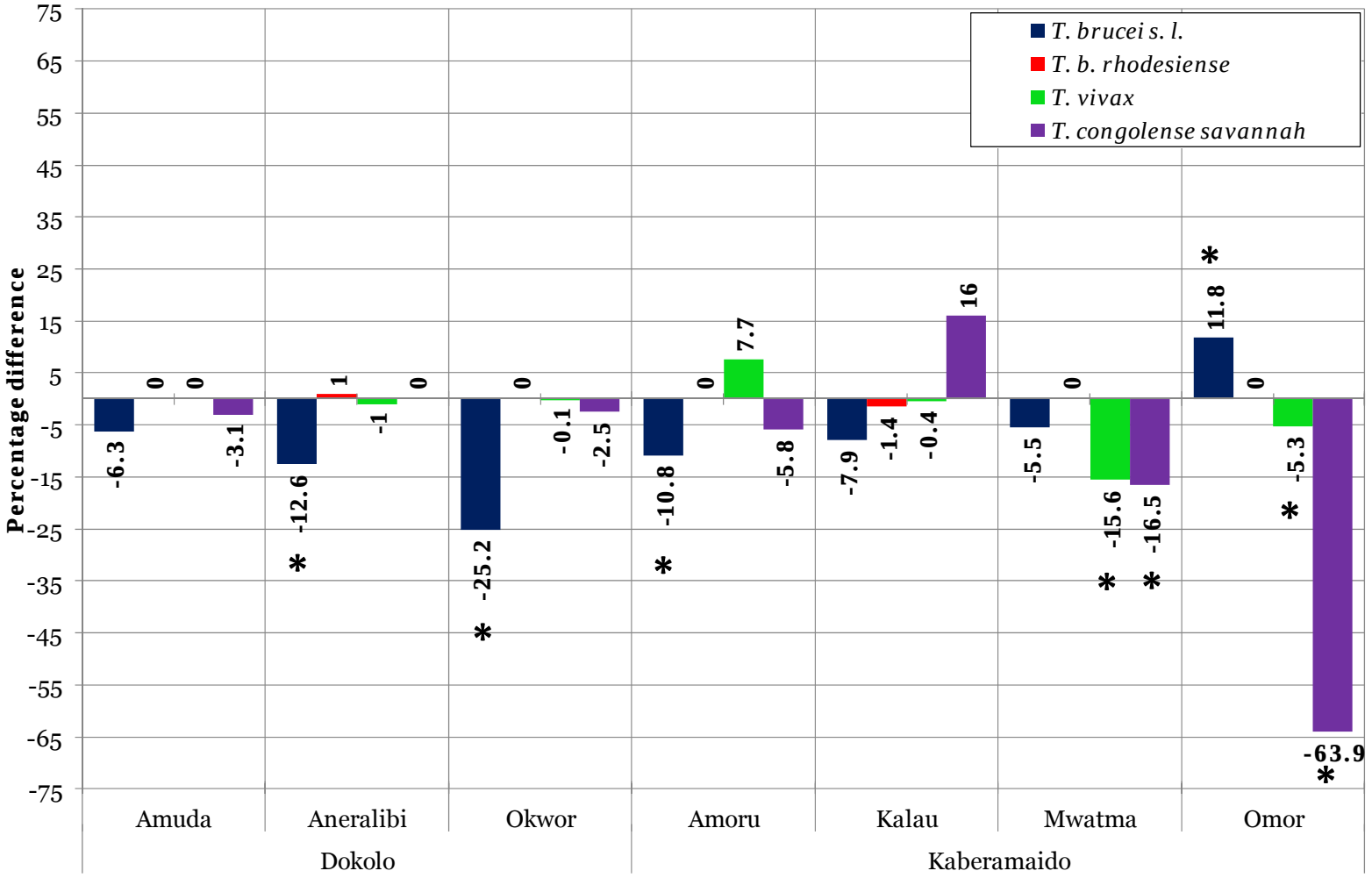

Figure 6.8- percentage difference in each species of trypanosome on an individual village basis between baseline and three months.

6.3.4.2 High prevalence villages in SOS phase one

In order to assess if particular villages had consistently higher prevalence than others, a rank analysis was carried out. For each species of trypanosome villages were ranked in order of their trypanosomiasis prevalence at each time point from high to low The top five villages were assigned points, the village with the highest prevalence received five points, second highest four points, etc. Villages outside the top five scored zero. Villages also scored one point for every time they appeared in the top five high prevalence villages at a given time point. Points were added up across all tables to determine the top five villages that routinely had high prevalence of trypanosomiasis. Individual tables for each trypanosome species are shown in Appendix I.

By assigning scores in this fashion the way in which villages were assessed was weighted to add importance to high prevalence as well as the frequency of each village having high prevalence. The aim of this analysis was to pick out villages that consistently had the highest prevalence of trypanosomiasis.

Olemai had the highest score out of all villages, this village also had the lowest farmer reported treatment coverage at three months. This suggests this village is suffering from high trypanosomiasis due to inadequate treatment coverage, a high number of un-treated animals moving into the area, or a combination of the two. In general there seems to be a correlation between treatment coverage and high trypanosomiasis prevalence, the average treatment coverage in the top five high ranking villages was 69.6%, in the remaining nine villages the average coverage rate was 83.1%. However the treatment coverage average in these high risk villages is brought down substantially by one village, Olemai. This information is displayed in Table 6.11.

Overall Ranking	Village name	Overall score	Treatment coverage
1	Olemai *	41	27.4%
2	Okwor	38	90.2%
3	Amoru	34	75.0%
4	Mwatma	32	78.3%
5	Olep	31	77.0%
6	Olio *	28	94.6%
7	Aneralibi *	27	78.8%
8	Amuda	24	83.7%
9	Omor	21	91.2%
19	Kalau	15	79.0%
11	Awinyiipany *	14	85.5%
12	Abyenek	12	81.3%
13	Amwoma	7	81.4%
14	Osirima	7	72.5%

Table 6.11 - ranking of high prevalence villages in the SOS phase one area alongside village level treatment coverage. Villages that also reported a case of HAT after April 2007 are indicated by *.

With the exception of Olep, the top five highest ranking villages from SOS phase one fall within the re-treatment area. The location of these villages is marked in Figure 6.9.

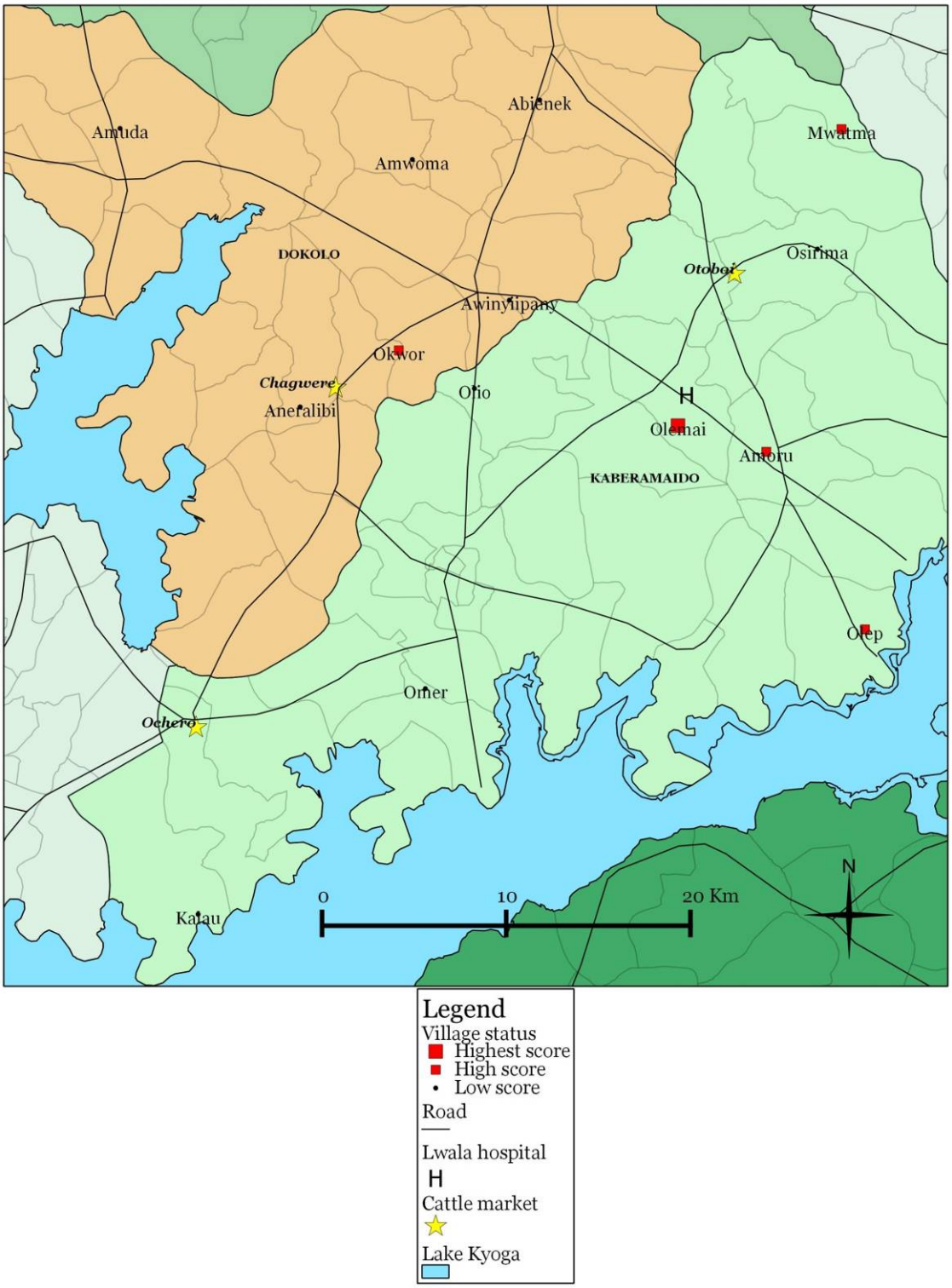

Figure 6.9 - location of high prevalence vilages identified during SOS phase one monitoring.

6.3.4.3 Change in trypanosome prevalence in re-treatment villages

Three case-positive villages experienced a significant drop in the prevalence of *T. brucei* s. l. between baseline and 6 month sampling; Chawgere ($p = 0.0008$), Kaburuburu ($p = 0.008$) and Kalere ($p = 0.0025$). A further three case-positive villages experienced similar sizable drops, but due to differences in sample numbers these drops were not statistically significant. Abat and Olumai had very small decreases in the prevalence of *T. brucei* s. l., and in Abongowoo and Otoro prevalence rose slightly. The prevalence of *T. b. rhodesiense* fell in all but two villages; Otoro and Olumai. For *T. congolense* savannah a significant drop in prevalence was not observed in any village. The prevalence of *T. congolense* savannah fell in Kalere, Otoro and Chagwere, but not significantly. In all other case-positive villages the prevalence of *T. congolense* savannah rose, however this rise was only statistically significant in Kaburuburu ($p = 0.0002$) and Katingi ($p = 0.0065$). The prevalence of *T. vivax* fell in all case-positive villages, and was only detected in the village of Abat. The drop was statistically significant in Kalere ($p < 0.0001$), Olumai ($p= 0.0289$) and Otage ($p = 0.0007$).

Seven case-negative villages experienced a drop in the prevalence of *T. brucei* s. l., this drop was statistically significant in Aswii ($p < 0.0001$), Agwingiri ($p < 0.0001$), Ilong ($p = 0.0009$), Ochukai ($p = 0.0024$), and Olabo ($p = 0.0028$). In the three remaining case-negative villages *T. brucei* s. l. prevalence rose, significantly so in the village of Amuli ($p = 0.0228$). The prevalence of *T. b. rhodesiense* either stayed the same (from a baseline prevalence of nothing) or fell. *T. congolense* savannah prevalence rose in seven villages, and this rise was statistically significant in five of these villages – Abwa ($p < 0.0001$), Akany ($p < 0.0001$), Agwingiri ($p = 0.0013$), Ochukai ($p = 0.0003$), and Olabo ($p < 0.0001$). Three villages experienced a drop in *T. congolense* savannah prevalence, and this drop was statistically significant in Amuli ($p = 0.0039$). This is summarised in Figure 6.10 and Figure 6.11.

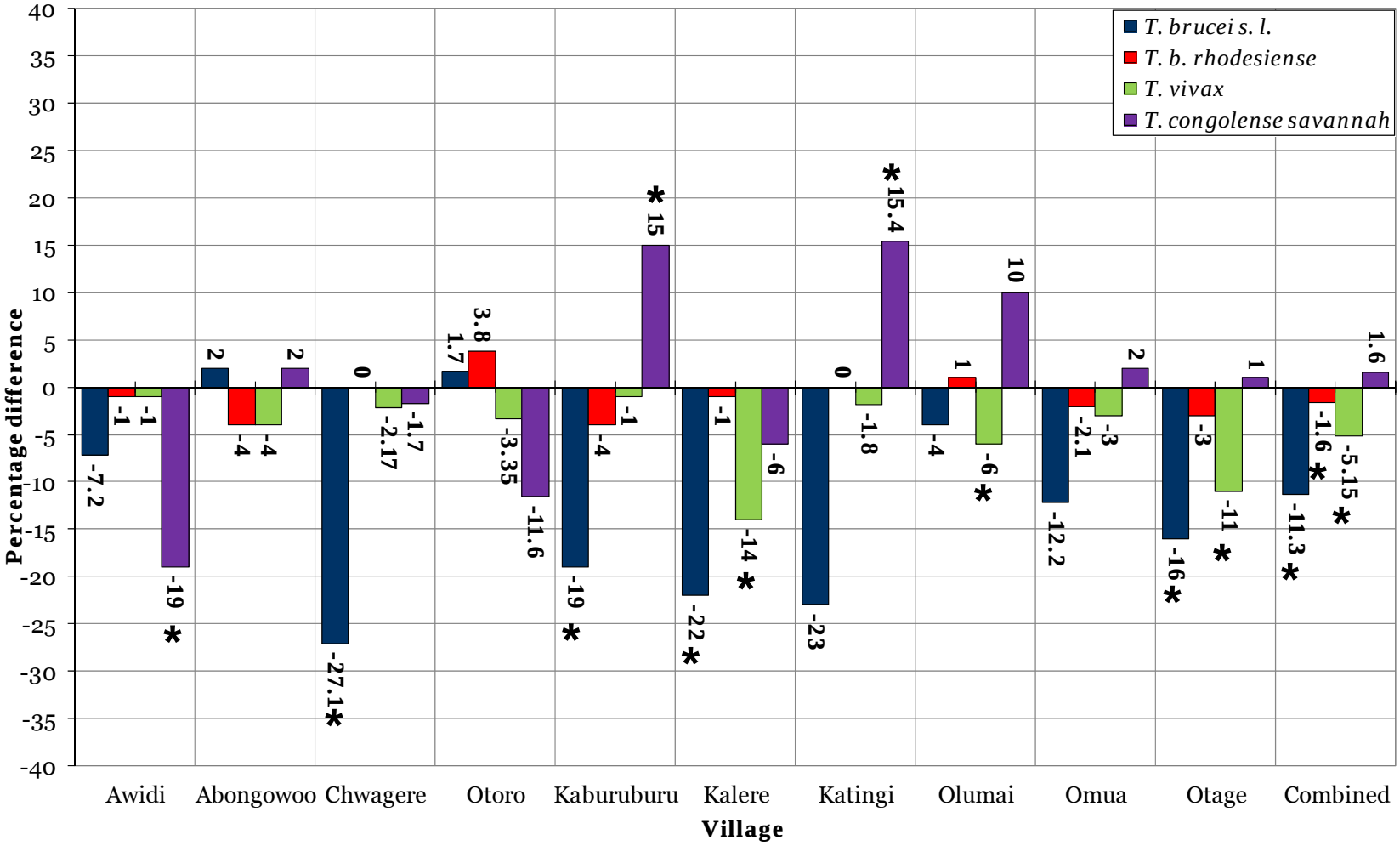

Figure 6.10 - percentage change in each species of trypanosome between baseline and six months in case-positive villages.

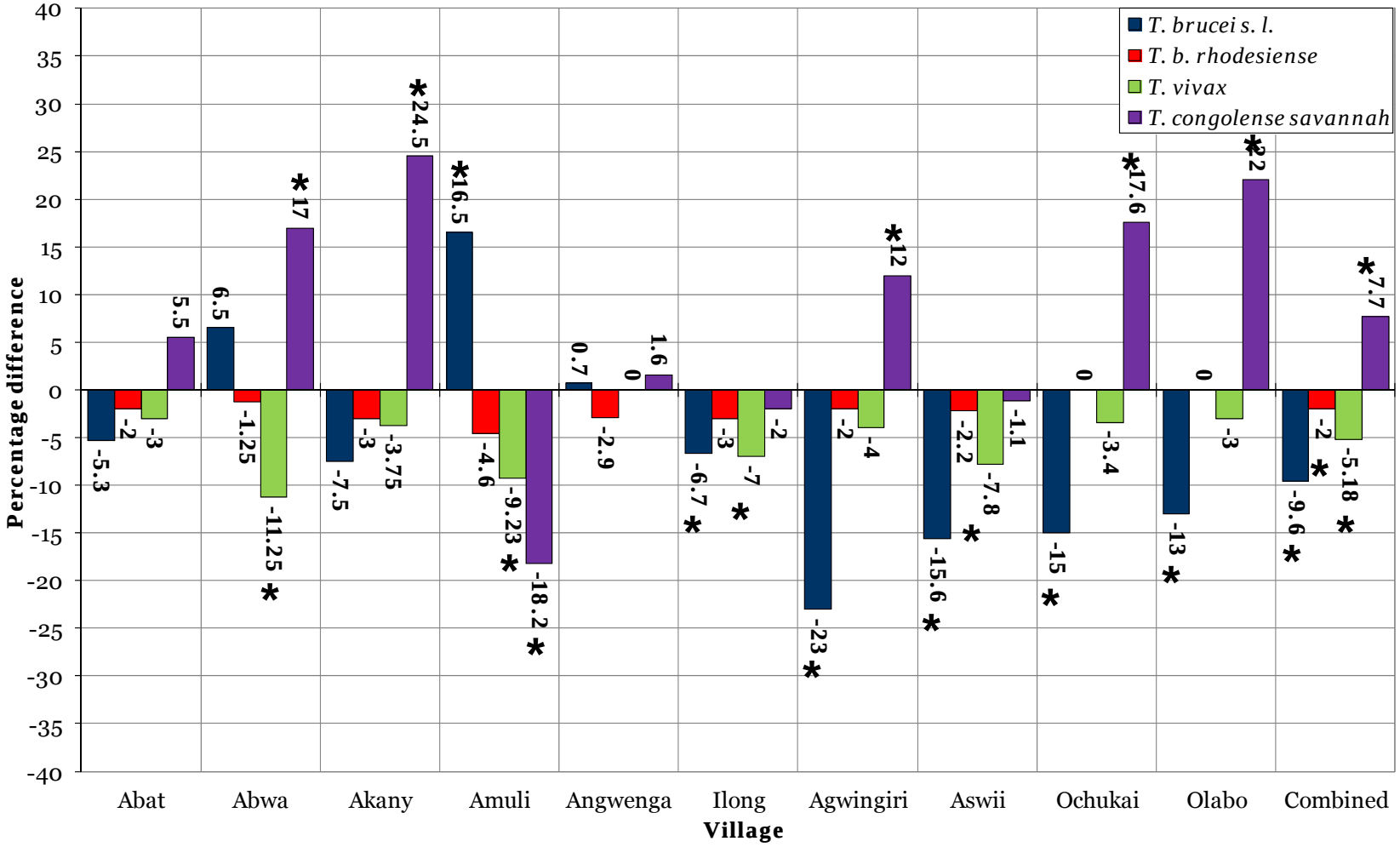

Figure 6.11 - percentage change in the prevalence of each species of trypanosome between baseline and six months in case-negative villages.

6.3.4.4 High prevalence villages in the re-treatment area

In order to assess if particular villages had consistently higher prevalence than others, a rank analysis was carried out. For each species of trypanosome villages were listed in order of their trypanosomiasis prevalence at each time point from high to low (see appendix one). The top five villages were assigned points, the village with the highest prevalence received five points, second highest four points, etc. Villages outside the top five scored zero. Villages also scored one point for every time they appeared in the top five high prevalence villages at a given time point. Points were added up across all tables to determine the top five villages that routinely had high prevalence of trypanosomiasis.

By assigning scores in this fashion the way in which villages were assessed was weighted to add importance to high prevalence as well as the frequency of each village having high prevalence. The aim of this analysis was to pick out villages that consistently had the highest prevalence of trypanosomiasis.

The case-negative village of Amuli has the highest overall trypanosomiasis prevalence. The association between high trypanosomiasis and low treatment coverage is week in re-treatment villages, with the top five villages having an average treatment coverage of 46.2%, and the average for the remaining villages being 39.8%. The percentage coverage in the top five is skewed by the high coverage in the village of Kalere.

Overall Ranking	Village name	Overall score	Treatment coverage
1	Amuli	29	39%
2	Kalere	28	82%
3	Kaburuburu	18	36%
4	Ilong	15	17%
5	Olumai *	13	57%
6	Abat	12	41.1%
7	Chwagere	12	71%
8	Otoro	12	10.3%
9	Akany	11	66%
10	Abwa	9	18%
11	Olabo	5	55%
12	Otage	4	40%
13	Abongowoo	3	53%
14	Omua	3	35.6%
15	Angwenga *	2	81%
16	Awidi	2	44.1%
17	Ochukai	2	25%
18	Agwingiri	0	15%
19	Aswii	0	30%
20	Katingi *	0	12%

Table 6.12 - ranking and treatment coverage percentage for every village in the re-treatment area. Case-positive villages are shown in red, case-negative villages in blue. Villages that reported a case of HAT in the year from April 2008 are indicated by *

The locations of these villages are shown in Figure 6.12.

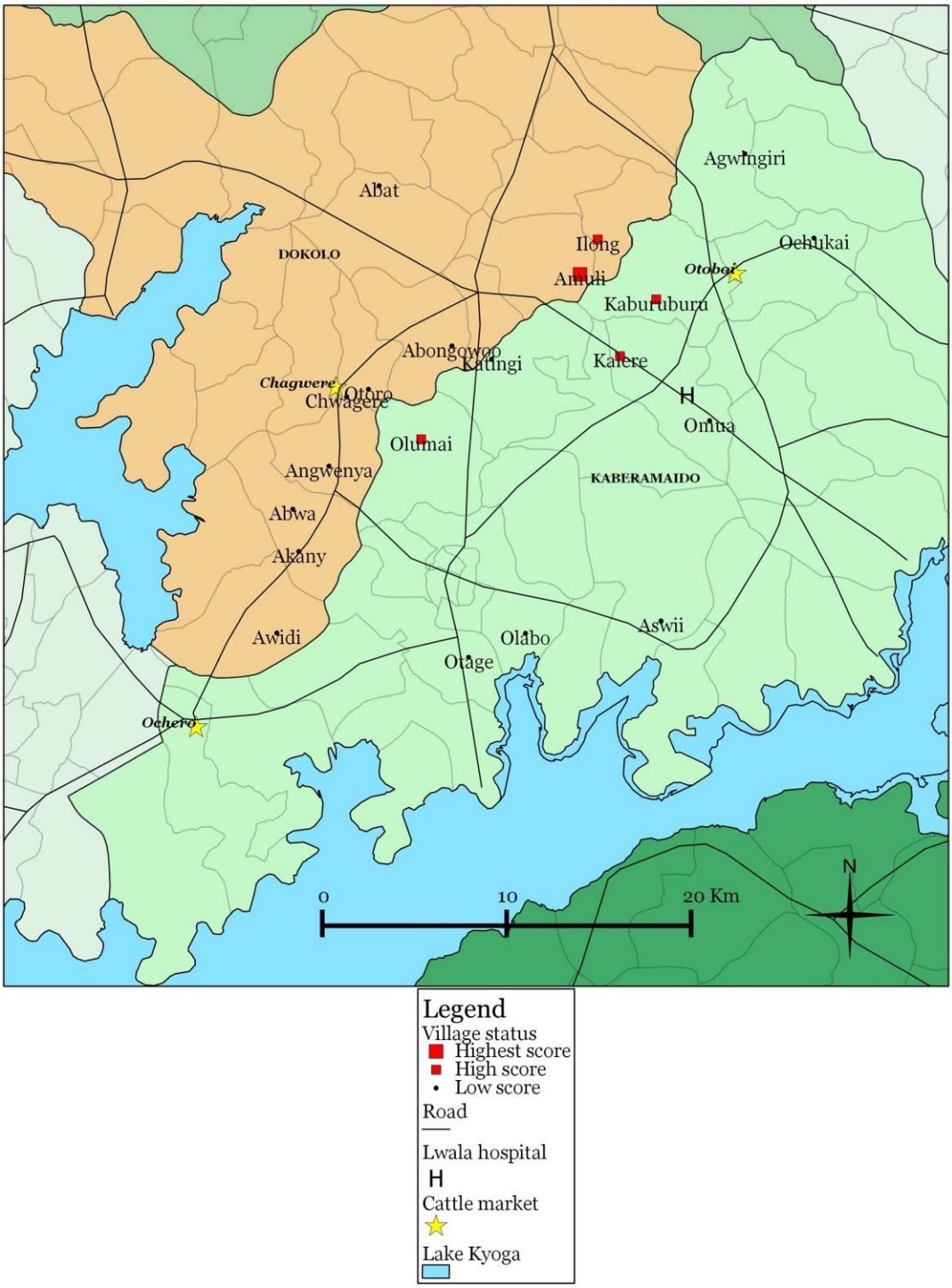

Figure 6.12 - location of high prevalence villages identified during SOS re-treatment monitoring.

6.4 Discussion

This chapter has collated detailed information from previous data chapters, mining a wealth of detailed epidemiological data for clues about factors affecting the transmission of trypanosomiasis. Each of the aims is considered separately below.

6.4.1 Mantel-Haenszel test results

No significant confounding was detected between age and sex at any of the sampling time points. This could indicate no interaction occurred, but it could equally represent the limitations of these data sets. The sampling for SOS was planned for monitoring and evaluation of the prevalence of *T. b. rhodesiense*, before and after treatment. The data is robust enough to fulfill this function. The study was not specifically designed to test for interactions between sex, age or any other variable relating to cattle. While it would be wrong to completely ignore the effect these variables may have on trypanosome prevalence, to try and use the data to unequivocally explain these variables and patterns within them would be to go beyond the boundaries of the data set.

Given that significant confounding was not detected between age and sex, their effect on trypanosome prevalence should be considered separately when making recommendations for future trypanosomiasis control efforts. Drawing on the results from the re-treatment, highest prevalence of *T. brucei* s. l. was detected in adult cattle at both time points. Therefore, where *T. brucei* s. l. is the primary concern of a mass treatment intervention, particular attention should be given to adult cattle to ensure the highest possible coverage in this group. With regards to *T. congolense* savannah, it is questionable whether a mass treatment to control this parasite could be recommended. SOS phase one had little discernible effect on the prevalence of *T. congolense* savannah, while the re-treatment significantly increased its prevalence. This could be because young treated animals are cured of infections and so do not get to build up tolerance or immunity as they normally would. Therefore, a treatment programme that focused on adult cattle would have the added benefit of minimizing the

potential impact upon the hypothesized development of tolerance, and would hopefully avoid increasing the prevalence of *T. congolense* savannah.

6.4.2 Treatment

Isometamidium did not appear to be prophylatically active three months post sampling, as there was no significant difference in the prevalence of trypanosomiasis in treated and untreated cattle at that time point. There was also little evidence remaining of the clearance of trypanosomiasis from treated cattle, suggesting both a short prophylactic period and high tsetse challenge. A shortened period of prophylaxis under conditions of high tsetse challenge has been observed in other studies (Dolan *et al.*, 1992).

Similarly there was no significant difference in the prevalence of trypanosomiasis in diminazene treated or untreated animals at SOS re-treatment six month sampling. This is not overly surprising given diminazene is purely curative.

During the re-treatment programme, cattle were sampled at baseline and six months. There is not a post sampling time point in common between SOS phase one and the re-treatment. In order to aid the comparison between isometamidium and diminazene, the predicted level of treatment coverage at six months for SOS phase one is shown in Figure 6.13.

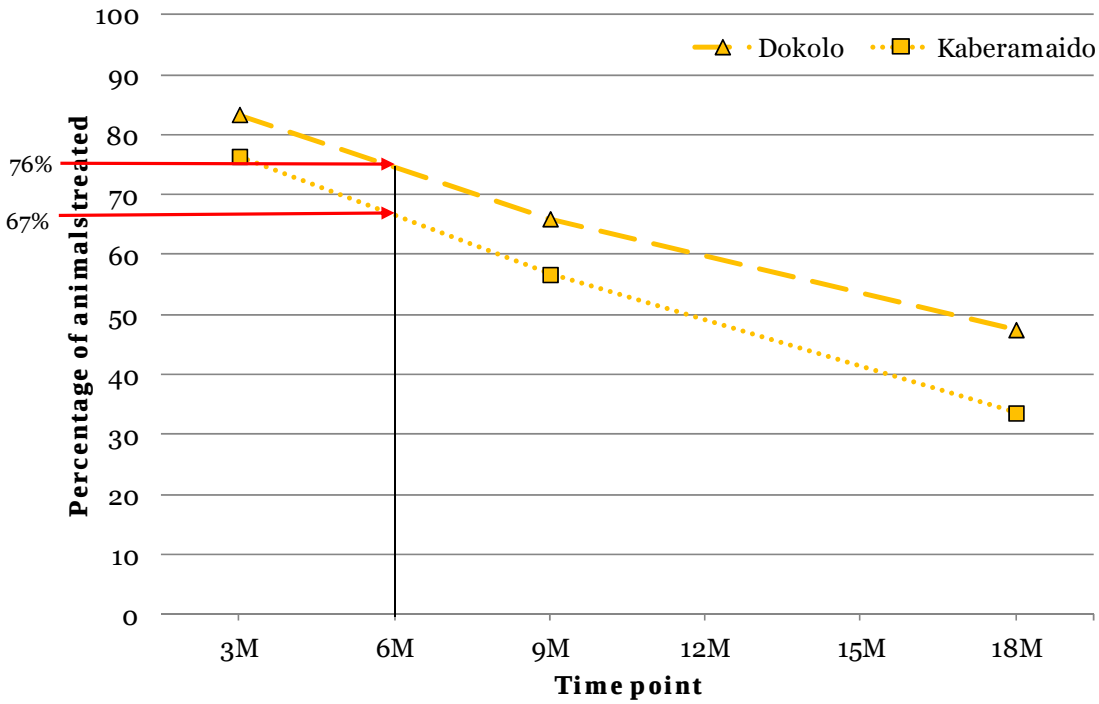

Figure 6.13 - reported treated coverage of cattle in Dokolo and Kaberamaido for the duration of SOS phase one monitoring. The predicted coverage six months post treatment is indicated by the red arrows.

As Figure 6.13 shows, the estimated coverage rate for SOS phase one at six months was 76% in Dokolo and 67% in Kaberamaido. This is considerably higher than the farmer reported diminazene coverage at six month re-treatment sampling. This suggest the re-treatment achieved a lower overall coverage level than SOS phase one. This apparent difference in treatment coverage levels between SOS phase one and the re-treatment could be another factor contributing to the differing impact these treatments had.

6.4.3 Mixed infections

Mixed infections are frequently overlooked when it comes to epidemiological surveys of trypanosomiasis, given how difficult mixed species trypanosomal infections are to detect using microscope based techniques and how often these techniques are employed in the field due to necessity. Parasitological techniques detected 2.9% of cattle with mixed species trypanosomal infections in Kenya (Tarimo-Nesbitt *et al.*, 1999). In contrast, using PCR on a small number (n = 103) of cattle samples from

western Kenya detected mixed trypanosomal infections at a prevalence of 10% (Thumbi *et al.*, 2010). The results detailed in this chapter represent perhaps one of the largest (n = 7, 556) analyses of mixed trypanosome infections in field samples using molecular techniques. The scale and detail of this work has revealed interesting trends.

6.4.3.1 Mixed infections in Dokolo

At baseline and eighteen month sampling there was no significant difference in the number of mixed infections observed and the number predicted by chance. At three months in Dokolo, the time point closest to the treatment, no mixed infections were detected whatsoever. It seems as if the treatment has had a particularly profound effect on mixed infections in Dokolo, completely clearing them from the sample population. At nine month sampling in Dokolo there are significantly more double infections than predicted by chance, and less single infections of all species alone. It must also be noted that between three and nine months in Dokolo the overall prevalence of *T. brucei* s. l. rose, the only two consecutive time points where a rise in *T. brucei* s. l. prevalence was detected in Dokolo. Between nine and eighteen months there is no significant change in the prevalence of *T. brucei* s. l., and no significant deviation away from the expected number of mixed infections. Therefore, it is possible to speculate an increase in the number of mixed infections in Dokolo facilitates a rise in the prevalence of *T. brucei* s. l. While this theory cannot be proven or disproved on the basis of this study alone, it indicates mixed trypanosome infections should be given more attention in control programmes of the future, as they could be profoundly influencing the prevalence of *T. brucei* s. l., a key target of the SOS programme.

6.4.3.2 Mixed infections in Kaberamaido

In Kaberamaido at baseline the results of the exact binomial test indicate there were significantly less than expected mixed infection with *T. brucei* s. l. and *T. congolense* savannah. All other trypanosome species were distributed normally, and there was not a significant difference between the number of observed and predicted number of single *T. brucei* s. l. or *T. congolense* savannah infections, therefore this could be something of an anomaly and nothing more.

In contrast to the situation observed in Dokolo, in Kaberamaido the percentage of mixed infections in the sample population remains unchanged between baseline and three months. Although the percentage of mixed infections remains the same overall, the species of trypanosome involved in the mixed infections differ. At three month sampling there is an association between *T. vivax* and *T. congolense* savannah, with these species occurring together significantly more often as well as being present in single infections less than predicted the exact binomial test. Mixed infections with *T. vivax* and *T. congolense* savannah are not altogether unsurprising, as these species are pathogenic to cattle, and a tsetse population feeding predominantly on cattle could be expected to pick up these infections fairly frequently. The health and production effects on cattle concurrently infected with two pathogenic strains of trypanosome could be severe, given the extent of disease each species is capable of causing separately. The prevalence of mixed *T. vivax*/*T. congolense* savannah infections at three months is cause for concern. At this time point, both *T. vivax* and *T. congolense* savannah general prevalence is higher than the prevalence of *T. brucei* s. l. in the sample population.

At nine month sampling the percentage of mixed infections within the sample population again remains static at 2.9%. There are no significant interactions between different species of trypanosome, except *T. vivax* being less prevalent in single infections than expected.

At eighteen month sampling the prevalence of mixed infections rises in the sample population in Kaberamaido for the first time, to 7.0%. At this time point there are significantly more *T. brucei* s. l./*T. congolense* savannah and *T. vivax*/*T. congolense* savannah than expected, a higher number of triple infections than expected and less single infections with both *T. vivax* and *T. congolense* savannah. This is against suggestive of an association between these two cattle pathogenic species of trypanosome. Between nine and eighteen months the overall prevalence of *T. congolense* savannah rose for the first time in this district.

6.4.3.3 Mixed infections in re-treatment case-positive villages

In case-positive villages at baseline there were no associations between particular species of trypanosome in the sample population. 4.3% of cattle were carrying mixed trypanosome infections. There were less than the expected number of single *T. vivax* infections, but no significant increase in the number of mixed infections with *T. vivax* and any other species of trypanosome.

Between baseline and six months there was a slight reduction in the percentage of mixed infections in the sample, falling slightly to 3.9%. There was also significantly more mixed *T. brucei* s. l. and *T. congolense* savannah than expected by chance. The prevalence of both these species dropped significantly between baseline and six months. It is important to note no *T. vivax* was detected at the six month time point, which could account for the slight reduction in the prevalence of mixed infections overall.

6.4.3.4 Mixed infections in re-treatment case-negative villages

The trend of mixed infections in case-negative villages is very similar to the situation in case-positive villages. At baseline there were no significant deviations from the expected number of mixed infections, and overall 2.1% of cattle were carrying a mixed infection. By six months this had risen to 3.1%, and just as was observed in case-positive villages, there were significantly more mixed infections with *T. brucei* s. l. and *T. congolense* savannah than expected by chance. Between baseline and six months in case-negative villages the prevalence of *T. brucei* s. l. and *T. vivax* had dropped significantly while the prevalence of *T. congolense* savannah had risen. The exact role played by mixed infections in these fluctuations in prevalence remains unclear. What is clear is the fact mixed infections are occurring more frequently than expected by chance in the re-treatment area in general. *T. brucei* s. l. and *T. b. rhodesiense* were the primary targets of the SOS programme. However, given the high number of mixed infections occurring, particularly after treatment, it is not feasible to propose control or eradication of this species without considering interactions between other trypanosome species.

6.4.3.5 Mixed infections in Dokolo and Kaberamaido, 2006 – 2008

The highest baseline prevalence of mixed infections was detected in re-treatment case-positive villages, were 4.3% of animals were carrying a mixed infection. This suggests that areas that are favourable for HAT transmission are also favourable for the acquisition of mixed infections. However post treatment both Dokolo and Kaberamaido had much higher prevalence of mixed infections at the SOS phase one eighteen month time point. As the eighteen month SOS phase one sampling and re-treatment baseline sampling were conducted at roughly the same time, these results could indicate a general situation of the prevalence of mixed infections increasing over time. Why this should be the case is unclear. Mixed infections are relatively common in tsetse, with 29% (Adams *et al.*, 2006), 12% (Jamonneau *et al.*, 2004), or 0.8% (Woolhouse *et al.*, 1994) of infected flies found to be carrying more than one species of trypanosome. There is some evidence tsetse may not easily transmit mixed infections under natural conditions (Moloo *et al.*, 1982). Elsewhere in Uganda, 0.7% of cattle in Tororo had mixed infections (Magona *et al.*, 2008), and similarly 0.7% of cattle had mixed infections in Mukono, Kamuli and Tororo districts (Waiswa *et al.*, 2003). These figures are markedly lower than the mixed infection rates recorded here. Further investigation is needed to determine the reason for the high prevalence of mixed infections in Dokolo and Kaberamaido, and the effect this may have on trypanosomiasis control in the area.

6.4.4 Village level trypanosomiasis

There is a clear link between high cattle trypanosomiasis, low SOS treatment coverage and HAT. Villages in Kaberamaido seem to be more heavily affected by trypanosomiasis, as this district has the majority of high prevalence villages in the rank analysis for both SOS phase one and the re-treatment. The rank analysis revealed some villages had scores that were many times higher than the majority of other villages, suggesting cattle in these villages were at consistently increased risk of trypanosomiasis and this risk was constant with the passing of time.

In the re-treatment area the change graphs seem to reveal a certain pattern in the change of trypanosomiasis prevalence between baseline and six months. Villages that experienced a fall in the prevalence of *T. brucei* s. l. often had also experienced a rise in the prevalence of *T. congolense* savannah. Similarly, where the prevalence of *T. congolense* savannah had fallen, in several instances the prevalence of *T. brucei* s. l. has risen in these same villages. This is suggestive of a link or an interaction between these two species of trypanosome, a suggestion to which further weight can be added by considering the uneven distribution of these species in the sample population, as described in sections 6.4.3.3 and 6.4.3.4.

In general the village level analysis of trypanosomiasis underlines previous assertions that HAT and AAT are focal diseases, the prevalence and transmission of which vary greatly on a small geographical scale. Given this variation, control programmes must be designed with a certain amount of flexibility, with frequent monitoring to detect small pockets where suboptimal control is allowing transmission of trypanosomes to continue.

6.5 Conclusions

No interaction between sex and age on the prevalence of trypanosomiasis was discovered. However data from previous chapters indicates cattle age and sex significantly affect trypanosome prevalence separately. *T. brucei* s. l. tends to be particularly prevalent in males and adult cattle, therefore HAT control programmes aiming to incorporate control of *T. b. rhodesiense* in the cattle reservoir should pay particular attention to ensuring high treatment coverage in these groups of cattle.

Analysis of the efficacy of isometamidium and diminazene treatment revealed, as could be reasonably expected, young animals have the highest ratio of treated to untreated cattle, presumably because many of these animals were not born when treatment occurred. Furthermore, anecdotal information suggests juvenile cattle are the most frequently bought and traded age group at market, if the animal is not subsequently

going to be slaughtered (R. Selby, personal communication). Therefore the low treatment coverage in younger age groups also highlights the impact of cattle movements on SOS treatment efficacy.

Analysis of mixed infections revealed at several time points trypanosome species were not evenly distributed in the sample population, with associations between different species detected. The associations detected were not constant, in that an association in the distribution of two species that was detected at one time point had vanished by the next, without any obvious explanation. The situation in Dokolo and Kaberamaido in relation to mixed infections was markedly different, highlighting the importance of small variations in the ecological conditions for the transmission of trypanosomiasis. Given the high number of mixed infections occurring, particularly after treatment, it is not feasible to propose control or eradication of *T. brucei* s. l. without considering interactions between other trypanosome species. It is highly likely the prevalence of mixed infection has adversely affected the efficacy of both SOS phase one and the re-treatment.

Chapter 7 – General Discussion

The focus of this thesis is one of the largest molecular epidemiological studies of AAT ever conducted, with molecular analysis of 7, 556 blood samples collected over a period of 24 months. The data sets analysed illustrate the effect of a two stage, mass trypanocidal drug treatment intervention on the prevalence of *T. vivax* and *T. congolense* savannah within the districts cattle herds of Kaberamaido and Dokolo in Uganda.

The re-treatment programme successfully reduced the prevalence of both *T. brucei* s. l. and *T. b. rhodesiense*. However the prevalence of *T. brucei* s. l. six months after re-treatment is considerably higher than that detected during baseline monitoring of SOS phase one. In general the prevalence data collected between 2006 and 2008 show a general trend of increasing prevalence, which is temporarily quelled at two points by the application of mass treatment. That the re-treatment did not elicit as large a reduction in *T. brucei* s. l. prevalence than the original treatment is hardly surprising, given that during SOS phase one all districts surrounding Dokolo and Kaberamaido were also treated, acting as a buffer against re-infection of the cattle population. Both rounds of the SOS intervention have undoubtedly reduced the prevalence of *T. brucei* s. l. and *T. b. rhodesiense*, further work needs to be done to develop strategies to sustain this reduction over time.

While the impact of the SOS campaign was successful in terms of its primary objective to control the northward spread of zoonotic trypanosomiasis, the effect of the mass trypanocidal drug treatment on AAT causing trypanosomes *T. vivax* and *T. congolense* savannah is questionable and variable, as set out in the preceding chapters of this thesis.

The dramatic decrease in the prevalence of *T. vivax* in the wake of the SOS re-treatment can be considered a particular triumph in terms of cattle herd health and productivity. *T. vivax* in East Africa often manifests as an acute, debilitating disease, cause of considerable morbidity and mortality (Losos and Ikede, 1972). As discussed in chapter five, the observed prevalence reduction strongly suggests genetic variability in

the *T. vivax* population was low, and that all strains present were extremely susceptible to diminazene treatment. The anomaly between the effect of mass isometamidium treatment on *T. vivax* prevalence and that of diminazene mass treatment is not easy to resolve.

There were no long term effects noted with regards the prevalence of *T. congolense* savannah following SOS phase one treatment, however following the re-treatment overall prevalence of this trypanosome rose. In both phases of monitoring *T. congolense* savannah was shown to have an epidemic character, having particularly high prevalence in some locations while in other neighbouring areas its prevalence is low or non-existent. It could be this epidemic character that allowed *T. congolense* savannah to rebound so quickly after treatment. However given the rise the prevalence observed during the re-treatment monitoring, the possibility of drug resistance must be given some consideration.

In recent years much work has been done elucidating the genetic determinants of drug resistance in trypanosomes. Although there are still many questions that remain unanswered, PCR specific for some of the common molecular markers related to drug resistance has been developed (Delespaux *et al.*, 2005, Delespaux *et al.*, 2008). Determining drug resistant *T. congolense* savannah at the time of sampling would ideally have involved taking isolates from infected cattle for culturing and *in vitro* drug resistance testing. Given the scale and remit of this intervention, not to mention the added technical and logistical considerations and costs this would have added, isolating live trypanosomes from infected cattle was not a viable option at that juncture. Therefore future work should involve the application of these PCR techniques to *T. congolense* savannah positive samples from Dokolo and Kaberamaido, to determine the presence or absence of these common resistance markers.

7.1 Diagnosis of AAT: future prospects

The sensitive molecular diagnostic techniques employed here set this study apart from the majority of other studies, which for reasons of ease of application and cost have been mainly reliant on parasitological diagnosis. Many of the barriers to sensitive field diagnosis of AAT were outlined in chapter one. The discrepancy between parasitological and molecular techniques has been well described in other studies (de Almeida *et al.*, 1998, Mugittu *et al.*, 2001, Picozzi *et al.*, 2002). The benefit of having used molecular techniques in this study is highlighted by comparing it to a similar investigation into the prevalence of bovine trypanosomiasis conducted in Dokolo and Kaberamaido. Using microscopy of wet smears in July 2008 (three months post re-treatment) a prevalence of 2.1% (9/435 cattle) was detected in parts of the re-treatment area (Enyaru *et al.*, 2009), with samples taken from some of the same villages analyzed in this thesis. Although re-treatment monitoring was not conducted in July 2008 so there are no direct comparisons to be made, the prevalence reported by Enyaru and colleagues was substantially lower than any prevalence detected by molecular means at any time point in the SOS phase one or re-treatment monitoring. Furthermore the identity of the trypanosome species observed in July 2008 remain a mystery, resulting in the loss of valuable data regarding zoonotic *T. b. rhodesiense* and mixed species infections.

Despite the advent of PCR more than twenty years ago, and the numerous species specific and *Trypanozoon* specific primers for which published protocols are readily available, molecular diagnostics are rarely, if ever, deployed in the field. Constraining the adaptability of PCR and other molecular techniques for field use are the requirement for:

- a constant source of electricity
- sterile conditions
- purification of sample genetic material before PCR analysis
- cold chain requirements for heat liable reagents
- expensive electrophoresis and imaging equipment for the visualization of results.

However there are many new advances that may soon see the application of molecular diagnostics to the field setting. One technique which many authors cite as being particularly field adaptable is loop-mediated isothermal amplification (LAMP) (Notomi *et al.*, 2000). The combination of four primer sets targeting six distinct regions of target DNA reportedly give LAMP much higher sensitivity and specificity than standard PCR, alongside a specialist DNA polymerase which allows the reaction to proceed rapidly under isothermal (65^{o}C) conditions (Notomi *et al.*, 2000). These conditions are readily achieved using a simple water bath or heating block. Several LAMP primer sets have been designed for the detection of pathogenic trypanosomes (Njiru *et al.*, 2004b, Njiru *et al.*, 2011). Their validation on a large set of field derived samples has yet to be reported in the peer-reviewed literature.

Since its inception end point detection has been a problem for LAMP, requiring measurements of turbidity (Mori *et al.*, 2001) or florescence emitted by expensive dyes (Njiru *et al.*, 2008). A recent comparative study of several dye-based end point detection techniques found none to be completely satisfactory when compared to visualization of the products using electrophoresis (Wastling *et al.*, 2010a). The most field applicable end point detection method to date comes in the form of the application of a lateral flow dipstick and specially labeled primers (Jaroenram *et al.*, 2009, Njiru *et al.*, 2010). There are also studies suggesting LAMP is much less susceptible to interference from inhibitory components within the sample than PCR is, and that it can be used to test samples with minimal preparation (Thekisoe *et al.*, 2009).

Ready-made, lyophilized PCR master mix kits are available various different PCR techniques, and are reported to be stable at a range of temperatures between 4 – 37^{o}C for up to three months (Klatser *et al.*, 1998), negating the requirement for the maintenance of cold chain conditions. If this freeze drying technology could be applied to trypanosome LAMP protocols in tandem with lateral flow dip sticks, the first truly field friendly molecular diagnostic for trypanosomiasis would be born. Such a tool would be invaluable to researchers, epidemiologists, medics and veterinarians alike. A field applicable molecular diagnostic would be essential if *T. b. rhodesiense* and *T. b. gambiense* were ever to overlap in Uganda, similarly if global elimination of HAT is to

be achieved by 2020, as WHO proposes, then field friendly molecular diagnostics are essential.

7.2 Movement of livestock and AAT

The movement of people and their animals has been proven to be crucial in the transmission and spread of many diseases, not least trypanosomiasis (Fèvre *et al.*, 2006a). In East Africa an estimated 210, 000 cattle were traded between Kenya, South Sudan, Uganda Tanzania and Ethiopia in the final three months of 2011 alone (MAS, 2012), over the course of a year close to one million cattle could be moved vast distances across East Africa. The number of livestock traded between East African countries has been rapidly increasing since 1991 and is expected to continue to do so (Little, 2009). The bulk of cattle traded are moved via the "informal" route (MAS, 2012), meaning these animals are moved without proper checks by government officials or other regulatory agencies to avoid taxes and other associated costs incurred with the movement of livestock. Some estimates put the volume of cross border trade in East Africa conducted through official channels as low as 10% (Di Nardo *et al.*, 2011). Even when cattle are traded through official channels, this often means the application of bribe money rather than subjecting to proper scrutiny and checks (Karugia *et al.*, 2009). For Uganda, this means the majority of cattle entering the country from neighbouring East African countries, through official or unofficial routes, will have circumnavigated the requirement for trypanocide treatment before sale currently required by the government of Uganda (Wendo, 2002).

Previous work reviewed in chapter one of this thesis beautifully illustrated the ease with which cattle can transport trypanosomes into previously unaffected areas (Enyaru *et al.*, 1993, Enyaru *et al.*, 1992, Fèvre *et al.*, 2001, Fèvre *et al.*, 2005, Hide *et al.*, 1994). Therefore the majority of cattle imported into Uganda have the potential to carry trypanosomes with them. This is a huge problem, not only in terms of *T. b. rhodesiense* but also when considering AAT. There have been few reports of drug resistant *T. congolense* or *T. vivax* (Mwambu and Mayende, 1971) in Uganda in the past, although it should also be noted drug resistance is not formally monitored or tested for. However

in Tanzania, Ethiopia and Kenya drug resistant AAT is becoming an increasing problem (Gray *et al.*, 1993, Rowlands *et al.*, 2001, Stevenson *et al.*, 1995, Tewelde, 2004). Therefore, in much the same way HAT was introduced into previously disease free areas, drug resistant AAT could become an increasing problem in areas without a long standing history of trypanocide use.

This phenomenon has been observed in developed countries in battle against methicillin resistant *Staphylococcus aureus*, MRSA, typically an hospital acquired infection, but from the early 1990's onwards an increasing proportion of resistant strains were community acquired, i.e., picked up outside the hospital environment by individuals without recent antibiotic exposure or previous ill health (Bassetti *et al.*, 2009, Nimmo and Coombs, 2008, Skov *et al.*, 2012, Walraven *et al.*, 2012). Furthermore it is recognized antibiotic resistance rates in developing countries are disproportionate to the level of antibiotic use (Raza *et al.*, 2004), meaning compounds seldom used in developing countries are already obsolete.

7.3 The current HAT situation in newly endemic areas of Uganda

Two mass cattle trypanocidal drug treatments were conducted in Dokolo and Kaberamaido under the auspices of the SOS campaign in Northern Uganda. In October 2006; over a quarter of a million cattle in the most northerly or newly affected areas were mass treated using trypanocides to reduce the burden and spread of zoonotic *T. b. rhodesiense*. In chapter two, the four aims of the SOS control programme were set out, in the following sections each of these aims will be examined separately to assess if the SOS control programme has successfully met its aims.

1. To maintain a barrier zone between the two forms of HAT in Uganda

There has been no detected convergence between the two forms of HAT in Uganda. To date screening of cattle in *T. b. gambiense* districts has failed to find any *T. b. rhodesiense* in addition no *T. b. rhodesiense* HAT cases have been reported north of Lira (Simarro *et al.*, 2010). The 150km barrier between *T. b. rhodesiense* and *T. b. gambiense* districts reported in 2005 still stands in 2012 (Balyeidhusa *et al.*, 2012). After a succession of newly affected districts between 1995 and 2005, eight years have passed without a new district becoming infected with *T. b. rhodesiense* HAT. The northerly extent of *T. b. rhodesiense* in Uganda in 2012 is as it was in 2005, as shown in Figure 7.1. Overlap between acute and chronic HAT has this far been prevented.

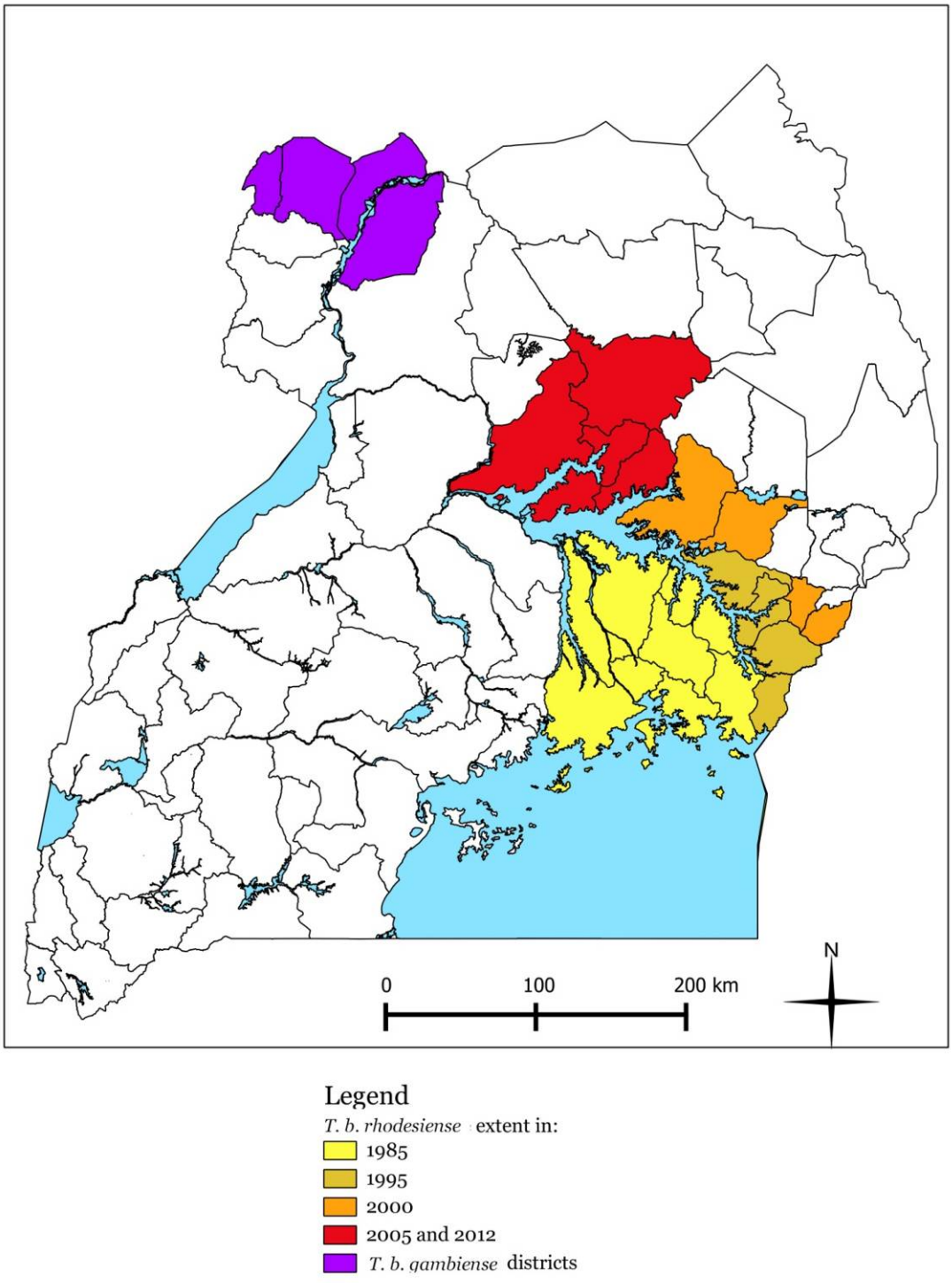

Figure 7.1 - map showing the extent of *T. b. rhodesiense* HAT in Uganda in 2012.

2. To reduce the prevalence of *T. b. rhodesiense* in cattle by treating more than 86% of the cattle population with trypanocides.

Both SOS phase one and the SOS re-treatment significantly reduced the prevalence of *T. b. rhodesiense* in cattle. The information set out in this thesis regarding the re-treatment and by Selby (2011) regarding SOS phase one show that mass treatment is an effective means to reduce the prevalence of *T. b. rhodesiense* within the cattle reservoir. In doing so, both rounds of treatment undoubtedly reduced the number of people at risk of cattle HAT from the cattle reservoir in the short term.

Official figures collected at the time of treatment indicate well over 86% of cattle were treated in Dokolo and Kaberamaido during both rounds of treatment. Post treatment coverage levels reported by farmers were below 86%. However accounting for young animals newly born or bought after treatment occurred, coverage reported by farmers for the remainder of their animals reaches and in some areas surpasses 86%.

Therefore it can be concluded the SOS campaign successfully reduced the prevalence of *T. b. rhodesiense* by treating more than 86% of cattle in intervention areas.

3. To maintain a reduction in the prevalence of *T. b. rhodesiense* in cattle via repeated insecticidal spraying

Over the eighteen months of monitoring during SOS phase one *T. b. rhodesiense* prevalence rebounded to levels not significantly different from the pretreatment baseline. Sub optimal spraying coverage has been indicated as a likely cause for the failure to sustain the reduction in *T. b. rhodesiense* prevalence, as well as the possible role of domestic pigs as reservoirs of infection, which were not included in SOS control efforts (Selby, 2011). In other *T. b. rhodesiense* endemic areas of Uganda pigs have been found harbouring this parasite (Waiswa *et al.*, 2003).

To focus solely on pigs as potential reservoirs of *T. b. rhodesiense* infection would be overlooking the many other domestic livestock and poultry kept in the area. In 2008 a country wide livestock census was conducted by the government of Uganda, the number of different livestock resident in Dokolo and Kaberamaido are shown in

Table 7.1.

	Cattle	Goats	Sheep	Pigs	Poultry
Dokolo	58, 902	71, 815	33,566	13,602	291,027
Kaberamaido	76, 109	97, 516	16 361	31,607	367,924

Table 7.1 - total number of cattle, goats, pigs, sheep and poultry resident in Dokolo and Kaberamaido in 2008 (UBOS and MAAF, 2009).

Unsurprisingly, given their small size and low cost, poultry are the most commonly kept livestock species in Dokolo and Kaberamaido. Across Uganda as a whole 40% of all rural households keep poultry, while just 20% of all rural households own cattle (Chilonda, 2005). Chickens have been shown to be capable of harbouring *T. b. rhodesiense* experimentally (Minter-Goedbloed, 1981). Some species of tsetse feed on avian hosts, and tsetse feeding patterns have been shown to be specific to a given area and able to evolve rapidly with time (Farikou *et al.*, 2010). Therefore poultry, the most numerous livestock species in both Dokolo and Kaberamaido, cannot be discounted as potential reservoir hosts for *T. b. rhodesiense*. Goats are also more numerous than cattle in both districts, and they too have been found harbouring *T. brucei* s. l. in other disease foci (Katunguka-Rwakishaya, 1996). Sheep and pigs, although less numerous than goats, cattle or poultry, have also been found harbouring *T. brucei* s. l. (Waiswa *et al.*, 2003).

In terms of longevity and their large size, which is very attractive to tsetse, cattle still most likely constitute the most important reservoir of *T. b. rhodesiense* in Dokolo and Kaberamaido. However given the failure of two mass treatments to control HAT in the area or eliminate *T. b. rhodesiense* from cattle, it is possible they are not the only

reservoir. Before further HAT or trypanosomiasis control is attempted in Dokolo or Kaberamaido a baseline survey of all livestock species present, to determine their reservoir status, is highly recommended by this author.

4. To reduce HAT prevalence in newly affected areas

Assessing the true burden of HAT is problematic due for many reasons, and under-reporting is an ongoing issue (Welburn *et al.*, 2009). The non specific symptoms of early HAT can lead to misdiagnosis; poor health care facilities and lack of a rapid and sensitive field diagnostic technique also play a part in misdiagnosis. Social stigma surrounding ill health may lead infected individuals to delay seeking treatment, as well as financial priorities. Modeling the level of HAT under reporting in South Eastern Uganda predicted for every recorded HAT fatality twelve go unreported (Odiit *et al.*, 2005). However due to the activities of the SOS campaign within Dokolo and Kaberamaido, alongside government education drives, awareness of HAT is likely to have increased since the outbreak began. This could led to a higher proportion of infected individuals successfully seeking treatment and receiving an accurate diagnosis, leading to an overall reduction in under reporting.

Furthermore, HAT cases tend to peak periodically in association with rainy and dry seasons, meaning short term assessment of case reduction can sometimes be misleading. As figure 2.11 shows, the initial peak in the number of HAT cases occurred at the beginning of the outbreak, when overall community awareness of HAT was low. Under reporting is likely to have been most prevalent in the early stages of the epidemic. Given that awareness has since increased, if SOS had not had an effect on HAT prevalence in newly affected areas an increase in the number of recorded cases would be expected. This has not been observed, with the number of HAT cases never reaching the number recorded at the beginning of the epidemic, before control and education activities began. Therefore it is likely the SOS campaign successfully controlled the prevalence of HAT in newly affected areas.

7.4 Prospects for global elimination of HAT

The WHO outlined plans to eliminate HAT in 2001 (WHO, 2002), and recently set the date for this target to be achieved: 2020 (Simarro *et al.*, 2008). The exact criteria determining elimination is to be defined by a special committee convened by the WHO at the end of 2012 (Savioli *et al.*, 2012). To propose global elimination of any disease is a daunting prospect, and history reveals the global eradication of an infectious disease has so far been achieved only once in the history of mankind, that disease was smallpox. Similarly, for disease of animals, rinderpest is the only pathogen to have been eradicated on a global scale (OIE, 2011). Attempts have been made to eradicate several other diseases: hookworm, malaria, yaws, polio and dracunculaisis (Tomori, 2011). Of these diseases, dracunculaisis and polio are close to eradication; eradication of hookworm, malaria and yaws is ongoing and arguably has a long way to go. Both rinderpest and smallpox were viral pestilences with no vector and no reservoirs of infection. The epidemiological situation for HAT is very different, and although eradication is not at this stage proposed, commonalities between these and other disease control programmes can be drawn on to inform the fight against HAT.

Across sub-Saharan Africa as a whole reported cases of HAT are at a 50 year low (Simarro *et al.*, 2010), this is interpreted by many as a promising indicator elimination is close at hand. However it must be remembered the world has before been on the brink of eradicating HAT, nearing the end of the colonial era in the 1960s it was generally accepted HAT had been consigned to the history books, so few were the reported cases, and control ceased to be a priority for most post-colonial governments (Nimmo, 2010). In spite of this by the early 1990's a resurgence in the number of HAT cases were recorded in many countries (Barrett, 1999). Lessons can be learnt from recent control efforts in Uganda that are applicable to the fight towards global elimination of HAT.

Elimination of HAT is not a task to underestimate; despite the sustained, highly successful control efforts by the SOS campaign with the dual focus of the reduction of *T. b. rhodesiense* in the cattle reservoir using trypanocidal drugs and reduction of the tsetse population through insecticidal cattle spraying, *T. b. rhodesiense* persists in the

cattle population of Dokolo and Kaberamaido and HAT cases are still recorded in these areas. Elimination was never a proposed outcome of the SOS campaign.

In order to propose elimination of sleeping sickness, several issues must be addressed. The unanimously fatal nature of *T. b. gambiense* HAT has been called into question, as a study which followed infected individuals for fifteen years after initial HAT diagnosis and refusal of treatment found individuals survived for the entire period of follow up, with trypanosomes undetectable by microscopy or PCR (Jamonneau *et al.*, 2012). Since death is not the only outcome following infection with HAT, the ethical considerations of programmes that use active screening to detect and treat infected individuals are greatly complicated, especially in light of the toxic and unpleasant stage two treatments. Given this added dimension of ambiguity, an accurate, sensitive field applicable diagnostic technique is needed now more than ever. While there are several promising avenues of research currently being conducted, such as the LAMP techniques outlined in 7.1, the chance of any delivering a finished product in time for the 2020 deadline seems slim. Without a sensitive field diagnostic detection of elimination will prove problematic, whatever criteria are set.

In Uganda the epidemiological situation regarding *T. b. rhodesiense* can be regarded as comparatively simple, in that various species of domestic livestock constitute the only non-human reservoirs in the majority of locations. Yet given this apparent simplicity it has been impossible to successfully control the parasite despite numerous attempts at doing so, and its geographical range has expanded. In other countries, where trypanosomiasis is a trans-boundary disease and wild animals act as reservoir hosts the epidemiological situation is much more complex, as too are the prospects for control or elimination. In northern Tanzania warthogs are thought to be important reservoirs of *T. b. rhodesiense* (Auty, 2008, Kaare *et al.*, 2007), as are lions (Welburn *et al.*, 2008). In Kenya many wild animal species as also recognized as important reservoir hosts of *T. b. rhodesiense*, such as the reedbuck (Allsopp, 1972, Heisch *et al.*, 1958) and hyena (Njiru *et al.*, 2004b). Wild animals cannot be easily treated with trypanocides or sprayed with insecticides, in these areas tsetse control is the main weapon against trypanosomiasis.

Rabies, another neglected zoonotic disease with wild and domestic hosts, is also problematic to control in situations where wild animals act as reservoirs of infection for the domestic dog population, which in turn are the main reservoir for human infection. Many species of wild animals are affected by rabies; commonly infected species including foxes, wild dogs, raccoons and bats (Chomel, 1993).

Much of the already achieved reduction in HAT cases have been reduction in the number of *T. b. gambiense* cases, with *T. b. rhodesiense* case numbers actually increasing in Uganda. Added to this are the countries such as the DRC, rife with civil instability to such an extent the full burden of *T. b. gambiense* is not easily quantifiable.

Many have lauded tsetse control, and in particular sterile insect technique as the key to eliminating tsetse and trypanosomiasis from the African continent (Kabayo, 2002, Vreysen *et al.*, 2000, Vreysen *et al.*, 2012). However the technique is expensive and requires extensive logistical support (Holmes, 2012). Furthermore, as illustrated in chapter one, the sheer number of species of tsetse fly thought to be present in each country, the level of international co-operation and co-ordination needed to prevent re-invasion of cleared areas and the huge costs associated with rearing and sterilizing sufficient numbers of male flies calls into question the feasibility of such an approach.

In short, there are many methods of trypanosomiasis control that have been proven to be effective, the question is not whether elimination can be achieved, more how it can be achieved. Which methods, in which areas, and for how long? Future trypanosomiasis research need not develop new tools and tests for the control or understanding of the epidemiology of this parasite, rather future research should focus on the best strategies for deploying existing control tools to achieve control and eventual elimination in sub-Saharan Africa.

As illustrated by the findings within this thesis, the pattern of trypanosome prevalence at the village level is varied and diverse, and in many ways highlights the scale and scope any future control or elimination intervention would have to take. In Dokolo and

Kaberamaido, two small districts in South Eastern Uganda, the impact of mass treatment varied greatly at the village level. The proposal of trypanosomiasis elimination on a pan African scale needs to account for the myriad of differing climatic and ecological situations present across the continent, since even within a 50km radius studied here, the impact of treatment varies greatly.

Chapter 8 – References

Adams, E. R., Hamilton, P. B., Rodrigues, A. C., Malele, I. I., Delespaux, V., Teixeira, M. M. G. & Gibson, W. (2010) New *Trypanosoma (Duttonella) vivax* genotypes from tsetse flies in East Africa. *Parasitology,* 137**,** 641-650.

Adams, E. R., Malele, I. I., Msangi, A. R. & Gibson, W. C. (2006) Trypanosome identification in wild tsetse populations in Tanzania using generic primers to amplify the ribosomal RNA ITS-1 region. *Acta Tropica,* 100**,** 103-109.

Agyemang, K. (2005) Trypanotolerant livestock in the context of trypanosomiasis intervention strategies. *PAAT Technical and Scientific Series,* 7**,** 1 - 73.

Ahmed, H. A. A. (2009) 'African Trypanosomes; a model for the improvement of molecular diagnosis of blood borne parasites' PhD Thesis, University of Edinburgh

Allsopp, R. (1972) The role of game animals in the maintenance of endemic and enzootic trypanosmiases in the Lambwe Valley, South Nyanza District, Kenya. *Bulletin of the World Health Organization,* 47**,** 735 - 746.

Anon (2010) Stamp Out Sleeping Sickness [webpage] Available from: http://www.stampoutsleepingsickness.com/ Accessed: 26/03/2010

Apted, F. I. C. (1970) Clinical Manifestations and Diagnosis of Sleeping Sickness.661 - 683 in: Mulligan, H. W. *The African Trypanosomiases.* London, George, Allen and Unwin LTD.

Assefa, E. & Abebe, G. (2001) Drug-resistant *Trypanosoma congolense* in naturally infected donkeys in north Omo Zone, southern Ethiopia. *Veterinary Parasitology,* 99**,** 261-271.

Assoku, R. K. G. & Gardiner, P. R. (1989) Detection of antibodies to platelets and erythrocytes during infection with haemorrhage-causing *Trypanosoma vivax* in ayrshire cattle. *Veterinary Parasitology,* 31**,** 199-216.

Auty, H., Mundy, A., Fyumagwa, R. D., Picozzi, K., Welburn, S. & Hoare, R. (2008) Health management of horses under high challenge from trypanosomes: A case study from Serengeti, Tanzania. *Veterinary Parasitology,* 154**,** 233-241.

Auty, H. K. (2008) 'Ecology of a vector-borne zoonosis in a complex ecosystem: Trypanosomiasis in Serengeti, Tanzania' PhD Thesis, University of Edinburgh

Balyeidhusa, A. S. P., Kironde, F. A. S. & Enyaru, J. C. K. (2012) Apparent lack of a domestic animal reservoir in Gambiense sleeping sickness in northwest Uganda. *Veterinary Parasitology,* 187**,** 157-167.

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

Bassetti, M., Nicco, E. & Mikulska, M. (2009) Why is community-associated MRSA spreading across the world and how will it change clinical practice? *International Journal of Antimicrobial Agents,* 34, Supplement 1**,** S15-S19.

Batchelor, N. A., Atkinson, P. M., Gething, P. W., Picozzi, K., Fèvre, E. M., Kakembo, A. S. L. & Welburn, S. C. (2009) Spatial Predictions of Rhodesian Human African Trypanosomiasis (Sleeping Sickness) Prevalence in Kaberamaido and Dokolo, Two Newly Affected Districts of Uganda. *PLoS Neglected Tropical Diseases,* 3**,** e563.

Baylis, M. & Nambiro, C. O. (1993) The effect of cattle infection by *Trypanosoma congolense* on the attraction, and feeding success, of the tsetse fly *Glossina pallidipes*. *Parasitology,* 106**,** 357-361.

Behnke, R. & Nakirya, M. (2012) The Contribution of Livestock to the Ugandan Economy FAO Rome

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

Bett, B., Irungu, P., Nyamwaro, S. O., Murilla, G., Kitala, P., Gathuma, J., Randolph, T. F. & McDermott, J. (2008) Estimation of tsetse challenge and its relationship

with trypanosomosis incidence in cattle kept under pastoral production systems in Kenya. *Veterinary Parasitology,* 155**,** 287-298.

Bett, B., Orenge, C., Irungu, P. & Munga, L. K. (2004) Epidemiological factors that influence time-to-treatment of trypanosomosis in Orma Boran cattle raised at Galana Ranch, Kenya. *Veterinary Parasitology,* 120**,** 43-53.

Bett, B., Randolph, T. F., Irungu, P., Nyamwaro, S. O., Kitala, P., Gathuma, J., Grace, D., Vale, G., Hargrove, J. & McDermott, J. (2010) Field trial of a synthetic tsetse-repellent technology developed for the control of bovine trypanosomosis in Kenya. *Preventive Veterinary Medicine,* 97**,** 220-227.

Biryomumaisho, S., Melville, S. E., Katunguka-Rwakishaya, E. & Lubega, G. W. (2009) Detection of natural *Trypanosoma vivax* infections in pigs with microhaematocrit centrifugation and amplification of ITS1 rDNA. *Onderstepoort Journal of Veterinary Research,* 76**,** 285 - 289.

Bouyer, J., Stachurski, F., Kaborè, I., Bauer, B. & Lancelot, R. (2007) Tsetse control in cattle from pyrethroid footbaths. *Preventive Veterinary Medicine,* 78**,** 223-238.

Bruce, D. (1895) Preliminary Report on Tsetse Fly Disease or Nagana in Zululand Durban

Bruce, D. (1908) Sleeping-Sickness in Africa. *Journal of the Royal African Society,* 7**,** 249-260.

Bruce, D. (1915) The Croonian Lectures on Trypanosomes: Causing Disease in Man and Domestic Animals in Central Africa II. *The Lancet,* 186**,** 1-6.

Bruce, D., Hamerton, A. E., Bateman, H. R. & Mackie, F. P. (1909) Sleeping Sickness in Uganda.-Duration of the Infectivity of the Glossina palpalis after the Removal of the Lake-Shore Population. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character,* 82**,** 56-63.

Bruce, D., Hamerton, A. E., Bateman, H. R. & Mackie, F. P. (1910a) The Development of Trypanosomes in Tsetse Flies. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character,* 82**,** 368-388.

Bruce, D., Hamerton, A. E., Bateman, H. R. & Mackie, F. P. (1910b) 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, Containing Papers of a Biological Character,* 82**,** 480-484.

Bruce, D., Hamerton, A. E., Bateman, H. R. & Mackie, F. P. (1910c) Trypanosome Diseases of Domestic Animals in Uganda II: *Trypanosoma brucei* (Plimmer and Bradford). *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character,* 83**,** 1 - 14.

Burri, C., Stich, A. & Brun, R. (2004) Current Chemotherapy of Human African Trypanosomiasis.403 - 419 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases*. Wallingford, CABI Publishing.

Buscher, P. & Lejon, V. (2004) Diagnosis of Human African Trypanosomiasis.203 - 218 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases.* Wallingford, CABI Publishing.

Butcher, C. (2009) From Research into Use: Monitoring and Evaluation of a Public-Private Partnership, Stamp Out Sleeping Sickness Case Study Development, D. f. I. London

Challier, A. & Laveissière, C. (1973) Un nouveau piège pour la capture des glossines (*Glossina: Diptera, Muscidae*): description et essais sure le terrain. *Cahiers O.R.S.T.O.M., sèrie Entomologie Mèdicale et Parasitologie,* 11**,** 251 - 262.

Checchi, F., Filipe, J., Barrett, M. P. & Chandramohan, D. (2008) The Natural Progression of Gambiense Sleeping Sickness: What Is the Evidence? *PLoS Neglected Tropical Diseases,* 2**,** e303.

Chemuliti, J. K., Mugunieri, G. M., Okoth, S., Munene, S. & NDung'u, J. M. (2005) Area-wide Pan African tsetse and trypanosomosis eradication campaign for the Central South of Mount Kenya belt - prospects and Challenges IN *28th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)*

Chilonda, P. (2005) Livestock Sector Brief: Uganda. Food and Agriculture Organization of the United Nations: Livestock Information Sector Analysis and Policy Branch FAO Rome.

Chomel, B. B. (1993) The modern epidemiological aspects of rabies in the world. *Comparative Immunology, Microbiology and Infectious Diseases,* 16**,** 11-20.

Clausen, P. H., Sidibe, I., Kaborè, I. & Bauer, B. (1992) Development of multiple drug resistance of *Trypanosoma congolense* in Zebu cattle under high natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. *Acta Tropica,* 51**,** 229-236.

Codjia, V., Mulatu, W., Majiwa, P. A. O., Leak, S. G. A., Rowlands, G. J., Authié, 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**,** 151-163.

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

Cox, A., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G. & Welburn, S. (2010) Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. *Parasites & Vectors,* 3**,** 82.

de Almeida, P. J. L. P., Ndao, M., Goossens, B. & Osaer, S. (1998) PCR primer evaluation for the detection of trypanosome DNA in naturally infected goats. *Veterinary Parasitology,* 80**,** 111 - 116.

Delespaux, V., Geysen, D., Majiwa, P. A. O. & Geerts, S. (2005) Identification of a genetic marker for isometamidium chloride resistance in *Trypanosoma congolense*. *International Journal for Parasitology,* 35**,** 235 - 243.

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

Di Nardo, A., Knowles, N. J. & Paton, D. J. (2011) Combining livestock trade patterns with phylogenetic analysis to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia. *Revue Scientique et Technique-Office International Des Epizooties,* 30**,** 63 - 85.

Dolan, R. B. (1998) *The Orma Boran: A trypanotolerant East African Breed. Fifteen years of research on Galana Ranch in Kenya,* Nairobi, Kenya, KETRI (Kenya Trypanosomiasis Research Institute).

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

Duffy, C. W., Morrison, L. J., Black, A., Pinchbeck, G. L., Christley, R. M., Schoenefeld, A., Tait, A., Turner, C. M. R. & MacLeod, A. (2009) *Trypanosoma vivax* displays a clonal population structure. *International Journal for Parasitology,* 39**,** 1475-1483.

Eisler, M. C., Arowolo, R. O. A., Gault, E. A., Moloo, S. K., Holmes, P. H. & Peregrine, A. S. (1994) Isometamidium concentrations in the sera of Boran cattle: Correlation with prophylaxis against tsetse-transmitted *Trypanosoma congolense*. *Acta Tropica,* 56**,** 39-50.

Eisler, M. C., Dwinger, R. H., Majiwa, P. A. O. & Picozzi, K. (2004) Diagnosis and Epidemiology of African Animal Trypanosomiasis.269 - 282 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases*. Wallingford, CABI Publishing.

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

Enyaru, J. C. K., Abila, P., Rutaro, R. K., Odyek, G., Okello, A. & Opio, P. (2009) Animal trypanosomiasis surveillance in the new focus of sleeping sickness in Dokolo and Alwa sub counties, North Eastern Uganda IN *30th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) Uganda*

Enyaru, J. C. K., Allingham, R., Bromidge, T., Kanmogne, G. D. & Carasco, J. F. (1993) The isolation and genetic heterogeneity of *Trypanosoma brucei gambiense* from north-west Uganda. *Acta Tropica,* 54**,** 31-39.

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

Fairlamb, A. H. (2003) Chemotherapy of human African trypanosomiasis: current and future prospects. *Trends in Parasitology,* 19**,** 488-494.

Farikou, O., Njiokou, F., Simo, G., Asonganyi, T., Cuny, G. & Geiger, A. (2010) Tsetse fly blood meal modification and trypanosome identification in two sleeping sickness foci in the forest of southern Cameroon. *Acta Tropica,* 116**,** 81-88.

Fèvre, E. M., Bronsvoort, B. M. d. C., Hamilton, K. A. & Cleaveland, S. (2006a) Animal movements and the spread of infectious diseases. *Trends in Microbiology,* 14**,** 125 - 131.

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

Fèvre, E. M., Coleman, P., Welburn, S. C. & Maudlin, I. (2004) Reanalyzing the 1900–1920 Sleeping Sickness Epidemic in Uganda. *Emerging Infectious Diseases,* 10**,** 567-573.

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

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

Ford, J. & Clifford, H. R. (1968) Changes in the Distribution of Cattle and of Bovine Trypanosomiasis Associated with the Spread of Tsetse-Flies (*Glossina*) in South-West Uganda. *Journal of Applied Ecology,* 5**,** 301-337.

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

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

Gall, Y., Woitag, T., Bauer, B., Sidibe, I., McDermott, J., Mehlitz, D. & Clausen, P.-H. (2004) Trypanocidal failure suggested by PCR results in cattle field samples. *Acta Tropica,* 92**,** 7-16.

Gardiner, P. R., Assoku, R. K. G., Whitelaw, D. D. & Murray, M. (1989) Haemorrhagic lesions resulting from *Trypanosoma vivax* infection in ayrshire cattle. *Veterinary Parasitology,* 31**,** 187-197.

Gardiner, P. R. & Wilson, A. J. (1987) *Trypanosoma (Duttonella) vivax*. *Parasitology Today,* 3**,** 49-52.

Gashumba, J. K., Komba, E. K., Truc, P., Allingham, R. M., Ferris, V. & Godfrey, D. G. (1994) The persistence of genetic homogeneity among *Trypanosoma brucei rhodesiense* isolates from patients in north-west Tanzania. *Acta Tropica,* 56**,** 341-348.

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

Gibson, W. C., Stevens, J. R., Mwendia, C. M. T., Makumi, J. N., Ngotho, J. & Ndung'u, J. (2001) Unravelling the phylogenetic relationships of African trypanosomes of suids. *Parasitology,* 122**,** 625-631.

Goossens, B., Mbwambo, H., Msangi, A., Geysen, D. & Vreysen, M. (2006) Trypanosomosis prevalence in cattle on Mafia Island (Tanzania). *Veterinary Parasitology,* 139**,** 74-83.

Gray, A. R. (1970) A Study of the Antigenic Relationships of Isolates of *Trypanosoma brucei* Collected from a Herd of Cattle Kept in One Locality for Five Years. *Journal of General Microbiology,* 62**,** 301-313.

Gray, A. R. & Roberts, C. J. (1971) The cyclical transmission of strains of *Trypanosoma congolense* and *T. vivax* resistant to normal therapeutic doses of trypanocidal drugs. *Parasitology,* 63**,** 67-89.

Gray, M. A., Kimarua, R. W., Peregrine, A. S. & Stevenson, P. (1993) Drug sensitivity screening in vitro of populations of *Trypanosoma congolense* originating from cattle and tsetse flies at Nguruman, Kenya. *Acta Tropica,* 55**,** 1-9.

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

Hide, G., Angus, S. D., Holmes, P. H., Maudlin, I. & Welburn, S. C. (1998) *Trypanosoma brucei*:Comparison of Circulating Strains in an Endemic and an

Epidemic Area of a Sleeping Sickness Focus. *Experimental Parasitology,* 89**,** 21-29.

Hide, G., Tait, A., Maudlin, I. & Welburn, S. C. (1996) The origins, dynamics and generation of *Trypanosoma brucei rhodesiense* epidemics in East Africa. *Parasitology Today,* 12**,** 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**,** 95-111.

Hoare, C. A. (1970) Systematic Description of the Mammalian Trypanosomes of Africa.24 - 59 in: Mulligan, H. W. *The African Trypanosomiases.* London, George, Allen and Unwin LTD.

Hoare, C. A. (1972) *The Trypanosomes of Mammals,* Oxford, Blackwell Scientific Publications.

Holmes, P. (2012) Tsetse-transmitted trypanosomes: Their biology, disease impact and control. *Journal of Invertebrate Pathology***,** *Article in press*.

Holmes, P. H., Eisler, M. C. & Geerts, S. (2004) Current Chemotherapy of Animal Trypanosomiasis.431 - 444 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases*. Wallinford, CABI Publishing.

Hudson, J. R. (1944) Acute and Subacute trypanosomiasis in cattle caused by *T. vivax*. *Journal of Comparative Pathology,* 44**,** 108 - 119.

Ilemobade, A. A. (2009) Tsetse and trypanosomosis in Africa: the challenges, the opportunities. *Onderstepoort Journal of Veterinary Research,* 76**,** 35-40.

Jamonneau, V., Ilboudo, H., Kaboré, J., Kaba, D., Koffi, M., Solano, P., Garcia, A., Courtin, D., Laveissiére, C., Lingue, K., Büscher, P. & Bucheton, B. (2012)

Untreated Human Infections by *Trypanosoma brucei gambiense* Are Not 100% Fatal. *PLoS Neglected Tropical Diseases,* 6**,** e1691.

Jamonneau, V., Ravel, S., Koffi, M., Kaba, D., Zeze, D., Ndri, L., Sane, B., Coulibaly, B., Cuny, G. & Solano, P. (2004) Mixed infection of tryanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Cote d'Ivoire. *Parasitology,* 129**,** 693 - 702.

Jaroenram, W., Kiatpathomchai, W. & Flegel, T. W. (2009) Rapid and sensitive detection of white spot syndrome virus by loop-mediated isothermal amplification combined with a lateral flow dipstick. *Molecular and Cellular Probes,* 23**,** 65-70.

Jolles, A. E., Ezenwa, V. O., Etienne, R. S., Turner, W. C. & Olff, H. (2008) Interactions between Macroparasites and Microparasites Drive Infection Patterns in Free-Ranging African Buffalo. *Ecology,* 89**,** 2239-2250.

Jones, T. W. & Davila, A. M. R. (2001) *Trypanosoma vivax* - out of Africa. *Trends in Parasitology,* 17**,** 99-101.

Kaare, M. T., Picozzi, K., Mlengeya, T., Fèvre, E. M., Mellau, L. S., Mtambo, M. M., Cleaveland, S. & Welburn, S. C. (2007) Sleeping sickness: A re-emerging disease in the Serengeti? *Travel Medicine and Infectious Disease,* 5**,** 117-124.

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

Kalu, A. U., Edeghere, H. U. & Lawani, F. A. (1986) Comparison of diagnostic techniques during subclinical single infections of trypanosomiasis in goats. *Veterinary Parasitology,* 22**,** 37-47.

Karugia, J., Wanjiku, J., Nzuma, J., Gbegbelegbe, S., Macharia, E., Massawe, S., Freeman, A., Waithaka, M. & Kaitibie, S. (2009) The impact of non-tariff barriers on maize and beef trade in East Africa Nairobi, Kenya

Katunguka-Rwakishaya, E. (1996) The prevalence of trypanosomosis in small ruminants and pigs in a sleeping sickness endemic area of Buikwer County, Mukono district, Uganda. *Revue Élevage Médecine Vétérinaire des Pays Tropicaux,* 49**,** 56 - 58.

Kauta, N., Opio, J., Otim, G., Rutebarika, C., Emwanu, T., Muhwezi, B. J., Chiozza, F., Robinson, T. P., Baltenweck, I., Kristjanson, P., Ochungo, P., Okwi, P., Owour, J., Henninger, N. & Lansberg, F. (2010) Mapping a Better Future: Spatial Analysis and Pro-Poor Livestock Strategies in Uganda Institute, W. R. Washington DC

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

Kennedy (2006) Diagnostic and neuropathogenesis issues in human African trypanosomiasis. *International Journal for Parasitology,* 36**,** 505-512.

Kennedy, P. G. E. (2008) Diagnosing central nervous system trypanosomiasis: two stage or not to stage? *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 102**,** 306-307.

Killick-Kendrick, R. (1971) The low pathogenicity of *Trypanosoma brucei* to cattle. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 65**,** 104.

Klatser, P., Kuijper, S., van Ingen, C. & Kolk, A. (1998) Stabilized, Freeze-Dried PCR Mix for the Detection of Mycobacteria. *Journal of Clinical Microbiology,* 36**,** 1798 - 1800.

Koerner, T., de Raadt, P. & Maudlin, I. (1995) The 1901 uganda sleeping sickness epidemic revisited: A case of mistaken identity? *Parasitology Today,* 11**,** 303-306.

Kristjanson, P. M., Swallow, B. M., Rowlands, G. J., Kruska, R. L. & de Leeuw, P. N. (1999) Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agricultural Systems,* 59**,** 79-98.

Leak, S. G. A. (1999) *Tsetse biology and ecology,* London, CABI publishing.

Leeflang, P., Buys, J. & Blotkamp, C. (1976) Studies on *Trypanosoma vivax*: Infectivity and serial maintenance of natural bovine isolates in mice. *International Journal for Parasitology,* 6**,** 413-417.

Levine, R. A., Wardlaw, S. C. & Patton, C. L. (1989) Detection of haematoparasites using quantitative buffy coat analysis tubes. *Parasitology Today,* 5**,** 132-134.

Little, P. D. (2009) Hidden value on the Hoof: Cross Border Livestock Trade in Eastern Africa Nairobi, Kenya

Lloyd, L. & Johnson, W. B. (1924) The Trypanosome Infections of Tsetse-flies in Northern Nigeria and a new Method of Estimation. *Bulletin of Entomological Research,* 14**,** 265-288.

Losos, G. J. & Ikede, B. O. (1972) Review of Pathology of Diseases in domestic and Laboratory Animals Caused by *Trypanosoma congolense*, *T. vivax*, *T. brucei*, *T. rhodesiense* and *T. gambiense*. *Veterinary Parasitology,* 9**,** 1 - 71.

Low, G. C. (1929) A retrospect of tropical medicine from 1894 to 1914. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 23**,** 213-232.

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

Luckins, A. G. (1992) Methods for diagnosis of trypanosomiasis in livestock. *World Animal Review,* 70/71**,** 15 - 20.

Lumsden, W. H. R., Kimber, C. D., Evans, D. A. & Doig, S. J. (1979) *Trypanosoma brucei*: miniature anion-exchange centrifugation technique for detection of low parasitaemias: adaptation for field use. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73**,** 312-317.

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

Machila, N., Wanyangu, S. W., McDermott, J., Welburn, S. C., Maudlin, I. & Eisler, M. C. (2003) Cattle owners' perceptions of African bovine trypanosomiasis and its control in Busia and Kwale Districts of Kenya. *Acta Tropica,* 86**,** 25-34.

MacKichan, I., W. (1944) Rhodesian sleeping sickness in Eastern Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 38**,** 49-60.

Magnus, E., Vervoort, 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. *Annales de la Societe belge de medecine tropicale,* 58**,** 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 trypanosomiasis. *Veterinary Parasitology,* 128**,** 1 - 9.

Magona, J. W., Walubengo, J. & Odimin, J. T. (2008) Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. *Acta Tropica,* 107**,** 186-191.

Maloo, S. H., Thorpe, W., Kioo, G., Ngumi, P., Rowlands, G. J. & Perry, B. D. (2001) Seroprevalences of vector-transmitted infections of small-holder dairy cattle in coastal Kenya. *Preventive Veterinary Medicine,* 52**,** 1-16.

Mamoudou, A., Zoli, A., Mbahin, N., Tanenbe, C., Bourdanne, Clausen, P. H., Marcotty, T., Van den Bossche, P. & Geerts, S. (2006) Prevalence and incidence of bovine trypanosomosis on the Adamaoua plateau in Cameroon 10 years after the tsetse eradication campaign. *Veterinary Parasitology,* 142**,** 16-22.

MAS (2012) East Africa Cross-border Trade Bulletin: October-December 2011. The Market Analysis Subgroup Of The Food Security And Nutrition Working Group, F. A. O. Rome

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.

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**,** 908 - 918.

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

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

McDermott, J., Woitag, T., Sidibè, I., Bauer, B., Diarra, B., Ouèdraogo, D., Kamuanga, M., Peregrine, A., Eisler, M., Zessin, K.-H., Mehlitz, D. & Clausen, P.-H. (2003) Field studies of drug-resistant cattle trypanosomes in Kènèdougou Province, Burkina Faso. *Acta Tropica,* 86**,** 93-103.

McDonald, J. H. (2009) *Handbook of Biological Statistics,* Baltimore, Sparky House Publishing.

Mihret, A. & Mamo, G. (2007) Bovine Trypanosomosis in three districts of East Gojjam Zone bordering the Blue Nile River in Ethiopia. *The journal of Infection in Developing Countries,* 1**,** 321-325.

Minter-Goedbloed, E. (1981) The susceptibility of chickens to *Trypanosoma brucei* subspecies. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 75**,** 345-349.

Moloo, S. K. (1985) Distribution of *Glossina* species in Africa. *Acta Tropica,* 42**,** 275 - 281.

Moloo, S. K., Dar, F. & Kamunya, G. W. (1982) The transmission of mixed infections of pathogenic *Trypanosoma* species to susceptible hosts by *Glossina morsitans morsitans*. *Acta Tropica,* 39**,** 303 - 306.

Moloo, S. K., Losos, G. J. & Kutuza, S. B. (1973) Transmission of *Trypanosoma brucei* to cats and dogs by feeding on infected goats. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 67**,** 286-287.

Moloo, S. K., Sabwa, C. L. & Baylis, M. (2000) Feeding behaviour of *Glossina pallidipes* and *G. morsitans centralis* on Boran cattle infected with *Trypanosoma congolense* or *T. vivax* under laboratory conditions. *Medical and Veterinary Entomology,* 14**,** 290 - 299.

Molyneux, D. H. (1975) Diagnostic methods in animal trypanosomiasis. *Veterinary Parasitology,* 1**,** 5-17.

Mori, Y., Nagamine, K., Tomita, N. & Notomi, T. (2001) Detection of Loop-Mediated Isothermal Amplification Reaction by Turbidity Derived from Magnesium Pyrophosphate Formation. *Biochemical and Biophysical Research Communications,* 289**,** 150-154.

Morrision, W. I., Murray, M. & McIntyre, W. I. M. (1981) Bovine Trypanosomiasis.469 - 498 in: Ristic, M. & McIntyre, I. *Diseases of Cattle in the Topics: Economic and Zoonotic Relevance.* London, Martinus Nijhoff Publishers.

Morrison, L. J., Tweedie, A., Black, A., Pinchbeck, G. L., Christley, R. M., Schoenefeld, A., Hertz-Fowler, C., MacLeod, A., Turner, C. M. R. & Tait, A. (2009) Discovery

of Mating in the Major African Livestock Pathogen *Trypanosoma congolense*. *PLoS ONE,* 4**,** e5564.

Moser, D. R., Cook, G. A., Ochs, D. E., Bailey, C. P., McKane, M. R. & Donelson, J. E. (1989) Detection of *Trypanosoma congolense* and *Trypanosoma brucei* subspecies by DNA amplification using the polymerase chain reaction. *Parasitology,* 99**,** 57-66.

Msangi, A. R., Whitaker, C. J. & Lehane, M. J. (1998) Factors influencing the prevalence of trypanosome infection of *Glossina pallidipes* on the Ruvu flood plain of Eastern Tanzania. *Acta Tropica,* 70**,** 143-155.

Mugittu, K. N., Silayo, R. S., Majiwa, P. A. O., Kimbita, E. K., Mutayoba, B. M. & Maselle, R. (2001) Application of PCR and DNA probes in the characterisation of trypanosomes in the blood of cattle in farms in Morogoro, Tanzania. *Veterinary Parasitology,* 94**,** 177-189.

Mulligan, H. W. (1970) *The African Trypanosomiasis,* London, George Allen & Unwin.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P. A. O., Masake, R. & Peregrine, A. S. (1997) Long-term occurrence of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium in cattle at Ghibe, Ethiopia. *Acta Tropica,* 64**,** 205-217.

Muraguri, G. R., McLeod, A., McDermott, J. J. & Taylor, N. (2005) The incidence of calf morbidity and mortality due to vector-borne infections in smallholder dairy farms in Kwale District, Kenya. *Veterinary Parasitology,* 130**,** 305-315.

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

Murray, M. & Trail, J. C. M. (1987) Comparative epidemiology and control of trypanosomes. *International Journal for Parasitology,* 17**,** 621-627.

Mwambu, P. M. & Mayende, J. S. P. (1971) Occurrence of Berenil resistant strains of *T. vivax*. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 65**,** 254-255.

Mwangi, E. K., Stevenson, P., Gettinby, G., Reid, S. W. J. & Murray, M. (1998) Susceptibility to trypanosomosis of three *Bos indicus* cattle breeds in areas of differing tsetse fly challenge. *Veterinary Parasitology,* 79**,** 1-17.

Mwebaze, S. M. N. (2006) Country Pasture/Forage Resource Profiles: Uganda FAO Rome

NG (2010) *National Geographic Atlas of the World,* New York, National Geopgraphical Society.

Nicholson, M. J. & Butterworth, M. H. (1986) A Guide to Condition Scoring of Zebu Cattle International Livestock Centre for Africa, Adddis Ababa, Ethiopia

Nimmo, C. (2010) Time to put out the lights on sleeping sickness? *Travel Medicine and Infectious Disease,* 8**,** 263 - 268.

Nimmo, G. R. & Coombs, G. W. (2008) Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) in Australia. *International Journal of Antimicrobial Agents,* 31**,** 401-410.

Njagu, Z., Mihok, S., Kokwaro, E. D. & Verloo, D. (1999) Isolation of *Trypanosoma brucei* from the monitor lizard (*Varanus niloticus*) in an endemic focus of rhodesian sleeping sickness in Kenya. *Acta Tropica,* 72**,** 137 - 148.

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

Njiru, Z. K., Makumi, J. N., Okoth, S., Ndungu, J. M. & Gibson, W. C. (2004a) Identification of trypanosomes in *Glossina pallidipes* and *G. longipennis* in Kenya. *Infection, Genetics and Evolution,* 4**,** 29-35.

Njiru, Z. K., Mikosza, A. S. J., Matovu, E., Enyaru, J. C. K., Ouma, J. O., Kibona, S. N., Thompson, R. C. A. & Ndung'u, J. M. (2008) African trypanosomiasis: Sensitive and rapid detection of the sub-genus Trypanozoon by loop-mediated isothermal amplification (LAMP) of parasite DNA. *International Journal for Parasitology,* 38**,** 589-599.

Njiru, Z. K., Ndung'u, K., Matete, G., Ndungu, J. M. & Gibson, W. C. (2004b) Detection of *Trypanosoma brucei rhodesiense* in animals from sleeping sickness foci in East Africa using the serum resistance associated (SRA) gene. *Acta Tropica,* 90**,** 249-254.

Njiru, Z. K., Ouma, J. O., Bateta, R., Njeru, S. E., Ndung'u, K., Gitonga, P. K., Guya, S. & Traub, R. (2011) Loop-mediated isothermal amplification test for *Trypanosoma vivax* based on satellite repeat DNA. *Veterinary Parasitology,* 180**,** 358-362.

Njiru, Z. K., Ouma, J. O., Enyaru, J. C. & Dargantes, A. P. (2010) Loop-mediated Isothermal Amplification (LAMP) test for detection of *Trypanosoma evansi* strain B. *Experimental Parasitology,* 125**,** 196-201.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. & Hase, T. (2000) Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research,* 28**,** e63.

O'Gorman, G., Park, S., Hill, E., Meade, K., Coussens, P., Agaba, M., Naessens, J., Kemp, S. & MacHugh, D. (2009) Transcriptional profiling of cattle infected with *Trypanosoma congolense* highlights gene expression signatures underlying trypanotolerance and trypanosusceptibility. *BMC Genomics,* 10**,** 207.

Odiit, M., Coleman, P., Liu, W. C., McDermott, J., Fèvre, E. M., Welburn, S. & Woolhouse, M. E. J. (2005) Quantifying the level of under-detection of *Trypanosoma brucei rhodesiense* sleeping sickness cases. *Tropical Medicine & International Health,* 10**,** 840 - 849.

Ogwal, L. M., Kalyebi, A. & Kaddu, J. B. (2007) The diurnal activity, movement and trypanosome infection rates of *Glossina fuscipes fuscipes* (Diptera: Glossinidae) in Buvuma Island, Lake Victoria, Uganda. *Insect Science,* 14**,** 477 - 484.

Ohaga, S. O., Kokwaro, E. D., Ndiege, I. O., Hassanali, A. & Saini, R. K. (2007) Livestock farmers' perception and epidemiology of bovine trypanosomosis in Kwale District, Kenya. *Preventive Veterinary Medicine,* 80**,** 24-33.

OIE (2009) Trypanosomosis (Tsetse Transmitted) Technical Disease Card. *World Organization for Animal Health*, Paris

OIE (2011) Declearation of Global Eradication of Rinderpest and Implementation of Follow-up Measures to Maintain World Freedom from Rinderpest IN *79th Session of the World Assembly of Delegates, Paris,* World Organization of Animal Health

Okuna, N. M., Mayende, J. S. P. & Guloba, A. (1986) *Trypanosoma brucei* infection in domestic pigs in a sleeping sickness epidemic area of Uganda. *Acta Tropica,* 43**,** 183 - 184.

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**,** 175-182.

Ormerod, W. E. (1961) The epidemic spread of Rhodesian sleeping sickness 1908 - 1960. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 55**,** 525-538.

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

Penchenier, L., Alhadji, D., Bahèbèguè, S., Simo, G., Laveissière, C. & Cuny, G. (2005) Spontaneous cure of domestic pigs experimentally infected by *Trypanosoma brucei gambiense*: Implications for the control of sleeping sickness. *Veterinary Parasitology,* 133**,** 7-11.

Pentreath, V. W. & Kennedy, P. G. E. (2004) Pathogenesis of Human African Trypanosomiasis.283 - 302 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases*. Wallingford, CABI Publishing.

Phiri, B. J., Benschop, J. & French, N. P. (2010) Systematic review of causes and factors associated with morbidity and mortality on smallholder dairy farms in Eastern and Southern Africa. *Preventive Veterinary Medicine,* 94**,** 1-8.

Picozzi, K., Carrington, M. & Welburn, S. C. (2008) A multiplex PCR that discriminates between *Trypanosoma brucei brucei* and zoonotic *T. b. rhodesiense*. *Experimental Parasitology,* 118**,** 41-46.

Picozzi, K., Févre, E., Odiit, M., Carrington, M., Eisler, M. C., Maudlin, I. & Welburn, S. (2005) Sleeping sickness in Uganda: a thin line between two fatal diseases. *British Medical Journal,* 331**,** 1238-1241.

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

Raza, M. W., Kazi, B. M., Mustafa, M. & Gould, F. K. (2004) Developing countries have their own characteristic problems with infection control. *Journal of Hospital Infection,* 57**,** 294-299.

Richardson, U. F. (1928) Notes on trypanosomiasis of cattle in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 22**,** 137-146.

Robson, J. & Ashkar, T. S. (1972) Trypanosomiasis in domestic livestock in the Lambwe Valley area and field evaluation of various diagnostic techniques. *Bulletin of the World Health Organization,* 47**,** 727 - 734.

Rogers, D. J., Hay, S. I. & Randolph, S. E. (2000) Satellites, space, time and the African trypanosomiases. *Advances in Parasitology,* 47**,** 129-171.

Rogers, D. J. & Robinson, T. P. (2004) Tsetse distribution.139 - 179 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases.* Wallingford, CABI Publishing.

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

Savioli, L., Daumerie, D. & Crompton, D. (2012) Accelerating work to overcome the global impact of neglected tropical diseases: A roadmap for implementation Rome

Scott, D. (1970) The Epidemiology of Gambian Sleeping Sickness.614 - 644 in: Mulligan, H. W. *The African Trypanosomiases.* London, George, Allen and Unwin LTD.

Selby, R. (2011) 'Limiting the northerly advance of *Trypanosoma brucei rhodesiense* in post conflict Uganda' PhD Thesis, University of Edinburgh

Simarro, P., Cecchi, G., Paone, M., Franco, J., Diarra, A., Ruiz, J., Fèvre, E., Courtin, F., Mattioli, R. & Jannin, J. (2010) The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. *International Journal of Health Geographics,* 9**,** 57.

Simarro, P., Diarra, A., Ruiz Postigo, J., Franco, J. & Jannin, J. (2011) The Human African Trypanosomiasis Control and Surveillance Programme of the World

Health Organization 2000 - 2009: The Way Forward. *PLoS Neglected Tropical Diseases,* 5**,** e1007.

Simarro, P. P., Jannin, J. & Cattand, P. (2008) Eliminating Human African Trypanosomiasis: where do we stand and what comes next? *PLoS Medicine,* 5**,** e55.

Skov, R., Christiansen, K., Dancer, S. J., Daum, R. S., Dryden, M., Huang, Y.-C. & Lowy, F. D. (2012) Update on the prevention and control of community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). *International Journal of Antimicrobial Agents,* 39**,** 193-200.

Smith, D. H. & Bailey, J. W. (1997) Human African trypanosomiasis in south-eastern Uganda: clinical diversity and isoenzyme profiles. *Annals of Tropical Medicine and Parasitology,* 91**,** 851-856.

Sow, A., Sidibé, I., Bengaly, Z., Marcotty, T., Séré, M., Diallo, A., Vitouley, H. S., Nebié, R. L., Ouédraogo, M., Akoda, G. K., Van den Bossche, P., Van Den Abbeele, J., De Deken, R. & Delespaux, V. (2012) Field detection of resistance to isometamidium chloride and diminazene aceturate in *Trypanosoma vivax* from the region of the Boucle du Mouhoun in Burkina Faso. *Veterinary Parasitology***,** Article in Press.

Stefano, P. & Ezio, B. (2007) The Mantel-Haenszel procedure in epidemiological studies: An intoduction. *Annali Della Facolta Di Medicina Veterina Del Studi Di Parma,* XXVII**,** 17 - 32.

Stephen, L. E. (1970a) Care and Treatment of Animals Suffering from Trypanosomiasis.795 - 798 in: Mulligan, H. W. *The African Trypanosomiases.* London, George, Allen and Unwin LTD.

Stephen, L. E. (1970b) Clinical Manifestations of the Trypanosomiases in Livestock and Other Domestic Animals.775 - 794 in: Mulligan, H. W. *The African Trypanosomiases.* London, George, Allen and Unwin LTD.

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

Stevenson, P., Sones, K. R., Gicheru, M. M. & Mwangi, E. K. (1995) Comparison of isometamidium chloride and homidium bromide as prophylactic drugs for trypanosomiasis in cattle at Nguruman, Kenya. *Acta Tropica,* 59**,** 77-84.

Tarimo-Nesbitt, 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**,** 107-117.

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

Taylor, K. A. (1998) Immune responses of cattle to African trypanosomes: protective or pathogenic? *International Journal for Parasitology,* 28**,** 219-240.

Tewelde, N. (2004) Application of field methods to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia. *Acta Tropica,* 90**,** 163-170.

Thekisoe, O., Bazie, R., Coronel-Servian, A., Sugimoto, C., Kawazu, S. & Inoue, N. (2009) Stability of Loop-Mediated Isothermal Amplification (LAMP) Reagents and its Amplification Efficency on Crude Trypanosome DNA templates. *Journal of Veterinary Medical Science,* 71**,** 471 - 475.

Thrusfield, M. (2005) *Veterinary Epidemiology,* Oxford, Blackwell Scientific.

Thumbi, S. M., Jung'a, J. O., Mosi, R. O. & McOdimba, F. A. (2010) Spatial distribution of African Animal Trypanosomiasis in Suba and Tesco districts in Western Kenya. *BMC Research Notes,* 3**,** 1 - 6.

Tilley, A., Welburn, S. C., Fèvre, 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**,** 26-32.

Tomori, O. (2011) From smallpox eradication to the future of global health: Innovations, application and lessons for future eradication and control initiatives. *Vaccine,* 29, Supplement 4**,** D145-D148.

Toro, M., Leon, E. & Lopez, R. (1981) Haematocrit centrifugation technique for the diagnosis of bovine trypanosomiasis. *Veterinary Parasitology,* 8**,** 23-29.

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

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

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

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

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

Turner, D. A. (1986) Tsetse and trypanosomiasis in the Lambwe Valley, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 80**,** 592-595.

UBOS (2010) Uganda Bureau of Statistics 2010 Statistical Abstract Kampala

UBOS & MAAF (2009) A summary report of the national livestock census, 2008 Kampala, Uganda

Uilenberg, G. (1998) A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis. Nations, F. a. A. O. o. t. U. Rome

Uzoigwe, N. R. (1986) Self-cure in Zebu calves experimentally infected with *Trypanosoma vivax*. *Veterinary Parasitology,* 22**,** 141-146.

Vale, G. A. (1987) Prospects for tsetse control. *International Journal for Parasitology,* 17**,** 665-670.

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

Van den Bossche, P., De Deken, R., Brandt, J., Seibou, B. & Geerts, S. (2004) Recirculation of *Trypanosoma brucei brucei* in cattle after *T. congolense* challenge by tsetse flies. *Veterinary Parasitology,* 121**,** 79-85.

Van den Bossche, P. & Mudenge, D. (1999) The Effect of Short-interval Deltamethrin Applications to Control Tsetse on the Seroprevalence of Babesiosis in Cattle. *Tropical Animal Health and Production,* 31**,** 215-222.

Van den Bossche, P., Shumba, W. & Makhambera, P. (2000) The distribution and epidemiology of bovine trypanosomosis in Malawi. *Veterinary Parasitology,* 88**,** 163 - 176.

von Wissmann, B., Machila, N., Picozzi, K., Fèvre, E. M., de C. Bronsvoort, B. M., Handel, I. G. & Welburn, S. C. (2011) Factors Associated with Acquisition of Human Infective and Animal Infective Trypanosome Infections in Domestic Livestock in Western Kenya. *PLoS Neglected Tropical Diseases,* 5**,** e941.

Vreysen, M. J. B., Saleh, K. M., Ali, M. Y., Abdulla, A. M., Zhu, Z.-R., Juma, K. G., Dyck, V. A., Msangi, A. R., Mkonyi, P. A. & Feldmann, H. U. (2000) *Glossina austeni*

(Diptera: Glossinidae) Eradicated on the Island of Unguja, Zanzibar, Using the Sterile Insect Technique. *Journal of Economic Entomology,* 93**,** 123-135.

Vreysen, M. J. B., Seck, M. T., Sall, B. & Bouyer, J. (2012) Tsetse flies: Their biology and control using area-wide integrated pest management approaches. *Journal of Invertebrate Pathology*.

WAHID-OIE (2012) Distribution of AAT in Africa [webpage] Available from: http://www.oie.int/wahis_2/public/wahid.php/countrymapinteractive Accessed: 17/07/2012

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**,** 149 - 155.

Waiswa, C., Picozzi, K., Katunguka-Rwakishaya, E., Olaho-Mukani, W., Musoke, A. J. & Welburn, S. (2006) *Glossina fuscipes fuscipes* in the trypanosomiasis endemic areas of south eastern Uganda: Apparent density, trypanosome infection rates and host feeding preferences. *Acta Tropica,* 99**,** 23-29.

Walraven, C. J., Lingenfelter, E., Rollo, J., Madsen, T. & Alexander, D. P. (2012) Diagnostic and Therapeutic Evaluation of Community-acquired Methicillin-resistant *Staphylococcus Aureus* (MRSA) Skin and Soft Tissue Infections in the Emergency Department. *The Journal of Emergency Medicine,* 42**,** 392-399.

Wardrop, N. A. (2011) Distribution of HAT in Dokolo and Kaberamaido, 2004 - 2010 *Personal communication*

Wastling, S. L., Picozzi, K., Kakembo, A. S. L. & Welburn, S. C. (2010a) LAMP for Human African Trypanosomiasis: A Comparative Study of Detection Formats. *PLoS Neglected Tropical Diseases,* 4**,** e865.

Wastling, S. L., Picozzi, K., Wamboga, C., Von Wissmann, B., Amongi-Accup, C., Wardrop, N. A., Stothard, J. R., Kakembo, A. & Welburn, S. C. (2010b) Latent

Trypanosoma brucei gambiense foci in Uganda: a silent epidemic in children and adults? *Parasitology,* 138**,** 1480-1487.

Wastling, S. L. & Welburn, S. C. (2011) Diagnosis of human sleeping sickness: sense and sensitivity. *Trends in Parasitology,* 27**,** 394-402.

Welburn, S., Coleman, P. G., Maudlin, I., Fèvre, E., Odiit, M. & Eisler, M. C. (2006) Crisis, what crisis? Control of Rhodesian sleeping sickness. *Trends in Parasitology,* 22**,** 123-128.

Welburn, S., Picozzi, K., Coleman, P. G. & Packer, C. (2008) Patterns in Age-Seroprevalence Consistent with Acquired Immunity against *Trypanosoma brucei* in Serengeti Lions. *PLoS Neglected Tropical Diseases,* 2**,** e347.

Welburn, S. C., Fèvre, E., Coleman, P., Odiit, M. & Maudlin, I. (2001a) Sleeping sickness: a tale of two diseases. *Trends in Parasitology,* 17**,** 19-24.

Welburn, S. C., Fèvre, E. M., Coleman, P. & Maudlin, I. (2004) Epidemiology of Human African Trypanosomiasis.219 - 232 in: Maudlin, I., Holmes, P. H. & Miles, M. A. *The Trypanosomiases.* Wallingford, CABI Publishing.

Welburn, S. C., Maudlin, I. & Simarro, P. P. (2009) Controlling sleeping sickness: a review. *Parasitology,* 136**,** 1943 - 1949.

Welburn, S. C. & Odiit, M. (2002) Recent developments in human African trypanosomiasis. *Current Opinion in Infectious Diseases,* 15**,** 477-84.

Welburn, S. C., Picozzi, K., Fèvre, E., Coleman, P., Odiit, M., Carrington, M. & Maudlin, I. (2001b) Identification of human-infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum-resistance-associated (SRA) gene. *The Lancet,* 358**,** 2017-2019.

Wellde, B., Chumo, D., Adoyo, M., Kovatch, R., Mwongela, G. & Opiyo, E. (1983) Haemorrhagic syndrome in cattle associated with *Trypanosoma vivax* infection. *Tropical Animal Health and Production,* 15**,** 95-102.

Wellde, B., Lötzsch, R., Deindl, G., Sadun, E., Williams, J. & Warui, G. (1974) *Trypanosoma congolense*: I. Clinical observations of experimentally infected cattle. *Experimental Parasitology,* 36**,** 6-19.

Wellde, B., Reardon, M. J., Kovatch, R., Chumo, D., Williams, J., Boyce, W. L., Hockmeyer, W. T. & Wykoff, D. E. (1989) Experimental infection of cattle with *Trypanosoma brucei rhodesiense*. *Annals of Tropical Medicine and Parasitology,* 83**,** 133 - 150.

Wellde, B. T., Hockmeyer, W. T., Kovatch, R. M., Bhogal, M. S. & Diggs, C. L. (1981) *Trypanosoma congolense*: Natural and acquired resistance in the bovine. *Experimental Parasitology,* 52**,** 219-232.

Wendo, C. (2002) Uganda revises cattle treatment to protect humans from sleeping sickness. *The Lancet,* 359**,** 239.

WHO (2002) WHO programme to eliminate sleeping sickness: Building a Global Alliance. WHO Document Number: WHO/CDS/CSR/EPH/2002.13 Geneva,

WHO (2010) Human African trypanosomiasis: number of new cases drops to historically low level in 50 years [webpage] Available from: http://www.who.int/neglected_diseases/integrated_media/integrated_media_hat_june_2010/en/ Accessed: 08/05/2012

Willett, K. C. (1972) An observation on the unexpected frequency of some multiple infections. *Bulletin of the World Health Organization,* 47**,** 747 - 749.

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

Wooff, W. R. (1969) A review of current tsetse control in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 61**,** 125 - 126.

Woolhouse, M. E. J. (1998) Patterns in Parasite Epidemiology: The Peak Shift. *Parasitology Today,* 14**,** 428-434.

Woolhouse, M. E. J., Bealby, K., McNamara, J. J. & Silutongwe, J. (1994) Trypanosome infections of the tsetse fly *Glossina pallidipes* in the Luangwa Valley, Zambia. *International Journal for Parasitology*, 24, 987-993.

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

Appendix I – SOS phase one ranking tables

In each table, villages are ranked in order of prevalence. The five villages with the highest prevalence score 5 – 1 points, with the highest prevalence village at each time point scoring the most points. Each village gets one point for the number of appearances it makes in the top five. The total score for each village in each table is given in the total column. At the end the scores across all tables were added up, and those scores were used to decide the final ranking, which is shown in the final table here and also in chapter six.

Overall trypanosomiasis								
Rank	Baseline		Three months		Nine Months		Eighteen months	
	Village	Score	Village	Score	Village	Score	Village	Score
1	Omor	5	Olio	5	Oleumai	5	Aneralibi	5
2	Okwor	4	Olep	4	Amuda	4	Mwatma	4
3	Mwatma	3	Kalau	3	Olep	3	Olemai	3
4	Aneralibi	2	Oleumai	2	Awingyiipany	2	Amoru	2
5	Amoru	1	Kalaki	1	Kalau	1	Olio	1
6	Amuda	0	Amwoma	0	Abienek	0	Osirima	0
7	Kalau	0	Omor	0	Olio	0	Okwor	0
8			Aneralibi	0	Aneralibi	0	Kalau	0
9			Amuda	0	Amoru	0	Amuda	0
19			Awingyiipany	0	Osirima	0	Abienek	0
11			Osirima	0	Okwor	0	Olep	0
12			Okwor	0	Mwatma	0	Omor	0
13			Abienek	0	Amwoma	0	Amwoma	0
14			Mwatma	0	Omor	0	Awingyiipany	0

Village	No.of appearences	Rank score	Total
Abyenek	0	0	0
Amoru	2	2	4
Amuda	1	4	5
Amwoma	0	0	0
Aneralibi	2	7	9
Awingyiipany	1	2	3
Kalau	1	1	2
Mwatma	4	7	11
Okwor	1	4	5
Olemai	3	10	13
Olep	2	7	9
Olio	2	6	8
Omor	1	5	6
Osirima	0	0	0

T. brucei s. l.								
Rank	Baseline		Three months		Nine Months		Eighteen months	
	Village	Score	Village	Score	Village	Score	Village	Score
1	Okwor	5	Olemai	5	Olemai	5	Amoru	5
2	Aneralibi	4	Omor	4	Awingyiipany	4	Olemai	4
3	Amoru	3	Aneralibi	3	Olio	3	Olio	3
4	Kalau	2	Okwor	2	Olep	2	Okwor	2
5	Mwatma	1	Amwoma	1	Osirima	1	Osirima	1
6	Amuda	0	Awingyiipany	0	Aneralibi	0	Mwatma	0
7	Omor	0	Kalau	0	Abyenek	0	Abyenek	0
8			Amoru	0	Kalau	0	Omor	0
9			Abyenek	0	Okwor	0	Kalau	0
19			Osirima	0	Amoru	0	Olep	0
11			Olep	0	Amuda	0	Amuda	0
12			Olio	0	Amwoma	0	Amwoma	0
13			Mwatma	0	Omor	0	Awingyiipany	0
14			Amuda	0	Mwatma	0	Aneralibi	0

Village	No. Of appearences	Rank score	Total
Abyenek	0	0	**0**
Amoru	2	7	**9**
Amuda	0	0	**0**
Amwoma	1	1	**2**
Aneralibi	2	7	**9**
Awingyiipany	1	4	**5**
Kalau	1	2	**3**
Mwatma	1	1	**2**
Okwor	3	9	**12**
Olemai	3	14	**17**
Olep	1	2	**3**
Olio	2	6	**8**
Omor	1	4	**5**
Osirima	2	1	**3**

T. b. rhodesiense * = human case								
Rank	Baseline		Three months		Nine Months		Eighteen months	
	Village	Score	Village	Score	Village	Score	Village	Score
1	Kalau	5	Aneralibi	5	Okwor	5	Awingyiipany *	5
2	Amuda	0	Amuda	0	Amuda	0	Abyenek	4
3	Aneralibi	0	Amwoma	0	Amwoma	0	Okwor	3
4	Okwor	0	Okwor	0	Aneralibi	0	Olemai*	2
5	Amoru	0	Abyenek	0	Abyenek	0	Olio*	1
6	Mwatma	0	Awingyiipany	0	Awingyiipany	0	Amuda	0
7	Omor	0	Amoru	0	Amoru	0	Amwoma	0
8			Kalau	0	Kalau	0	Aneralibi	0
9			Mwatma	0	Mwatma	0	Amoru	0
19			Olemai	0	Olemai	0	Kalau	0
11			Olep	0	Olep	0	Mwatma	0
12			Olio	0	Olio	0	Olep	0
13			Omor	0	Omor	0	Omor	0
14			Osirima	0	Osirima	0	Osirima	0

Village	No. of appearences	Rank score	Total
Abyenek	1	4	**5**
Amoru	0	0	**0**
Amuda	0	0	**0**
Amwoma	0	0	**0**
Aneralibi	0	0	**0**
Awingyiipany	1	5	**6**
Kalau	0	0	**0**
Mwatma	0	0	**0**
Okwor	2	8	**10**
Olemai	1	2	**3**
Olep	0	0	**0**
Olio	1	1	**2**
Omor	0	0	**0**
Osirima	0	0	**0**

T. vivax								
Rank	Baseline		Three months		Nine Months		Eighteen months	
	Village	Score	Village	Score	Village	Score	Village	Score
1	Mwatma	5	Olio	5	Amuda	5	Mwatma	5
2	Amoru	4	Olep	4	Olep	4	Amoru	4
3	Omor	3	Amoru	3	Abyenek	3	Okwor	3
4	Aneralibi	2	Amwoma	2	Kalau	2	Olemai	2
5	Kalau	1	Osirima	1	Olemai	1	Osirima	1
6	Okwor	0	Olemai	0	Okwor	0	Amwoma	0
7	Amuda	0	Aneralibi	0	Mwatma	0	Kalau	0
8			Awingyiipany	0	Amoru	0	Aneralibi	0
9			Mwatma	0	Amwoma	0	Omor	0
19			Abyenek	0	Awingyiipany	0	Olep	0
11			Okwor	0	Olio	0	Abyenek	0
12			Kalau	0	Aneralibi	0	Amuda	0
13			Amuda	0	Osirima	0	Olio	0
14			Omor	0	Omor	0	Awingyiipany	0

Village	No. Of appearences	Rank score	Total
Abyenek	1	3	4
Amoru	3	11	14
Amuda	1	5	6
Amwoma	1	2	3
Aneralibi	1	2	3
Awingyiipany	0	0	0
Kalau	2	3	5
Mwatma	2	10	12
Okwor	1	3	4
Olemai	2	3	5
Olep	2	8	10
Olio	1	5	6
Omor	1	3	4
Osirima	2	2	4

T. congolense savannah								
Rank	Baseline		Three months		Nine Months		Eighteen months	
	Village	Score	Village	Score	Village	Score	Village	Score
1	Omor	5	Kalau	5	Amuda	5	Aneralibi	5
2	Mwatma	4	Olep	4	Kalau	4	Okwor	4
3	Amuda	3	Olio	3	Olep	3	Mwatma	3
4	Amoru	2	Amuda	2	Abyenek	2	Olemai	2
5	Okwor	1	Amoru	1	Amwoma	1	Amoru	1
6	Aneralibi	0	Amwoma	0	Amoru	0	Olep	0
7	Kalau	0	Abyenek	0	Okwor	0	Kalau	0
8			Awingyiipany	0	Aneralibi	0	Abyenek	0
9			Omor	0	Awingyiipany	0	Omor	0
19			Mwatma	0	Mwatma	0	Amuda	0
11			Aneralibi	0	Olemai	0	Amwoma	0
12			Okwor	0	Olio	0	Awingyiipany	0
13			Olemai	0	Omor	0	Olio	0
14			Osirima	0	Osirima	0	Osirima	0

Village	No. Of appearences	Rank score	Total
Abyenek	1	2	3
Amoru	3	4	7
Amuda	3	10	13
Amwoma	1	1	2
Aneralibi	1	5	6
Awingyiipany	0	0	0
Kalau	1	4	5
Mwatma	2	7	9
Okwor	2	5	7
Olemai	1	2	3
Olep	2	7	9
Olio	1	3	4
Omor	1	5	6
Osirima	0	0	0

Sum of all tables	
Abyenek	12
Amoru	34
Amuda	24
Amwoma	7
Aneralibi	27
Awingyiipany	14
Kalau	15
Mwatma	34
Okwor	38
Olemai	41
Olep	31
Olio	28
Omor	21
Osirima	7

Final ranking	
1	Olemai
2	Okwor
3	Amoru
4	Mwatma
5	Olep
6	Olio
7	Aneralibi
8	Amuda
9	Omor
19	Kalau
11	Awingyiipany
12	Abyenek
13	Amwoma
14	Osirima

Appendix II – Re-treatment ranking tables

In each table, villages are ranked in order of prevalence. The five villages with the highest prevalence score 5 – 1 points, with the highest prevalence village at each time point scoring the most points. Each village gets one point for the number of appearances it makes in the top five. The total score for each village in each table is given in the total column. At the end the scores across all tables were added up, and those scores were used to decide the final ranking, which is shown in the final table here and also in chapter six.

Overall trypanosomiasis				
	Baseline		6 months	
Rank	Village	Score	Village	Score
1	Kalere	5	Amuli	5
2	Chwagere	4	Olumai	4
3	Ilong	3	Abwa	3
4	Amuli	2	Otoro	2
5	Kaburuburu	1	Kaburuburu	1
6	Otage	0	Katingi	0
7	Otoro	0	Abat	0
8	Awidi	0	Kalere	0
9	Akany	0	Ochukai	0
10	Abat	0	Chwagere	0
11	Ochukai	0	Ilong	0
12	Olumai	0	Akany	0
13	Omua	0	Olabo	0
14	Katingi	0	Omua	0
15	Angwenga	0	Abongowoo	0
16	Agwingiri	0	Otage	0
17	Abwa	0	Angwenga	0
18	Abongowoo	0	Agwingiri	0
19	Aswii	0	Awidi	0
20	Olabo	0	Aswii	0

	No of appearences	Rank score	Total
Abat	0	0	0
Abongowoo	0	0	0
Abwa	1	3	4
Agwingiri	0	0	0
Akany	0	0	0
Amuli	2	7	9
Angwenga	0	0	0
Aswii	0	0	0
Awidi	0	0	0
Chwagere	1	4	5
Ilong	1	3	4
Kaburuburu	2	2	4
Kalere	1	5	6
Katingi	0	0	0
Ochukai	0	0	0
Olabo	0	0	0
Olumai	1	4	5
Omua	0	0	0
Otage	0	0	0
Otoro	1	2	3

T. brucei s. l.				
	Baseline		6 months	
Rank	Village	Score	Village	Score
1	Kalere	5	Amuli	5
2	Kaburuburu	4	Kalere	4
3	Chwagere	3	Otoro	3
4	Akany	2	Kaburuburu	2
5	Ilong	1	Akany	1
6	Katingi	0	Abat	0
7	Omua	0	Abwa	0
8	Ochukai	0	Katingi	0
9	Abat	0	Abongowoo	0
10	Otage	0	Olumai	0
11	Olumai	0	Angwenga	0
12	Agwingiri	0	Omua	0
13	Otoro	0	Chwagere	0
14	Angwenga	0	Ilong	0
15	Abongowoo	0	Ochukai	0
16	Amuli	0	Otage	0
17	Abwa	0	Awidi	0
18	Awidi	0	Agwingiri	0
19	Olabo	0	Olabo	0
20	Aswii	0	Aswii	0

	No of appearences	**Rank score**	**Total**
Abat	0	0	**0**
Abongowoo	0	0	**0**
Abwa	0	0	**0**
Agwingiri	0	0	**0**
Akany	2	3	**5**
Amuli	1	5	**6**
Angwenga	0	0	**0**
Aswii	0	0	**0**
Awidi	0	0	**0**
Chwagere	1	3	**4**
Ilong	1	1	**2**
Kaburuburu	2	6	**8**
Kalere	2	9	**11**
Katingi	0	0	**0**
Ochukai	0	0	**0**
Olabo	0	0	**0**
Olumai	0	0	**0**
Omua	0	0	**0**
Otage	0	0	**0**
Otoro	1	3	**4**

T. b. rhodesiense				
	Baseline		6 months	
Rank	Village	Score	Village	Score
1	Kaburuburu	5	Otoro	5
2	Amuli	4	Kalere	4
3	Abat	3	Olumai	3
4	Abongowoo	2	Omua	2
5	Ilong	1	Angwenya	1
6	Omua	0	Ilong	0
7	Angwenga	0	Kaburuburu	0
8	Akany	0	Abat	0
9	Kalere	0	Abongowat	0
10	Otage	0	Abwa	0
11	Aswii	0	Akany	0
12	Agwingiri	0	Amuli	0
13	Abwa	0	Awidi	0
14	Awidi	0	Chwagere	0
15	Olumai	0	Agwingiri	0
16	Chwagere	0	Aswii	0
17	Otoro	0	Ochukai	0
18	Ochukai	0	Olabo	0
19	Olabo	0	Katingi	0
20	Katingi	0	Otage	0

	No of appearences	**Rank score**	**Total**
Abat	1	3	**4**
Abongowoo	1	2	**3**
Abwa	0	0	**0**
Agwingiri	0	0	**0**
Akany	0	0	**0**
Amuli	1	4	**5**
Angwenga	1	1	**2**
Aswii	0	0	**0**
Awidi	0	0	**0**
Chwagere	0	0	**0**
Ilong	1	1	**2**
Kaburuburu	1	5	**6**
Kalere	1	4	**5**
Katingi	0	0	**0**
Ochukai	0	0	**0**
Olabo	0	0	**0**
Olumai	1	3	**4**
Omua	1	2	**3**
Otage	0	0	**0**
Otoro	0	0	**0**

T. vivax				
	Baseline		6 months	
Rank	Village	Score	Village	Score
1	Kalere	5	Abat	5
2	Abwa	4	Kalere	0
3	Otage	3	Abwa	0
4	Amuli	2	Otage	0
5	Abat	1	Amuli	0
6	Aswii	0	Aswii	0
7	Ilong	0	Ilong	0
8	Olumai	0	Olumai	0
9	Abongowoo	0	Abongowoo	0
10	Agwingiri	0	Agwingiri	0
11	Otoro	0	Otoro	0
12	Akany	0	Akany	0
13	Ochukai	0	Ochukai	0
14	Omua	0	Omua	0
15	Olabo	0	Olabo	0
16	Chwagere	0	Chwagere	0
17	Katingi	0	Katingi	0
18	Awidi	0	Awidi	0
19	Kaburuburu	0	Kaburuburu	0
20	Angwenga	0	Angwenga	0

	No of appearences	**Rank score**	**Total**
Abat	2	6	**8**
Abongowoo	0	0	**0**
Abwa	1	4	**5**
Agwingiri	0	0	**0**
Akany	0	0	**0**
Amuli	1	2	**3**
Angwenga	0	0	**0**
Aswii	0	0	**0**
Awidi	0	0	**0**
Chwagere	0	0	**0**
Ilong	0	0	**0**
Kaburuburu	0	0	**0**
Kalere	1	5	**6**
Katingi	0	0	**0**
Ochukai	0	0	**0**
Olabo	0	0	**0**
Olumai	0	0	**0**
Omua	0	0	**0**
Otage	1	3	**4**
Otoro	0	0	**0**

T. congolense savannah				
	Baseline		6 months	
Rank	Village	Score	Village	Score
1	Amuli	5	Akany	5
2	Otoro	4	Olabo	4
3	Ilong	3	Olumai	3
4	Chwagere	2	Ilong	2
5	Awidi	1	Ochukai	1
6	Olumai	0	Chwagere	0
7	Otage	0	Katingi	0
8	Kalere	0	Abwa	0
9	Angwenga	0	Kaburuburu	0
10	Abongowoo	0	Agwingiri	0
11	Katingi	0	Otage	0
12	Ochukai	0	Otoro	0
13	Olabo	0	Amuli	0
14	Omua	0	Angwenga	0
15	Akany	0	Abongowoo	0
16	Aswii	0	Abat	0
17	Agwingiri	0	Omua	0
18	Kaburuburu	0	Kalere	0
19	Abat	0	Awidi	0
20	Abwa	0	Aswii	0

	No of appearences	**Rank score**	**Total**
Abat	0	0	**0**
Abongowoo	0	0	**0**
Abwa	0	0	**0**
Agwingiri	0	0	**0**
Akany	1	5	**6**
Amuli	1	5	**6**
Angwenga	0	0	**0**
Aswii	0	0	**0**
Awidi	1	1	**2**
Chwagere	1	2	**3**
Ilong	2	5	**7**
Kaburuburu	0	0	**0**
Kalere	0	0	**0**
Katingi	0	0	**0**
Ochukai	1	1	**2**
Olabo	1	4	**5**
Olumai	1	3	**4**
Omua	0	0	**0**
Otage	0	0	**0**
Otoro	1	4	**5**

Sum of all totals	
Abat	12
Abongowoo	3
Abwa	9
Agwingiri	0
Akany	11
Amuli	29
Angwenga	2
Aswii	0
Awidi	2
Chwagere	12
Ilong	15
Kaburuburu	18
Kalere	28
Katingi	0
Ochukai	2
Olabo	5
Olumai	13
Omua	3
Otage	4
Otoro	12

Final rank	
1	Amuli
2	Kalere
3	Kaburuburu
4	Ilong
5	Olumai
6	Abat
7	Chwagere
8	Otoro
9	Akany
10	Abwa
11	Olabo
12	Otage
13	Abongowoo
14	Omua
15	Angwenga
16	Awidi
17	Ochukai
18	Agwingiri
19	Aswii
20	Katingi