

# **EVALUATION OF A NOVEL METHOD FOR CONTROLLING BOVINE TRYPANOSOMIASIS**

A LONGITUDINAL STUDY IN SOUTH-EAST UGANDA

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## DECLARATION

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

Andrew C. Brownlow

Edinburgh, 2007

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## Abstract

The problem of controlling tsetse flies in Africa is an old one. The tsetse fly transmits the trypanosome parasites which cause sleeping sickness in humans and disease in cattle. Because cattle are a favoured food source for tsetse much work has been done looking at the use of insecticide treated cattle as a control strategy for the tsetse fly. Such treatment methods possess many advantages; they are safe and relatively environmentally benign, they can be applied by individual farmers without the need for logistically demanding and costly traditional control programmes and, in addition to tsetse flies the insecticides are effective against a wide range of other harmful cattle parasites. The cost of the insecticide is however a significant constraint to the number of livestock keepers who can afford to employ the technique and as a result many cattle remain untreated. Following the discovery that tsetse had a significant predilection for feeding on the legs and belly of cattle, it was hypothesised that restricting the insecticide to only those areas could offer comparable protection to treating the whole animal. Such an approach would use up to 80% less drug and thus make the treatment per animal much cheaper. In addition, preferentially targeting areas favoured by tsetse, and leaving the rest of the animal untreated, preserves some important ecological balances between cattle and their parasites which traditional treatment methods destabilise.

This thesis describes the design, implementation and analysis of a longitudinal study run over 8 months in south east Uganda that sought to compare the effect of applying insecticide to cattle only on the regions favoured by tsetse flies. Cattle were recruited to the study and assigned one of four treatment groups; a whole body application of deltamethrin insecticide pour-on; a restricted application of deltamethrin spray, applied to the front legs, ears and belly; a prophylactic trypanocide injection of isometamidium chloride, and a control group, that received no further treatments. All animals in the study were however cleared using twin doses of a trypanocide diminazene aceturate at the start of the study.



Animals were blood sampled every 28 days for the duration of the study, clinically examined and tested for anaemia using a portable haemoglobinometer. Samples were subsequently screened in the UK using a molecular diagnostic technique (ITS-PCR).

In terms of incident trypanosome infections as measured by ITS-PCR, there was no significant difference between the restricted application spray method and whole-body treatment using pour on, with both conferring significant protection against reinfection with trypanosomes compared with the control group. In terms of more general indicators of animal health, such as anaemia, only the pour on group showed a significant improvement in haemoglobin values over time. There were indications that the prophylactic use of isometamidium was in some cases detrimental to the health of the animals and reasons for this are explored.

Finally, in terms of clinical signs, the pour-on group showed significant improvements in terms of tick burden and condition score during the study. Beneficial effects were also apparent but less pronounced in the restricted spray group. The study concluded that the restricted application had the potential to be of use in controlling trypanosomiasis but required a shortened treatment interval. The restricted spray technique was estimated to be approximately one-fifth of the cost of whole-body spray treatment and one-eighteenth the cost of using pour-on insecticide. The long term sustainability of trypanosomiasis control using this method in the current framework of veterinary service provision in Africa is discussed.

*“Either there is no tsetse fly anywhere in the Ugandan Protectorate, or it is not able to introduce into the bodies of domestic animals the malarial germs which cause tsetse fever, therefore theoretically there is no part of the Protectorate in which cattle, sheep goats and horses cannot be productively kept.”*

Sir Harry Johnston, Special Commissioner to the Ugandan Protectorate, 1899-1902; in *The Ugandan Protectorate Vol 1* (1902). Page 288

*“It is the unexpected which always happens in Africa”*

Sir Harry Johnston, Special Commissioner to the Ugandan Protectorate, 1899-1902; in *The Ugandan Protectorate Vol 1* (1902) Page 302

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## List of Abbreviations

AHO	Animal Husbandry Officer
AIC	Akaike Information Criteria
BCT	Buffy Coat Technique
CATT	Card Agglutination Test for Trypanosomiasis
CI	Confidence Interval
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
CTVM	Centre for Tropical Veterinary Medicine, Edinburgh University
DDT	Dichloro-Diphenyl-Trichloroethane insecticide
DF	Degrees of Freedom
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleoside triphosphates
DVO	District Veterinary Officer
ECF	East Coast Fever
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FITCA	Farming in Tsetse Controlled Areas
GDP	Gross Domestic Product
GIS	Geographical Information System (s)
GLM	Generalised linear models
GLMM	Generalised linear mixed effect model
HCT	Haemocrit Centrifugation Technique
ITS-PCR	Internal Transcribed Spacers PCR
LIRI	Ugandan Livestock Research Institute
LME	Linear mixed effect models
LRA	Lords Resistance Army
ML	Maximum likelihood
NGO	Non-Governmental Organisation
Obs	Observed
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PCR	Polymerase Chain Reaction
PCV	Packed cell volume
PQL	Penalised-quasi likelihoods
REML	Restricted Maximum likelihood
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RSP	Restricted Spray Protocol
SAT	Sequential Aerosol technique
SIT	Sterile insect technique
SOS	Stamp Out Sleeping Sickness
SRA	Serum resistance-associated protein
TBD	Tick-borne diseases
TBE	Tris Borate EDTA
TE	Tris EDTA
Th	Theoretical
USD	United States Dollars

UV	Ultraviolet
VAT	Variable Antigen Type
VO	Veterinary Officer
VSG	Variable Surface Glycoprotein
WEC	Worm Egg Count
WHO	World Health Organisation

The following abbreviations are commonly used throughout diagrams and charts in this thesis and refer to the intervention groupings of the study:

Pour-on	Use of a concentrated formulation of insecticide applied to the animal in the hair along the dorsum
Spray	Use of a restricted application protocol of normal concentration aqueous deltamethrin applied to defined areas of the animal
Control	No further treatments given during the intervention period
Iso	Single dose isometamidium at 1mg/kg administered at day 0

### Overview

Epidemic diseases can be dramatic, but the insidious attrition caused by endemic diseases is more of an economic burden (Tisdell *et al.*, 1999). Seventy percent of the rural poor in Africa depend on livestock (LID, 1999) and cattle play a fundamental role in the provision of a route out of poverty. Cattle are a source of food, manure, draught power for arable agriculture, as well as functioning as an investment and credit source, to be sold when sudden expenses arise. Furthermore, they fill numerous social functions and are a display of status (Moll, 2005).

Parasitic diseases represent many of the important endemic diseases that afflict African livestock, of which the most economically damaging are those vectored by ticks and tsetse flies (McLeod & Kristjanson, 1999; Swallow, 2000). Ticks transmit a number of blood parasites whose impacts range from debilitating to rapidly fatal. Tsetse flies vector trypanosomiasis, a blood borne parasite that causes severe production losses in indigenous cattle breeds and high mortalities in introduced potentially more productive, breeds. In addition trypanosomes cause sleeping sickness in humans, an invariably fatal disease without treatment and currently afflicts between 30,000 and 300,000 people per year (Barrett *et al.*, 2003a). The uncertainty in the above estimate is due to issues with unreporting of the disease (Odiit *et al.*, 2005) (World Health Organization, 1998).

Control of trypanosomiasis dominated the imperial colonial agenda, at one time taking a quarter of colonial research spending (Rogers & Randolph, 2002) and for many years focused on the control of the tsetse vector. With the decline of government services in the 1970's and 1980's, the disease increasingly became managed by individual farmers as a private good. Although drug control has been historically effective, the compounds have been in use for over 40 years, resistance is manifest and there are no replacement compounds on the horizon. Consequently, emphasis has shifted to look for alternative methods of control, and one such option has been the use of insecticides on cattle. In addition to providing tsetse control, insecticides offer control of ticks, and although this is usually beneficial, it has the potential to cause problems due to differing host-parasite ecologies of trypanosomiasis and tick-borne diseases. Moreover, conventional insecticide

use remains too costly for the majority of rural livestock keepers to adopt. Following on from the discovery that tsetse have a predilection for feeding mainly on the legs and belly of the cattle (Vale *et al.*, 1999), it was hypothesised that restricting the application of insecticide to just these sites could reduce the costs of insecticide whilst still providing adequate protection from disease. This thesis describes the background to, design, implementation and subsequent analysis of a longitudinal study conducted in south-east Uganda, the aim being to compare this novel approach of restricted insecticide use with conventional treatments currently available to livestock keepers.

## **Chapter 1 :- Introduction**

## 1.1 Livestock and poverty

Sub-Saharan Africa is home to some of the world's most impoverished people. There are an estimated 790 million people globally who do not have adequate access to food, of which 25 % reside in sub-Saharan Africa (Pretty *et al.*, 2003). Put another way, over 70% of the rural population of Africa live with food insecurity (Rosegrant, 2005). It is sadly ironic that smallholder farmers, the producers of over 90 % of the continent's food supply, make up half of this population. The rest of the food insecure population consists of the landless poor in rural areas (30 %) and the urban poor. For comparison, throughout the developing world agriculture accounts for around 9 % of the gross domestic product (GDP) and more than half of total employment. In countries where over a third of the population is considered undernourished, agriculture represents 30 % of GDP and nearly 70 % of the population relies on agriculture for their livelihood (Pinstrup-Andersen, 2002).

Current development paradigms admit the links between disease, poverty and hunger hence eradication of extreme poverty occupies the top position in the United Nation's Millennium Development Goals (United Nations, 2006). The raising of national levels of food production to meet increasing domestic demand and the concomitant reduction in dependency of foreign imports of food is a primary objective.(World Bank, 2005) Key to this expansion is better use of animal traction, and the main constraint on this goal is tsetse-transmitted trypanosomiasis.

### 1.1.1 Current state of veterinary provision

The veterinary service industry in the developing world is in a state of flux. Although largely decimated by the structural reform programmes of the last 15 years (Umali *et al.*, 1994), it is about to be met with some very interesting challenges. While the international donor community is increasingly reticent about funding large scale, top-down veterinary intervention projects, the current emphasis on poverty reduction places livestock in a key role (Commission for Africa, 2005). On a global stage, population growth, urbanisation and an increasing amount of disposable income are rapidly expanding the market for

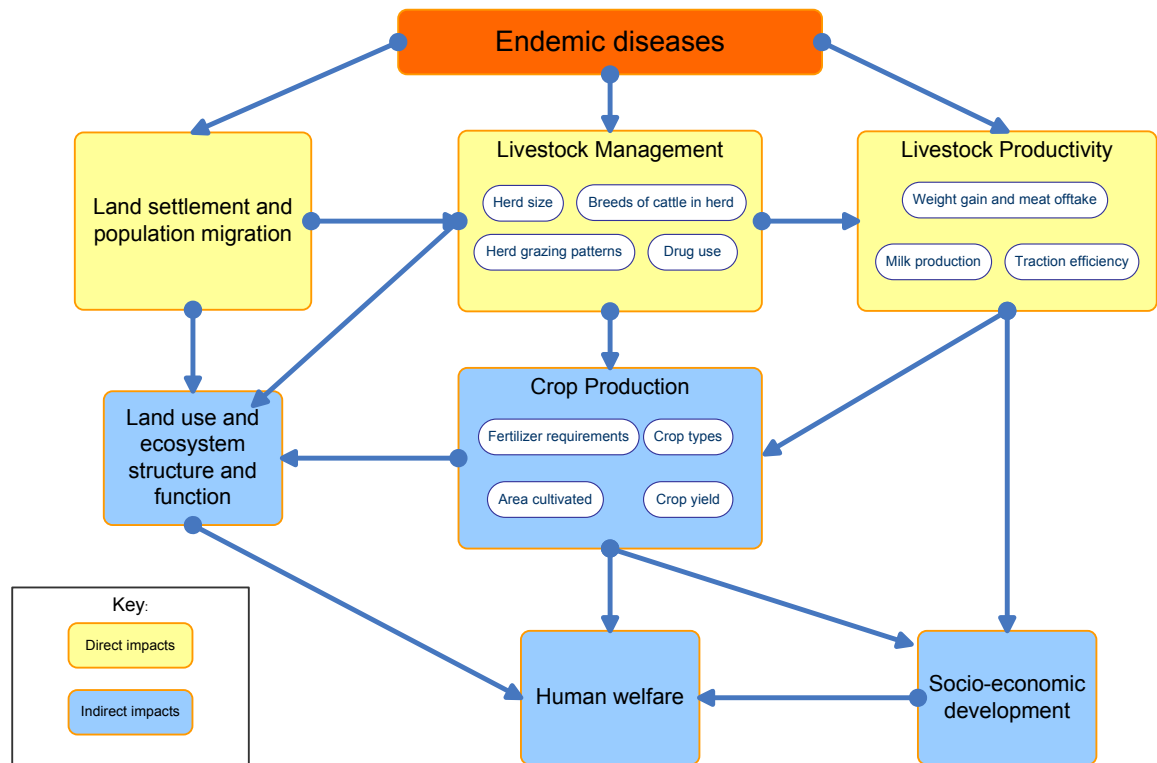


livestock products (Scoones & Wolmer, 2006), with climate change, land tenure and environmental degradation promising to starkly alter current agricultural zones. Finally, expanding globalisation of trade and travel is increasing the risk of zoonotic diseases and threatening food security (de Haan, 2004; Turner, 2005).

## 1.2 Trypanosomiasis

African animal trypanosomiasis places a severe constraint on agricultural production in Africa as it prevents the use of livestock in some of the continent's potentially most fertile areas (WHO, 1998). In high challenge areas, or in trypanosusceptible livestock, the disease can be rapidly fatal, and animals living with even moderate risks of trypanosomiasis are less productive, suffer from lower milk yields, slower weight gain, lower calving rates and elevated rates of calf mortality (Swallow, 2000). Livestock owners are faced with the necessity to control the impact of the disease, either through treatment of their animals with curative or prophylactic trypanocidal drugs, or through vector control.

Any assessment of the influence of trypanosomiasis on African livelihoods is often confounded by other concurrent production constraints, for example livestock health problems e.g. tick borne diseases, or social factors, e.g. civil insecurity. Various analyses that draw the distinction between direct and indirect impacts and their interactions are highlighted in Figure 1-1



**Figure 1-1:-Framework of the impacts of endemic cattle diseases. Based on Swallow (2000)**

Trypanosomiasis has a direct impact on the average number of livestock kept by farmers. Using geographical information systems to spatially link cattle density data with economic model data, Kristjanson et al. showed that cattle density is highest in tsetse free areas in all agro-ecological zones, except those classified as arid. (In arid areas tsetse distribution is solely along water courses where cattle and human population also cluster). A summary of the data for East Africa is shown in Figure 1-2. This model demonstrated that the costs of trypanosomiasis alone are immense. Tsetse-free areas produce 83% more milk and 97% more meat than the equivalent infested areas. This alone translates to a potential economic surplus of US\$700 million should trypanosomiasis control be possible. The model estimated the costs of trypanosomiasis from the associated reduction in meat and milk production compared with a simulated, tsetse free herd. The estimated annual cost of trypanosomiasis in terms of foregone meat and milk is around US\$1.3 billion per year (Kristensson & Bentivoglio, 1999).

Whilst this is possibly an over-simplistic way of quantifying the cost of trypanosomiasis, it perhaps serves to give some indication of the magnitude of the problem.

Region and agro-ecological zone	Total number of cattle (million)	Number of cattle in tsetse infected areas (million)	Percent of total	Tsetse infected area Cattle/km <sup>2</sup>	Tsetse-free area Cattle/km <sup>2</sup>
Eastern Africa					
Arid	15.5	1.5	10%	13.7	5.4
Semi-arid	17.9	5.09	28%	23.7	16.9
Subhumid	10.2	6.19	61%	9.9	13.7
Humid	0.9	0.59	66%	7.9	8.6
Highlands	31.7	7.96	25%	21.5	34.5
Total: East Africa	76.2	21.32	28%	15.3	15.8
Total: All sub-Saharan Africa	<b>149.8</b>	<b>47.75</b>	<b>32%</b>	<b>5.3</b>	<b>7.2</b>

**Figure 1-2:-Number and density of cattle in tsetse areas of east Africa by region and agro-ecological zone. Adapted from Kristjanson et al. (1999).**

Aside from the direct effects of trypanosomiasis on reproductive efficiency and herd mortality, the disease requires that farmers structure and manage their herds to ameliorate its potentially devastating effects. Breeds of animal well suited to a particular function, e.g. traction or dairying, can be so susceptible to trypanosomiasis that, even when they are under prophylaxis treatments, they are non-viable in some areas (Marples, 1967). This has particular impact in mixed crop/livestock agricultural systems where livestock provide a form of transport, manure fertilizers and traction. A study in the Ghibe valley of Ethiopia showed oxen in areas with a high risk of trypanosomiasis were 38% less efficient providers of traction than oxen in low-risk areas (Swallow, 1998). Animal traction has been shown to be instrumental in increasing the agricultural productivity of smallholder farmers (Savadogo *et al.*, 1998). With economic policy placing agricultural productivity

key to raising the income of Africa's rural poor, (Thirtle *et al.*, 2003; Bahiigwa *et al.*, 2005) the requirement for cost-effective solutions to the constraints of livestock production increases.

### 1.2.1 Outline of trypanosome ecology, biology and distribution

The trypanosomiasis are a group of human and animal diseases caused by a unicellular flagellate protozoan parasite of the genus *Trypanosoma*. Trypanosomes are obligate parasites and the group infects most vertebrate genera, including man. In sub-Saharan Africa two subspecies of *Trypanosoma brucei* cause human African trypanosomiasis, or sleeping sickness, and in the Americas *Trypanosoma cruzi* causes Chagas' disease. These parasites are collectively responsible for profound human misery, yet remain probably one of the most neglected human diseases of our history (Coleman, 2002; Molyneux, 2004). The African trypanosomiasis are cyclically transmitted by the tsetse fly. Following a bite from an infected fly, the parasites proliferate in the circulation, evading the host's immune response by continuously changing their antigenic coat of variant surface glycoproteins. This enables a trypanosome population to continually evade removal by the immune system whilst simultaneously immunosuppressing and destabilizing the immune system by the release of other inflammatory mediators (Cook, 1996). Pathology stems from these trypanosome-induced derangements of the host immune system. Additionally, *Trypanosoma brucei* species can leave the circulation and invade tissues or enter the cerebrospinal fluid (CSF). In humans, the presence of parasites in the CSF and the concomitant meningitis lead to the somnolent state that gives the disease its common name of sleeping sickness (Stich *et al.*, 2002).

Trypanosomiasis infections in livestock are important for two reasons. Firstly, some trypanosome species are pathogenic to livestock in their own right and secondly *T. brucei* infections in livestock are important in the epidemiology of human sleeping sickness (Hide *et al.*, 1996; Fevre, 2002; Waiswa *et al.*, 2003). In south east Uganda, livestock serve as asymptomatic reservoirs for the *T. brucei rhodesiense* zoonosis that causes human sleeping sickness and thus pose a risk to the human population. As such, management of the cattle reservoir must be included in any attempt to control *T. b. rhodesiense* sleeping sickness (Welburn *et al.*, 2001a).

Trypanosome classification		Human	Cattle	Goats	Sheep	Pigs	Horses	Donkeys
Trypanozoon	<i>T.b.brucei</i>	-	+	++	++	+	+++	++
	<i>T.b.brucei</i>	<i>T.b.rhodesiense</i>	+++	- <sup>1</sup>	-	-	- <sup>2</sup>	-
	<i>T.b.gambiense</i>	+++	-	-	-	-	-	-
	<i>T.evansi</i> <sup>3</sup>	-	++	+	+	++	+++	++
	<i>T.equiperdum</i> <sup>4</sup>	-	-	-	-	-	+++	++
Nannomonas	<i>T.congolense</i>	-	+++	++	++	+	++	++
	<i>T.simiae</i>	-	-	+	+	+++	-	-
Duttonella	<i>T.vivax</i>	-	+++	++	++	-	++	+
Pycnomonas	<i>T.suis</i>	-	-	-	-	++	-	-

**Table 1-1: Pathogenicity of clinically important trypanosomes. - Not pathogenic, + mild, ++, moderate, +++ severely pathogenic. Adapted from (Connor, 1992)**

### 1.2.2 Animal trypanosomiasis

Nagana, or African bovine trypanosomiasis, is considered to be the most important constraint on livestock production in Africa (Budd, 1999). Cattle can be infected with *T. vivax*, *T. congolense* and *T. brucei.*, with the most important pathogenic effects confined to bloodstream infections with *T. vivax* and *T. congolense* (Uilenberg, 1998). *T. congolense* is divided into subtypes dependent on geographical distribution and pathogenicity and is considered the most significant cause of bovine trypanosomiasis in East Africa (Stephen, 1986). In West Africa, *T. vivax* infection in cattle is often an acute form with high mortality. In contrast, with the exception of a sporadic hemorrhagic syndrome, East African strains of *T.vivax* tend to produce mild infections which are self-limiting in healthy animals (Gardiner, 1989).

<sup>1</sup> Significant zoonotic reservoir

<sup>2</sup> Significant zoonotic reservoir

<sup>3</sup> Mechanical transmission

<sup>4</sup> Venereal transmission

Trypanosomiasis clinically manifests as a wasting disease with progressive loss of condition, increasing anaemia and weakness to the point of extreme emaciation, collapse and death (Uilenberg, 1998). *T. brucei* infections usually cause a mild or asymptomatic disease with a prolonged parasitaemia but with low morbidity, although cases showing central nervous system involvement infections of *T. brucei* in Zebu cattle (Wellde *et al.*, 1989a; Wellde *et al.*, 1989b), and Ankole breeds (Clausen *et al.*, 1999) have been recorded.

### 1.2.3 Varying susceptibility to trypanosomiasis and trypanotolerance

Trypanosomes can circulate in a variety of domestic and wildlife hosts; in the latter case co-evolution has arguably allowed these infections to be tolerated with little pathology. The existence of many alternative hosts, often living in close contact with each other, makes control of the disease problematic and elimination potentially impossible. An individual ox will exhibit different susceptibilities to trypanosomiasis dependent on age, previous exposure and immune status. However, the most important factor when assessing susceptibility is breed. *Bos taurus* African cattle breeds were likely domesticated from northern African herds of the, now extinct, wild ox, or aurochs, (*Bos primigenius*) (Bradley *et al.*, 1996). These cattle have shared the continent with trypanosomes for at least 10,000 years. In contrast, the *Bos indicus* cattle, the Zebu, have spread westward across Africa from a population of cattle which evolved independently to *Bos taurus* in south Asia, and have probably only existed in Africa since the Arab invasions post 670 A.D. (Bradley *et al.*, 1998). West African *Bos taurus* breeds, for example N'Dama, exhibit a degree of trypanotolerance allowing them to be productive under a disease challenge destructive for *Bos indicus* cattle (Kemp & Teale, 1998). Trypanotolerant animals have been shown to be more competent at limiting parasitaemias and controlling the anaemia associated with trypanosomiasis (Dwinger *et al.*, 1992), rather than demonstrating an acquired immunity. Of the two, anaemia control is the most important for controlling pathology (Naessens, 2006). However, in SE Uganda, some Nkedi Zebu performed better than Ankole cattle when exposed to the same challenge, indicating a degree of acquired resistance may be evident even in the 'trypanosusceptible' Zebu breeds (Magona *et al.*, 2004c). Conversely, although N'Dama cattle may be more

productive than Zebu in areas of high challenge (Itty, 1996), they can still succumb to the disease if under stress (Mattioli *et al.*, 1999).

### 1.2.4 Trypanosomiasis as a production disease

Various studies based on longitudinal monitoring, case-control or pre- and post-trypanocidal intervention methodologies have investigated the impact of trypanosomiasis on animal productivity. Trypanosomiasis infection most consistently impacts on birth rates and mortality of young stock. Calving rates were shown to be reduced by 1-12% in tolerant cattle (Trail *et al.*, 1990b) and between 11-20% in susceptible breeds (Fox *et al.*, 1993). Similar percentages were seen in the increase in calf mortality, with a 0-10% increase in tolerant breeds of cattle and 10-20% in susceptible breeds (Fall *et al.*, 1999; Ganaba *et al.*, 2002). From a clinical perspective, most studies show infection with pathogenic trypanosomes results in an increase in anaemia, a decrease in weight gain and a lowered immunity to concurrent infections (Agyemang *et al.*, 1993; Dwinger *et al.*, 1994; Mattioli *et al.*, 1999). It is worth highlighting that even in controlled field trials, the presence of concurrent infections, seasonal variation in management practices, and a multitude of other confounders make precise and unbiased quantification of the biological effect of trypanosomiasis difficult (Rowlands *et al.*, 1996). However, stress, due to concurrent disease, malnutrition or dehydration, work, pregnancy or lactation are likely to precipitate and exacerbate any pathology (Holmes *et al.*, 2000).

### 1.2.5 Trypanosomiasis in humans

Human African trypanosomiasis is caused by two subspecies of *Trypanosoma brucei*. Both manifest with different clinical presentations, epidemiology and geographical location. Infection with either subspecies is uniformly fatal if untreated (Barrett *et al.*, 2003a). In West and Central Africa, *T. brucei gambiense* causes a chronic form of the disease, and transmission occurs directly from person to person via the tsetse vector. In east and southern Africa however, *T. brucei rhodesiense* is a zoonosis, causing an acute form of the disease and involves an animal reservoir. Both infections are classified as either early (stage 1) or late (stage 2) dependent on whether parasites have passed into the CNS and become manifest in the cerebrospinal fluid.

### 1.2.5.1 *T. brucei rhodesiense*: Eastern and Southern African Sleeping Sickness

*T. b. rhodesiense* sleeping sickness is characterised by a rapid progression to severe disease, and over 80% of deaths occur within 6 months of the onset of illness (Welburn & Odiit, 2002). The first symptoms can be seen at the site of the tsetse fly bite. Around five days after inoculation, the proliferating parasites cause a localised skin reaction termed a chancre. Parasites then spread to the draining lymph node, causing a regional lymphadenopathy, and on to the bloodstream. The haemolymphatic stage of the disease is characterised by general malaise, headache and an undulating fever following the ‘waves’ of parasite multiplication in the blood. At this stage parasites are present in blood, lymph and tissue but are often below levels of detection (Barrett *et al.*, 2003a). The second or late stage of the disease involves the parasites invading the internal organs such as the CNS and can occur within a few weeks of infection. Headaches become severe and, with progressive CNS involvement, mental impairment, sleep dysfunction and ataxia begin to manifest. This culminates in coma and death in untreated cases (Stich *et al.*, 2002).

### 1.2.5.2 *T. brucei gambiense*: West and Central African Sleeping Sickness

*T. b. gambiense* sleeping sickness is often described as a chronic infection as the disease progresses over a matter of months and years. Patients can remain asymptomatic or with only mild clinical signs for long periods before progressing to severe disease. As a result, early symptoms in *T. b. gambiense* infection are usually mild and often unnoticed. A generalised lymphadenopathy with swellings in the posterior triangle of the neck, known as Winterbottom’s sign, is a typical indication of early stage *T. b. gambiense* infection. Other non-specific symptoms are rash and hepatosplenomegaly. However, after a few months the parasites invade the CNS and the disease progresses to the late stage with the same sequelae as *T. b. rhodesiense* infections, with chronic encephalopathy and death if untreated (Cook, 1996).

## 1.2.6 Sick people? Treat the cows

Unlike *T. b. gambiense* which is directly vectored between humans, *T. b. rhodesiense* transmission involves an animal reservoir. In SE Uganda the most epidemiologically



significant species in this cycle are cattle (Maudlin *et al.*, 1990). In recent years, outbreaks of sleeping sickness occurring north of their traditional foci have been demonstrated to be caused by the movement of infected cattle from regions endemic for *T. b. rhodesiense* (Fèvre *et al.*, 2001; Welburn *et al.*, 2001b; Fèvre *et al.*, 2005). In response to this, government policy is to treat all translocated cattle at point of sale with a trypanocidal drug (Wendo, 2002; Hutchinson *et al.*, 2003). However, the implementation of this strategy is probably not yet achieving coverage necessary to reduce transmission (Fèvre *et al.*, 2006).

### 1.2.7 Diagnosis of trypanosomiasis

The most basic way to detect trypanosome infection is by direct observation of the blood under a microscope, using medium magnification (usually a dry objective of 40x and eye pieces of 5-10x) (Uilenberg, 1998). Trypanosomes are either seen directly moving between the blood cells or indirectly by the movement of the blood cells around them. Alternately, preparation of thick and thin blood smears allows the samples to be collected and stored. Examined under a microscope using oil immersion and 100x objective, this method allows the identification of different species by morphology (Uilenberg, 1998). However, *T. b. brucei*, *T. b. rhodesiense* and *T. b. gambiense* are morphologically identical so cannot be visually distinguished. The above techniques are straightforward but, due to the trypanosomes ability to alter their antigenic coat, the concentration of trypanosomes in the blood can fluctuate below detection levels resulting in a low sensitivity of the microscopic techniques (Paris *et al.*, 1982; Stich *et al.*, 2002).

#### 1.2.7.1 Concentration methods

Trypanosomes in a blood sample can be concentrated to give increased sensitivity. Trypanosomes have a specific gravity between that of erythrocytes and white blood cells. By either allowing a whole blood sample to settle and clot or by centrifugation the parasites tend to concentrate on the border between the two layers. This border is called the buffy coat, and removal and analysis of this layer under phase contrast increases the probability of detection. Methods of applying these principles are detailed in (Uilenberg, 1998), and form the basis of the haematocrit centrifugation technique (HCT) (Bennett, 1962; Woo, 1970) and buffy coat technique (BCT) (Murray *et al.*, 1977).

### 1.2.7.2 Card agglutination test for trypanosomiasis (CATT)

This method can be used for the diagnosis of *T. b. gambiense* trypanosomiasis in humans (Magnus *et al.*, 1978). This is currently the only serological test that is used in control programmes (Pépin & Méda, 2001). It relies on the detection of host antibodies using a variable antigen type (VAT) from the trypanosome. This test can be done very simply and cheaply, with good sensitivity and specificity (Chappuis, 2002). However, there is no similar test for *T. b. rhodesiense* or any of the pathogenic animal trypanosomes.

### 1.2.7.3 Molecular diagnosis of trypanosomiasis

Molecular assays which demonstrate the presence of parasite DNA in the host are very sensitive, and specific tests exist for trypanosomiasis. A positive result will indicate an active infection in the host at the time of sampling, as the DNA does not survive in the host long after the death of the parasite (Picozzi, 2001). In comparison with microscopic techniques, molecular diagnosis is 2-3 times more sensitive (Solano *et al.*, 1999; Picozzi *et al.*, 2002). Techniques such as DNA probing and polymerase chain reaction (PCR) (Desquesnes & Dávila, 2002) are used for diagnosis and species identification in the laboratory. However, due the specialised nature of these techniques, the equipment needed and the cost, they are currently not practical for widespread field use. In addition, molecular techniques, such as PCR, do not distinguish active infections that will produce clinical signs in an animal from infections at a subclinical, endemic level. The actual clinical impact of infection tends to be low in trypanosomiasis endemic areas, despite the recorded high levels of incidence in susceptible cattle breeds that are affected by tsetse, and form a reservoir for trypanosomes (Van den Bossche, 2001). As such, interpretation of molecular diagnosis results must consider that active infection is not the same as clinical disease. These techniques do however allow for reliable differentiation between *T. b. rhodesiense* and *T. b. brucei*. *T. b. rhodesiense* possesses a gene which confers resistance human serum. Identification of the serum resistance associated (SRA) gene can therefore be used as a diagnostic test to differentiate between these two forms. This is a useful tool for screening the animal reservoir to find the portion of animals infected with *T. b. rhodesiense* (Welburn, Picozzi *et al.* 2001).

#### 1.2.7.4 Diagnosis of trypanosomiasis in livestock

Although definitive diagnosis of the disease can be achieved using the techniques listed above, these are not widely available to farmers or animal health assistants. Field diagnosis depends on the clinical signs, which include anaemia, pyrexia, weight loss, abortion and, if left untreated, death (Stephen, 1986).

The development of anaemia is an inevitable consequence of trypanosome infection, and is the best indicator of the presence of the disease (Murray, 1988). Measurement of anaemia is commonly done by assessment of the packed red-blood cell volume (PCV), and average PCV has been shown to correlate closely with trypanosome prevalence (Eisler, 1998). However, measurement of PCV requires a centrifuge and therefore a power source and therefore not ideal for field diagnosis. Other techniques such as those which use ocular mucous membrane pallor, or a visual comparison of blood spots against standardised colour scales, are possibilities where standard laboratory facilities are limited (Montresor *et al.*, 2003).

Progressive cachexia and emaciation of infected animals are good indicators of the disease. A simple scoring system which has been shown to be a repeatable and reproducible method of quantifying the body condition of cattle has been developed (Nicholson & Butterworth, 1986). Used in conjunction with an assessment of anaemia, they offer the best possibility for reliable diagnosis of trypanosomiasis in a field setting. Both of these techniques are employed in the study examined in this thesis and will be described in more detail in the relevant section.

#### 1.2.8 Trypanosomiasis control using drugs

There is no vaccine for trypanosomiasis. The vast variation in surface antigens does not present a constant target for antibody-mediated immunity and therefore negates the use of a vaccine. However, the costs in terms of human health and livestock production have given this parasite a prominent position on Africa's development agendas for over a century. The main control strategies include use of trypanotolerant livestock, control of the vector or control of the parasite using drugs. The oldest method of dealing with trypanosomiasis is simply to avoid the areas infested with tsetse, and, combined with

strategic use of trypanocidal drugs, constitutes the control strategy employed by the vast majority of livestock keepers. Treatment relies on the use of curative (chemotherapy) and preventive (chemoprophylaxis) drugs (Brightwell *et al.*, 2001).

#### 1.2.8.1 History of drug control of trypanosomiasis

In the 1920's the successful treatment of human patients suffering from sleeping sickness prompted the search for veterinary analogues for treating animal trypanosomiasis. The first compound, Sodium antimony tartrate was only relatively successful: it was difficult to use, was irritant to tissues so could only be administered intravenously and clinical resolution of disease frequently required repeated treatments (Hoppe, 2003).

In the late 1930's the phenanthridinium class of drugs appeared (Browning *et al.*, 1938) and in 1949, quinapyramine was introduced. The formulation, known as Antrycide Pro-Salt was the first chemoprophylactic drug (Willett, 1963). These compounds effected a cure with usually one treatment and offered significant periods when cattle were free from the disease, providing a potential for keeping cattle in areas of high tsetse challenge productively for the first time. It was not long, however, before the reappearance of the disease in treated cattle led to recognition that the development of drug resistance was severely curtailing the effectiveness of these compounds (Finelle & Yvone, 1962). In 1948 another phenanthridinium compound, homidium chloride, was released. In 1955, the German pharmaceutical company Hoechst brought out a *Berenil*®, whose active ingredient was diminazene aceturate, a diminadine developed as a spin-off to the dye industry (Fussganger, 1995). In the early 1960's, May and Baker isometamidium chloride was released, and in contrast to the solely trypanocidal properties of diminazene, could also be given at a elevated dose to provide prophylaxis (Sutherland *et al.*, 1991; Mdachi, 1999).

#### 1.2.8.2 Current drugs

Three drugs are currently available for the treatment of bovine trypanosomiasis: isometamidium chloride, homidium (chloride and bromide) and diminazene aceturate. Table 1-2 shows the current range of drugs, and some trade names, that can be used to control trypanosomiasis. With such a small number of drugs on offer in the therapeutic

armoury, the need to manage the development and propagation of drug resistance has become increasingly apparent over the four decades since the last novel trypanocide agent was introduced for African livestock.

Compound	Trade names <sup>a</sup>	Dose (mg/kg)	Activity	Route	Type
Diminazene aceturate	Berenil®, Veridium®, Numerous others	3.5-7	T. congolense, T.vivax T. brucei at higher dose	i.m i.m	T
Homidium chloride	Novidium®	1.0	T. congolense	i.m	T
Homidium bromide	Ethidium®	1.0	T.vivax	i.m	T
Isometamidium chloride	Samorin®, Trypamidium®, Veridium®	0.25-0.5	T. congolense	i.m	T
		0.5-1.0	T.vivax	i.m	P
		0.5-1.0	T.brucei	i.m	P
i.m., intramuscular; T, therapeutic; P, prophylactic					
<sup>a</sup> non exhaustive list					

**Table 1-2:- Currently available trypanocidal drugs for cattle**

### 1.2.8.3 Mode of action of trypanocides

Since its introduction in 1958, isometamidium chloride has remained the only agent available for the chemoprophylaxis of trypanosomiasis in animals. Assay of the original formulation, Samorin®, reveals a mixture of isomers with isometamidium 8-(3-m-amidinophenyl-2-triazeno)-3-amino-5-ethyl-6-phenylphenanthridinium chloride hydrochloride, mercifully abbreviated to ISM II, as the major active component. Synthesis of this particular active isomer requires rigid control of reaction temperature and pH to avoid the formation of other, less biologically active compounds (Geerts *et al.*, 2001). Assays of generic isometamidium formulations have shown large variability in their isomeric constituents and hence pharmacological efficacy (Tettey *et al.*, 1999). The main mode of action of both isometamidium and diminazene is cleavage of kDNA-topoisomerase complexes (Burri *et al.*, 1996). The process of resistance is uncertain, but

is thought to involve a decrease in the uptake or increased efflux of the drug by the parasite (Barrett & Fairlamb, 1999). It has been shown that in arsenical resistant *T. b. brucei* accumulation of diminazene was markedly reduced owing to alterations in the nucleoside transporter system (P2) which prevented uptake of the drug (Carter & Fairlamb, 1993).

Isometamidium is administered by deep intramuscular injection. The solution is irritant and forms a sterile abscess in the tissue which acts as a depot from which the drug is released into circulation. Work done using an ELISA showed considerable individual variation in the pharmacodynamics of ISMM, an interesting observation being that the duration of detection of the drug in the serum seems to be dependent upon the intensity of challenge and resistance status of the challenging trypanosomes (Eisler *et al.*, 1994; Mdachi, 1999). This could be explained by considering that each trypanosome 'consumes' a small amount of drug before it is killed, hence higher challenge, or more tolerant clones of trypanosomes will use up more drug (Torr *et al.*, 2002).

#### 1.2.8.4 Drug resistance

It is estimated that 35 million doses of trypanocide are administered each year in the 37 African countries afflicted with trypanosomiasis (Geerts & Holmes, 1998). Historically, trypanocides were controlled and largely administered by government departments. With the decline in centralised veterinary services and the deregulation of the pharmaceutical sectors, trypanocides, along with many other pharmaceuticals are now traded on the open market and can be purchased directly by farmers (Van den Bossche *et al.*, 2000). With this large increase in availability came the risk of inappropriate use. Trypanocides are considered to be one of the most commonly bought veterinary products in Africa and, in the absence of access to diagnostics, are often used by livestock keepers as the empirical treatment for *all* manifestations of disease (Geerts *et al.*, 2001). How and why an individual farmer chooses to treat their animals depends on a variety of factors including breed of cattle, management practices, knowledge about the disease and financial ability, but interestingly not always actual trypanosomiasis risk (Van den Bossche *et al.*, 2000; Machila *et al.*, 2003).

Given these patterns of usage, and the fact that all three trypanocides are used in cattle and small ruminants, it is not surprising that resistance has emerged.

It is said that ‘drug resistance attends chemotherapy like a faithful shadow’, resistance in this case defined as the loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible (Uilenberg, 1998). The mechanisms for how trypanosomes acquire drug resistance are not clear. Other organisms generally acquire resistance in their populations by the selection pressure favouring pre-existing genetic resistance in a small number of individuals (Anene *et al.*, 2001). Some authors attribute trypanosomes with an adaptive capability to respond to environmental insults, either by up regulating processes which ameliorate the toxic effect of the drug, or by leaving the bloodstream and hiding in tissues the drug cannot sufficiently penetrate (Uilenberg, 1998).

The development of resistance to trypanocides has been considered to come with an associated fitness cost, and the removal of the selection pressure from the drug will eventually lead to the disappearance of the resistance trait (Berger *et al.*, 1995; Mutugi *et al.*, 1995). Investigation in Ghibe, Ethiopia, showed that, once established, multiple resistant trypanosomes were maintained in the population for several years (Mulugeta *et al.*, 1997). Additionally, recent work with isogenic clones of *T.congolense* showed significantly higher infection rates in tsetse flies infected with the *T. congolense* clone with the *highest* level of drug resistance (Van den Bossche *et al.*, 2006).

Using *Plasmodium spp.* as a general model for the behaviour of clonal parasites, it is thought that drug resistance is likely to occur under the circumstances of: i) large-scale drug use; ii) inadequate dosing; and iii) using adequate dosing with drugs that are slowly eliminated from the body (Geerts & Holmes, 1998). It is also ironically possible that, given the mutagenic nature of some of the trypanocidal agents, chemotherapy could be accelerating the development of resistance in the trypanosome populations.

#### 1.2.8.5 Where are the new drugs?

Only three trypanocidal drugs are currently available - isometamidium chloride, diminazene aceturate and homidium salts. These compounds have been on the market for

over 40 years. There are currently no novel compounds in the pipeline because, despite an annual market for trypanocides by African farmers and national veterinary/livestock services of over US\$35 million, trypanosomiasis is not considered sufficiently financially important to justify investment by large pharmaceutical companies in the development and licensing of new trypanocides. Developing a novel drug from initial screening results to registration typically costs over US\$160 million, with no guarantee of a marketable product at the end of it (Pecoul *et al.*, 1999). Additional costs of drug licensing at national levels, combined with the laxity of patent laws have not inspired pharmaceutical companies to believe there is any economic return in innovating new compounds for this disease.

Given the absence of any novel compounds in the pipeline, the requirement to maintain the lifespan of the current drug arsenal is paramount. The following points have been recommended to delay the development of trypanocidal drug resistance.

The selection pressure generated by exposing the parasites to the drug drives the acquisition of resistance. Consequently, reducing the number of treatments reduces the selection pressure. Exclusive reliance on drugs for the control of trypanosomiasis, especially in areas of high challenge, and frequent, block treatments of a whole herd are practices to be discouraged. Integrating chemoprophylaxis with other modalities, e.g. vector control, or land use management offer the best chance of delaying resistance, and integrated approaches are now widely advocated as the best way of managing trypanosomiasis in cattle (Holmes, 1997; Eisler, 2003).

### 1.2.8.6 One size does not fit all

Sub-therapeutic drug concentrations exert a strong selective pressure for propagation of clones that have any pre-existing resistance (Mdachi, 1999). Inadequate dosing, from errors in weight estimation, deliberate under-dosing or poor drug preparation and administration will contribute to this problem. Incorrect weight estimation, for example by calculating the weight of the animal from the amount paid for similar sized dead weight carcasses, will underestimate the weight by about 50% (Personal observation). Use of weigh bands not standardised to the local cattle or adoption of a simple 'one



individual sachet fits all' approach to dosage are all likely to lead to under dosing. The individual sachets of diminazene aceturate sold throughout Africa contain the correct dose for an animal weighing 300kg. In the study herds considered in this thesis, 20% of cattle weighed over this threshold, so unless multiple sachets are used under-dosing is a risk. As the drugs are relatively expensive there is a temptation to over dilute the drug and hence under dose. Education of livestock keepers about treatment regimes and development of weigh bands calibrated for the relevant breeds could significantly reduce this problem (Eisler *et al.*, 2003).

#### 1.2.8.7 Selective treatments of specific animals

Block treatment of an entire herd has been effectively used for many years at keeping animals productive in the face of high trypanosome challenge (Trail, 1985). However, increasing the percentage of trypanosomes exposed to a drug will increase the probability that resistance will emerge. Limiting treatment to individual clinical cases reduces exposure of the trypanosome population to the drug. Models for anthelmintic resistance in sheep suggest that leaving 20% of the herd untreated significantly decreases the rate of development of resistance (Barnes *et al.*, 1995).

There are other reasons to attempt to minimise mass treatments. Aside from the obvious economic benefits to the livestock keeper from strategically minimising drug use, there are production benefits from reducing the blanket treatment of a herd. Although isometamidium has been well proven to provide prophylaxis in areas of high challenge, work done in E Kenya demonstrated that, in the absence of parasitaemia, isometamidium prophylaxis could have a deleterious effect on both PCV and body weight when administered to cattle in the absence of significant tsetse challenge (Mdachi, 1999).

Strategic drug use however relies on adequate disease detection methods and also has to be balanced against the benefit of treating subclinical cases. It is obviously unsuitable if the aim of treatment is eradication of a trypanosome population from a herd, for example to prevent the spread of human infective sleeping sickness through the cattle reservoir (Fevre *et al.*, 2003).

Trypanosome resistance is not binary, and even when prevalent in an area, chemotherapy can be, and in many places has to remain, a possible treatment option. Early use of a 'sanative pair'; a combination dose regime of isometamidium/homidium and diminazene, can theoretically remove clones before they acquire multiple resistance (Sinyangwe *et al.*, 2004). Cattle kept in the Ghibe valley, southwest Ethiopia, an area of high trypanosomiasis risk where resistance to all available trypanocidal drugs was established, were productively maintained on a combination of tsetse control measures and drug therapy (Rowlands *et al.*, 1994). Above all, the immunological status of the patient plays a important role in the outcome of chemotherapy, as an example from human medicine demonstrates; malnourished children in a refugee camp in the Congolese town of Goma, considered to be immuno-compromised, showed a higher risk from treatment failure resulting from drug-resistant *Plasmodium falciparum* than well-nourished children (Wolday *et al.*, 1995). Host-parasite dynamics at the level of the individual are thus influential, not only on the outcome of a given drug treatment, but also on the development of drug resistance.

#### 1.2.8.8 The problems with drug use

The privatisation of veterinary services is shifting animal treatment campaigns from top down project-driven approaches to schemes demand-driven by livestock keepers. In addition, financial support is moving away from external international donor funding to a situation where increasingly projects are expected to recover their costs, ultimately from the livestock owners (Leonard, 1998). This has resulted in a largely unsupervised delivery system of animal health care which coupled with the zoological ignorance of the farmers, can lead to inappropriate drug administration. (Machila *et al.*, 2003). Drugs are often not used properly, resistance is developing and there are few prospects on the horizon for novel 'wonder drugs'. Therefore, a system exists where a reliance on an age-old technique for the control of trypanosomiasis remains: the control of the biological vector of the trypanosome - the tsetse fly.

### 1.3 Tsetse control

African trypanosomiasis is transmitted between the vertebrate hosts by tsetse flies, genus *Glossina*, family *Glossinidae* and order *Diptera*. The genus *Glossina* is made up of 31

known species, subdivided into three subgenera (Table 1-3). Classification is based largely on morphological differences in genital structure; however, the subgenera can be broadly divided by habitat preference. The *fusca* group are a savannah species tolerant of arid conditions, the *palpalis* group are riverine flies found along watercourses and gallery forests of Central and West Africa, and the *morsitans* group contain predominantly forest flies of East Africa (Leak, 1999). *G. brevipalpis*, *G. pallidipes* and *G. fuscipes fuscipes* are the main species of tsetse fly found in Uganda (Okoth, 1986).

Subgenus (group)	Species (subspecies)	Preferred habitat
<i>Glossina (morsitans)</i>	<i>G. morsitans</i> <i>G. m. submorsitans</i> , <i>G. m. centralis</i> , <i>G. m. morsitans</i> <i>G. pallidipes</i> <i>G. swynertoni</i> <i>G. austeni</i> <i>G. longipalpis</i>	Savannah
<i>Nemorihina (palpalis)</i>	<i>G. tachinoides</i> <i>G. palpalis</i> <i>G. p. palpalis</i> , <i>G. p. gambiensis</i> <i>G. fuscipes</i> <i>G. f. fuscipes</i>	Riverine, lacustrine and forest
<i>Austenina (fusca)</i>	<i>G. brevipalpis</i> <i>G. longipennis</i> <i>G. fusca</i> <i>G. medicorum</i> <i>G. tabaniformis</i>	Lowland forests, gallery forests

**Table 1-3: Tsetse classification**

The tsetse's role in trypanosomiasis transmission has been known for over a century (Bruce & Nabarro, 1903; Pépin & Méda, 2001). For most of this time, efforts of the scientific community have focussed on developing ways of protecting, at current estimates, some 29 million people, 45 million cattle and unknown millions of sheep and goats at risk from contracting trypanosomiasis from the tsetse fly (Reid *et al.*, 2000).

Tsetse ought to be simple to control. Tsetse flies are highly mobile and have an obligate requirement for regular blood meals. They exist within a relatively narrow ecological range of temperature, humidity and vegetation and have a reproduction strategy usually associated with small mammals rather than insects. Ecologically, tsetse are K-strategists; they have a low reproduction rate, produce a single offspring with a high chance of survival. By comparison, mosquitoes are R-strategists, they produce many offspring but with a low probability of survival. K strategists are highly adapted to exploit their habitat but are sensitive to interventions that alter their environment or lower their reproductive rate (Barrett *et al.*, 2003b). Control strategies only have to remove 3-4% of the total number of female tsetse each day to cause the population to decline (Hargrove, 2003).

### 1.3.1 Game clearance

Tsetse flies are unusual amongst insects in that both sexes feed exclusively on vertebrate blood. They cannot survive without regular blood meals; remove the hosts and tsetse populations should plummet. This tsetse control technique was employed with great success by colonial administrations, such as in Shinyanga, Tanzania, where between 1945 and 1951, more than 8000 game animals were shot (Potts & Jackson, 1952). During this time mean catches of non-teneral (fed at least once) male tsetse declined from 180 to zero. Nowadays such deliberate game clearance is anathema, not only from an ecological standpoint but because, as can be seen in south east Uganda, in an area with virtually no wild animals, tsetse populations can be happily supported on domestic livestock (Waiswa *et al.*, 2003).

### 1.3.2 Bush clearance

According to Ford *et al* (1970) '*Total, sheer or ruthless clearing means the destruction of all trees and shrubs in the area treated. It is a completely effective method of eliminating Glossina and the oldest*' (Hargrove, 2003). There is much debate as to the reasons bush clearance was effective. The opinion that tsetse have a particular 'home' vegetation type which, if removed, causes the population to crash, is now considered too simplistic (Hargrove, 2003). Certainly total clearance is effective, although this can possibly be attributed to its impact on the hosts as much as on the flies themselves.

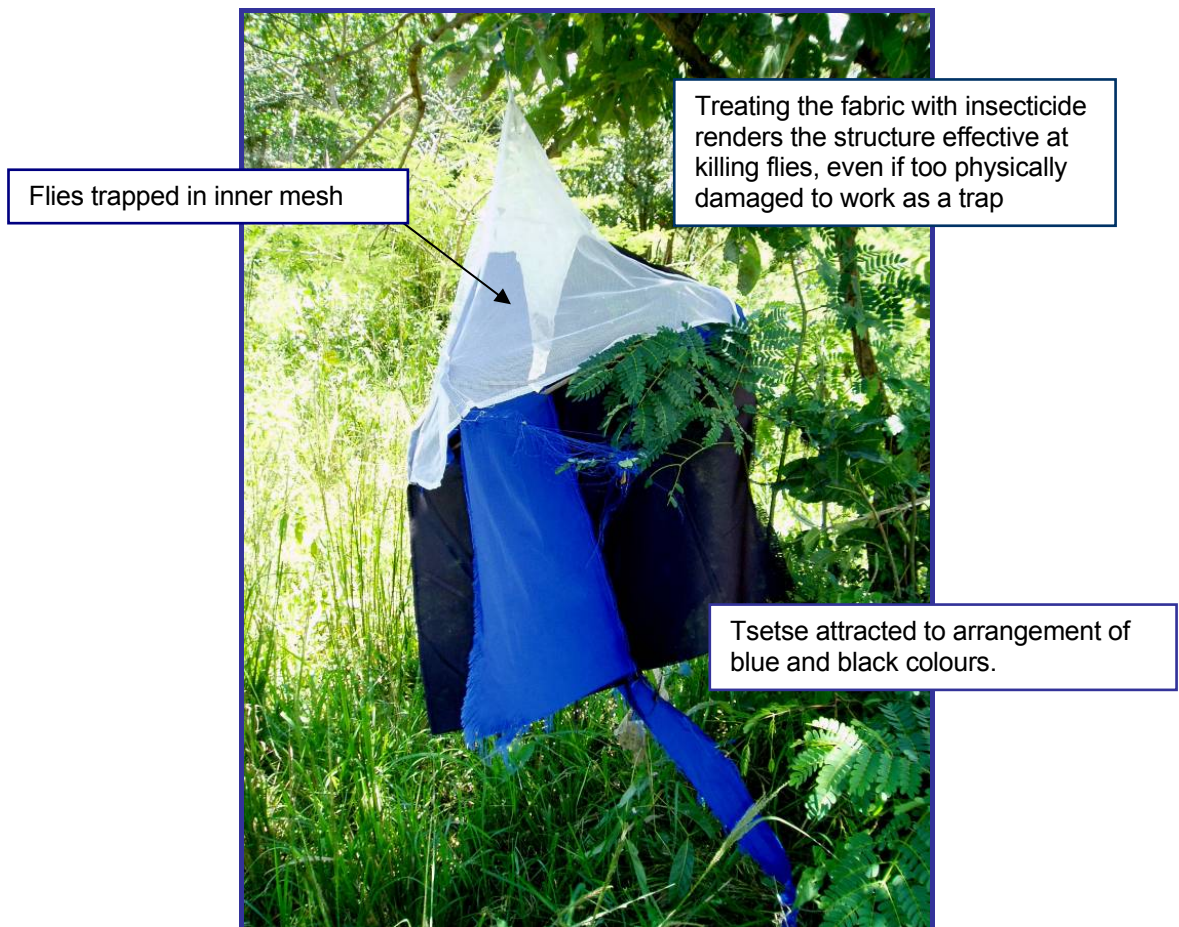
Although *purposive* ecological destruction is abhorred nowadays, it does serve to highlight the effectiveness of the method. As Bourne pointed out, '*the greatest changes in tsetse distribution have resulted simply from the expansion of human populations into tsetse habitats with the consequent reduction in game numbers and land clearance for agriculture*' (WHO, 1998; Bourne, 2001; Hargrove, 2003).

### 1.3.3 Ground and Aerial Spraying

Tsetse are exceptionally sensitive to insecticides, moreover, due to their low dispersion and reproductive rate, they do not make strong candidates for developing resistance (Krafsur, 2003). Application of insecticide to vast tracts of land by either ground or aerial spraying has been used in the past to successfully eradicate tsetse from northern Nigeria and Zimbabwe; between 1955 and 1978 approximately 570 tonnes of DDT was sprayed over some 200,000km<sup>2</sup> of northern Nigeria (Jordan, 1986). The use of DDT fell out of favour, partly due to valid ecological concerns about insecticide residues, but also because its application made logistical demands beyond that of the increasingly resource-deprived tsetse control departments (Robertson, 1971; Okoth, 1999). In an attempt to overcome the problems associated with ground spraying, aerial spraying with synthetic pyrethroids was increasingly adopted in the 1980's (Holmes, 1997). Compared with ground spraying, ultra low doses of insecticide can be used, applied as aerosols and released from helicopters or low flying aircraft. Although effective on adult tsetse, this spray is not effective on flies in the pupal stage as they live underground. Therefore, careful attention needs to be paid to the spraying interval of 19 days in order to ensure that the emerging females are killed before they can replenish the protected pupal population. It is also vital to have reliable geo-positioning information of the treatment area to avoid 'islands' of tsetse remaining in untreated patches which can quickly reinvade a treated area (Hargrove, 2003). The recent successful eradication of tsetse from the Okavango Delta in Botswana is attributed to GIS guided application of an adequate dose of deltamethrin and the effective use of targets to prevent tsetse re-invasion (Kgori *et al.*, 2006).

### 1.3.4 Bait technology: Traps and targets

Tsetse can be controlled by exploiting their predisposition to visual and olfactory cues. Traps, essentially coloured fabric and netting constructions, have been developed to capture most tsetse species and provide a way of both controlling and monitoring tsetse populations. Targets work by the same principle but are impregnated with insecticide and are often simple and cheaper to construct. Fly capture was first used in the 1900's to eradicate tsetse from the island of Principie by simple hand netting or attaching sticky panels to people and sending them into a tsetse area (Hargrove, 2003). The first bi-conical traps were developed in the early 1970's by Challier and Laveissiere and were shown to be effective against riverine tsetse species (Allsopp, 1984). In the early 1980's, a decline in the use of environmentally applied insecticides caused by concern over their ecological impact stimulated research into other control strategies, such as traps and targets (Grant, 2001). Initial work demonstrated that traps failed to catch many of the flies visiting them, but highlighted the importance of odour as a tsetse attractant (Vale, 1980). A demonstration trial followed on Antelope Island using odour baited traps and target devices and successfully eradicated a closed population of *G.m.mortisans* and *G. pallidipes* (Vale *et al.*, 1988). Since then, carbon dioxide, acetone, butanone, 1-octen-3-ol, p-cresol, 4-methylphenol, 3-n-propylphenol and cow urine have been shown to act as attractants. Impregnating the fabric of tsetse traps and targets with insecticides kills alighting flies even if structural damage prevents physical trapping. These augmentations can increase the efficacy of traps and targets by 200% (Vale *et al.*, 1988).



**Figure 1-3: Version of a pyramidal tsetse trap used in south east Uganda. Damage to mesh probably renders this trap ineffective unless impregnated with insecticide.**

Several types of trap have been developed, but a cheap and effective design employed in south east Uganda is a version of the pyramidal trap (Lancien, 1986), demonstrated in Figure 1-3. Maintenance of traps is critical, and theft or vandalism is a risk (Kioy & Mattock). When such traps are distributed at a density  $>4$  per  $\text{km}^2$  they are sufficient to reduce transmission (Lancien, 1986). Due to the low cost of materials and the low level of technology used, traps and targets have been thought to be potentially the most affordable and sustainable method of tsetse control (Holmes, 1997). Traps can be very cheap to

make, and a study in 1991 in Uganda estimated the cost of materials at US\$4.70 (Okoth *et al.*, 1991).

In the 1990's, researchers across east Africa began to implement 'community based' tsetse control projects. These projects typically aimed to transfer tsetse control to the rural communities, enabling the rural population to implement and maintain their own traps and targets. Local communities were eventually to hold responsibility for the cost and maintenance of the traps, including re-treating and protecting them from damage and theft. It was found that, as the levels of tsetse decreased following a successful control program, the obvious need for action decreased and commitment from the local community became difficult to maintain (Kamara *et al.*, 1995; Machila *et al.*, 2003). Consequently, whilst these projects were often initially very successful, with dramatic reduction of the tsetse population, maintenance was often not sustained and the cleared areas were reinvaded by the fly (Okoth *et al.*, 1991; Budd, 1999; Okoth, 1999). The lack of long term project sustainability essentially lay in how the projects were implemented, confusion over ownership and financial responsibility of the projects, and the intrinsic problems associated with demanding a private investment for a public good (Swallow, 2000; Brightwell *et al.*, 2001; Catley *et al.*, 2002).

### 1.3.5 Bait technology: Insecticide treated cattle

A logical alternative to the use of artificial baits is the use of natural ones. In areas where there are large numbers of cattle, application of the insecticide to the animal has been shown to be a cheap, simple and effective method of vector control in a number of countries; Zimbabwe (Thompson *et al.*, 1991), Burkina Faso (Bauer *et al.*, 1992a), Tanzania (Fox *et al.*, 1993), Ethiopia (Leak *et al.*, 1995) and Kenya (Baylis & Stevenson, 1998b). Although the principle was trialled in the 1940's, the lack of persistence of the insecticide (DDT) did not make it a viable control option at the time (Whiteside, 1949). In the 1980's however, synthetic pyrethroid insecticides, specifically deltamethrin, were tested and shown to be effective for up to a month (Thompson, 1987). Cattle are treated with synthetic pyrethroid insecticides in either spray, pour-on or dip formulations and these compounds had a 'knockdown effect', causing alighting flies to be incapacitated before taking a feed (Bauer *et al.*, 1992a). Spray and dip formulations are aqueous and



require 100-1000 times further dilution before application. Application by spray requires equipment that can deliver the insecticide in fine droplet form. In the case of dips, cattle are fully immersed in a solution of insecticide. A description of the logistics of dipping is given in (Norval *et al.*, 1992) and (Okello-Onen *et al.*, 1994).

Pour-on applications in contrast are low volume insecticide applications. The first pour-on application of an insecticide was reported in 1957 when aldrin, a systemic insecticide was used to control body lice in sheep and poultry (McCosker, 1957; McCosker & Osborne, 1957). Applied to a small area of skin, the drug was systemically absorbed, distributed in the blood stream and excreted to reach all skin surfaces (Brimer *et al.*, 1994). The use of organophosphates such as rulene (Rogoff & Kohler, 1960) were successful at controlling the cattle grub and revolutionised the control of cattle lice (Nickel, 1971). Following their introduction in the early 1970's (Mitchell, 1996), synthetic pyrethroids, namely deltamethrin, cypermethrin and  $\alpha$ -cypermethrin, became widely employed, initially to control the cattle louse *Damalinia ovis* (Kettle & Lukies, 1979). In comparison with the mode of action described for organophosphates, these compounds do not become systemic. Application is by a concentrated formulation of the active ingredient mixed with a lipophilic dispersal agent, poured along the spine of the animal. The insecticide rapidly diffuses through the sebaceous layer between the coat and skin surface, but is not absorbed into the tissues (Magnusson *et al.*, 2001). The concentration of active agents decreases with distance from the application site (Magnusson *et al.*, 2001). However, using radio-labelled [<sup>14</sup>C] cypermethrin it was found that redistribution is rapid, with pour-on formulations spreading radially across the skin within the stratum corneum at a rate of approximately 11 cm/h (Jenkinson *et al.*, 1986).

Use of pyrethroid insecticides confers other benefits to animal health by controlling nuisance flies (e.g. *Stomoxes* spp.) and ticks (Vale *et al.*, 1999). This technique offered several advantages over existing control strategies - it was cheaper (than using a fly control and an acaricide), it could utilise the dipping infrastructure already in place for the control of tick borne diseases, and adoption of the technique was good within smallholder livestock keeper communities due to the more obvious benefits to animal health (Torr *et al.*, 2002). The pour-on formulations were easy to use and did not require large volumes

of water, although they were expensive (mainly due to the cost of the dispersal agent (Vale *et al.*, 1999)).

However, there are problems with the widespread use of insecticide treated cattle for tsetse control. Cattle do not equally disperse themselves across a landscape, so many tsetse habitats can go unexposed to the insecticide control (Hargrove *et al.*, 2000). Insecticide formulations are so costly they are largely beyond the budget of the majority of rural livestock keepers (Shaw, 2004) and, due to the mobility of tsetse and the risk of reinvasion, these techniques were only successful at controlling fly populations if deployed over areas of several hundred square kilometres (Vale *et al.*, 1999). Concern has also been raised about the ecological effects of this treatment method, for example its impact on dung fauna (Vale *et al.*, 1999) and on the endemic stability of tick-borne diseases ((Eisler *et al.*, 2003) and section 1.4.6. below).

### 1.3.6 Sterile insect techniques

At the other end of the spectrum of tsetse control comes the sterile insect technique (SIT). This involves the release of irradiated sterile males into the wild. Matings between these males and wild females produce no offspring. As female tsetse usually only mate once, have a low reproductive rate and produce only one larva every 10 days, SIT can significantly affect the fly population. Application of SIT methodology to a trial on the island of Unguja, Zanzibar successfully eradicated *G. austeni*. However, successful application of SIT to mainland Africa is a topic of vigorous debate in the literature (Vreysen *et al.*, 2000; Allsopp, 2001; Rogers & Randolph, 2002). The eradication of *G. austeni* from Zanzibar took over two years and the release of more than 8.5 million sterile males to complete the eradication of a population whose male component comprised only 1000 flies at the start of the release programme (Hargrove, 2003). Extending this technique to areas of Africa supporting 22 tsetse species and with a constant risk of re-invasion raises feasibility issues, in addition to the huge financial and logistical commitments such an undertaking requires. The sterile insect technique is however the only mode of tsetse control where efficacy increases with decreasing tsetse density, and if used in conjunction with other forms of control could provide a final *coup de grace* to eradicate a suppressed or resistant tsetse population.

### 1.3.7 Eradication or control?

As the above section shows, the toolbox of tsetse control options is far from empty. And the pervasive persistence of tsetse is arguably due to the misapplication of the technologies rather than their design. Although ‘eradication’ has been achieved in many areas by various modalities, re-invasion remains the main problem for tsetse control (Molyneux, 2001). Without effective barriers and reliable monitoring, tsetse populations can recover alarmingly rapidly to their original levels. Donor agencies, jaded by the financial demands of large scale programmes are looking towards a more farmer-driven approach to tsetse control (Leonard, 1998). However, modelling of tsetse population dynamics suggests that the scale of operations needed for these attempts to succeed is beyond the financial or organisational means of community-driven organisations (Hargrove, 2003; Torr *et al.*, 2005). Increasingly, the problem of effective trypanosomiasis control is being managed in a climate of decentralising health care, decreasing capacity for ‘top-down’ interventions and the increasing acceptance that, for animal disease control, the livestock farmer is being left to ‘go-it alone’ (Eisler, 2003). Trypanosomiasis control has to be contextualised as just one of the many endemic diseases afflicting domestic livestock, in particular diseases vectored by ticks. Additionally, treatment methodologies such as the use of insecticide on cattle to control tsetse can have an impact on the tick populations, which due to significant epidemiological and immunological differences between the two vectors, may not always be beneficial (Torr *et al.*, 2002).

## 1.4 Ticks and tick-borne diseases

Ticks, and the diseases they vector, are a major constraint on livestock production. Worldwide, estimates of their cost to livestock production are in excess of US\$15 billion (Griffiths & McCosker, 1990; McLeod & Kristjanson, 1999) and on the African continent tick-borne diseases (TBD) are overshadowed only by trypanosomiasis in terms of importance as a livestock disease (Kivaria, 2006).

Ticks can damage their hosts by the direct effects of attaching, by injection of toxins (tick pyaemia, or, in large enough numbers, by causing anaemia (de Castro, 1997). It is the

indirect effect of ticks that are the most damaging, however, through the morbidity and mortality associated with the diseases they vector.

The major tick-borne diseases of cattle can be classified in terms of the genera of their vectors:

*Boophilus* ticks transmit *Babesia*, a protozoon, and *Anaplasma*, a rickettsial, species of pathogen. The respective diseases, babesiosis and anaplasmosis, are the most widely distributed TBD worldwide, and particularly affect highly producing exotic dairy and beef cattle.

*Amblyomma* ticks transmit the rickettsia *Ehrlichia* (formerly *Cowdria*) *ruminantium* which causes heartwater, a serious disease that is often fatal in small ruminants and exotic cattle. *Amblyomma* ticks can also transmit the protozoa *Theileria mutans* and the actinomycete *Dermatophilus congolensis*. Adult *Amblyomma* ticks have a predilection site for the ventrum and axilla regions of the animal.

*Rhipicephalus* ticks transmit the protozoa *Theileria parva* which cause East Coast fever (ECF) a hugely destructive disease in sub-Saharan and southern Africa. In a recent study in Tanzania, ECF accounted for 68% of the 1.3 million cattle lost each year to tick borne diseases, at an estimated cost of US\$364 million (Kivaria, 2006). Adult *Rhipicephalus* ticks have predilection sites for the perimeum and ears, giving them the common name of the brown ear tick.

### 1.4.1 Anaplasmosis

The anaplasma group of diseases are mainly transmitted by ticks, but can also be transmitted mechanically by biting *Dipteran* flies or iatrogenically through contaminated needles (Minjauw & McLeod, 2003). The parasite enters the host's erythrocytes early in infection. The two *Anaplasma* species, *Anaplasma centrale* and *Anaplasma marginale*, are morphologically indistinguishable but occupy different locations in the red blood cell, from which they take their names. *A. marginale* is the more pathogenic of the two species with up to 50% mortality in susceptible animals (de Castro, 1997).

Anaplasmosis is characterised by pyrexia, anaemic and jaundice. Morbidity and mortality are higher in exotic animals although local breeds can be affected under conditions of stress, brought about for example by poor nutrition, parturition (Richey, 1992) or concurrent disease (Fox *et al.*, 1993). Mixed infection with *Babesia spp.* is common (Eisler *et al.*, 2003).

### 1.4.2 Babesiosis

Babesiosis is caused by two protozoan parasites *B.bigemina* and *B.bovis*, both transmitted by *Boophilus* ticks. *B.bigemina* is vectored by *Boophilus decoloratus* (the blue ear tick) which is widespread across Africa. *B.bovis* is more pathogenic but is only vectored by *Boophilus microplus* and *Boophilus annulatus*, both with geographical foci around south-east and western Africa, and not present in S.E Uganda (Minjauw & McLeod, 2003).

Clinical signs of babesiosis result from the severe intravascular haemolysis brought about by the sequential invasion and destruction of red blood cells by the parasite. Babesiosis' common name of 'redwater fever' comes from the often stark haemoglobinuria as a result of this process. Peracute infections can also exhibit neurological signs. As with anaplasmosis, the severity of disease can be exacerbated in animals with a compromised immune system.

The disease can be controlled by chemotherapy using diminazene aceturate or imidocarb dipropionate. Unlike the other TBD discussed here, *Babesia spp.* can be transmitted transovarially in the vector, i.e. through the eggs of the infected tick to its progeny. This is epidemiologically significant as the disease can be maintained in the environment in the absence of infected hosts (Eisler *et al.*, 2003).

### 1.4.3 Heartwater

Vectored by *Amblyomma spp.* ticks, heartwater, caused by the rickettsia *Ehrlichia ruminantum*, is a disease mainly found in small ruminants and exotic cattle. However, as with the previous two disease cases, health, nutrition and immune competence are

influential, and when these parameters are low indigenous cattle can also be affected. Clinical signs are largely neurological and linked to increased vascular permeability; the name heartwater is derived from the hydropericardium often seen on post mortem examination. The disease is very difficult to diagnose; Giemsa stained brain smears are the most reliable way of demonstrating the organism's presence. Consequently, it was not possible to investigate the prevalence of heartwater in this study, although the disease is considered to be the second most important TBD after ECF in East Africa, so its control is of some relevance (Mukhebi *et al.*, 1999; Minjauw & McLeod, 2003).

#### 1.4.4 Theileriosis

The protozoan parasite species *Theileria* is responsible for the most pathogenic TBD of cattle (Perry *et al.*, 2002). *Theileria parva*, transmitted by the brown ear tick *Rhipicephalus appendiculatus*, is the causative agent of East Coast fever. *Theileria annulata* is a widespread problem in the countries bordering the Mediterranean, along the Nile and throughout Asia, although not, at present, in Uganda.

East Coast fever is predominantly a lymphoproliferative disease, with high mortality resulting from leucopaenia and oedema from invasion of alveolar tissue by parasitized lymphocytes (Urquhart *et al.*, 1996). Pyrexia is common and although the piroplasmic form of the parasite subsequently invade erythrocytes, anaemia is not a common clinical feature (Norval *et al.*, 1992). Clinical signs are lymphadenopathy, fever, lacrimation, diarrhoea and petechial haemorrhages, in addition to respiratory signs consistent with pulmonary oedema (Shannon, 1977).

East Coast fever can be treated with parvoquone, buparvaquone and halguginone, although the drugs are expensive and much less effective in the advanced stages of the disease when clinical signs are more obvious (Dolan *et al.*, 1984; Musoke *et al.*, 2004). Most prevention is by an 'infect and treat' method of establishing immunity, whereby cattle are challenged with live sporozoites and simultaneous administration of a tetracycline drug. Tetracyclines ameliorate the effect of the challenge and usually allow for the establishment of a solid immunity (Torr *et al.*, 2002). The technique is not without its problems however, since immunity can be strain specific, vaccination teams require a

cold-chain delivery and, given the technique relies upon live vaccination of pathogenic organisms, any failure of the tetracycline can have serious consequences (Minjauw & McLeod, 2003).

#### 1.4.5 Diagnosis of tick borne diseases

Given the endemic nature of TBD (Perry *et al.*, 1991), it can be difficult to accurately assess their clinical impact because infection is not synonymous with disease. Identification of parasites from thin film blood smears may merely indicate that the animal is a carrier rather than diseased (Norval *et al.*, 1992). Clinical assessment of lymph node size can give a indication of lymphadenopathies associated with early infection of *Theileria* parasites although for accurate assessment of tick-borne diseases, microscopic identification of stained lymph node aspirates (Norval *et al.*, 1992), antibody detection (Katende *et al.*, 1998; Rubaire-Akiiki *et al.*, 2004) or parasitic DNA amplification (Skilton *et al.*, 2002) are preferred.

#### 1.4.6 Endemic stability

Endemic stability is an important aspect of the control of tick-borne diseases in indigenous cattle. Whilst the diseases are often severe and associated with sudden and high mortality in exotic breeds, indigenous cattle are able to exist in areas where the diseases are endemic and suffer much lower losses. A major reason for this is the establishing of a state of endemic stability; an epidemiological state where clinical disease is rare despite high levels of infection in the population. This relies on an inverse age-related immunity, whereby younger animals are more resistant to developing clinical disease following infection than older animals (Norval *et al.*, 1992; Torr *et al.*, 2002). If calves and young animals acquire the infection whilst young and are innately protected, they will develop a long lasting immunity which protects them from disease when challenged as older animals. The concept of endemic stability was first used to describe *Babesia* infections in Australian cattle (Mahoney & Ross, 1972) and it is well accepted to occur with babesiosis and heartwater, is sometimes seen in anaplasmosis, but only occasionally appears to be the case with ECF. There is very limited evidence of inverse age immunity in ECF, and indeed unlike other TBDs in endemic areas disease tends to affect calves (Bruce *et al.*,

1910) On the other hand, the phenomenon of 'quantum of infection' is recognised in ECF, whereby the severity of disease depends upon the size of the inoculum (Jarret *et al.*, 1969). Together these features of the disease suggest that a different type of stability may exist for ECF (Eisler, 2005). This idea fits with the significant ECF induced mortality in calves and young stock in endemic areas that is not seen with the other tick-borne diseases (Gitau *et al.*, 2000).

#### 1.4.7 Control of ticks in crop-livestock systems of east Africa

Control of TBD is achieved in three main ways; control of the tick vectors, immunisation and chemotherapy. Acaracides, applied by running cattle through plunge dips, were the mainstay of the colonial approach to tick control. The Ugandan administration made dipping compulsory in the mid 1960's (Ministry of Animal Industry Game and Fisheries, 1968), in some cases at intervals of every 2-3 days. Increasing resistance among the tick populations to acaracides, capacity reduction of the veterinary infrastructure, the increased cost of dip chemicals and a reversion of cattle demography towards indigenous breeds have diminished the number of working dips by more than 70% (ICIPE, 1997; Okello-Onen *et al.*, 1998a). In the current management systems of south east Uganda, the largely indigenous cattle population are managed with a non interventionist approach where vaccination is seldom practised and acaracides infrequently used (Okello-Onen *et al.*, 2003). The low incidence of clinical disease arises from an innate resistance of Zebu cattle raised in such endemic areas combined with the development of acquired immunity to infection as a result of the low but virtually continuous exposure of calves to ticks (Perry & Young, 1995). Calves are protected from the detrimental effects of anaplasma and babesiosis infection by their endemic stability and losses due to ECF are, to some degree, an accepted part of farmers' management strategy (Rubaire-Akiiki *et al.*, 2006). It is possible the low infection rates of *T.parva* in ticks, acquired from low parasitaemias in immune carrier cattle, control the disease more than traditional endemic stability seen with other TBD (Kariuki *et al.*, 1995). In contrast, studies conducted in south west Uganda, a more pastoralist farming region, found a much less *laissez-faire* towards TBD, and most farmers engaged in proactive tick control, mainly through regular spraying with insecticide (Mugisha *et al.*, 2005).



Method	Advantages	Disadvantages
Bush clearance	<ul style="list-style-type: none"> <li>• Low technology method of control</li> </ul>	<ul style="list-style-type: none"> <li>• Requires maintenance</li> <li>• Ecologically damaging</li> <li>• Potential source of disagreement between communities</li> </ul>
Trypanocidal drugs	<ul style="list-style-type: none"> <li>• Effective</li> <li>• Obvious 'private good' benefit to individual</li> <li>• Affordable to many farmers</li> </ul>	<ul style="list-style-type: none"> <li>• Drug resistance</li> <li>• Drug availability</li> <li>• Drug residues in meat</li> </ul>
Cattle movement	<ul style="list-style-type: none"> <li>• Simple</li> <li>• Well known method of control</li> </ul>	<ul style="list-style-type: none"> <li>• Conflict over land use with other communities</li> </ul>
Community based targets and traps	<ul style="list-style-type: none"> <li>• Environmentally friendly</li> <li>• Relatively inexpensive</li> <li>• Effective</li> <li>• Easy to use and train</li> </ul>	<ul style="list-style-type: none"> <li>• Require sufficient numbers and adequate spacing of traps to be effective</li> <li>• Easily stolen or damaged</li> <li>• Need strong community organisation to manage</li> </ul>
Insecticide Pour-on formulation	<ul style="list-style-type: none"> <li>• Effective, insecticide supplied in a oil emulsion. Insecticide disperses through sebaceous layer of skin from an application of 10-20ml along dorsum.</li> <li>• Benefit to individual</li> <li>• Controls of ticks and biting flies</li> <li>• Requires no additional equipment to apply</li> </ul>	<ul style="list-style-type: none"> <li>• Very expensive due to cost of dispersal agent.</li> <li>• Insecticide can be licked off and contaminate faeces -&gt; impact on dung beetles -&gt; soil ecology disrupted</li> </ul>
Insecticide Dips	<ul style="list-style-type: none"> <li>• Effective</li> <li>• Provide a central point to acquire advice/treatments from AHW(Cohen &amp; Uphoff, 1980)</li> </ul>	<ul style="list-style-type: none"> <li>• Require expensive building of dip tanks</li> <li>• Can cause problems collecting and manage costs of insecticide.</li> <li>• Require a minimum throughput to be cost effective</li> <li>• Ecological concerns over disposal</li> </ul>
Insecticide spraying	<ul style="list-style-type: none"> <li>• Effective</li> <li>• Benefit to individual</li> <li>• Control of ticks and biting flies</li> </ul>	<ul style="list-style-type: none"> <li>• Relatively expensive</li> <li>• Requires pump equipment and water source</li> <li>• Persistence variable, may require regular treatments for effective control</li> </ul>
Sterile Insect Technique	<ul style="list-style-type: none"> <li>• No drug residues in environment</li> <li>• Efficacy improves as tsetse populations decrease.</li> </ul>	<ul style="list-style-type: none"> <li>• Requires large financial, logistical and political commitments</li> <li>• Many species of fly to control</li> <li>• Questionable reproductive fitness of released sterile males</li> </ul>

**Table 1-4: Summary of tick borne disease and trypanosomiasis control methods.**

## 1.5 Integrated control of tick and tsetse

Table 1-4 summarises the various control methods for tick and tsetse vectored diseases, along with comparative advantages and disadvantages.

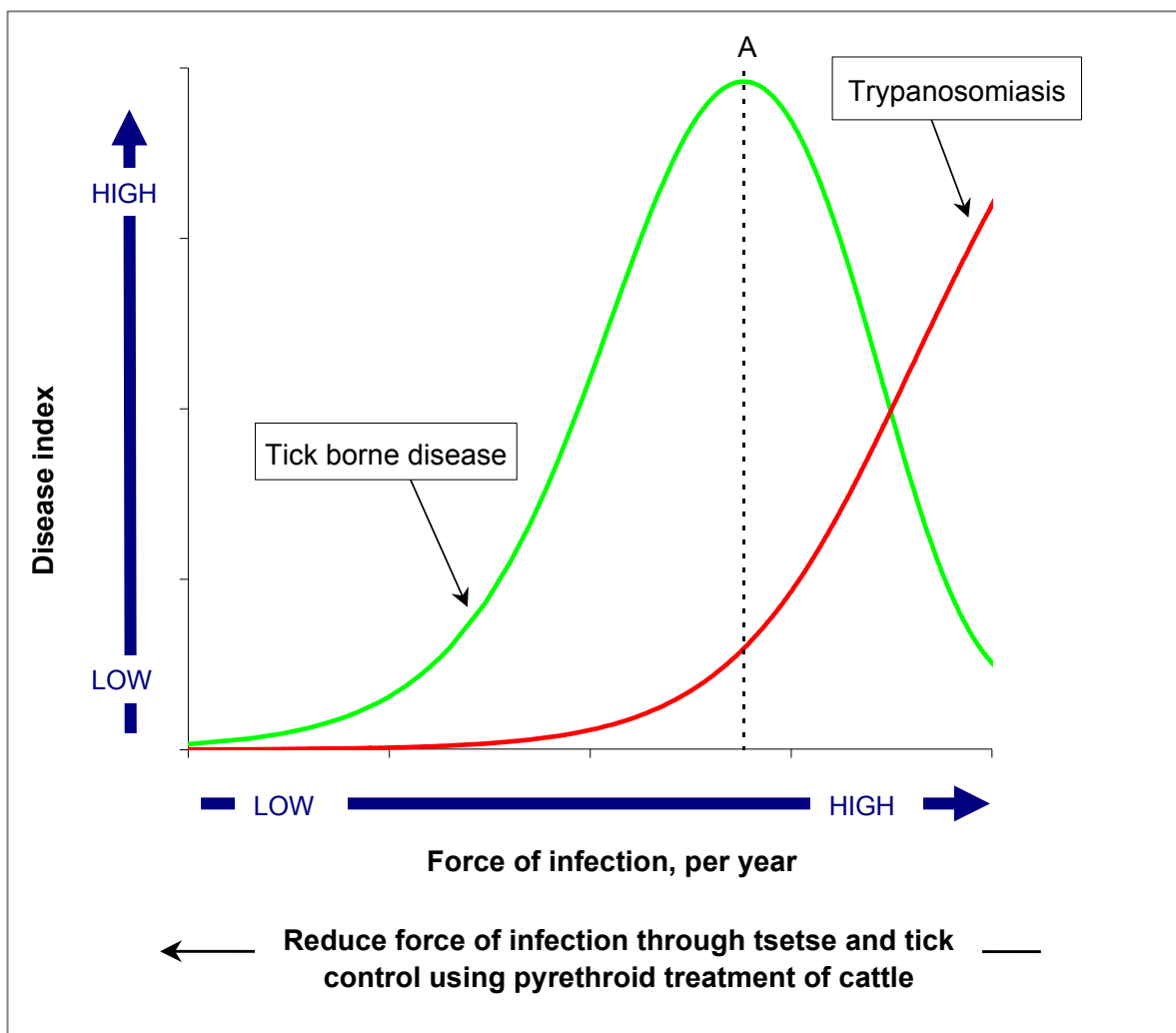
### 1.5.1 Summary of tick and tsetse control methods applicable to south-east Uganda

In south east Uganda domestic cattle are continually challenged by a variety of endemic and epidemic diseases, of which TBD and trypanosomiasis, along with helminthiasis and malnutrition, are the major constraints to productivity (Magona *et al.*, 2003). Farmers are precluded from owning more productive breeds due to their high susceptibility to these diseases, and the predominantly Zebu herds are maintained in a state of equilibrium with endemic tick-borne diseases. Endemic stability does not exist for trypanosome infections however, and trypanosomiasis has severe impacts on livestock health and productivity. Additionally, cattle in south east Uganda are a significant reservoir for human infective trypanosomiasis, making control of the disease in cattle a development priority (Fèvre *et al.*, 2001). Disease control methods are largely devolved to private investment from the individual livestock keeper and control options have to be demonstrably functional at this level. Insecticide treated cattle offer an alternative to the use of traps and targets for tsetse control. Livestock keepers are more likely to adopt technologies that show a visible effect on the tick burdens compared with the suppressed *G. fuscipes* tsetse population (Okiria *et al.*, 2002a).

### 1.5.2 Integrated control and endemic stability

Insecticide treated cattle are proving to be the only control method rural farmers are likely to adopt without continuous external input, although several of the drawbacks (Section 1.3.5) need to be addressed for it to be successful. Tsetse and tick control protocols in indigenous cattle populations are designed with different agendas. The aim with tick control is to allow the exposure of animals, particularly young stock, to ticks so endemic stability can be established and maintained, whilst minimising the damage from the direct effects of ticks and the excessive inoculation of *T.parva* sporozoites. Conversely, there is

no benefit to be gained from infection with trypanosomiasis early in life so the aim of tsetse control programmes is simply to prevent transmission of the disease to livestock animals, in this case by the use of insecticides to knockdown tsetse. There is a potential situation where excessive use of insecticides provides protection against trypanosomiasis but prevents exposure to TBD during the period of innate resistance, leaving animals vulnerable to develop extreme pathology should they meet tick-borne diseases later in life. This was seen when unrest in Zimbabwe stopped tick control programmes and over a million cattle died of TBD (Peter *et al.*, 2005). This theoretical relationship has been modelled (Eisler *et al.*, 2003) and explained in Figure 1-4.



**Figure 1-4:- Conceptual model of clinical disease over increasing forces of infection (adapted from Eisler *et al.* (2003))**

Figure 1-4 shows the output for a conceptual model linking the force of infection to disease index for TBD (green) and trypanosomiasis (red). The y-axis is a representation of the disease incidence or morbidity in a population. The x-axis shows the force of infection, given for example by the number of feeds from an infected vector. Trypanosomiasis exhibits a logarithmic relationship where increased force of infection leads to increase disease. Tick-borne diseases in contrast exhibit a climax relationship at the level of disease challenge shown by line A. If animals exist in an environment where the disease challenge is higher, to the right of line A, then any reduction in the challenge will be beneficial at reducing trypanosomiasis but may, paradoxically, cause an increase of TBD. This is because as the force of infection drops, so does the probability that animals are infected by TBD as young-stock. Consequently the disease index can be higher in adult cattle, even though the overall force of infection is lower.

### 1.5.3 Restricted application of insecticide to cattle

In order to conserve the endemic stability in a cattle herd, yet still supply protection from trypanosomiasis, some elegant entomological research has offered several options for control. Both techniques use less insecticide by selecting certain application areas. Whilst this has obvious financial benefits, these techniques also minimise some of the environmental contamination that is associated with blanket applications, such as the detrimental effect on dung beetles (Vale *et al.*, 1999; Sommer *et al.*, 2001; Vale & Grant, 2002).

#### 1.5.3.1 Size matters

Tsetse show a bias towards feeding on older and larger animals, to the extent that in herds comprising a mixture of two oxen, four cows/steers and two calves, 80% (range, 67% - 91%) of meals were from the two largest animals and < 3 % of tsetse fed on the calves (Torr *et al.*, 2001). Consequently, avoiding treating the calves allows exposure to ticks and hence immunity to be acquired to TBD without compromising the efficacy of the herd at controlling tsetse. This can be taken a stage further, whereby only treating the two largest animals in the herd is was thought to kill 75% of the tsetse feeding off that herd (Torr *et al.*, 2002). This would at least halve insecticide costs and, given many livestock

owners tend to bias treatment towards the larger, more productive and valuable animals (Machila, 2004), may be widely adopted.

### 1.5.3.2 Sick cows? Treat their legs

Tsetse have preferences for where they feed on an animal. From observing cattle exposed to natural tsetse populations, various work has shown that 80% of *G.pallidipes* feed on the legs, and mostly the front legs, of cattle (Thompson, 1987; Torr & Hargrove, 1998; Vale *et al.*, 1999). Further work showed that 55% of tsetse feeds were from the lower third of the legs, or to put another way, over half the tsetse fed on about 2% of the body surface (Vale, 2003). Application of insecticide only to those areas of the animal favoured by tsetse offers many advantages. It can reduce the amount of insecticide used by 90% saving money, labour time, and, if spray insecticide is used, water. Not covering the entire animal with insecticide potentially mitigates the effect on tick populations, by allowing some challenge for ticks to establish and maintain endemic stability. The restricted application also puts less active ingredient on the animal and places it in a location unlikely to be licked off which may minimise dung contamination and unwanted environmental effects of insecticide.

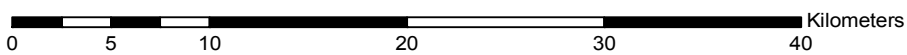
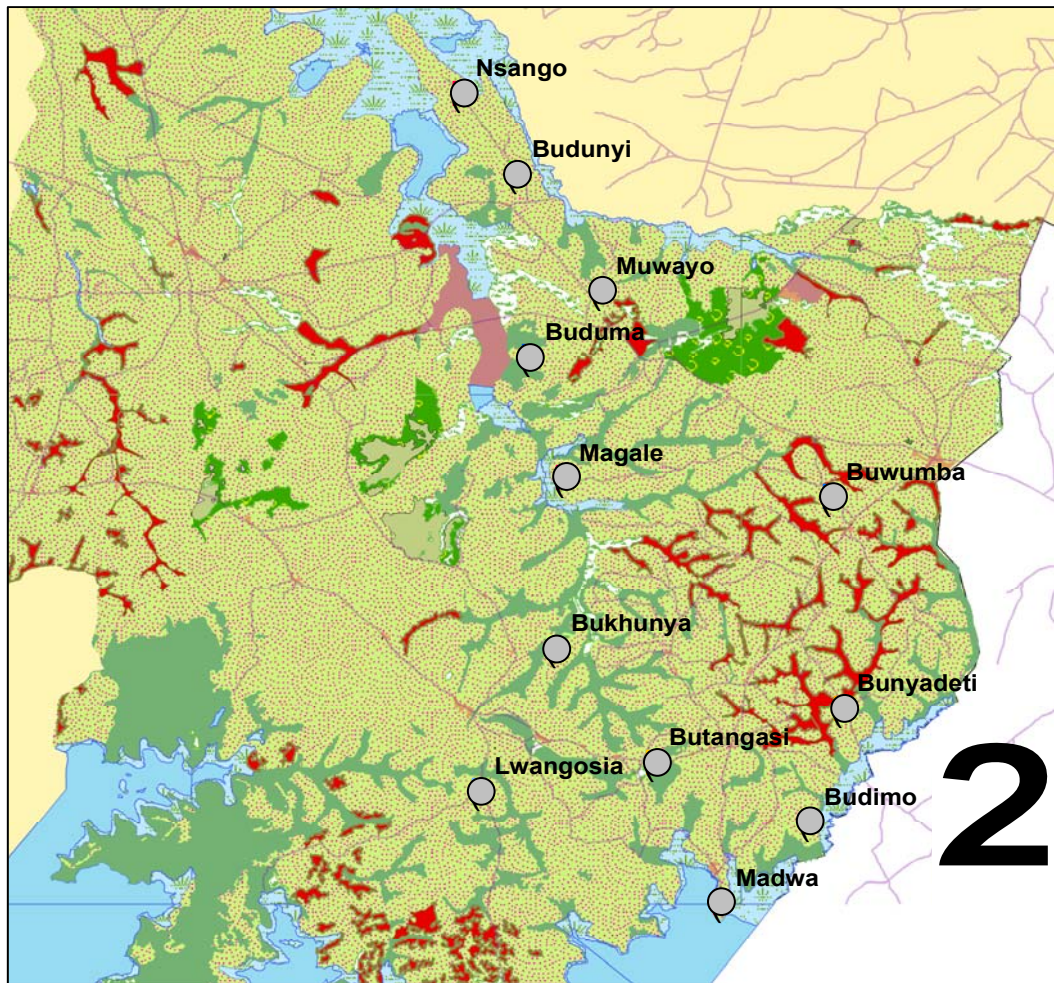
## **Chapter 2 :- Study design**

## 2.1 Design of the longitudinal study

The aim of this longitudinal study was to investigate the use of a restricted insecticide application protocol as a novel technique for the integrated control of endemic trypanosomiasis and tick-borne diseases under typical smallholder farming conditions in Uganda. The study aimed to evaluate the effect on parasite burden and animal health of chemotherapeutic interventions applied to cattle over a six-month time period in agro-pastoral production systems in villages in SE Uganda, and in specific to assess the effect of restricting the application of insecticide to those parts of the animal favoured by tsetse flies. This was compared with treating animals with the same insecticide in a pour-on formulation and with a single prophylactic dose of injectable trypanocide.

## 2.2 Study area

Two districts in the south-east of Uganda were chosen for the study. The study covered an area of approximately 1000km<sup>2</sup>, extending north from the shores of Lake Victoria to the southernmost swamps of the Lake Kyoga drainage system. Mixed crop-livestock farming is practised in these areas, with cotton forming the main cash crop and maize, finger millet, cassava, beans and sweet potatoes grown for home consumption (Okiria *et al.*, 2002b). The farming systems were deemed typical of the peri-lake regions of eastern Uganda. The area was known to be endemic for both human and animal trypanosomiasis (Hide, 1999; Fevre, 2002), and tick-borne diseases (Okello-Onen *et al.*, 1998b). The predominant tsetse species is *Glossina fuscipes fuscipes* (Moloo *et al.*, 1980; Lancien, 1991) although recent evidence suggested *Glossina pallidipes* is present in increasing densities (Magona *et al.*, 1997); (Magona *et al.*, 2005). The topography of the area was uniform high plain between 1050-1200m above sea level and dissected with a network of slow streams and ephemeral swamp. Mean annual rainfall ranged from 1250-1500mm on the shores of Lake Victoria to 100-1500mm around Tororo. Rainfall was generally well distributed over the year with peaks in April and September and relatively drier periods in January and July (Director of Lands and Surveys, 1962; Ndyabahinduka, 1993). Figure 2-1 shows a land use map of the area.



**Legend**

- Roads/Tracks
- Study villages
- Grassland, rangeland, open savannah, pastureland

**Landuse**

**Classification**

- Deciduous plantation or woodlot
- Coniferous plantation or woodlot
- Fully stocked trop. high forest
- Depleted tropical high forest
- Woodland (av. height > 4 metres)
- Bushland
- Wetland (swamps)
- Subsistence farmland (mixed, in use or fallow)
- Uniform farmland (tea or sugar estates)
- Urban or rural built up area
- Open water (large rivers, ponds and lakes)
- Impediments (bare rock and barren soil)
- Kenya
- No data

**Figure 2-1:-Landuse map of study area in S.E.Uganda. (Source data from (Uganda Forest Department, 1996)**



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Technical support for sample processing was available at the Ugandan Livestock Research Institute in Tororo (LIRI).

### 2.3 Cattle demographics

The vast majority of cattle in the area were East Africa shorthorn Zebu farmed by traditional management practices. Cattle owners in the area were sedentary agro-pastoralists who undertook no regular drug treatments to manage tick, tsetse or pasture-vector diseases. The cattle were grazed on communal pastures during the day, and tethered or corralled close to homesteads at night. The extent of daytime ranging was highly seasonal. During the dry seasons cattle may be driven several hours each day for water and grazing whereas in the wetter seasons cattle were permitted to graze in smaller herds closer to the homesteads. In both cases however, exposure to the ticks, tsetse and pasture transmitted helminths highly prevalent in swamp areas pose a considerable hazard to both cattle (de la Rocque *et al.*, 1999) and cattle drivers (Odiit *et al.*, 2006; Zoller *et al.*, in press).

### 2.4 Study site selection

The study required the comparison of three interventions with a control. Each intervention was to be repeated at three separate sites, and consequently twelve sites needed to be recruited. Potential sites had to meet these requirements:

- Established herds of cattle in the area and livestock management practices consistent across all sites.
- No recent, current or planned animal health interventions from government or non-governmental organisations, nor any community based programmes of vector control.
- Logistically possible to reach the site by 4 wheel-drive vehicle, within two hours of daybreak, to ensure the cattle could be sampled before they were taken to graze.
- Sites, and their potential grazing areas, had to be physically isolated from each other so that there would be no contact between groups.

In October and November 2002, 22 sites meeting these criteria were identified with the help of the local veterinary services. A minimum of 50 animals were sampled from each site and screened for trypanosomes by the haematocrit centrifugation technique, HCT,

(Bennett, 1962; Woo, 1970) and buffy coat technique, BCT (Murray *et al.*, 1977). In addition, 20 samples were randomly selected for subsequent processing for *T.brucei* by polymerase chain reaction (PCR) assay (described in section 3.4.2 ).

Table 2-1 shows the geographical coordinates and screening details from the initial visits, mapped in Figure 2-4. In general, the twelve villages showing the highest prevalence of trypanosomiasis by PCR were selected for the study, contingent on the sites remaining free of planned interventions when revisited and recruited in March 2003. Figure 2-2 shows trypanosome prevalences for the 22 screened sites and the selected villages.

Village name	District	Latitude WGS84	Longitude WGS84UTM	Number screened by microscopy	Number positive - HCT	Number positive - BCT	Total positive micro	Prevalence of all trypanosomes by microscopy	Number screened by PCR	Number positive for <i>T.brucei</i> by PCR	Prevalence of <i>T.brucei</i>	Selected for study?
Budimo	Busia	N0.28715	E34.03305	80	1	3	3	0.04	20	5	0.25	Yes
Buduma	Bugiri	N0.34749	E33.67280	50	2	3	3	0.06	21	3	0.14	Yes
Budunyi	Bugiri	N0.30726	E33.95576	100	4	4	5	0.05	20	3	0.15	Yes
Bukhunya	Busia	N0.37268	E33.91748	81	9	6	9	0.11	20	2	0.10	Yes
Bunyadeti	Busia	N0.34310	E34.04790	66	7	7	7	0.11	20	1	0.05	Yes
Butangasi	Busia	N0.53671	E33.89739	53	3	3	4	0.08	18	2	0.11	Yes
Buwumba	Busia	N0.44711	E34.04290	51	7	7	7	0.14	20	1	0.05	Yes
Lwangosia	Bugiri	N0.30239	E33.88262	50	5	8	8	0.16	20	1	0.05	Yes
Madwa	Busia	N0.24748	E33.99279	61	2	2	2	0.03	20	3	0.15	Yes
Magale	Busia	N0.43711	E34.03290	50	9	7	8	0.16	20	3	0.15	Yes
Muwayo	Bugiri	N0.54833	E33.93465	80	5	5	5	0.06	20	5	0.25	Yes
Nsango	Bugiri	N0.64531	E33.87403	80	2	3	3	0.04	20	3	0.15	Yes
Budola	Bugiri	N0.40459	E33.90210	81	4	4	4	0.05	20	1	0.05	No
Bufasi	Bugiri	N0.56718	E33.90976	56	5	6	6	0.11	20	0	0.00	No
Bufunda A	Bugiri	N0.59700	E33.87008	52	2	2	2	0.04	20	0	0.00	No
Bwoya East	Bugiri	N0.27323	E33.89039	80	9	9	9	0.11	20	1	0.05	No
Lugano	Bugiri	N0.54892	E33.95205	58	2	3	3	0.05	20	0	0.00	No
Maanga	Busia	N0.35943	E34.08184	53	1	1	1	0.02	20	0	0.00	No
Namaji	Bugiri	N0.44083	E33.87805	67	5	4	5	0.07	20	0	0.00	No
Nayenda Swamp	Busia	N0.34871	E33.93431	58	1	1	1	0.02	20	0	0.00	No
Rukaba	Bugiri	N0.26775	E33.95321	72	7	7	7	0.10	20	1	0.05	No
Sironyo	Bugiri	N0.56563	E33.94383	68	2	2	2	0.03	20	0	0.00	No

**Table 2-1:-Results of initial screening for sample selection.**

## Prevalence of trypanosomiasis in potential study sites

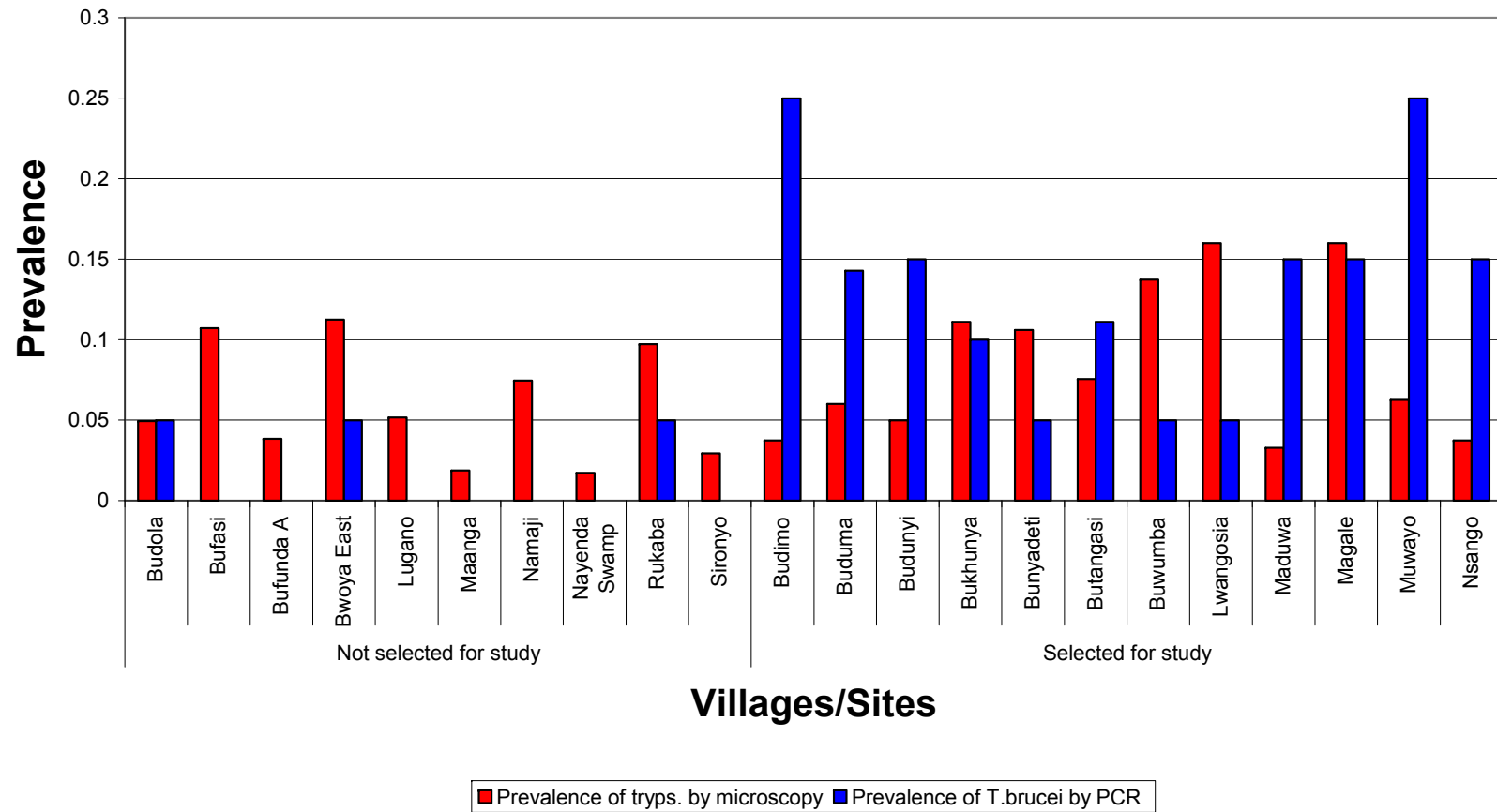
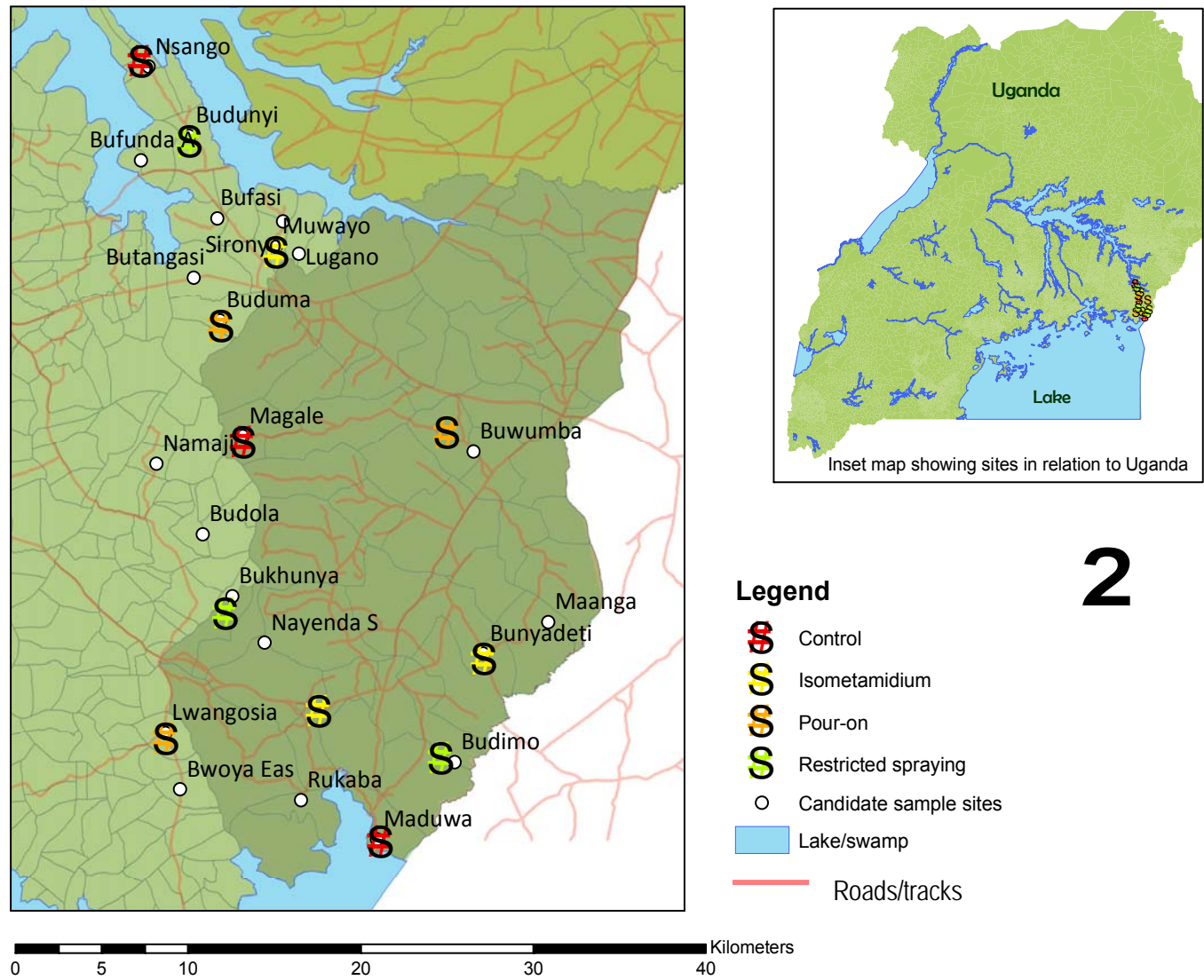
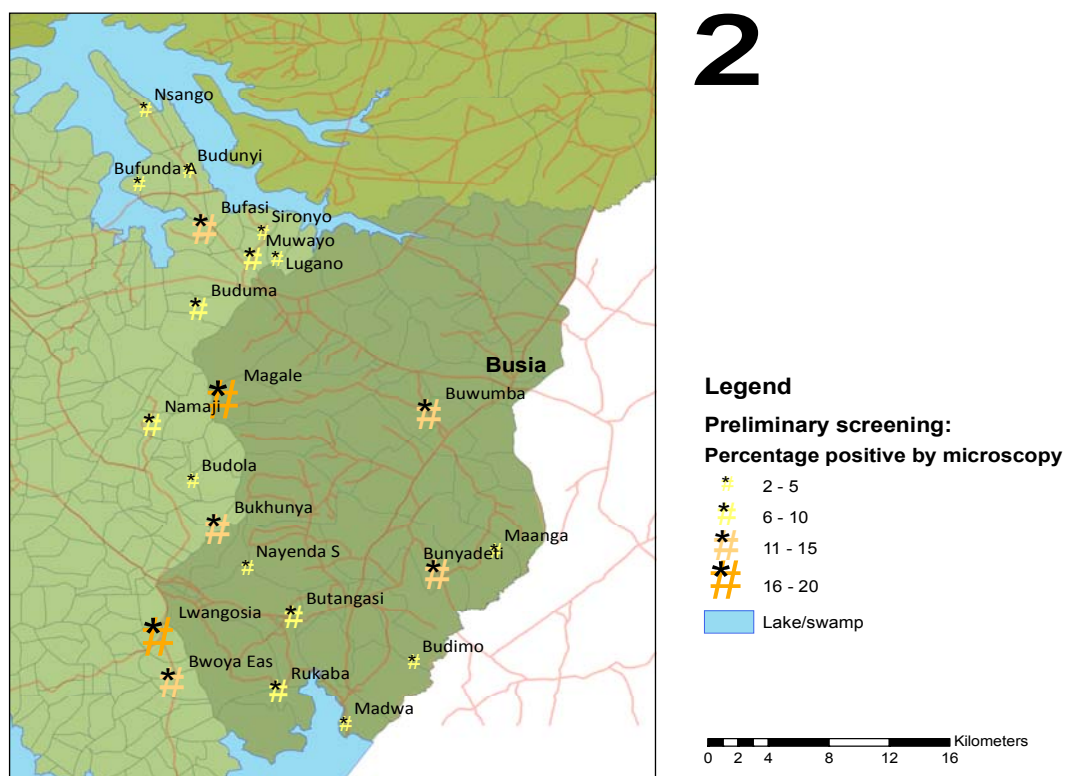
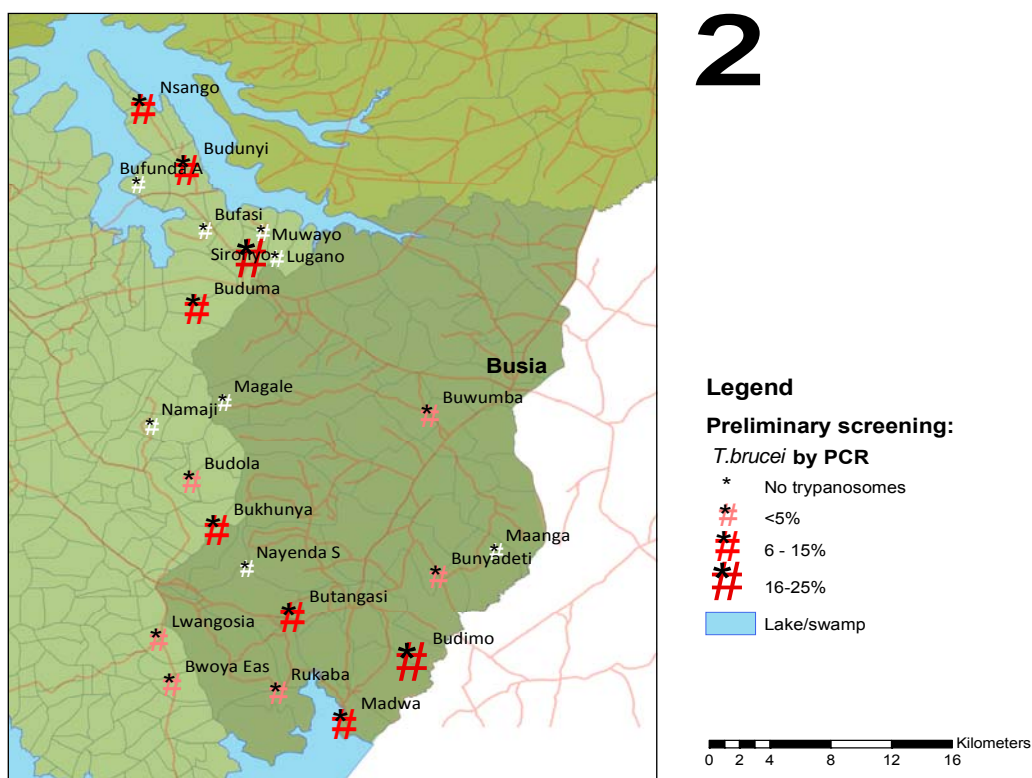


Figure 2-2:- Trypanosome prevalence in screened villages



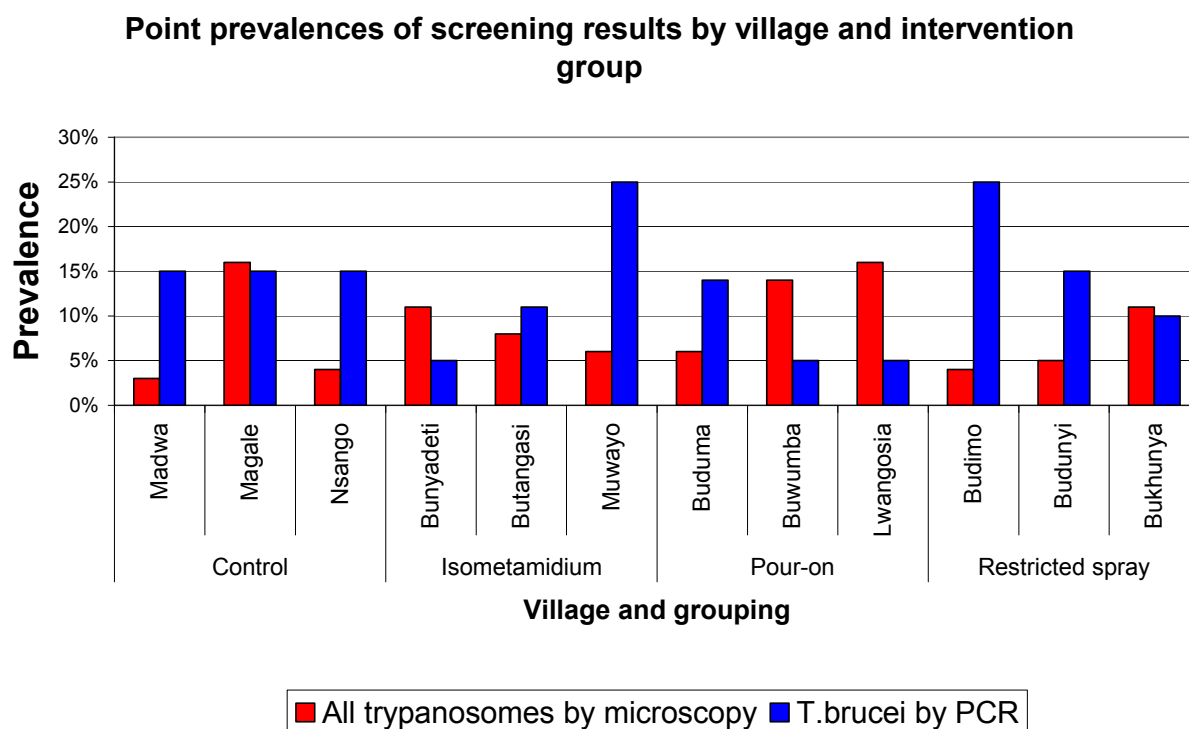
**Figure 2-3:- Location of study sites coded by intervention grouping.**



**Figure 2-4:- Map of trypanosome prevalence in potential study villages**

## 2.5 Village groupings

Initial baseline sampling revealed a fair degree of heterogeneity between the villages in terms of trypanosome prevalences (Figure 2-2). The study area covered the veterinary departments of two districts, Busia and Bugiri. It was decided therefore to stratify the villages by trypanosome prevalence and administrative district and form uniform groups based on this information. Each group was then allocated an intervention at random. Figure 2-5 shows the trypanosome prevalence by intervention group.



**Figure 2-5: Trypanosome prevalences by intervention group**



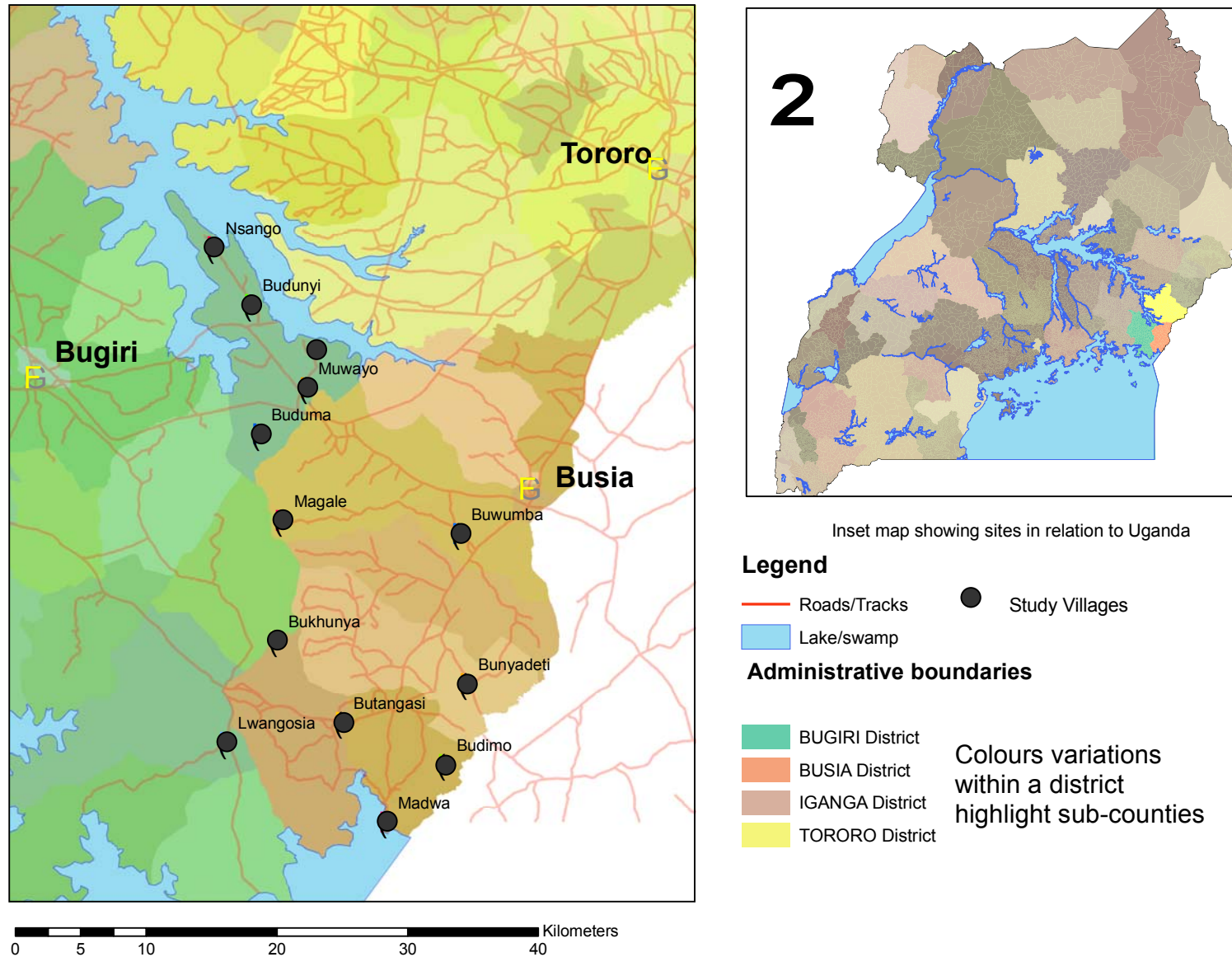


Figure 2-6:-Administrative boundary map of study area in S.E.Uganda. (Source data from UBOS 2002)



## 2.6 Calculation of sample size

In an ideal scenario, calculating the number of animals required to effectively test the hypotheses of interest are primary considerations in the design of a study. In actuality, the number of animals recruited is often constrained by the resources available and a more pragmatic approach need to be adopted (Crawley, 2005). In this study, factors such as stocking density, staff availability, sampling time, socio-political norms of host villages and the attrition expected from longitudinal studies all influenced the number of animals we recruited. The issue is whether the eventual sample size gave a reasonable chance of identifying the impacts under investigation.

The minimum number of animals,  $n$ , required to be recruited in each group can be estimated using the WinEpiscope program (Thrusfield *et al.*, 2001) using to the following equation for comparison of prevalences:

$$n = \left( t \frac{\sqrt{P(1-P)}}{l} \right)^2$$

Where  $n$ = sample size

$t$ = students T value, taken to be 1.96 for a 95% confidence level

$P$ = prevalence, taken to be 0.15 from screening results

$l$ = absolute level of precision or power, taken to be 5%

Given these parameters the minimum number of animals in each group has to be:

$$n = \left( 1.96 \frac{\sqrt{0.15(1-0.15)}}{0.05} \right)^2 = 196$$

Despite this admittedly post-hoc calculation of sample size, it appears that the decision to recruit between 70-80 cattle in each village, giving a group size of at least 230 animals, was sufficient to pick up the key impacts under investigation. It was recognized that in many cases a significant proportion of the total number of animals present in the area would be recruited although technically the above equation is only valid for sample size of < 10% of the population. Table 2-2 shows the demographics of the recruited villages and shows the percentages of cattle from each village recruited to the study. Figure 2-5 shows the age and sex structure of the cattle in each intervention group.

## **2.7 Recruitment of cattle into the study**

Prior to recruiting cattle for the study, a meeting was held in each selected site attended by local livestock keepers and members of the sampling team. The nature of the study was explained in addition to the intervention each village would receive. This provided an opportunity for cattle keepers to ask questions and decide if they wished to take part in the study. A communal grazing area was identified in each of the 12 villages which would serve as a sampling point and a local person was nominated to organise the farmers presenting their animals for sampling and act as a liaison between the village and the sampling team. A house to house questionnaire was carried out to establish the size of the village (represented by number of households) and the total number of cattle owned by the village. Results are tabulated in Table 2-2.

At the first sampling visit, cattle were selected for inclusion into the study. In situations where more cattle were presented than required for the study, animals were selected to attempt to represent the age and sex structure of the entire herd and include all cattle keeping households. This was done by requesting each livestock keeper presented animals they were not intending to sell of varying age and sex. All study cattle were ear tagged with a unique identifier consisting of the village code (Table 2-2) prefixing a two digit number between 01-80.

	Village Name	Village Code	Total of households	Total number of cattle kept in village	Total number of cattle recruited to study	Percentage	Number of livestock keepers in study
Control	Madwa	WA	42	406	70	17.2%	11
	Magale	MG	54	212	80	37.7%	33
	Nsango	NS	63	234	80	34.2%	22
	<b>Total</b>		<b>159</b>	<b>852</b>	<b>230</b>	<b>27.0%</b>	<b>66</b>
Isometamidium	Bunyadeti	YA	42	213	80	37.6%	28
	Butangasi	BT	30	267	80	30.0%	17
	Muwayo	YO	45	265	80	30.2%	38
	<b>Total</b>		<b>117</b>	<b>745</b>	<b>240</b>	<b>32.2%</b>	<b>83</b>
Pour-on	Buduma	DU	30	390	80	20.5%	20
	Buwumba	BW	44	245	80	32.7%	29
	Lwangosia	LW	39	164	75	45.7%	10
	<b>Total</b>		<b>113</b>	<b>799</b>	<b>235</b>	<b>29.4%</b>	<b>59</b>
Spray	Budimo	IM	32	362	80	22.1%	14
	Budunyi	BI	35	169	80	47.3%	26
	Bukhunya	BK	50	223	80	35.9%	15
	<b>Total</b>		<b>117</b>	<b>754</b>	<b>240</b>	<b>31.8%</b>	<b>55</b>
<b>Overall total</b>			506	3150	945	30.0%	263

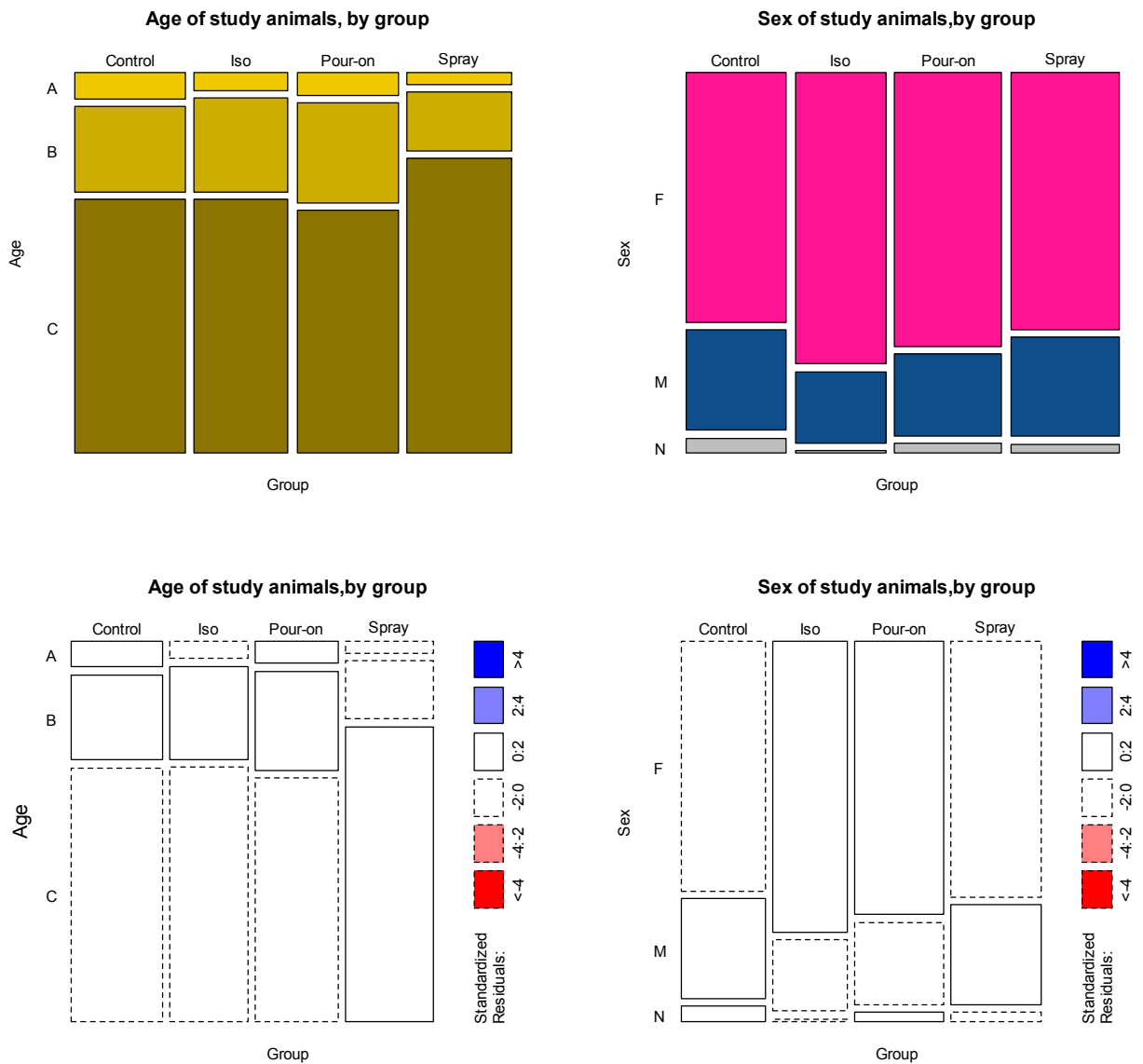
Table 2-2:-Demographics of recruited villages

## 2.8 Aging cattle

Cattle were aged using a combination of dentition scoring and information provided by the farmer. Adult cattle have 8 pairs of permanent incisors that begin erupting from 25-27 months of age and are complete by 4 years old. Dentition scoring measures are not precise as they are dependent on nutrition and inter-breed variability (Kikule, 1953). However they provided a useful indicator to support the information provided by the owner.

Age, in months	Terminology	Number of permanent incisor pairs	Age coding
0-8	Calf	0	A
9-27	Juvenile	0	B
25-35	Young adult	1	C
33-39	Adult	2	C
40-46	Adult	3	C
47 onwards	Adult	4	C

**Table 2-3: Age of cattle by teeth eruption. Age coding refers to classifications used in the study. Eruption times show considerable variability hence the overlap of age brackets.**



**Figure 2-7: Mosaic plot of age and sex structure of herd**  
Mosaic plots and their residuals are described in section 3.18. Age groupings are defined in Table 2-3, Sex F=female, M=Male, N=Neutered male

Figure 2-7 shows a mosaic plot of the age and sex structure of the animals recruited into the study. Group codings are as described in Table 3-1. The larger proportion of female to male animals in the study was representative of the overall herd structure apparent in the selected villages.

## 2.9 Initial treatment of all cattle with trypanocide.

All study cattle were treated with two doses of a chemotherapeutic trypanocide, diminazene aceturate, 42 (day -42) and 14 (day -14) days prior to beginning the interventions. This was done to attempt to clear any existing trypanosome infection. Diminazene aceturate was supplied as sachets of *Veriben*<sup>TM</sup> (CEVA Sante Animale) containing 1.05g active ingredient. The drug was reconstituted with distilled water according to the manufacturer's instructions and administered at a dose rate of 7mg/kg by deep intramuscular injection.

## 2.10 Interventions

Interventions began on day 0, 14 days after the second diminazene treatment, and each village was sampled every 28 days until day 112. Due to the availability of the field team, there was then a 35 day interval before the final visit on day 147. At each visit, presented animals were cast in lateral recumbency to aid clinically examination and sample collection. Samples collected are outlined in section 3.2.

Interventions details are given below and summarised in Table 2-4:

### 2.10.1 'Spray Group': Restricted application of a deltamethrin insecticide spray

All cattle were sprayed with a 1:1000 aqueous solution of 5% deltamethrin, (Decatix<sup>®</sup>, Coopers) using a 10 litre pump-pressurized knapsack sprayer capable of delivering the solution in fine droplet form.

20% of the volume advised for whole body treatment was applied. For a 200kg animal this was 400ml of solution. Saturation of the hair coat to the point of runoff was achieved with this dosage volume.

The application was only applied to the front legs, belly and ears of the animal. Figure 2-8 shows a cartoon of the area sprayed.

Cattle were retreated every 28 days for the duration of the study.

### 2.10.2 'Pour-on Group': Application of a pour-on deltamethrin insecticide

All cattle were treated with a 1% deltamethrin pour-on formulation (Spot-On<sup>TM</sup>, Coopers)

Application was at the recommended rate of 1ml/10kg applied topically along the spine of the animal.

Cattle were retreated every 28 days for the duration of the study.

### 2.10.3 'Isometamidium Group': Single administration of a prophylactic trypanocide

All cattle treated on day 0 with the prophylactic trypanocide isometamidium chloride hydrochloride 2% solution (Veridium®, Ceva.)

Animals were treated at a dose rate of 1mg/kg by deep intramuscular injection into the middle third of the neck region.

In an attempt to minimise the tissue reactions, no more than 15ml of solution was injected at any one site. Animals over 300kg thus require a divided dose.

No further routine treatments were administered on subsequent visits.

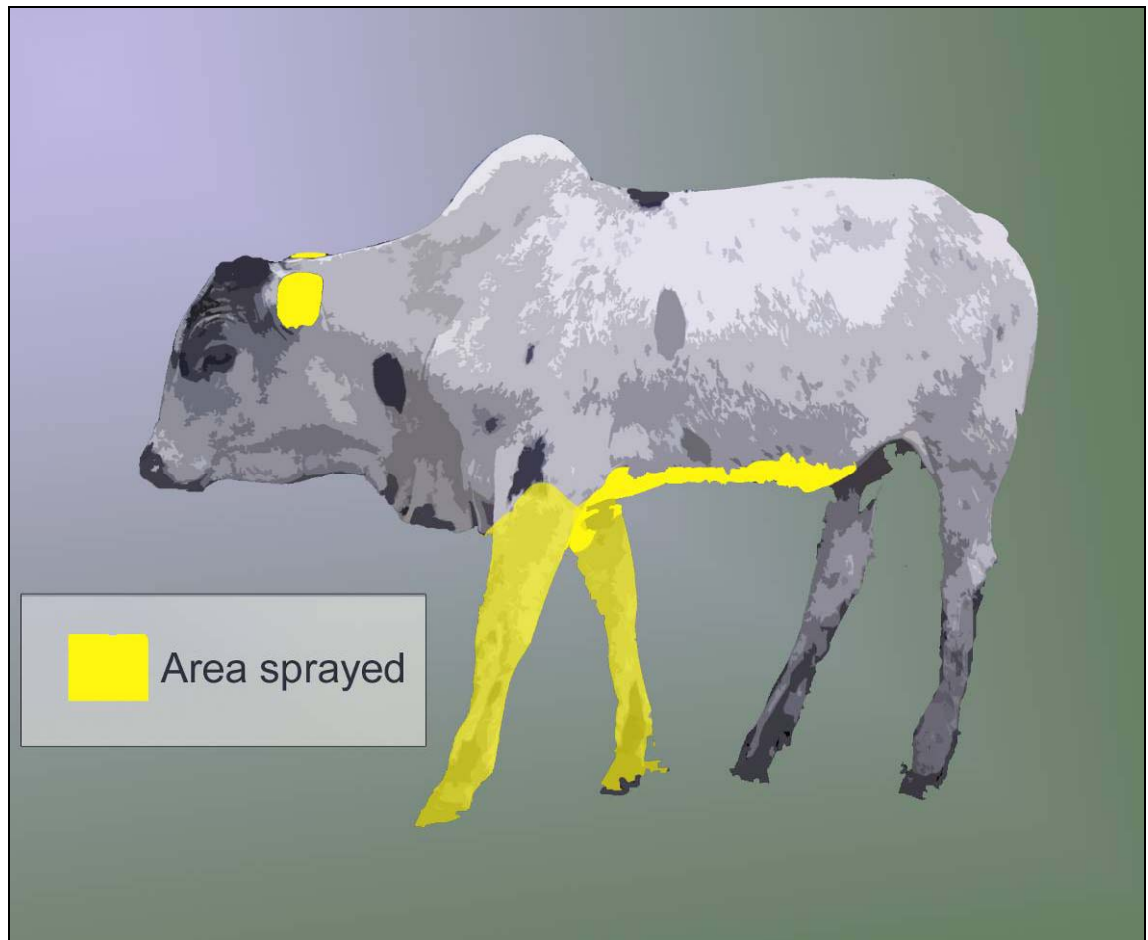
### 2.10.4 'Control Group': No treatments

From Day 0, the control group received no further interventions.

Visit number		1 (Baseline)	2	3	4	5	6	7	8	
Day of study		-42	-14	0	28	56	84	112	147	
Group	Village									
Control	Madwa	All cattle treated with diminazene aceturate @ 7mg.kg	All cattle treated with diminazene aceturate @ 7mg.kg	No treatment	No treatment	No treatment	No treatment	No treatment	No treatment	
	Magale									No treatment
Nsango	Injection of isometamidium chloride @ 1mg/kg			No treatment	No treatment	No treatment	No treatment	No treatment		
Iso									Bunyadeti	Application of deltamethrin pour-on
	Butangasi			Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	
Pour-on	Buduma	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray							Restricted application of deltamethrin spray
	Buwumba			Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	
Spray	Lwangosia	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray							Restricted application of deltamethrin spray
	Budimo			Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	
Budunyi	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray							Restricted application of deltamethrin spray
Bukhunya				Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	Restricted application of deltamethrin spray	

Table 2-4: Outline of drug treatments applied during the study





**Figure 2-8:-Cartoon showing extent of sprayed areas for restricted application on front legs, ears and belly.**

## **Chapter 3 :-** Methods used for data collection and analysis

### 3.1 Data collection

A general clinical examination was conducted on all cattle presenting during the visits. Examinations were made by a veterinarian, either the author or Dr Charles Waiswa, on animals cast in lateral recumbency prior to sampling. The list of clinical variables assessed and the levels used to record the data are given in Table 3-1.

### 3.2 Field based examination techniques

#### 3.2.1 Body weight

Body weight was recorded for all cattle at the beginning (day -42) and end (day 147) of the study, and for the isometamidium group at day 0 to ensure correct dosing. Weight was estimated using a weighband.

#### 3.2.2 Clinical assessment

Each animal was assessed for signs of a rough or 'staring' coat, an indication of poor condition linked to chronic trypanosomiasis (Machila *et al.*, 2003). In addition, the skin was assessed for indications of trauma, burns, photosensitisation, evidence of *Dermatophilus congolense*, *Parafilaria bovicola* or capripoxviruses (lumpy skin disease). Superficial lymph nodes (femoral, prescapular, parotid and sunbmadibular) were palpated and an overall assessment of lymphadenopathy made on a four point scale (normal, or mild, moderate or severe lymph node enlargement). Any discharges were noted in terms of site (ocular, nasal, vaginal), type (clear, sero-sanguineous, purulent) and severity (mild, moderate, or severe). Diarrhoea was graded on a similar four point scale.

#### 3.2.3 Tick counts

The number of skin-attached adult stages of *Amblyomma variegatum*, *Boophilus decoloratus* and *Rhipicephalus appendiculatus* on half the body surface was recorded. The information

was coded according to a tick score for each species: 0 = no ticks, 1=1-10 ticks, 2=11-50 ticks, 3=more than 50 ticks.

### 3.2.4 Condition scoring

Condition scoring is a well established indication of production performance. Changes in an animal's weight do not necessarily provide consistent information about the condition of an animal due to variations attributable to skeletal size, hydration levels, gut fill, or pregnancy status (Bartholomew *et al.*, 1994; Moran, 2005). Although of particular relevance to dairy cattle, the technique is equally applicable to any breed. It is a visual assessment of the amount of fat and musculature covering the bones of the animal and has been shown to be highly repeatable and consistently reproducible between sufficiently trained scorers (Nicholson & Sayers, 1987). Condition scoring was performed as described by Nicholson & Butterworth (1986) using a nine point scale specifically designed for Zebu cattle. An individual is scored to one of three main categories, lean (L), medium (M) or fat (F), each grading further subdivided into three (-, or ., or +), thus forming a nine point scale. This is coded with integers from 1-9, 1 representing the lowest condition ration of L-, 9 the highest F+. This scale can approximate to a continuous distribution for modelling purposes.

Identification variables	Description of factor levels	Factor name in models	Type of variable	Coded factor levels
Tag Number	Alphanumeric code	Tag.No	Nominal	Unique to animal
Village Name	Text	Village.Name	Nominal	Text
Intervention Group	No intervention, Single isometamidium treatment, Pour-On deltamethrin application, Restricted application of deltamethrin	Group	Nominal	Control, Iso-1, Pour-on, Spray
Cattle owner's name	Text	Owners.Name	Nominal	Text
Sampling visit number	1 to 8	Visit.Number	Ordinal	1-8
Time from start of interventions	-42 to 147	Day	Continuous	-42,-14,0,28, 56,84,112,147
Predominant coat colour	Black/Grey/ Black+White/ Brown/ Brown+White/Mixed	Colour	Nominal	Text
Age	Calf/ Juvenile/ Adult	Age	Nominal	A, B, C
Sex	Female/Male/ Male Neuter	Sex	Nominal	F,M,N

**Table 3-1: Data recorded for each animal in study**

Clinical examination	Description of factor levels	Factor name in models	Type of variable	Coded factor levels
Weight	kg	Weight	Continuous	Numeric
Condition score	L-/L/L+/M-/M/M+/F-/F/F+	Cond.Score	Continuous	1,2,3,4,5,6,7,8,9
Coat condition	Normal/ Staring	Staring.Coat	Nominal	0,1
Skin condition	Normal/ <i>Dermatophilus congolensis</i> traumatic wound, /burns, photosensitisation/ lumpy skin	Dermatophilus	Nominal	0,1
		Skin	Nominal	Text
Lymph node enlargement	Normal/Mild/Moderate/Severe	LN	Ordinal	0,1,2,3
Half body tick count, <i>Rhipicephalus appendiculatus</i>	No ticks/ 1-10 ticks/ 11-50 ticks/ >50 ticks	Rh	Ordinal	0,1,2,3
Half body tick count, <i>Boophilus decoloratus</i>	No ticks/ 1-10 ticks/ 11-50 ticks/ >50 ticks	Amb	Ordinal	0,1,2,3
Half body tick count, <i>Amblyomma variegatum</i>	No ticks/ 1-10 ticks/ 11-50 ticks/ >50 ticks	Boo	Ordinal	0,1,2,3
Blood haemoglobin level	g/dl	Hemocue	Continuous	Numeric
Presence of diarrhoea	No diarrhoea, Mild/moderate/severe	Diarrhoea	Ordinal	0,1,2,3
Presence of discharge	No discharge, Mild,/moderate/severe Site of discharge	Severity	Ordinal	0,1,2,3
		Site	Nominal	Text

Table 3-2: Data collected from each animal at each sampling point

### 3.2.5 Measurement of Haemoglobin

A handheld digital haemoglobin meter developed for use in the human medical field (HemoCue, AB, Angelholm, Sweden) was recently positively evaluated for its application as a field tool to quantify bovine haemoglobin (Magona *et al.*, 2004b). The device was used in this study with a set method. A small amount (10µl) of whole blood from a lancet prick to an auricular vein was drawn into a customised microcuvette by capillary action. The microcuvettes required for the test are produced to provide a fixed (0.13mm) light path between parallel, i.e. non-refractive, walls. Additionally, the inner cavity of the microcuvette contains the dry reagents necessary to convert haemoglobin into azide methaemoglobin. Following visual inspection to ensure the cuvette contained no air bubbles or external contaminants, it was placed into the HemoCue instrument for analysis. The HemoCue calculates the concentration of haemoglobin from the differential absorption of two wavelengths of light (565 and 880nm) shone through the sample and displays the result (in g/dl) within a few seconds. Although the device needed to be used out of direct sunlight, it provided a robust and technically simple method of measuring the haemoglobin of bovine blood samples in the field.

## 3.3 Laboratory diagnostic techniques

### 3.3.1 Faecal analysis

A worm egg count (WEC) was carried out for each animal at each sampling point. Samples were taken from the rectum and placed in separate plastic bags, labelled and transported to LIRI for analysis. Samples were examined for strongylid eggs using the McMaster technique described in Hansen & Perry (1994). In summary, a salt solution containing 4g sodium chloride and 5g sucrose per 10ml water was added to a faecal sample, thoroughly mixed and strained. Examination of the filtrate under 10x objective in a Macmaster chamber allowed for a quantification of the egg burden. Counts below 400 eggs/g were considered subclinical, over 1000 eggs/g a heavy nematode infection (Hansen & Perry, 1994). Trematode egg

burden was assessed by the sedimentation qualitative technique, also described in Hansen & Perry (1994). Trematode eggs are dense, and the technique removes the lighter supernate of faecal material and the eggs are stained with 5% methylene blue and visualised by microscopy. An animal was deemed to be infected if any *Fasciola spp.* or *Schistosoma spp.* eggs were found.

### 3.3.2 Blood smear analysis for haemoparasites

A blood sample was collected using a microhaematocrit capillary tube and a lancet prick to the marginal ear vein. A drop of blood was applied to a clean microscope slide and thick and thin smears made on the same slide. The slide was labelled with the animal's tag number using an indelible marker and transported to LIRI for analysis that day.

The thin blood film was stood in a fresh solution of methanol for 3 minutes to fix the sample, followed by the immersion of the thick blood film area of the slide in distilled water for 5 minutes to cause erythrolysis. The blood smears were dried and stained in a 10% Giemsa solution phosphate buffered to pH 7.2. Following rinsing, air drying and covering with a mountant and coverslip, the samples were examined by microscopy under 100x oil immersion objective. Fifty microscopic fields were examined for each sample.

Samples were screened for *Theileria spp.*, *Anaplasma spp.*, *T.brucei*, *T.congolense* and *T.vivax*. Trypanosome species were identified according to morphological forms (Uilenberg, 1998) and the intensity of parasitaemia scored: 0=no parasites detected, 1=1 organism found every 10 or more fields, 2= one organism found every 2-10 fields, 3=one organism found per field.

### 3.4 Molecular diagnostic techniques

Diagnostic methodologies of haemoparasites using molecular techniques based on the polymerase chain reaction (PCR) are able to demonstrate the presence of DNA sequences specific to the organism in question. Polymerase chain reaction techniques present a diagnostic option with potentially excellent diagnostic specificity and sensitivity. The



question of what a positive result means in epidemiological and clinical terms is discussed in Chapter 4. In molecular terms, a positive result is taken to mean viable parasites were present in the blood of the host at the time of sampling.

Polymerase chain reaction techniques involve the iterative amplification of a particular DNA sequence specific to the parasite such that over the course of 20 cycles a single strand of parasitic DNA can undergo a  $10^6$  amplification. The DNA can then be separated by size using electrophoresis and the resultant banding pattern stained and visualised.

The ability of this technique to amplify minute amounts of parasite material allow for the identification of low parasitaemias with high analytical specificity. The test can also be very sensitive; of the order of 1 trypanosome per 10 ml of cattle blood (Masake *et al.*, 2002). Targeting multiple copy genes or using a nested PCR can increase the sensitivity of these techniques, as can using concentration techniques such as DNA extraction (Chomczynski *et al.*, 1997) or centrifugation and amplification of the buffy coat layer (Picozzi *et al.*, 2002).

### 3.4.1 Preparation of samples

Whole blood samples were collected from an auricular vein using a lancet and a 100 $\mu$ l microhaematocrit capillary tube and immediately applied in a spiral pattern on Whatman FTA<sup>®</sup> filter paper. The cards were allowed to dry thoroughly at ambient temperature. Four samples could be collected per card. Each card was labelled with the animal IDs, location, date, and visit number of the sample, placed in foil pouches with a silica desiccant and mailed back to the UK.

### 3.4.2 FTA<sup>®</sup> Cards

FTA<sup>®</sup> cards (Whatman BioScience, Cambridge, UK) are a storage medium designed to simplify the collection, transport, analysis, and archiving of DNA from a range of sources, including whole blood and tissue. The card is a paper matrix impregnated with protein denaturants, chelating agents and free radical traps. When a sample is applied to FTA<sup>®</sup> Cards, cells are lysed and nucleic acids immobilised and stabilised within the FTA<sup>®</sup> Card's matrix.

Once the sample is dry, the cards can be shipped, by mail if necessary, without the requirement for refrigeration or the hazard packaging normally associated with the transportation of biological materials. The cards can be stored at room temperature without sample degradation for at least 14 years (Whatman, 2004).

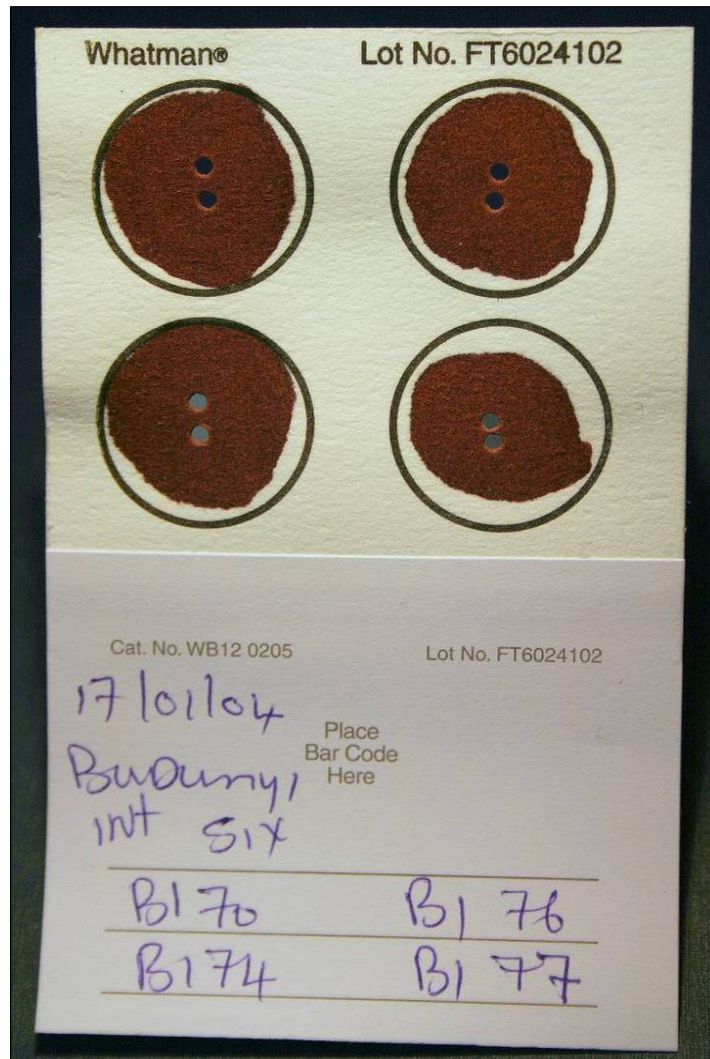


Figure 3.1: FTA<sup>®</sup> card with four blood samples. Holes show the discs punched out for screening.

### 3.4.3 Sample processing

Deoxyribonucleic acid (DNA) is bound to the FTA<sup>®</sup> card so PCR amplification is performed directly on small discs cut from the card, washed, dried and placed in the reaction tube. The FTA<sup>®</sup> card was placed on a mat and a 2mm circular disc of sample saturated FTA<sup>®</sup> matrix cut from the card using a Harris Micro Punch<sup>™</sup> tool (Whatman Bioscience, Cambridge). Each disc was transferred into a 1.5ml eppendorf tube for washing. Two discs were cut out from each sample and each disc was transferred to a separate eppendorf tube. To prevent cross contamination the tool was cleaned by punching a blank filter card between samples.

Whole blood contains potent PCR inhibitors; immunoglobulin G in plasma, haemoglobin in erythrocytes and lactoferrin in leukocytes (Al-Soud, 2000). Prior to DNA amplification, each disc had to be washed to remove these compounds from the sample. Discs were prepared according to the manufacturer's instructions. Each disc was washed three times for 10 minutes in 200µl of FTA<sup>®</sup> purification reagent (Whatman Bioscience, Cambridge, UK) and rinsed twice for 5 minutes in 200µl of 1 mM Tris-EDTA buffer (Sigma Aldrich, Dorset, UK) to remove traces of FTA<sup>®</sup> buffer. Each sample was then transferred into a clean PCR tube and dried at 35°C for 45 minutes before performing the PCR reaction. A blank disc was included in the washing and in the PCR to act as a negative control.

Target species	Code	Primer Sequence	Supplier	Size (bp)	References
<i>Trypanozoon (T. brucei)</i> <i>T. vivax</i> <i>T. congolense</i> (River/Forest) <i>T. congolense</i> (Kilifi) <i>T. congolense</i> (Savannah) <i>T. congolense</i> (Tsavo) <i>T. simiae</i> <i>T. evansi</i> <i>T. theileri</i>	ITS-1 ITS-2 ITS-3 ITS-4	5' GAT TAC GTC CCT GCC ATT TG-3' 5'- TTG TTC GCT ATC GGT CTT CC-3' 5'- GGA AGC AAA AGT CGT AAC AAG G-3' 5'- TGT TTT CTT TTC CTC CGC TG-3'	MWG Biotech	1215 620 1501 1430 1408 951 847 550 998	(Cox <i>et al.</i> , 2005)
Amplification conditions : 95°C for 7 minutes (1 cycle)→ 94°C for 60s, 55°C for 60s, 72°C for 120s (35 cycles)					

Table 3-3: Data for ITS-PCR screening protocol

Target species	Code	Primer Sequence	Supplier	Size (bp)	References
<i>T. brucei</i>	TBR1 TBR2	5' AGA ACC ATT TAT TAG CTT TGT TGC-3' 5'-CGA ATG AAT ATT AAA CAA TGC GCA GT-3'	MWG-Biotech	177	(Moser <i>et al.</i> , 1989), (Artama <i>et al.</i> , 1992)
Amplification conditions : 94°C for 3 minutes (1 cycle)→ 94°C for 60s, 55°C for 60s, 72°C for 30s (30 cycles)					

Table 3-4: Data for PCR protocol for screening *T. brucei*

#### 3.4.4 Detection of *T. brucei* trypanosome prevalence by PCR

The individual prevalence of *T. brucei* was investigated during the set up of the longitudinal study (section 2.4). *T. brucei* alone was amplified using a set of species specific primers as listed in Table 3-4. The target is a non coding 177bp satellite DNA repeat sequence (Sloof *et al.*, 1983). Post washing and drying, discs were ready for PCR amplification. PCR amplifications were carried out in 25µl reactions, each containing the following reagents: Sigma Aldrich PCR buffer (10mM TrisHCl pH 8.3, 50mM KCl, 1.5mM MgCl<sub>2</sub>, and 0.001% gelatine), 200µm of each of the 4 deoxynucleotide triphosphates, dNTPs (Sigma Aldrich), 1 Unit of *REDTaq* DNA polymerase recombinant (Sigma Aldrich) and 0.4 µM of each TBR primer. Positive control was provided by 1 µl of *T. brucei* (ILtat 1.2). Thermal cycling conditions were as listed in Table 3-4. An initial denaturation step of 3 minutes at 94°C followed by 30 cycles of 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 30 seconds. The reaction concluded with a final extension step of 5 minutes at 72°C. Reactions were carried out in a DNA Engine DYAD™ Peltier Thermal Cycler. Twenty microlitres of the resultant product was visualised by electrophoresis as detailed below.

#### 3.4.5 Detection of all trypanosome prevalences by ITS-PCR

The internal transcribed spacer polymerase chain reaction (ITS-PCR) protocol targets the internal transcribed spacers region located within the genes encoding the ribosomal RNA subunits. It is able to identify and differentiate all clinically relevant African trypanosome species listed in Table 3-3. A high copy number combined with inter-species variation in the length of the ITS region allows for identification and, importantly, species differentiation of trypanosomes. Sensitive enough to detect a single parasite, a unique size of PCR product is produced for each species of trypanosome. Initially demonstrated by (Desquesnes *et al.*, 2001), (Cox *et al.*, 2005) refined the technique to improve the detection of *T. vivax* and the resultant protocol uses a simple nested PCR optimised for whole blood samples collected on FTA® cards. Figure 3.3 shows the amplification regions of three hypothetical trypanosome species.

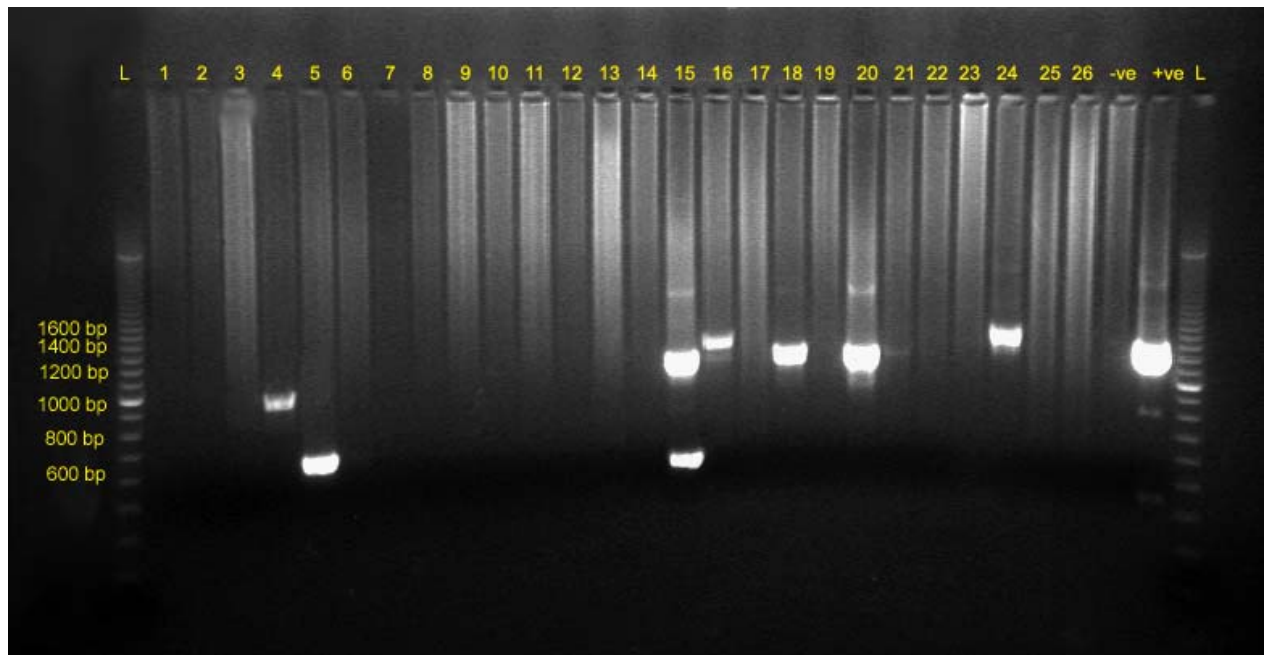
### 3.4.6 ITS-PCR methodology

Primer sequences for the ITS protocol are listed in Table 3-3. Each washed and dried disc was placed directly in PCR tubes. The reaction volume of 25 µl reactions each contained the following reagents: Super Taq PCR buffer from HT Biotechnologies, Cambridge (10mM TrisHCl pH 9.0, 50mM KCl, 1.5mM MgCl<sub>2</sub>, and 0.1% Triton X-100 and 0.01% (w/v) stabilizer), 200µm of each of the 4 deoxynucleotide triphosphates, dNTPs (Sigma Aldrich), 1.25 Units of *REDTaq* DNA polymerase recombinant (Sigma Aldrich) and 0.2µM of each outer primer ITS1 and ITS2. Positive control was provided by 1 µl of *T.brucei* (ILtat 1.2) and *T.vivax* (ILDat 1.2). Thermal cycling conditions were as listed in Table 3-3. An initial denaturation step of 7 minutes at 95°C was followed by 35 cycles of 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 120 seconds. Thermal cycling was carried out on a Strategene Robocycler.

For the second (nested) reaction, 1µl of the PCR product from the first round reaction was placed in a fresh tube and 24µl of the reaction mixture as listed for the outer primers above, with the exception of the substitution of the outer primers ITS1 and ITS2 with the inner primers ITS3 and ITS4. Twenty micro-litres of the resultant product was visualised by electrophoresis as detailed below.

### 3.4.7 Amplicon visualisation using electrophoresis

Samples were electrophoresed on 30x20cm 1.5% agarose gels. 10ml of Tris Borate EDTA 10x concentrate (Sigma Aldrich) was added to 90ml of distilled water, to which 0.005mg of ethidium bromide was added. This formed the buffer solution. 1.5g of agarose (Sigma Aldrich) was added to the buffer solution, dissolved by heating and allowed to cool until set. Twenty micro-litres of 100bp molecular weight marker 'ladder' (Bioline) was dry loaded into the first, middle and last well followed by 20µl of each PCR reaction, the positive and negative controls. The gel was placed in a gel tank (Sigma Aldrich) containing buffer solution and electrophoresed at 100v and visualised using a Flowgen Alpha 1220 gel imaging system and BioRad Gel Doc 2000 imaging software. Figure 3.2 shows a representative gel with illustrated band sizes.



**Figure 3.2: Representative gel showing bands obtained from PCR amplification using ITS protocol.**

**L represents a marker graduated in 100bp intervals (band sizes illustrated). Samples 15, 18 and 20 are positive for *T.brucei*, Samples 16 and 24 positive for *T. congolense*, samples 5 and 15 positive for *T.vivax* and sample 4 for *T. theileri*. Sample 15 thus shows a mixed T.b./Tv. Infection.**

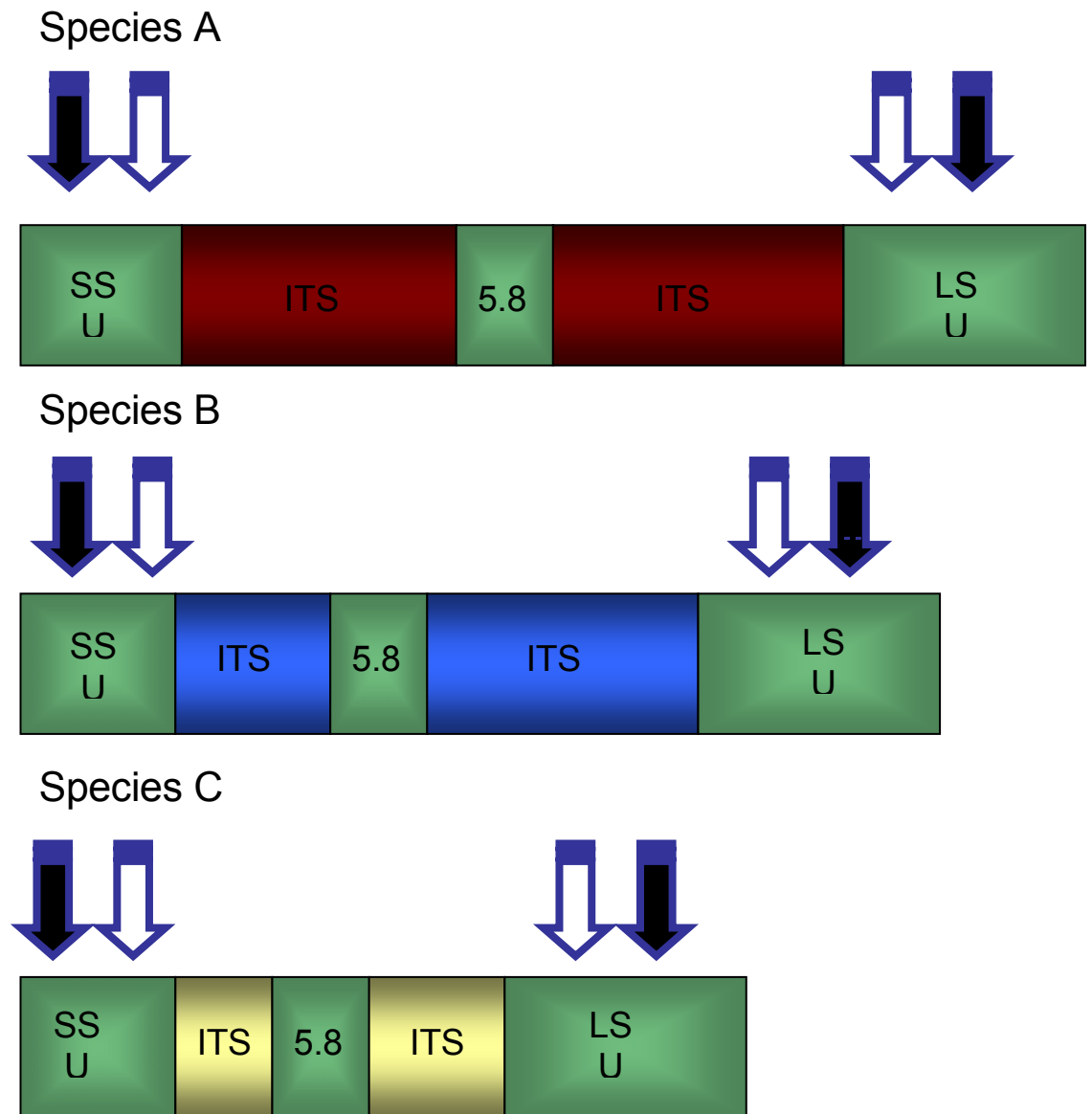


Figure 3.3: Cartoon showing the structure of part of the ribosomal RNA gene locus.

Present in tandem arrays of 100-200 copies, each gene consists of conserved coding regions (green) and non coding spacer regions (ITS $\alpha$  & ITS $\beta$ ). The ITS regions vary between species. Nested primers designed to the conserved regions are represented by the black arrows (outer primers) ITS1 and ITS2 and the white arrows (inner primers) ITS3 and ITS4



## 3.5 Techniques for data analysis

### 3.5.1 “All models are wrong, some are useful”

This quote by industrial statistician George Box (Box, 1979) is a tongue-in-cheek appraisal of the issues with modelling, and is of particular relevance to data generated by observational longitudinal studies. An unbalanced dataset, animal drop out and diagnostic variability are just three factors that promised to make a very ‘noisy’ dataset. There were two simple aims in analysing the data in this thesis 1) to test the significance of various biological and management factors on the prevalence and incidence of parasitic infections, and 2) to quantify the efficacy of different treatment protocols in terms of a range of diagnostic and clinical variables. The models used have been selected to answer these questions.

## 3.6 The R statistical environment

All modelling was carried out in using the R statistical environment (R Foundation for Statistical Computing, 2006). R is a language and environment for statistical analysis and graphics production. It is an open source version of the S language and environment and, although there are some significant differences, much code written for S runs unaltered under R. R is freely available through the internet as a General Public Licence. Some specific statistical processes require additional packages. Known as libraries, these add-on functions are freely downloadable and, when used in the thesis, are specified by name. For an introduction to the R programming language there is copious information available through <http://www.R-project.org> or in the introductory reviews (Dalgaard, 2002; Venables & Smith, 2006). Throughout the thesis, salient extracts of code are included as follows:

Text in this font represents commands or code executable in the R environment.

## 3.7 Types of models used

Different types of models were fitted depending on the structure of the data. Generalised linear models (GLM) were fitted to non-clustered data. Linear mixed effect models (LME) provided a method of analysing clustered data with a continuous outcome

variable. Generalised linear mixed effect models were employed to model clustered count or binary data.

### **3.8 Structure of the data from longitudinal studies**

Longitudinal studies are defined by the repeated measurements of the same subjects over time, thereby allowing for the direct study of change in an individual. Investigation of the way a subject changes is indeed only possible by repeatedly sampling the individual through time. Cross sectional studies in contrast can only provide information about the differences between individuals, and provide no way of separating variation within a individual from variation between different individuals (Fitzmaurice *et al.*, 2004).

Data derived from longitudinal studies are very likely to exhibit clustering (Liang & Zeger, 1986). In the context of this thesis, samples were taken from the same animals at different time points, and from animals in villages based in different geographical areas. Consequently this data is clustered in both space and time. Temporal clustering is likely due to the positive correlation of repeated measures from the same individual, i.e. measurements taken from the same individual are likely to be more similar than measurements taken from different individuals. Spatial clustering is likely because it is reasonable to expect animals from the same geographical area will be exposed to similar environmental conditions and livestock management to the degree that variation within villages will be less than variation between villages. Analysis of the data has to take these sources of clustering into account.

#### **3.8.1 Sources of variation in longitudinal studies**

Mixed effect models provide a method of analysing clustered data. The purpose of the analysis of longitudinal data is to separate the effect due to treatment from the ‘noise’ generated by the intrinsic random variation of subjects in time and space. In order to model the effect of the treatment effectively, it was necessary to account for the following three sources of random variation:

1. Animal effects: The animals in the study have been sampled from a population, and their response to an intervention may show a stochastic variation between individuals. The response is likely to vary between individuals as a result of a number of unmeasured variables, genetics, immune status, clinical history etc., which make some animals intrinsically more, or less, capable of responding to a treatment.
2. Serial correlation: Some of the observed responses are likely to be attributable to intrinsic biological processes which vary through time. Two samples taken from the same animal are therefore correlated, and typically, this degree of association becomes weaker as the time separation between samples increases. A low blood haemoglobin reading at one timepoint is likely to influence the probability of an animal presenting with a low reading at the next sampling time, but will be much less influential on a reading six months later.
3. Measurement error: The measurement process itself may introduce a component of variation to the data. Using the haemoglobin example to again illustrate the point, two samples taken simultaneously from a cow may have different blood haemoglobin values because the small variations in the assay technique, i.e. the haemoglobinometer, may add a component of random variation.

These three sources of error can be thought of as incorporation of two distinct levels of variation; *between* subjects and variation *within* subjects. Because the cattle are grouped by intervention, a third level of random variation has to be added to describe the variability between *groups*. In the context of this study, we could consider the random variation between villages assigned to a treatment group, between animals within a single village, and between samples taken through time from a single animal. These issues can be approached using a methodology of multi-level random effect models which has largely been adopted for analysis of this dataset (Goldstein, 1986; Paterson & Lello, 2003; Faraway, 2006).

### 3.9 Generalised Linear Modelling

The presence of spatial and temporal serial correlation disturb two of the most important assumptions of linear modelling, constant variance and normal distribution of errors. In addition, some of the outcome variables are of a binomial or count nature which also require particular modelling techniques. To address the specific question in this thesis it has been necessary to use generalised linear models appropriate for these types of data.

#### 3.9.1 Modelling count data

Count data has several attributes which negate the use of standard linear regression models. It refers to data where an event has happened a known number of times, but there is no way of knowing how many times it did not happen. Models using count data have to account for the following attributes:

- The model cannot predict negative values (a negative count is nonsensical)
- Variance of the response is not constant and likely to increase with the mean
- Non-normal errors
- Transforming data is difficult with zero's

GLM's are fitted using R and the above factors accounted for by specifying a poisson error structure

```
glm(Response variable~ Explanatory variables) family=Poisson.
```

#### 3.9.2 Modelling proportion data

Proportion data modelling is a specific example of count data for situations where we know how many subjects have responded in a particular way, and how many have not, for example infection rates. Models using proportion data have to account for the following attributes:

- Predictions are strictly bounded between 0% and 100%.
- Variance is not constant, in fact it is an n-shaped function greatest at 50%, and converges to equal the mean at the extremes of 0% and 100%.

Modelling with a GLM, specifying a response,  $y$ , as a two variable vector where:

```
y<-(number of successes, number of failures)  
glm(y~ Explanatory variables), family=binomial.
```

This method has considerable advantages over the traditional method of modelling proportions by fitting models to percentage data because it takes into account the sample size from which the proportion is taken.

### 3.9.3 Modelling binomial data

Binomial data modelling is used where the individual is the unit of interest. Examples of such data are infected/not infected, dead/alive, above threshold/below threshold. The response variable is a single column of 1 or 0, and models share the similar variance as proportion data as only two outcomes are possible, 1 or 0.

`glm(y~Explanatory variables), family=Binomial.`

### 3.10 Components of generalised linear models (GLM's)

GLM's have three important components, the error structure, the linear predictor, and the link function.

The structure of a generalised linear model relates each observed  $y$  value to a predicted value. This predicted value is obtained by transforming the value emerging from a *linear predictor*. The *linear predictor*,  $\eta$ , is the sum of the explanatory variables in the model and contains as many terms as there are parameters to be estimated from the data. The outcome of the linear predictor is not itself a value of  $y$ , but is related to it by a *link function*. This use of a link function to relate the observed value of  $y$  to the outcome of the linear predictor allows for the extension of linear modelling techniques to data otherwise unsuitable for normal regression, i.e. data described in sections 3.9.1 to 3.9.3. The link function is chosen to make sure the fitted values, are within meaningful bounds. For example, a log link is appropriate for count data because fitted values therefore become antilogs of the linear predictor and all antilogs are greater than or equal to zero. This avoids the nonsensical prediction of a negative count. Binomial errors similarly use the "logit" link to bound the predicted values between 0 and 1, appropriate for proportion data.

Estimates obtained from the directive summary (modelx) represent the difference between the means for the different factor levels. Back transformation from logits to more conventional proportion estimates is made by using the directive `predict(modelx, type="response")`. The models in this thesis output the difference between the factor level in question and the control. The difference between the logits of two probabilities is the logarithm of the odds ratio, hence the antilog of the model coefficients gives the odds ratio.

### 3.11 Parameter estimation

Parameters are estimated in linear mixed effect models by maximum likelihood (ML) or restricted maximum likelihood (REML) algorithms. REML are preferred in the statistical literature ((Diggle & Heagerty, 2002; Crawley, 2005) because the ML ignores the degrees of freedom used by the fixed effects and thus underestimates the size of the variance components. Parameter estimation for generalised linear models is by iterative, weighted least squares (Faraway, 2006), similar to the conventional estimation using maximum likelihood estimations which are not usually available for GLMs. Parameters in generalised linear mixed effect models fitted using the function `glmmPQL` are estimated by penalised-quasi likelihood methods. This was demonstrated to be a robust method for the type of longitudinal data analysed in this thesis (Breslow & Clayton, 1993).

### 3.12 Notation and model building

The classes of linear models used in this thesis share a general notational format:

$$y \sim a + b$$

models the response,  $y$ , against main terms  $a$ ,  $b$ .

$$y \sim a * b$$

models the response,  $y$ , against the main terms of  $a$  and  $b$  in addition to the interaction between  $a$  and  $b$ . Also written as  $y \sim a + b + (a:b)$ , interaction terms essentially ask the question, does the response of  $a$  depend on the level of  $b$ ?

### 3.12.1 Simplifying models

The assessment of the significance of a main or interaction term is carried out as specified in Crawley (2005) Initially a full model is fitted and simplified using the Akaike information criteria (AIC). The AIC is given as

$$AIC = -2 \times \log \text{likelihood} + 2(p+1)$$

where  $p$  is the number of parameters in the model. The log likelihood is the log of the maximum likelihood, itself simply the value of the parameters of the model which make the data most likely. The AIC is a useful way of balancing the complexity of an estimated model against how well the model fits the data; if the model included a parameter for every data point the fit would be perfect but the explanatory power zero. Superfluous parameters in the model are penalised by the AIC by adding  $2p$  to the deviance so the smaller the AIC, the better the fit of the model.

### 3.13 Mixed effect models

The general structure of mixed effect models was proposed by Laird & Ware (1982). Mixed models are a subset of linear models designed to analyse observations structured into groups and have two components; fixed and random effects. Fixed effects are those common to any group of observations and represent the mean value of the response variable, regardless of the group of animals sampled. Random effects represent the consistent deviations from the predicted value (given by the fixed effects) attributable, for example, to innate differences between the susceptibility of animals or disease challenge. (Laird & Ware, 1982) Pseudoreplication from temporal and spatial groupings can be handled successfully with a mixed model approach (Paterson & Lello, 2003). The non independence of errors associated with pseudoreplicated data is managed by modelling the covariance structure, i.e. the correlation between individuals in a particular group. This approach of aggregating the variation of a group into a single term carries the major benefit of economising on the degrees of freedom used up by the factor levels. For example in this study, modelling the village ( $n=12$ ) as a fixed effect uses 11 degrees of freedom, whereas fitting village as a random effect uses only a single degree of freedom, increasing the explanatory power of the model. In addition, whilst the variability between villages is of interest, the level of a particular village is not; villages studied were selected

at random from a larger number of potential villages. These models are particularly suited for longitudinal data; they can handle balanced or unbalanced datasets, missing data values and, by specifying a more complex random effect structure, can simultaneously account for the temporal correlation between repeated measures from the same individual as well as spatial correlation between individuals from a particular site (Pinheiro & Bates, 2000). Model specification in this thesis has been in accordance with Pinheiro & Bates (2000) using the `lme` function in the `nlme` library of R.

### 3.14 Generalised linear mixed effect models

Extensions of mixed effect models for binomial or count data were fitted using the `glmmPQL` directive available in the `MASS` library of R. `GlmmPQL` allows for the specification of a non normal error structure and estimate parameters using a penalised quasi-likelihood (PQL) method (Breslow & Clayton, 1993; Heagerty, 1999). PQL is an approximation to the maximum likelihood tests and as such AIC or anova tests **cannot** be used to simplify different models. Comparison and simplification of models products by `glmmPQL` is still unclear (Venables & Ripley, 2002). As a result models fitted using `glmmPQL` were ‘compared’ by fitting the same fixed effect to variety of different random effects as given in Table 3-5. If the results were robust when tested against the range of biologically plausible random effects, the fixed effect was considered to be significant.



Random effect structure	Interpretation
<code>~1 Tag.No</code>	<ul style="list-style-type: none"> <li>Animals have <b>different</b> intercepts</li> <li>Villages have a <b>common</b> intercept</li> <li>Villages and animals within villages have a <b>common</b> time slope</li> </ul>
<code>~ 1 Village.Name/Tag.No</code>	<ul style="list-style-type: none"> <li>Villages have <b>different</b> intercepts</li> <li>Individual animals nested within villages have <b>different</b> intercepts.</li> <li>Villages and animals within villages, have <b>common</b> time slope</li> </ul>
<code>list(Village.Name=~Visit.Number,Tag.No=~1)</code>	<ul style="list-style-type: none"> <li>Villages have <b>different</b> intercepts</li> <li>Individual animals nested within villages have <b>different</b> intercepts.</li> <li>Villages have <b>different</b> time slopes.</li> <li>Time slopes are <b>common</b> to all animals within a village.</li> </ul>
Either; a) <code>~Visit.Number Village.Name/Tag.No</code>  Or,b) <code>list(Village.Name=~Visit.Number,Tag.No=~Visit.Number)</code>	<ul style="list-style-type: none"> <li>Villages, and animals within villages, have <b>different</b> intercepts.</li> <li>Villages, and animals within villages, have <b>different</b> time slopes</li> </ul>

**Table 3-5:-Random effect structure fitted in mixed effect models**

### 3.15 Interpreting model output

In this thesis several output tables or plots include the R code as an annotation. This hopefully augments the table and gives insight into the data sources and error structure of the objects under discussion. A sample table is included here to allow for an explanation of the terms.

```
demomodel<-(glmmPQL(YESNO~Group ,random=~Visit.Number|Village.Name/Tag.No,
data=dataset, family="binomial", na.action=na.omit.
```

	Coefficient	Std. Error	DF	t-value	p-value	OR	$^1X$	Value +x	Value -x	Upper CI	Lower CI
(Intercept)	-0.667	0.107	5096	-6.24							
Group B	-0.248	0.228	933	-1.09	0.28	0.78	0.45	0.20	-0.69	1.22	0.50
Group C	-0.408	0.154	933	-2.65	0.01	0.66	0.30	-0.11	-0.71	0.90	0.49
Group D	-0.281	0.150	9	-1.87	0.09	0.75	0.29	0.01	-0.58	1.01	0.56

$^1X = \text{Std.Error} * (\text{qt}(0.975, \text{degrees of freedom}))$

Lower 95% CI =  $\exp(\text{coefficient} - x)$

Upper 95% CI =  $\exp(\text{coefficient} + x)$

**Table 3-6: Summary of fixed effects from a GLM.**

Table 3-6 shows the fixed effects from model object ‘demomodel’ fitted using glmmPQL, the form of output is however generally applicable to GLM’s. The binomial explanatory variable ‘YESNO’ is a vector containing a value for each animal as either Y (positive) or N (negative). This is fitted as a fixed effect to a single categorical explanatory variable ‘Group’, which has four levels, A,B,C or D. The random effects are fitted with the structure Visit.Number|Village.Name/Tag.No, explained in Table 3-5. The data under analysis exists in a dataframe called dataset and family=“binomial” defines a binomial error structure and a log link function. na.action=na.omit ignores data rows for which there is no data.

The output is a comparison between the means or group of means as specified by the contrasts used in the model. R by default uses treatment contrasts, which are the same as the factor levels, hence in this example the model asks the question ‘is there a significant difference between levels A, B, C and D in the factor Group in terms of the explanatory variable YESNO?’ The output compares the *difference* between factor levels and is interpreted as follows.

- Intercept in row 1 is the intercept for Group A. The standard error in this row is the standard error of the mean.
- The subsequent rows display coefficients of the *differences* between the intercepts and standard errors of the *differences* between the means. For example row 2, labelled Group B, is the difference in intercept between Groups A and B.
- The p value is the probability that the observed difference will arise due to chance when the means are actually the same, and for significance at the 95% confidence level has to be <0.05.
- Coefficients are back transformed for presentation of odds ratios with corresponding 95% confidence intervals. The odds ratio is the reverse of the log link, i.e. the exponent of the coefficient.

### 3.16 Odds ratio

Odds ratios are measures of effect size. They are defined as the ratio of the odds of an event occurring in the first group, to the odds of it occurring in a second group. If the probabilities of the event in each of the groups are  $p$  (first group) and  $q$  (second group), then the odds ratio is:

$$\frac{p/(1-p)}{q/(1-q)} = \frac{p(1-q)}{q(1-p)}$$

For most of the models used in this thesis, the probabilities were not calculated explicitly, instead the odds ratios are calculated through back transformation of model estimations. An odds ratio of 1 indicates that the condition or event under study is equally likely in both groups. An odds ratio greater than 1 indicates that the condition or event is more likely in the first group; an odds ratio less than 1 indicates that the condition or event is less likely in the first group. Consequently, confidence intervals that cross 1 are not significant. In the case of odds ratios connected with disease prevalences, odds ratios less than 1 confer a protective effect, greater than 1 a risk. For example, an odds ratio of 10 from prevalence data between groups A and B is interpreted as animals in the group A have 10 times higher odds of being positive than animals in group B. Note, this is not the same as saying that animals in the group A are 10 times more likely to be positive than those in group B; this involves the calculation of relative risks which is less straightforward with GlimmPQL outputs (R helpgroup [r-help@stat.math.ethz.ch](mailto:r-help@stat.math.ethz.ch)).

### 3.17 Tree models

Although it is theoretically possible to fit generalised linear models to all the possible combinations of factors measured in the study (over 5000), it is not always clear what high level interaction terms actually mean, and, due to the unbalanced nature of the data in this study, such models are often impossible to fit. In contrast, tree based models offer an alternative way of tackling multi-factor data analysis, and are increasingly being used as a graphical alternative to the generalised linear modelling techniques described above (Breiman *et al.*, 1984; Cappelli *et al.*, 2002).

Tree models are computationally intensive methods that are useful for datasets containing a large number of explanatory variables. As with generalised linear models, tree-based methods compare a dependent variable with a series of independent factors. They are able to cope with both dependent and explanatory binary, categorical or continuous variables. Tree models are fitted by binary recursively partitioning the data on the basis of the lowest deviance. Each explanatory variable is assessed in turn and the variable explaining the greatest amount of the deviance in the response variable is selected. That variable is then split into two partitions at a particular threshold; the threshold value calculated as the value which *minimises* the deviance *within* the partition and *maximises* the deviance *between* the partitions. The process is then repeated for the values associated with low values of the first explanatory variable, and again with the high values, to form the second bifurcation. The process is further repeated until there is no residual explanatory power. Trees are then pruned at points where comparisons are no longer significant (Fisher's exact test), as trees are arguably clinically meaningless at the ends of the branches. A good introduction to tree based methods is given in (Crawley, 2005) and a more detailed explanation in (Chambers & Hastie, 1992).

Tree models have a great advantage in data inspection as they give as a clear picture of the structure of the data and an insight into the interactions between the variables. They

also deal well with missing data and will exclude cases only if all the explanatory variables are missing, thus increasing the data points in the model (Venables & Ripley, 2002). Tree models also have the considerable advantage that it doesn't matter which order the explanatory factors are put into the model. As such they do not allow for interactions between factors, but they do allow for a graphical illustration of the entire interaction structure which can be analysed subsequently using linear modelling techniques.

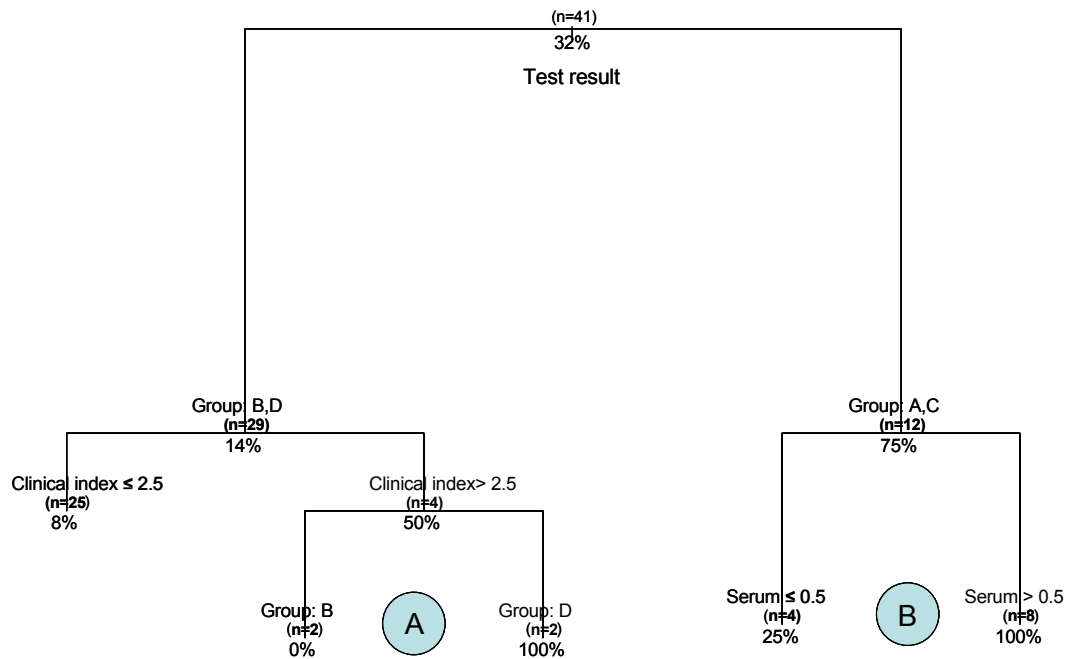
Tree models are fitted using package library (tree) and the directive tree.

```
treedemo<-tree(testresult~ Clinical.Index+Group+Age+Serum,
data=treedemo,na.action=na.omit)
```

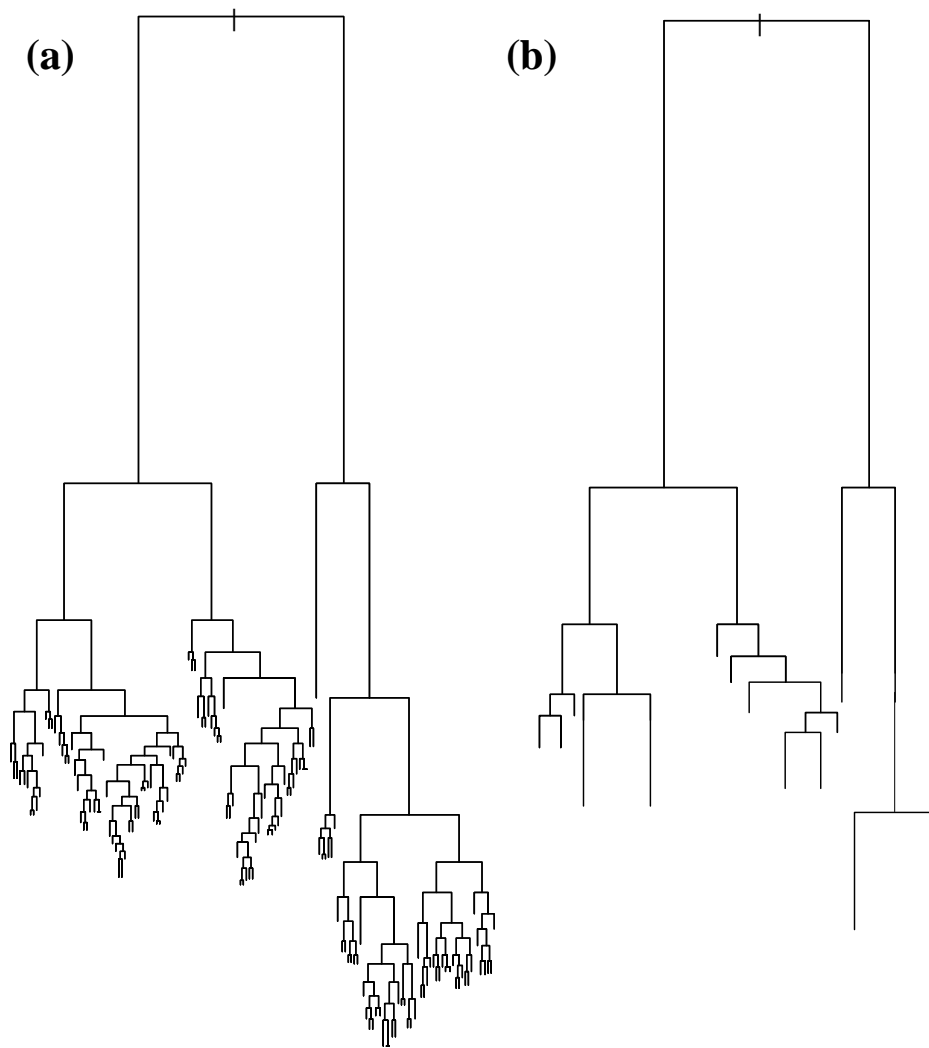
Figure 3.4 shows the plotted output of the treedemo model; the binary outcome 'testresult' modelled against explanatory variables 'Clinical.Index', 'Group', 'Age' and 'Serum'. Of an overall prevalence of 32%, the first partition is made on the basis of the categorical variable 'Group', of whose levels B and D show a prevalence of 14%, compared with a prevalence of 75% for A and C. Following the left hand side down, the next split is made on the basis of the continuous variable 'clinical index'. Individuals below 2.5 have a prevalence of 8%, compared with 50% for those with a clinical index above 2.5. The split marked 'A' on Figure 3.4 is a terminal node and shows that, for a clinical index of above 2.5, group B are all negative and group D all positive. Considering the right hand branch, individuals in groups A and C, the clinical index variable does not appear, instead the variable serum is used to partition the variance ('B' in Figure 3.4). Finally, the variable Age in the model does not appear at all in the final tree diagram; no partition of age explains the variance in the treedemo dataset.

The significance of the splits marked A and B can be established by chi squared tests, in this example given the low sample size this is unlikely. In general, comparisons of interest were tested for significance using a chi-squared contingency table. Tables were constructed using the function `x<- table(a,b)` with *a* representing the split of the factor and *b* the population under test. The function 'chisq.test(x)' was then applied to tests

where the expected values were  $>5$ , or the function 'fisher.test(x)' for values less than 5, to test for significance.



**Figure 3.4:- Tree model output for the example described in text. Percentages refer to value of each partition. Comparisons A and B refer to regions of the tree described in the text.**



**Figure 3.5:- Example of a full (a) and pruned (b) tree.**

## 3.18 Comparing categorical data using Mosaic plots

### 3.18.1 Log linear models

Log linear models are specialised cases of generalised linear models for Poisson-distributed data (Friendly, 1994; Crawley, 2005). Log linear analysis extends the two-way contingency table to higher dimensional data where the conditional relationship between two or more discrete, categorical variables is analysed by taking the natural logarithm of the cell frequencies within a contingency table. In this case however, the models are only fitted to a two way contingency table and residual outputs are the same as those calculated by chi-square values testing the hypothesis of independence. Log-linear models consider all variables as response variables, so only the association and not the dependence can be distinguished by these techniques.

### 3.18.2 Mosaic plots

Mosaic plot graphs represent a contingency table, and are essentially grouped bar charts where width and height of the bars show the relative frequencies of the two variables (Hartigan & Kleiner, 1984). The tiles in a mosaic plot are proportional to the observed cell frequencies. Extended mosaic displays (Friendly, 1994) use a colour coding of the tiles to visualise deviations (residuals) from a given log-linear model fitted to the table. Negative residuals are shaded red and with broken outlines; positive residuals are blue with solid outlines.

Analyses using mosaic plots have been done by time point. Significant differences are between the other groupings at that time point, so the plots do not directly provide information about change over time. To interpret the association between treatment group and pathogen count, consider the pattern of positive (Blue) and negative (Red) tiles in the mosaic display. A cell is coloured blue if its observed frequency is significantly greater than that which would be found under independence, and red if its frequency is



significantly less. Threshold residuals correspond to  $p < 0.05$  and  $p < 0.001$  for the standardized Pearson residuals of 2-4 and  $>4$  of a Chi-squared statistic.

**Chapter 4 :-** The use of molecular techniques to assess the impact of control strategies

## 4.1 Introduction

An accurate classification of an animal's infection status is arguably the most important step in the design and validation of a disease control strategy. Analysis of the data discussed in this thesis primarily aims to assess the effect of the different treatment regimes with particular reference to trypanosomiasis. There is no pathognomic clinical indication of trypanosomiasis (Uilenberg, 1998), and parasitological detection methods lack sensitivity. Traditional methods of epidemiological screening rely on the visual identification of the parasite in the blood sample by microscopy. Although widely used, microscopy can suffer from poor sensitivity due to characteristically low parasitaemia in endemic cattle (Paris *et al.*, 1982; Picozzi *et al.*, 2002). In addition such techniques are labour intensive, require a skilled and dedicated microscopist and can be difficult to manage in a field situation away from a reliable power source.

Immunological techniques to demonstrate active infection have so far not proved sensitive enough to be of diagnostic value (Eisler *et al.*, 1998). Over the last few years, DNA based diagnostic tools have greatly improved, and are now sensitive, specific, robust and cheap enough to be employed as screening techniques for epidemiological studies (Duvallet *et al.*, 1999; Desquesnes & Davila, 2002; Picozzi *et al.*, 2002; Gasser, 2006).

## 4.2 What is a pathogen?

The aetiology of an infectious disease has traditionally been investigated through the establishment of Koch's postulates, namely

1. The organism must be found in all animals suffering from the disease, but not in healthy animals.
2. The organism must be isolated from a diseased animal and grown in pure culture
3. The cultured organism should cause disease when introduced into a healthy animal.
4. The organism must be re-isolated from the experimentally infected animal (Koch, 1882)

Many diseases do not comfortably fit into these postulates; Koch himself saw the limitations in the second part of first postulate when he discovered asymptomatic carriers of cholera (Koch, 1893). Although it was rapidly recognised that some infectious agents were causal for disease despite the fact that they did not fulfil all of the criteria, Koch's postulates provided a logical framework for convincing sceptics that micro-organisms caused disease and a robust methodology for studying the aetiology of infectious diseases (Falkow, 2004). Indeed, as noted by Fredericks and Relman,

“The power of Koch's postulates comes not from their rigid application, but from the spirit of scientific rigour that they foster. The proof of disease causation rests on the concordance of scientific evidence, and Koch's postulates serve as guidelines for collecting this evidence.”(Fredericks & Relman, 1996)

To redefine Koch's postulates in the context of contemporary disease diagnosis and therapy raises an interesting question - what is a pathogen? Given the increasingly apparent sophistication and subtlety of interactions between host and parasite, for most clinical cases demonstrable proof of the presence of a parasite within its host is no longer sufficient to account for a disease. The reservoirs of many common, and often quite deadly, infections persistently infect asymptomatic individuals (Falkow, 2004), and in many cases the transition from asymptomatic carrier to clinically sick is more to do with a failure of the host's immune capabilities than an increase in pathogenicity on the part of the parasite (Evans, 1991; Poxton, 2005).

This is of particular relevance when considering the use of molecular techniques to analyse samples collected in the longitudinal study covered in this thesis. The molecular screening techniques discussed in this chapter provide the capacity to identify minute amounts of parasitic genomic DNA. The longevity of dead parasite DNA in the host's bloodstream is not known, but most work suggests that it does not survive for long in the blood. Using a very sensitive PCR and DNA hybridization assay, Clausen *et al.* (1999) found it possible to detect a positive signal 4 days after treatment with a known effective trypanocide although they noted that the signal strength was notably weaker after the trypanocidal treatment. In contrast, Lo *et al* (1999) monitored the clearance of circulation foetal DNA postpartum in twelve women in order to assess the survival of extra cellular

DNA in blood. Eighty-seven percent of women had undetectable foetal DNA after 2 hours and further investigation yielded a mean half-life for circulating foetal DNA of 16.3 minutes. In the context of this study it is probably fair to assume that a positive result indicates the presence of live trypanosomes in the circulation at the moment the sample was taken. However, these results should be taken in context. The molecular techniques employed here are not quantitative, and unlike real time PCR the response is a binary yes or no. Whilst PCR techniques are of great benefit for their specificity, it could be argued they are too sensitive for some analyses. A positive result does not distinguish between ‘fortuitously’ taking a punch containing trypanosomal DNA from an animal with one circulating trypanosome and a punch taken from an animal with a parasitaemia six orders of magnitude higher. Given the endemic status of trypanosomiasis in the sample population, many animals appear to exist in a continual state of infection, resolution and re-infection (Waiswa, 2003). In trypanosomiasis endemic areas where susceptible cattle breeds constitute the main host of tsetse and are the reservoir of trypanosomes, the clinical impact of infection tends to be low (Van den Bossche, 2001). Consequently, relating PCR results to the actual clinical impact that the disease has to be done with caution. Care should also be used when making inferences about the epidemiological significance, i.e. transmissibility, of the treatment options due to the difficulties in quantifying infection burden using these tools.

With these caveats in mind, this chapter aims to assess the impact of the different interventions applied to cattle during a longitudinal study run in south east Uganda, based upon ITS-PCR test for trypanosomiasis. The study design was described in chapter 2 and the material and methods for the molecular analysis are described in section 3.4.

### 4.3 Methodology

Whole blood samples were collected and stored on FTA® cards as described in section 3.1. Four timepoints were selected from the study for analysis of trypanosomal DNA by a polymerase chain reaction protocol described in 3.4.5. To attempt to improve the sensitivity of the technique, each sample was screened twice by taking two punches from the FTA® card, with each punch processed in parallel using identical reagents and

equipment. The selected timepoints were the baseline, and the first, third and sixth (final) intervention on day -42, 0, 56 and 147 respectively. All animals presenting at a timepoint were screened by PCR however particular analysis may involved subsets of this data.

#### 4.4 Results overview

Table 4-1 shows the results of the PCR screening arranged by timepoint and treatment group. To ensure consistency within treatment groups, subsequent analysis is restricted to those animals that received two treatments of trypanocide prior to day 0. In addition animals in the villages receiving isometamidium that did not receive the drug on day 0 have been removed from subsequent analysis. An animal was recorded as positive if either of the punches gave a positive result. Figure 4.1 is a graphical representation of Table 4-1, included to aid the descriptions. 95% confidence intervals for binomial probabilities were calculated using the `binconf` function in R as described in section 3.9.3.

It should be noted that up to and including samples taken on Day 0, all groups received identical treatments (see section 2.9). Classification of villages into groups at day -42 and day 0 has been done to show the future allocation of interventions from Day 0 onwards.

#### 4.5 Trypanosome prevalence for each intervention group

Initial trypanosome infection at the baseline varies between groups but, as can be seen from the overlapping binomial exact confidence intervals, this is not significant. At day 0, following two treatments with diminazene aceturate at a dose rate of 7mg/kg, the prevalence in all the groups has dropped to zero. Fifty-six days later trypanosome prevalence has increased to between 2.3% and 8.2% however overlapping 95% confidence intervals indicate this is not statistically significant. 147 days after beginning the interventions, the prevalence of the control and isometamidium treated villages have returned to  $\approx 15\%$ ; within the range of the baseline values. In contrast, intervention groups receiving monthly insecticide treatment have a prevalence of 3.8% and 1.3% for the pour-on and spray groups respectively. Error bars do not overlap, demonstrating significance at  $p=0.05$ .

	n	Prevalence	Lower 95% CI	Upper 95% CI
<b>Baseline:- Day -42</b>				
Control	274	20.8%	16.4%	26.0%
Isometamidium	197	13.2%	9.1%	18.6%
Pour	235	17.0%	12.7%	22.4%
Spray	240	19.2%	14.6%	24.6%
<b>Visit 3:- Day 0</b>				
Control	183	0.0%	0.0%	2.1%
Isometamidium	175	0.0%	0.0%	2.3%
Pour	203	0.0%	0.0%	1.7%
Spray	210	0.0%	0.0%	1.8%
<b>Visit 5:- Day 56</b>				
Control	184	8.2%	5.0%	13.0%
Isometamidium	150	3.3%	1.4%	7.6%
Pour	172	2.9%	1.2%	6.6%
Spray	176	2.3%	0.9%	5.7%
<b>Visit 8:- Day 147</b>				
Control	110	15.5%	9.9%	23.3%
Isometamidium	117	15.4%	9.9%	23.0%
Pour	134	3.8%	1.6%	8.5%
Spray	149	1.3%	0.3%	4.8%

**Table 4-1:- Trypanosome prevalence for pathogenic trypanosome species with binomial 95% confidence intervals.**

## 4.6 Modelling prevalence data

Initial data inspection suggests there are differences between the treatment groups, so further analysis is warranted. This data is investigated to ask two specific questions.

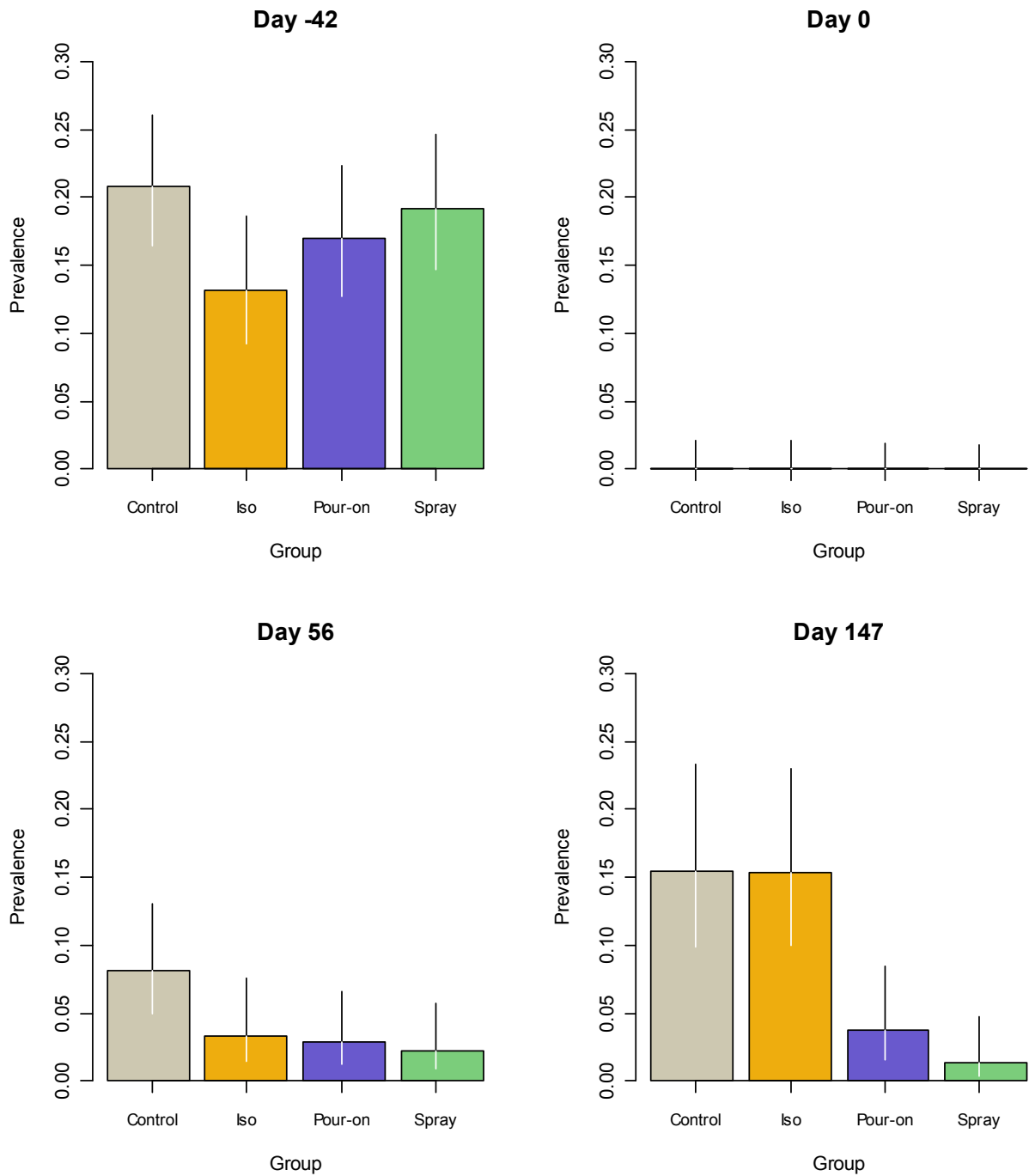
- Is there a difference in trypanosome prevalence among different treatment groups?

- Could this difference be explained by other factors other than the treatment protocols?

Primarily, it of interest to model the change in trypanosome infection over time in order to quantify differences between the groups, secondly it is interesting to compare the differences between the samples in terms of other clinical and diagnostic indicators.

The incidence of trypanosome infections could be used as an assessment of the degree of protection to disease transmission conferred by the different interventions. Consequently, results were amalgamated across trypanosome species and coded as a binary variable representing the outcome of the ITS-PCR screening. A positive state was recorded if the animal was positive for any pathogenic (*T brucei*, *T.vivax*, *T.congolense*) trypanosome species, by either one of the screening punches, at a given time. Descriptions of the molecular techniques used are given in section 3.4





**Figure 4.1:-Plot of overall trypanosome prevalence by visit number and intervention group. Error bars represent 95% binomial confidence intervals.**

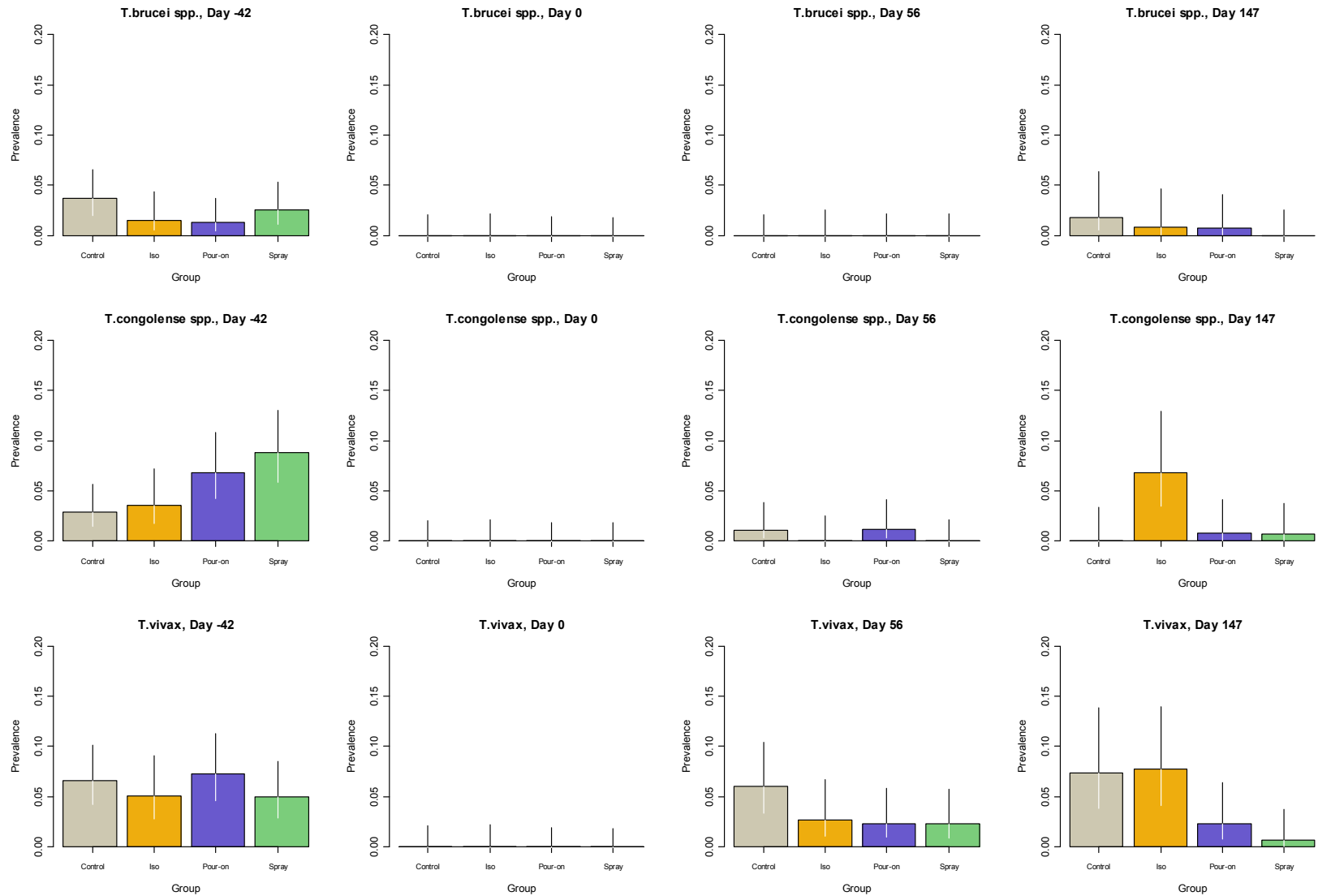


Figure 4.2:- Barplot of trypanosome prevalence by treatment group and individual species. Vertical bars represent binomial 95% confidence intervals.

### 4.6.1 Trypanosome prevalence by individual species

Figure 4.2 shows the prevalence by ITS-PCR of three clinically important trypanosome species; *T.brucei*, *T.congolense* and *T.vivax*. Prevalence was modelled using a generalised linear model for count data described in section 3.9.1. At the baseline there is a significant difference ( $p < 0.01$ ) between the prevalence of *T.brucei* and the other two species; *T.brucei* approximately half the prevalence of the other two. (Table 4-2). This pattern appears to be broadly maintained over the incidence of infections emerging after day 0, although these were not significant ( $p > 0.05$ )

Species	Positive	Negative	Prevalence	Significance
<i>T.congolense</i>	52	893	5.50%	
<i>T.brucei</i>	22	924	2.33%	$p > 0.01$
<i>T.vivax</i>	57	889	6.03%	

**Table 4-2:- Difference between prevalence for individual trypanosome species at baseline. Significance calculated from a glm with binomial errors**

## 4.7 Univariate analysis of PCR results

Initial analysis involved comparing the trypanosome prevalence to a range of clinical and 'signalment' (individual descriptive variables, such as age, sex, breed) to look for potentially significant associations. This is of relevance in its own right, but was also employed at this stage to look for associations that may confound analysis of the treatment effect.

Multiple, univariate analyses were carried out using a generalised linear mixed effect model as described in 3.14.

```
glmmPQL(PCRresult~VariableX ,random=~Visit.Number|Village.Name/Tag.No,
,family="binomial")
```

PCRresult is the binary response variable, VariableX the univariate in question. Random effects allowed for different intercepts for villages within treatment groups and a different slope over time. Variables were coded as outlines in section 3.2.2 and included the results of the laboratory and field analysis.

Table 4-3 and Table 4-4 show the outcome for each of the univariate analysis, with concomitant p value and odds ratios. Most of the factors are not significant, the exception being the gender and the infection status of the animal for the tick-borne parasite *Anaplasma marginale*. Compared with female animals, male animals have significantly ( $p=0.02$ ) higher odds of becoming infected than females, (Odds ratio 1.75, (CI1.1-2.8)). Animals found positive for *Anaplasma* by microscopy show significantly ( $p<0.001$ ) higher odds of also being found positive for trypanosomes by PCR (Odds ratio 2.45, (1.54 3.87)). There also is a significant association ( $p<0.05$ ) between *Boophilus* tick counts and positive trypanosome signal.

These findings are consistent with previous work. Analysis of longitudinal data set of trypanosome infections in cattle in the Ghibe Valley, Ethiopia using transition models showed a similar odds ratio (1.46, CI 1.30-1.63) between male and female cattle (Schukken, 2004). Similar results were found in Boran cattle in Kenya (Dolan, 1997) This effect was attributed to the extra demands placed on male cattle for provision of draught power, this would also be consistent with the management systems in the Ugandan study villages.

The significance of concurrent anaplasmosis increasing the odds of trypanosomiasis are consistent with other studies in S.E. Uganda (Magona & Mayende, 2002) in addition to work in other management systems. Mkwaja ranch in N.E Tanzania employed deltamethrin dips to control trypanosomiasis. Following the introduction of deltamethrin insecticide, the percentage of cattle mortality attributed to anaplasmosis declined. It was not considered this was due to tick control although the possibility of a reduction in mechanical transmission from biting flies was considered. A possible explanation was chronic, subclinical

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trypanosomiasis caused sufficient physiological stress to cause patent parasitaemia, or even clinical disease, in *Anaplasma* carrier animals (Fox *et al.*, 1993).

It is also important, although perhaps unsurprising, to note that there is a significant ( $p < 0.01$ , OR 3.03) association between anaemia (blood haemoglobin of below 8.5 g/dl), and a positive trypanosome diagnosis by ITS-PCR. The link between *Boophilus* and trypanosome infections is investigated in section 6.3 but this result could be attributable to the apparent low recovery rate of *Boophilus* tick species to insecticide and thus serve as an indicator for the effect of the pour and spray interventions.

Variable	Factor level	Value	Std. Error	t-value	p-value	OR	Upper 95% CI	Lower 95% CI	Signif?
Signalment									
Sex	(Intercept) Female	-4.657	0.245	-19.03					
	Male	0.562	0.239	2.35	0.02	1.75	2.80	1.10	*
	Male, Neuter	-0.706	0.904	-0.78	0.44	0.49	2.91	0.08	NS
Colour	(Intercept) Black	-4.309	0.314	-13.71					
	Black & White	0.603	0.435	1.39	0.17	1.83	4.28	0.78	NS
	Brown	-0.394	0.295	-1.34	0.18	0.67	1.20	0.38	NS
	Brown & White	-1.671	0.905	-1.85	0.07	0.19	1.11	0.03	NS
	Grey	-0.356	0.680	-0.52	0.6	0.70	2.66	0.18	NS
	Mixed	-0.083	0.366	-0.23	0.82	0.92	1.89	0.45	NS
	White	-0.392	0.395	-0.99	0.32	0.68	1.46	0.31	NS
Age	(Intercept) A	-3.910	0.455	-8.59					
	Age B	-0.240	0.463	-0.52	0.6	0.79	1.95	0.32	NS
	Age C	-0.730	0.445	-1.64	0.1	0.48	1.15	0.20	NS

**Table 4-3:- Result of multiple, univariate analyse of signalment factors against trypanosome prevalences from ITS-PCR screening**  
 \*=significant, NS= not significant

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Variable, x	Factor level	Value	Std.Error	t-value	p-value	OR	Upper 95% CI	Lower 95% CI	Sig
<b>Clinical Parameters</b>									
Cond.Score	(Intercept) >4	-4.676	0.751	-6.22					
	<4 (Thin)	0.007	0.151	0.05	0.96	1.01	1.35	0.75	NS
Lymph node size	(Intercept) Normal	-4.747	0.264	-17.98					
	Enlarged	0.373	0.232	1.61	0.11	1.45	2.29	0.92	NS
	Severely enlarged	0.098	0.978	0.1	0.92	1.10	7.50	0.16	NS
<b>Laboratory outcomes</b>									
T.parva positive?	(Intercept) No	-4.690	0.283	-16.56					
	Yes	-0.454	0.236	-1.93	0.05	0.63	1.01	0.40	NS
Anaplasma positive?	(Intercept) No	-5.054	0.288	-17.54					
	Yes	0.897	0.233	3.86	<0.01	2.45	3.87	1.56	*
WEC	(Intercept)	-4.457	0.227	-19.67					
	WEC score	-0.022	0.051	-0.43	0.67	0.98	1.08	0.88	NS
Coccidia?	(Intercept) No	-4.472	0.227	-19.73					
	Yes	-0.123	0.642	-0.19	0.85	0.88	3.11	0.25	NS
Fluke eggs	(Intercept) No	-4.813	0.270	-17.51					
	Yes	0.170	0.270	0.63	0.53	1.19	2.01	0.70	NS
Anaemic? (Hb<8.5g/dl)	(Intercept) No	-4.623	0.331	-13.95					
	Yes	1.107	0.273	4.06	<0.01	3.03	5.16	1.77	*
<b>Tick burden</b>									
<i>Boophilus spp</i>	(Intercept) No ticks	-5.100	0.309	-16.5					
	1-10 ticks	0.601	0.250	2.4	0.02	1.82	2.98	1.12	*
	11-50 ticks	0.969	0.345	2.81	0.01	2.64	5.18	1.34	*
	>50 ticks	0.689	0.993	0.69	0.49	1.99	13.96	0.28	NS
<i>Amblyomma spp</i>	(Intercept) No ticks	-4.526	0.305	-14.86					
	1-10 ticks	-0.482	0.273	-1.76	0.08	0.62	1.05	0.36	NS
	11-50 ticks	-0.042	0.304	-0.14	0.89	0.96	1.74	0.53	NS
	>50 ticks	1.143	0.593	1.93	0.05	3.14	10.03	0.98	NS
<i>Rhipicephalus spp.</i>	(Intercept) No ticks	-0.996	0.067	0					
	1-10 ticks	-0.106	0.060	0	0.87	0.90	1.01	0.80	NS
	11-50 ticks	-0.009	0.067	0	0.65	0.99	1.13	0.87	NS
	>50 ticks	0.252	0.131	0	0.78	1.29	1.66	1.00	NS

**Table 4-4:- Result of multiple, univariate analyse of clinical and laboratory variables against trypanosome prevalences from ITS-PCR screening \*=significant, NS= not significant**

## 4.8 Generalised linear model with binomial errors

Univariate analysis of signalment variables (Table 4-3) and clinical parameters (Table 4-4) suggest that as many of the factors are not significant they could be left out of further models. Nonetheless, initially a full model was fitted and compared with a model only containing variables significant at the 95% confidence level in the univariate analysis (Section 4.7). The model was fitted to all data collected during the interventions, i.e. after day 0.

```
model1<-glm(PCR ~all variables, family="binomial")
model2<-glm(PCR ~Group*Sex*Boophilus burden*Anaplasma status ,
family="binomial")
```

Comparing the two models using anova showed no significant difference ( $\chi^2=0.178$ ) between the two models. Inspection of the models showed no significant interactions so the simpler model2 was selected for further analysis. Term deletion of model2 resulted in the final model, model3, which only contained the group variable. All other factors could be removed from the model as they were not significant.

```
model3<-glm(PCR~Group, family="binomial")
```

	Estimate	Std.Error	p-value	Signif?
(Intercept) Control	-1.695	0.263	<0.001	
Isometamidium treatment	-0.005	0.367	0.9883	
Pour-on treatment	-1.550	0.526	0.0032	*
Spray treatment	-2.597	0.759	0.0006	*

**Table 4-5:- Output from minimum model of PCR result against intervention group**

Table 4-5 shows the output of model3. Initial inspection suggests there is a significant difference between both the pour-on and spray groups and the control. However, this analysis is potentially unsound. Figure 4.3 shows the prevalences listed in Table 4-5, plotted by individual village. It can be seen that there is a significant variation between villages allocated to a treatment group. This indicates there may be a significant effect of village. Model4 is a variation on model3 but with village as a fixed effect. Comparing the two models with anova allows for an assessment of the residuals, hence the fit of the model:

```
model 3: PCRYN ~ Group
model 4: PCRYN ~ Group + Village Name
anova(model 3,model 4, test="Chi")
```

	Resid. Df	Resid. Dev	Df	Deviance	P(> Chi )	Signif?
Model 3	494	249.53				
Model 4	487	226.59	7	22.95	0.002	*

**Table 4-6:- Anova result of comparing model3, with no village effect, to model4, including village as a fixed effect**

Table 4-6 shows that model4 is significantly better at explaining the data than model3, strong evidence for a significant village effect. However when the model4 was examined, none of the variables remained significant (data not shown) It is difficult to separate the effect of village from the effect of the interventions when village is fitted as a fixed effect. Models fitted to this data will have to account for the variation in villages within intervention groups to effectively analyse the treatment effects.



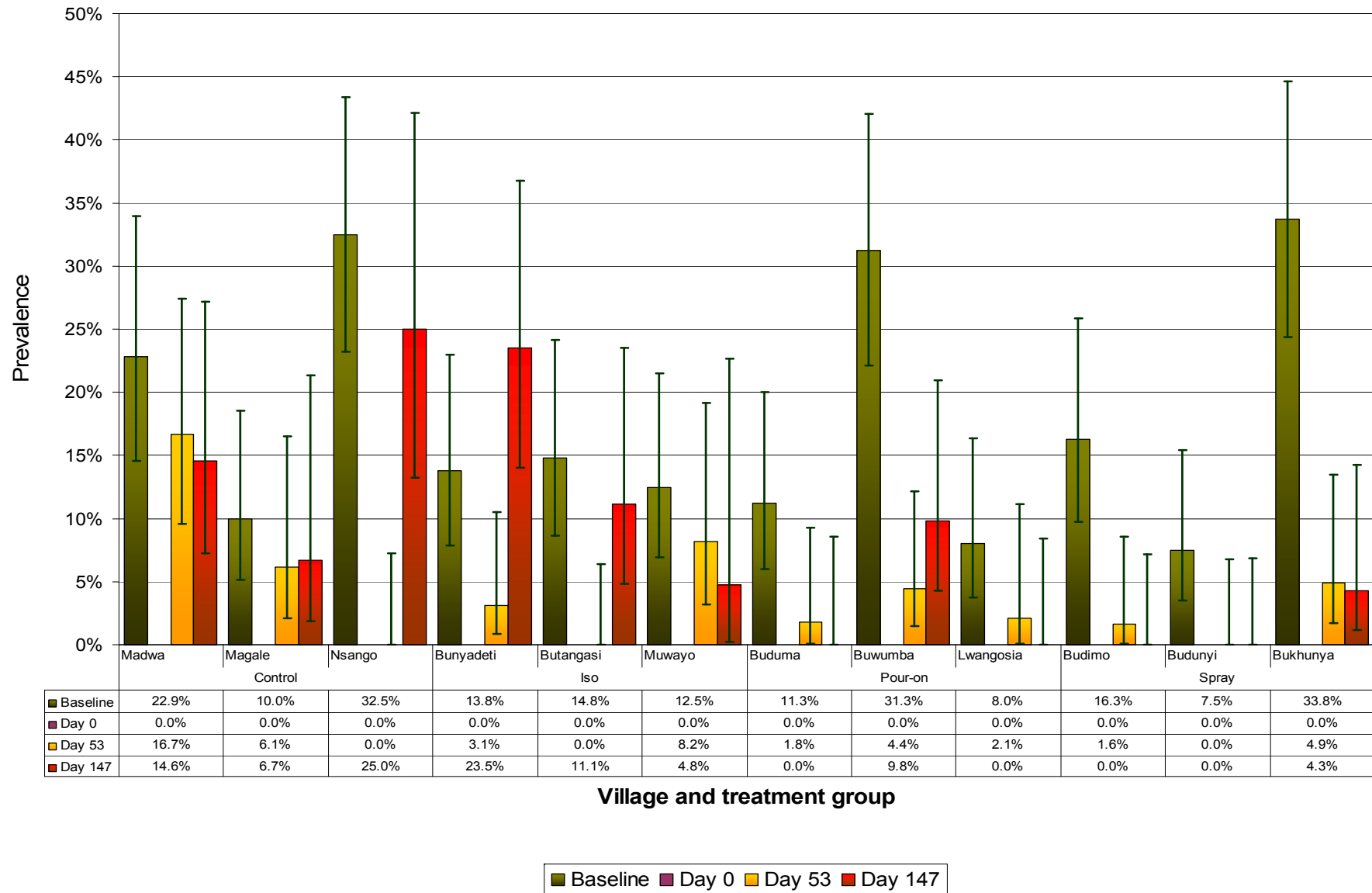


Figure 4.3:-Trypanosome prevalences in individual study villages over time. Error bars represent exact binomial 95% confidence intervals

## 4.9 Demonstration of heterogeneity between villages

It is useful to quantify the variation in villages highlighted in Figure 4.3 in order to show why a more complex model is needed than outlined in section 4.8. Table 4-7 shows the output when village is fitted as a fixed effect, the coefficients relate to the difference of the names village and the village of Budimo as explained in section 3.15. Villages showing a significant difference in trypanosome prevalences are highlighted in yellow. Whilst the prevalence in particular villages is not of interest per se, it does highlight the need for models able to account for the significant heterogeneity between villages. For example, in this case animals in Buwumba village have a 2.34x higher chance of having a trypanosome infection than those in the comparison village of Budimo (95% CI 1.09-5.03). The important point here is that these twelve villages were selected from a pool of potentially several thousand villages, and as such there is little point in estimating the means of a particular village, and no point at all in comparing individual means for two villages. Instead, they need to be recognised for what they are; random samples from a much larger population. It is the additional variation caused by differences between the villages that we are interested in and hence the use of generalised linear mixed effect models for the rest of the data analysis.

	Coefficien t	Standard error	DF	p-value	OR	Upper CI	Lower CI
(Intercept) (Budimo)	-1.64	0.31	934	<0.001			
Buduma	-0.43	0.47	934	0.36	0.65	1.64	0.26
Budunyi	-0.87	0.52	934	0.10	0.42	1.17	0.15
Bukhunya	0.97	0.39	934	0.01	2.63	5.60	1.23
Bunyadeti	-0.20	0.45	934	0.66	0.82	1.97	0.34
Butangasi	-0.11	0.44	934	0.80	0.90	2.12	0.38
Buwumba	0.85	0.39	934	0.03	2.34	5.03	1.09
Lwangosia	-0.80	0.53	934	0.13	0.45	1.26	0.16
Madwa	0.42	0.42	934	0.31	1.53	3.47	0.67
Magale	-0.56	0.48	934	0.25	0.57	1.48	0.22
Muwayo	-0.31	0.46	934	0.50	0.74	1.80	0.30
Nsango	0.91	0.39	934	0.02	2.48	5.31	1.16

**Table 4-7:- Output from a glm model relating trypanosome prevalence to village during intervention period. Significant differences are highlighted in yellow.**

#### 4.10 Generalised linear mixed effect models (GLMM)

The use of generalized mixed effect models with binomial errors (GLMM) allows the village variation to be accounted for by fitting village as a random effect. As before, PCR status is fitted as a binary outcome variable (PCRresult) and treatment group as a fixed effect (Group). Village is fitted as a random effect. In this section, the analysis considers each timepoint separately, the question of interest the difference between the different treatment groups at several snapshots through the study. The inclusion of time as a random effect is investigated later.

The random terms in the model are important as they allow for uncontrollable influences due to the temporal and spatial autocorrelation. Adjusting for these sources of variability allows any true effects of intervention to be disentangled from otherwise ‘noisy’ data, and are described in section 3.8.1.

Models were fitted using the glmmPQL directive as described in section 3.14. All models used in this section have the structure of binary infection status as the response variable, intervention group fitted as a fixed effect and village as a random effect:

```
glmmPQL(PCRresult~Group, random=~1|Village.Name, data=Visitx,
family="binomial")
```

#### 4.10.1 Comparison of treatment groups at the baseline

Selection and allocation of the villages to a group is outlined in section 2.5. Groups were randomly allocated a treatment protocol. Village allocation was made on the basis of preliminary screening analyses, however it is important however to assess any variation in trypanosome prevalence which might suggest inequality in village allocation and potentially bias the rest of the study.

	Coeff	Std.Error	DF	t-value	p-value	OR	Upper CI	Lower CI
(Intercept) Control	-1.43	0.28	933	-5.02	<0.001	0.24		
Spray group	-0.11	0.45	9	-0.24	<b>0.82</b>	0.90	2.49	0.33
Iso.group	-0.40	0.37	9	-1.09	<b>0.28</b>	0.67	1.55	0.29
Pour-on group	-0.26	0.45	9	-0.58	<b>0.58</b>	0.77	2.15	0.28

**Table 4-8:- GLMM output comparing PCR prevalence to future intervention group at the baseline.**

Table 4-8 shows the model for the baseline sampling point (Day -42). There is no significant difference between groups in the prevalence of trypanosomiasis at the start of the study,

indicating that although heterogeneity exists between the villages, these differences cancel out at the group level. Consequently, it has been assumed that there is no bias towards a particular group in terms of the risk of trypanosomiasis and analysis can continue accordingly.

#### 4.10.2 Comparison of treatment groups at start of the interventions:- effect of two doses of diminazene aceturate

The effect of two doses of the trypanocidal drug diminazene aceturate (Berenil®, Intervet) was assessed. All animals were treated 42 and 14 days prior to the start of the interventions as described in section 2.9. At day 0, the trypanosome prevalence was zero for all animals screened (Table 4-1) With zero prevalence, it is not possible to fit a model to this time point however it is fair to conclude that two doses of the drug effectively lowered the parasitaemias to below the detection threshold of PCR. Although the absence of a comparison group, i.e. one that did not receive the diminazene treatments, strictly prevents any causal conclusions about the drug effect, it is very likely that the diminazene was responsible for the decrease in trypanosome prevalences. It is assumed therefore that the effect is real and that there is no evidence of to suggest drug resistance is a problem in the study villages of south east Uganda.

#### 4.10.3 Reinfection, recrudescence or retest?

The sensitivity threshold of detection of trypanosome infection is of the range 1-20 parasites/ml of blood, the ITS protocol able to detect DNA concentrations as low as 55pg/ml; less than a single trypanosome. (Cox *et al.*, 2005). Assuming procedural safeguards minimised laboratory error, a positive result is fairly clearly interpreted: trypanosomal DNA was detected in the blood thus indicating an active infection at the point of sampling. A negative result is more ambiguous however. Trypanosome parasitaemia show immense fluctuation over 9 orders of magnitude, from more than  $10^6$  parasites per ml to less than 1 parasite per litre of blood. (Desquesnes & Dávila, 2002). It is thus still possible that PCR techniques fail to detect low parasitaemias, simply because there is no DNA in the analysed sample. In the context of this study, it is being assumed that the study animals were clear of

trypanosome parasites at day 0 and any animals emerging as positive after this point are being taken to be new infections. It is however important to consider that the technique may have failed to identify parasitaemic animals or that infections are recrudescing from animals which were aparasitaemic at the point of sampling.

#### 4.10.4 Comparison of treatment groups 8 weeks into the interventions.

Day 56 corresponds to the second visit after the group interventions began on day 0. The interventions up to this point were as follows:

- Control group:- No interventions
- Isometamidium group:- a single dose of isometamidium chloride 8 weeks earlier.
- Insecticide groups:- Treatment with their respective protocols 8, and again 4, weeks previously.

	Value	Std.Error	DF	t-value	p-value	OR	Upper CI	Lower CI
(Intercept) Control	-2.80	0.44	669	-6.43	<0.001	0.06		
Spray	-1.05	0.75	9	-1.39	0.20	0.35	1.93	0.06
Isometamidium	-0.48	0.64	9	-0.75	0.45	0.62	2.65	0.14
Pour-on	-0.75	0.72	9	-1.04	0.32	0.47	2.41	0.09

**Table 4-9:- GLMM output comparing PCR prevalence to intervention group on day 56.**

By 8 weeks into the study, at day 56, all groups are showing some trypanosome infections by ITS-PCR (Table 4-1) Table 4-9 shows an output from a GLMM showed no significant difference between the trypanosome prevalences ( $p > 0.05$ ) of the four intervention groups. There is a suggestion that the interventions are protective in comparison to the control (Odds ratio  $< 1$ ), however this is not significant.

#### 4.10.5 Comparison of treatment groups at 21 weeks into the interventions.

Day 147 corresponds to the final visit of the study, 21 weeks after the interventions began. The interventions up to this point were as follows:

- Control villages:- No interventions
- Isometamidium villages:- a single dose of isometamidium chloride 21 weeks earlier.
- Insecticide villages:- Treatment with their respective protocols every 4 weeks from day 0, last treatment 5 weeks previously.

Twenty-one weeks into the study there are clear differences emerging between the groups in terms of trypanosome prevalences. Table 4-10 shows the model output. There are significant differences between the control group and the insecticide treated groups ( $p < 0.01$ ), however no significant difference exists between the between the control and the isometamidium treated groups ( $p = 0.96$ ).

	Value	Std.Error	DF	t-value	p-value	OR	Upper CI	Lower CI
(Intercept) Control	-7.368	0.55	498	-13.35	0.000			
Spray	-4.853	0.73	8	-6.67	<0.001	0.01	0.03	0.00
Isometamidium	-0.032	0.77	8	-0.04	0.968	0.97	4.37	0.21
Pour-on	-3.582	0.74	8	-4.81	0.002	0.03	0.12	0.01

From this it is possible to conclude that the insecticide treated animals have significantly

**Table 4-10:- GLMM output comparing PCR prevalence to intervention group day 147. Groups significantly different to the control are highlighted in yellow.**

lower trypanosome prevalence than either the control villages or the isometamidium treated villages.

#### 4.10.6 Quantification of the difference in treatment effects between groups

It is possible from the model output to calculate the odds ratios for the different treatment options. The antilog of the coefficient in the column marked 'Value' gives odds ratio relative to the first coefficient, as explained in section 3.16. By default comparisons are made with control group, however by re-specifying the factor levels other comparisons are possible. The following directive reorders the levels of the group variable so coefficients now relate to the 'spray' group:

```
Visit1$GroupS <- factor(Visit1$Group, levels=c('Spray','Iso','Pour-on','Control'))
```

Rerunning the GLMM model for day 147 with the appropriate factor structures allowed for the calculation of all the pair-wise odds ratios given in Table 4-11.

	<b>Control</b>	<b>Pour-on</b>	<b>Spray</b>	<b>Iso</b>
<b>Control</b>	-	<b>5.88</b> (1.34-25.8)	<b>13.61</b> (2.44-75.78)	1.15 (0.32-4.20) Not Sig.
<b>Pour-On</b>	<b>0.17</b> (0.04-0.75)	-	2.31 (0.56-15.05) Not Sig.	<b>0.20</b> (0.04-0.87)
<b>Spray</b>	<b>0.07</b> (0.01-0.41)	0.43 (0.07-2.81) Not Sig.	-	<b>0.08</b> (0.02-0.48)
<b>Iso</b>	0.87 (0.04-3.16) Not Sig.	<b>5.10</b> (1.3-22.7)	<b>11.80</b> (2.10-66.39)	-

**Table 4-11: Odds-ratios for interventions at day 147.**  
**Bracketed numbers refer to the 95% confidence intervals.**



Table 4-11 shows the odds ratios for the different groups 28 weeks after the commencing the different interventions. The numbers in brackets refer to the 95% confidence intervals. Confidence intervals that cross 1 are not significant, consequently numbers in bold indicate significance. Odds ratios less than 1 confer a protective effect, greater than 1 a risk. Animals in the control group have 5.88 (1.34-25.8) and 13.6 (2.44-75.78) higher odds of being positive for trypanosomes by PCR than animals in the pour-on or spray groups respectively. Similarly, animals in the isometamidium group have 5.10 (1.3-22.8) and 11.8 (2.10-66.39) higher odds of being positive for trypanosomes by PCR than animals in the pour-on or spray groups respectively. There is however no significant difference between control and isometamidium groups or between pour on and spray treated groups.

### 4.10.7 Summary of treatment effect at 8 and 21 week time-points.

There are indications 8 weeks into the study that all three interventions are offering different levels of protection compared with the control group, however none of these are significant. As the study progresses however, by day 147, there are significant differences in the detected parasitaemias between control villages and pour-on villages ( $p=0.002$ ) and between control villages and restricted spray villages ( $p<0.001$ ). There is no difference between control and isometamidium treated villages ( $p=0.83$ ), or between pour-on and spray villages ( $p=0.23$ ). The control and isometamidium groups show no significant difference between the prevalence of trypanosomiasis at day 147 compared with the baseline ( $p=0.46$ ), suggesting the disease prevalence has returned to pre-study levels. In contrast, both the pour-on and spray villages show a significant ( $p<0.01$ ) decrease in trypanosome prevalences compared with baseline values throughout the study, suggesting the insecticides are having a protective effect on the transmission of trypanosomiasis.

#### 4.11 Overall effect of treatments over time.

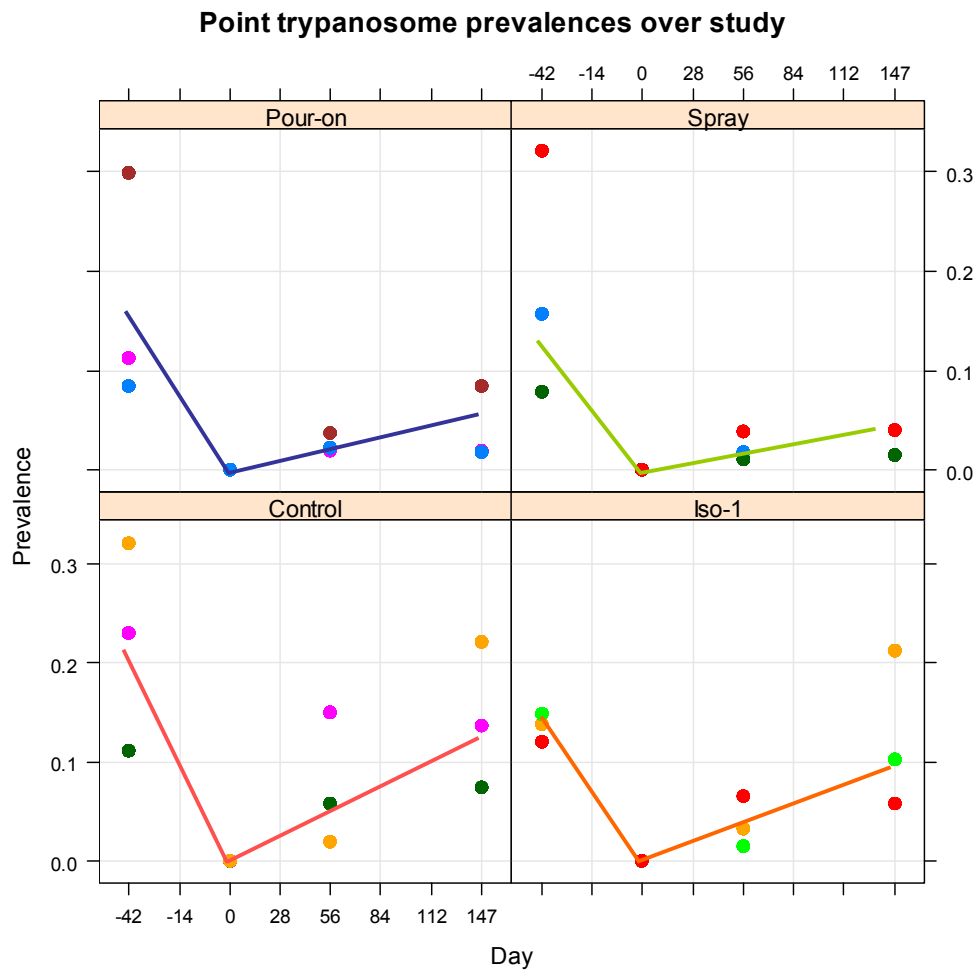
So far, analysis has concentrated on discrete time points. Whilst these temporal snapshots imply a differential rate of disease incidence between the treatment groups, they do not explicitly quantify it. Generalised linear mixed effect models allow for investigation of the overall effect of the interventions by inclusion of different time slopes into the model.

A GLMM model is fitted with time as an additional random effect with the structure  $\text{random} \sim \text{Time} | \text{Village} / \text{Animal}$ . This allows for the villages, and animals within villages to have different time slopes, i.e. the rate of change of infection prevalence can differ between villages

To reiterate the study design, the study is essentially has two parts;

- Establishing a baseline prevalences and clearing all animals of trypanosome infection trypanocidal drug.
- Investigating the incident infections in a cleared population exposed to different interventions

In this section of analysis, it is only the incidence of infection which is of interest. For the purpose of this analysis therefore only data from Day 0 was modelled. Including the baseline data values gives a 'V' shaped response curve (Figure 4.4), which is both awkward to model and clinically not important because the decrease to zero prevalence in response to the diminazene is a different question. Figure 4.4 shows the individual village prevalences over time, a best-fit line showing the change over time.



**Figure 4.4:-Dotplot showing point prevalences for all trypanosome species over time. Coloured dots represent villages, line represent mean prevalences for group.**

The following model structure was fitted to all a dataset containing all values from day 0 onwards.

`glmmPQL(PCRresult~Group, random=~Day|Village.Name/Animal ID, family="binomial"`

Table 4-12 shows the output from the model, Table 4-13 displays the odds ratio calculated for the contrasts in the model. The use of a larger dataset has reduced the variance of the odds ratios in comparison with Table 4-11. There remains a significant effect difference between the control and the spray and pour on groups, suggesting a protective effect of 0.22 (0.08-0.60) and 0.30 (0.12-0.74) respectively. Although isometamidium has a protective effect, it is not significant 0.48 (0.21-1.10).

	Value	Std.Error	DF	t-value	p-value	OR	Upper CI	Lower CI
(Intercept)	-3.262	0.24	1950	-13.63	<0.001			
Group:Spray	-1.512	0.51	9	-2.98	0.016	0.22	0.60	0.08
Group:Iso	-0.738	0.43	9	-1.73	0.083	0.48	1.10	0.21
Group:Pour-on	-1.208	0.47	9	-2.60	0.029	0.30	0.74	0.12

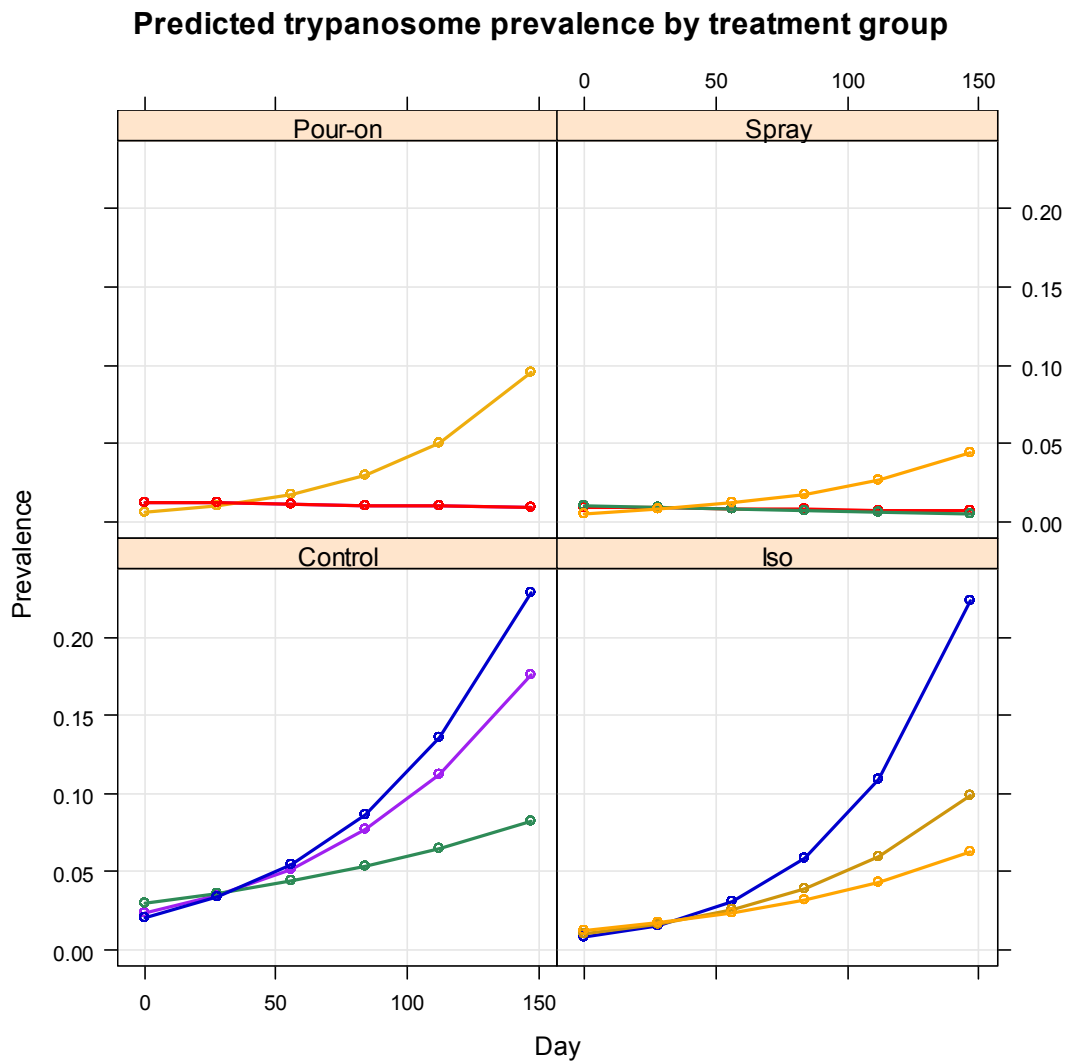
**Table 4-12:- GLMM output comparing PCR prevalence with intervention group for all intervention timepoints. Groups significantly different to the control are highlighted in yellow**

	<b>Control</b>	<b>Pour-on</b>	<b>Spray</b>	<b>Iso</b>
<b>Control</b>	-	<b>3.35</b> (1.35-8.33)	<b>4.54</b> (1.68-12.27)	2.09 (0.91-4.82) Not Sig.
<b>Pour-On</b>	<b>0.30</b> (0.12-0.74)	-	1.36 (0.42-4.39) Not Sig.	0.62 (0.22-1.78) Not Sig.
<b>Spray</b>	<b>0.22</b> (0.08-0.60)	0.74 (0.23-2.39) Not Sig.	-	0.46 (0.15-1.42) Not Sig.
<b>Iso</b>	0.48 (0.21-1.10) Not Sig.	1.60 (0.56-4.57) Not Sig.	2.17 (0.71-6.67) Not Sig.	-

**Table 4-13:-Odds ratio of different interventions for the whole intervention period**

#### 4.11.1 Predicted models of trypanosome prevalence for different groups

The output of GLMM models can be back-transformed to prevalences and plotted for each group and village. The following graphs show the predicted best-fit lines calculated from the data and can be used to illustrate the effect of the different interventions. Figure 4.5 shows the predicted values for the prevalence over time in the different villages. Each panel represents a intervention group, each line the trypanosome incidence for each village. Lines representing pour-on and spray villages overlap, hence only two lines are visible. Two out of the three insecticide treated villages do not register an increase in trypanosome prevalence from after day 0, whereas all of the villages in the control and pour on group show an increase.



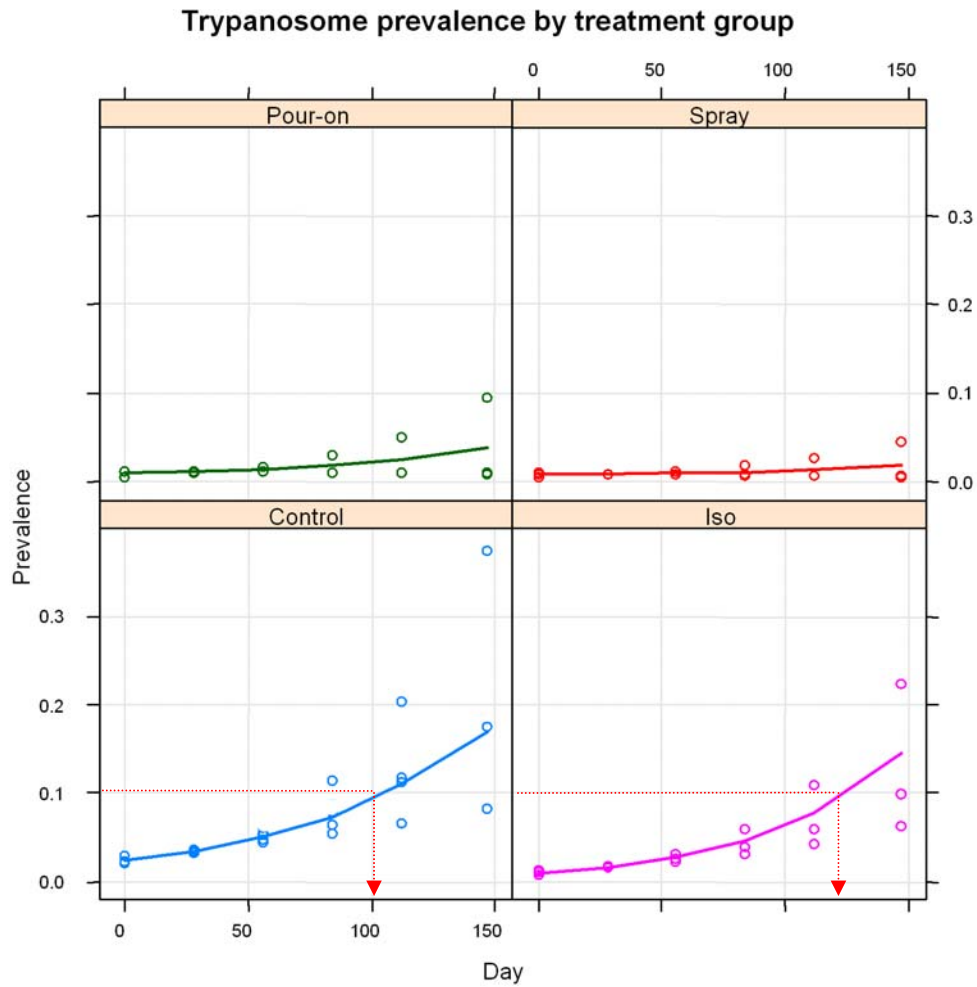
**Figure 4.5:- Predicted values from GLMM, showing the change in trypanosome infection over time. Each panel represents a intervention group, each line the trypanosome incidence for each village.**

#### 4.11.2 Predicted duration of isometamidium prophylaxis

Figure 4.6 shows the same model predictions as Figure 4.5 but averaged out per group. If it is assumed that the control and isometamidium groups have a similar disease challenge, and hence share a similar incidence curve, then it is possible to estimate the prophylactic effect of the isometamidium. Taking a point prevalence, such as 10%, it can be seen that isometamidium villages take approximately 25 days longer than control villages to reach this threshold. (red dotted line, Figure 4.6).

It should be emphasised that these calculations rest on many assumptions, such as the rate at which the two groups acquire infections, and are based on the mean predicted values from a model fitted to a small number of time points. With that in mind however, it could be suggested that the prophylactic period for isometamidium chloride is of the order of 3-4 weeks in the sample villages. Work done in neighbouring Kenya concluded the duration of prophylaxis of 7-10 weeks and attributed demonstrable parasitaemias within 28 days as evidence of resistance. (Stevenson *et al.*, 2000). Earlier work done under a very similar protocol to this study demonstrated an average time to a 10% incidence of 7.5 $\pm$  1.9 weeks. (Stevenson *et al.*, 1995). These earlier studies used phase contrast microscopy to detect infections so it is very possible that the more sensitive PCR detecting lower emerging parasitaemias, rather than resistant infections. Strain-typing could be employed to investigate this further (Hide & Tilley, 2001; Tilley *et al.*, 2003), however for the purposes of assessing the interventions this result would be consistent with the absence of any noticeable effect of the isometamidium at the end of the study.



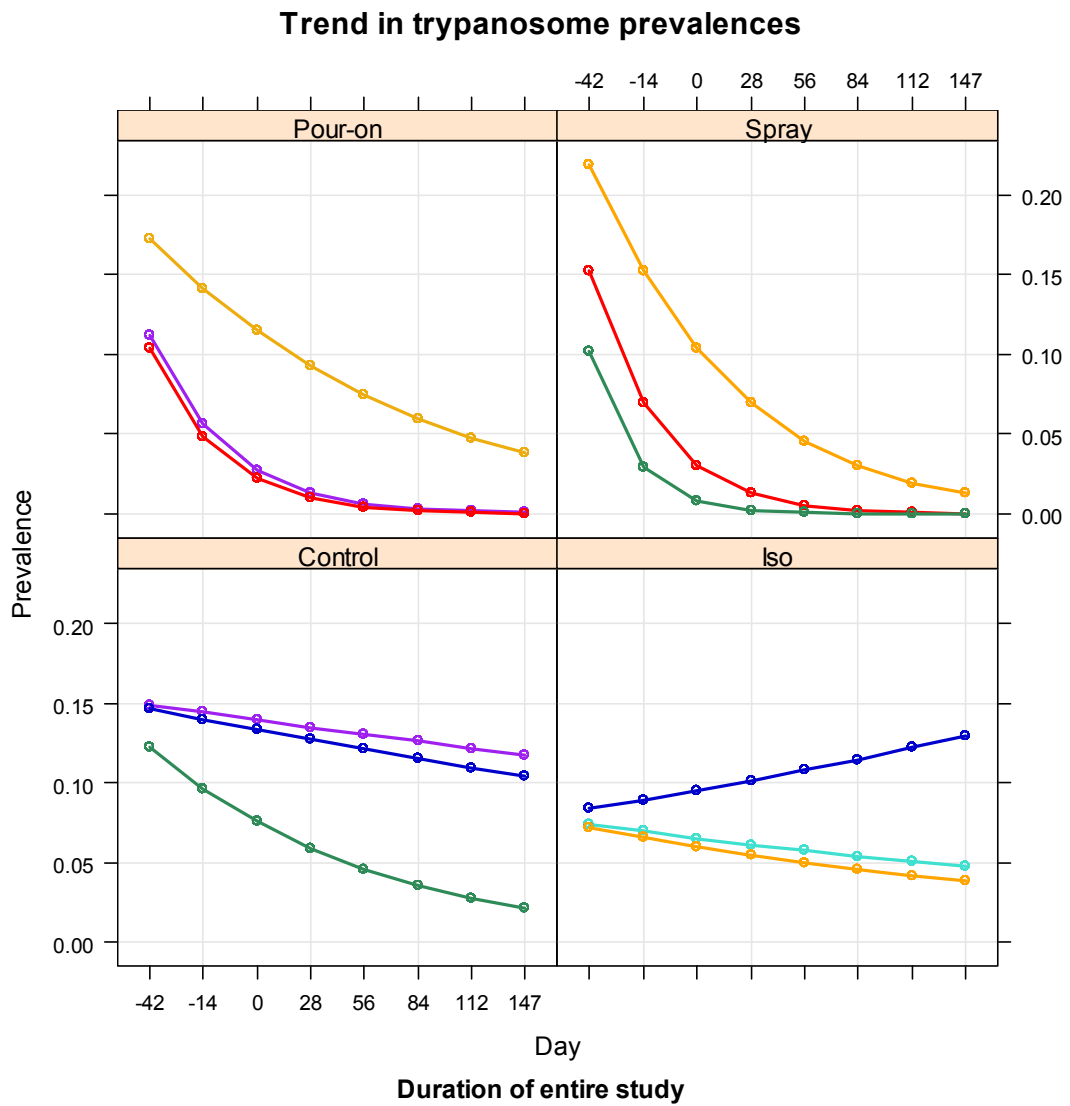


**Figure 4.6:- Predicted values from GLMM showing change in trypanosome infection over time per group. Red dotted line shows predicted time for control and isometamidium villages to regain a 10% prevalence.**

### 4.11.3 Overall effect of study treatments

Finally, a model was fitted to assess the impact of both the common diminazene treatments and specific interventions. Figure 4.7 shows the predicted values for the entire study, and shows the overall effect of the common treatments of diminazene, in addition to the group specific interventions. With one exception in the isometamidium group, there is an overall decrease in prevalence for all villages, however this effect is more profound in insecticide treated villages.

The use of multiple drug treatments offers a plausible way of implementing disease control programmes. An example from S.E. Uganda is the recent EU funded FITCA (Farming in Tsetse Controlled Areas) initiative (FITCA, 2005). Cattle were block-treated with an injectable trypanocide (either diminazene and isometamidium) and farmers were left a quantity of deltamethrin insecticide for subsequent treatment. Figure 4.7 could be interpreted as the overall effect of a typical control programme where twice yearly animals are given a block treatment with a trypanocide by a central agency, and interim treatments are provided by farmers groups. Furthermore, as an approximation, the area under each curve can be interpreted as the burden of infection for the village in question, and potentially as an indication of the transmission risk. It has to be emphasised that such interpretation assumes many things about the disease transmission, and in specific the epidemiological significance of PCR positive animals, but it provides an indication of the level of protection conferred by each intervention protocol.



**Figure 4.7: Predicted values from GLMM, showing the change in trypanosome infection over the whole study. Each line represents a village.**

## 4.12 Tree models

There was an abundance of data generated in the study and the choice of analysis method was dependent on the hypothesis under scrutiny. GLMMs were employed as a way of accounting for the heterogeneity of individuals and pseudoreplication implicit in longitudinal studies. In contrast, when it comes to investigating the relationship between an response and a list of potentially significant factors, GLMM models did not prove amenable to multiple explanatory variables. In addition, the algorithms used by the function `glmmPQL` to fit the mixed effect models to binary data have to approximate the fit, and as such models cannot be directly compared, for example by the `anova` function (B.Ripley, per comm.) As a result the comparison of different models with different fixed effects is not possible. Without robust methods of model comparison and simplification, it is difficult to coherently take these models forward. Additionally, penalised quasi-likelihood estimation techniques have been shown to produce biased estimates for multi-factorial models, especially so when the fitted probabilities are close to 0 or 1. (Lin & Breslow, 1996). Methods of resolving are a matter of active debate in the statistical literature, (Ng *et al.*, 2006) however for the purpose of analysing this dataset, other avenues were required.

Tree models offer an alternative way of analysing the data generated from molecular analysis, and are particularly well suited to large, multi-factorial and unbalanced datasets typical of longitudinal studies. A classification tree can readily be grown with a binary response variable and numerous explanatory variables, either categorical or continuous. The tree algorithms examine each variable and the initial bifurcation is made at the level of the variable that best partitions the dataset to minimise the deviance. This is recursed until no remaining variance can be explained. In comparison with the generalised linear models used above, no interactions between variables are considered and thus the order of the variables are put in the in the model is not significant. Tree models are used in this chapter to explore the broad patterns of variation between the groups and highlight any associations not readily

apparent from a linear modelling perspective. A more detailed explanation of tree models is given in section 3.17

### 4.12.1 Growing a tree model of all the data

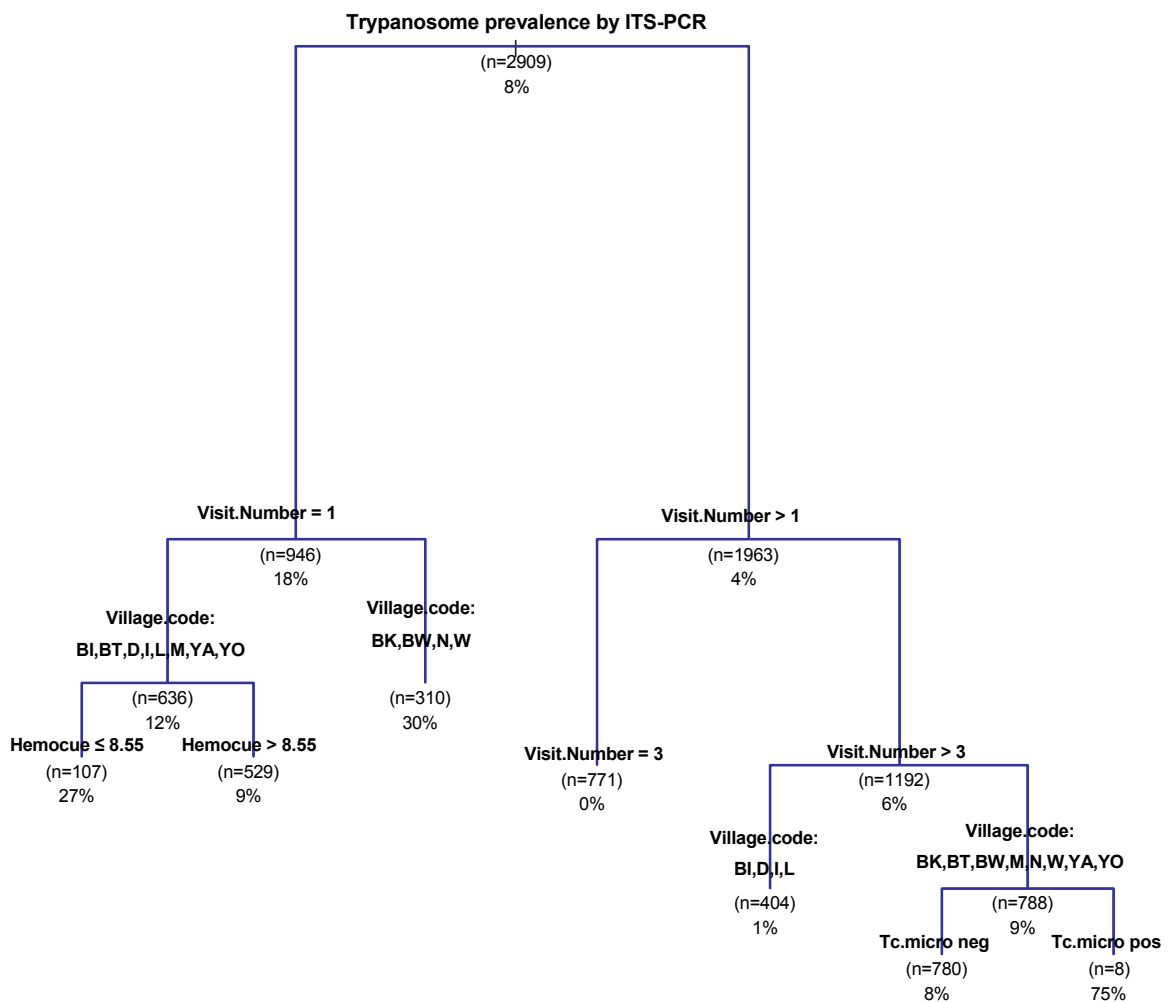


Figure 4.8:- Tree model fitted to all variables in entire dataset

Figure 4.8 shows the output from a tree model relating the ITS-PCR screening result for trypanosomiasis against all the geographical, individual, clinical and diagnostic independent variables, in specific;

PCR~Visit.Number+Village.Code + Treatment group + Age + Weight + Sex+ Condition Score + Hemoglobin level+ Faecal consistency+ T.parva status + Anaplasma status+ Lymph node size+ Skin condition + Coat condition + Faecal egg count + Faecal coccidia status + Fluke egg status + Tick count, *Rhipicephalus* species + Tick count, *Amblyomma* species + Tick count, *Boophilus* species + Tick-borne disease status (by microscopy) +Trypanosome status (by microscopy).

Despite the inclusion of all these variables in the model, it can be seen from the output in Figure 4.8 that not all variables are displayed. If an explanatory variable does not provide a way of partitioning the variance in the response variable, it will not be included.

The first partition is the visit number; this is not surprising given that the intervention of double dose trypanocide prior to day 0 which reduced the observed prevalence to zero. The prevalence of animals at the baseline is 18% compared with 4% thereafter. Continuing down the right hand branch, the next split is between visits prior to day 53, where the prevalence as seen is zero, and 53-end of study, where the incidence of trypanosomiasis was increasing.

Given the variation due to time, village accounts for the next biggest split in variation. This serves to justify the use mixed effect models, allowing for random variation between villages, but also suggests that variation at the village level, rather than the intervention group, is the significant division.

Considering the left branch, after time and village variations, haemoglobin partitions the remaining variance at a threshold of 8.55g/dl. Cattle with a haemoglobin level below 8.55g/dl

have a trypanosome prevalence of 27%, compared with 9% for those less anaemic animals above the threshold.

The right branch has no haemoglobin split, but given time and village, the microscopy status of the animal for *T.congolense* spp. is significant.

Although none of the above results is particularly surprising, it is interesting to note the position trypanosome prevalence by microscopy has in relation to all the other variables. Although more significant than any other clinical variable, trypanosome prevalences by microscopy do not appear strong indicators of trypanosome prevalences by PCR. This is as expected and found by other comparisons(Picozzi *et al.*, 2002)

#### 4.12.2 In search of subtlety

For the next section, tree models are being used to look for associations that are not as strong as the known effects of visit number or village. By removing variables from the model, variance attributable to those variables is returned to the model. This confounds the data, but can be useful for highlighting associations that otherwise would be ignored by the model.

Figure 4.9 shows the output for an identical model to Figure 4.8 however with the village effect removed. Visit number is still the most important partition, however for samples taken after day 0 (Visit 3) it can be seen that insecticide groups have a trypanosome prevalence of 3% compared with 10% for the non insecticide groups 10%.

On both sides of the tree, lower haemoglobin levels are associated with higher trypanosome prevalence which is consistent with expectations. The area highlighted A shows a potentially clinically incongruous result however. Animals with a haemoglobin of over 8.05 g/dl and a condition score of >4.5 have a 17% prevalence of trypanosomes, compared with 7% for those with a condition score of <4.5. Chi-squared tests found this to be significant ( $\chi^2 = 11.26$ , df = 1, p-value = <0.001). If the effect is genuine, it may reflect either the clandestine treatment of thin animals by farmers or indicate the degree of subclinical infections in the population.

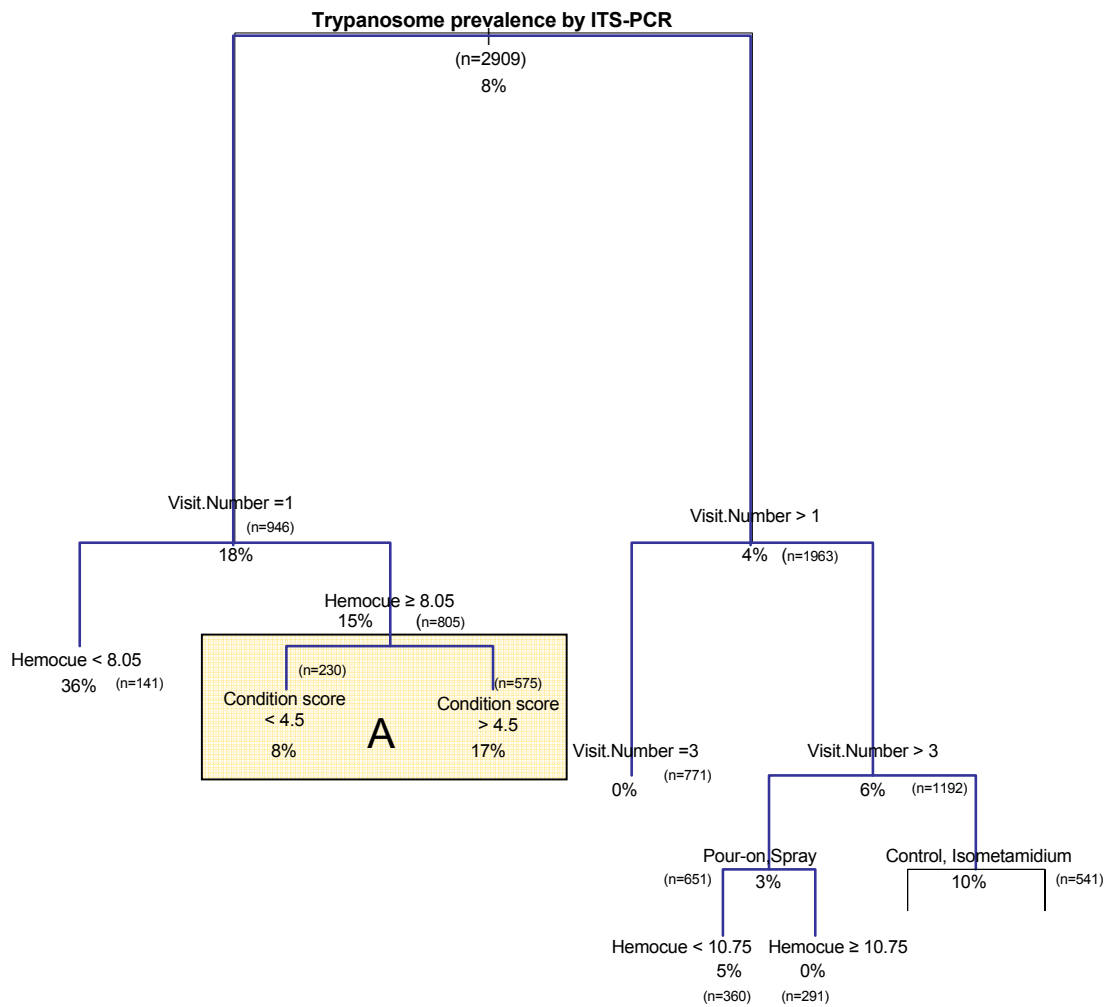


Figure 4.9:- Tree model fitted to all variables in entire dataset excluding village. Area highlighted 'A' indicate  $\chi^2$  comparisons indicated in the text.



### 4.12.3 What factors best explain the incidence of trypanosomiasis?

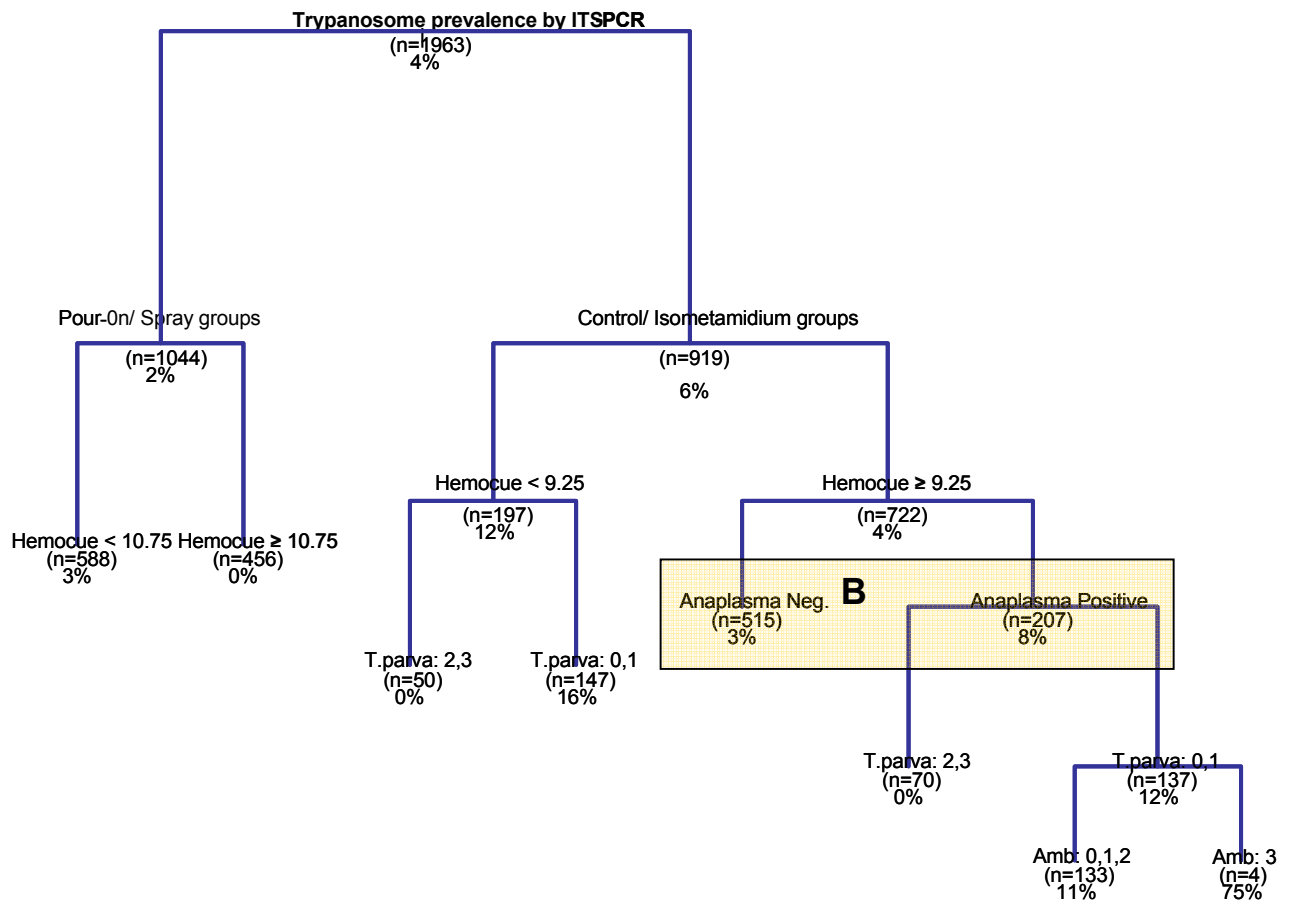


Figure 4.10:- Tree model fitted to all clinical variables from day 0, excluding time and village. Highlighted area 'B' refers to  $\chi^2$  comparisons indicated in the text

The purpose of this section is to investigate if any of the study variables can explain the differences in trypanosome incidence during the intervention period. Figure 4.10 shows a model fitted to data from Day 0 excluding time and village as variables. The most significant variation is between treatment groups, with the division between insecticide and non-insecticide treated villages. Haemoglobin also remains a significant variable, again indicating that that anaemia and trypanosome prevalences are positively correlated.

There is an indication that anaplasmosis (diagnosed by microscopy) and trypanosomiasis are also positively correlated. For animals in the non-insecticide treated groups and with a blood haemoglobin over 9.25g/dl, anaplasma positive animals have a trypanosome prevalence of 8%, compared with 3% for their uninfected counterparts.(highlighted B on Figure 4.10) This is a significant association ( $\chi^2 = 9.55$ ,  $df = 1$ ,  $p\text{-value} = 0.002$ ), similar to the result of the univariate analysis covered in section 4.7 above suggests.

### 4.12.4 Summary of tree models results

Table 4-14 summarised the relative positions of the variables fitted to the tree models. A '+' indicated the variable was included in the model; a number shows the position the variable came in the tree, and a '-' indicates the variable did not explain any of the variance so was not included in the tree. Included in the table are the outcomes for two further tree models fitted to timepoints 53 and 147 respectively.

Tree models offer some insight into the relative importance of explanatory factors. Table 4-14 shows temporal and spatial variables to be the best way of partitioning the trypanosome prevalences. This is consistent with the findings from the generalised linear mixed effect modelling earlier in the chapter.

The importance of haemoglobin as a significant indicator of trypanosome infection (Sekoni *et al.*, 1990; Taylor & Authié, 2004) is supported by this data analysis. The correlation between anaplasmosis and trypanosomiasis also appears significant. This supports the findings from generalised linear mixed effect modelling, and the previous findings as discussed by (Fox *et al.*, 1993; Magona & Mayende, 2002).

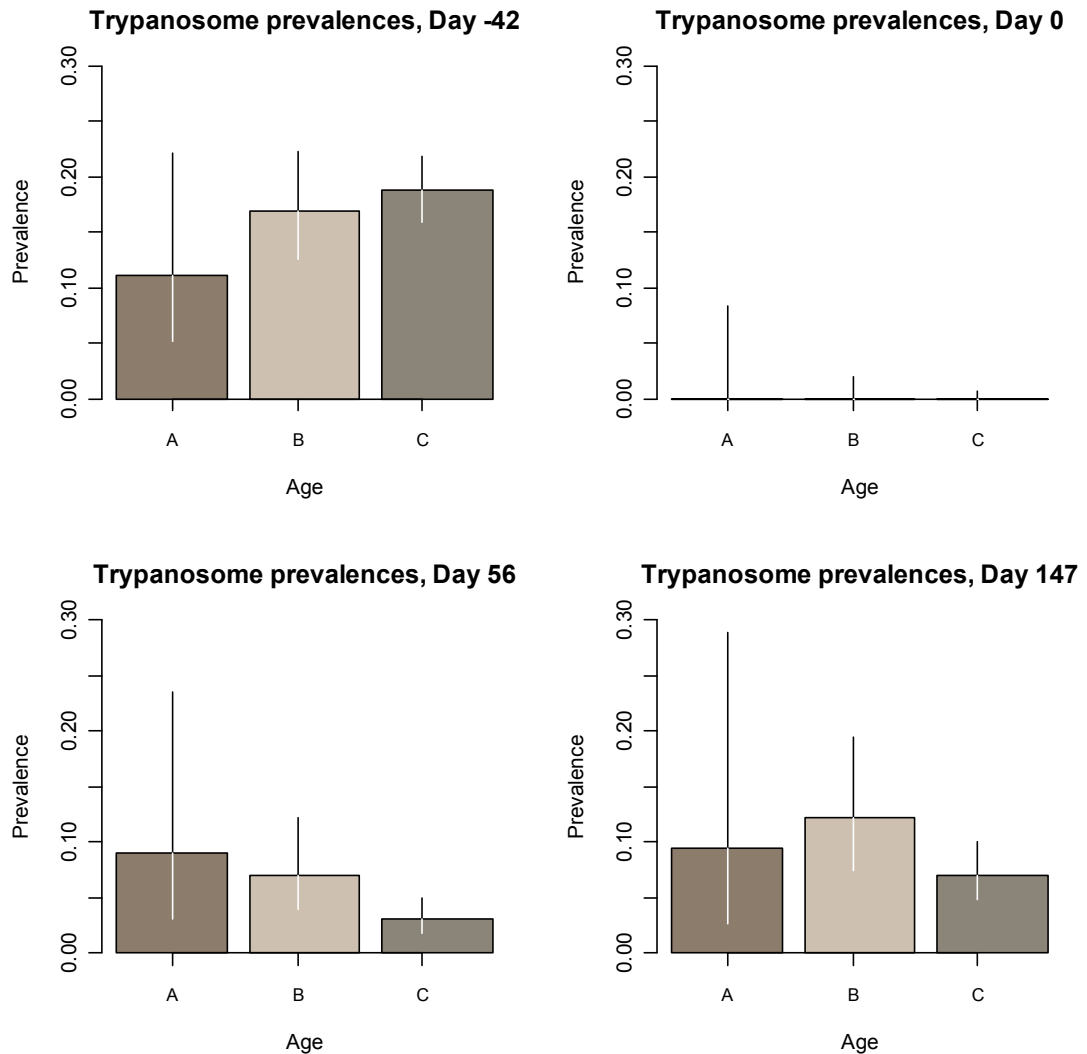
	Figure 4.8		Figure 4.9		Figure 4.10		Not shown		Not shown	
	All time points		All time points		Day 0 onwards		Day 56		Day 147	
<b>Temporal and spatial factors</b>										
Village	+	2	-	NA	-	NA	-	NA	-	NA
Visit number	+	1	+	1	-	NA	-	NA	-	NA
Intervention group	+	-	+	4	+	1	+	-	+	1
<b>Animal factors</b>										
Sex	+	-	+	-	+	-	+	-	+	-
Breed	+	-	+	-	+	-	+	-	+	-
Age	+	-	+	-	+	-	+	-	+	-
Colour	+	-	+	-	+	-	+	-	+	-
Weight	+	-	+	5	+	3	+	-	+	4
<b>Clinical factors</b>										
Condition Score	+	-	+	3	+	-	+	-	+	-
Staring Coat	+	-	+		+	-	+	-	+	-
Lumpy skin	+	-	+		+	-	+	-	+	-
Lymph node size	+	-	+		+	-	+	-	+	-
Tick count:- <i>Rhipicephalus spp</i>	+	-	+		+	-	+	4	+	5
Tick count:- <i>Amblyomma spp</i>	+	-	+		+	5	+	-	+	-
Tick count:- <i>Boophilus spp</i>	+	-	+		+		+	3	+	-
<b>Laboratory factors</b>										
Haemoglobin level	+	4	+	2	+	2	+	1	+	2
Positive for T.parva by micro.	+	-	+	-	+	4	+	-	+	-
Positive for Anaplasma by micro.	+	-	+	-	+	3	+	2	+	3
Faecal WEC count	+	-	+	-	+	-	+	-	+	-
Positive for Tc/Tv/Tb by micro.	+	-	+	-	+	-	+	-	+	-

**Table 4-14:- Explanatory variables fitted to tree models. ‘+’ = factor included in model. Numbers represent position in tree ‘-’= variable not significant.**

It is necessary to remember when interpreting the nodes that tree models do not directly consider correlations between variables, and do not allow for an interpretation of causality. For example, weight appears as a partitioning variable in three of the models, where increased size is associated with lower trypanosome prevalences. This could be explained in several ways; heavier animals could plausibly have lower trypanosome prevalences due to preferential treatment by farmers of their heavier, more valuable, traction animals. Probably much more likely is the correlation between weight and clinical indicators of disease, trypanosomiasis causing weight loss for example, and the chance that a continuous variable, such a weight, offers more ways to partition the variance than a variable with a small number of factors or a binary split.

It is also of interest to see what variables do not appear as a node in tree diagrams. For example, none of the 'signalment' variables, (age, sex, breed, and colour) appears at any level of the tree models. This is broadly in line with the result from the univariate analysis in section 4.7, although that analysis suggested the sex of the animal was significant. Just because a factor doesn't form a partitioning node does not necessarily indicate it is not important or significant; it may be for example that it's effect is more comprehensively explained by another variable. It is useful as an indication however, and in this case there are no indications that there is a particular signalment demographic that is at a higher risk of testing positive for trypanosomiasis.

To illustrate this, Figure 4.11 shows a barplot of trypanosome prevalences for different age groups over the course of the study. Error bars represent 95% confidence intervals and as they overlap it can be seen there is no significance difference between the different age groups in terms of trypanosome prevalence by ITS-PCR.



**Figure 4.11: Bargraph showing trypanosme prevalences by age grouping over time. A=Calf (0-8 months) B=Juvenile (8-28 months) C=Adult (>32 months). Error bars show exact 95% confidence intervals.**

### 4.13 Tree models: - So why branch out?

Tree models offer a graphical way of by highlighting interesting patterns in the data. They offer a complementary perspective on the data to the mixed effect models and show broad patterns and highlight interaction mixed effect models cannot. For example, a full mixed

effect models with more than 5 interaction terms fails to converge, mixed effect models are order specific and interaction terms with 3 or more interaction terms provide reams of output which are difficult to interpret. Tree models however do not directly assess the significance of the associations, or consider interactions, and as such can over interpret the data.

#### 4.14 General summary and conclusions

Generalised mixed effects model have been used in this study to model the effect of group on the trypanosome prevalences obtained from PCR analysis of whole blood samples using an ITS-protocol. Village and individual animals within village were fitted as a random effect to account for the spatial heterogeneity of village and autocorrelation from repeated measurement of a single individual. The following conclusions may be drawn:

Although significant variation existed between villages at the baseline, taken on a group level there were no significant difference between the treatment groups at the start of the study. The grouping of villages appears to have been adequate at averaging out individual village variations, to the extent that no statistical difference existed between groups at the start of the study. The heterogeneity of individual villages however necessitated the use of mixed effect models for subsequent analysis.

Treatment of all cattle with two doses of diminazene aceturate at a dose of 7.0mg/kg prior to commencing the interventions at day 0 reduced the prevalence in all the villages to zero. Subsequent prevalences were attributed to new infections and used as an indication of the degree of protection to incident infections offered by allocated treatment.

Separate analysis of the individual time points shows no significant difference between the prevalences 56 days into the study, and although there was a suggestion that the control group has higher prevalence of trypanosomiasis than the other groups (8.15% compared with 3.15%) this is not statistically significant ( $p=0.56$ ). By day 147 however, there is a significant difference between intervention groups. Trypanosome prevalence in the control

and isometamidium treated groups is 15.5% and 15.4% whereas the prevalence of the pour-on and spray groups is 3.7% and 1.4% respectively. There is a highly significant difference between the control and pour on groups and the control and spray groups ( $p < 0.001$ ).

Incorporating time into a generalised linear mixed effect models model and fitted to all the intervention timepoints still maintains a significance difference between the insecticide and non-insecticide treated groups ( $0.01 < p < 0.05$ ). No significant difference exists between the control and isometamidium groups or between the pour-on and spray groups.

In conclusion therefore, once temporal and spatial clustering are accounted for by fitting a generalised linear mixed effect model with binomial error structure, there is a significant protective effect of both insecticide treatments compared with either the control or the isometamidium treated animals. Animals in the pour-on and spray groups have 3.4 and 4.5 respectively lower odds of becoming infected with trypanosomiasis than those in the control group (Table 4-13) There is no significant difference between the two insecticide treatments, suggesting that, on the basis of trypanosome prevalence elucidated by molecular techniques able to identify parasite DNA, either treatment protocol is protective and statistically indistinguishable.



**Chapter 5 :-** Use of a pen-side test for haemoglobin to assess the impact of the intervention protocols

## 5.1 Introduction

This chapter explores the change in cattle health during the longitudinal study carried out in south east Uganda, using one of the tools available for pen-side diagnosis; a portable haemoglobinometer. The measurement of an animal's haemoglobin offers a method of clinically quantifying the health of the animal, using a tool that could potentially be available to rural livestock keepers or animal health workers. In this chapter, changes in an animal's haemoglobin will be assessed in relation to its intervention protocol, with the aim of quantifying the impact of the interventions on this important determinant of animal health.

The data collection methods and study design are outlined in chapter 3. For the purposes of analysis in this chapter the dataset can be effectively split into two time periods. From the first baseline visit (day -42) up to and including visit 3 (day 0), the data can be used to assess the impact of two diminazene treatments on a population of 947 animals roughly equally spread between 12 villages. After day 0, the data can be used to assess if there is a significant difference between the different intervention protocols tested in this study.

## 5.2 Anaemia as an indicator of disease

The normal range of haemoglobin in healthy cattle is 8-15g/dl (Schalm *et al.*, 1980). Anaemia can be defined as a pathological state in which the quantity or quality of the circulating red blood cells is below normal levels. This state has multiple aetiologies, broadly classified into three causal categories: increased destruction of red blood cells (haemolytic anaemia), decreased production of red blood cells or haemoglobin (non, or inadequate, regenerative anaemia) or simply from extra vascular loss (haemorrhage) (Eddy *et al.*, 2003).

Anaemia is a major clinical sign of many parasitological infections, and its assessment remains an essential indicator in the diagnosis and monitoring of many endemic African diseases. Common examples of diseases with anaemia as a clinical symptom, in both humans and animals, include malaria (Pasvol, 2005), schistosomiasis (King *et al.*, 2006), malnutrition (Perry *et al.*, 2002), intestinal helminthiasis (Brown, 2005), tick infestation

(Perry *et al.*, 2002), anaplasmosis (Gale *et al.*, 1996), babesiosis (Vial & Gorenflot, 2006) and trypanosomiasis (Maudlin *et al.*, 2004).

Studies comparing cattle populations with and without an anaemia causing disease (gastric ulceration) found healthy animals had a haemoglobin level of 10.4g/dl compared with 7.2g/dl for diseased animals (Ok *et al.*, 2001). In samples investigated by microscopy for tick-borne diseases, it was found that animals positive for *Babesia spp.* had haematological parameters (haemoglobin  $5.9 \pm 1.54$  g/dl) significantly lower ( $P < 0.05$ ) than in those with *Theileria spp.* (haemoglobin  $9.7 \pm 0.79$  g/dl) or negative (erythrocyte haemoglobin  $10.4 \pm 1.78$  g/dl) (Garcia-Sanmartin *et al.*, 2006). Of all the above diseases, trypanosomiasis is considered to induce the most severe anaemia, (Taylor & Authié, 2004), and anaemia is widely used as a primary indicator of the degree of pathology of this disease (Trail *et al.*, 1990a; D'Ieteren *et al.*, 1998; Taylor, 1998). It is the control of anaemia, more than control of parasitaemia, that allows trypanotolerant cattle to remain productive in areas of high trypanosome challenge (Naessens, 2006). In field conditions, cattle are exposed to multiple disease challenges that can exacerbate anaemia, for example concurrent trypanosome and helminth infections (Dwinger *et al.*, 1994) or trypanosome and anaplasmosis (Fox *et al.*, 1993) infections can exacerbate the susceptibility of animals. The severity of anaemia in bovine trypanosomiasis is also associated by nutritional status, with animals on a poor plane of nutrition experiencing a more acute and profound anaemia (Agyemang *et al.*, 1990; Osaer *et al.*, 2000).

### 5.3 Pathogenesis of anaemia in bovine trypanosomiasis

Although anaemia is a clinical indication of a number of livestock diseases, its marked severity in trypanosomiasis makes it a particularly important indicator. The onset of anaemia is correlated to the first appearance of trypanosomes in the blood, although the magnitude and severity of the pathology is contingent upon such factors as the species of trypanosome, the innate and acquired resistance of the host animal, the nutritional status of the animal, and presence or absence of other concurrent infections (Murray, 1988). The initial infection period is also characterised by pyrexia and an activated and expanded population of mononuclear phagocytes. It is currently believed that erythrocyte destruction as a result of

opsonisation and removal by monocytes causes haemolytic anaemia to be seen in the early phases of infection, whereas in chronic infections (>1 month) animals tend to suffer a non regenerative anaemia from ineffective erythropoiesis (Taylor & Authié, 2004). Chronic anaemia hugely undermines an animal's productive capacity and resistance to other diseases, as well as negatively affecting its ability to cope with concurrent stressors, such as parturition, lactation or exhaustion, for example that caused by extensively foraging or by being used for traction. As such, mortalities due to trypanosomiasis can be as attributable to immunocompromised animals succumbing to infections they could otherwise resist as to direct trypanosomal induced pathology, such as heart failure (Taylor & Authié, 2004).

### 5.3.1 Finding the pale cow

Measuring anaemia has traditionally involved assessing the animal's packed cell volume (PCV) by centrifuging a blood-filled microhaematocrit tube at 12000rpm for 3 minutes and measuring the proportion of blood volume occupied by red blood cells (Uilenberg, 1998). This technique is reliable and cheap to run, but requires a power source and costly equipment to set up. Low technology systems have been developed to assess anaemia in the field context. The WHO colour scale provides a standardised colour chart against which blood samples can be compared by eye (Lewis *et al.*, 1998; Critchley & Bates, 2005). From a clinical perspective, assessment of the pallor of mucous membranes allows for the subjective assessment of anaemia, jaundice and hydration status of the animal as well as signs of disease, such as Foot and Mouth disease or rinderpest. The FAMACHA© chart (Bath *et al.*, 1996) was developed to quantify the burden of *Haemonchus contortus* in small ruminants by comparing the pallor of mucous membranes to a 5 point colour scale, and has recently been used as an objective measurement of anaemia in these species (Sissay *et al.*, 2007). A recent review compared three existing technologies for measuring haemoglobin in the field, a visual colour scale and two instruments capable of quantifying haemoglobin concentration from the optical density of the sample (Magona *et al.*, 2004b). Although all techniques were advocated as acceptable for application in the field, each technique suffered in terms of either accuracy, cost, or ease of use. For the purposes of this study, the most simple and accurate (hence also

the most expensive) technology was chosen, a *HemoCue* digital haemoglobinometer system (Angeholm, Sweden). The use of this system and a description of the sampling methodology is given in section 3.2.5.

## 5.4 Do the drugs still work?

As explained in section 2.9, all cattle in the study received two doses of diminazene aceturate at 7mg/kg on the first and second visits (Day -42 and -14 respectively). The two doses were administered with the aim of clearing all existing trypanosome infections by Day 0. Assessing the impact of the diminazene treatments themselves is however worthwhile for several reasons. Assessment of the homogeneity of the cattle population at the start of the interventions on day 0 is necessary to contextualise differences in treatment effects. As a treatment in its own right, diminazene aceturate represents a mainstay of the therapeutic options available to livestock keepers (Machila *et al.*, 2003; Magona *et al.*, 2004a). A study, conducted in the same area of south east Uganda as the research discussed in this thesis, found 48% of cattle owners knew about and used diminazene aceturate as a treatment option, comparable to 55% for isometamidium chloride. Farmers were significantly more aware of these treatment options than of the alternative options presented, such as pour-on insecticide (1%) or fly trapping technologies (8%) (Magona *et al.*, 2004d).

### 5.4.1 Drug resistance to diminazene aceturate

Although there is no evidence of resistance to diminazene aceturate in south east Uganda, the widespread use of the drug as described above makes resistance a possibility (Geerts & Holmes, 1998). Aside from the obvious implications resistance development would have on the control of animal disease, there have been concerns raised about cross resistance with the human trypanocide melarsoprol. Both drugs rely on the same P2 aminopurine transporter in the trypanosome membrane for uptake of drug by the parasite. Trypanosome strains lacking the P2 transporter exhibit reduced uptake and increased resistance to the trypanocidal agent (Carter & Fairlamb, 1993; Barrett, 2001; Geerts *et al.*, 2001). In light of the increasing awareness of the role of cattle in the spread of human sleeping sickness (Fèvre *et al.*, 2001),

and the desire to reduce the prevalence of human-infective trypanosomes in the cattle reservoir host (Welburn *et al.*, 2001a), any nascent indicators of resistance are vital. Although *in vitro* work has demonstrated the presence of a diminazene and isometamidium resistant isolate of *T. b. rhodesiense* in SE Uganda (Matovu *et al.*, 1997), the status of resistance among *T. congolense* and *T. vivax* is not clear. The assessment of the twin doses of diminazene on the prevalence of trypanosomes was covered in the previous chapter. In this chapter the effectiveness of diminazene treatments is assessed in terms of haemoglobin.

In addition to its trypanocidal effect, diminazene aceturate is a viable treatment for babesiosis caused by *Babesia bigemina* or *Babesia bovis* endemic to SE Uganda (Okello-Onen *et al.*, 1998b; Vial & Gorenflot, 2006). However, it is worth noting that in the areas of south east Uganda, trypanosomiasis is considered to be a significantly more prevalent and important pathogen (Okello-Onen *et al.*, 1998b; Magona & Mayende, 2002).

### 5.5 Methodology of data collection and visualisation

The structure of the longitudinal study is described in section 2.1, but to briefly summarise here: 945 cattle were selected and tagged from 12 villages in south east Uganda and sampled monthly for 8 months. Villages were allocated into four equal groups and each group randomly given an intervention protocol for the duration of the study. Interventions are detailed in Table 2-4, each representing a method of controlling trypanosomiasis. At each sampling point, a number of clinical and laboratory variables were collected from each animal. Analysis in this chapter focuses on the haemoglobin values measured for each animal at each sampling point. Values range between 3-16 g/dl, and for the purposes of analysis are considered as a continuous variable. There are two sources of pseudoreplication to be considered; a temporal source from repeated samples on the same animal and a spatial source from clustering animals within villages. Aside from normal line graphs, the following plots have been used to visualise and describe the data.

### 5.5.1 Box and whisker plots

Box and whisker plots provide a condensed way of visualising continuous data for multiple categories. Each vertical plot represents the haemoglobin distribution for the grouping variable in question. The horizontal line shows the median response for each group, the top and bottom of the box delineate the 25 and 75 percentiles respectively, i.e. the middle 50% of the data. The horizontal line at the end of the dotted 'whisker' shows 1.5 times the interquartile range of the data; points beyond this are drawn individually as open circles. These plots are useful for showing the spread of the data and any asymmetry in the data (different sizes of the two halves of the box). In addition, the box width is proportional to the sample size for each group to indicate the amount of data contribution to each plot.

### 5.5.2 Multiple comparisons

The purpose of initial data exploration is to highlight interesting comparisons for further investigation, more precisely, significant differences between villages or treatment protocols. The problem with making multiple significance tests of every potential combination is that the probability of finding a "significant" difference just by chance increases. Comparing the means for all the different villages using t-tests will inflate the probability of declaring a significant difference when it is not actually present (Type I error) (Crawley, 2005). Confidence intervals are calculated with a given coverage probability for each interval, but the interpretation of the coverage is usually with respect to the entire family of intervals (Miller, 1981; Venables & Ripley, 2002). John Tukey introduced intervals based on the range of the sample means rather than the individual differences and these can be used to more accurately create a set of confidence intervals on the differences between the means of the levels of a factor. The `multcomp` function in the R statistical package has been used to generate the subsequent outputs and provides more information in a set of simultaneous confidence intervals than achieved from a set of individual significant tests of differences (Crawley, 2005).

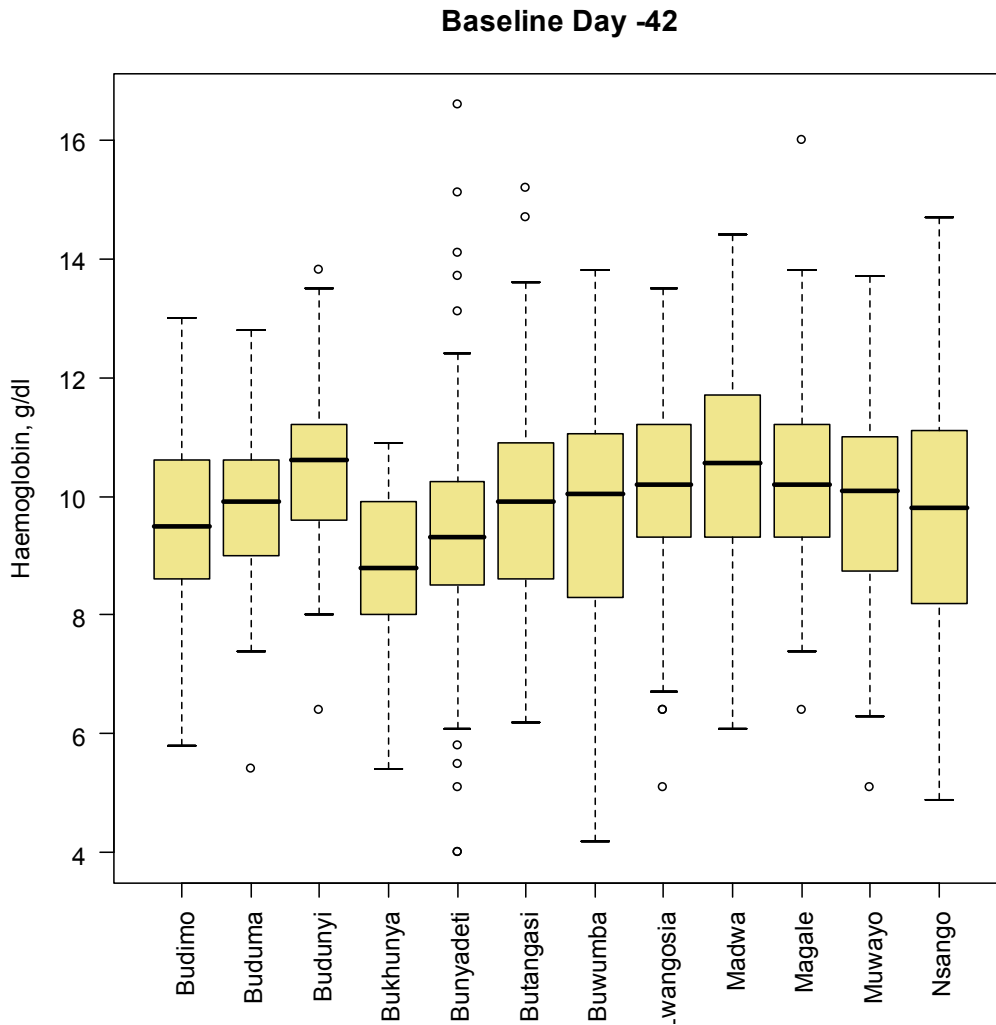
## 5.6 Overview of analysis and results

The analysis of haemoglobin in this chapter is structured as follows. Initial comparisons are made between the different grouping structures of the data (groups and villages within groups) with the aim to quantify any heterogeneity that may bias the interpretation of the treatment effects. The effect of diminazene is then investigated which leads to an assessment of the anaemia status of the groups at the beginning of the interventions. Quantification of the effect of the interventions is made by linear mixed effect modelling and the evolution of the model that best fits the data is described. Assessment of the mean haemoglobin and the change of haemoglobin over time are made with respect to the intervention administered. Interpretation of the haemoglobin values is then considered in relation to absolute thresholds of anaemia for each intervention group. Finally, analysis investigates a specific question asked by the livestock keepers enrolled in the study, namely the improvement seen in their animals compared with the previous visit.

## 5.7 Differences between villages at baseline

The techniques described in above can be illustrated by comparing the differences between villages at the baseline. Villages, and animals within villages, were recruited with as much care as possible to ensure treatment groups were as homogenous as possible (Chapter 2). How successful this attempt was can be assessed by comparing the haemoglobin levels between villages. Figure 5-1 is a box and whisker plot of the haemoglobin values at the baseline sampling point. It can be seen that there is some variation in the mean haemoglobin value in different villages. Figure 5-2 is the output from the multiple comparison analysis. Each row shows the 95% confidence limits of the comparison of two village's mean haemoglobin. Significantly different comparisons are those that do not intersect the zero line. Figure 5-2 shows that most villages are not significantly different, with the exception of the village of Bukhunya which has a significantly lower mean value. Figure 5-3 is a similar plot for the haemoglobin values at day 0 after two diminazene treatments. Rather than normalise the villages, the diminazene treatments appear to have exacerbated the differences between the villages, with 17 out of 66 comparisons proving significantly different.



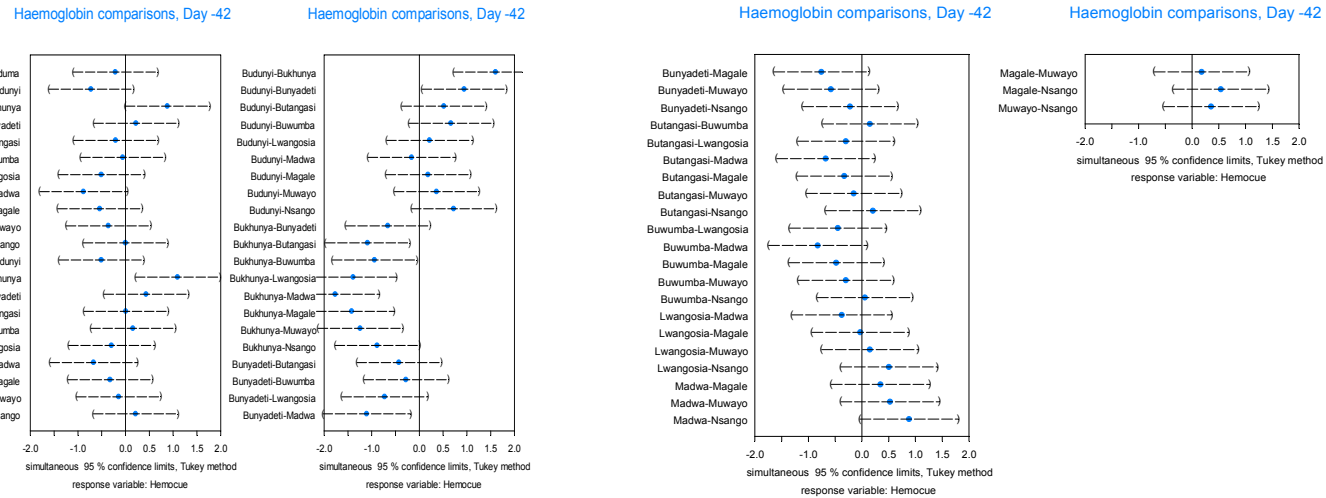


**Figure 5-1:- Box and whisker plot of haemoglobin values by village at baseline sampling point. 74>n>80 for each village.**

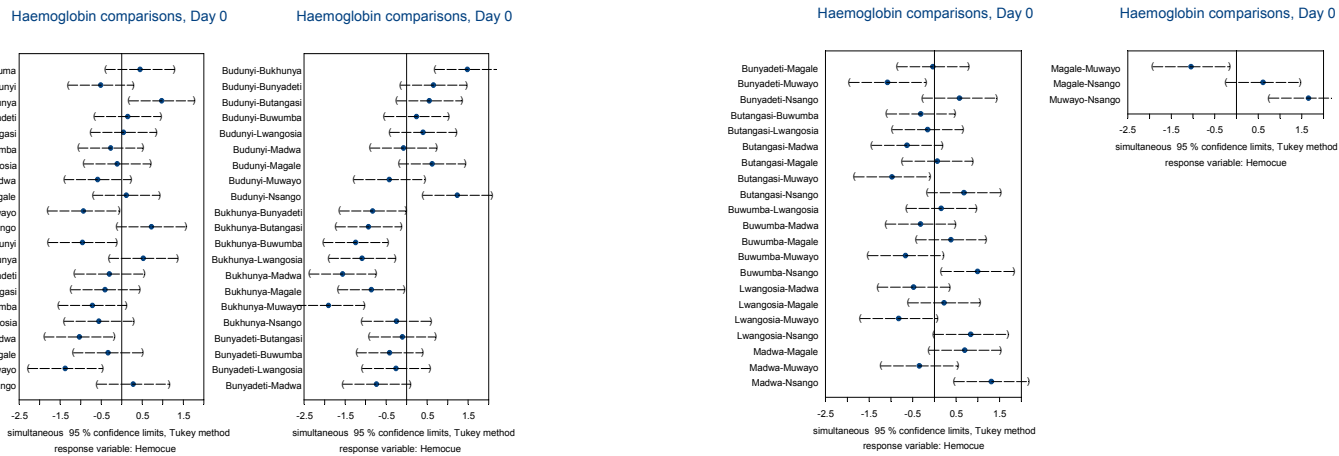
### 5.8 Differences between groups at baseline

Figure 5-4 shows multiple comparisons made between the intervention groupings, rather than the villages themselves. At the baseline there is a significant difference between villages allocated into the control and restricted spray groups. However, by day -14 all significant

differences have disappeared and, at day 0 when the interventions begin, there is no significant difference between any of the groupings.



**Figure 5-2: Multiple comparisons of average haemoglobin in study villages between villages at baseline. Significant comparisons do not intersect the zero line.**



**Figure 5-3: Multiple comparisons of average haemoglobin in study villages between villages at day 0**

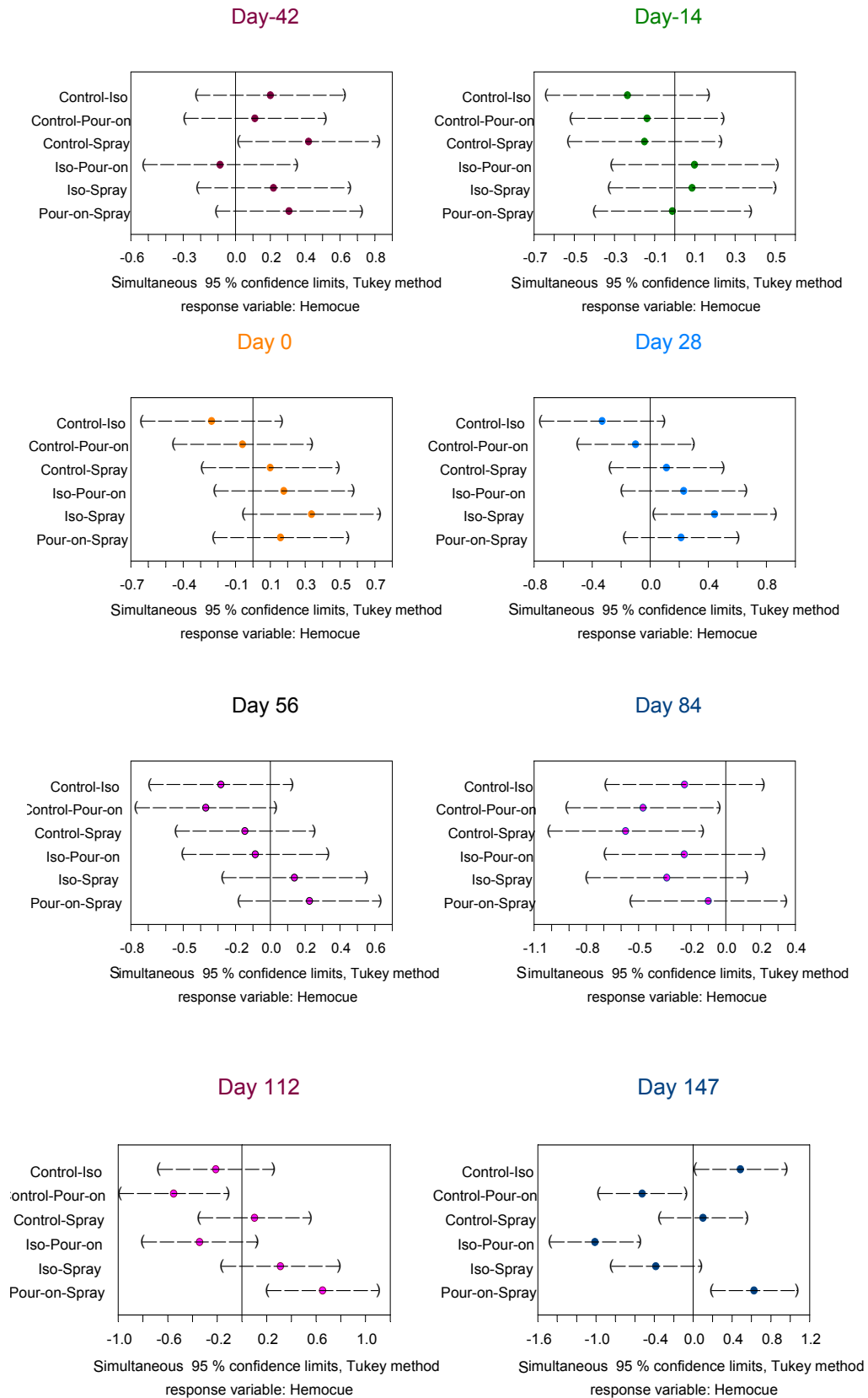
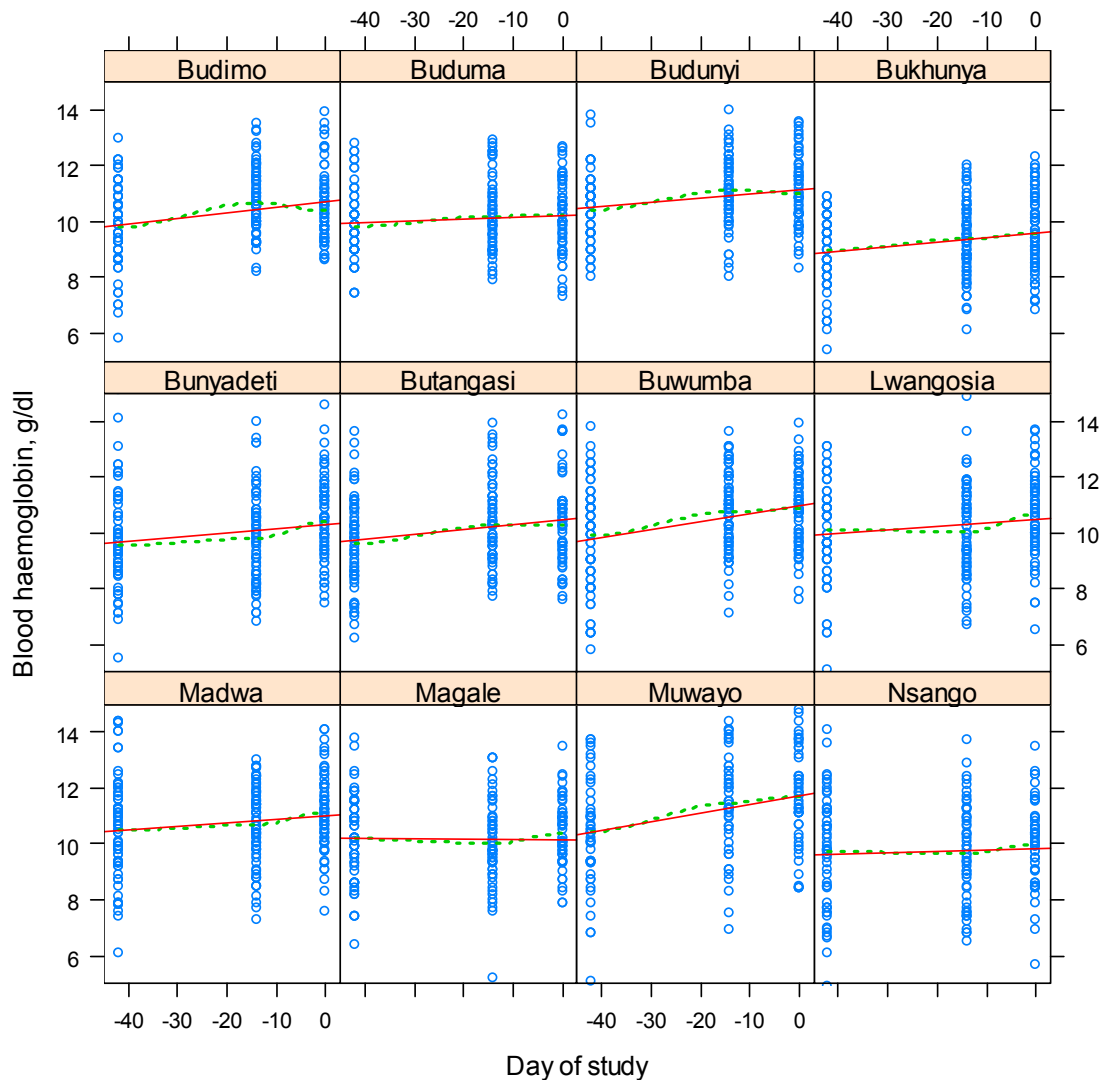


Figure 5-4:-Multiple comparisons for intervention groups for each sampling visit.

### 5.9 Change in haemoglobin during diminazene treatments

Figure 5-5 shows a series of plots of blood haemoglobin over time for the first three sampling visits for each village. The fitted lines show the change in the blood haemoglobin per village from Baseline to Day 0 and represent the change from baseline up to the start of the interventions. Between Day -42 and Day 0 there is the suggestion of an increase in average haemoglobin which can be investigated by modelling.

A linear mixed effect model was used to assess the variation of haemoglobin between



**Figure 5-5:-Trellis plot of haemoglobin against time, plotted for each individual village. Solid red line represents a linear regression, dotted green line a non-parametric smoothed line**

day-42 and day 0. Haemoglobin was fitted as the response variable Hemocue with time as a fixed effect. Random effects are Day|Village.Name/Tag.No

$\text{lme}(\text{fixed}=\text{Hemocue}\sim\text{Day}, \text{random} = \sim \text{Day}|\text{Village.Name}/\text{Tag.No})$

	Value	Std.Error	DF	t-value	p-value
(Intercept)	10.5202	0.1647	1682	63.858	
Day	0.01588	0.0027	1682	5.843	<0.001

**Table 5-1:- Linear mixed effect model of haemoglobin fitted against day of study for timepoints up to Day 0**

Table 5-1 shows that there is a highly significant positive change in haemoglobin with time between the baseline and the start of the interventions across all villages.

	Value	Std.Error	DF	t-value	p-value
Baseline (Intercept)	9.8514	0.13311	1681	74.007	
Visit 2, Day-14	0.4501	0.08189	1681	5.496	<0.001
Visit 3, Day 0	0.6658	0.11437	1681	5.821	<0.001

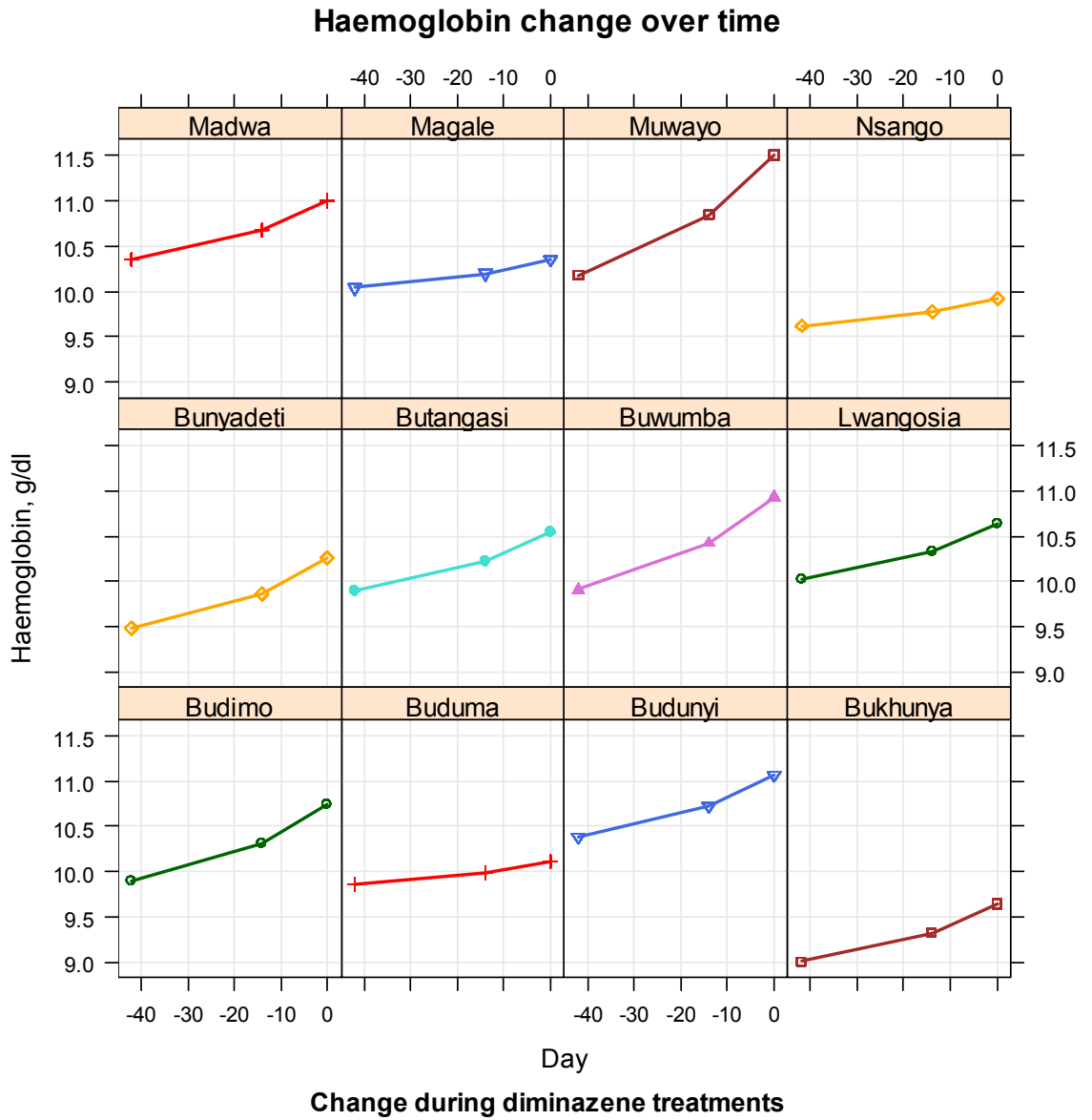
**Table 5-2:- Linear mixed effect model of haemoglobin fitted against day of study as a factor for timepoints up to Day 0**

Table 5-2 shows day fitted as a factor for the same time points and it can be concluded that there was a significant positive difference between both Day -14 and Day 0 sampling points and the baseline at day -42. Figure 5-6 shows the predicted values from this model plotted for village. It can be seen that all villages show an improvement. In some cases (e.g. Muwayo) this is clinically considerable, in others (e.g. Buduma) the impact is less noticeable. The degree of improvement does not seem to be related to initial values - Bukhunya village, for example, with a low mean haemoglobin, does not show disproportionately more improvement than villages with higher mean starting values.

### 5.9.1 Summary of haemoglobin up to day 0

It is not strictly possible to attribute the change in haemoglobin to diminazene treatments because we have no animals that were not treated for comparison. However, it is possible to say that between the baseline and day 0, all villages showed a highly significant increase in blood haemoglobin and it is very probable that this was caused by the treatments of diminazene aceturate.

Assuming that the improvement of haemoglobin is largely due to the trypanocidal activity of diminazene aceturate clearing pre-existing infections, would suggest that there is no evidence of resistance to diminazene aceturate in the pathogenic trypanosome population circulating in the south east Uganda. This result would agree with findings of the ITS-PCR analysis in the previous chapter.



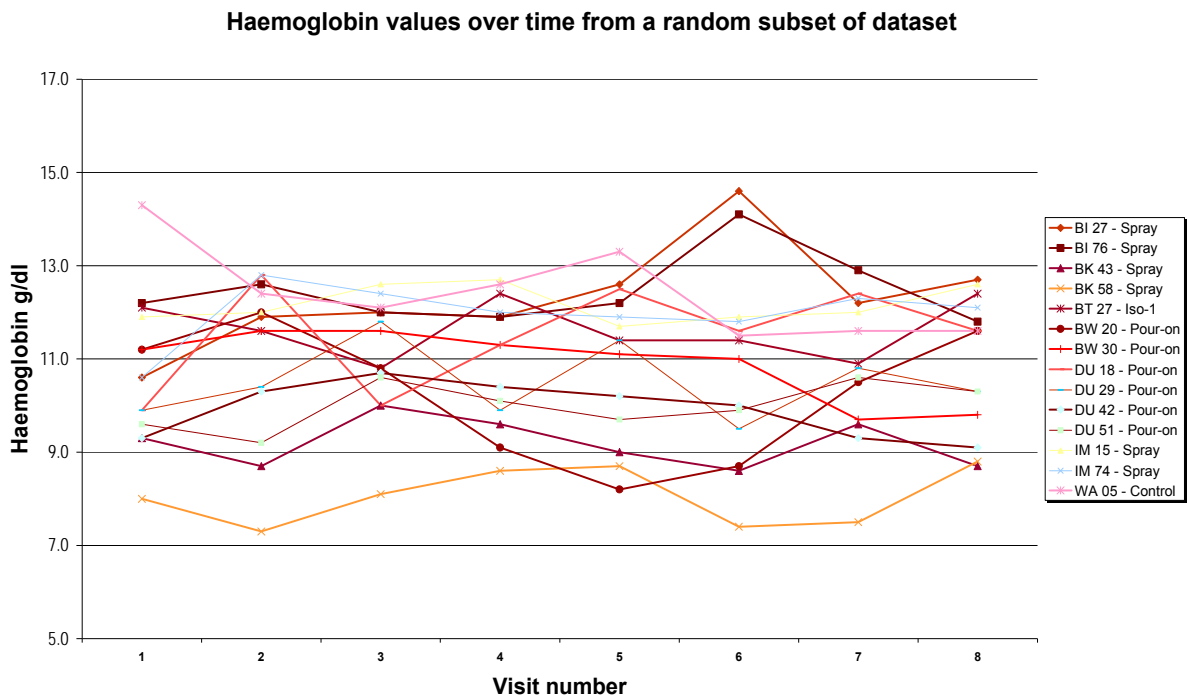
**Figure 5-6:-Plot of mean haemoglobin values by village up to Day 0, showing the change in haemoglobin by village up to the start of the interventions**



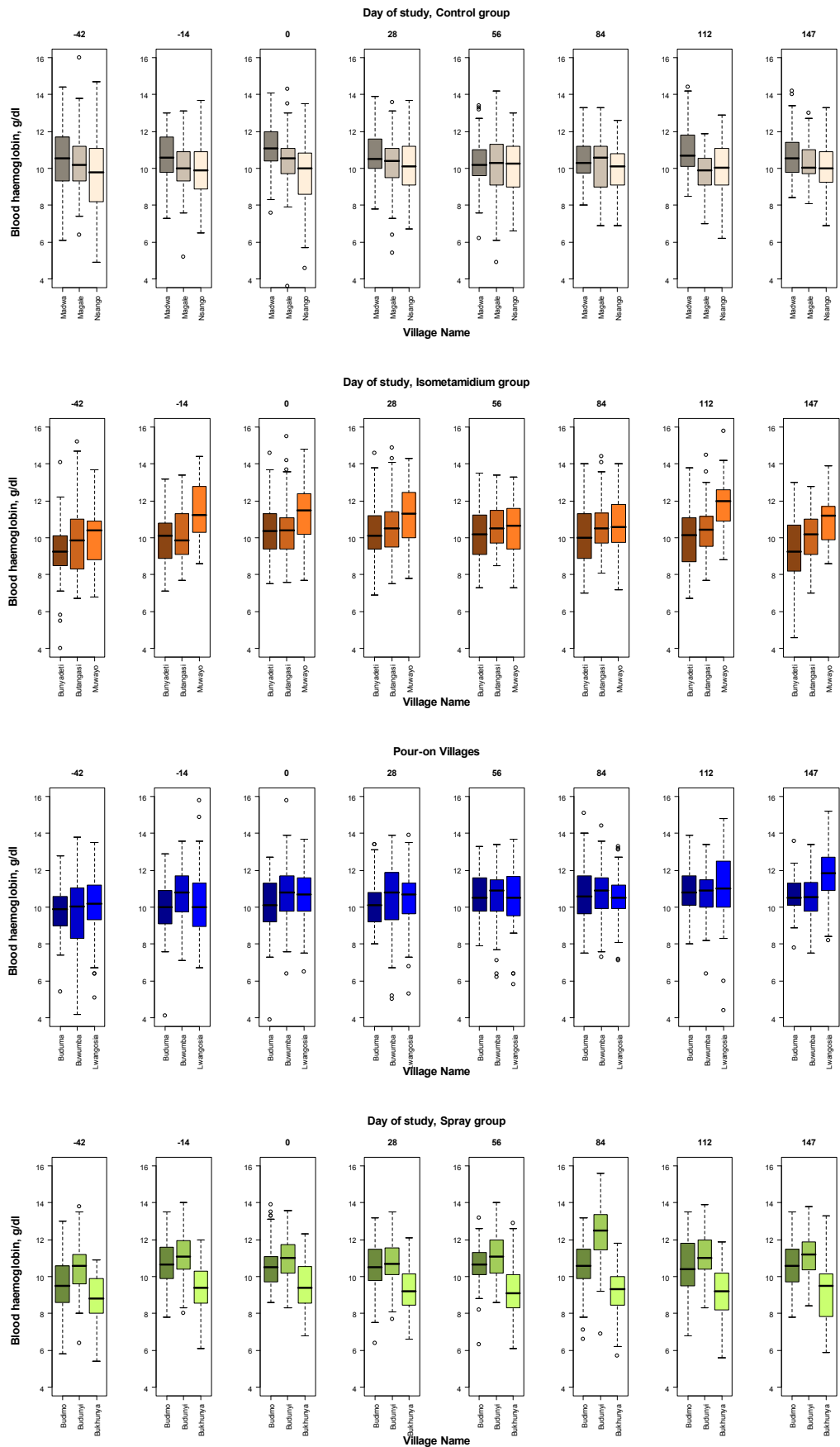
## 5.10 Change of haemoglobin during the study

This section is concerned with displaying and quantifying changes in haemoglobin occurring over the duration of the study and relating these to the treatment protocols applied to the groups of villages. The haemoglobin values for each animal over time arranged by village and intervention group are plotted in graphs 9.1 to 9.4 in the appendix. Although not very concise, these plots are useful not only for initial data inspection and to identify possibly anomalous values, but also to highlight possible patterns in the data.

After the increase in haemoglobin at the start of the study haemoglobin values follow a less obvious trend. From first inspection, between animal variability appears to be between 8-13g/dl. Figure 5-7 demonstrates the within animal variability in haemoglobin values from a randomly selected subset of the study animals. It can be seen that some animals fluctuate around a mean haemoglobin value of 7g/dl, others at over 12g/dl. It may be difficult to separate variation attributable to pathology from the normal variation in cattle.



**Figure 5-7: Haemoglobin values over time for a randomly selected subset of animals**



**Figure 5-8:- Boxplots of haemoglobin by village over time. Coloured themed plots represent intervention groups; grey=control, orange=isometamidium, blue=pour-on and green=spray**

Figure 5-8 shows box plots for each village over the duration of the study. Each individual panel contains three box plots representing the haemoglobin values for each village in an intervention group at single time points. Rows of panels show the change over time. The variation between villages is apparent, particularly for the spray group. Trends in haemoglobin are less obvious, although there is a suggestion that the lower interquartile range of pour-on villages increases over time. There is an obvious heterogeneity between villages however, particularly in the restricted spray group where the village of Bukhunya shows little change over time compared with the other villages in the group. This suggests that modelling this data will have to account for village heterogeneity.

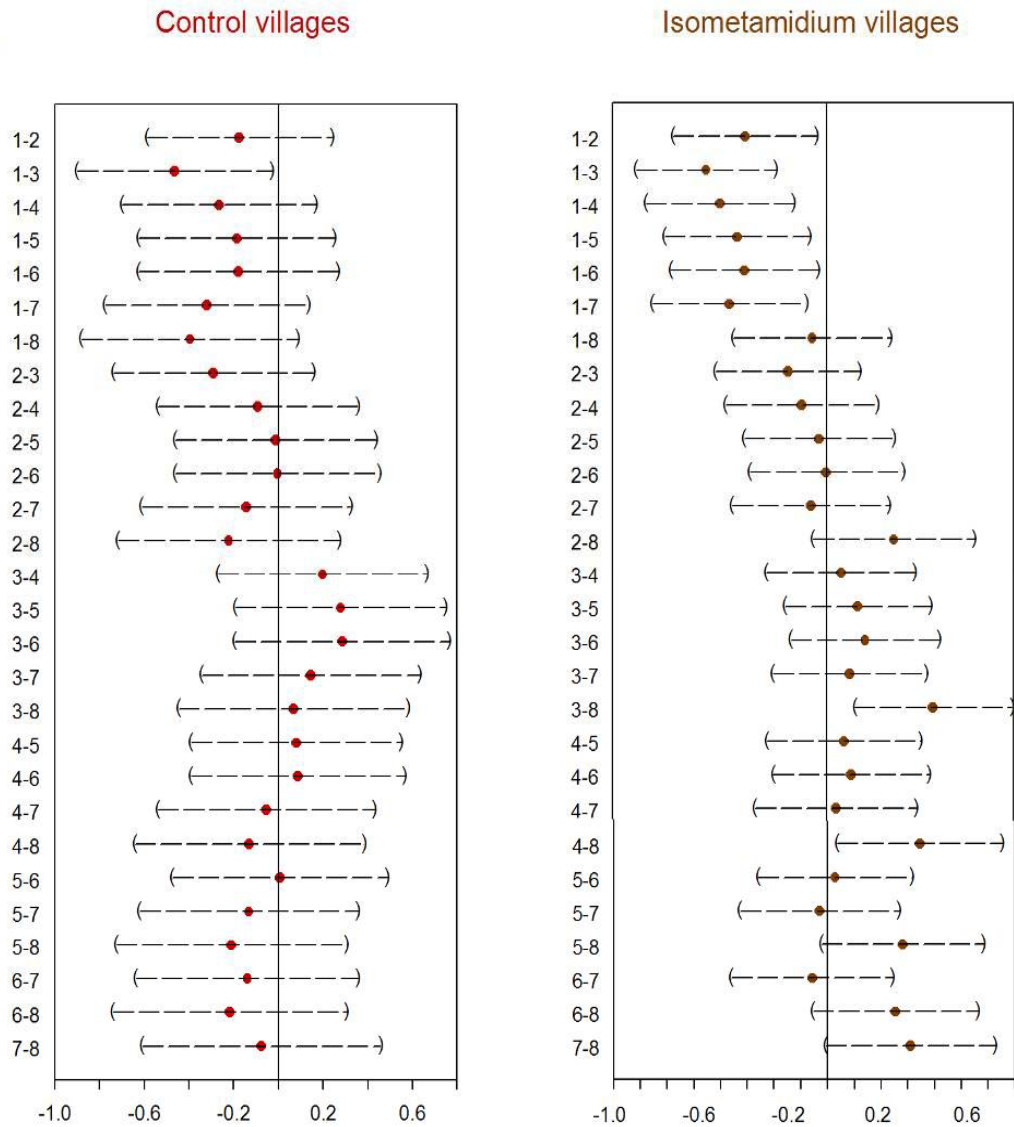
### 5.11 Multiple comparisons over study

Figure 5-4 shows multiple comparisons for the intervention groupings and Figure 5-9 and Figure 5-10 show multiple comparison plots of the mean haemoglobin for each visit, plotted by intervention. These plots are useful to identify significant patterns in the data. Pairs of numbers on the y-axis represent the two time points compared in that row. Confidence intervals not crossing the zero line are significant ( $p=0.05$ ).

Consider Figure 5-9 and Figure 5-10. As demonstrated in section 5.9, all villages show a significant difference between day -42 (Visit1) and day 0 (Visit3). From day 0 onwards, the control group shows no significant differences between haemoglobin levels over time. The pattern in the other treatment groups is slightly different - with the exception of the Visit1-Visit8 comparison for isometamidium villages, all groups show a significant improvement between the baseline and subsequent visits. The isometamidium group shows a significant decrease between visits 3 and 4 and the final visit. If this decrease in haemoglobin is due to the re-emergence of trypanosomiasis then it indicates the prophylactic period of isometamidium is shorter than the usual duration of 4-6 months (Eisler *et al.*, 1994; Magona *et al.*, 2004a).

Pour-on groups in contrast show significant improvements over the study. Considering Figure 5-10 there is a very obvious trend showing improved haemoglobin values over time. This trend is not seen in the spray villages suggesting that the pour-on confers a

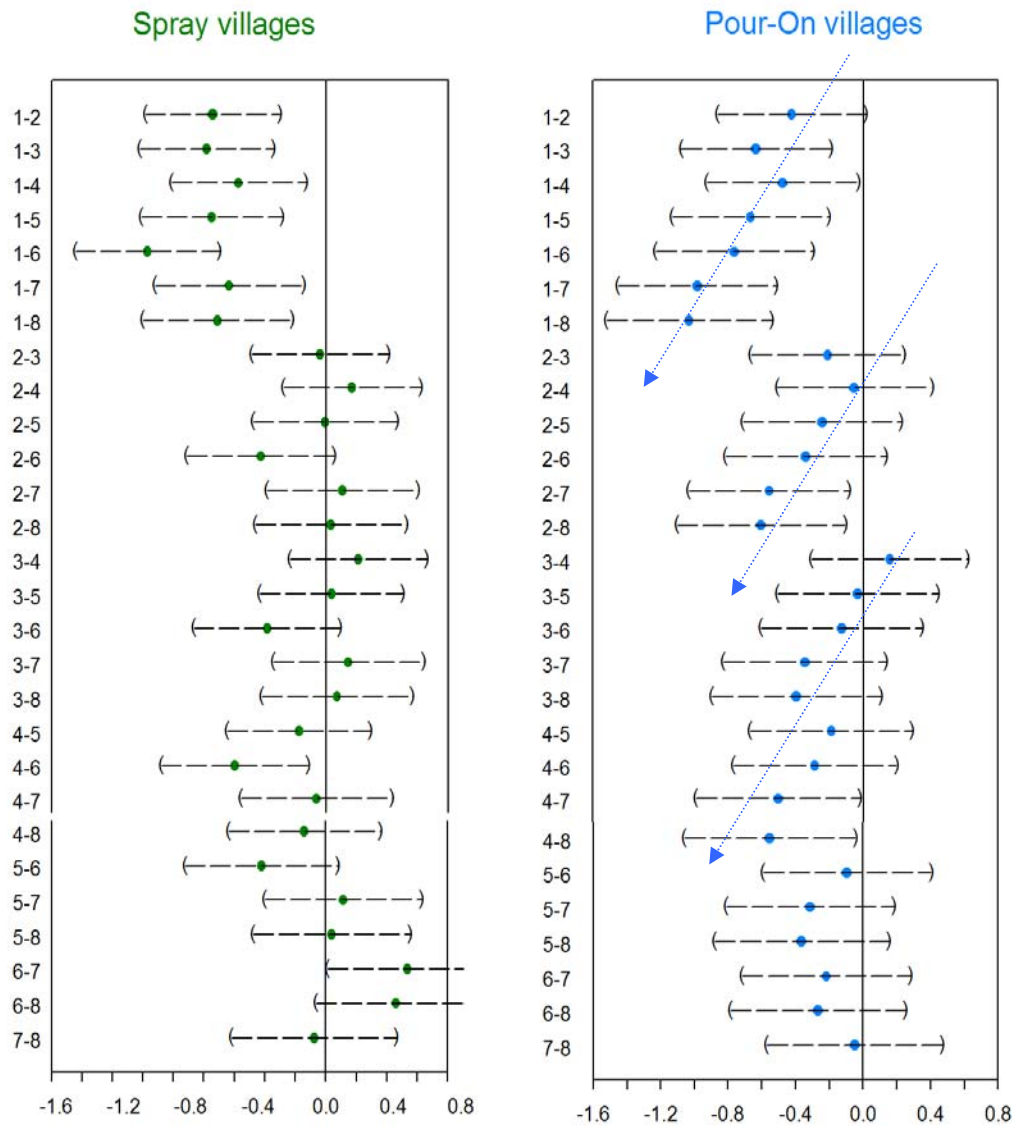
protective effect against diseases contributing to anaemia that is not provided by the spray protocol. Referring back to Figure 5-4, the pour-on group has significantly higher haemoglobin levels than the control group from day 84 onwards and at day 147, the mean haemoglobin of the study animals is significantly higher than all other treatment groups. In contrast, with the exception of day 84, the restricted spray group shows no difference from the control during the intervention periods.



Simultaneous 95% confidence limits, Tukey method.

Response variable: Hemocue

**Figure 5-9:- Multiple comparisons of mean haemoglobin between sampling visits for control and isometamidium groups.**



Simultaneous 95% confidence limits, Tukey method.

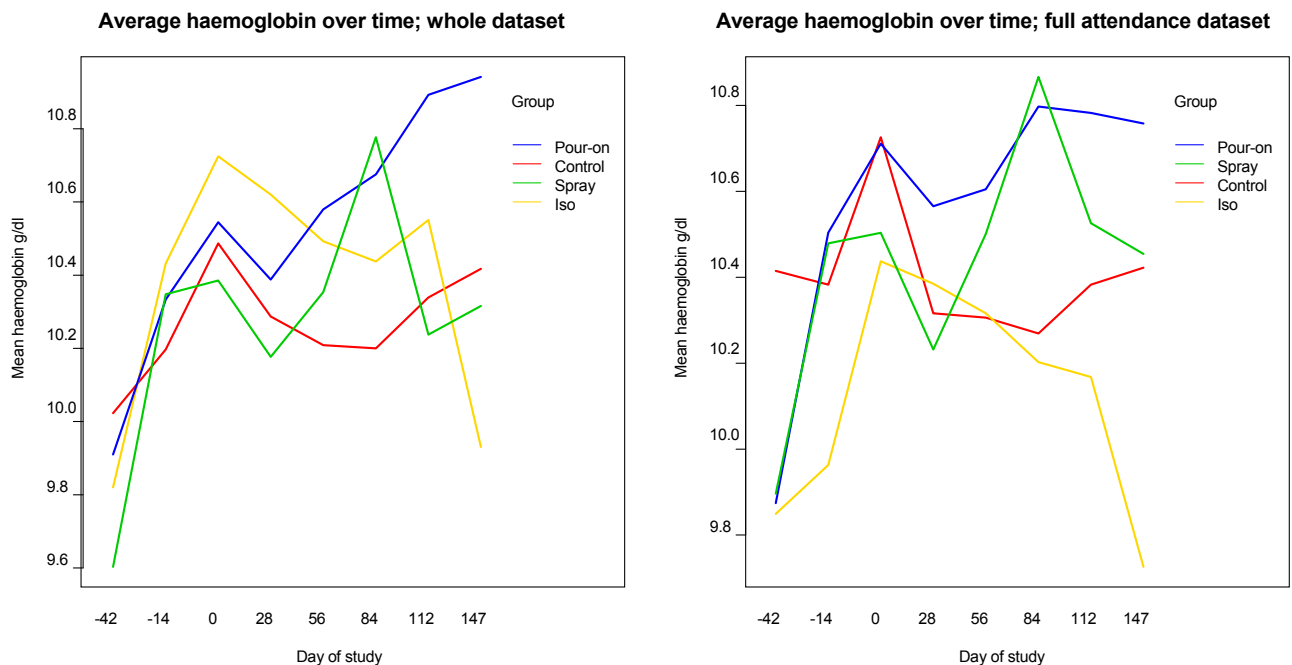
Response variable: Hemocue.

**Figure 5-10:- Multiple comparisons of mean haemoglobin between sampling visits for insecticide treated groups Dotted blue arrows highlight the trend of improving haemoglobin values over time.**

## 5.12 Modelling the change in haemoglobin over time

### 5.12.1 Considerations of data analysis

It appears there are significant differences in the haemoglobin values some of the intervention groups, and this section aims to quantify these differences. The modelling algorithms have the capacity to cope with missing data points so the models could be fitted to the full dataset, including animals that did not attend some sampling visits. There is an argument to exclude animals that failed to attend all the visits because, for the insecticide groups, that may mean they missed a re-treatment. Conversely however, this subset would be biased against the animals that did not attend due to disease. Figure 5-11 shows the mean haemoglobin levels for the entire dataset next to the mean haemoglobin levels for a dataset of animals that attended all visits. In general the shape of the curves are similar and pairwise comparisons showed differences to be non-significant at  $p > 0.05$  (data not shown). On balance therefore it was felt that analysis of the full dataset containing the missing values was justified.



**Figure 5-11: Comparison of haemoglobin change over time between entire dataset and a full attendance subset**

Correct analysis of the haemoglobin data collected from the longitudinal study requires temporal pseudoreplication to be taken into account, i.e. repeated measurements taken from the same animal over time. Failure to consider pseudoreplication results in too many degrees of freedom for the error variance and thus risks incorrectly rejecting the null hypothesis (Type 1 error) (Crawley, 2005).

### 5.13 ANOVA models of haemoglobin data

The purpose of this section is to model the differences in the haemoglobin levels among treatment groups. Traditionally, temporal pseudoreplication can be eliminated by only analysing data for a single time point. This can be done with a simple analysis of variance model

`aov(Hemocue~Group,subset=(Visit.Number==x))` where x= Visit number

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.?
Baseline						
Group	3	23.78	7.93	2.5721	0.052	Not significant
Residuals	942	2902.79	3.08			
Day 0						
Group	3	12.41	4.14	1.7311	0.159	Not significant
Residuals	825	1971.48	2.39			
Day 56						
Group	3	14.84	4.95	2.1961	0.087	Not significant
Residuals	728	1639.92	2.25			
Day 147						
Group	3	73.46	24.49	11.164	<0.001	Significant
Residuals	571	1252.41	2.19			

**Table 5-3:- Analysis of variance tables showing difference in haemoglobin levels between intervention groups for four timepoints throughout the study.**

Table 5-3 shows the output from an analysis of variance calculation run for four different time points. It suggests that there is a close to significant difference between the groups at



the baseline ( $p=0.053$ ), however that disappears by Day 0 and only reappears again by the final visit.

Day	Control	Isometa midium	Pour-on	Spray
-42	10.41	9.85	9.87	9.90
-14	10.38	9.96	10.50	10.48
0	10.73	10.44	10.71	10.50
28	10.31	10.39	10.57	10.23
56	10.31	10.32	10.61	10.50
84	10.27	10.20	10.80	10.87
112	10.38	10.17	10.78	10.53
147	10.42	9.72	10.76	10.45

**Table 5-4: Mean haemoglobin values by time and treatment group**

Table 5-4 tabulates mean haemoglobin values by group and time point and demonstrates that mean haemoglobin levels differ by very little, especially given the normal range highlighted in Figure 5-7.

Although analysis of covariance (ANCOVA) models can be used to fit regression lines to describe haemoglobin change over time for each intervention group. Investigation of the output from Table 5-3 indicates too many degrees of freedom for the nested structure of the dataset. In addition, the heterogeneity at the village level needs to be accounted for, as apparent from Figure 5-8. Further analysis will therefore necessitate the use mixed effect models, as described in 3.13.

#### 5.14 Fitting a linear mixed effect model (lme) to the haemoglobin data

This section seeks to fit a linear mixed effect model to the haemoglobin data collected from day 0 onwards to assess to see if there is any difference in the haemoglobin values between the intervention groups. To account for the nested structure of the data, the following structure was specified:

- Fixed effects (Haemoglobin ~Intervention group)
- Temporal random effect (random = ~Visit.Number)
- Spatial random effects (Village.Name)
- Nesting structure for the repeated measures (Visit.Number|Village.Name/Group)

Initially this creates an object which shows the haemoglobin over time, accounting for the grouped and nested structure of the data; animals are repeatedly sampled and belong to villages themselves belonging to a treatment group.

```
groupedData(Hemocue~Visit.Number|Group,  
outer=~Visit.Number|Village.Name/Tag. No
```

Figure 5-12 shows the separate linear regressions for haemoglobin against visit number for each group from day 0 (Visit 3). The coefficients (obtained by the function `lmList`) of haemoglobin against visit are shown in Table 5-5. The intercept represents the mean haemoglobin value on day 0 and the visit column contains the slope of the regression line, thus representing change over time. As can be seen from the almost horizontal lines, even accounting for the nested structure of the dataset, there are not big changes in mean haemoglobin over time for any of the groups.

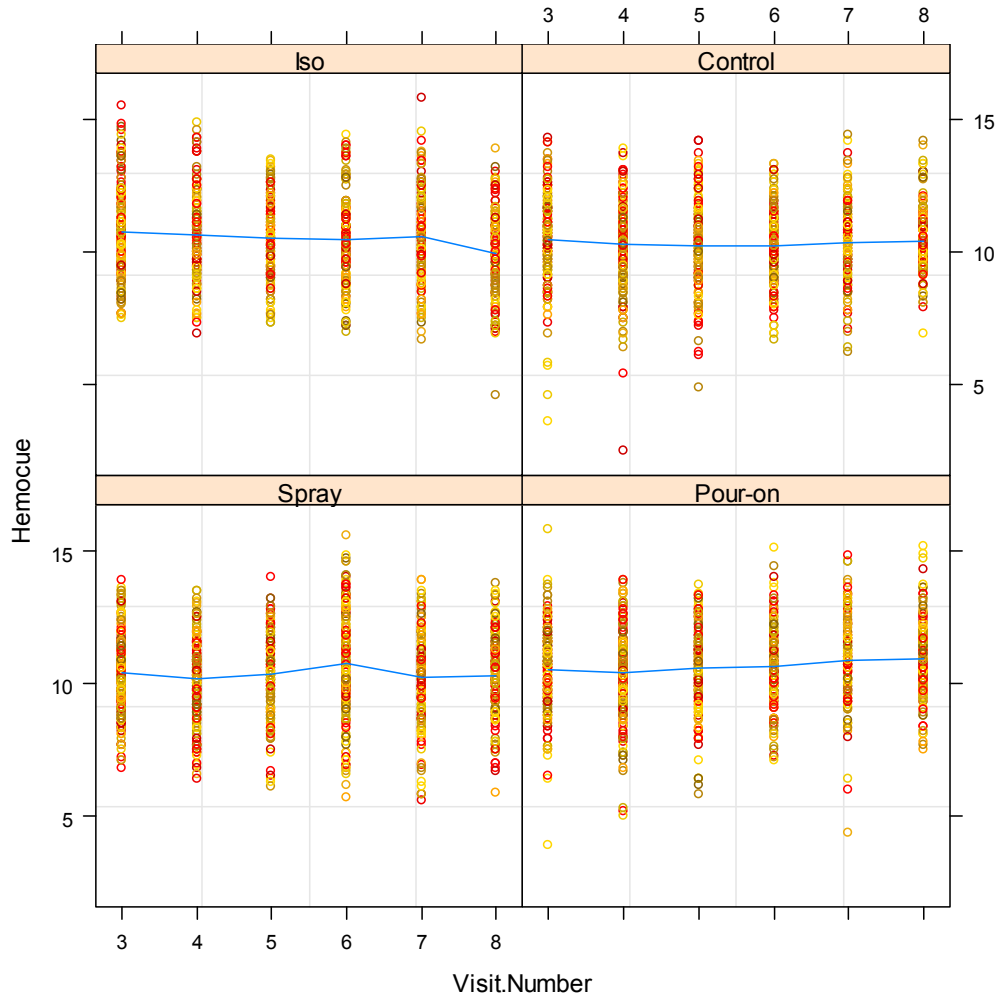


Figure 5-12:- Plot of haemoglobin over time, blue line representing regression fit random effects Visit.Number|Village.Name/Tag.No

	(Intercept)	Visit
Spray	10.30	0.012
Pour-on	10.11	0.100
Isometamidium	11.09	-0.116
Control	10.37	-0.010

Table 5-5: Coefficients for Figure 5-12 showing the mean baseline haemoglobin for each group (Intercept) and the change over time per visit (g/dl/visit)

Table 5-5 shows a positive time slope for the insecticide treated animals, indicating improvement in haemoglobin as the study progresses, and a negative time slope for the control and isometamidium villages. The rate in the pour-on group is an order of magnitude more than the restricted spray (0.1 g/dl/visit compared with 0.01 g/dl/visit for pour-on and spray groups respectively). In comparison, the isometamidium and control groupings show a similar pattern, control villages showing a change of -0.01g/dl/visit compared with -0.11g/dl for the isometamidium villages.

Further analysis of the data by linear mixed effect models using the same grouped data structure as outputted in Figure 5-12 showed no significant difference ( $p$  values $>0.05$ ) between the intervention groups.

It is possible that there is an interaction effect between groups and time, as suggested by the multiple comparison plot Figure 5-9. This model allowed for different slopes of haemoglobin change for each intervention group. This interaction term was specified by the directive Visit.Number\*Intervention:

```
Model1<-lme(fixed=Hemocue~Visit.Number*Group, random = ~
Visit.number | Village.Name/Tag.No
```

Random effects			
Formula: ~Visit.number   Village.Name			
	StdDev	Correlation	Variance
Intercept (i)	(a) 0.4537	(Intr)	0.2059
Visit.Number	(b) 0.0340	0.746	0.0011
Formula: ~Visit.number   Tag.No in Village.Name			
	StdDev	Correlation	
Intercept (ii)	1.4445	(Intr)	2.0868
Visit.Number	0.1887	-0.615	0.0356
Residual	0.9543		0.9107

**Table 5-6: Random effects output for linear mixed effect model model1 Random effects show which components of the random structure explain the most variance of the data**

Table 5-6 summarises the random effects of the model. The random effects refer to the variance attributable to different parts of the model and can be used to see which terms are contributing most of the explanatory power to the model. Variance is calculated as the square of standard deviation (StdDev). Output row (i) shows the differences between intercepts of different villages, row (ii) shows difference between intercepts of animals within villages. The differences introduced by the different time slopes are in the row coded Visit.Number. Output (i) shows that the variation attributable to differences in slope (b) (s.d.=0.034), is small compared with the variance attributable to differences in village (a) (s.d =0.45). This is the same case in (ii) where variance attributable to differences in slope (s.d=0.189) is small compared with the variation between animals nested within villages (s.d =1.44).

Table 5-6 table can simply be interpreted as showing that the variance attributable to different villages or different animals within different villages has much more bearing on the model than the variance attributable to fitting a different slope for each time point. Consequently, it was investigated to see if the model could be simplified by investigated by fitting a common time slope

```
Model1<-lme(fixed=Hemocue~Visit.Number*Group, random = ~
Visit.number|Village.Name/Tag.No
```

```
Model2<- lme(fixed=Hemocue~ Visit.Number*Group, random = ~
1|Village.Name/Tag.No...)
```

	df	AIC	BIC	logLik	L.Ratio	p-value
Model1, with random effects Visit.Number Village.Name/Tag.No	16	13347	13581	-6657	18.82	
Model2, with random effects 1 Village.Name/Tag.No	12	13358	13434	-6667		<0.001

**Table 5-7: Comparison model1 and model2. Lower Akaike information criteria (AIC, see section 3.12.1) indicates better fit of the model**

Comparing model1 and model2 using anova showed there was a significant difference between them, and a lower Akaike information criteria (AIC) than in the current model. Simplifying the model further by removal of the animal effect gave much larger AIC values and hence model1 is considered the best fit.

Fixed effects: Hemocue ~ Visit.Number * Intervention						
	Value	Std.Error	DF	t-value	p-value	Sig?
(Intercept)	10.177	0.2839	5091	35.83	<0.001	
Time	0.0129	0.0265	5091	0.48	0.62	
Isometamidium	0.0864	0.4040	933	0.21	0.831	
Pour-On	-0.2051	0.4011	9	-0.51	0.621	
Spray	-0.2695	0.4008	9	-0.67	0.518	
Time: Isometamidium	0.0363	0.0378	5091	0.96	0.337	
Time: Pour-On	0.1038	0.0371	5091	2.79	0.005	**
Time: Spray	0.0606	0.0370	5091	1.63	0.102	

**Table 5-8: Fixed effects from model1**

Table 5-8 lists the fixed outputs from model1. Main effects of group are not significant. However the Time:Pour-On interaction indicates that the pour-on group has a significantly different timeslope. In other words, although there is no difference in the mean haemoglobin levels of the different intervention groups, there is a significant difference in the improvement of haemoglobin shown over time by animals in the pour-on group.

#### 5.14.1 Refining the model: accounting for the correlation structure of the data

It is reasonable to believe that individual animal haemoglobin values are autocorrelated throughout time. That is to say, the value obtained at time  $t$  is likely to be correlated to the reading at  $t_{-1}$ , and to a lesser extent by  $t_{-2}$ ,  $t_{-3}$ ... $t_0$ . This can be investigated for the haemoglobin values

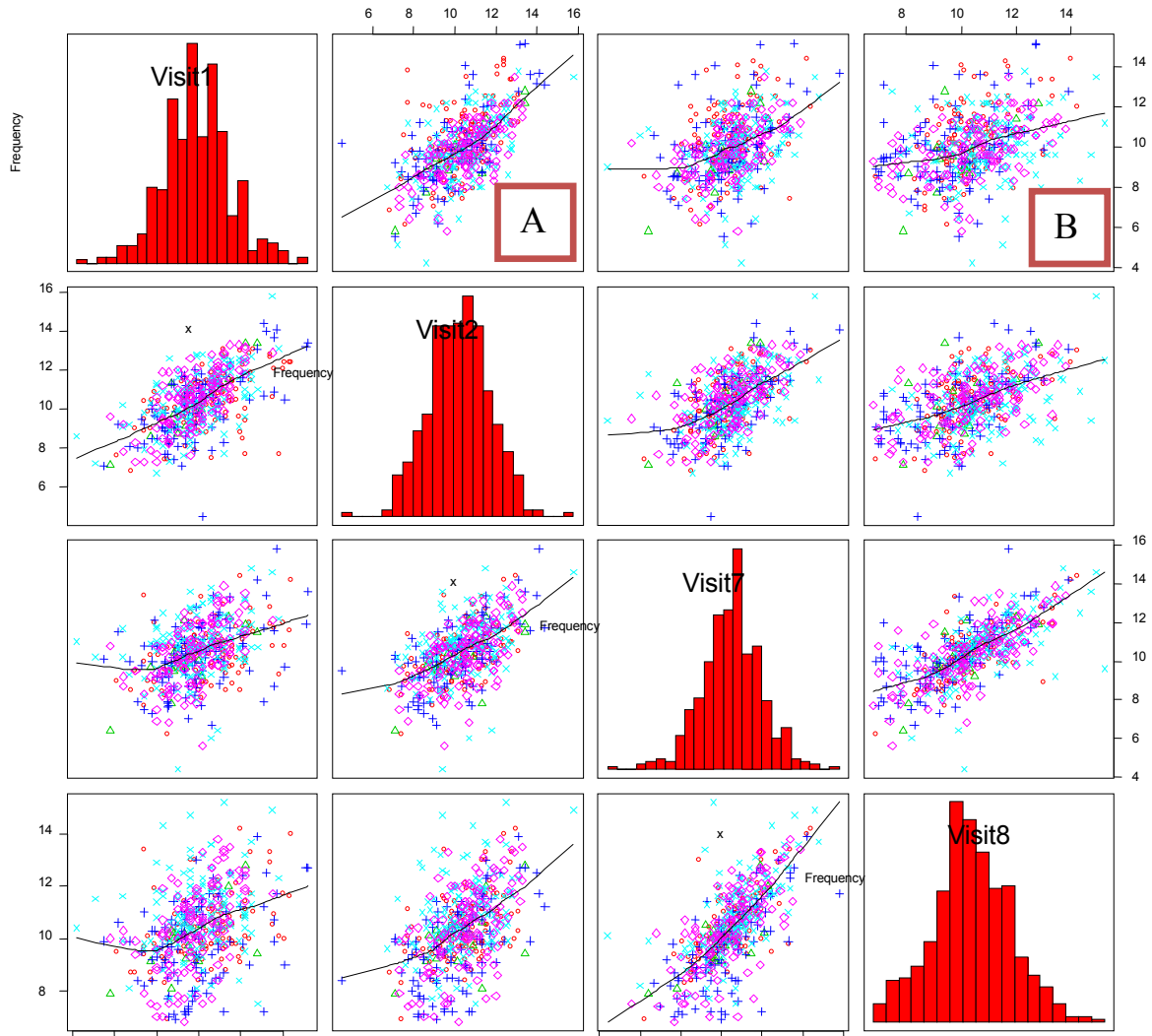


Figure 5-13 shows the autocorrelation between different time points. The histograms on the diagonal indicate the time points graphed on the bisecting horizontal and vertical panels. For example, the panel marked A shows an individual's haemoglobin value at visit 1 plotted against those for visit 2, taken 28 days later. As can be seen from the slope of the fitted line, there is evidence of correlation between these two values. Panel B shows the correlation between Visit 1 and Visit 8 taken over six months later. The regression slope for this panel is much flatter, indicating a much weaker correlation for values taken six months apart. This pattern is consistent for all time points, although, for ease of

**Figure 5-13:- Correlation structure of haemoglobin data, showing association between four timepoints ( on diagonal) Steeper lines indicate stronger correlation**

interpretation only four are shown here.



The model can be updated to take account of temporal autocorrelation. The function `corAR1` fits an autocorrelation of lag 1, i.e. correlation between a value and its immediate temporal predecessor. The new model can be compared with model 2 using ANOVA:

```
Model2<-update(model1, corr=corAR1())
anova(model1, model2)
```

	Model	df	AIC	BIC	logLik	L.Ratio	p-value
Model1	1	16	13473	13581			
Model2	2	18	13360	13474	-6662	114.82	<.0001

**Table 5-9: Comparison of models with and without correlation structure**

Model2 is significantly different with a lower AIC and BIC. Thus, there is strong evidence of autocorrelation in this data, and models have to take this correlation structure into account.

### 5.14.2 Final model

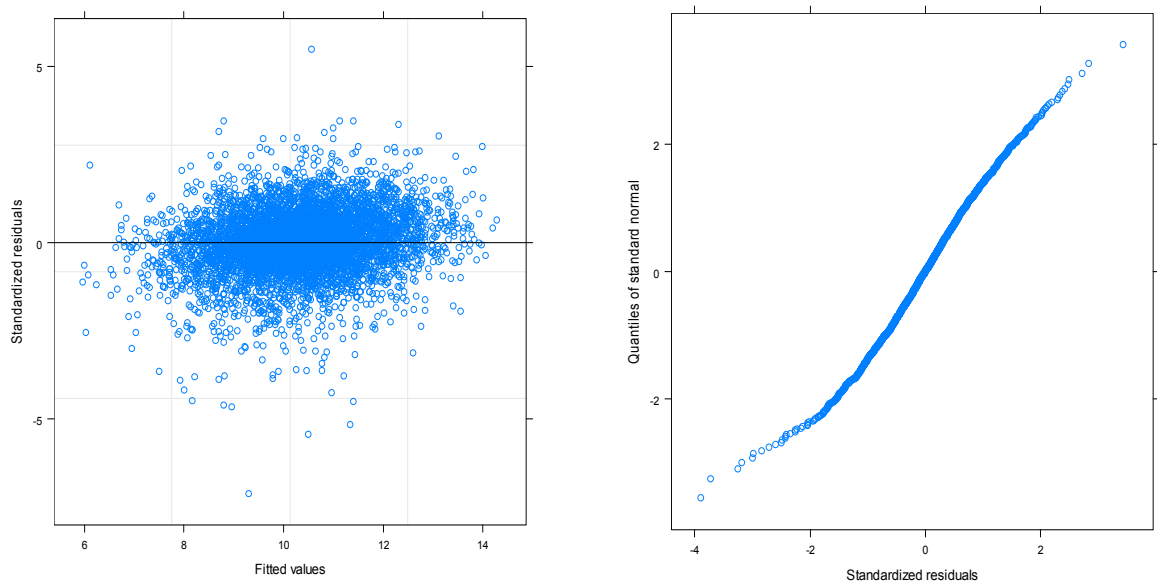
The most parsimonious model to be fitted is as follows. Day has been substituted for visit number to give the rate of change on a daily basis.

```
Model3<-lme(fixed=Hemocue~Day*Group, random = ~ Day|Village.Name/Tag.No,
na.action=na.omit, data = OBnot1,corr=corAR1())
```

	Value	Std.Error	DF	t-value	p-value
(Intercept)	10.368	0.233	3326	44.428	
Day	-0.001	0.001	3326	-1.129	0.259
Isometamidium	0.414	0.239	899	1.735	0.083
Pour-On	0.076	0.375	9	0.204	0.843
Spray	-0.009	0.374	9	-0.023	0.982
Time: Isometamidium	-0.002	0.002	3326	-1.344	0.179
Time: Pour-On	0.005	0.002	3326	2.297	0.022
Time: Spray	0.001	0.002	3326	0.624	0.533

**Table 5-10:- Final model fixed effects, indicating there is a significant effect of pour-on compared with control in terms of change over time.**

Figure 5-14 shows diagnostic plots for the model3 and proves that, largely speaking, it is properly specified.



**Figure 5-14: Diagnostic plots for model3**

### 5.14.3 Summary on haemoglobin change during the interventions

Table 5-10 shows fixed effect output from the most parsimonious mixed effect model that described haemoglobin values over time, taking into account temporal and spatial pseudoreplication, and allowing for temporal autocorrelation of values. There was no significant difference between the intervention groups in terms of mean haemoglobin values. Accounting for the effect of time showed a significant difference in the rate of change of haemoglobin values between the pour-on group and the control. Animals in the pour-on villages show on average a 0.005 g/dl/day (or 0.13g/dl/visit) improvement in haemoglobin. There is no significant difference between either the mean, or the rate of change, of haemoglobin between the control group and the isometamidium villages, or the control and restricted spray villages.

## 5.15 Haemoglobin change during the entire study

The analysis described in section 5.14 was repeated for all the time points, including the diminazene treatments prior to day 0. Model specification and simplification was carried out in exactly the same way. For the sake of brevity the intermediate stages are not shown. The most parsimonious model reduced to the same structure:

```
Model4<-lme(fixed=Hemocue~Day*Intervention, random = ~
Day|Village.Name/Tag.No, na.action=na.omit, corr=corAR1())
```

Table 5-11 shows the output of the model4 fitted to all time points in the dataset. Similarly to model3, there is no significant difference in the mean haemoglobin values among interventions. In contrast to the above section however, the pour-on and spray groups show an improvement over time which is significantly different to the control group. Both insecticide groups show a positive change in haemoglobin compared with the control villages. Animals in the pour-on group show an average rate of improvement of 0.004g/dl/day, twice that of the spray group's 0.002g/dl/day. There is no significant effect of isometamidium compared with the control.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	10.21	0.34	5091	30.27	0.00
Day	0.000	0.001	5091	0.64	0.52
Isometamidium	0.134	0.478	933	0.28	0.78
Pour-On	0.055	0.477	9	0.12	0.91
Spray	-0.151	0.477	9	-0.32	0.76
Time: Iso.	0.001	0.001	5091	0.88	0.38
Time: Pour-On	0.004	0.001	5091	5.13	<0.001
Time: Spray	0.002	0.001	5091	2.74	0.01

**Table 5-11:- Final model output on all time points. Considering the change from baseline, rather than from day 0 (above), indicated a significant change over time for both insecticide treated groups**

## 5.16 Overall summary of haemoglobin change

Model3 and model4 characterised the change of haemoglobin values over time for the intervention period and entire study period respectively. Figure 5-16 to Figure 5-17 show panel plots of these models. The mean haemoglobin value at each timepoint was calculated using the predict directive, and plotted against time to show the rate of change. Lines show either mean response per group or per village. Figure 5-16 and Figure 5-15 show model outputs for changes since Day 0, Figure 5-17 and Figure 5-18 show the outputs for the whole study, including the diminazene treatments.

### 5.16.1 Haemoglobin change during intervention period

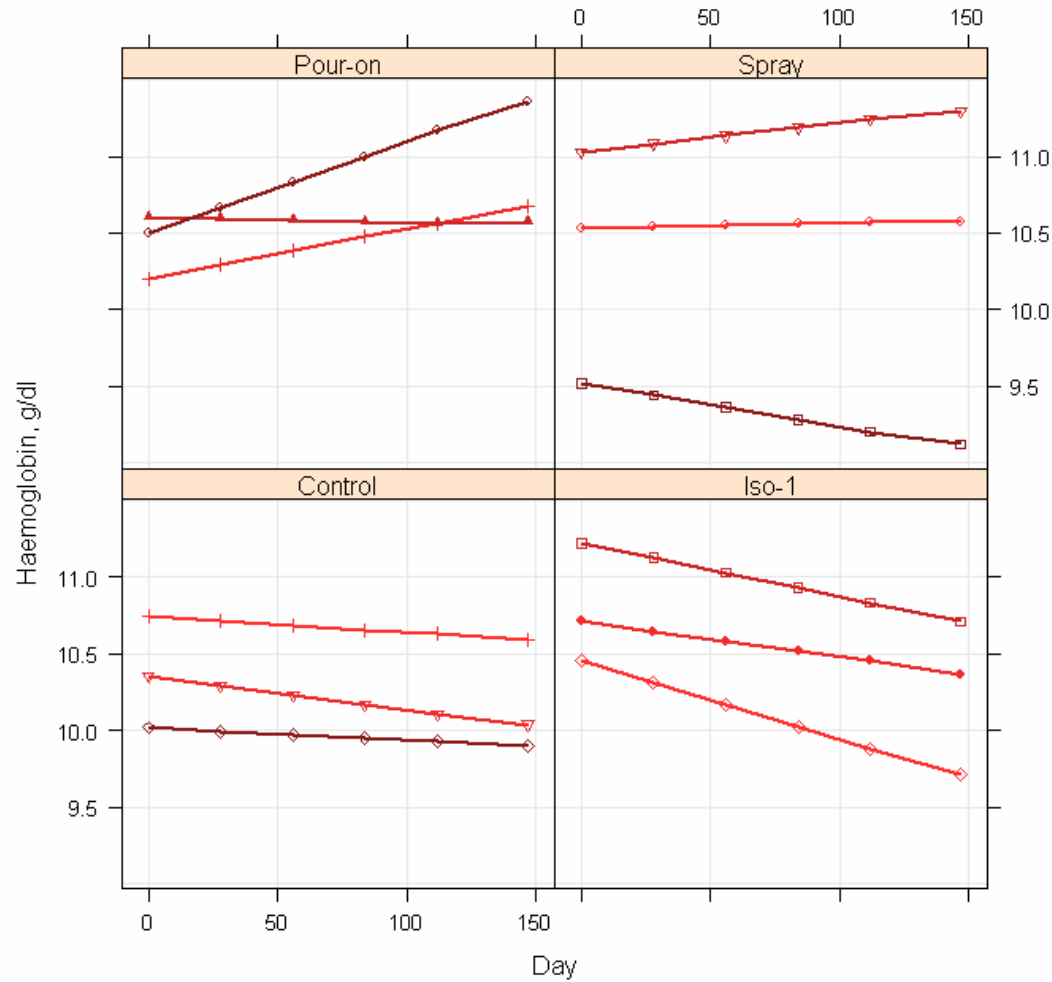
Figure 5-16 and Figure 5-15 suggest that, from day 0, animals in isometamidium treated villages show a decrease in haemoglobin over time, although not significantly different from the control. All villages in the control and isometamidium groups exhibit similar slopes, indicating a generally consistent response over time. In contrast, insecticide treated villages show quite variable effects from day 0. For example, the pour-on group exhibits a steep increase in haemoglobin in two of its villages yet the third showing a slight decrease. As discussed above, this is statistically significant. Spray villages show much

greater variation in the intercepts (day 0 values) and slopes, with one village exhibiting an increase, one showing no change and one displaying a decrease. Overall, the spray groups are not statistically significant from the control.

### 5.16.2 Haemoglobin change during entire study period

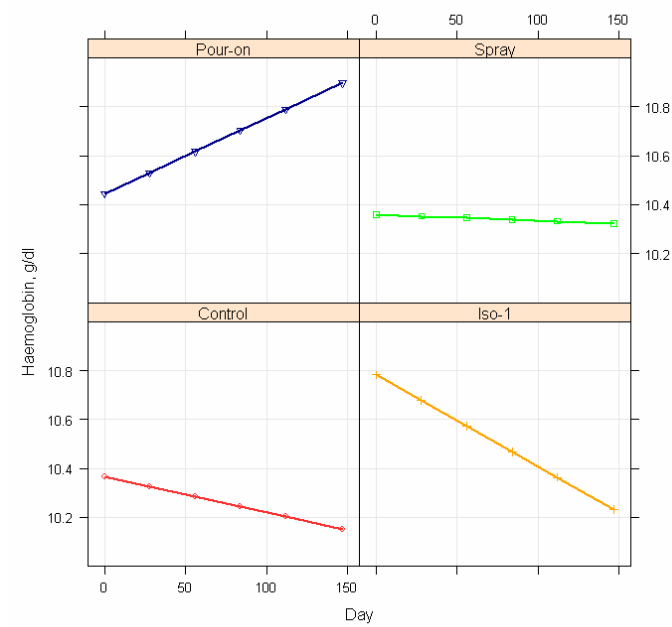
Figure 5-17 shows the overall change in haemoglobin values, including the period of diminazene treatments. All villages show an increase in haemoglobin values, although this is only statistically different from the control in the pour-on and spray villages. The heterogeneity of the spray villages is pronounced, showing that one of the spray villages, Bukhunya, began with and remained with low haemoglobin values that were not responsive to diminazene treatments.

**Predicted haemoglobin values over time, by group and village**



**Chapter 5:- Impact of interventions on haemoglobin**

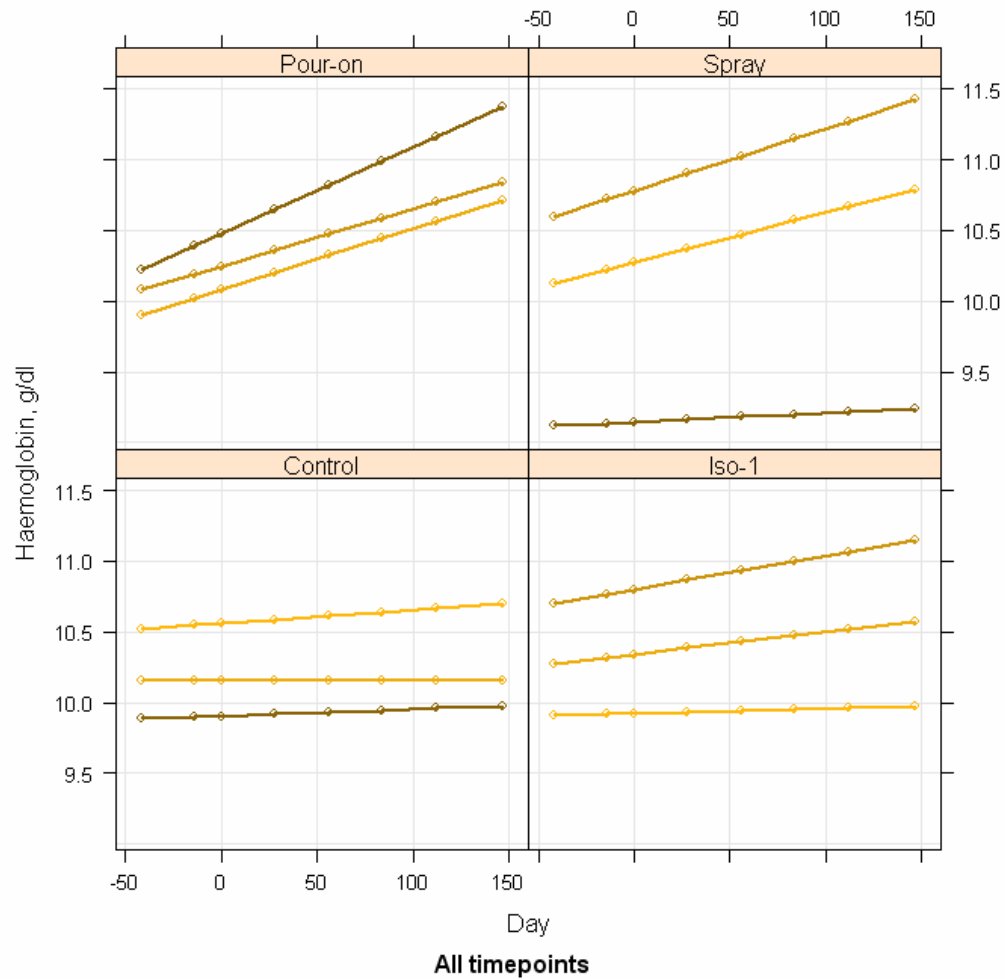
**Predicted haemoglobin values over time, by group**



**Figure 5-15:- Model output showing change in haemoglobin over time from day 0. Panels are arranged by group and show predicted lines for each village**

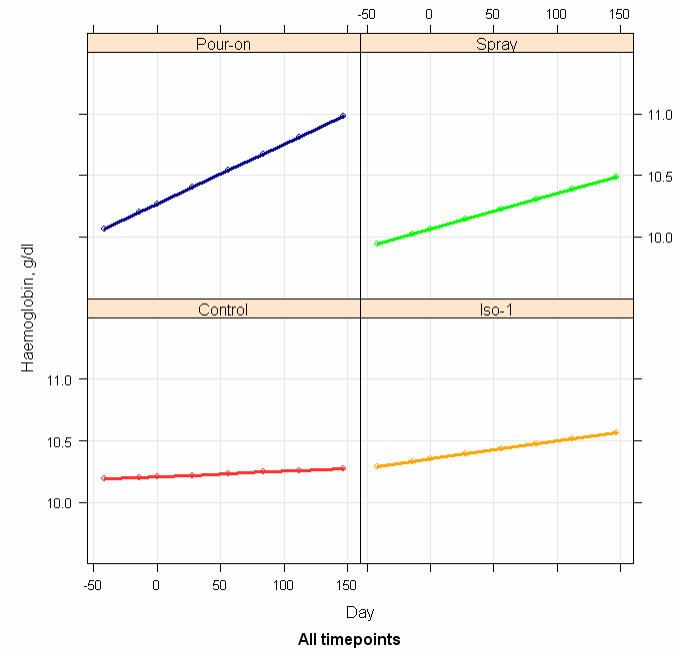
**Figure 5-16:- Model output showing change in haemoglobin over time from day 0. Panels show mean response by group**

**Predicted haemoglobin values over time, by group and village**



**Figure 5-18: Model output showing change in haemoglobin over time from baseline. Panels are arranged by group and show predicted lines for each village**

**Predicted haemoglobin values over time, by group**



**Figure 5-17: Model output showing change in haemoglobin over time from baseline. Panels show mean response by group**

### 5.17 Improvement thresholds

So far this chapter has discussed the change in haemoglobin levels over time and attempted to relate differences to intervention groups. This has involved models which can characterise the change in haemoglobin values over time for each individual animal. However, there is a different way of looking at haemoglobin values, embedded in the models of the first section but not made explicit. On a herd level, it is interesting to identify the number of animals above a particular haemoglobin threshold and to measure the degree to which they improve from one visit to the next. The degree of improvement of individual herd members within a treatment group was of greatest interest to the farmers participating in the study, and investigation of this is worthwhile as it displays some interesting patterns in the data.

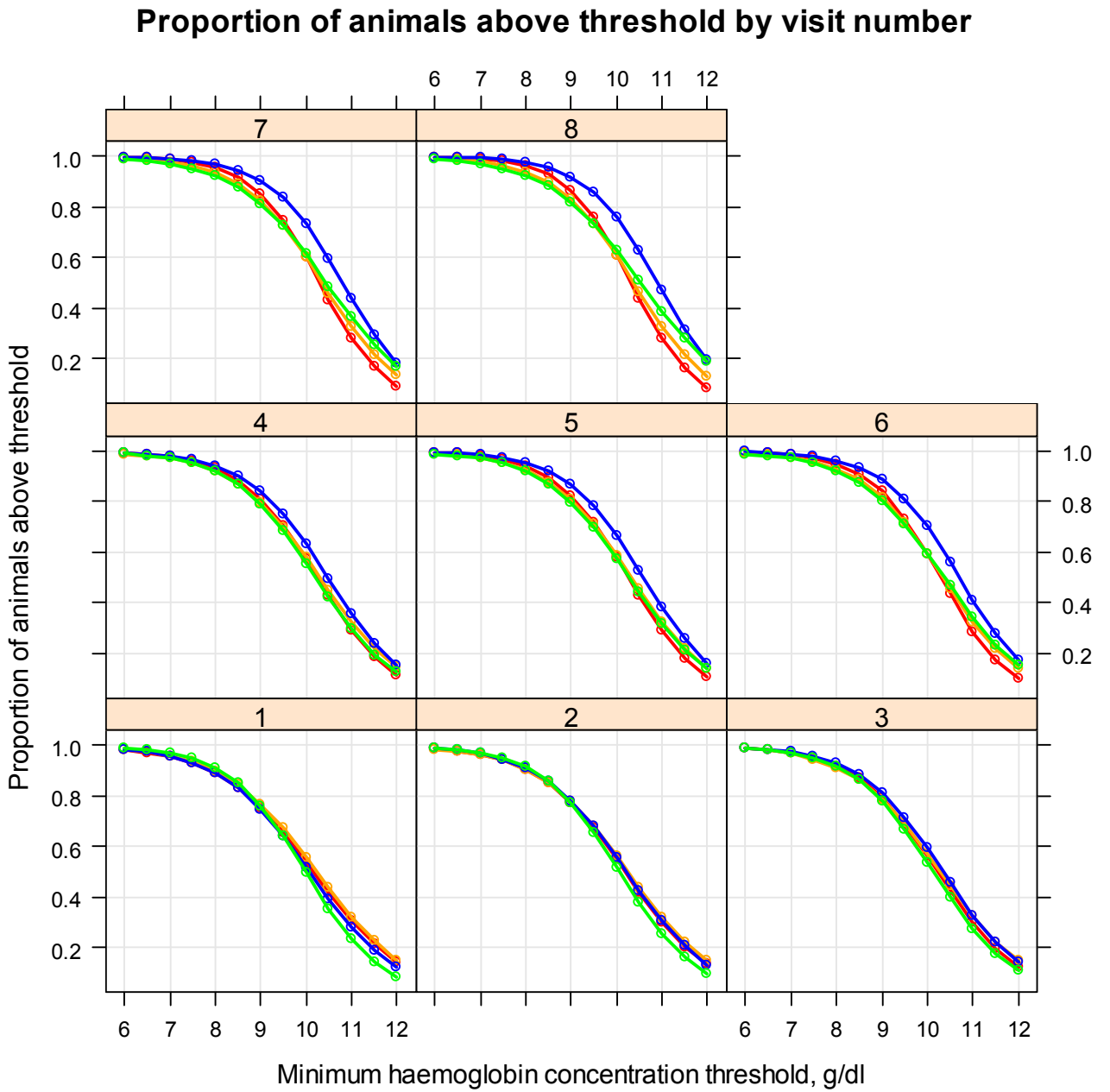
### 5.18 Number of animals above a specific haemoglobin threshold

A good indicator of the health of a herd would be an assessment of the percentage of animals over a minimum level of haemoglobin. This was assessed by building a generalised mixed effect model for proportion data. Methodologies for modelling count data is given in section 3.9.1. The response object, Counts were formed by binding together two vectors containing the count of animals above and the count of animals below a nominated threshold. Modelling using a generalised linear modelling function allowed for specifying a binomial error structure. The final model fitted included intervention grouping and the visit number. The threshold of haemoglobin was fitted as a random effect:

```
Model6<-lme(fixed=Counts~Intervention*Visit.Number, random = ~ 1|Threshold,  
family=binomial....)
```

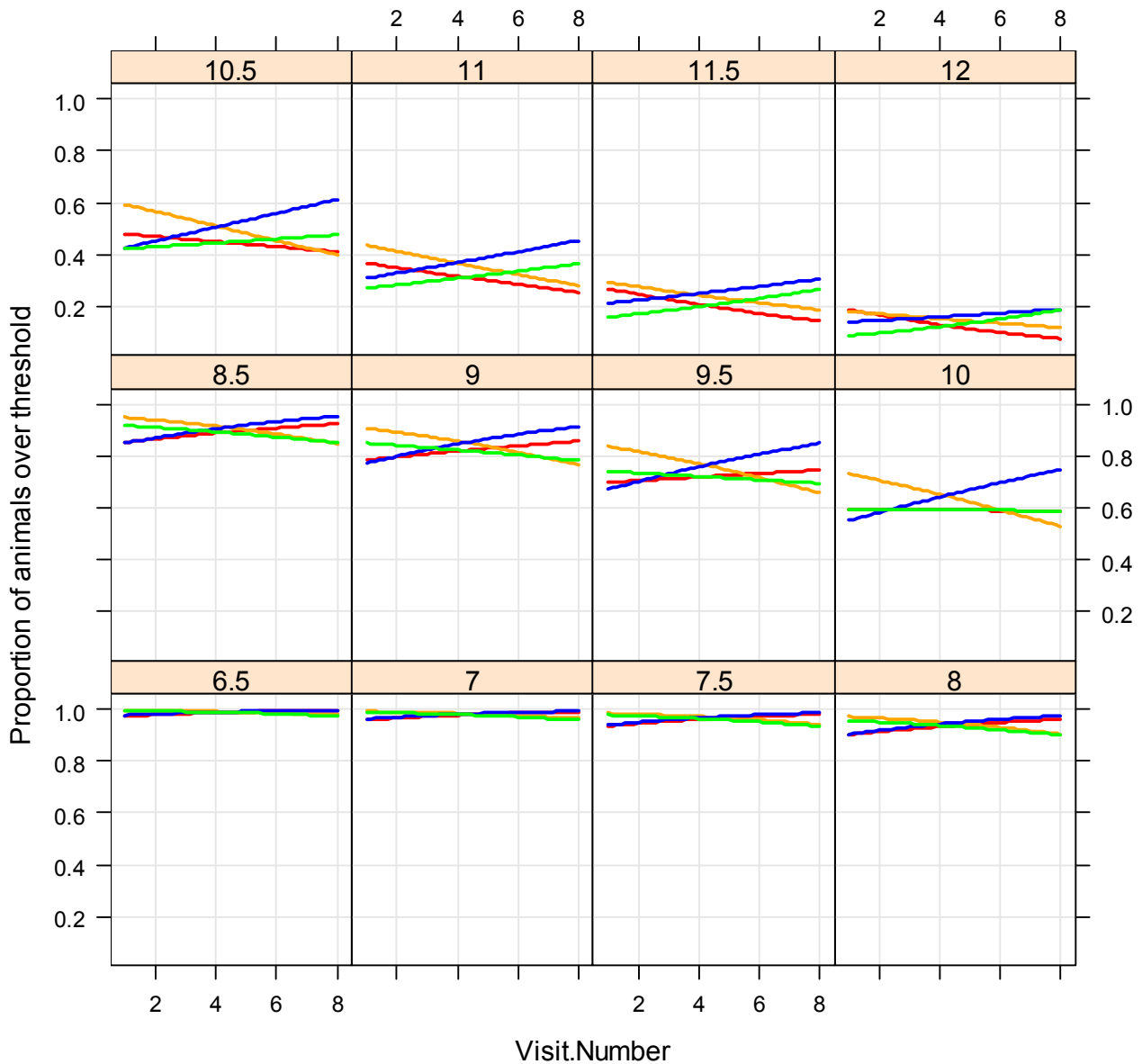
Interactions were significant and the model was over dispersed, residual deviance is much greater than residual degrees of freedom. Predicted values were back transformed to proportions and plotted in Figure 5-20 and Figure 5-20.





**Figure 5-19: Proportion of animals above haemoglobin thresholds, arranged by by visit number (1-8). Each panel shows the proportion of animals over the minimum level of haemoglobin displayed on the x axis.**

**Proportion of animals above haemoglobin threshold over time**



**Figure 5-20: Proportion of animals above particular haemoglobin thresholds over the duration of the study. Each panel shows the change in the proportion of animals above the stated minimum threshold of haemoglobin over time (visit number).**

### 5.18.1 Outcome of threshold analysis

Figure 5-19 and Figure 5-20 show the predicted values for model6. Figure 5-19 shows the proportion of animals over the minimum level of haemoglobin displayed on the x axis. Panels are arranged by visit number (1-8). It can be seen that at the start of the study the lines mainly overlapped, indicating the structure of the groups was very similar. After visit 5 however, the pour-on group shows increasingly higher haemoglobin values through the whole range of threshold values, and by visit 8 this is highly significant ( $p < 0.01$ ).

Figure 5-20 shows the model visualised differently, with the proportion of animals above particular haemoglobin thresholds over the duration of the study. Each panel shows the change in the proportion of animals above a particular minimum threshold. Table 5-12 shows the output for the model6 and there are significant differences in the proportion of animals in the pour-on and isometamidium groups. The overall pattern during the study is that the pour-on groups show progressive, significant improvement in haemoglobin over the study whereas the isometamidium villages show a decrease. Table 5-12 shows the trends for the pour-on and isometamidium villages are significantly different to the control ( $p < 0.001$ ). There is no difference between the control and spray groups.

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	0.816	0.063	12.990	< 2e-16	***
Isometamidium	0.415	0.091	4.580	<0.001	***
Pour-On	-0.159	0.088	-1.799	0.072	.
Spray	-0.016	0.086	-0.188	0.851	
Visit.number	-0.004	0.011	-0.335	0.737	
Time: Isometamidium	-0.068	0.016	-4.161	<0.001	***
Time: Pour-On	0.071	0.016	4.439	<0.001	***
Time: Spray	0.004	0.016	0.229	0.819	

**Table 5-12: Model output for proportion data.**

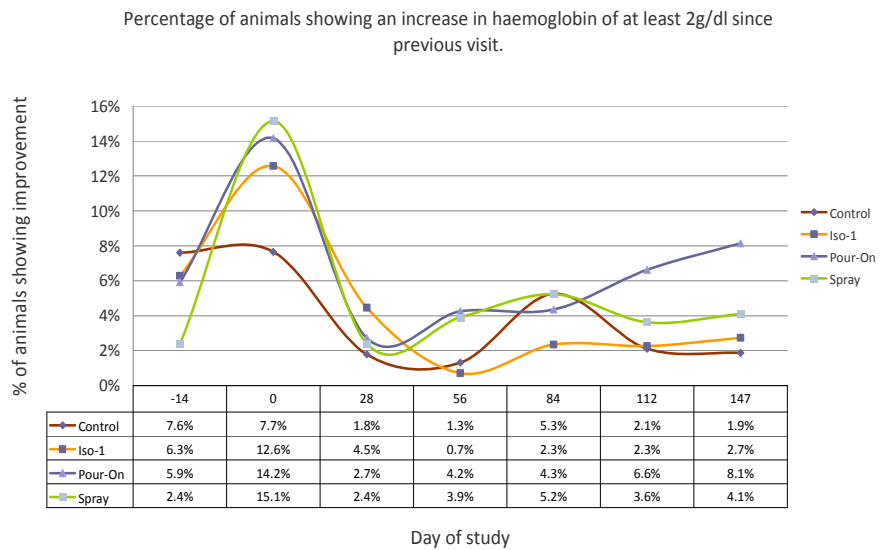
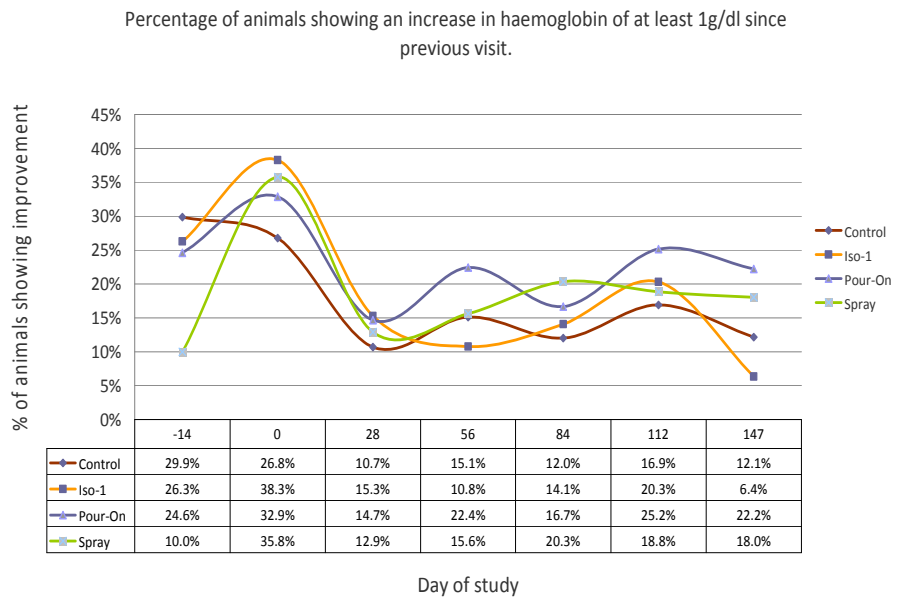
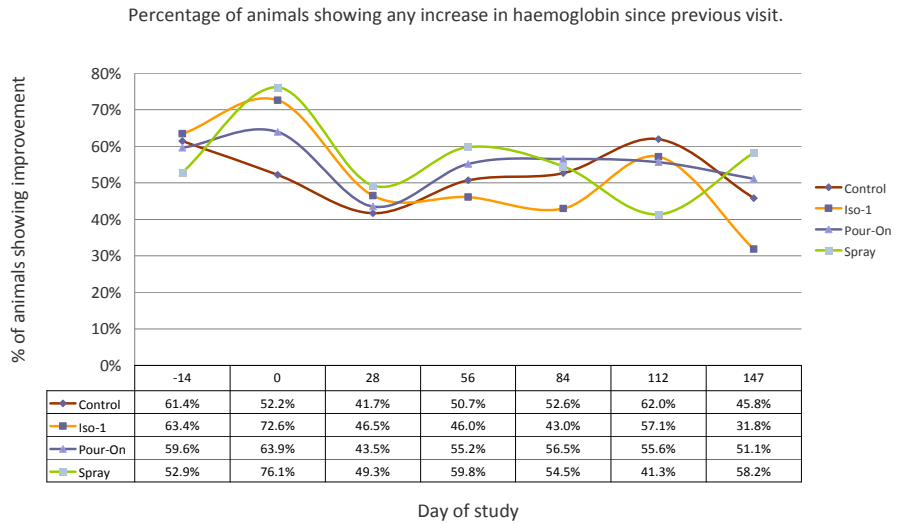
### 5.19 Improvement of individual animals over time

Finally, a slightly different way to look at the data is an assessment of how much an animal has improved since the last visit. The broad interpretation of this is a rising plane of haemoglobin that is indicative of improving health and absence of, or recovery from, many of the anaemia causing diseases. In contrast to the previous analysis, this method looks at the difference in haemoglobin between subsequent sampling times and provides no information about the absolute values. This is arguably as clinically relevant as absolute values, especially considering the range in which animals appear to maintain their haemoglobin (Figure 5-7). There are obvious issues with interpretation in this analysis; a moribund animal increasing its haemoglobin from 5 to 7 g/dl will show huge improvement but the animal is still arguably worse off than an animal in haemoglobin homeostasis around 11 g/dl.

Nonetheless, the visit by visit improvement of the study cattle was considered an important indicator by the livestock keepers who took part in the study, and although simplistic and clinically inaccurate, the ‘more is better’ approach to haemoglobin represented a fundamental aspect of how the technology was interpreted by livestock keepers.

Figure 5-21 contains three plots showing the proportion of animals presenting with haemoglobin values improved from the previous visit. In sequential order the minimum level of haemoglobin improvement is 0, 1 and 2 g/dl on the previous visit.

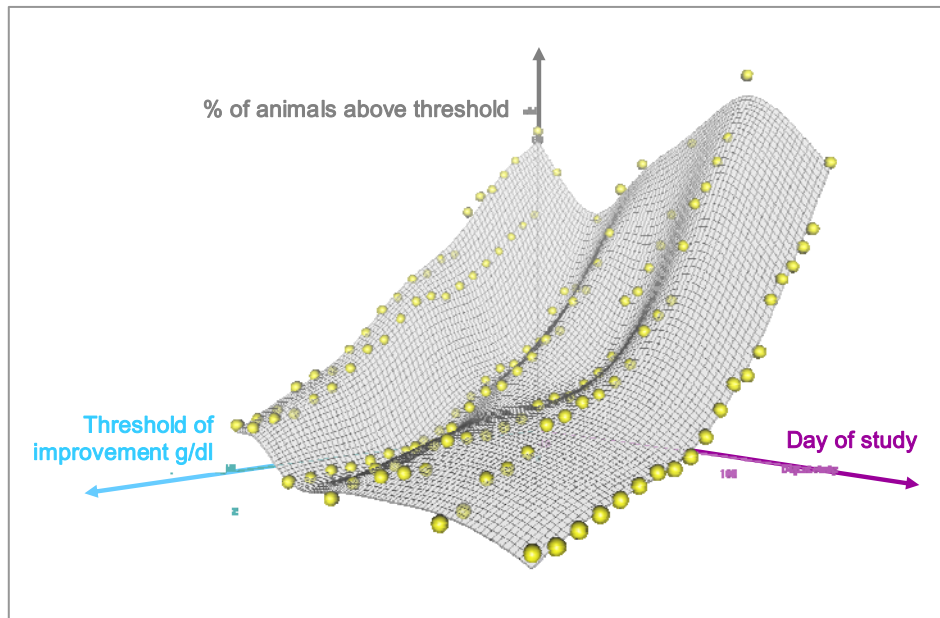
The most improvement is seen at day 0, and as highlighted in previous sections, following day 0, the trends become less clear, although there are indications that animals receiving pour-on treatments show the most improvement. There are indications that the isometamidium group is deteriorating towards the end of the study, with 32% of animals showing any improvement at all on the previous visit, (compared with over 45% for the other groups). However, without further timepoints it is difficult to attach clinical significance to this finding.



**Figure 5-21: Percentage of animals showing increases in haemoglobin over time**

### 5.19.1 Visualising thresholds

The thresholds chosen in Figure 5-21 are arbitrary ‘slices’ through a three dimensional plane with proportion of animals on the y axis, time on the x axis and the minimum threshold of haemoglobin on the z axis. An example of a 3D surface plot is shown below in, and surface plots for each intervention group are shown in the appendix. The 3D plots are fitted using the `scatter3d` directive in R and provide an excellent way of visualising data on screen as the function allows for the user to freely move around and zoom into the object. Such facilities translate less well to printed diagrams but are possible nonetheless and are useful to get a feel of graphs fitted with two explanatory variables. The surfaces are fitted with a non-parametric smoothed loess curve (Chambers & Hastie, 1992; Crawley, 2005). A non-parametric smooth method was chosen as the best way of exploring the landscape of y values. **Error! Reference source not found.** shows the relative positions of the four surfaces fitted with the proportion of animals above the threshold value on the grey y axis, bounded from 0 to 1, time fitted along the purple x axis ranging from day -14 to day 147 and the threshold of improvement on the light blue axis with values from 0 to 2 g/dl. A threshold value of 0, at the origin, shows the proportion of animals that did not deteriorate their haemoglobin values between this and the previous visit. A threshold value of 2 shows animals that improved by at least 2 g/dl between this and



previous visit.

**Figure 5-22:- Example of a 3D plot**



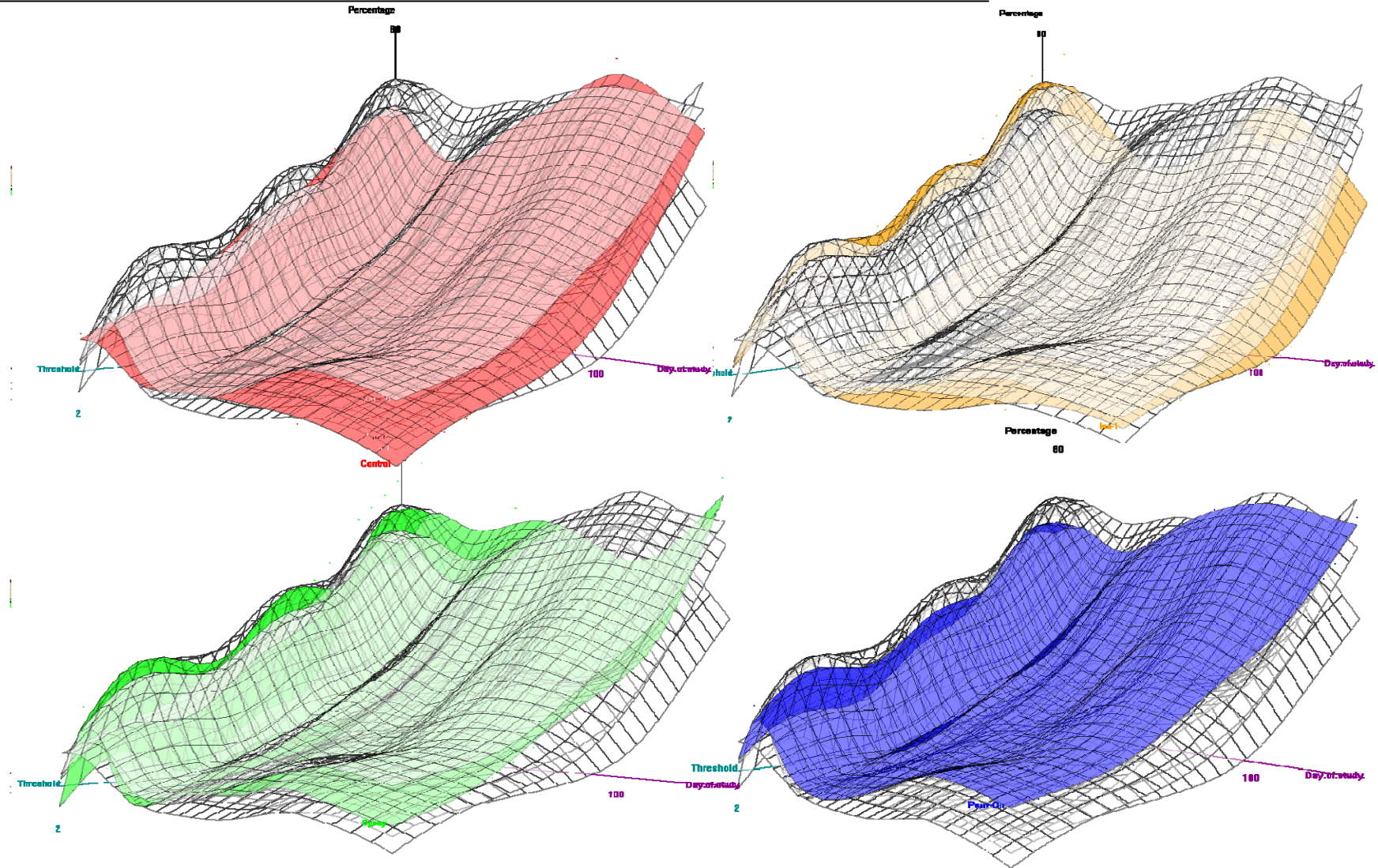


Figure 5-23:- Individual surface plots showing the relative positions of treatment group surfaces. Y axis (grey) shows the percentage of animals improving, x-axis (purple) indicates time (day of the study) and the z axis (light blue) shows the amount of improvement, in haemoglobin, between visits, range 0-2 g/dl. Surfaces are coloured for each group; red=control, orange=isometamidium, blue=pour-on, green = restricted spray.



## 5.20 Interpretation of surface plots

So what do the surface plots tell us that is not shown Figure 5-21? Overall, the percentage of animals showing an improvement in haemoglobin is higher in the pour-on group (blue surface), particularly during the latter part of the study (RHS of diagram in this view). The surface plots also show how the other three surfaces are interwoven and do not present a clear difference. The significance of these differences can be assessed by adding the `model.summary=TRUE` command to the plot function, with the output displayed in Table 5-13:- Difference in groups represented by 3D surface plots

which shows that in terms of visit to visit improvement of haemoglobin, the pour-on is the only intervention significantly different from the control.

Group	Estimate	Std. Error	z value	Pr(> z )	Sig?
(Intercept) Control	0.7482	0.0157	47.621		
Isometamidium	0.0256	0.0227	1.128	0.254	NS
Pour-On	0.1521	0.0221	6.879	<0.001	Sig
Spray	-0.0214	0.0216	-0.988	0.323	NS

**Table 5-13:- Difference in groups represented by 3D surface plots**

## 5.21 Overall conclusion

The purpose of this chapter was to investigate the impact of the interventions in terms of an animal's blood haemoglobin, measured every 28 days for 8 months. Whilst anaemia is a clinical indicator of a number of endemic cattle diseases, namely parasitic nematode infections (Parkins & Holmes, 1989) and anaplasmosis (Ristic *et al.*, 1972; Richey, 1992), it is the main characteristic of trypanosomiasis (Murray, 1988) as it begins with the first wave of infection and is progressive thereafter. With the privatisation of veterinary services and associated decline in the use of veterinary diagnostic laboratories (de Haan, 2004), the responsibility for disease diagnosis and treatment is devolving to the individual farmers,

animal health assistants, community animal health workers and veterinary drug vendors (Van den Bossche *et al.*, 2000; Machila, 2004). Field based diagnostic techniques that provide support to these resource- and information-deprived groups are thankfully being developed, examples ranging from decision support aids for disease diagnosis (Cockcroft, 1999; Eisler *et al.*, 2007) to diagnostic tools robust enough to be used in the field. Such tools provide information can be used to differentiate infected animals and monitor treatment success and include the portable haemoglobinometer reviewed in (Magona *et al.*, 2004b) and the FAMANCA system for identifying anaemic animals suffering from gastrointestinal nematode parasites (Bath *et al.*, 1996). The assessment of haemoglobin is thus important for two reasons in this study, primarily it provided an quantification of overall animal health that was an indication not of infection, but of disease. Given the endemic nature of disease and continual challenge and re-challenge nature of disease in south east Uganda, the populations of disease resistant livestock, the significance of interactions and co-infections and the carrier status associated with many of these diseases, a overall indicator of pathology is arguable more useful than demonstration of infection. Anaemia is a good indicator of this, especially in the case of monitoring trypanosomiasis. For example, productivity in trypanotolerance is attributable mainly to a ability to control anaemia rather than a parasitaemia (Naessens, 2006).

Discussion has focussed on quantifying the changes in haemoglobin that were seen in a population of 947 cattle recruited to a longitudinal study in south east Uganda, and roughly equally spread between 12 villages. The study aimed to assess three animal health interventions that are in use, or potentially available for use, by cattle keepers in this area as methods of controlling endemic tick and tsetse borne diseases, in comparison to a control group of animals that did not receive any intervention treatment.

The entire study population was given two doses of a trypanocide diminazene aceturate 42 and 14 days prior to beginning the interventions. Interventions consisted of either a) a single trypanoprophylactic dose of isometamidium chloride at day 0; b) monthly (q.28 days) application of a pour-on formulation of deltamethrin; c) monthly (q.28 days) application of a

spray formulation of deltamethrin to the front legs, ears and belly of the animal, and d) a control, which received no further intervention.

For the purposes of analysis the dataset can effectively be split into two time periods. From the first baseline visit (day -42) up to and including visit 3 (day 0), the data can be used to assess the impact of two diminazene treatments on the cattle population recruited for the study. After day 0 the data can be used to assess if there is a significant difference between the different intervention protocols tested in this study.

Initial pairwise comparisons were made between the different grouping structures of the data (groups and villages within groups) to quantify any heterogeneity that may bias the interpretation of the treatment effects. At baseline (day -42), comparing the mean haemoglobin value of villages at the 95% confidence level showed that villages were not significantly different, with the exception of the village of Bukhunya which had a significantly lower mean value. Haemoglobin values of the study population were therefore considered to be acceptably homogenous.

Multiple comparisons between intervention groups, rather than the villages themselves showed that at the baseline there was a significant difference between villages allocated into the control and restricted spray groups. However, by day -14 all significant differences had disappeared and, at day 0 when the interventions began, there was no significant difference between any of the groups.

Analysis of the haemoglobin values largely used linear mixed effect modelling to account for the nested and pseudoreplicated structure of the dataset. Between the baseline (day -42) and the start of the interventions (day 0) there was a highly significant ( $p < 0.001$ ) improvement in the mean haemoglobin of all twelve villages recruited to the study. Due to the nature of the study design it is strictly not possible to attribute this to the administration of diminazene, although this was considered to be highly probable.

During the interventions significant differences between the treatment groups become apparent. Insecticide treated villages showed a range of different responses during the interventions. Pairwise analysis shows that the pour-on group had a significantly higher mean haemoglobin value than the control group from day 84 onwards. Additionally, by the end of the study (day 147), the pour-on group had a significantly higher mean haemoglobin value than both of the other treatment groups. In contrast, with the exception of day 84, the restricted spray group was not statistically significantly different from the control group during the intervention periods. The isometamidium group showed a significant decrease in mean haemoglobin levels which may indicate that the prophylactic period of isometamidium was shorter than the anticipated 4-6 months, allowing the re-emergence of trypanosomiasis infection at a clinical level. The decrease in haemoglobin levels was not statistically significantly different from the control group. All villages in the control and isometamidium groups exhibit similar slopes, indicating a generally consistent response over time. From day 0 onwards, the control group displayed no significant change in haemoglobin levels, despite a slight decrease in the mean value.

Evidence of significant within animal as well as between animal variations in haemoglobin values was seen in the linear mixed effect models after the start of the interventions on day 0. Modelling required a linear mixed effect model with village and animal within village fitted as random effects and accounting for the temporal autocorrelation between samples. No significant differences in mean haemoglobin values between the intervention groups were identified in the models. However, the output of these models showed a significant difference in the rate of improvement of haemoglobin in the pour-on group compared with the control. When the model was fitted to data from the whole study, and therefore included the diminazene treatment period, the pour-on and spray groups both demonstrated a significantly higher rate of improvement compared with the control. There was no significant effect of isometamidium compared with the control.

Analysis of the percentage of animals over a particular haemoglobin threshold indicates that cattle in the pour-on groups show a consistent, progressive and highly significant improvement throughout the intervention period. Animals in isometamidium villages showed a highly significant decrease. No significant difference was evident between the control and spray groups.

In summary, over the intervention period, pour-on villages had a significantly higher rate of mean haemoglobin value improvement compared with the control. Although the spray groups also displayed a significantly higher *rate* of improvement than the control, the mean haemoglobin value was not significantly different to that of the control. In contrast, the isometamidium villages showed a significant decrease in mean haemoglobin values compared with the control, although the rate of change is not significantly different to that of the control group.

**Chapter 6 :-** Seen to be working:  
Impact of the interventions on clinical  
parameters

## 6.1 Introduction

The current trend in controlling livestock diseases in Africa of leaving the farmer to “go it alone” (Eisler, 2003) profoundly influences the way diseases should be managed. Moving the provision of veterinary healthcare from a public service to private choice alters fundamental aspects of health care. The choice of the individual, presented as one of the benefits of privatisation of healthcare (Sen & Chander, 2003), fragments the delivery of healthcare to the level of an individual farmer choosing to treat an individual animal. This has obvious implications in the management of infectious diseases where the scale of health management needs to be at the herd or the regional level. Fragmentation also impacts the type of healthcare options sought, and successful treatments are those that quickly confer an obvious, and preferably exclusive, benefit to the farmer (Umali *et al.*, 1994). In economic terms, successful health options are private goods with a minimum free rider principle. An example of healthcare as a private good could be seen as the small foil packet containing a single dose of trypanocide, of which 35 million are administered annually throughout Africa (Holmes *et al.*, 2004). Interventions such as these are simple, require no communal or governmental input and have a rapid, obvious benefit of immediate and important significance only to the implementer.

Over the past 15 years the scale of livestock health provision in Africa has devolved to the level where most of the health decisions are made by individual farmers on almost entirely short term economic grounds (Leonard, 2004). Although large, centrally funded, equipped and managed projects are continually in circulation they exist in tandem with this groundswell of private veterinary provision. The treatment options explored in this study can be treated as private goods available to the individual livestock keeper. Although many ‘top-down’ control programmes employ both insecticide spraying and trypanocidal drugs, for example the EU funded Farming in Tsetse Controlled Areas (FITCA, 2005) or the Regional Tsetse and Trypanosomiasis Control Programmes (Food and Agriculture Organization of the

United Nations, 1993), the technologies on trial function primarily as private goods at the level of the livestock keeper.

Synthetic pyrethroid insecticides are becoming important in the control of vector borne diseases in Africa (Vale & Torr, 2004). As patents expire and generic formulations appear on the market (Torr *et al.*, 2005) unit costs are decreasing. They have flexible methods of application, and the legacy of crop spraying and governmental spraying regimes make it a technology familiar to many rural farmers (Okoth, 1999). From an epidemiological standpoint, insecticide treated cattle are of particular interest because, despite individuals choosing to treat their animals as an entirely private good, the impact on the tsetse population of an insecticide treated cow confers a public benefit (Vale *et al.*, 1999).

The overall aim of this study is to investigate treatment options available to rural livestock keepers. Given the current state of veterinary provision in rural Africa, a successful treatment has to be efficient at providing a 'private good'. The current paradigm of health provision relies on individual farmers choosing to spend a proportion of their income on a treatment, and for that treatment to be adopted it needs to be demonstrably and reliably effective. SE Uganda has a suppressed tsetse population (Magona *et al.*, 2004a) and as such the presence of tsetse is probably not perceived by the livestock keepers as an obvious hazard. Coupled with an endemic disease state in the predominantly zebu population, cattle infected with trypanosomiasis are more likely to suffer chronic signs and incur long-term production losses than exhibit acute signs of the illness (Waiswa & Katunguka-Rwakishaya, 2004). As a result, farmers often do not understand the link between tsetse and trypanosomiasis (Kamara *et al.*, 1995; Machila *et al.*, 2003) making it difficult to persuade livestock keepers that it is worthwhile investing in technologies that only target the tsetse flies.

Synthetic pyrethroids offer a good way of controlling tsetse as a 'side effect' of controlling the very visible ticks on cattle. The tick burden carried by cattle can be immense and the presence of such parasites are widely disliked by farmers, as much for aesthetic reasons as their associated pathologies. The impact of the interventions on the visual appearance of the



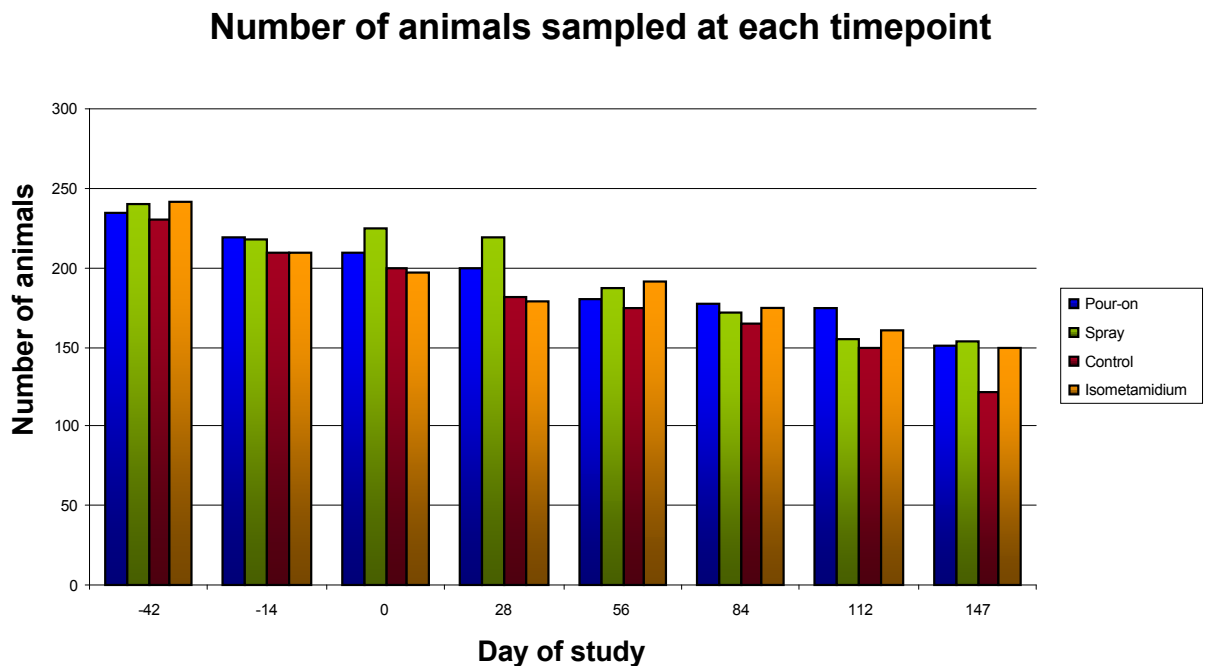
study animals is an important factor in the long term sustainability of these treatment methods, in specific the restricted spray methodology. This chapter will assess the impact of the study on the visual parameters available to rural livestock keepers, although some outcomes are further quantified with reference to ancillary diagnostic techniques. Specific clinical variables to be assessed are tick counts, condition scores, lymph node enlargement and weight.

## 6.2 Methods of data visualisation and exploration

The clinical data under analysis is ordinal. Half body tick counts and condition score methodologies are described in section 3.2. Analysis of the data will involve the use of log-linear modelling incorporated within mosaic plots. Analysis of deviance on the proportional tick counts offers an alternative method of analysis, however mosaic plots have been chosen because they provide a visual method of interpreting the differences. A detailed explanation of mosaic plots is given in section 3.18.2. To summarise they are graphical representations of a 2x2 contingency table, and are essentially grouped bar charts where the width and height of the bars show the relative frequencies of the two variables. The tiles in a mosaic plot are proportional to the observed cell frequencies. The panels presented in this chapter are arranged in two panels; the upper panel shows a mosaic plot for each timepoint, plotting the clinical variable of interest against treatment group. The lower panel is a mirror of the upper but the cells are shaded to represent significant differences between the treatment groups. Comparisons are made by *row*, hence a shaded cell indicates a group that has a significantly different proportion of animals with a level of clinical variable compared with the other three groups. Cells shaded red indicates that a cell is significantly lower, blue indicates significantly higher. The key indicates values for the standardised Pearson residuals of 2-4 and >4 of a Chi-squared statistic and correspond to  $p < 0.05$  and  $p < 0.001$  for residuals 2-4 (lighter colour, broken border line) and >4 (deeper colour, solid border line)

### 6.2.1 Full or restricted dataset?

Mosaic plots do not provide information about the number of samples. If the number of animals in each group at a particular time point is significantly different to that in another group this may introduce bias. Figure 6.1 shows the number of animals sampled in each group over time. Sample size of each group was not considered different enough to introduce

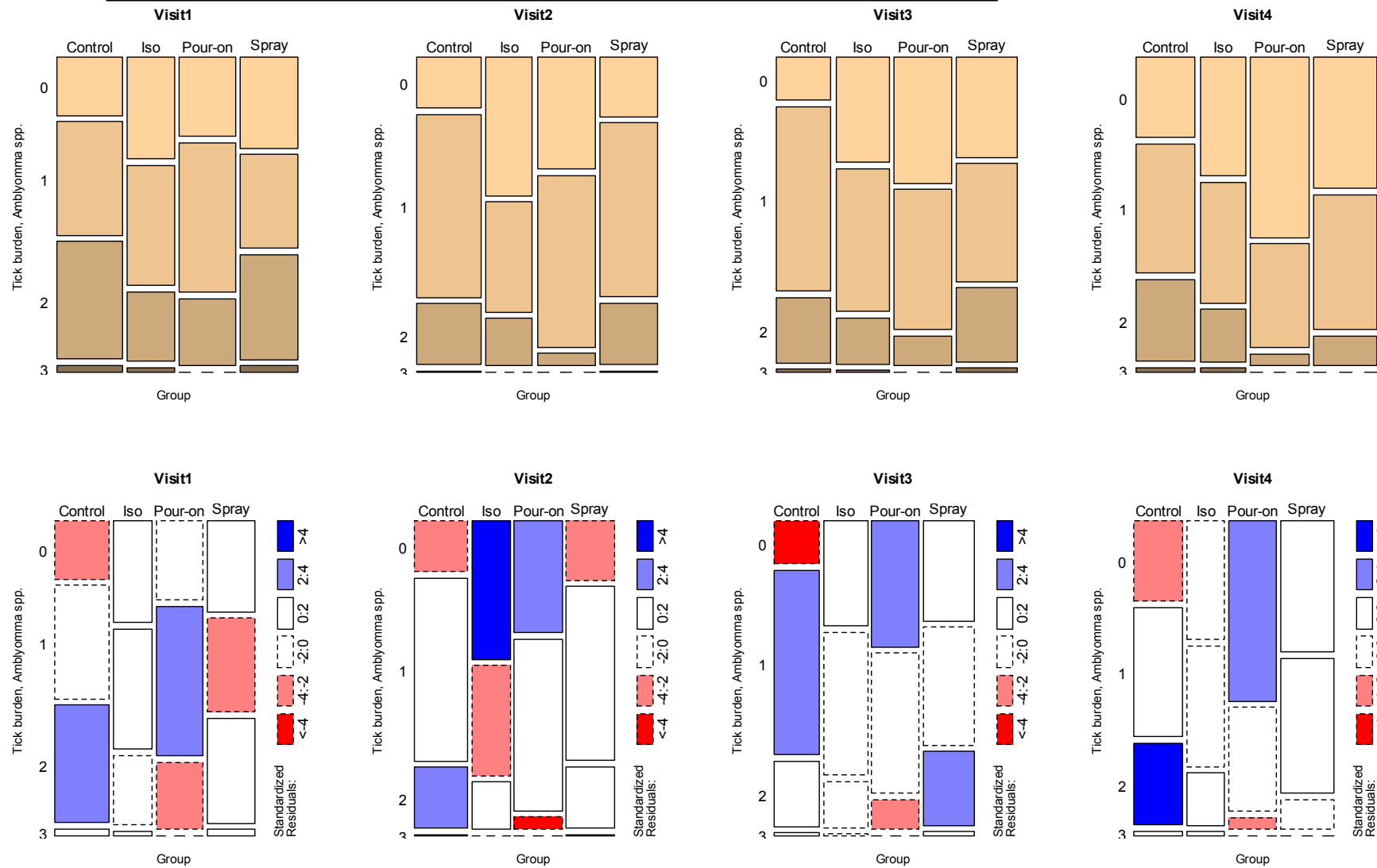


bias in this study.

### 6.3 Impact of interventions on tick burden

Figure 6.1 to Figure 6.6 show mosaic plots for the three species of ticks commonly found parasitizing cattle in the study; *Amblyomma variegatum*, *Rhipicephalus appendiculatus*, and *Boophilus decoloratus*. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50

ticks, 3 >50 ticks. All figures refer to half body tick counts made from a laterally recumbent animal as described in section 3.2.3. The upper row on each page shows a solid filled mosaic plot of intervention groups plotted against tick burden. Cells highlighted in the lower row identify which plots are significantly different when compared with others in the same row.



**Figure 6.1:- Mosaic plot showing burden of *Amblyomma* spp for visits 1-4. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50 ticks, 3 >50 ticks.**

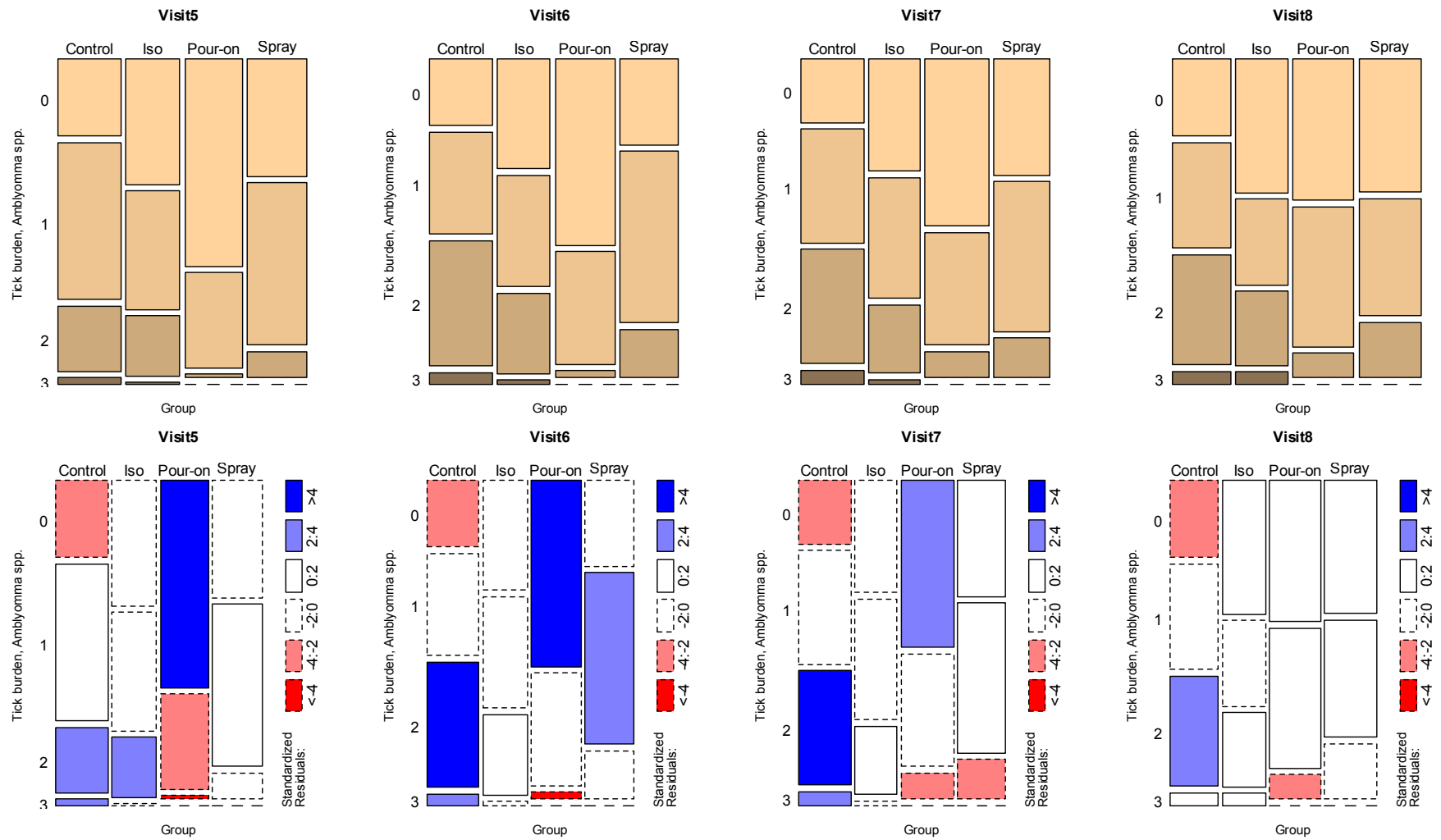


Figure 6.2:- Mosaic plot showing burden of *Amblyomma spp* for visits 5-8. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50 ticks, 3 >50 ticks.

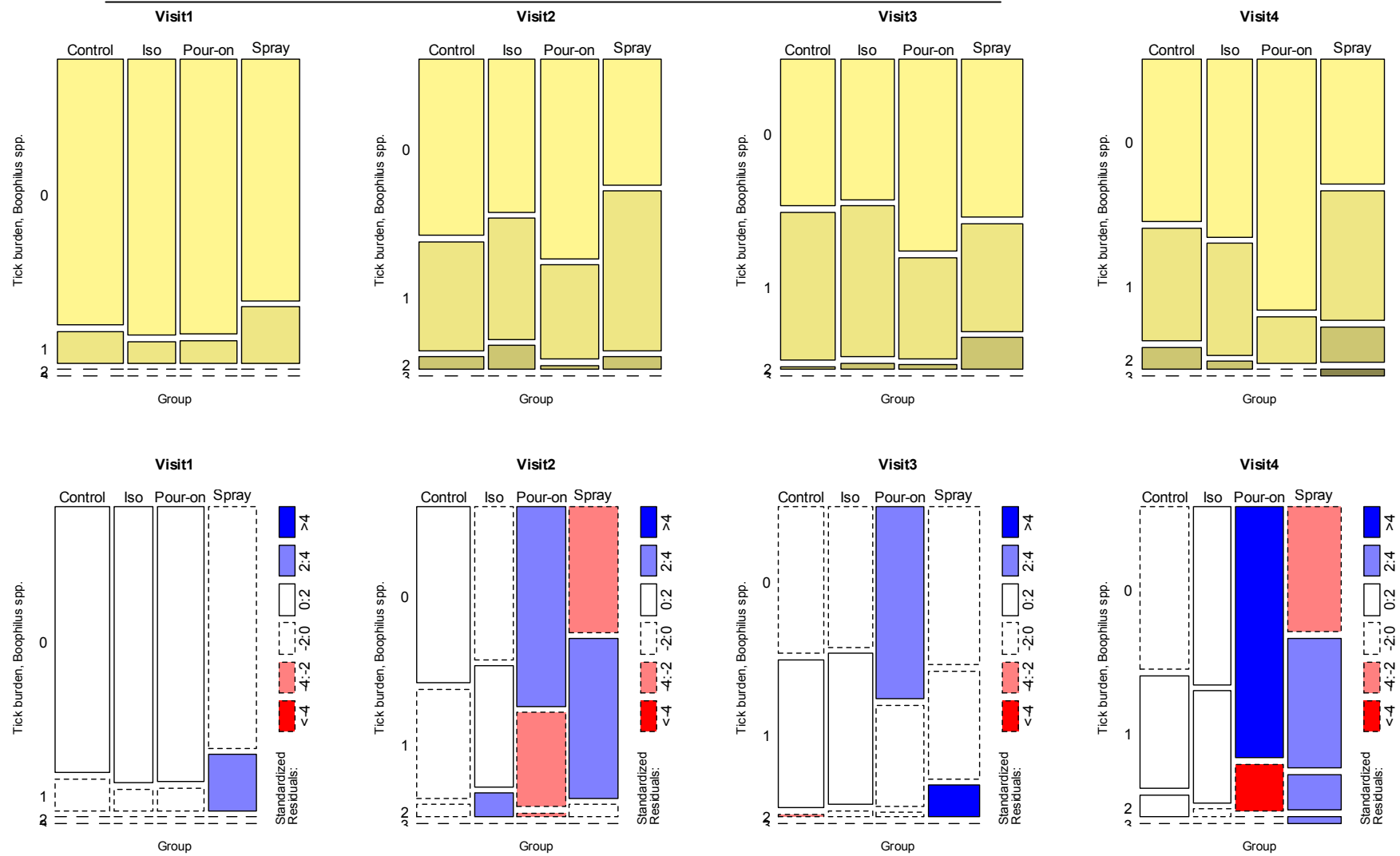
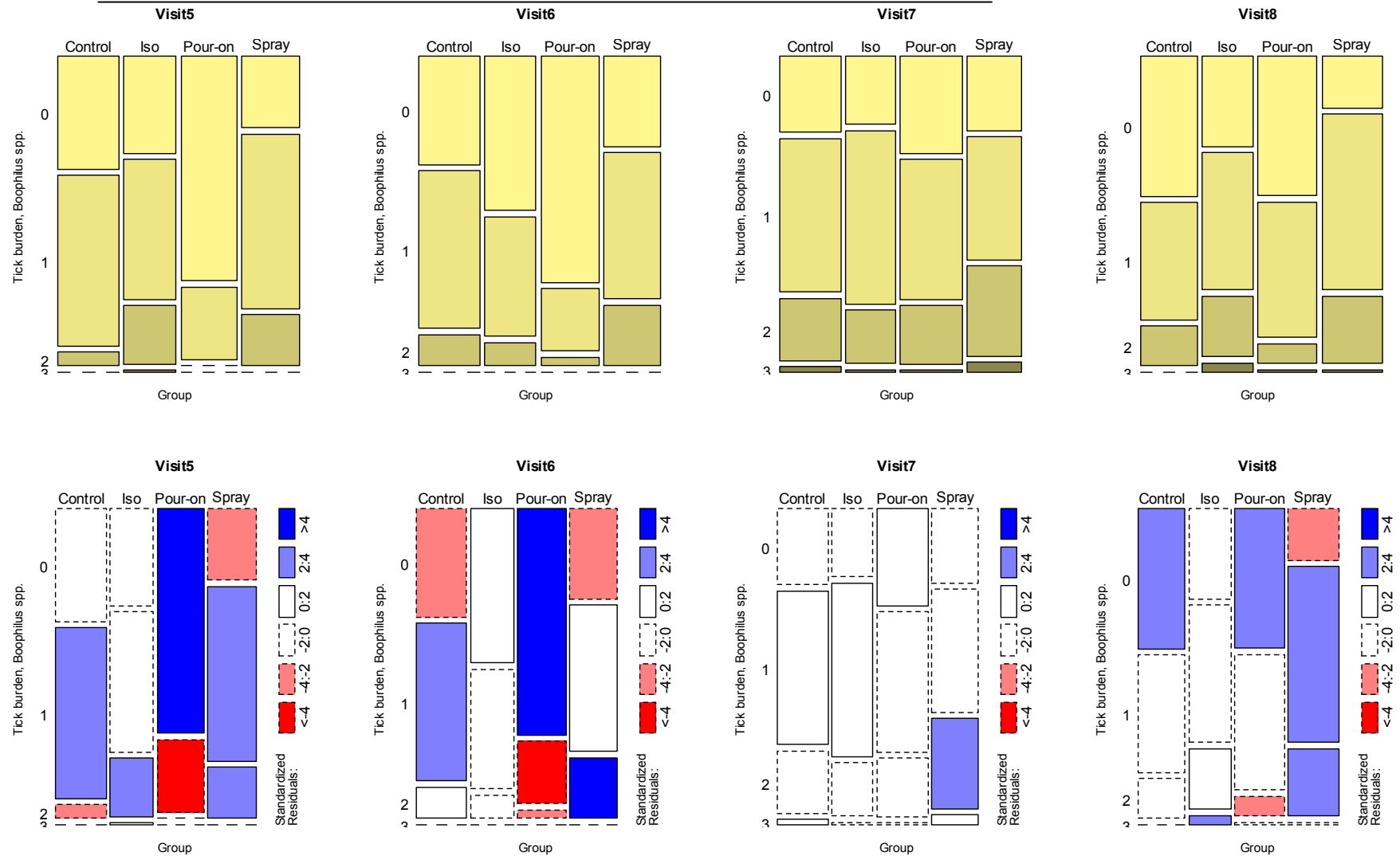
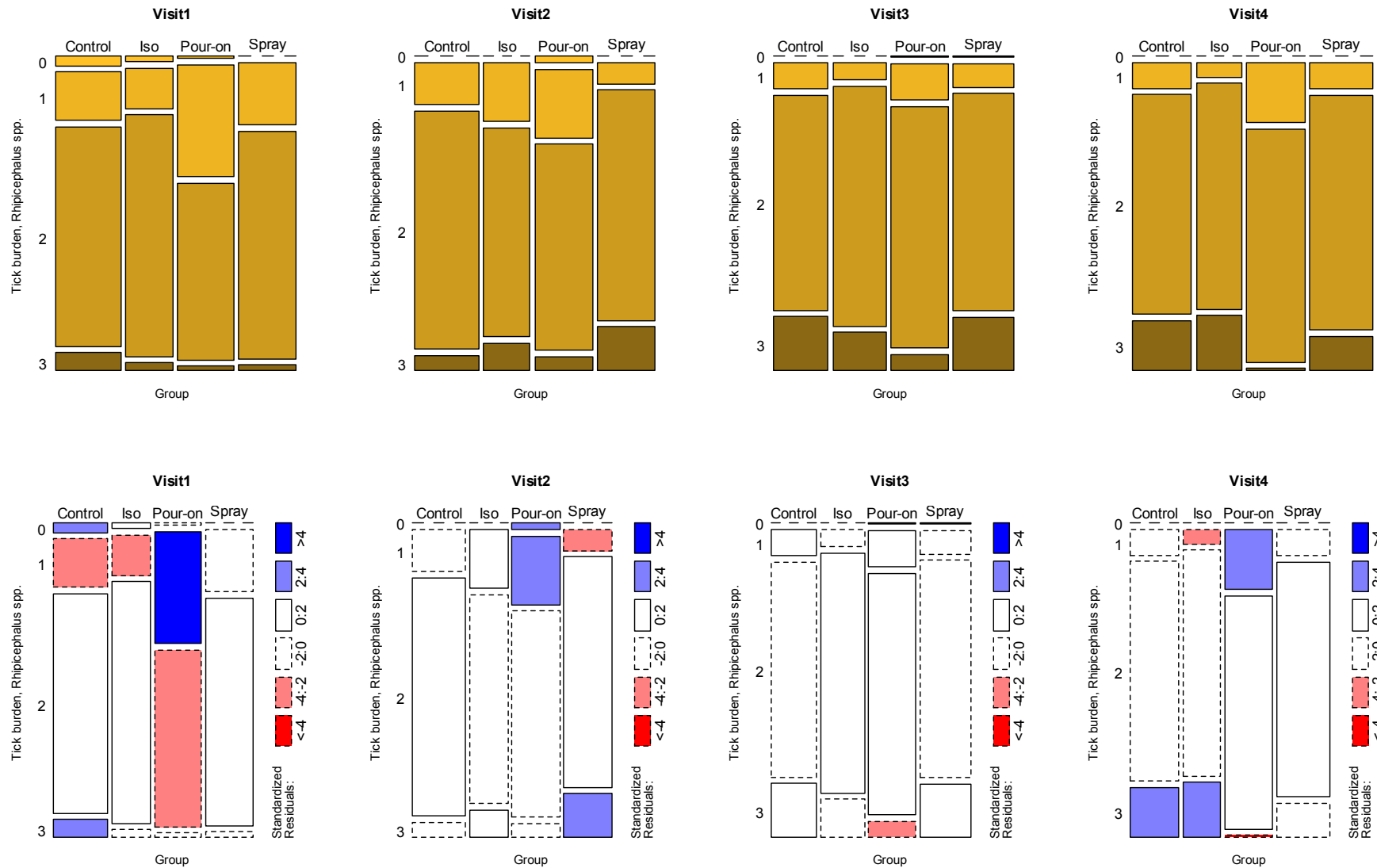


Figure 6.3:- Mosaic plot showing burden of *Boophilus spp* for visits 1-4. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50 ticks, 3 >50 ticks.



**Figure 6.4:- Mosaic plot showing burden of *Boophilus spp* for visits 5-8. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50 ticks, 3 >50 ticks.**



**Figure 6.5:- Mosaic plot showing burden of *Rhipicephalus spp* for visits 1-4. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50 ticks, 3 >50 ticks.**



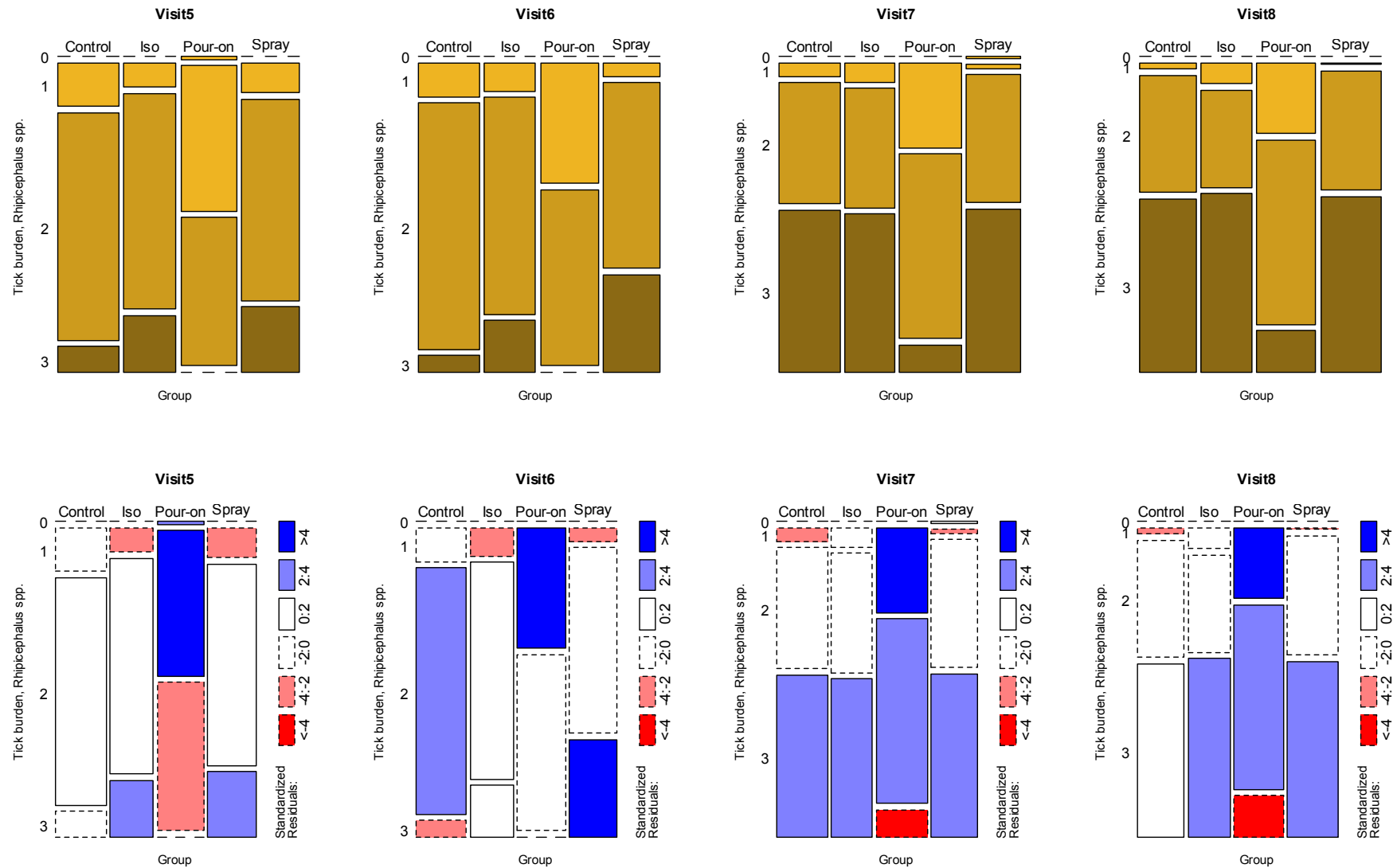


Figure 6.6:- Mosaic plot showing burden of *Rhipicephalus spp* for visits 5-8. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Tick burden is coded as follows: 0= no ticks, 1=1-10 ticks, 2=11-50 ticks, 3 >50 ticks.

#### 6.4 Tick burden of *Amblyomma* tick species

Figure 6.1 and Figure 6.2 show the differing burden of *Amblyomma* ticks throughout the study. At the baseline there are significantly more ticks on the control group than the other three and by visit 3 when the interventions began this pattern is more pronounced (Control groups have a significantly ( $p < 0.05$ ) smaller proportion in the 'no ticks' category, shaded red, and significantly more in the 10-50 category, shaded blue). This suggests significant differences in the environmental challenge which must be borne in mind when interpreting subsequent analysis. Considering time points 5-8, there are indications that a lower proportion of animals present with  $>50$  ticks (coded 3) in both pour-on and spray insecticide categories compared with the non-insecticide groups. Of the two insecticide treatments, the effect is more pronounced in the pour-on group. Given the possible differing challenge, however, this result ought to be interpreted with some caution. As a possible explanation, *Amblyomma* ticks localise on the ventrum, axilla udder and perineal regions of the animal and consequently the direct effect of the restricted spray, which avoided the caudal predilection sites, could be expected to be lower than the pour-on.

#### 6.5 Tick burden of *Boophilus* tick species

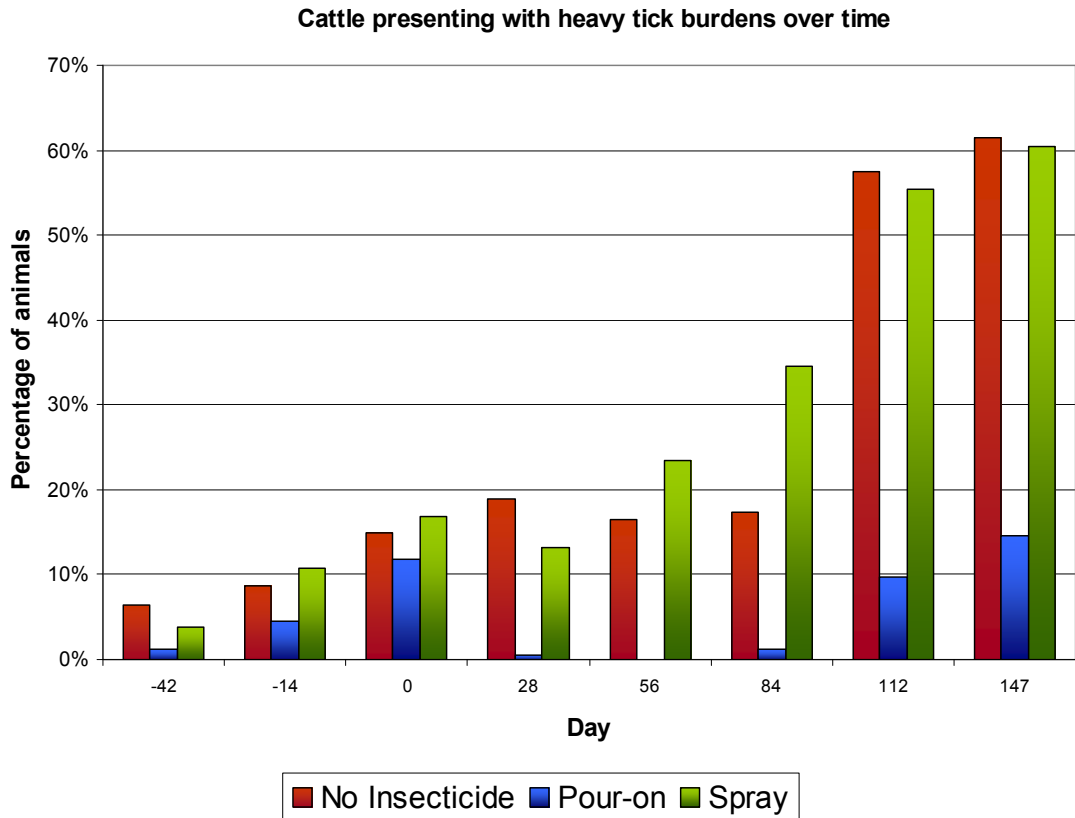
Figure 6.3 and Figure 6.4 show the differing burden of *Boophilus* ticks throughout the study. They show an overall increase in *Boophilus* tick counts throughout the study, for example, fewer animals have a tick burden of 2 at visits 1 & 2, compared with visits 7 & 8. This is most likely due to an increase in tick challenge with seasonal change over the eight months of the study. There is a strong indication that the pour-on group has significantly lower *Boophilus* tick counts for visits 4, 5 and 6 compared with the other groupings, although this difference is not apparent over the last two sampling points. Conversely, the spray group has a proportionally higher number of animals with high *Boophilus* tick counts compared with the other treatments. For this species, the acaracidal effect of the restricted spray is not particularly apparent, although this could partially be explained by the spray protocol avoiding some of the predilection sites of attachment such as flank and dewlap.

## 6.6 Tick burden of *Rhipicephalus* tick species

Figure 6.5 and Figure 6.6 show the differing burden of *Rhipicephalus* ticks throughout the study. Similarly to *Boophilus*, there appears to be strong indication that tick challenge increases over time. The pour-on villages have a significantly ( $p < 0.001$ ) lower number of animals carrying heavy *Rhipicephalus* tick burdens from visit 5 onwards, suggesting a protective effect from the pour-on. There appears to be no significant difference between spray and non insecticide treated groups, suggesting that in terms of this species there appears to be no effect from the restricted application. This is despite the restricted spray protocol targeting the predilection sites of the ear and axilla.

## 6.7 Proportion of animals with a heavy tick burden

An animal was coded as having a heavy tick burden if it had more than 50 ticks of any species or more than 10 of all three tick species. Although arbitrary, such coding represents what seemed to be a ‘noticeably heavy’ tick burden to the cattle owners. The proportion of animals with a heavy tick burden by intervention group is given in Table 6-1. Figure 6.7 highlights the difference between the pour-on and spray groupings. The control and isometamidium groups are amalgamated to simplify the graph. As described in section 2.10, the intervention effect applies after day 0, and differences between the groups prior to this are therefore due to intrinsic variation between the groups. As can be inferred from Figure 1-8, there is a highly significant difference between the spray group and the pour-on ( $\chi^2 = 39.77$ ,  $df = 1$ ,  $p\text{-value} = <0.001$ ) and no significant difference between the restricted spray group and groups that received no insecticide treatments ( $\chi^2 = 0.0019$ ,  $df = 1$ ,  $p\text{-value} = 0.9656$ ).



**Figure 6.7: Comparison of 'heavy' tick burdens between insecticides**

Heavy tick burden?	Control	Iso.	Pour-on	Spray
Yes	58.5%	60.9%	14.1%	59.4%
No	41.5%	39.1%	85.9%	40.6%
Sample size	121	128	151	154

**Table 6-1: Comparison of tick burdens by intervention group at day 147**

## 6.8 Investigation of the residual efficacy of the restricted application spray

The restricted application protocol involves a spray application of a 1:1000 aqueous solution of deltamethrin every 28 days. The concentration is the same as the manufacturer's recommendation, however the total volume applied is reduced by 80% as the spray is only applied to the legs, ears and belly of cattle.

In order to investigate the residual efficacy of the restricted application protocol at controlling tick burdens, a study was run on a separate population of zebu cattle whereby tick counts were made at 72 hour intervals for the duration of 30 days.

### 6.8.1 Residual efficacy study design and methodology

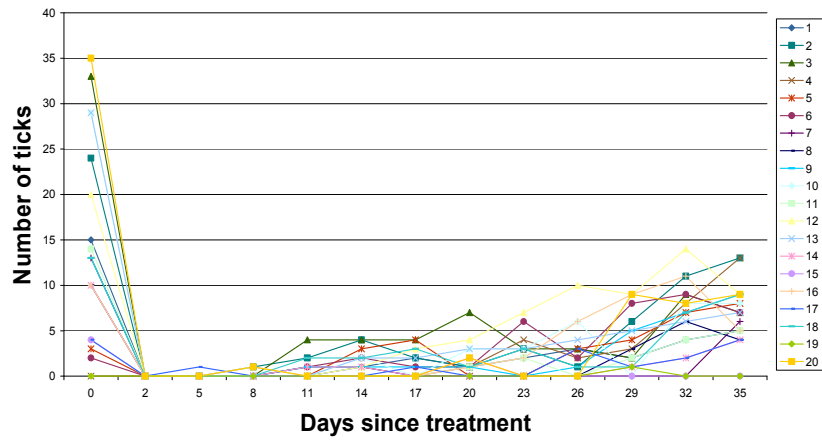
Twenty cattle from a site known to have similar cattle demographics and management systems as the study villages were recruited to this study. A site was chosen close to the Ugandan Livestock Research Institute in Tororo for logistical reasons. Twenty cattle were selected at random (by random number allocation) out of a herd of 97 animals for inclusion in the study. Selected cattle were identified by ear tags for subsequent follow-up.

Cattle were examined on day 0 and the tick burden counted for each species as described in Chapter 2. All cattle were sprayed once with a 1:1000 solution of 5% deltamethrin (Vectocid™, Ceva Sante Animale) using the same protocol employed in the longitudinal study. Cattle were returned to the herd, and for the duration of the study were managed by daytime extensive grazing and tethered or corralled around homesteads at night. The aim was to emulate the conditions of the longitudinal study animals as much as possible.

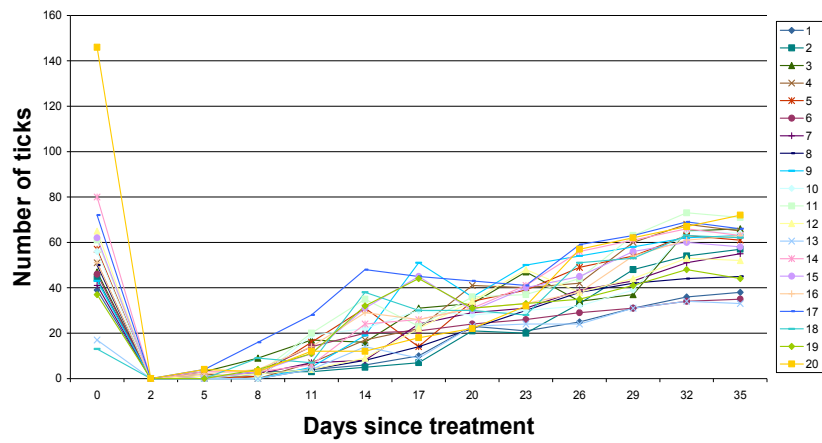
48 hours later the 20 tagged cattle were examined. None was found to have live, attached ticks, although in some cases dead ticks were found entrained in the animal's coat. These were not counted. Cattle were then re-examined every 72 hours to assess the incident tick burden. Absolute half-body tick counts were made for each species. The results are graphed in Figure 1-9 and Figure 6.9.

The study ran for 35 days.

*Amblyomma variegatum*



*Rhipicephalus appendiculatus*



*Boophilus decoloratus*

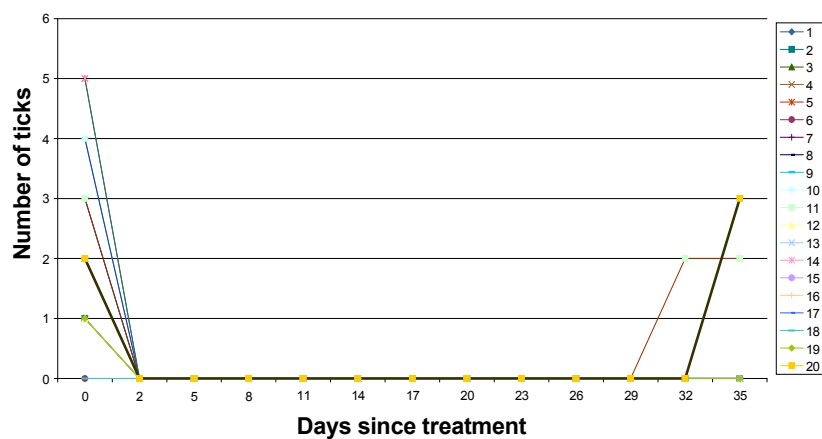
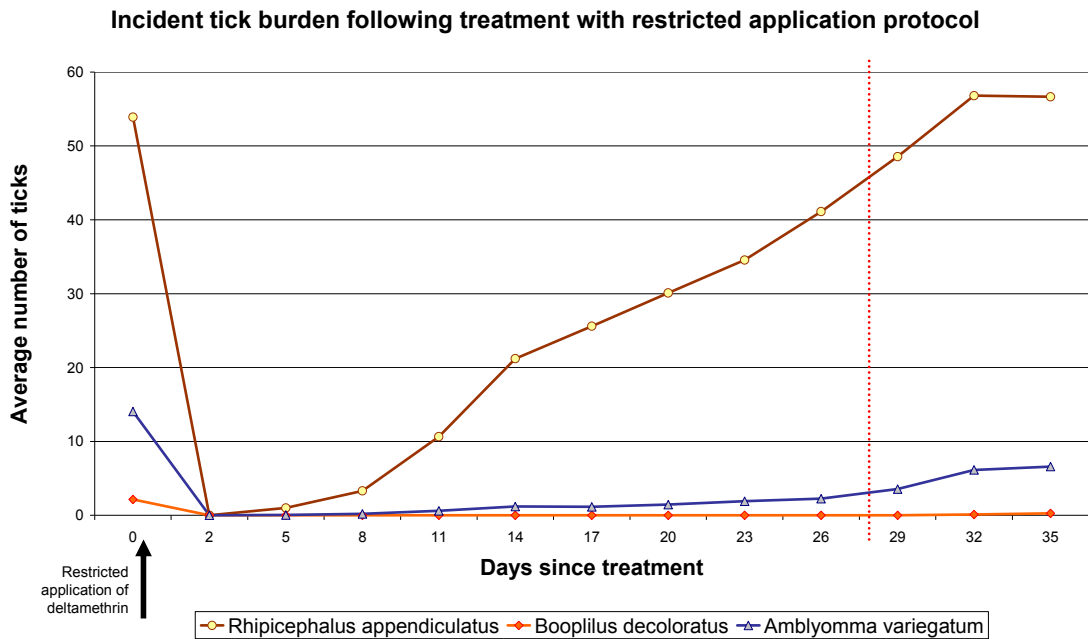


Figure 6.8: Burden of individual tick species following restricted spraying with deltamethrin on day 0. Each line represents a study animal.



**Figure 6.9: Tick burden following treatment with restricted application spray**

### 6.8.2 Results of residual efficacy study

Figure 6.9 shows the burden of ticks for each study animal by tick species. The amalgamated data is presented in Figure 6.8. The dotted red line represents 28 days after the application of spray, which coincides with the sampling interval in the longitudinal study. Following the application of insecticide, the tick prevalences dropped to zero. The reestablishment of parasite load was dependent upon tick species, *Rhipicephalus* demonstrating an almost linear recovery pattern to pre-treatment levels approximately 30 days later. *Amblyomma* burdens are lower, but also demonstrated a similar recovery pattern. On average, however, *Amblyomma* tick populations did not recover to pre-treatment levels by the end of the study. *Boophilus* ticks, in contrast do not seem to have a high prevalence. The maximum baseline level of *Boophilus* ticks is 5. Recovery to a pre-treatment level of prevalence is very prolonged, all cattle remaining free of *Boophilus* ticks for 29 days.

### 6.8.3 Interpretation

The tick burden patterns shown in Figure 1-9 and Figure 1-10 could usefully explain the tick counts measured in the longitudinal study. Animals in the longitudinal study were sampled every 28 days and, for animals in the spray group, re-treated with insecticide at the same time. It can be seen that re-spraying at an interval of 28 days allows considerable recovery of tick burdens, particularly with the *Rhipicephalus* species. As the tick burden fell to zero immediately after the application of spray there is little evidence of tick resistance to deltamethrin. Whilst it is possible that the re-establishment of ticks to almost pre-treatment levels of prevalence 28 days after each spraying is attributable to resistant populations, discussions with farmers did not suggest this was the case, and it is more likely that a similar pattern of prevalence recovery to that seen in Figure 6.8 is occurring in the longitudinal study. Assessment at a 28 day interval will therefore not show an impact of the spray insecticide. In contrast, the tick counts observed with the pour-on formulation suggest residual activity against ticks in excess of 28 days. This is in line with other studies in this area and the manufacturer's claims (Fox *et al.*, 1993; Okiria *et al.*, 2002a).

### 6.9 Tick burden reduction

It is possible to gain a crude assessment of the reduction in tick burden. The area under the curve for a given time interval represents the tick burden for that interval, and was calculated using the trapezoidal rule (Burden & Faires, 2000). If it is assumed that the tick populations would continue at their baseline levels in the absence of treatment, the area under the observed curves can be expressed as a percentage of the theoretical tick burden had the intervention not occurred. Tick burden is expressed as the cumulative number of ticks attached per day for a 28 day period following a spray event on day 0. Theoretical tick burden is simply the number of ticks observed at baseline multiplied by the number of days. Table 6-2 shows the percentage reduction over a 28 day period.



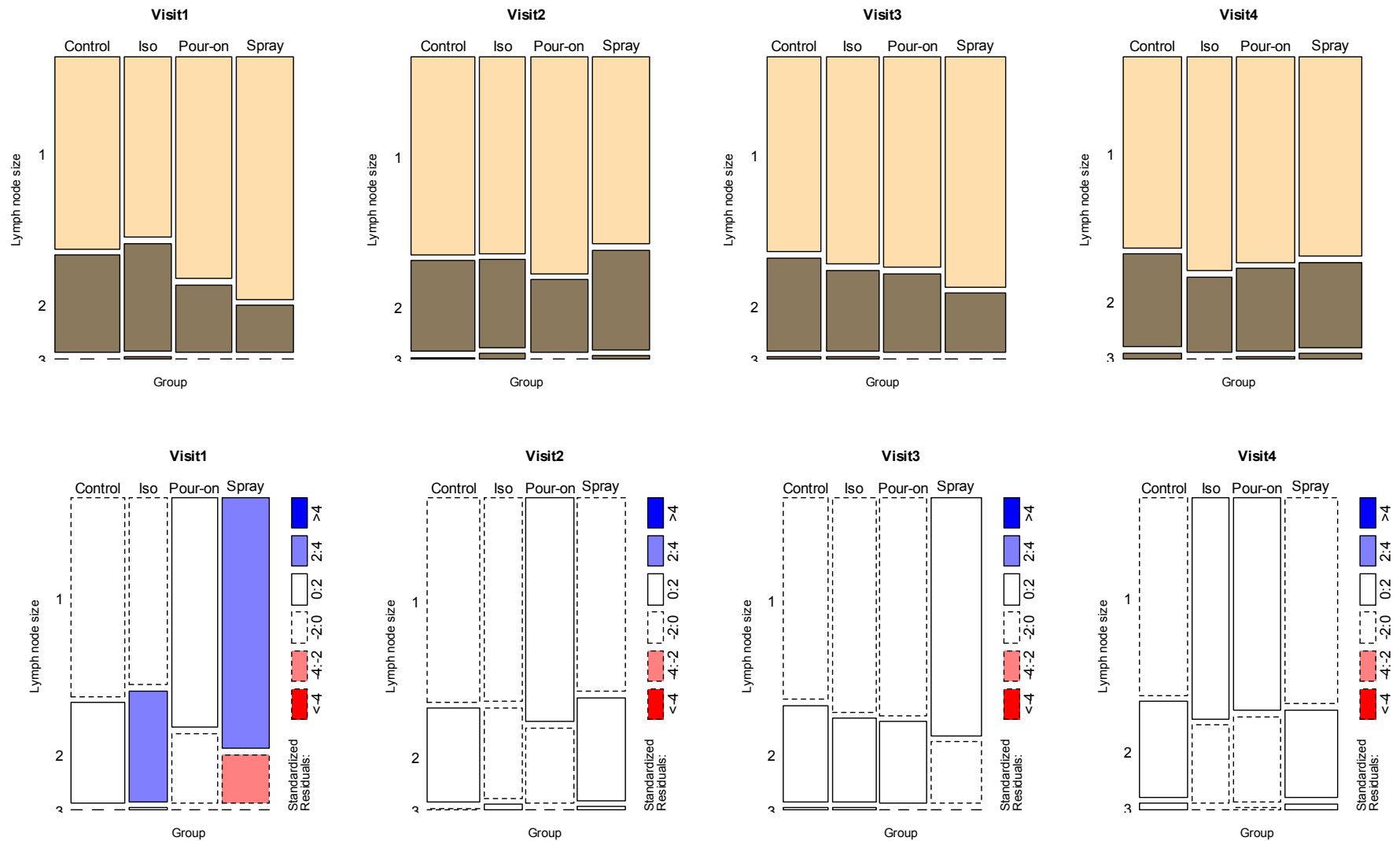
Tick species	Observed cumulative tick burden (Obs)	Theoretical cumulative tick burden (Th)	Percentage Obs/Th	Percentage reduction 1-(Obs/Th)
<i>Rhipicephalus spp.</i>	989.5	1886.5	52.40%	47.60%
<i>Boophilus spp.</i>	1.5	75	1.40%	98.60%
<i>Amblyomma spp.</i>	75.3	430	17.51%	82.49%
Total tick burden	1066.3	2391.5	44.59%	55.41%

**Table 6-2: Theoretical reduction in tick burden as a result of a 28 day spray interval.**

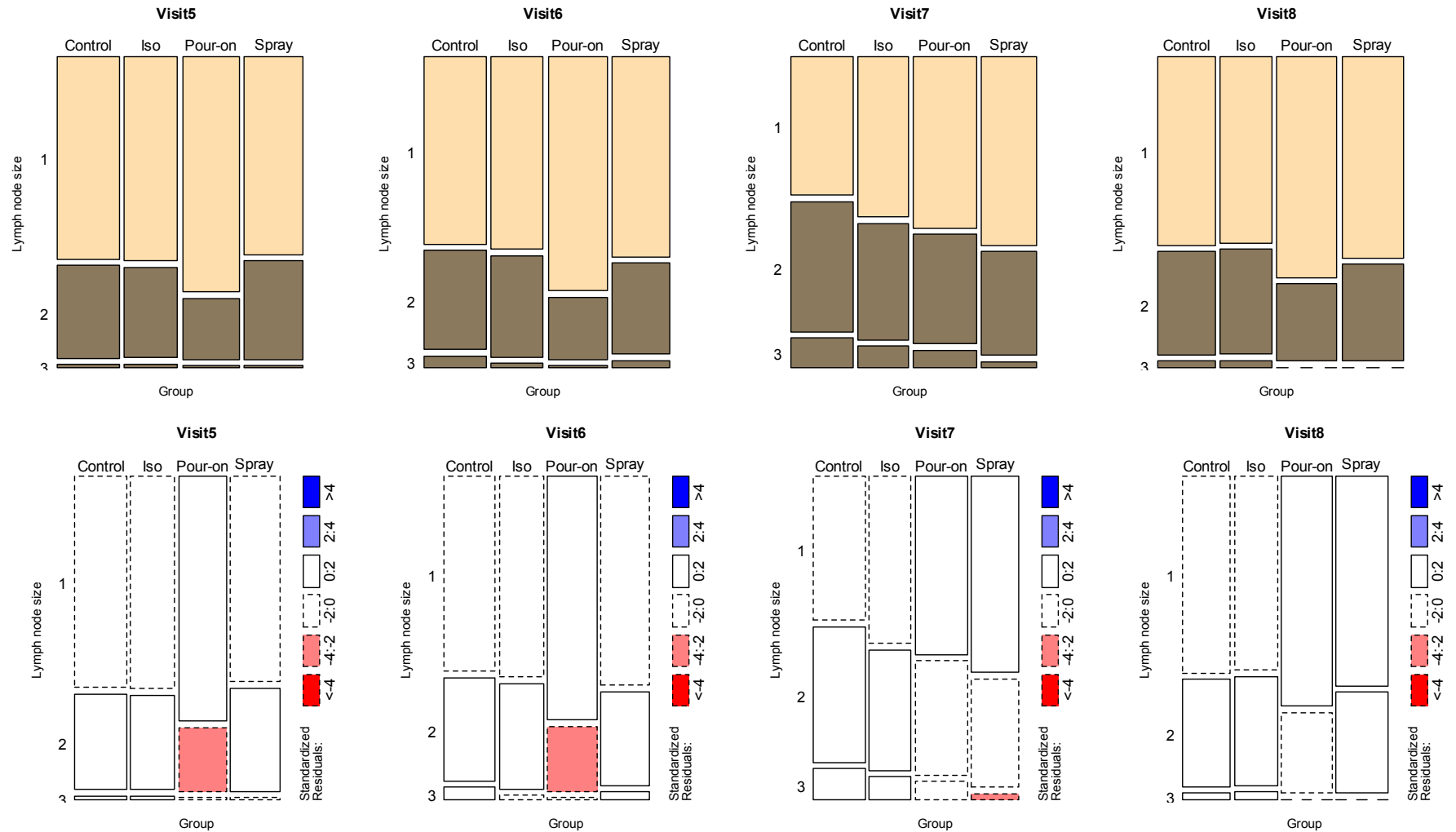
These predications rely on assumptions made about the tick burden had no intervention occurred, but go some way to quantify the effect of the restricted spray over the administered time interval. Given the small numbers involved, predictions about *Boophilus* species should probably be treated with caution. In general, however, spraying at a 28 interval reduces the burden of *Rhipicephalus* ticks by almost half and the burden from the other two species by over 80%. In terms of the impact on endemic stability of tick borne diseases, the restricted application protocol satisfies the criteria of minimising but not removing exposure to ticks (Coleman *et al.*, 2001). Additionally, such a protocol could be beneficial in terms of slowing the development of resistance by maintaining a larger population of ticks with a broader range of susceptibilities to the insecticide (Dolan, 1999). The effects these treatments have had on the pathogens the ticks transmit is also of interest, and is discussed below.

### 6.10 Impact of intervention on tick-borne diseases

Given the endemic nature of tick-borne diseases in SE Uganda (Perry *et al.*, 1991), the short duration of the study and the diagnostic technique employed, it is difficult to accurately assess the impact of the treatments on tick-borne diseases. Identification of parasites from thin film blood smears may merely indicate that the animal is a carrier rather than diseased (Norval *et al.*, 1992). Nonetheless, the microscopic and clinical indicators of infection are presented in the following section, but should be interpreted with this caveat in mind.



**Figure 6.10:- Mosaic plot showing lymph node sizes for visits 1-4. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Lymph node size coding: 1= normal, 2= mild enlargement, 3= profound enlargement**



**Figure 6.11:- Mosaic plot showing lymph node sizes for visits 5-8. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups. Lymph node size coding: 1= normal, 2= mild enlargement, 3= profound enlargement**

### 6.11 Assessment of lymphadenopathy

Enlarged lymph nodes are an indication that an animal is mounting an immune response to infection. In the early stages of East Coast Fever, *T.parva* schizonts proliferate in the lymphocytes, often causing profound enlargement of lymph nodes (Norval *et al.*, 1992). This is also an indication of acute trypanosomiasis (OIE, 2006). Assessment of the degree of lymphadenopathy was a routine part of the clinical examination of each animal as described in chapter 2. Lymph nodes were scored and coded to a four-point scale; 0 indicated the lymph node wasn't palpable, with 1, 2 and 3 for mild, moderate and severe enlargements respectively. Although clinically subjective, this coding system proved to have good repeatability within and between examiners and a previous study considered it to be as reliable as assessment using callipers (Tosas-Auguet, 2006).

Mosaic plots were used to highlight differences between treatment groups. Figure 6.10 and Figure 6.11 show the variation in lymph node enlargement over time. Indications of differences between the groups at baseline were not apparent by Visit 3. Visit 5 and 6 show a significantly lower degree of lymphadenopathy ( $p < 0.05$ ) in the pour-on group compared with the other groups, although this significance is not apparent in the final two sampling visits. There appears to be no difference between spray on and control villages in terms of this assessment of lymphadenopathy.

### 6.12 Microscopic prevalence of tick-borne diseases

It is worth reiterating that, used in isolation, thin whole blood smears are a relatively insensitive technique for assessing endemic tick-borne diseases because of their inability to differentiate diseased and carrier animals (Garcia-Sanmartin *et al.*, 2006). Additionally, in the case of *T.parva*, piroplasmic parasitaemias can be intermittent or below detection thresholds (Norval *et al.*, 1992). Assuming, however, that these constraints remained constant throughout the study, the microscopy results do give some indication of the burden of tick-borne diseases, and this can be compared between intervention groups.

### 6.13 Modelling tick-borne diseases

Results from the microscopy were coded as a binary variable for presence or absence of infection. A generalized linear mixed effect model was fitted following the same procedure as described in chapter 3. The error structure allowed for different intercepts and timeslope of village and animal within village. Outputs are odds ratios and the resultant models were plotted.

### 6.14 *Anaplasma spp.* prevalences

Figure 6.12 shows a barplot of *Anaplasma* prevalences for each timepoint of the study. Whilst there is no clear trend, it appears as if the prevalence in control villages is increasing over time, whereas the other intervention groups appear to remain more constant. Investigation proceeded by fitting the following models to the data:

```
model1<-glmmPQL(Anaplasma~Group ,random=~Day|Village.Name/Tag.No,
family="binomial", dataset= Full data)
```

```
model2<-glmmPQL(Anaplasma~Group ,random=~Day|Village.Name/Tag.No,
family="binomial", dataset= Intervention period)
```

Model1 and model2 share the same structure but are fitted to different datasets. Model1 is fitted to the whole dataset, model2 only to values from the start of the interventions.

	Value	Std.Error	DF	t-value	p-value	OR	Upper 95% CI	Lower 95% CI
(Intercept)	-0.667	0.107	5096	-6.24				
Isometamidium	-0.408	0.154	933	-2.65	0.01	0.66	0.90	0.49
Pour-on	-0.281	0.150	9	-1.87	0.09	0.75	1.01	0.56
Spray	0.050	0.149	9	0.33	0.75	1.05	1.41	0.78

**Table 6-3:- Output of model1:- Full dataset**

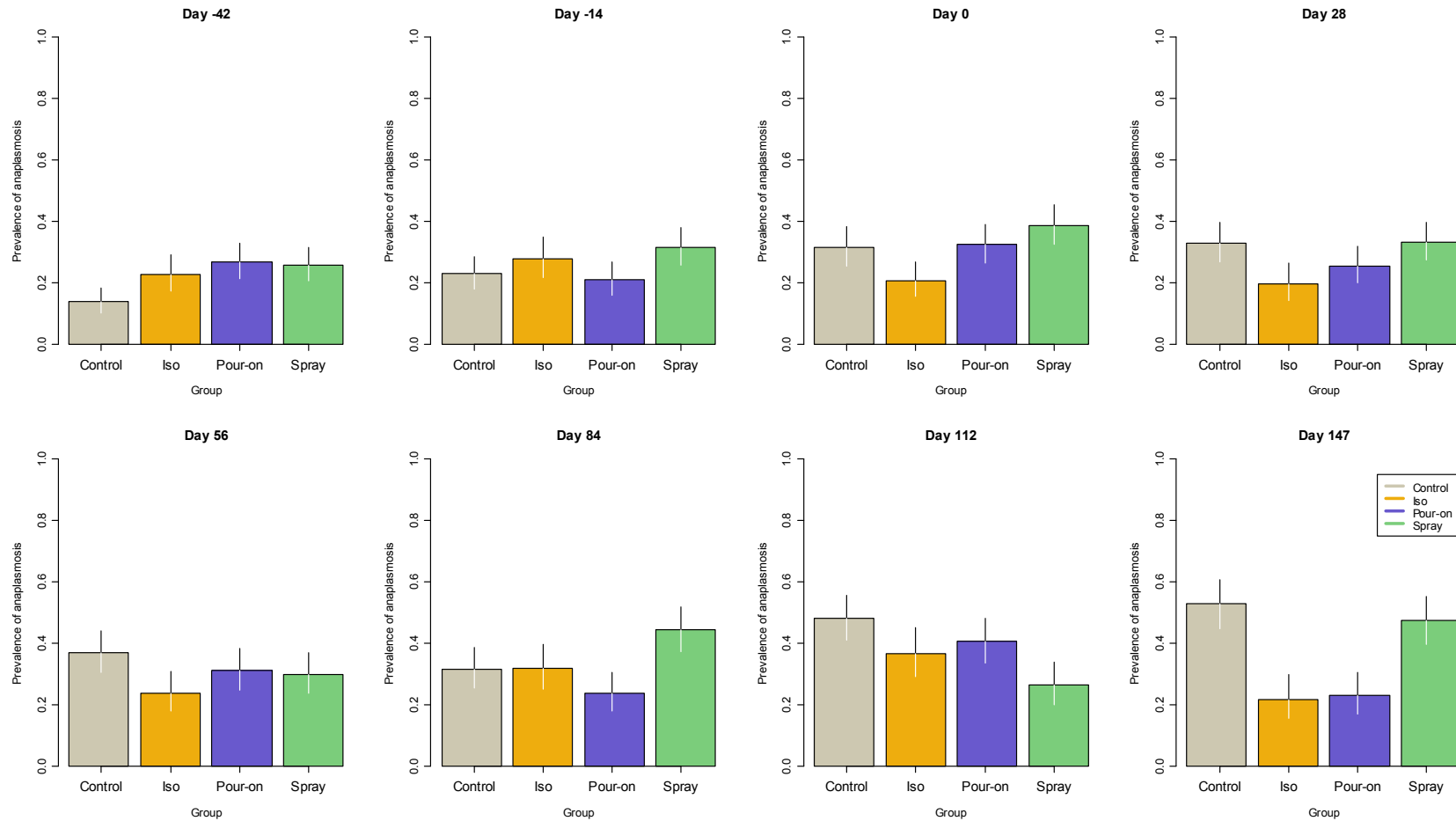
	Value	Std.Error	DF	t-value	p-value	OR	Upper 95% CI	Lower 95% CI
(Intercept)	-0.316	0.108	3330	-2.00				
Isometamidium	-0.772	0.157	898	-4.92	<0.001	0.46	0.63	0.34
Pour-on	-0.685	0.152	9	-4.51	<0.001	0.50	0.68	0.37
Spray	-0.283	0.150	9	-1.89	0.09	0.75	1.01	0.56

**Table 6-4:- Output from model2:- Intervention period only**

Table 6-3 shows the output from model1. There is a significant protective effect of isometamidium compared with the control ( $p=0.01$ ). Although there are indications that animals in the pour-on group have lower odds of being positive, this is not significant. Table 6-4 shows the output from model2. Considering only the time from the beginning of the interventions, there is a significant effect protective of both pour-on and isometamidium ( $p<0.001$ ).

Figure 6.13 and Figure 6.14 show the predicted values from model1 and model2 respectively. Figure 6.13 shows curves for all timepoints of the study. Whilst there is some evidence of variation between villages this is small compared with the overall trends. Comparing treatment options, it appears that for the control group the disease odds increase over the study, a trend which is reflected to a lesser extent in the spray group and resisted by the pour-on and isometamidium groups. Figure 6.14 shows the predicted curves for only the intervention timepoints. In contrast to the generalised increase in the odds of infection with time shown in Figure 6.13, Figure 6.14 clearly shows different trends between villages. Two villages in the pour-on group and one in the spray group show a decrease over time. Overall the effect of both the insecticide groups is fairly stationary, although the upward trend in the pour-on group is very pronounced.

## Prevalence of anaplasmosis by microscopy



**Figure 6.12:-Prevalence of anaplasmosis diagnosed by microscopy, by group, over time. Each panel represents a visit number reading from top left. Error bars represent exact binomial 95% confidence intervals**

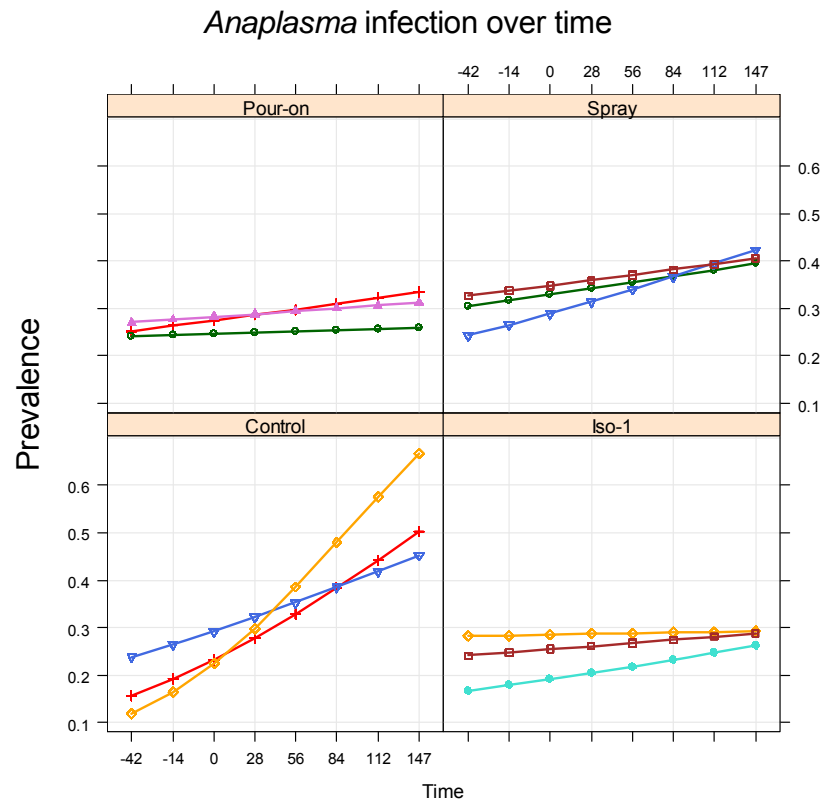


Figure 6.13: Predicted values from model1 showing change in *Anaplasma* infections over time by group and village

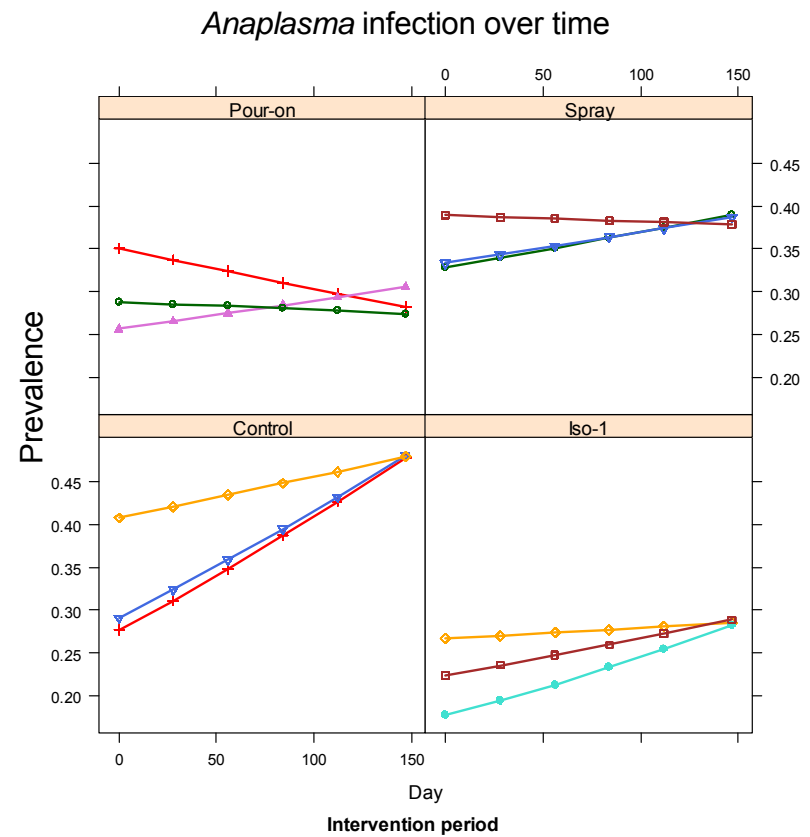


Figure 6.14: Predicted values from model2 showing change in *Anaplasma* infections over time by group and village from day 0.



### 6.15 *Theileria parva* prevalences

Figure 6.15 show bar plots of *Theileria parva* prevalences for each timepoint of the study. As with the prevalence of *Anaplasma spp*, there appears to be no obvious trend. There are some indications that isometamidium and pour-on groups have lower level of infection at the end of the study, although these differences are not pronounced.

The response to treatment was modelled as before, with two identical models run on full and intervention period only datasets.

```
Model3<-glmmPQL(TparvaYN~Intervention,random=~Day|Village.Name/Tag.No,
data=full dataset,family="binomial")
```

```
Model4<-glmmPQL(TparvaYN~Intervention,random=~Day|Village.Name/Tag.No,
data=Intervention period only, family="binomial")
```

	Value	Std.Error	DF	t-value	p-value	OR	Upper 95% CI	Lower 95% CI
(Intercept)	-0.479	0.163	5096	-2.95	0.003			
Isometamidium	-0.079	0.231	933	-0.34	0.732	0.92	1.45	0.59
Pour-on	-0.282	0.230	9	-1.22	0.252	0.75	1.18	0.48
Spray	-0.221	0.230	9	-0.96	0.361	0.80	1.26	0.51

**Table 6-5:- Output from model3:- Full dataset**

	Value	Std.Error	DF	t-value	p-value	OR	Upper 95% CI	Lower 95% CI
(Intercept)	-0.372	0.157	3330	-2.09	0.027			
Isometamidium	-0.421	0.224	898	-1.88	0.060	0.66	1.02	0.42
Pour-on	-0.558	0.222	9	-2.51	0.033	0.57	0.88	0.37
Spray	-0.524	0.222	9	-2.36	0.042	0.59	0.91	0.38

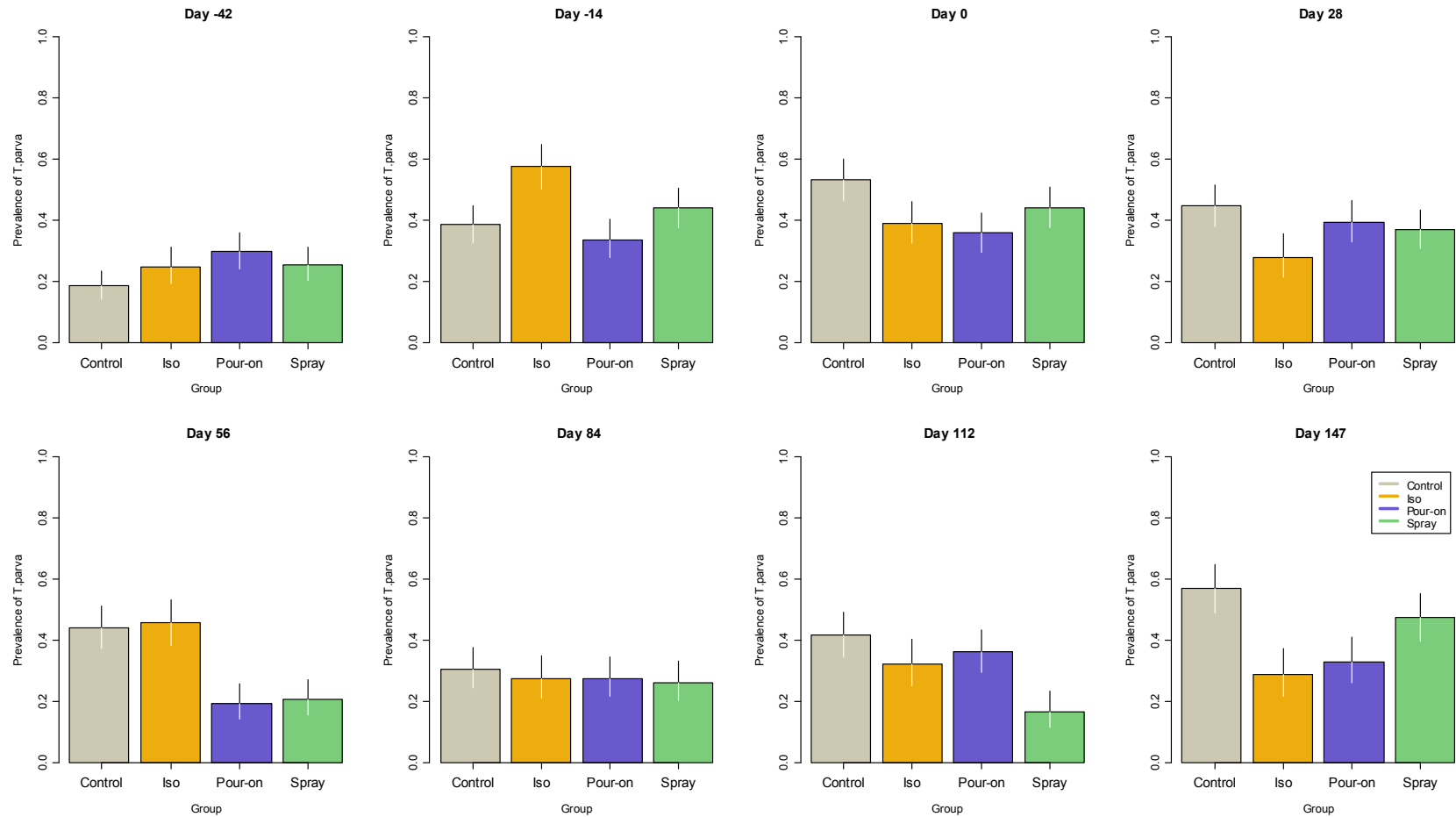
**Table 6-6:-Output from model4:- Intervention period only**

Table 6-5 shows the output from model3 fitted to the whole dataset. There is no significant difference between the groups in terms of prevalence of *Theileria* pathogens ( $p>0.05$ ).

Table 6-6 shows the output from model4 fitted to the intervention period only. There is a significant difference between the pour-on and spray groups and the controls ( $p<0.05$ ). There is no significant difference between the isometamidium treated group and the control.

Figure 6.16 and Figure 6.17 show the predicted values from model3 and model4 respectively. Figure 6.16 shows the output curves for all timepoints in the study. The response varies between groups and between villages within groups. There is not a general trend and therefore at this level there is no significant difference. Figure 6.17 shows the output for only the intervention period, and again there is large variation between villages, especially within the control and isometamidium groups. The spray and pour-on groups are slightly more consistent in response, and show little change over time.

## Prevalence of *Theileria parva* by microscopy



**Figure 6.15:-Prevalence of *T.parva* as diagnosed by microscopy, by group, over time. Each panel represents a visit number reading from top left. Error bars represent exact binomial 95% confidence intervals.**

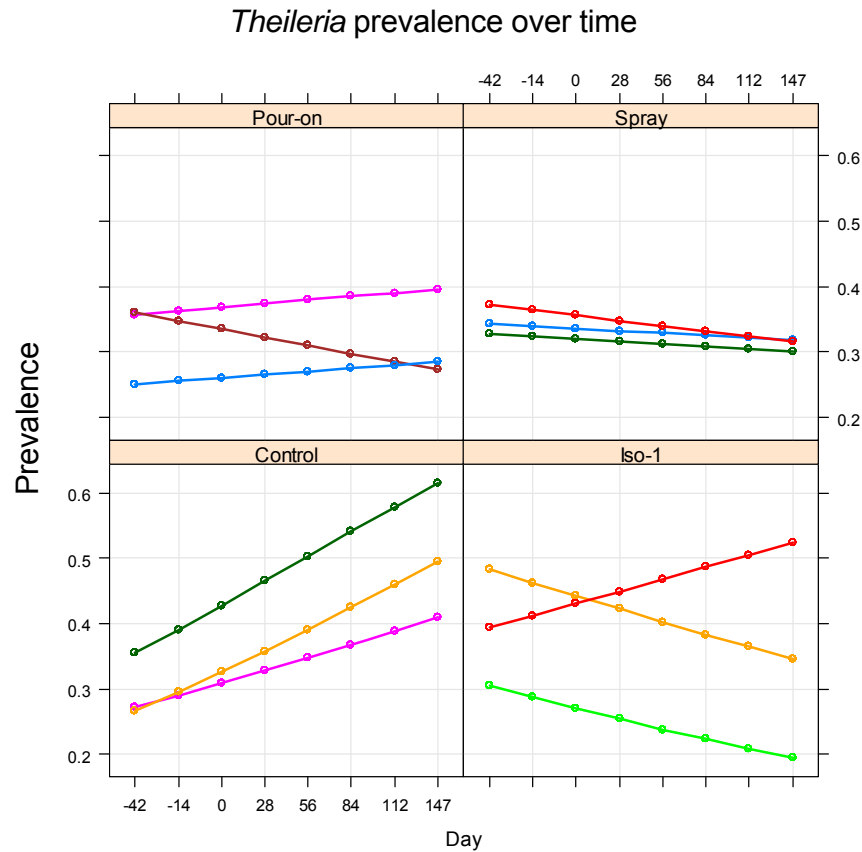


Figure 6.16: Predicted values from model3 showing change in *Theileria* infections over time by group and village

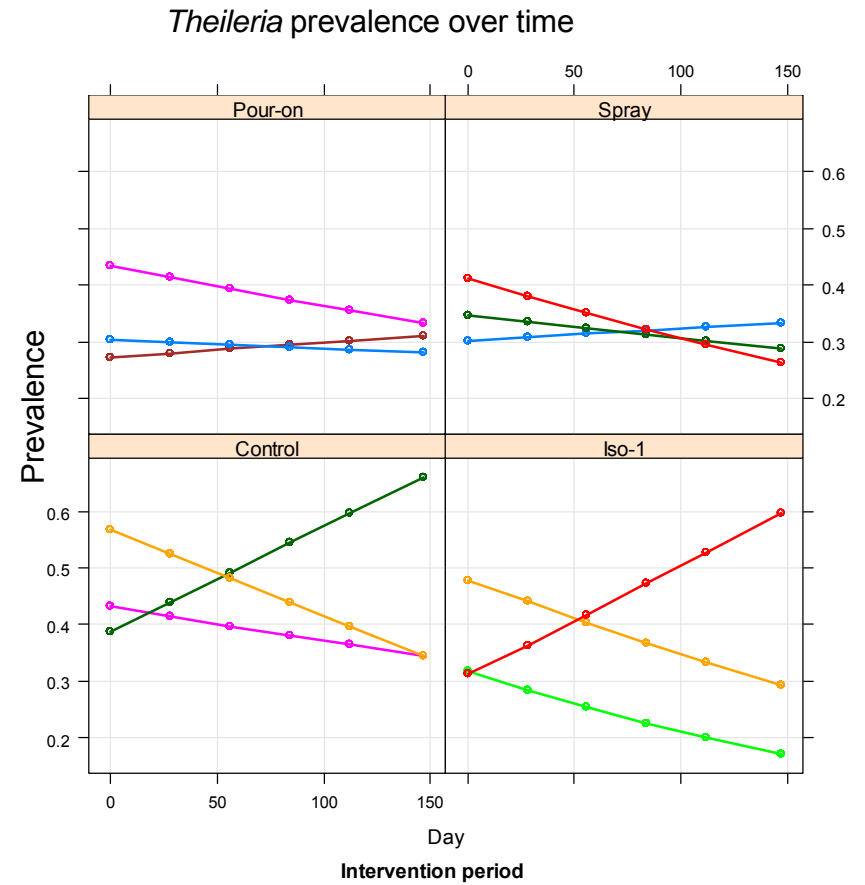


Figure 6.17: Predicted values from model4 showing change in *Theileria* infections over time by group and village from day 0

## 6.16 Weight change

One of the most insidious impacts of chronic trypanosomiasis is a loss of weight and condition, and a reduction in productivity (Agyemang *et al.*, 1991; Kristjanson *et al.*, 1999; Taylor & Authié, 2004). Weight, and specifically weight change, are also visible signs that are readily apparent to the livestock keeper. In a study examining the opinions of cattle owners, run in the same geographical area to this study, weight loss was ranked as the third most common sign of trypanosomiasis after inappetence and poor coat (Machila *et al.*, 2003).

### 6.16.1 Weight change over the study

For the purpose of the analysis of weight during the study, the dataset has been restricted to animals that were fully grown at the start of the study, i.e. had two permanent incisors and were therefore in age category C (see Table 2-3). This was done to minimise the effect of natural, age-related growth on any possible change in weight due to pathology. Weight was only recorded for all animals at the beginning and end of the study, so the measure of change includes the diminazene treatments in addition to the intervention periods. Weight difference was calculated as

$$\text{Weight difference} = \text{Weight, kg at Visit 8 (Day 147)} / \text{Weight, kg at Baseline, (Day -42)}$$

Figure 6.19 shows the absolute weight of animals at the baseline and Figure 6.19 the difference in weight between the beginning and end of the study.

Chapter 6:- Impact of interventions on clinical parameters

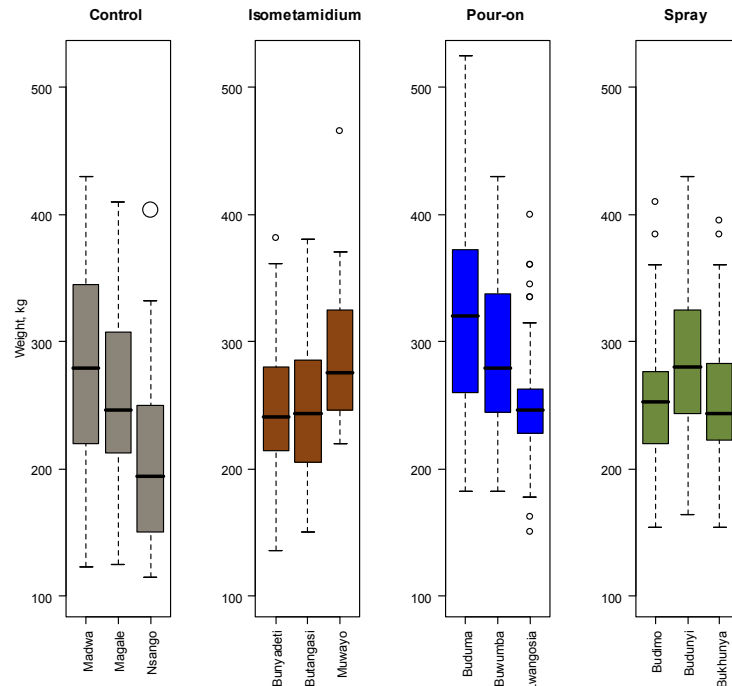


Figure 6.18:-Weights of adult cattle at baseline, by village

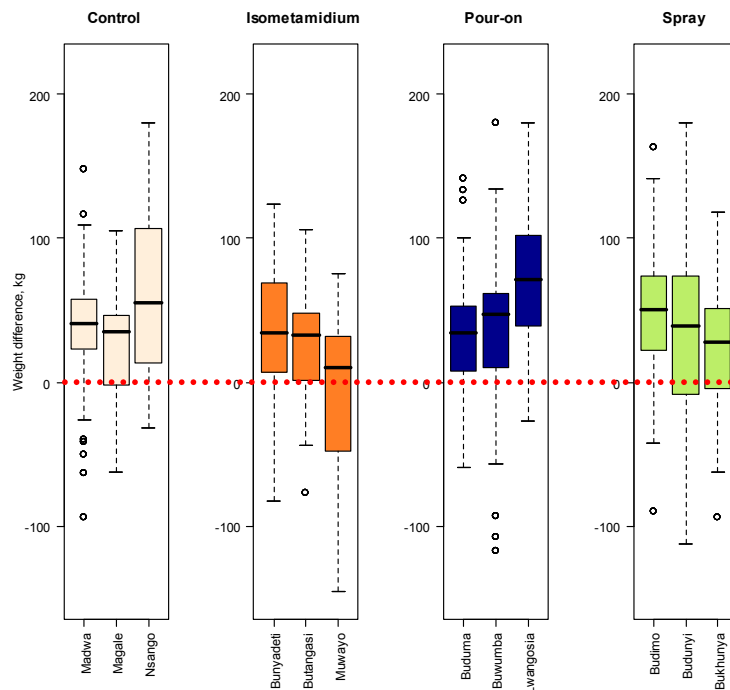


Figure 6.19:- Weight difference in cattle during study, by village. Red dotted line indicates zero line, indicating no change in weight over study.

### 6.16.2 Modelling weight over time

Weight was modelled using a linear mixed effect model with intervention group as a fixed effect and random effects as for earlier models. The outcome variable, weight, was fitted as a continuous variable so the model was fitted using the lme directive

```
Model5<-lme(fixed=Weight~Group, random = ~ Day|Village.Name/Tag.No,
na.action=na.omit, data = OB, subset=Age.42=='C')
```

	Value	Std.Error	DF	t-value	p-value
(Intercept)	281.4935	8.571825	1145	32.83939	0
Isometamidium	-6.80918	10.03759	649	-0.67837	0.4978
Pour-on	38.98973	13.4894	9	2.8904	0.0179
Spray	9.91383	13.2923	9	0.74583	0.4748

**Table 6-7: Output of model5**

Table 6-7 shows the output for model5. There is a significant difference in the mean weight of the pour-on group, although, referring to Figure 6.18, this is probably attributable to a difference in the baseline values. Consequently, an assessment of the rate of weight change is required, involving the interaction of weight with time:

```
Model6<-lme(fixed=Weight~Group*Day, random = ~ Day|Village.Name/Tag.No,
na.action=na.omit, data = OB, subset=Age.42=='C')
```

Chapter 6:- Impact of interventions on clinical parameters

	Value	Std.Error	DF	t-value	p-value
(Intercept)	246.45	13.61	1104	18.11	<0.001
Isometamidium	16.87	14.48	587	1.17	0.24
Pour-on	33.73	21.80	9	1.55	0.16
Spray	15.68	21.64	9	0.72	0.49
Day	5.25	1.60	1104	3.29	<0.001
Isometamidium:Day	-3.29	1.83	1104	-1.79	0.07
Pour-on:Day	1.13	2.49	1104	0.45	0.65
Spray:Day	-0.57	2.47	1104	-0.23	0.82

**Table 6-8: Output of model6 showing the change in weight over time**



Table 6-8 shows the output from model6 showing the predicted values of weight change over time. Figure 6.21 is a diagnostic plot of the model residuals, which are normal, suggesting the model is adequately specified. Although there is a significant positive effect of time, i.e. animals increase in weight throughout the study, there is no significant difference in this rate between the groups. Although not significant, Figure 6.20 suggests an improvement for insecticide treated groups, and a decrease in the isometamidium treated groups.

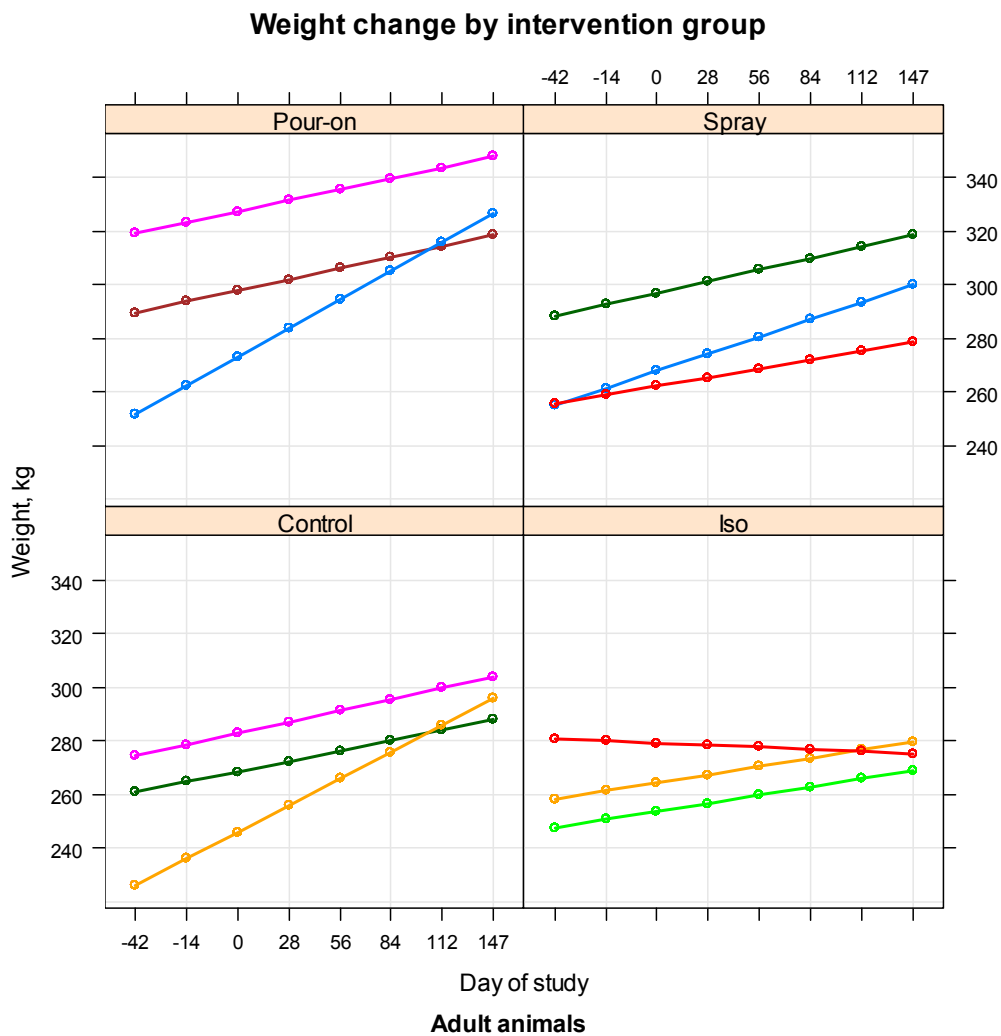
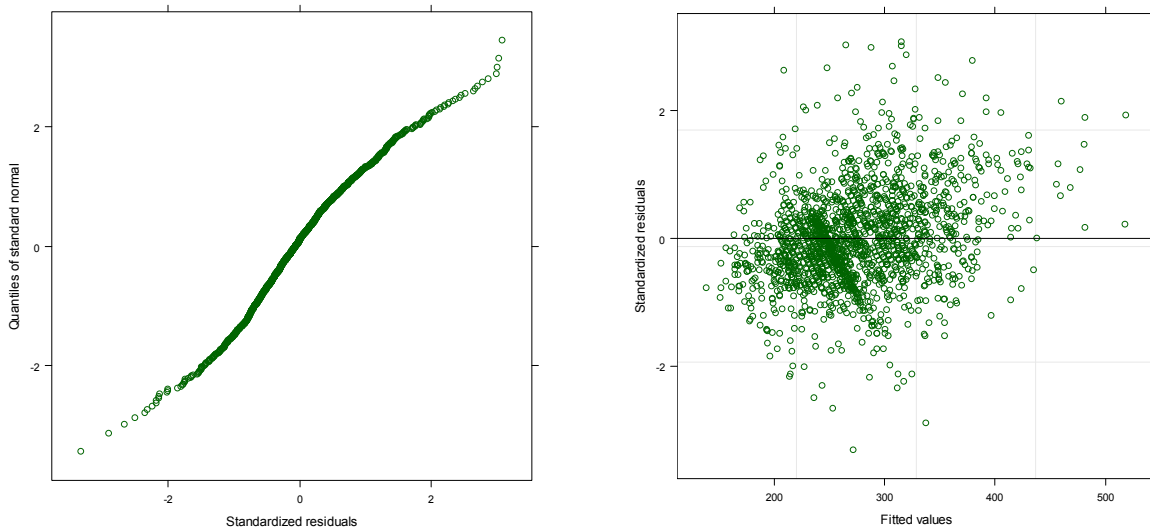


Figure 6.20:- Plot of model6 showing change in weight over time, arranged by group and villages



**Figure 6.21: Plot of residuals of model5**

### 6.16.3 Change in weight over study

Analysing weight change gives a slightly different result. Here each animal’s weight between the beginning and end of the study is modelled with respect to group.

Model7<-lme(fixed=Weightdiff~Group, random = ~ 1|Village.Name,  
na.action=na.omit, subset= Age.42=='C')

	Value	Std.Error	DF	t-value	p-value
(Intercept)	40.521	8.219	561	4.93	0.000
Isometamidium	-18.774	9.947	561	-1.89	0.060
Pour-on	5.852	12.809	9	0.46	0.659
Spray	-5.177	12.797	9	-0.40	0.695

**Table 6-9:-Weight change during study**

Table 6-9 shows the outcome of model7. The pour-on shows the greatest mean increase of 46.4 kg (40.5+5.9), 5kg more than the control. The spray performs 5kg worse than the control (not significant,  $p>0.05$ ), and the isometamidium groups gain 18.8kg less weight than the control, although this just fails to be significant ( $p=0.06$ ).

### 6.17 Condition score

As described in section 3.2.4, assessment of an animal's condition score is a well established indication of production performance. It is a visual assessment of the amount of fat and the musculature covering the bones of the animal, and can be assessed independently of weight, hydration, gut fill, or pregnancy status (Bartholomew *et al.*, 1994; Moran, 2005). Condition scoring was carried out as described in chapter 2 in accordance with (Nicholson & Butterworth, 1986).

Condition scores are integers from 1 to 9; animals in this study all scored between 2 and 8. Analysis can proceed with condition score as either an ordinal factor, or as an approximation to a continuous distribution. Although valid conclusions could be made from either method, it was decided to show both analyses here for comparison. Mosaic plots have been fitted to condition scores as discrete ordinal variables and a mixed effect model is fitted to values as a continuous variable.

#### 6.17.1 Condition score mosaic plots

Figure 6.22 shows a mosaic plot of condition scores for visits 1-4. At the beginning of the interventions, there are significantly higher condition animals in the isometamidium group compared with the others. However, by the subsequent visit the pour-on group shows a significantly higher proportion of high condition animals. Figure 6.23 shows a mosaic plot of

condition scores for visits 5-8. Overall, there are no obvious significant differences that maintain over time, although there are indications that the pour-on group gives higher and the control group lower condition scores compared with the other groups (dotted, but not shaded cells hence significance  $p>0.05$ ). Subtleties in any patterns of temporal effects may be made evident by modelling the response of condition score as a continuous variable.

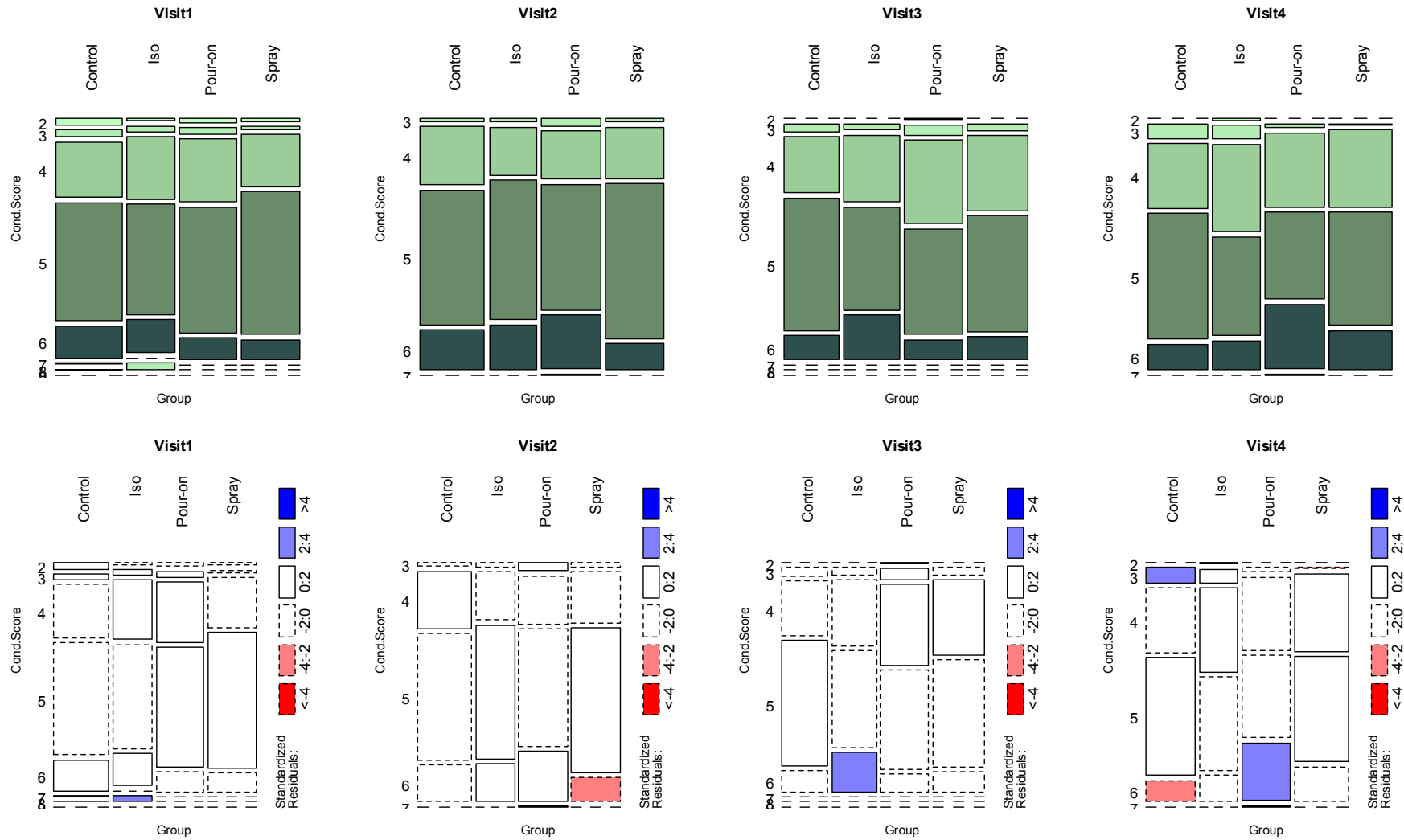


Figure 6.22: Mosaic plot showing condition scores for visits 1-4. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups

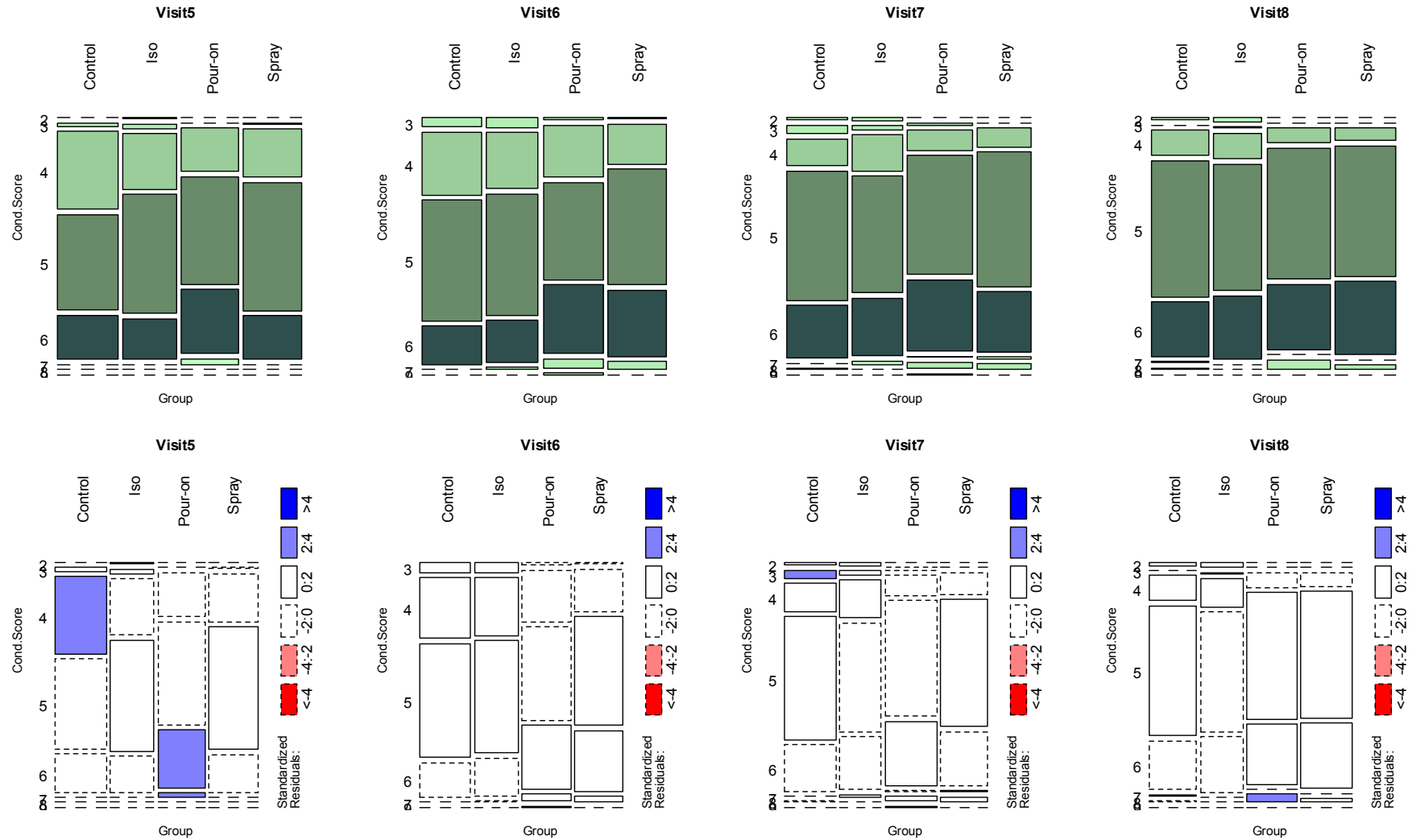


Figure 6.23:- Mosaic plot showing condition scores for visits 5-8. Upper row shows proportions of each category, lower row highlights significant differences among treatment groups

### 6.17.2 Condition score as a continuous variable

The initial model was fitted to condition score. Data was fitted only to the intervention period:-

```
Model8<-lme(fixed=Cond.Score.Number~Group, random = ~
Day|Village.Name/Tag.No, data = Intervention period))
```

Table 6-10 shows the output from model8. There is no significant difference among the mean condition score of the different intervention groups.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	4.750	0.071	3330	66.11	0
Isometamidium	-0.068	0.090	899	-0.75	0.451
Pour-on	0.013	0.110	9	0.12	0.903
Spray	-0.005	0.109	9	-0.04	0.963

**Table 6-10:Output from model8 showing mean condition score between treatment groups.**

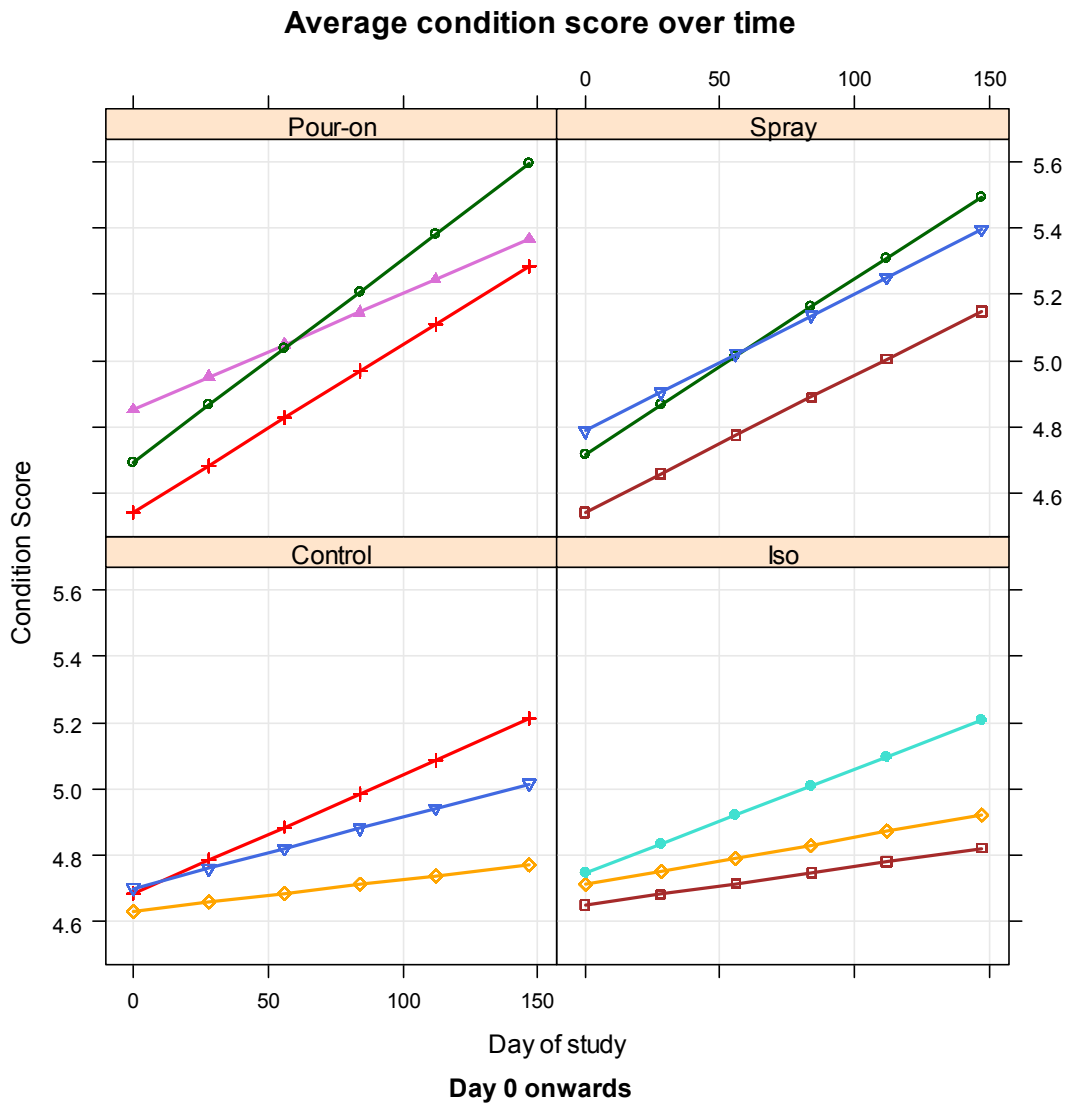
```
Model9<-lme(fixed=Cond.Score.Number~Group*Day, random = ~
Day|Village.Name/Tag.No, na.action=na.omit, data = Intervention period))
```

Considering time as a fixed effect, the rate of change of condition score can be assessed, giving different slopes for change in condition score.

	Value	Std.Error	DF	t-value	p-value
(Intercept)	4.700	0.073	3326	64.279	<0.001
Isometamidium	-0.033	0.096	899	-0.346	0.729
Pour-on	-0.010	0.110	9	-0.087	0.932
Spray	-0.023	0.110	9	-0.212	0.837
Day	0.030	0.001	3326	4.063	<0.001
Isometamidium:Day	0.001	0.001	3326	-0.808	0.420
Pour-on:Day	0.003	0.001	3326	2.805	0.005
Spray:Day	0.002	0.001	3326	2.385	0.017

**Table 6-11:-Output from model9 showing change in condition score between treatment groups over time.**

Table 6-11 shows the output from model9. The effect of time is significant, with all animals showing an improvement in condition score over time. However, the rate of change is significantly steeper for the pour-on ( $p>0.01$ ) and the spray village ( $p=0.02$ ) villages than the control villages. Figure 6.24 shows the predicted values for model9, and shows the difference in slopes between the villages within each intervention group.



**Figure 6.24:- Output of model showing the change in condition score by group and village**



### 6.17.3 Quantification of thin animals in each intervention group

A final way of assessing the difference between the intervention groups may be the proportion of thin animals present in each group. A thin animal was coded as having a condition score of 3 or less, so each animal received a binary coding of their status of thin or not thin at each visit. This threshold was chosen as it most closely corresponded to the classifications of the livestock keepers (personal observation).

Figure 6.26 shows the prevalence of thin animals over the study. Overall, the proportion of thin animals decreases throughout the study, although overlapping 95% binomial confidence intervals indicate non-significant differences. There does appear to be a separation between insecticide and non-insecticide treated groups evident throughout the intervention period.

A generalised linear mixed effect model with binomial errors was fitted as follows:

```
Model10<-glmmPQL(Thin~Intervention*Day , random=~Day|Village.Name/Tag.No,
data=OB, family="binomial")
```

	Value	Std.Error	DF	t-value	p-value	OR	Upper 95% CI	Lower 95% CI
(Intercept)	-0.729	0.193	5091	-3.78	0.000			
isometamidium	0.010	0.280	933	0.03	0.973	1.01	1.75	0.58
Pour-on	0.452	0.273	9	1.65	0.133	1.57	2.69	0.92
Spray	0.187	0.273	9	0.69	0.510	1.21	2.06	0.71
Day	-0.094	0.041	5091	-2.27	0.023	0.91	0.99	0.84
Isometamidium:Day	0.002	0.059	5091	0.04	0.967	1.00	1.13	0.89
Pour-on:Day	-0.185	0.059	5091	-3.13	0.002	0.83	0.93	0.74
Spray:Day	-0.154	0.059	5091	-2.59	0.010	0.86	0.96	0.76

**Table 6-12:-Output of model10 showing the proportion of thin animals (condition score  $\leq 3$ ) throughout the study.**

Table 6-12 shows the output of model10. There is no significant difference in the proportion of thin animals among the intervention groups, although there is a significant decrease in the number of thin animals in all groups over time ( $p=0.02$ ) Both pour-on and spray groups show a significant reduction in the number of thin animal over time ( $p<0.01$ ) compared with the control. Figure 6.25 shows the predicted values of model10. There is a reduction of the proportion of thin animals over time for all villages except one of the isometamidium villages which shows a small increase. The reduction in the number of thin animals in the spray and pour-on villages can be seen to be much more pronounced than in the non-insecticide treated villages.

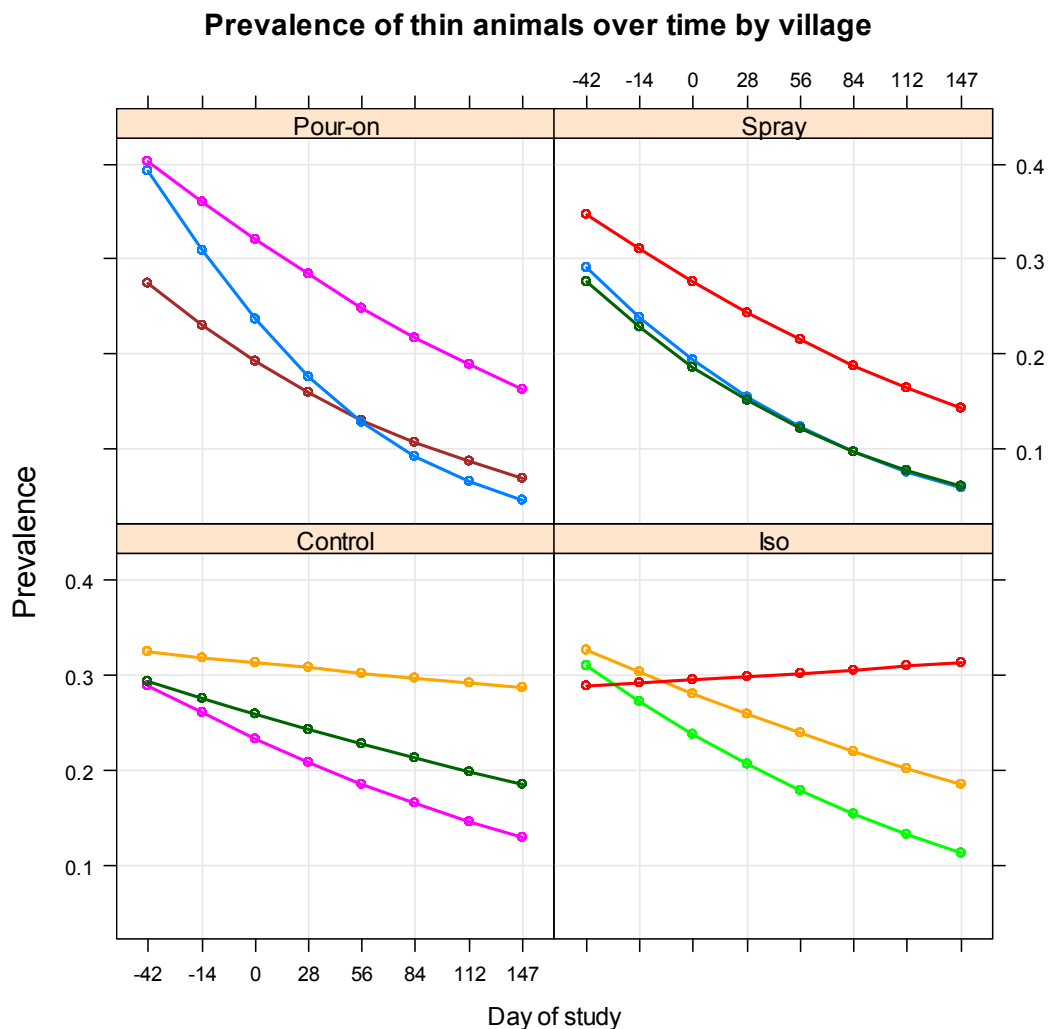
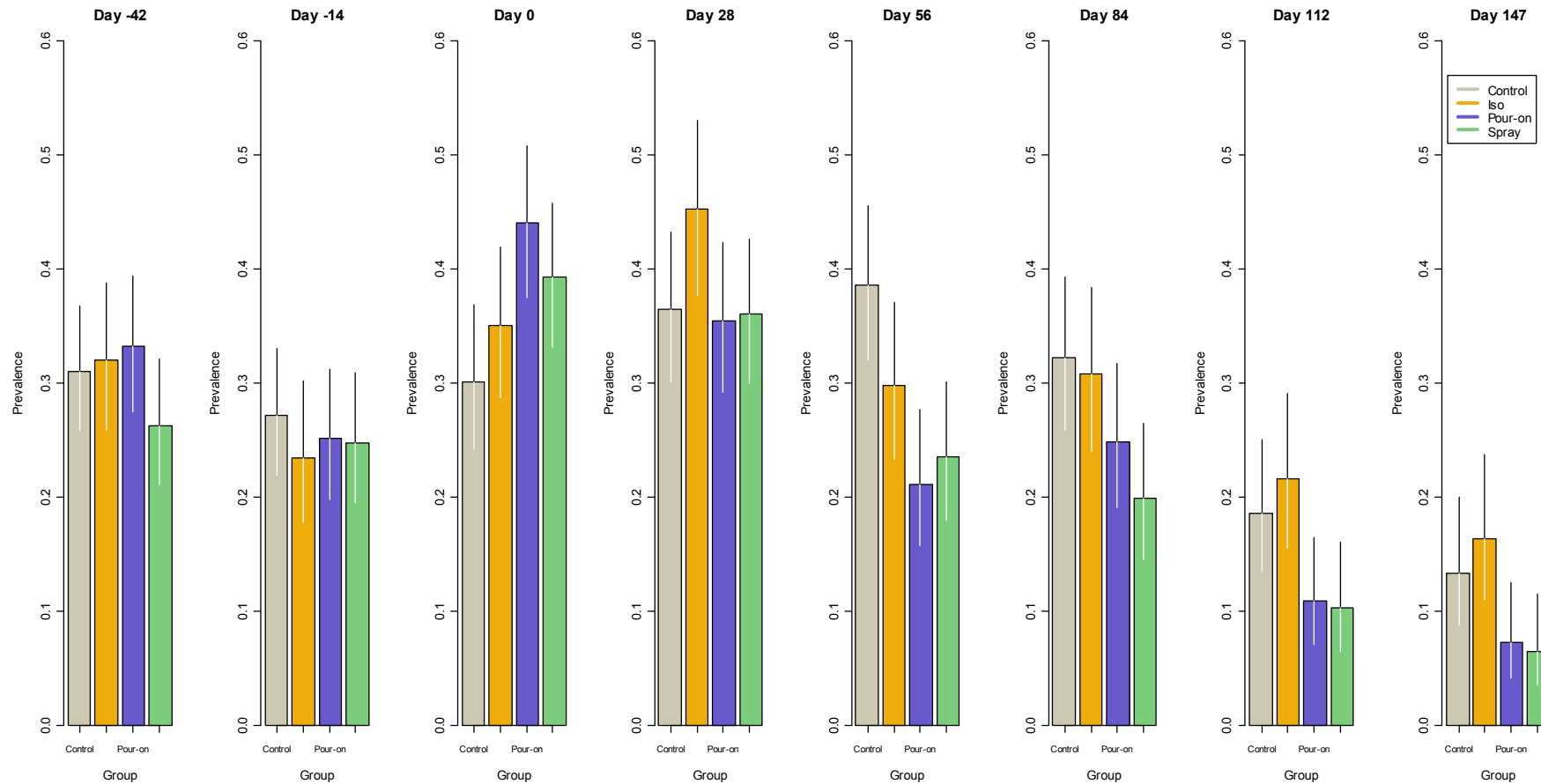


Figure 6.25: Fitted values for model10 showing the change in the number of thin animals during the study.

## Prevalence of thin animals throughout the study



**Figure 6.26: Proportion of animals deemed to be thin (condition score  $\leq 3$ ) during study. Error bars show exact 95% confidence intervals.**

## 6.18 Summary and discussion

This chapter has concentrated on two ways of assessing the impact of the interventions; firstly the impact of the interventions on ticks and tick-borne diseases and, secondly, the effect on some of the general indicators of animal health that can be assessed in the field as part of a clinical examination. Analysis of the ordinal data was made by a log-linear model fitted to a two-way contingency table, hence essentially a chi-squared test, and displayed using mosaic plots that graphically indicate cells that are statistically different from others in the same category. Assessment of binary or continuous data was made using linear mixed effect models or generalised linear mixed effect models to account for the grouped structure of the data.

### 6.18.1 Summary of changes in tick burden

Analysis of the tick burdens for each intervention group showed significantly lower tick burdens in the pour-on insecticide groups, and this was particularly prominent with the brown ear tick *Rhipicephalus appendiculatus*. In contrast, the restricted spray protocol was not consistently significantly different from either the control or isometamidium treated villages. The isometamidium group did not show any significant differences compared with the control for any of the tick species throughout the duration of the interventions. Any anecdotal effect of the drug causing ticks to detach is not evident.

### 6.18.2 Tick burden summary

The mosaic plots for individual tick species suggest that the tick challenge increases over the duration of the study. This is consistent with the seasonal rise in tick burden in this region, coinciding with a less arid climate (Randolph, 1994). In this context, the pour-on group tends to show a significantly lower tick burden than the other treatment groups. This is particularly apparent in the counts of *Rhipicephalus* ticks. The groups receiving the spray treatments show no reduction in tick burden compared with the control. The isometamidium group also shows no difference from the control groups.

The difference in application sites and dose probably account for the difference between the pour-on and spray groups. Pour-on insecticide formulations of synthetic pyrethroids are designed to rapidly disperse from the point of application through the sebum layer to cover the entire skin surface, and hair clipping analysis has shown this to take in the order of 48 hours (Allan *et al.*, 1998). However, studies found concentrations on the back to be six times higher than on the belly and 39 times higher than on the legs (Stendel *et al.*, 1992; Vale *et al.*, 1999), demonstrating that redistribution was not uniform. Although the same redistribution occurs with the spray formulations, the total amount of active ingredient applied in the restricted application protocol is an order of magnitude lower and is applied in an aqueous rather than a lipophilic vehicle. Redistribution to other sites is thus reduced and the local effect is probably more important.

A subsidiary study was also run to investigate the residual effect of insecticide. A population of 20 cattle were treated with the same spray protocol as applied in the longitudinal study and monitored every 72 hours to assess the residual effect of the insecticide at preventing tick attachment. Conditions of tick challenge were selected to emulate the tick challenge experienced by cattle in the longitudinal study as much as possible. The study showed that the insecticide spray reduced the tick burden of all species to zero. However, attached populations began to recover after 3 days and for *Rhipicephalus appendiculatus* were back to pre-treatment levels 30 days later. *Amblyomma variegatum* and *Boophilus decoloratus* did not recover to pre-treatment levels by the time the study was ended 35 days after spraying. Calculation of the cumulative tick attachment days during a 28 day interval was compared with a theoretical burden had no intervention occurred, and showed the restricted application protocol reduced the burden of *Rhipicephalus. spp* by 48%, *Boophilus* by 99% and *Amblyomma* by 82%. Overall, there was a 55% reduction in tick burden using the restricted application method.

### 6.18.3 Summary of *Anaplasma spp.* prevalences

It appears that there is a significant difference between the isometamidium villages and the control for both models, with isometamidium treated animals showing lower odds of a positive diagnosis for anaplasmosis by microscopy than control animals. This is unusual,

for isometamidium is not considered to have any direct effect on anaplasmosis. There are anecdotal reports of ticks dying or detaching from animals shortly after administration of isometamidium, hence a reduction in the transmission of the disease. An alternative interpretation however is an interaction between trypanosomiasis and anaplasmosis. Animals carrying a mixed infection of *T.congolense* and *Anaplasma* spp. were found to have significantly higher parasitaemias and clinical syndromes than expected if the pathological effect was simply additive (Tosas-Auguet, 2006) Consequently, prevention of the immunosuppressant effect of trypanosomiasis (Scott *et al.*, 1977) by isometamidium prophylaxis would allow for the anaplasmosis to be kept at a low level, both clinically and parasitologically. This hypothesis is similar to the effect observed in an outbreak of anaplasmosis in Switzerland. Forty-seven percent of cattle in a herd were found to be positive for the *Anaplasma marginale* by microscopy. However, it was concurrent infection with up to five other vector borne agents, detected in 90% of the clinically sick animals, which exacerbated the morbidity of the outbreak (Hofmann-Lehmann *et al.*, 2004). The observation that pour-on is significantly protective compared with the control could also be due also to the interaction hypothesis given above. By control of the vector, hence controlling incident trypanosome infections, the clinical impact of anaplasmosis is minimised. This result could also be attributable simply to the direct effect of the insecticide, reducing the tick populations and biting flies that vector *Anaplasma* spp.

### 6.18.4 Summary of *Theileria parva* prevalences

There are no major differences in *Theileria* prevalence between the groups, although the insecticide groups have a protective effect compared with the control significant at the 95% confidence limit. Given the issues with this diagnostic technique raised in Section 6.12 these results should be treated with a degree of caution. From a clinical perspective, given the ecology of the parasite and the time period of this study, it is probably unlikely the interventions would have had a direct noticeable effect. If it is assumed that a state of endemic stability to tick borne diseases exists in this area (Deem *et al.*, 1993; Rubaire-Akiiki *et al.*, 2004), a hypothesis reinforced by the high prevalence of the vector, *Rhipicephalus appendiculatus*, then diagnosis by microscopy is unlikely to be able to quantify any reduction in vectoral challenge due to the confounding effect of carrier

status. It is possible that a reduction in overall disease burden resulting, for example, from the impact of the interventions on trypanosome populations or TBD transmission, are having a significant effect. A more general assessment of cattle health may be of use, and this is covered in the following section

### 6.18.5 Summary of clinical signs

Comparison of the clinical signs indicated a lower level of lymphadenopathy in the pour-on group, although this was not consistent across time.

Analysis of the change in weight of the cattle in the study also did not reveal any significant differences between groups. On a group level, there was a general increase in weight over the study. This may be because the age of some animals was mis-categorised, and hence this change therefore represents natural age-related growth. Alternatively, the increased weight could be the result of management factors common to all sites, such as improved nutrition due to improved seasonal grazing. Analysis of the weight change of each animal indicated that the isometamidium group had approximately half the weight increase of the other groups, although this difference was not significant ( $p=0.06$ ).

Assessment of condition scores indicated pour-on and spray groups had a significantly higher rate of improvement over time than control villages ( $p>0.01$  and  $p>0.02$  for pour-on and spray groups respectively). There was no difference between the isometamidium and control villages in terms of condition score.

Overall, this indicates that there are significant positive animal health benefits that can be attributable to the monthly application of a pour-on formulation of deltamethrin. These changes are not as apparent for the restricted spray, at least not at a resolution of a 28 day sampling frame. Closer investigation of the residual activity of the restricted application protocol indicated the method had a significant effect on the tick populations, although the persistence was not sufficient to keep animals clear of ticks for the four week interval. Halving the spraying interval would probably be possible and still afford some tick challenge to maintain endemic stability.

**Chapter 7 :-** Summary and  
Discussion



### 7.1 The problem in context

Endemic diseases, especially tick and tsetse transmitted pathogens are severe constraints to animal productivity in Africa. Control of these diseases has in the past been the responsibility of government departments, involving specialist personnel funded and resourced centrally, often with a wide geographical sphere of operation. Following structural changes in the 1980's and 1990's, the capacity of government veterinary departments to deliver veterinary services declined (de Haan, 1991; Umali *et al.*, 1994), and donor policy shifted to a demand-driven cost recovery approach of veterinary service delivery (Leonard, 1998). The main policy document that catalysed the restructuring of health services, the Berg Report (World Bank, 1981), put faith in the economic principles of the market and had a minimalist view of the state. The philosophy that animal health was an economic service, therefore ought to be privatised, whereas human health was a welfare service that necessitated continual state support underpinned many of the changes made to health provision (Jeppsson & Okuonzi, 2000; Leonard, 2004). The recognition, however, that animal health was in many ways inexorably tied to human health, both in terms of a pathway out of poverty (Perry *et al.*, 2002; Kristjanson *et al.*, 2004), and in terms of zoonotic disease (Meslin, 1997; World Health Organization, 2005) took a further 20 years to influence policy. In the meantime, emphasis shifted from top-down approaches to market driven health provision (Chilonda & Van Huylenbroeck, 2001), putting the financial and logistical responsibility for healthcare into the hands of poor rural livestock keeper. Consequently factors such as the cost of treatment and proximity to vendors were found to be the two most important influences on the accessibility of animal healthcare in the new system (Heffernan & Misturelli, 2000). Furthermore, sustainability of programmes designed to improve animal health suffered due to the lack of long term commitment from the community (Barrett & Okali, 1998; Kamuanga *et al.*, 2001) and the perception that livestock health problems remained the responsibility of the state (Catley & Leyland, 2001; Kamuanga, 2003). In this economic paradigm, curative medications replaced prophylactic treatments, on the basic principle that not paying for a preventative

good entailed the risk of loss, whereas not paying for curative goods came with a certainty of loss (Grace, 2006).

The current state of a market driven veterinary service thus demands that successful animal health interventions are economically viable. Successful interventions are therefore those that are cheap, easy to acquire and rapidly effective at demonstrably providing a benefit to the individual that made the investment (Torr *et al.*, 2005). To control trypanosomiasis, the options are to treat the cattle with a trypanocide, or to control the tsetse vector. Although tsetse control options have been the understandable obsession of scientists and policy-makers for over a century, large scale tsetse eradication campaigns have only cleared 2% of tsetse-infested land since the 1970's (Budd, 1999) and only 1% of Africa's tsetse infested areas were under any vector control in the late 1990's (Allsopp, 1998).

In order for vector control options to be functional, they have to be deployed over a large area, often hundreds or thousands of square kilometres (Hargrove, 2003). In order for this degree of coverage to be attained in the current veterinary service paradigm, the chosen method has to provide an obvious benefit to each one of the individual livestock keepers who are being relied upon to invest in it. The only vector control option that is likely to offer sustainability in this context is the use of insecticide treated cattle (Hargrove *et al.*, 2000; Brightwell *et al.*, 2001) however current application methods still make insecticide more expensive than the use of trypanocidal drugs (Shaw, 2004). It has been estimated that 70% of cattle at risk from trypanosomiasis are treated with trypanocidal drugs (Allsopp, 1998), and the 35 million doses estimated to be used each year (Geerts *et al.*, 1997) indicate this is overwhelmingly the most common option of managing the disease. This is not sustainable in the long term however due to emerging resistance (Holmes *et al.*, 2004), livestock are still less productive than cattle raised in areas free of trypanosomiasis (Kristjanson *et al.*, 1999) and the proportion of household income spent on the drugs can be large; a study in the Central African Republic found that 80% of cash expenses went on trypanocides (Blanc *et al.* cited in Shaw, (2004)). Coupled with the tendency of livestock keepers to only invest treatment in their most valuable animals (Doran, 2000), there is little prospect that allowing the farmer to 'go it alone' (Eisler,

2003) is going to make any enduring difference to the burden trypanosomiasis in African livestock.. Additionally, due to the role of cattle as asymptomatic carriers of zoonotic *T.b.rhodesiense* parasites in east Africa, the benefits of mass treating cattle with trypanocides to remove the reservoirs of sleeping sickness (Fevre, 2002; Wendo, 2002) would be greatly augmented if the cattle could be kept free of reinfection. Consequently, there was a desire to investigate ways of making the use of insecticides cheaper and easier to use and thus more likely to be adopted.

### 7.2 What we did

This thesis has explored the outputs from a longitudinal study conducted in Uganda in 2003-2004 whereby existing trypanocidal and insecticidal treatment options were compared with a novel technique that reduced the amount of insecticide by 80% by only treating the areas of the animal shown to be preferential feeding sites for tsetse. Work done in Zimbabwe showed that *G.pallidipes* and *G.m.morsitans* fed mainly on the legs and belly of the cattle. By spraying insecticide only onto these areas, the amount of drug used could be reduced by 80%. If this conferred the same protection against tsetse then the technique could have three advantages. It would be cheaper, it could reduce the detrimental effect to seen in invertebrate dung fauna associated with high residues in the dung following ingestion of insecticide during allogrooming (Wardhaugh *et al.*, 1998; Vale & Grant, 2002), and it may provide less of a detrimental impact on the endemic stability to tick and tick borne diseases (Eisler *et al.*, 2003).

To briefly summarise the study design, 945 cattle were recruited from 12 villages in Busia and Bugiri administrative districts of south east Uganda. The entire study population were given two doses of a trypanocide diminazene aceturate at 42 and 14 days prior to beginning the interventions. Interventions were allocated to groups of three villages at random and consisted of one of the following four options:

1. A single trypanoprophylactic dose of isometamidium chloride at day 0;
2. A monthly (q.28 days) application of a commercially available pour-on formulation of deltamethrin;

3. A monthly (q.28 days) application of a spray formulation of deltamethrin, mixed to manufacturer's standard concentration but only applied to the front legs, ears and belly of the animal;
4. A control group, which received no further interventions.

The cattle were sampled over a period of 147 days after the interventions began on day 0. Sampling intervals were every 4 weeks apart from the last visit which had a 5 week interval due to staff availability. A variety of clinical and laboratory parameters were collected for each animal, with the aim of quantifying the impact of the interventions using a variety of indications of infection and disease.

### 7.3 What it means

Assessments of the efficacy of the interventions have been made on a number of levels. The output of an assessment is largely defined by the criteria used to quantify its success combined with the information available to validate those criteria. It was decided that it would be useful to look at the results of the study in terms of the levels of information available to different groups of people that are likely to be involved with this technology. The impacts of the different interventions were therefore assessed using techniques available to, and applicable for, the various stakeholders; namely external researchers/policy makers, local animal health workers and rural farmers. Although there is usually a decreasing gradient of resources between those three named groups, it is not a hierarchical system. For example, more information may be available to external researchers due to them being better resourced, however local survey teams are likely to have better knowledge about recent disease incidents and only the livestock keeper have experience of the husbandry and production history of their animals.

		Livestock keeper	Animal health worker	Local surveillance team	External research groups
History	Appetite	++	+	-	-
	Lactation status	++	+	-	-
	Calving interval	++	-	-	-
	Calf mortality	++	+	-	-
	Change in milk yield	++	-	-	-
	Interaction with herd	++	-	-	-
	Herd disease history	++	+	+	-
	Shade seeking behaviour	++	-	-	-
Field observations	Abnormal behaviour	++	+	+	-
	Loss of tail hair	++	-	-	-
	Lethargy or weakness	++	+	-	-
	Weight loss	++	+	-	-
	Diarrhoea/Constipation	++	++	-	-
	Coat condition	++	++	+	-
	Dehydration status	+	++	++	-
	Ectoparasite burden	++	++	++	-
	Mucous membrane colour	-	+	++	-
	Oedema	+	+	-	-
	Discharge	+	++	++	-
	Staring coat	+	++	++	-
	Lymphadenopathy	+	++	++	-
	Rectal temperature	-	++	++	-
	Condition Score	-	++	++	-
Haemoglobin	-	+	++	+	
Laboratory techniques	PCV	-	-	++	+
	Gross pathology (Post Mortem)	+	+	++	-
	Faecal egg analysis	-	-	++	-
	Dry thick and thin blood smears	-	-	++	+
	HCT Microscopy	-	-	++	+
	BCT Microscopy	-	-	++	+
Specialist techniques	Antigen ELISA	-	-	+	++
	Antibody ELISA/IFAT	-	-	+	++
	Mouse inoculation	-	-	-	++
	PCR	-	-	-	++
	Real-time PCR	-	-	-	++
	GIS Disease Risk map	-	-	-	++
	Epidemiological bulletins	-	-	+	++

**Table 7-1:- Observations relevant to different stakeholders. ‘-‘ :- Test unlikely to be used; ‘+’;- Test available but probably of restricted use. ‘++’ :- Test available and likely to be used**

Table 7-1 shows some of the observations that can be used to quantify disease and the stakeholders who are likely to access and interpret information on that level. The likely use of a variable is assessed on grounds of both knowledge and resource availability, a ‘-‘ indicates information at that level is unlikely to be available to that group, a ‘+’ indicates it is of limited availability and a ‘++’ indicates that information derived from that variable could form a significant part of the diagnosis. The purpose of the table is to highlight the differences in information that are available to different groups. For a treatment to be adopted it has to be seen as a success by all these groups.

### 7.4 Farmer level assessment

Analysis of the data from a clinical point of view, using visible signs that require no specialist equipment, is the most basic, but arguable the most important output of this study. In order for technologies to be adopted they not only have to work, they have to be seen to work, hence unless the end user, i.e. the livestock keeper, sees a difference in his or her animals as a result of an intervention, it is unlikely to succeed. In the case of the interventions trialled in this study, visually assessable criteria were tick burden, condition score and weight change.

#### 7.4.1 Tick burden reduction

In terms of tick attachment, the pour-on applications kept tick burdens at a significantly lower level for the four-week interval between applications. Spray formulations reduced the tick burden by 55%, but were not persistent enough to show any difference at four week intervals. These results were consistent with an investigation in the Gambia on the use of flumethrin insecticide applied only to the tick attachment areas. Compared with untreated controls, the restricted application gave a 61% reduction in tick burden compared with a 75% reduction for the whole body treatment, however used 14 times less insecticide and was 25 times cheaper (Mattioli *et al.*, 1999).

Researchers in West Africa have recently also looked at the use of restricted application techniques to control ticks and tsetse. Initially designed as a low-cost control method for the tick *Amblyomma variegatum*, it was discovered that just treating the feet of cows

conferred a significant beneficial effect on tick and tsetse burdens. Cattle walked through a 46x25 cm footbath, containing a normal concentration of synthetic pyrethroid insecticide, had a significantly reduced burden of *Amblyomma variegatum*. Previous work had found that this species of ticks initially attached to the interdigital areas and only moved to their preferential sites of attachment in the udder and belly when the animal lay down in the evening (Stachurski, 2006). By footbath-treating cattle every 3 days after returning from grazing, newly acquired tick burdens were prevented from attaching to their preferential sites (Stachurski & Lancelot, 2006). Although tick burdens were not removed entirely, they were reduced by over 80%. This technique used around 200ml of solution per animal treatment and was well received by farmers due to its ease of use. These results are also consistent with the results displayed in section 6.9 covering the re-attachment of ticks following restricted application of deltamethrin.

### 7.4.2 Healthier cows?

Assessment of production factors such as weight and condition score showed a general increase over the whole study for all groups, and a significant improvement in condition score for both insecticide treatments. Although this was more pronounced for the pour-on group, it was arguably a sufficient enough difference to be noticed by the farmers. This is supported from personal conversations held with the farmers receiving the spray application, who stated condition gain and tick control as the most noticeable outcomes of the study. It should also be noted however that when, at the close of the study farmers were gifted a choice of treatment, pour-on insecticide was the most requested item.

## 7.5 Animal health worker assessment

Chapter 5 looked at the impact of the interventions in terms of the effects potentially visible to local animal health workers. One of the diagnostic tools emerging as potentially cheap, robust and simple enough for field use are those able to quantify the anaemia status of the animal (Magona *et al.*, 2004b). Assessing the anaemia status of an animal provide an indication, not of infection status, but of how the individual is coping with any present infection. Of the endemic diseases afflicting cattle in East Africa, anaplasmosis, schistosomiasis, helminthiasis and, particularly, trypanosomiasis cause anaemia.

Consequently, an assessment of the effect of the interventions using this parameter was considered important as it provided a broad indication of health. Additionally, given the current paucity of laboratory services to most rural areas of Africa (Machila *et al.*, 2003), these tools are likely to be one of few resources that could be made available to the groups of people actually making treatment decisions (Magona *et al.*, 2003; Eisler *et al.*, 2007).

The most significantly noticeable positive effect of the interventions was attributed to the twin diminazene doses at the start of the study. After two doses of trypanocide the mean haemoglobin had increased by 0.7g/dl, with over 60% of cattle registering an improvement in haemoglobin after the first diminazene injection and 65% continuing to show an improvement after the second. During the intervention period there was a further significant increase in the rate of haemoglobin improvement of the animals treated with pour-on insecticide, however this was not seen with the group receiving the restricted spray protocol.

### 7.5.1 Effect of isometamidium

The isometamidium villages in contrast showed deterioration on haemoglobin both during the interventions and over the whole study. Explanations for this are open to speculation; however a possible hypothesis could be related to the toxic side effects of the drug. Isometamidium chloride is far from a benign drug, injection causes a sterile lesion which acts as a depot from which the drug diffuses over time (Hill & McFadzean, 1963; Dowler *et al.*, 1989). Side-effects to this lesion include infection of the depot site, often attributed to poor injection technique, lowered lactation rate, anorexia weight loss and decreased condition score (Mdachi, 1999). These effects has been attributed to the increased toxicity of the drug in areas of low trypanosome challenge (Mdachi, 1999); basically if there are insufficient circulating trypanosomes to actively take up the drug the side effects are more pronounced. This would fit with the evidence from the ITS-PCR analysis which showed trypanosome challenge, as interpreted by the rate of re-infection of the control groups, was relatively low and the evidence that cattle were clear of existing infections prior to the isometamidium treatment.



An alternative hypothesis involves the assumption that cattle in endemic areas become adapted to a particular strain of trypanosomes, which prevents establishment of further infections from different, possibly more pathogenic strains (Morrison *et al.*, 1982; Dwinger *et al.*, 1989; Sones *et al.*, 1989). Removal of this pathogen population allows for re-infection with a different strain to which the host could be less adapted, hence suffers greater pathology. If this is the case, then the policy of one-off block treatments of cattle (Wendo, 2002; FITCA, 2005) would perversely leave cattle more susceptible to trypanosomiasis in the long term.

### 7.5.2 Overall impression of anaemia status

From the point of view of a field clinician using a haemoglobinometer, the pour-on insecticide progressively makes animals less anaemic, the spray and control groups are generally equivocal, and the isometamidium treated animals slightly deteriorate. From personal observations in the field, animals did not respond well to the isometamidium, many farmers complained of a reduction in lactation, and swelling around the injection site to the extent that in one village several negotiations were required to maintain continued attendance in the study.

### 7.6 Epidemiological assessment

Samples collected from the cattle were screened using an ITS-PCR protocol to identify any trypanosomal DNA circulating in the animal's bloodstream at the point of sampling. PCR is a highly specific technique and is 2-3 times more sensitive than microscopy (Solano *et al.*, 1999; Picozzi *et al.*, 2002) however the results indicate an active infection but do not give a quantification of the level of parasitaemia. These results have been used to assess the effect of the treatments on the prevalence of pathogenic trypanosome infections in the bloodstream of study animals.

Chapter 4 analysed the ITS-PCR data using generalised linear mixed effect models to account for the grouping structure of the data. Initial baseline prevalences were 17.8% which was consistent with prevalences found by contemporaneous studies in the same

area (Tosas-Auguet, 2006). After two treatments with diminazene aceturate the prevalence had dropped to zero which was attributed to the effect of the diminazene.

### 7.6.1 Effect of diminazene aceturate

Several authors (Barrett & Fairlamb, 1999; Maser *et al.*, 2003) have commented on the potential for cross resistance to develop between diminazene aceturate and melarsoprol, a significant drug for the treatment of late stage *T.b rhodesiense* sleeping sickness, and thus questioned the advisability of block treatment of cattle (Barrett, 2001). This highlights a particular need for integrating control techniques in order to minimise reliance on any one methodology. Although the wisdom of widespread block treatment of cattle is questioned by some authors, on the basis that it may propagate drug resistance, in actuality the biggest risk of developing resistance comes from the widespread, deregulated use of trypanocidal drugs by livestock keepers who may not possess the knowledge or resources to correctly dose their animals (Welburn *et al.*, 2006). However, from the evidence of the PCR screening and, assuming the decrease in observed prevalences was attributed to the effect of the diminazene, there is no indication therefore that trypanosome resistance to diminazene aceturate is yet a problem in S.E. Uganda. This agrees with a similar study by (Magona *et al.*, 2004a)

### 7.6.2 Prophylactic period of isometamidium

There was no difference between the control and isometamidium groups at day 56 or day 147, however modelling the incident rate of infection suggested that the isometamidium conferred a prophylactic period of 3-4 weeks in the study villages. The reported duration of isometamidium prophylaxis ranges considerably in the literature from 36 weeks (Fairclough, 1963), 24 weeks (Eisler *et al.*, 1994), 16 weeks (Toro *et al.*, 1983), 8 weeks (Eisler *et al.*, 1997) or 3 weeks (Dolan *et al.*, 1992), dependent on the degree drug resistance exhibited by the challenging strain of trypanosomes. It should be emphasised that monitoring in these cases was in the main by phase contrast microscopy, thus the improved sensitivity of the PCR techniques do not make this result directly comparable. Nonetheless, the return to baseline levels of infection in the isometamidium group by day

147 suggests that use of the drug to reduce transmission would require retreatment at intervals of every 8-10 weeks.

### 7.6.3 Impact of insecticide protocols

#### 7.6.3.1 South-east Uganda study

Incident evidence of parasitaemias emerging in the cleared population during the course of the interventions were assumed to come from new infections and have been used to assess the degree of protection conferred by the respective treatments. This indicated a significant difference between the insecticide and non-insecticide treated groups which became more apparent over time. By the end of the study the prevalence of trypanosomiasis in the control and isometamidium groups had returned to baseline levels of approximately 15%. In contrast, the pour-on and restricted spray interventions kept below 4% for the whole duration of the study. There was no significant difference between the pour on or restricted spray groups indicating that they were both equally effective at reducing transmission of trypanosomiasis.

#### 7.6.3.2 West Africa study

Following on from the work described above on the use of footbaths to control ticks (Stachurski & Lancelot, 2006), the same technology was assessed as a control method for tsetse. A similar treatment interval of footbath dipping at 3 day intervals was found to give comparable knockdown rates as spraying the entire animal once a week, only using 75% less insecticide (Bouyer *et al.*, 2007) Additional work showed that 82% of the blood meals for *G. tachinoides* and 95% for *G. p. gambiensis* were from the legs of the animal. Investigation of the effect of treating 70% of a the herd (total n=96) with a restricted application of pyrethroid using a footbath showed a significant increase in the daily mortality rates of both marked and released reared flies and a closed population of wild flies, as inferred from a sharp decrease of their apparent density (Bouyer *et al.*, 2007). There was also evidence that the incidence of animal trypanosomiasis in these herds dropped from 20% to zero following this protocol (Jérémy Bouyer per comm.)

So in terms of the incidence of new infections, the results from the south east Uganda study explored in this thesis concur with similar studies that quantify the impact of the restricted applications in terms of the impact on the vector. Although neither of the studies explicitly assess the impact on the *G.f. fuscipes* vector predominant in south east Uganda, there are strong indications that insecticidal treatment of the legs and belly of cattle exposed to this subspecies offers protection against disease transmission.

### 7.6.4 Why it should not work

The study discussed in this thesis makes no direct assessment of the impact on tsetse flies; the indication of transmission reduction is inferred from lower trypanosome prevalences. There is however an interesting paradox here about the mechanism of control. The number of cattle treated in each site was small ( $n \approx 80$ ) and was surrounded by an area specifically chosen to be devoid of other control operations. Work done modelling the dynamics of tsetse populations however suggests this area and number of cattle is far too small to have any effect on the overall tsetse population, on account of fly re-invasion (Hargrove *et al.*, 2000; Vale & Torr, 2005). There *is* a significant decrease in the incidence of trypanosome infections in cattle treated with insecticide, inferring that the insecticide is having an impact on aspects of trypanosomiasis transmission. These are discussed below

### 7.6.5 Persistence of insecticide

This above effect could be explained if the insecticide achieved a consistent knockdown rate able to incapacitate the fly before it had chance to feed and transmit infections. Recent work done in Zimbabwe on a *G pallidipies* tsetse population found that both pour-on and spray formulations had a persistence period (whereby knockdown was >50% of landing tsetse) of between 9-20 days for whole body treatments with insecticide. Increasing temperature and rainfall both contributed to a decrease in the persistence period of the insecticide, as did restricting the application to smaller areas of the animal (Torr *et al.*, in press): The restricted application on the legs and belly persisted from between 15 days during the cool (20°C) dry season to 8 days in the warmer (>26 °C).

wetter season Results were the same regardless of the formulation used (spray or pour-on) (Torr *et al.*, in press). Data from the trial in south-east Uganda indicate there was not complete knockdown during the treatment period, given all groups have some incident infections, but suggest that the degree of knockdown offered a significant reduction in challenge. These results do counter the findings from Burkina Faso that suggested (pour-on) insecticide treatments were effective for ~75 days (Bauer *et al.*, 1992b).

### 7.6.6 Local depletion in tsetse population?

The alternative explanation to the effect of the insecticide is a local depletion in tsetse populations (Baylis & Stevenson, 1998a). This would be explicable given the slow mobility rate of *G.fuscipes fuscipes*, the predominant tsetse species in south east Uganda (Okiria *et al.*, 2002b; Magona *et al.*, 2005) and hence a low re-invasion rate. In terms of the study in south east Uganda, it would have been informative to sample non-treated cattle from the same herd as sprayed animals to see if these animals were similarly protected. Due however to the social implications of such an exercise it was decided not to adopt that type of study design. Other work in the same area however demonstrated that treating only 10% of the cattle population in an already suppressed tsetse area did not keep emerging infections an acceptable level (Okiria *et al.*, 2002b).

### 7.6.7 Repellent effect of synthetic pyrethroids

One of the ways now considered unlikely that deltamethrin works is as a repellent. The potential repellent or feeding inhibitory effect of deltamethrin (Bauer *et al.*, 1992b) has not been noted in extensive studies of fly behaviour comparing treated and non treated cattle (Baylis *et al.*, 1994; Vale *et al.*, 1999), nor does insecticide sprayed on a restricted area of the animal cause flies to alight on a non treated area (Torr *et al.*, in press).

### 7.6.8 Disease interactions

Although this study did not directly investigate interactions between diseases, there were several indications of their importance. There was a significant association between anaplasma positive animals and those positive by ITS-PCR. Additionally, there was evidence to suggest that animals that were protected from re-infection with

trypanosomiasis showed a lower prevalence of anaplasmosis. Due to the limitations of the diagnostic techniques used, such associations would benefit from more specific investigation, however analysis of a previous longitudinal study in south east Uganda investigated pathogen associations in more detail. It was shown that mixed parasite infections were the norm and interactions were often more than simply additive, in addition to highlighting the importance of considering the parasite community as a whole when exploring the pathogenicity of any of its individual components (Tosas-Auguet, 2006). It is known that good nutrition plays a significant role in the ability of an animal to manage morbidity associated with trypanosome infection (Holmes *et al.*, 2000) and the ability to manage helminth infections (Parkins & Holmes, 1989). In terms of improving animal health and production in indigenous livestock in areas of endemic disease, the emphasis is not on prevention of infection but on management of disease. The benefits of insecticide treatment on lowering overall disease challenge, the effect of this on interactions between different pathogens within a host, and the overarching role of nutrition and husbandry could all be influential in explaining the results.

### 7.6.9 Impact on environmental contamination

Work investigating dung residues for a variety of treatment options concluded that spray formulations gave significantly lower dung contamination than pour-on formulations. Additionally, this contamination was not due to systemic absorption of deltamethrin as both milk and blood samples were at negligible or sub-detection levels ( $<10^{-10}$  g/ml)(Bourne *et al.*, 2005).

## 7.7 Constraints of the study design, analysis or interpretation.

### 7.7.1 A lack of control

Investigating the change in clinical signs specifically highlighted a constraint of the chosen study design. Because all animals were given a twin dose of diminazene, the control group is not the same as a completely untreated group. Statistical analyses have thus made differential rather than absolute comparisons to an untreated population of cattle. Whilst this does not invalidate any of the findings, it makes identifying

significances harder. It is possible that the beneficial effect of the diminazene lasted for most of the duration of the study, which would be consistent with similar work that followed cattle in the same area of south east Uganda for 12 months following either isometamidium or diminazene treatments, and found no significant difference between the two treatments in terms of prophylaxis of new infections (Magona *et al.*, 2004a)

### 7.7.2 Length of study

Another criticism of the study is that at 8 months it was comparatively short. If resources had been available to continue the study for longer than potentially important information about seasonal changes in parasite burden could be investigated, for example the persistent effect of the insecticide in a hotter, wetter climate and the impact of seasonally burgeoning tick challenge. In addition, a longer timeframe would allow for an adequate assessment of the impact of this strategy on endemic stability in tick-borne diseases, for, although there were no negative indications from this study, requires a longer period of time to effectively assess.

### 7.7.3 Missing values

In terms of data analysis, the use of linear and generalised linear models was necessary to account for the clustered structure of the dataset. This also offered the advantage that modelling algorithms can analyse datasets with missing values, an almost inevitable consequence of this type of study design. As with all longitudinal studies however, the issue of whether drop out is random or due to some aspect of the treatment could be important. In this study full datasets were compared with subsets including only animals that had attended all samplings and found not to be significantly different, however more robust methods of interpolating missing data points are emerging (Carpenter *et al.*, 2002; Diggle & Heagerty, 2002; Fitzmaurice *et al.*, 2004) which could be of benefit to apply to future studies structured in this way.

## 7.8 Costs of control options

Calculating the actual costs of interventions is not straightforward due to disparities in published data as to the precise components that are included in the equation, such as

training, administration, initial research, monitoring and evaluation (Shaw, 2004). Nonetheless, Table 7-2 shows an estimate of the costs associated with different control options. It can be seen that by the cheapest option is the use of trypanocidal drugs, however as discussed above, this is not ideal. Traps offer a potentially economically feasible control method but also have disadvantages in terms of maintenance and sustainability (Holmes, 1997)

Technique	Costs per km <sup>2</sup> \$USD
Linear km of barrier using targets - barrier establishment - annual barrier maintenance	2 000 1 600
Ground spraying	265-390
Aerial spraying (Sequential Aerial Technique)	435-535
Sterile insect technique (SIT)	250-800
Low-density mono-pyramidal traps	26
Cattle insecticide treatment using pour-on (assuming 44 cattle per km <sup>2</sup> retreated every 30 days)	60-440
Prophylactic trypanocides, assuming 15 cattle and 3 treatments/yr	135
Curative trypanocides, assuming 15 cattle, 10% prevalence and eight week duration of disease	6.5

**Table 7-2:- Estimated costs of different control options for trypanosomiasis control, adapted from Shaw (2004).**

## 7.9 Less is more

This thesis has compared different control options mainly directed at the control of trypanosomiasis in endemic cattle. The restricted application of insecticide was indistinguishable from pour-on formulations in terms of protecting cattle from the incidence of new trypanosome infections, and manifested some benefits on levels of animal health and production that are likely to be apparent to rural livestock keepers and animal health workers. It has to be emphasised however that the pour-on insecticide gives the most beneficial results, at least over the timescale of this study. The chief advantage of the restricted spray protocol is its reduced cost. Table 7-3 shows the cost of each of the



interventions trialled in this study, calculated on a per dose basis, and additionally in terms of cost per animal per year. The restricted spray protocol (RSP) is approximately 18 times cheaper than pour-on, and 5 times cheaper than whole body spray application. At approximately \$0.60 per animal per year, the RSP represents the only economically viable alternative to the current option of curative drug use. Due to the apparent persistence of the RSP, it is probably advisable to halve the treatment interval to 14 days. This promises to provide additional protection from both ticks and tsetse, whilst protecting endemic stability and minimising drug, labour and environmental costs. If cost is not an constraint however, or if the pour-on can be made available cheaper to the end user, the use of pour-on insecticide appears to be the best option of those investigated in this study.

	Interval (days)	Cost (USD)
<b>Cost of curative trypanocide</b>		
Cost of diminazene aceturate ( <i>Veriben™, Ceva Sante-Animale</i> (10x 1.05g sachets))		\$5.21
Cost of diminazene aceturate (per treatment for a 240kg animal @ 7mg/kg)		<b>\$0.83</b>
<b>Cost of prophylactic trypanocide</b>		
Cost of isometamidium chloride ( <i>Veridium™, Ceva Sante-Animale</i> ) (10x1g sachet)		\$41.14
Cost of 240mg isometamidium chloride ( Treatment for 240kg animal @ 1mg/kg)		\$0.98
Treatment interval (days)	56	
Cost per animal per year		<b>\$6.38</b>
<b>Cost of pour-on insecticide applications</b>		
Cost of pour-on deltamethrin ( <i>Spot-On™ Coopers</i> ) per 200ml pack		\$6.86
Cost of pour-on (per treatment)		\$0.86
Treatment interval (days)	30	
Cost per animal per year		<b>\$10.43</b>
<b>Cost of whole body insecticide application</b>		
Cost of spray formulation of deltamethrin ( <i>Decatix™ Coopers</i> ) cattle dip/spray 1 litre		\$45.11
Cost of whole body treatment, (assuming using 2.5ml of concentrate diluted 1:1000))		\$0.10
Treatment interval (days)	14	
Cost per animal per year		<b>\$2.95</b>
<b>Cost of restricted insecticide application (front legs, belly and ears)</b>		
Cost of spray formulation of deltamethrin ( <i>Decatix™ Coopers</i> ) cattle dip/spray 1 litre		\$45.11
Cost of restricted application per treatment, (assuming using 0.5ml of concentrate diluted 1:1000))		\$0.02
Treatment interval (days)	14	
Cost per animal per year		<b>\$0.59</b>

**Table 7-3: Cost of individually treating an animal using the control options covered in this study. Costs calculated from wholesale market prices of drugs, Kampala, Uganda Sept. 2006**

The use of insecticide by rural livestock owners needs to evolve into a ‘little and often’ approach. In order to initiate this change, it is almost certain that the control of ticks, rather than the control of tsetse will be the driving force. In south-east Uganda, tsetse are not a particularly visible problem, and a study in western Uganda with similar challenge found that livestock keepers did not associate the fly with trypanosomiasis (Mugisha *et al.*, 2005). However, using the reappearance of tick populations as an indication that cattle need to be re-treated, it is possible that a consistent effect on the mortality of tsetse can be achieved.

### 7.10 Current and future work

“Prior to World War II, western medicine in Africa was either compulsory or unobtainable” was a comment made of the colonial approach to healthcare. (Austen & Headrick, 1983). The situation is now quite different, however the rural availability of drugs remains a serious constraint to healthcare delivery.(Heffernan & Misturelli, 2000). In order for insecticide use to be widely adopted, it has to be made available in appropriate volumes, with adequate information pertaining to its use. The hugely successful single dose trypanocide sachet has attributes of being well known, easy to use, cheap, small enough to be widely distributed, has a long shelf life and tamper-evident packaging. Competing technologies have to emulate this. In late 2006, an intervention trial was set up in by a consortium of Ugandan and UK based academic institutions, a drug company and a private equity firm to mass-treat cattle in northern Uganda in an attempt to reduce the reservoir of human infective trypanosomiasis.(Welburn *et al.*, 2006) By January 2007, approximately 111,000 cattle had been treated with diminazene aceturate and sprayed three times at 2 week intervals with deltamethrin using the restricted application protocol described above. Analyses of much of this trial is still pending, however it is apparent that sustainability of the technique will be highly dependant on the availability of the insecticide to rural communities. The health of African livestock has been placed in the hands of the market economy. Now it has to be seen how the market will respond.

## **Chapter 8 :- Appendix**

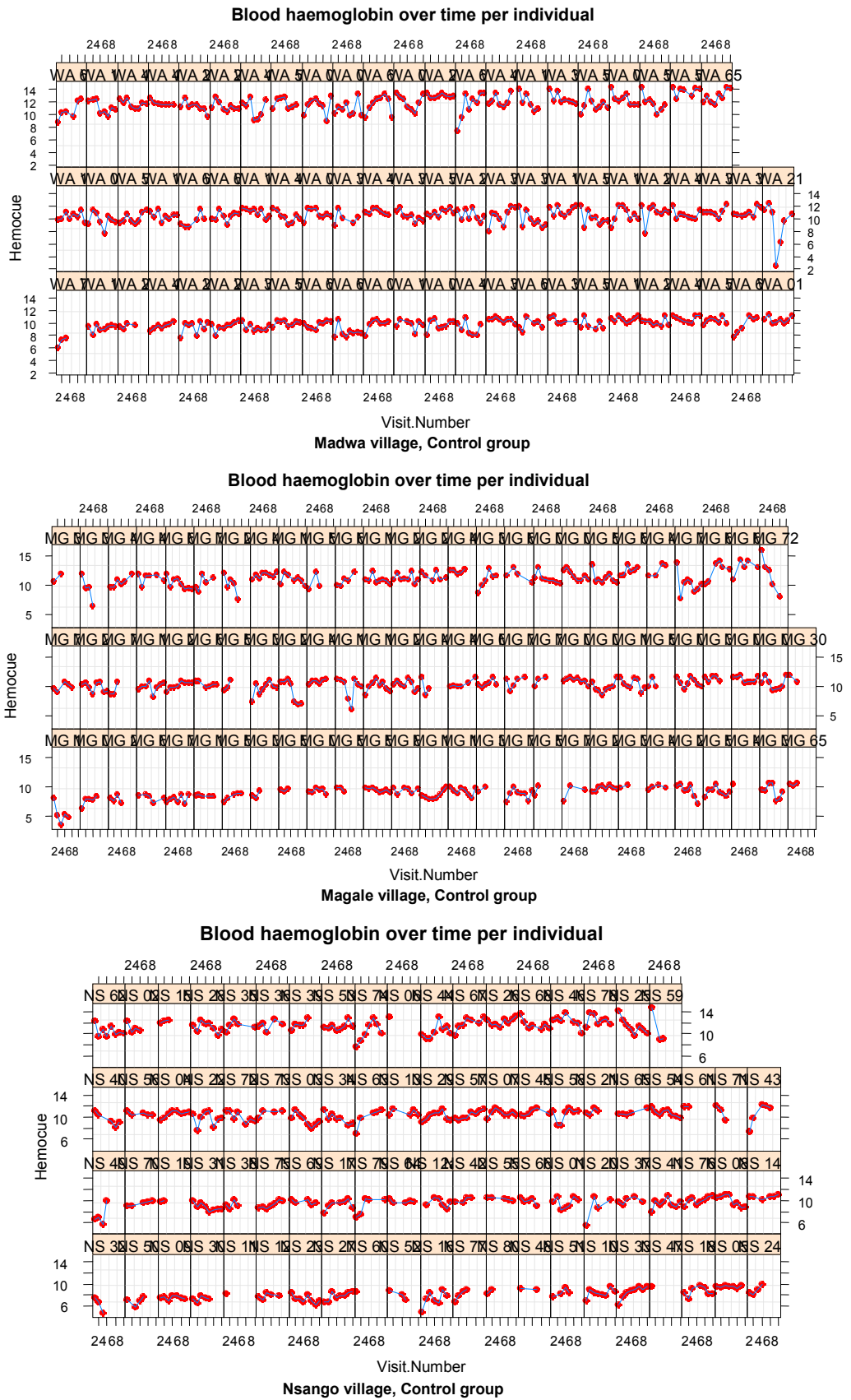


Figure 8-1:- Panel plot for each animal within control villages

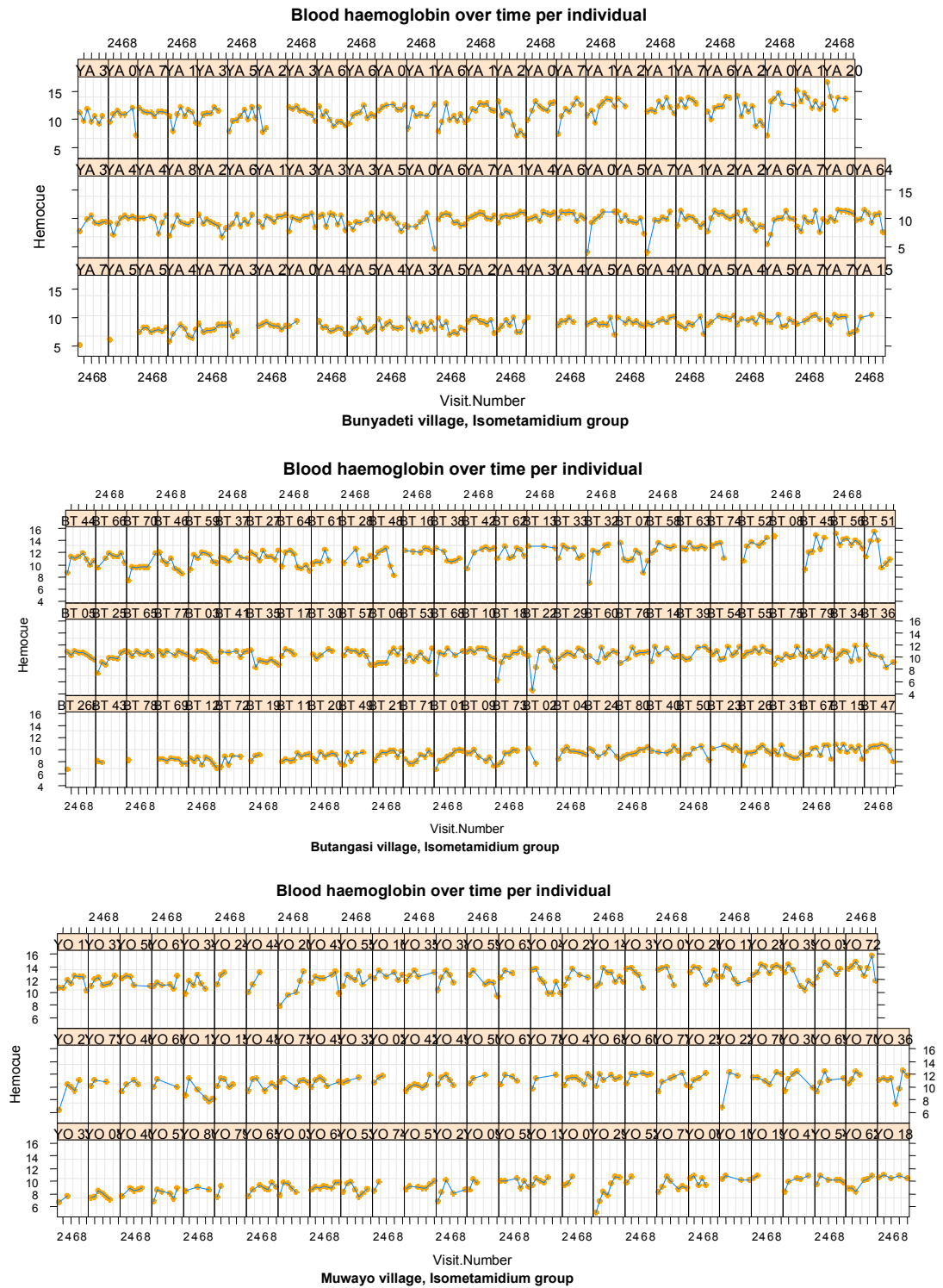


Figure 8-2:- Panel plot for each animal within Isometamidium villages



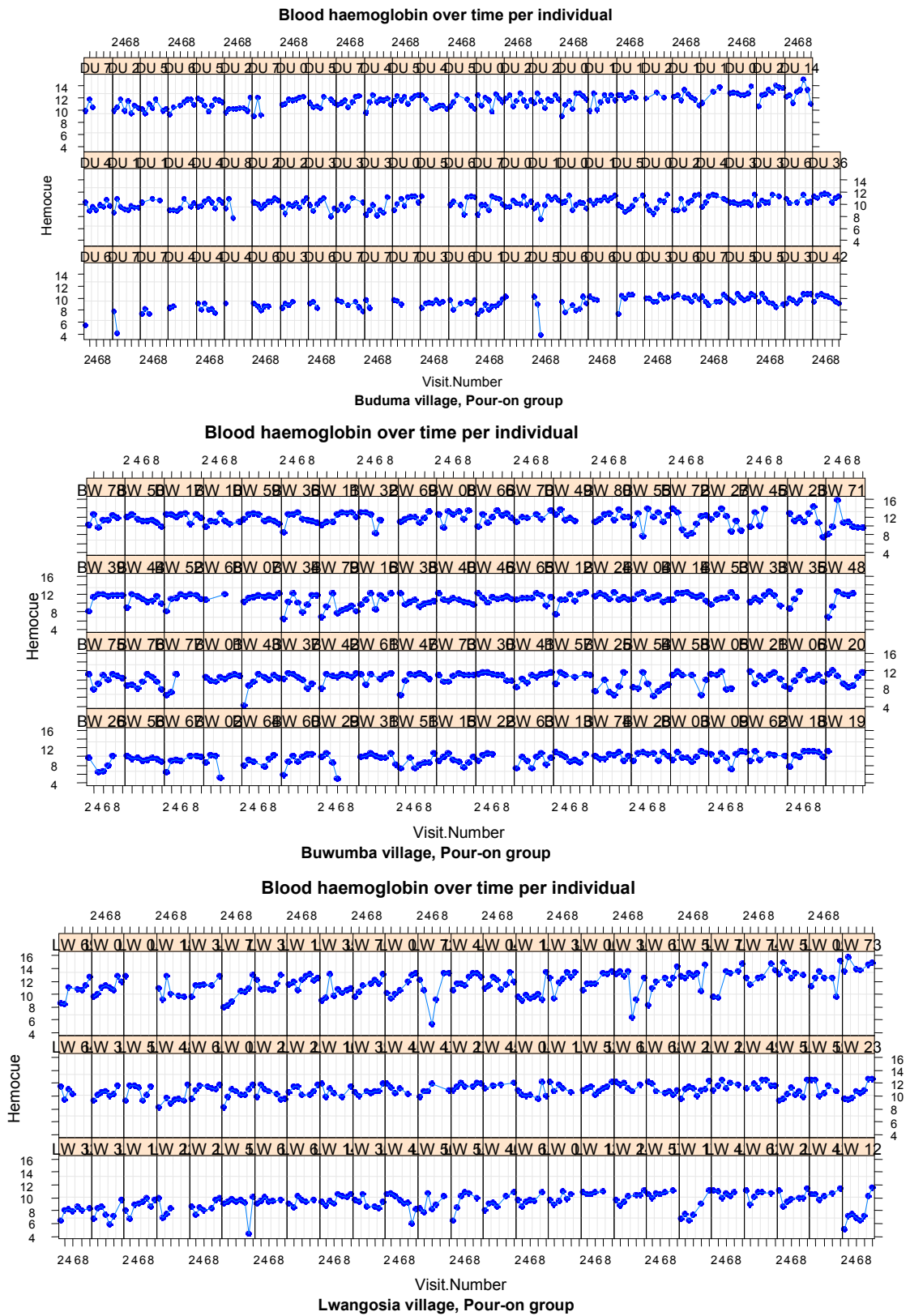
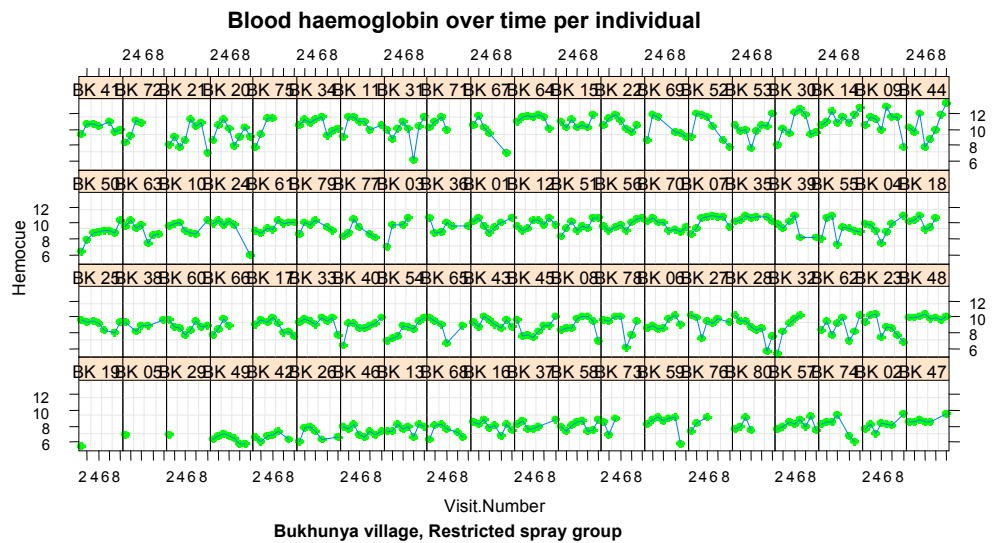
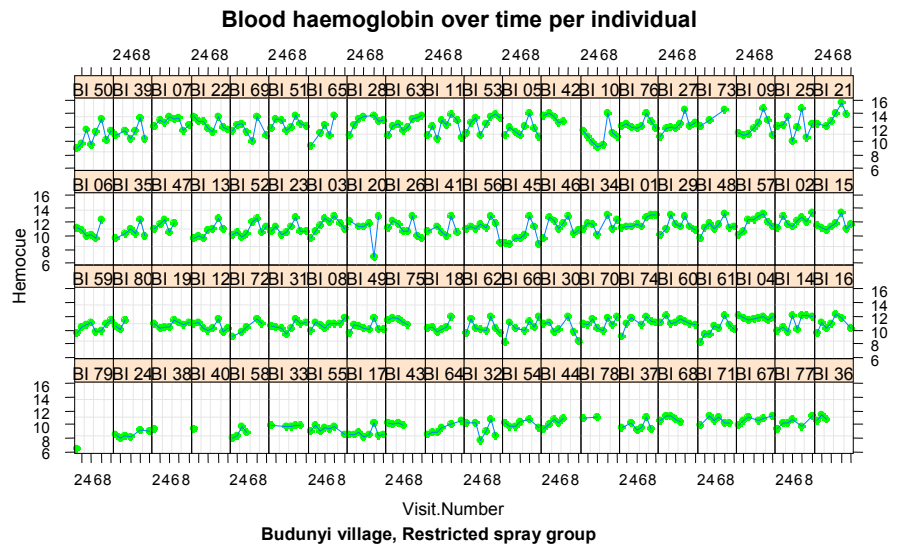
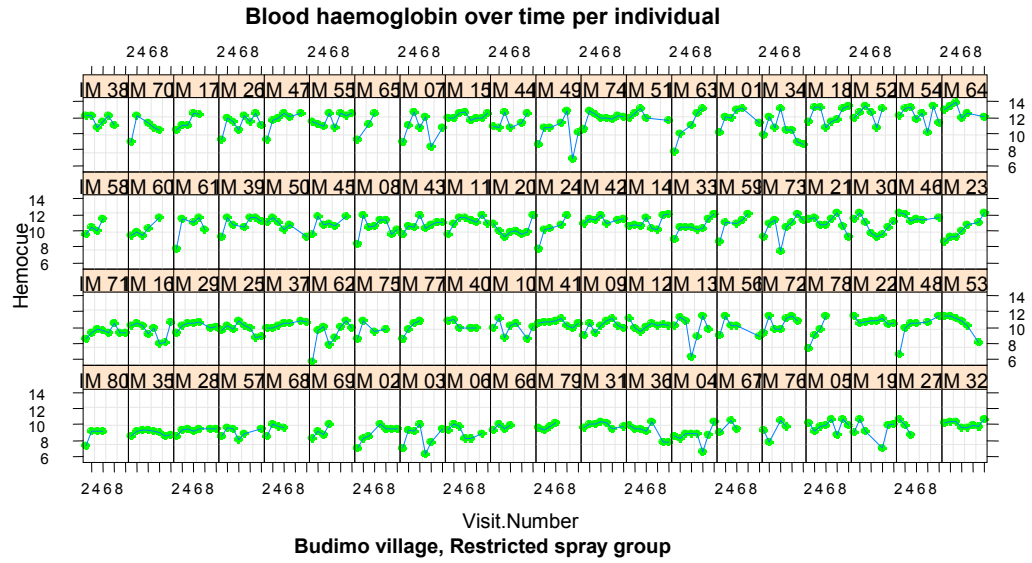
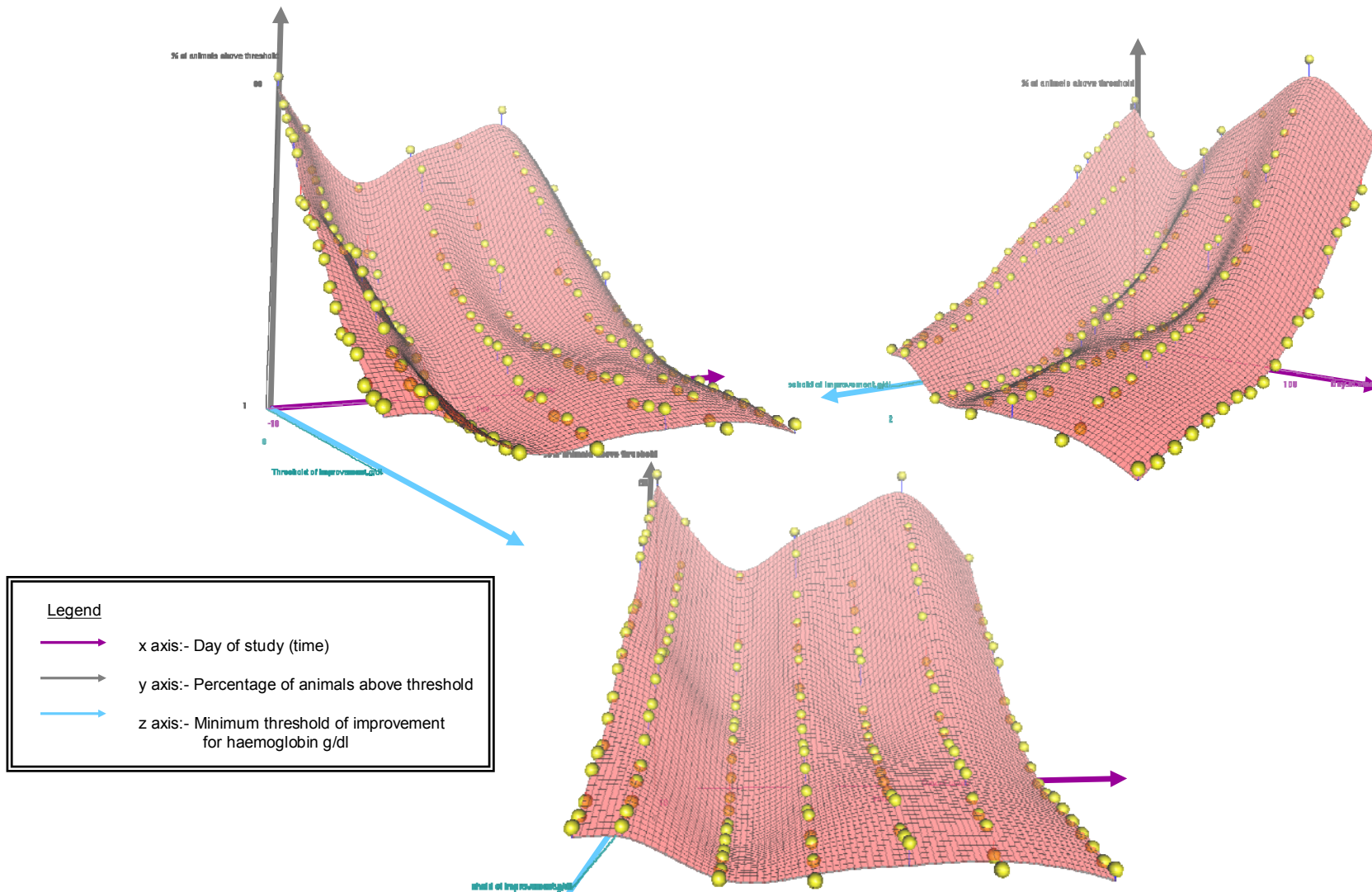


Figure 8-3:- Panel plot for each animal within pour-on insecticide villages

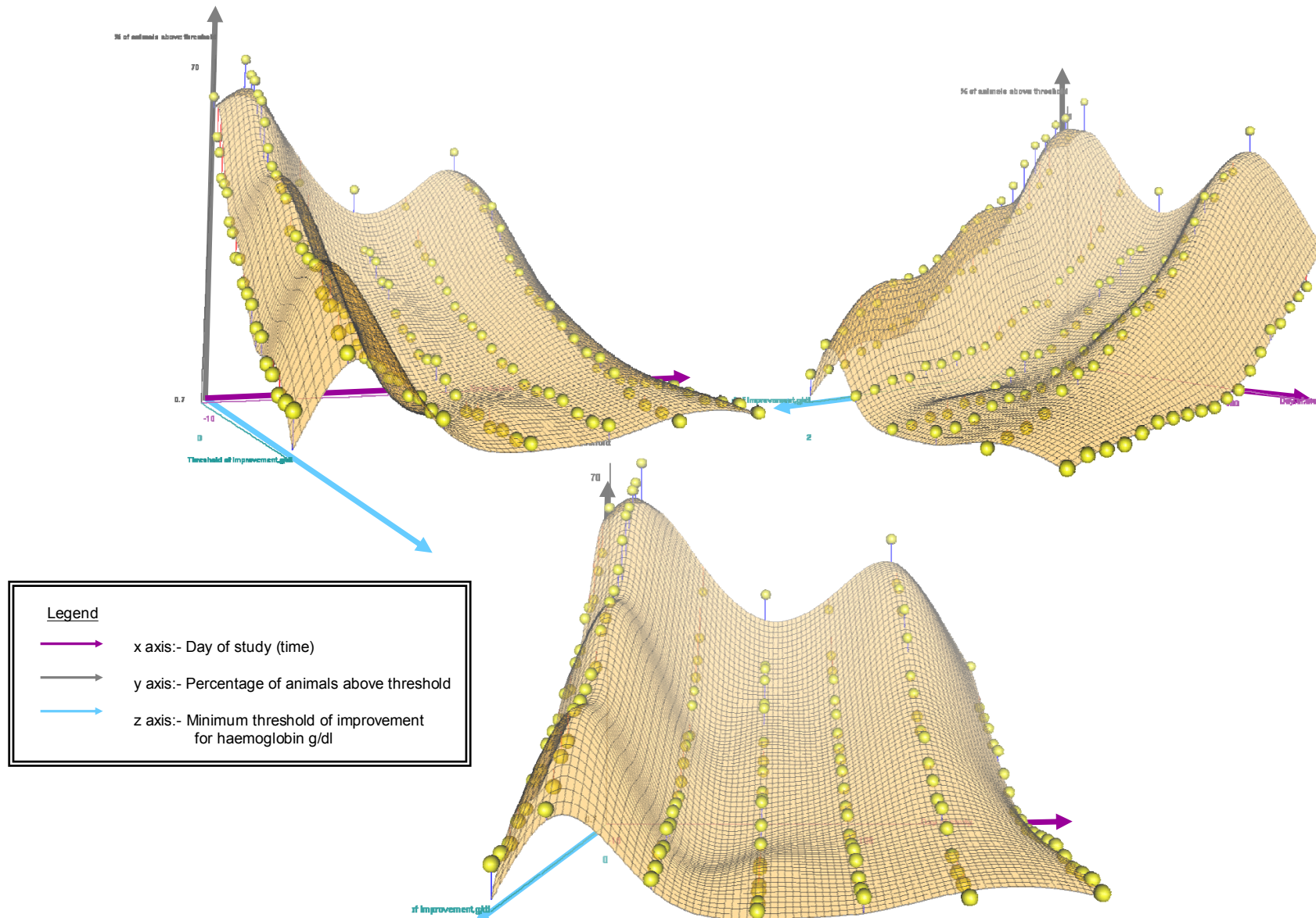




**Figure 8-4:- Panel plot for each animal within restricted spray insecticide villages**

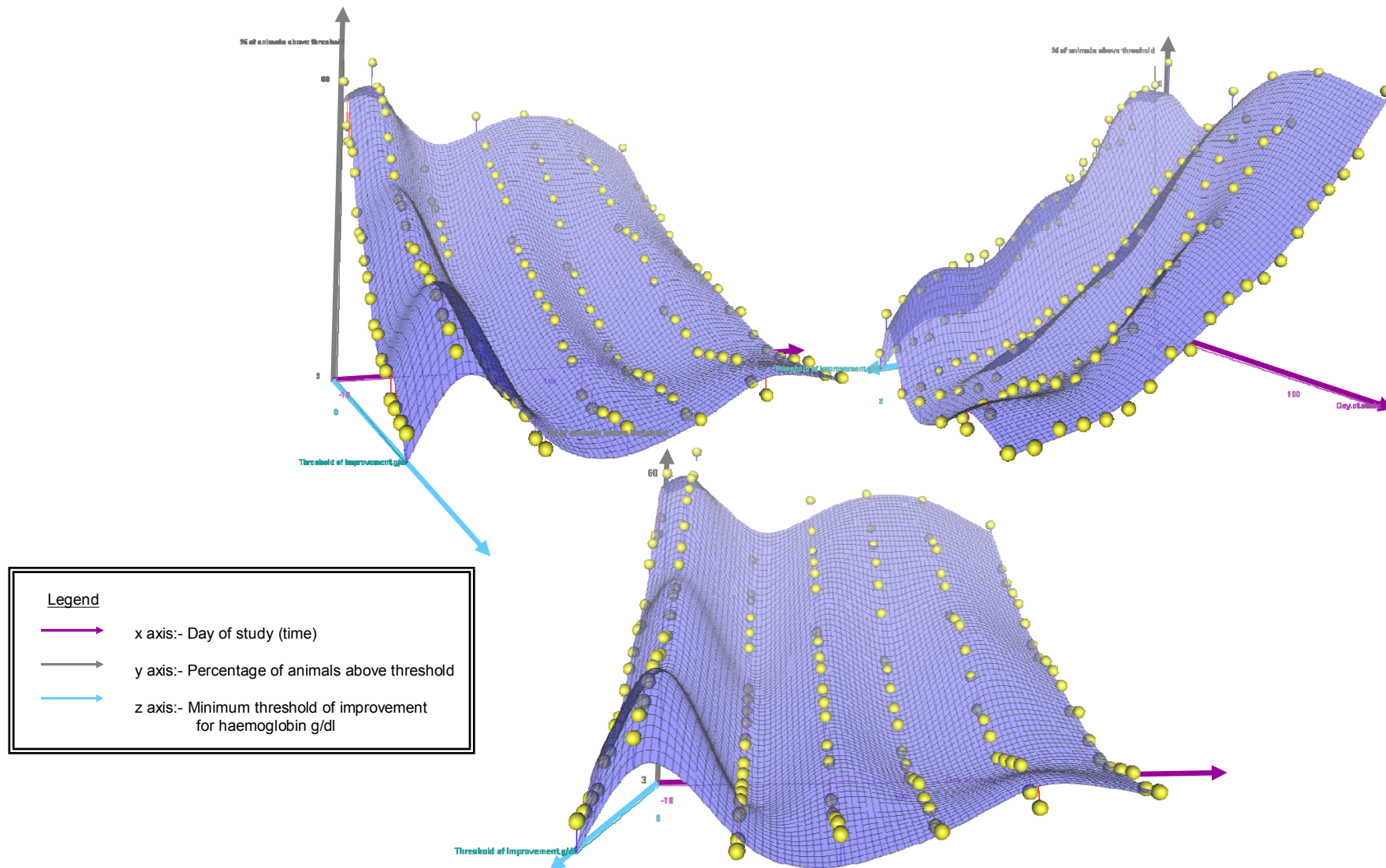


**Figure 8-5: Control group: 3D surface plot showing the change in the percentage of animals showing an improvement in blood haemoglobin from the previous visit, plotted over time. Y axis (grey) shows the percentage of animals improving, x-axis (purple) indicates time (day of the study) and the z axis shows the amount of improvement, in haemoglobin, between visits, range 0-2 g/dl.**

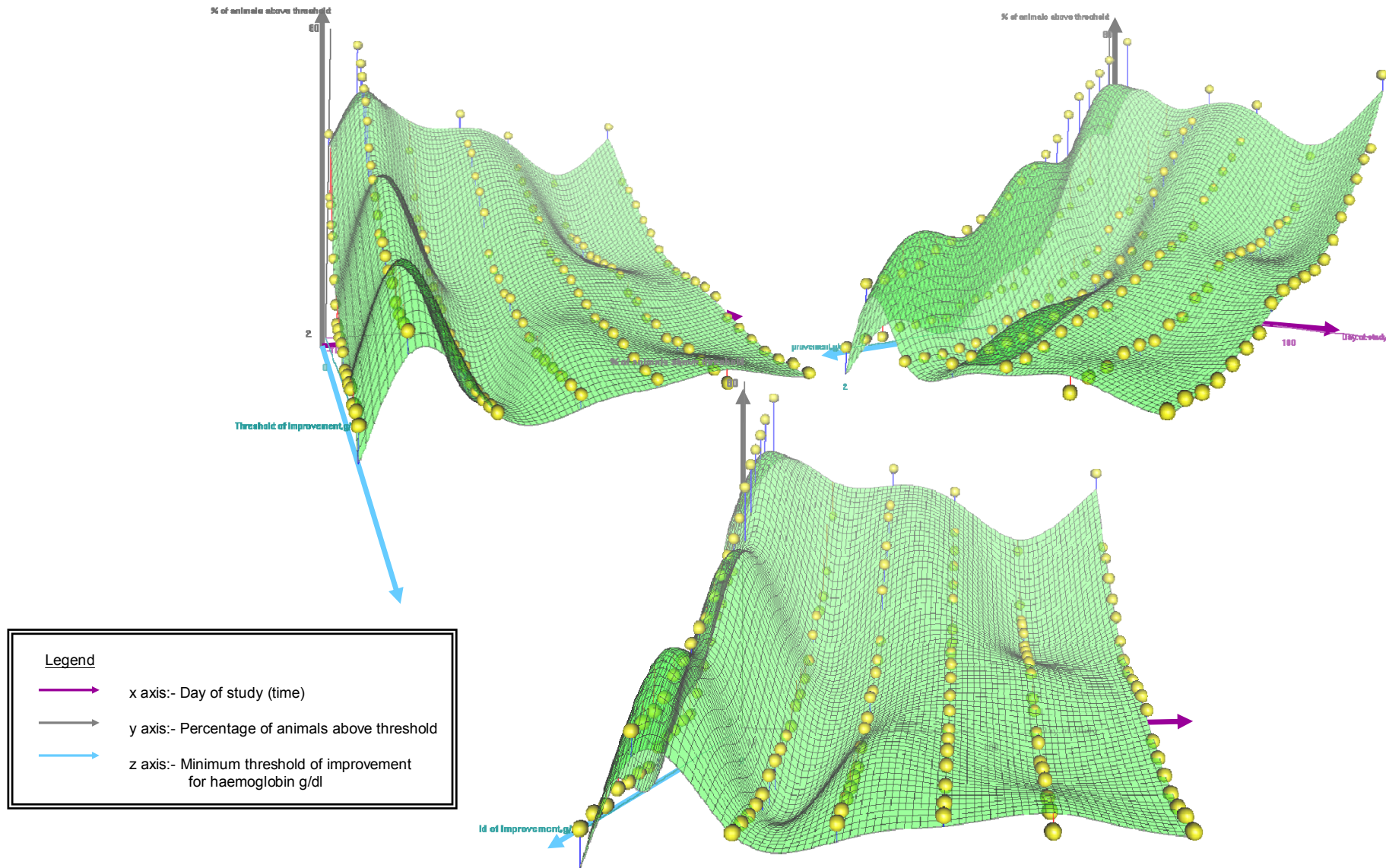


**Figure 8-6: Isometamidium group: 3D surface plot showing the change in the percentage of animals showing an improvement in blood haemoglobin from the previous visit, plotted over time. Y axis (grey) shows the percentage of animals improving, x-axis (purple) indicates time (day of the study) and the z axis shows the amount of improvement, in haemoglobin, between visits, range 0-2 g/dl.**



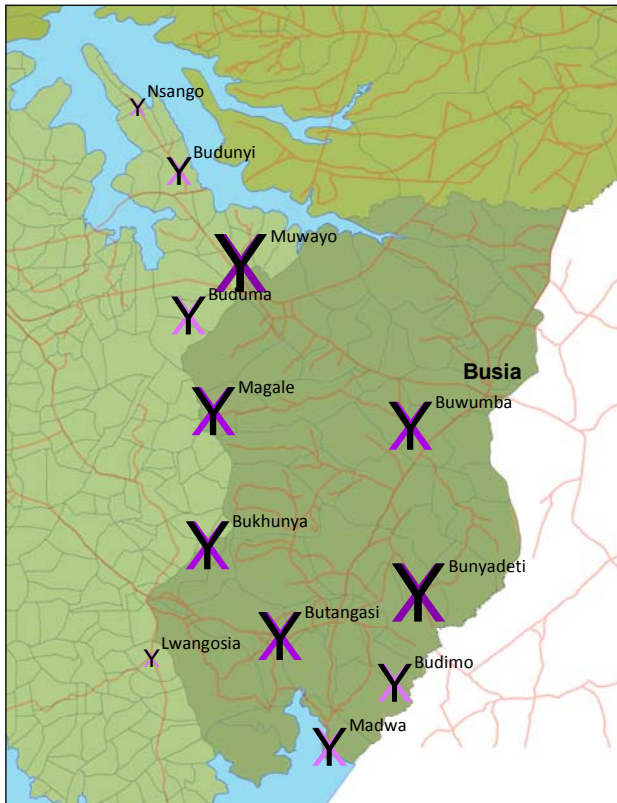


**Figure 8-7: Pour-on group: 3D surface plot showing the change in the percentage of animals showing an improvement in blood haemoglobin from the previous visit, plotted over time. Y axis (grey) shows the percentage of animals improving, x-axis (purple) indicates time (day of the study) and the z axis shows the amount of improvement, in haemoglobin, between visits, range 0-2 g/dl.**



**Figure 8-8: Spray group: 3D surface plot showing the change in the percentage of animals showing an improvement in blood haemoglobin from the previous visit, plotted over time. Y axis (grey) shows the percentage of animals improving, x-axis (purple) indicates time (day of the study) and the z axis shows the amount of improvement, in haemoglobin, between visits, range 0-2 g/dl.**

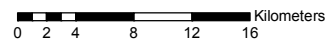
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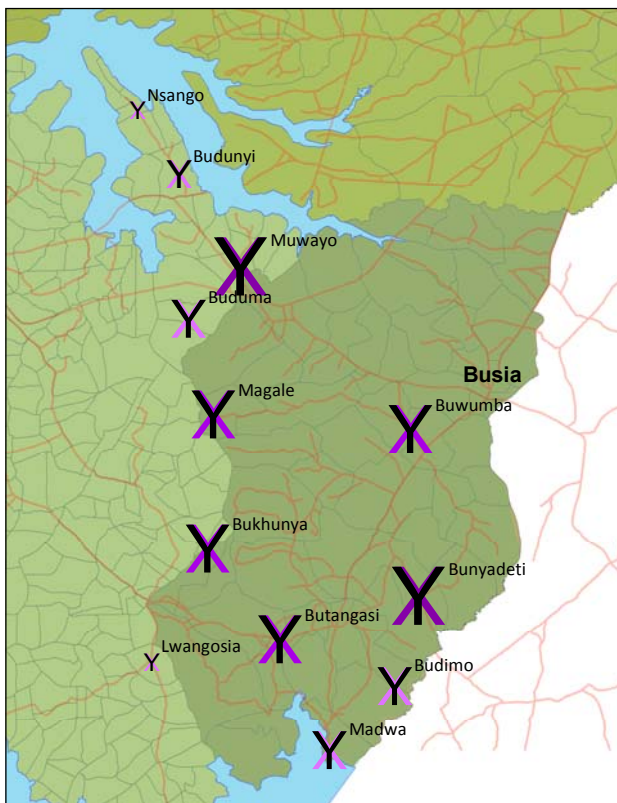
**Legend**

**Longitudinal study: Baseline**  
**Tick-borne diseases,**

- X <15%
- X 16 - 30%
- X 31 - 45%
- X 46 - 60%
- X 61 - 75%
- Lake/swamp



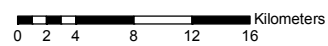
2



**Legend**

**Longitudinal study: Day 147**  
**Tick-borne diseases,**

- X <15%
- X 16 - 30%
- X 31 - 45%
- X 46 - 60%
- X 61 - 75%
- Lake/swamp



**Figure 8-9:- Map showing tick-borne disease prevalences for each study village at baseline (top) and day 147 (bottom)**



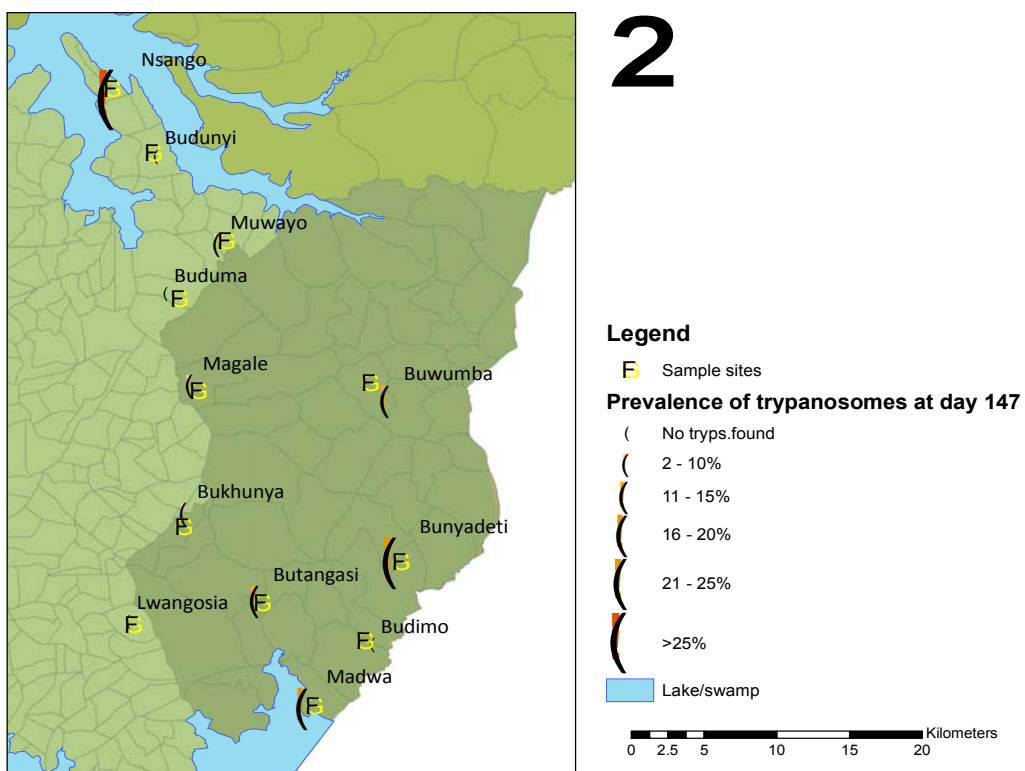
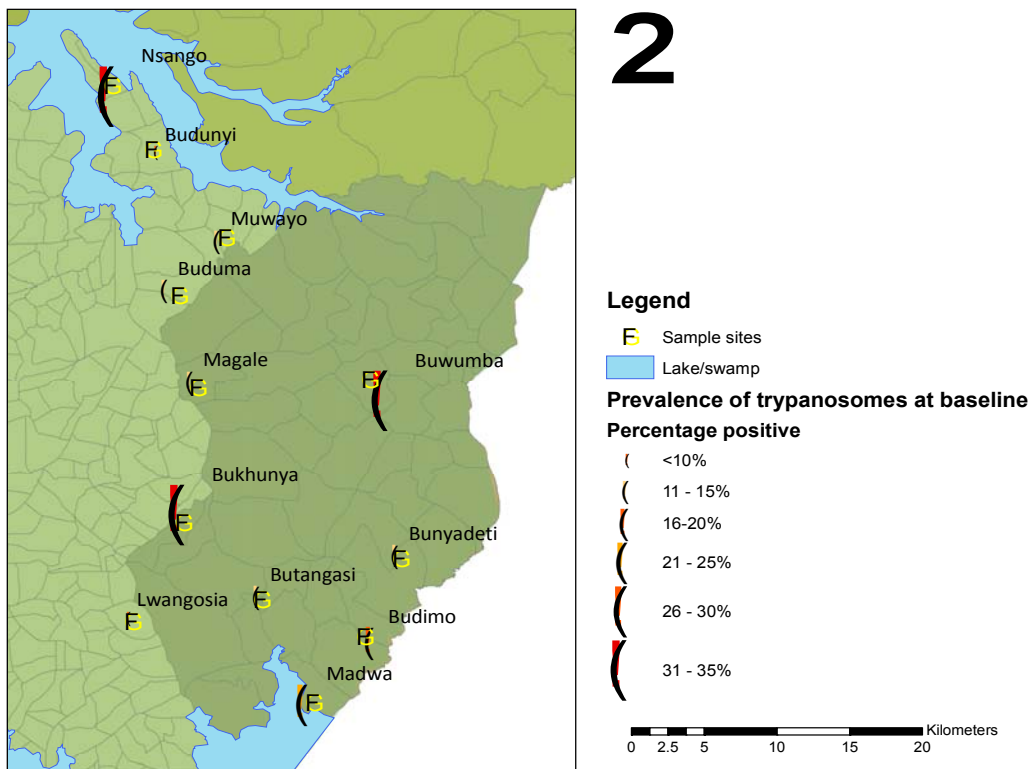


Figure 8-11:- Map showing tick-borne disease prevalences for each study village at baseline (top) and day 147 (bottom)

## **Chapter 9 :- References**



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