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Management of trypanocidal drug resistance in cattle in identified chemoresistance hot spots in the administrative District of Sikasso, south-east Mali

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First to GOD ALMIGHTY

and my family

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Abbreviations and acronyms

AAT	African animal trypanosomosis
Ab	Antibody
AT	African trypanosomosis
AR	Anthelmintic resistance
BCS	Body condition score
BCT	Phase contrast buffy coat' technique
bp	Base pairs
Bw	Body weight
º C	Degrees Celcius
CD	Curaive dose
CI	Confidence interval
DIIT	Drug incubation infectivity test
DIGIT	Drug incubation *Glossina* infectivity test
DIM	Diminazene aceturate
ED	Effective dose
EPG	Eggs per gramme faeces
ELISA	Enzyme linke immuno-sorbent assay
FAO	Food and Agriculture Organization
FEC	Feacal egg counts
FECRT	Faecal egg count reduction test
FTD	Flies per trap per day
GDP	Gross Domestic Product
GINs	Gastro-intestinal nematodes
GPS	Global positing system
HAT	Human African trypanosomosis
HCT	Haematocrit centrifugation technique
HOM	Homidium salts
IDR	Incidence density rate
IER, Sikasso	Institut d'Economie Rurale, Sikasso
IFAT	Indirect fluorescent antibody test
ILRAD	International Laboratory for Research on Animal Diseases
i.m.	Intra-muscular

ISCTRC	International Scientific Council for Trypanosomiasis Research and Control
ISMM	Isometamidium chloride
ISVEE	International Symposium for Veterinary Epidemiology and Economics
Kg	Kilogramme
Km^2	Square kilometres
LD	Lethal dose
LDCs	Least developed countries
m	Metre
MAFF	UK, Ministry of Agriculture Forestry and Fisheries
MEP	Mitochondrial electrical potential
mg	Milligramme
mm	Millimetre
ml	Millilitre
NaCl	Sodium chloride
ng	Nannogramme
PCR	Polymerase chain reaction
PCV	Packed cell volume
%	Percent
RR	Rate ratio
RDU	Rational Drug Use
RFLP	Restriction fragment length polymorphism
SADs	Stationary attractive devices
SAT	Sequential aerial technique
SD	Standard deviation
SIT	Sterile insect technique
SMT	Standard mouse test
SSA	sub-Saharan Africa
UN	United Nations
US$	US dollars
μm	Micro-metre
Vmax	Maximal uptake rates
WA	West Africa
WHO	World Health Organization
WAAVP	World Association for the Advancement of Veterinary Parasitology

1 Introduction

Thirty four out of 49 African countries are classified as Least Developed Countries (LDCs) and can therefore be considered among the poorest in the world (Otte and Knips, 2005). In sub-Saharan Africa (SSA), 50 per cent of the total population or 300 million people live on less than 1 US$ per day. Worse, the number of poor people grew in the 1990s, causing SSA's share of the world's absolute poor to increase from 25 to 30 per cent (Otte and Knips, 2005).

The SSA is the only region in the world where the average food production per person has been declining over the past 40 years (UN, 2001). Hunger, the most extreme manifestation of poverty, remains acute in rural SSA, with 34% of the population being undernourished (Reichard, 2002). Alleviation of poverty can only start with reduction of hunger and this will be achieved through the development of sustainable agricultural systems, in which livestock plays a key role (Vreysen, 2006).

As in many countries in other regions of SSA, agriculture is the backbone of the economies of countries in West Africa (WA). Approximately 50-80% of the workforce in several of these countries is engaged in the agricultural sector (FAO, 2005). Agricultural production contributes 30-50% to the gross domestic products (GDPs), of which livestock production contributes 20-25% (Ehui et al., 2002). Ruminant livestock products, namely meat and milk, are important components in the diet of most people. Due to an increase in human population and projected urbanization, growth in demand for livestock products will outstrip that for crop-based food (FAO, 2005). Nicholson et al. (1999) projected a doubling of the demand for dairy products in WA by 2010. Most of these increases in demand will have to be met from indigenous production using local animals.

Despite the central role which livestock agriculture plays as an engine for rural development and sustainable food and for nutritional security for the rural and peri-urban households, animal disease stresses, feed inadequacy and low genetic potential still prevail to be factors that constrain the productivity of this sub-sector in SSA. Of these, infectious diseases stand out as key constraints to increased livestock production.

Tsetse transmitted trypanosomosis is arguably the single most important constraint to animal agriculture in the semi-arid, subhumid, and non-forested portions of SSA (FAO, 2005).

Modernization of agriculture through rearing of animals with superior productive genotypes will hence remain a pipe dream for as long as trypanosomosis is not controlled (Feldman et al., 2005). Tsetse and trypanosomosis can therefore be rightfully considered one of the root causes of hunger, food insecurity and poverty in SSA, as is exemplified by the remarkable correlation and overlap between the 37 tsetse-infested countries and the 34 heavily indebted poor countries in Africa (Feldmann et al., 2005). Efforts to alleviate food insecurity and poverty must consider tackling tsetse and trypanosomosis menace as a way of modernising agricultural systems.

The Sikasso region, covering an area of 240,000 km^2 or 16% of Mali, is the most agriculturally productive area in Mali. Human demographic factors have exerted significant pressure on the cultivable land, resulting in reduced fallow periods and degradation in some instances (FAO, 2005). To sustainably increase land productivity, intensification of the agricultural process through a greater integration of crop and livestock production or by mixed crop-livestock farming has been adopted (Winrock, 1992). Consequently, the traditional role of livestock in providing animal draught power for cultivation, weed control and harvesting has expanded. Similarly, the collection and storage of manure and urine from livestock for crop production has been made more efficient (Fernández-Rivera et al., 1995).

The trypanosusceptible zebu cattle introduced for draught power needs in Sikasso in the 1960s (Diall, 2001) are continuously under high trypanosomosis risk, leading to an increased over-reliance on trypanocidal drugs to maintain them. This increased use of trypanocides led to trypanocidal drug resistance reported from the neighbouring Burkina Faso in the early 1980s (Authie 1984; Pinder and Authie 1984). This was followed by multiple drug resistance reports in the same country (Clausen et al., 1992; Bauer et al., 1995; McDermott et al., 2003) and in Mali (Diall et al., 2003; Grace, 2005). The high trypanosomosis risk, coupled with trypanocidal drug resistance, threatens the cattle-based livelihoods of the rural poor in this region and, if not addressed, will further exacerbate rural poverty.

Although there is a consistent demand for trypanocidal drugs by African farmers, the animal market value of about US$ 30 million per year is not considered sufficient to justify investment by large pharmaceutical companies in the development and licensing of new trypanocide molecules, the costs of which may exceed US$ 250 million for a single compound (Sones, 2001). The challenge, therefore, remains to make optimal use of the 3 currently available compounds, until new methods of treatment emerge, possibly through

serendipitous cross-reactivity with new broad-spectrum anti-protozoal compounds such as those currently being developed for the treatment of malaria and cryptosporidiosis (Holmes et al., 2004).

With no hope for a conventional anti-infection vaccine in the near future, efforts to prolong the efficacy of the existing drugs are urgently required (Geerts et al., 2001). The rearing of trypanotolerant cattle breeds known to survive and remain productive in tsetse infested areas with minimal use of trypanocidal drugs is likely to be of help in the medium to the long term (Murray et at., 1982; d'Ieteren et al., 1998). Unfortunately, these animals are less preferred (smaller and less tractable) in subhumid SSA, where animal traction is critical to the farming systems (Kamuanga et al., 2001). Additionally, they fetch lower sale prices. Consequently, the trypanotolerant breeds remain a minority choice due to the continued intromission of zebu genotype (Grace, 2003).

The concept of Rational Drug Use (RDU) has been hailed as a cornerstone for controlling antimicrobial resistance in human medicine (WHO, 2001). The World Health Organization (WHO) Global Strategy on Microbial Resistance stresses appropriate use of antimicrobials to maximize clinical therapeutic effect, while minimizing both drug related toxicity and antimicrobial resistance (WHO, 2001). The successful application of RDU to combat resistance in humans has been documented (Ross-Degnan et al., 1997; Radyowijati and Haak, 2002). Although RDU has rarely been applied to livestock systems in developing countries, it is well documented that most animal health treatments in Africa are given at community level, and that with basic training, farmers can competently make straight forward diagnoses and give treatments (Martin, 2001; Grace, 2008).

Vector control is another feasible option that is increasingly being adopted for controlling AAT and hence trypanocidal drug resistance (Geerts et al., 2001). However, without externally supported interventions, vector control is not sustainable due to the associated high transaction costs required in setting up and maintaining the control efforts. The success of vector control in reducing trypanosomosis risk and resistance is documented in several reports (Fox et al., 1993, Peregrine et al., 1994, Bauer et al., 1995). On its own, vector control does not completely address the problem of trypanocidal drugs resistant trypanosome populations. This leads to a high risk of their spread in case of reinvasion of tsetse controlled areas.

Integrated trypanosomosis control packages consisting of vector control, strategic helminth control and strategic feeding components, are necessary for clean-up of resistant trypanosomes from an area (Holmes et al., 2004). Due to immuno-suppression associated with trypanosome infections, it is desirous to boost the immunological competence of animals particularly in the trypanocide resistance hot spots for them to overcome the resistant-trypanosome populations through self-cure. Additionally, a pathological synergy between *T. congolense* and *Haemonchus* species (Kaufmann et al., 1992) has been demonstrated. This justifies the need for integrated trypanosomosis control approaches aimed at reducing trypanosome transmission and improving the general health of animals. A health package including tsetse control and strategic helminth control is likely to achieve the above stated objective of improving animal performance. Studies on malaria have demonstrated that well nourished children have less incidences of treatment failures as compared to malnourished ones (Hess et al., 1992). This reasoning further justifies the need for supplementing animals in chemo-resistance hot spots with high protein and energy feeds in order to improve their immunological competence to be able to utilize self-cure against the resistant-trypanosomes.

While the role of vector control and of trypanotolerant cattle breeds is appreciated in the control of AAT, as described before, little is known on the role of RDU, strategic drenching and of feed supplementation in AAT management. Rational drug use was tested on a sample of farmers in Mali and resulted in improved knowledge, practices and animal health outcomes (Grace et al., 2008). Although there are some reports on the interaction of trypanosome and helminth parasites (Kaufmann et al., 1992; Dwinger et al., 1994; Faye et al., 2002), none of these has addressed the impact of helminth control on trypanosomosis management. In order to bridge this existing knowledge gap, an integrated AAT management package including vector control, strategic helminth control and RDU (treatment of trypanosome positive cattle) was tested in this study in an identified chemoresistance hot spot area for its ability to contain the resistance.

2 Objectives

2.1 Broad objective

To design, execute and analyze the parasitological and epidemiological components of a tsetse and strategic helminth control package in the chemo-resistance hot-spot environment of Sikasso, south-east Mali, in order to contain and /or reverse trypanocidal drug resistance.

2.2 Specific objectives

1. To identify the pre-intervention, intervention and post-intervention tsetse challenge
2. To estimate the pre-intervention, intervention and post-intervention frequencies and distributions of trypanosome infections
3. To appraise the pre-intervention and intervention trypanocidal drug use practices
4. To estimate the pre-intervention trypanocidal drug resistance and monitor its evolution until post-intervention
5. To measure the effect of strategic helminth control on gastro-intestinal nematode infections
6. To estimate the anthelmintic resistance situation indicative for the cotton belt of West Africa
7. To explore developments of herd dynamics under control scheme

3 Literature review

3.1 Tsetse transmitted African animal trypanosomosis (AAT)

3.1.1 Impact of AAT on African agriculture

Trypanosomosis is a disease complex caused by several species of protozoan parasites of the genus *Trypanosoma*. It is estimated that about 37% of the African continent or approximately 8-11 million km² is infested by tsetse flies (Jordan, 1986; Mattioli et al., 2004). About 65% of this area (7 million km^2) could be used for livestock or mixed agriculture development without stress to the environment if trypanosomosis was controlled (MacLennan, 1980). Estimates put the number of cattle at risk from trypanosomosis between 50-70 million animals (Geerts and Holmes, 1998; Swallow, 2000).

The economic impacts of trypanosomosis in Africa are diverse and complex, with direct effects on animal production and human health, as well as indirect effects on settlement patterns, land use, draught power use, animal husbandry and farming (Swallow, 2000; Bourn et al., 2005). Quantifying these wide-ranging effects has proven to be difficult, but a considerable body of evidence has been gathered through numerous studies of specific situations (Swallow, 2000; Shaw 2004). Aggregating these results to the African content as a whole is problematic because of general uncertainities about cattle numbers, infection rates and the extent of actual, as opposed to potential, tsetse infestation. However, direct aggregate losses due to animal trypanosomosis are estimated to exceed US$ 1.3 billion annually, calculated by Kristjanson et al. (1999), which excludes losses from reduced efficiency of draught oxen and manure use. These authors estimated that the potential benefits of improved trypanosomosis control in terms of meat and milk productivity alone amount to US$ 0.7 billion a year in Africa. To this figure, the expenditure on trypanocides, estimated at around US$ 30 million per annum for some 35 million doses, needs to be added for a more encompassing assessment (Holmes et al., 2004). Cattle mortality associated with trypanosomosis is another example of direct loss. It was, for instance, estimated through a questionnaire survey that the high trypanosomosis risk in the pastoral area of Yalé in Burkina Faso caused herd mortality of between 75% and 85% (Kamuanga et al., 2001a).

Each year in SSA, milk and meat offtake are 10-40% lower due to trypanosomosis infections (Swallow, 1997). Likewise, cattle numbers would increase by 37% in the sub-humid and 70% in humid zones if trypanosomes were to be eradicated. Trypanosomosis also reduces total agricultural production by between 2% and 10% (the crude relationship is that a 50% increase in livestock numbers would increase total agricultural output by 10%) (Swallow, 1997). Shaw (2004) reported that livestock under trypanosomosis challenge have a 6-20 percentage point higher annual calf mortality, a 6-19 point lower calving rate and a 20% decrease in milk yield.

3.1.2 Epidemiology of bovine trypanosomosis

3.1.2.1 Pathogen (trypanosomes)

Trypanosomes are unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae, and genus *Trypanosoma* (Levine et al., 1980). Three principal parasites namely, *T. congolense, T. vivax* and *T. brucei brucei* are known to transmit trypanosomosis in bovines normally via the bite of an infected tsetse. In some rare instances, trypanosome species like *T. vivax* and *T. congolense* are transmitted mechanically (Desquesnes and Dia, 2003a; 2003b). The different trypanosome species differ in morphological characteristics as described by Maudlin et al. (2004).

Trypanosoma congolense is divided into subtypes, with different distributions and pathogenicity: savannah type, forest type, Tsavo type, and Kilifi type (Majiwa et al*.,* 1993). *Trypanosoma congolense* savannah type is the most pathogenic of the four and is capable of causing severe anaemia and even death of infected cattle (Bengaly et al., 2002). Other *T. congolense* types cause mild disease that in certain instances does self-cure.

Trypanosoma vivax shows variable levels of virulence and distinct pathogenicity in West African isolates, causing an acute disease in cattle often accompanied by weight loss, reduced milk yield, abortions and mortality, whereas the East African isolates largely cause chronic infection (Gardiner and Mahmoud, 1992). In East Africa, there are two types of *T. vivax* isolates: the haemorrhagic *T. vivax* that causes an acute haemorrhagic syndrome and the mild strain (Mwongela et al., 1981; Bett et al., 2004; Magona et al., 2008). Cattle infected with the haemorrhagic *T. vivax* produce auto-antibodies to red blood cells, a phenomenon that is not observed in the non-haemorrhagic *T. vivax* (Bett et al., 2004).

Infections with *T. brucei*, on the other hand, have been described as being chronic and sub-patent, with minimal impact on the health of the infected cattle (Killick-Kendrick, 1971). It is thus not surprising that cattle may act as important reservoirs of human pathogenic *T. brucei* species and can play an important role in the epidemiology of human sleeping sickness (Fevre et al., 2001). The importance of a bovine carrier in the epidemiology of *T. brucei* trypanosomosis will be determined to a large extent by the behaviour of the parasite. Of particular interest is the behaviour of the *T. brucei* when their reservoirs are challenged and become infected with other trypanosome species such as *T. congolense*. This is likely to occur frequently in the field, considering the high infection rates of tsetse flies with *T. congolense* compared to *T. brucei* (Woolhouse et al., 1994).

3.1.2.2 The vector of African trypanosomosis (AT)

Tsetse flies, the vectors for AT, belong to the family *Glossinidae*, order *Diptera* – the two winged flies. There are 31 recognized *Glossina* species and sub-species, divided into three groups (*morsitans*, *palpalis* and *fusca*) which have been given sub-generic status (Solano et al., 2010). Recently, comparative gene sequence analysis and geometric wing morphometry have been proposed to help in the *Glossina* group identification (Patterson and Schofield, 2005). The *morsitans* group that includes *G. morsitans morsitans*, *G. m. submorsitans, G. pallidipes, G. longipalis* and *G. austeni* is found mainly in the savannah ecosystems. They are the most important vectors of bovine trypanosomosis (Leak et al., 1999). The *palpalis* group is found mainly in the riverine galleries of West and Central Africa but sometimes extends into savannah regions between the river systems (Hendrickx et al., 2004). The *palpalis* fly species are less mobile than the *morsitans* group*,* often relying on sight rather than smell to locate their hosts (Leak, 1999). In West Africa, important bovine trypanosomosis vectors among the *palpalis* group include *G. palpalis palpalis, G. p. gambiensis* and *G. tachinoides* (Hendrickx et al., 2004; Raiyaisse et al., 2010; Solano et al., 2010). The *fusca* group flies settle mainly in forests and are therefore less important vectors of bovine trypanosomosis. *Glossina longipennis* and *G. brevipalpis* found in the drier areas of Kenya are exceptions among the *fusca* group, as they have been demonstrated to transmit trypanosomes (Makumi et al., 2000).

Adult *Glossina* species are dull in appearance, varying in colour from light yellowish brown to dark blackish brown (Leak, 1999). In some species the abdomen may have alternate darker

and lighter bands. The smallest species is 6-8 mm long and the largest 10-14 mm (Jordan, 1986).

The adult female produces a single egg, which hatches to first stage larva in the uterus. After a period of development and moulting, a third stage larva is deposited on the ground. Females produce one full grown larva every 8-10 days which pupates in light clay or sandy soil. The adult fly will emerge after a puparial period that varies according to temperature but may be around 30 days at 24^0 C. Consequently, tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. This reproductive method of tsetse flies is known as adenotropic viviparity (Leak, 1999). Females are receptive to males as soon as they start seeking food and are often mated soon after taking their first blood meal (Leak, 1999). They are usually mated once with viable sperms remaining stored in the spemathecae throughout the life of the female from where the sperms get nourished by secretions from a layer of cells surrounding the cuticular lining of the lumen of each spermathecae. In certain instances, mating may happen more than once. Male flies may not mate soon after emergence from the pupa and they are not fully fertile until they are a few days old.

Other than tsetse flies, other haematophagous insects like tananids and *Stomoxys* species also transmit trypanosomosis mechanically as has been demonstrated by Desquesnes and Dia (2003a).

3.1.2.3 Hosts

Trypanosomosis is known to affect a number of mammalian vertebrates, either as African animal trypanosomosis (AAT) and or as human African trypanosomosis (HAT) (Leak, 1999). *Trypanosoma vivax, T. congolense* and *T. brucei,* for example, affect various ungulates including cattle, sheep, goats, horses, pigs and camels (Maudlin et al., 2004). Other animals like dogs, cats and the wild carnidae are also affected (Hoare, 1972). *Trypanosoma evansi,* principally a parasite of camels and equines, also infects other animals like water buffaloes, sheep, goats, cattle and deers. *Trypanosoma vivax* and *T. evansi* by virtue of their transmission by haematophagous biting flies occur in SSA, Asia, Central and North and Southern America (Hoare, 1972). The silvatic cycle that involves wild animals is known to greatly influence the epidemiology of trypanosomosis since wild animals serve as reservoirs for both human and animal trypanosomosis (Taylor and Authié, 2004). Trypanosomosis

maintains large areas of Africa (so-called 'fly belts') free of livestock, and it is presumed that wildlife have developed an evolutionary immuno-tolerance to these parasites with which they have cohabitated for millennia (Reichard, 2002).

A number of factors contribute to the severity of disease in its various hosts. Exotic animals (dairy cattle) are more severely affected by trypanosomosis than the local genotypes, which exhibit a range of breed and individual animal susceptibility. The West African taurine breeds, like the N'Dama, Baoule and their crosses with zebu (d'Ieteren et al., 1998), and certain zebu cattle in East Africa (Njogu et al., 1985) can survive and remain productive under trypanosomosis risk. This phenomenon is called trypanotolerance and involves the ability of the animals to control parasitaemia, maintain weight and resist anaemia (Murray et al., 1982). Clausen et al. (1993) established through experimental work that animals within the trypanotolerant breeds with previous exposure to trypanosomosis suffer less severe trypanosomosis effects as compared to those without previous exposure (naïve animals).

Suckling calves are also known not to suffer from serious attacks of trypanosomosis, possibly because of the influence of maternal antibodies in their systems (Dwinger et al., 1992). There is also evidence from studies that tsetse get attracted mostly to larger cattle from which they feed rather than smaller ones (Torr and Mangwiro, 2000; Vale and Torr, 2005). Torr and Mangwiro (2000) estimated that a large ox was bitten ~ 10 times more often by tsetse than a calf. Within herd differences have also been established (Torr et al., 2001) where ~ 75% of the tsetse feed from ~ 25% of the herd. The physiological status of the host, as well as nutritional and environmental factors, further play important roles in modulating the severity of the disease (Taylor and Authié, 2004). It would be expected that animals with concomitant infections with other parasites like *Haemonchus* species would develop serious disease when infected by trypanosomes, particularly *T. congolense,* as was reported in the Gambia (Kaufmann et al., 1992).

3.1.2.3 Environment

The environment allows for the interaction between the *Glossina* species, vertebrate hosts and the trypanosomes in order for trypanososmosis to be produced. In West Africa, tsetse habitats have been sub-divided along distinct north-south climatic gradients, with predominantly riverine tsetse species in the north and a mixture in the south (Hendrickx et al., 2004). In the

north, arid conditions prevent fly spread and riparian vegetation constitutes suitable niches for the localized, well-demarcated pockets of tsetse populations. Outside these favourable micro-climates, tsetse hardly survives and it would appear that no links exist between pockets, except occasionally and in spatially limited neighbouring areas during the rainy seasons. In the intermediary band, climatic conditions and vegetation become gradually more suitable. Distinct fly pockets tend to merge and tsetse distribution patterns become more linear along main streams. Tsetse populations still remain concentrated in pockets during the dry season, but disperse (Cuisance et al., 1985; Bouyer et al., 2006) during the rainy season over large parts of the river systems, including important tributaries and savannah buffers. In the humid south, there are no climatic limitations to fly distribution and flies are present along river sytems and even the surrounding humid woodlands and forests.

Due to increasing human population and consequently the opening up of more land for crops, the *morsitans* group is disappearing in most places of West Africa (Djiteye et al., 1997). Riparian tsetse species on the other hand are more versatile and can co-exist with human development. They are opportunistic feeders; where agricultural intensity is low; they feed on wild reptiles and rarely carry pathogenic trypanosomes (de la Rocque et al., 2001a).

3.1.3 Diagnosis of AAT

3.1.3.1 Parasitological tests

3.1.3.1.1 Blood films

The examination of wet blood films and Giemsa-stained thick and thin fixed blood films with the aid of a light microscope have been used as diagnostic methods ever since they were first used to identify the aetiological agents of trypanosomosis. With a wet smear, a drop of blood can be examined next to the animal, provided that a microscope is available. Thin and thick blood smears fixed in methanol or acetone and stained with Giemsa may be used in the laboratory to detect blood parasites and determine trypanosome species involved, respectively. However, these techniques are not sensitive enough to detect low parasite levels, characteristic of the disease in large animals (Eisler et al., 2004). In order to have a high chance of detecting parasites, it is often considered best to collect blood samples early in the

morning and from peripheral capillaries like the ear or underside of the tail where parasite concentration is high.

3.1.3.1.2 Parasite concentration techniques

These include the haematocrit centrifugation technique (HCT) described by Woo (1970) and its improved version, the buffy coat technique (BCT) described by Murray et al. (1977). The two methods are associated with increased diagnostic sensitivity following concentration of parasites in the buffy coat after centrifugation. The packed cell volume (PCV %) can be determined as a measure of anaemia. With the BCT, the three most important trypanosome species in ruminants can be identified due to their characteristic movement patterns, with a possibility for estimation of parasitaemia through a scoring system (Paris et al., 1982).

The analytical sensitivity of BCT depends on the species of trypanosome as has been demonstrated by Paris et al. (1982), with the smallest numbers detectable per millilitre of blood being $2.5*10^2$, $5*10^2$ and $5*10^3$, for *T. congolense*, *T. vivax* and *T. brucei,* respectively. On the other hand, HCT is the most sensitive microscopic technique to detect *T. brucei* in bovine blood.

3.1.3.1.3 Sub-inoculation

These methods involve the transfer of trypanosomes from a suspected case to another vertebrate or invertebrate host. These methods have the advantage to conserve (stabilate) isolated trypanosomes for further investigations (Eisler et al., 2004). Laboratory rodents are inoculated with 0.2-0.5 ml (depending on size of rodent) of freshly collected trypanosome-positive blood. Artificial immuno-suppression of recipient animals by irradiation or drug treatment (cyclophosphamide) greatly increases the chances of isolating the parasite (F.W. Jennings, Personnal communication, 1992 Glasgow cited by Eisler et al., 2004). The method is particularly appropriate for *Trypanozoon* species (Robinson and Ashkar (1972). On the other hand, only 50% of *T. congolense* can be conserved in mice. *Trypanosoma vivax* rarely establishes in mice, and, if it does, the resultant parasitaemia is quite transient.

3.1.3.1.4 Xenodiagnosis

Xenodiagnosis is the feeding of a clean susceptible vector species on a suspected case of trypanosomosis, after which it is either dissected and examined for trypanosomes or allowed to feed on a clean animal which is itself examined for the development of infection (Eisler et al., 2004). Because of the scarcity of laboratory-reared *Glossina* species in Africa, this method of diagnosis is rarely attempted for tsetse-transmitted bovine trypanosomosis. The method is extremely sensitive and may be used if the presence of a trypanosome infection in a particular animal or cattle population is suspected on the basis of surrogate tests (tests that do not conclusively demonstrate the presence of a parasite, such as antigen-ELISA) but cannot be conclusively demonstrated by other means (Eisler et al., 2004). The differential susceptibility of species of *Glossina* should be taken into account if this technique is used; for example, *Glossina palpalis* is unlikely to become infected with *T. congolense* (Stephen, 1986).

3.1.3.1.5 In vitro culture methods

Detection of trypanosomes through inoculation of culture medium with blood is possible but, due to the low success rates, the in vitro culture is seldomly applied in trypanosome detection. The technique consists of inoculating aseptically a large volume (10 ml) of venous blood into culture medium in order for the bloodstream trypanosomes to transform into procyclics and start to multiply. Zweygarth and Kaminsky (1990) described a method for isolating *T. brucei brucei* and *T. evansi* directly in culture from host animals with low parasitaemias, in which in some cases parasites were not detected on wet blood film or HCT.

3.1.3.2 Serological techniques

A number of serological techniques are available for diagnosing trypanosomes. The indirect fluorescent antibody test (IFAT) is both sensitive and specific in the detection of bovine anti-trypanosomal antibodies (Luckins and Mehlitz, 1978). The antibody-enzyme linked immmuno-sorbent assay (Ab-ELISA) or the indirect ELISA (Luckins and Mehlitz, 1978) detect more serogically positive cattle than the IFAT. Antigen-ELISAs (Ag-ELISA) developed at the International Laboratory for Research on Livestock Diseases (ILRAD), using the sandwich ELISA methodology, incorporated trypanosome species-specific monoclonal

antibodies (Mabs), which reacted with determinants of *T. brucei, T. congolense* or *T. vivax* (Nantulya et al., 1987). The test has increased sensitivity (small quantity of analyte – trypanosome – can be detected) as compared to the Ab-ELISA or IFAT. For epidemiological analysis, a greater proportion of trypanosome infected animals does react to the (Ag-ELISA) test.

3.1.3.3 Polymerase chain reaction (PCR)

Several studies have shown that PCR is a specific and more sensitive method in the diagnosis of trypanosomosis in experimental as well as natural infections (Desquesnes, 1997; Clausen et al., 1998; Masake et al., 1997, 2002; Bengaly et al., 2002; Gall et al., 2004). In actual fact, two periods of the trypanosome infection process must be distinguished; early in an infection, parasitological and PCR techniques show a very similar sensitivity (80%), but during the chronic phase of infection, parasitological examination exhibits a very low sensitivity (<10%) and PCR then is two to three times more sensitive than the parasitological methods; the test is still of low sensitivity, since 70–80% of the tests remain negative for infected animals (Desquesnes, 1997).

In field samples, both acute and chronic infections are present in different proportions; infection rates depend on the epidemiological situation (epidemic/endemic). In the Sideradougou area of Burkina Faso, an epidemiological survey carried out in 1000 herds of cattle indicated a parasitological prevalence of 5.3%; a representative sub-sampling of 260 samples tested with PCR indicated a prevalence of 11.5% (Desquesnes and Davila, 2002). In another study, carried out in 76 goats in The Gambia, the parasitological prevalence was 8% against 24% with PCR (Perreira De Almeida et al., 1998). In such studies, the rate of positive samples by PCR is generally two to three times higher than that by the buffy coat method (Desquesnes and Davila, 2002; Gall et al., 2004). These field observations in endemic areas are in agreement with results obtained during the chronic phase of experimental infections.

In an experimental study, the PCR did detect trypanosomes in infected cattle as early as 5 days post-infection (Masake et al., 2002). The detection and identification of trypanosomes by molecular means should as a principle always be based upon stable, parasite-specific genetic characteristics (Eisler et al., 2004).

3.1.4 Management of bovine trypanosomosis

3.1.4.1 Trypanocidal drugs

In the 37 African countries where animal trypanosomosis is endemic, trypanocides are used for the control of the disease (Geerts and Holmes, 1998). Drugs have proven sustainably and sufficiently attractive to the livestock keepers. Three compounds - isometamidium chloride (ISMM), homidium salts (homidium bromide (Ethidium®) and homidium chloride (Novidium®)) and diminazene aceturate (DIM) have been and are still in use more than 50 years since they were released in the market (Holmes et al., 2004).

3.1.4.1.1 Prophylactic treatments

Prophylactic treatments target all animals in a herd or a particular group of valuable or 'at-risk' animals (Holmes et al., 2004). Isometamidium (ISMM) administered intramuscularly (i.m.) at a dose rate of 0.5-1mg/kg b.w provides up to 3 months' (range of between 2-22 weeks) protection against pathogenic trypanosomes of cattle and against *T. vivax* especially in small stock, *T. brucei* in equidae and *T. evansi* in camels (Peregrine, 1994; Geerts and Holmes, 1998; Geerts et al., 2001). Isometamidium is given either as routine block treatment (pre-determined intervals) or as strategic block treatment (when challenge reaches a pre-determined threshold). It is recommended that once a year, additional to ISMM, the animals are separately treated with DIM in order to delay the development of resistance, following the concept of 'sanative pair' (Whiteside, 1962).

3.1.4.1.2 Curative treatments

Diminazene aceturate (DIM) and Homidium (HOM) salts are the main therapeutic drugs used in the management of clinical trypanosomosis in animals (Holmes et al., 2004). Diminazene aceturate is administered i.m. at a dose rate of 3.5-7mg/kg bw the lowest dose being effective against *T. congolense* and *T. vivax* and the highest dose against *T. brucei* (Peregrine, 1994; Geerts and Holmes, 1998). At these dose rates, DIM, in addition to its curative uses, also offers short term protection of up to 2 weeks (Geerts et al., 2001). Homidium on the other hand is administered at a dose rate of 1mg/kg bw in cattle. In low tsetse challenge areas, a prophylactic effect of HOM salts has also been observed (Peregrine, 1994).

3.1.4.2 Tsetse control

Controlling the vector remains theoretically the most desirable way of containing trypanosomosis (Leak, 1999). Jordan (1986) shared the same view by stating that 'only by removal of the tsetse fly can a disease-free environment be created. It is a strategy that has worked well in many areas where multiple drug resistance has been reported before (Fox et al., 1993; Peregrine et al., 1994; Bauer et al., 1995). Vector control methods available include:

3.1.4.2.1 Sequential aerosol technique (SAT)

The SAT involves the ultra-low volume spraying of non-residual insecticides 10-15 metres above tree canopy by fixed wing aircraft or helicopter (in more difficult terrain) in 5-6 subsequent spraying cycles, separated by 16-18 days depending on temperature (Allsopp and Hursey, 2004). The goal is to kill all adult tsetse flies in the first spraying cycle and then kill all emerging flies in the subsequent cycles before they start reproducing. Insecticide application occurs during periods of temperature inversion, i.e. at night. It remains a perfect method if done under global positioning system navigation (GPS), especially for effective area-wide tsetse suppression (the efficacy of this technique still needs to be assessed in dense humid forest ecosystems) or even eradication (in open savannah-type ecosystems) (Allsopp and Hursey, 2004). The disadvantage with the method is that insecticides sprayed may also kill non-target insects.

3.1.4.2.2 Stationary attractive devices (traps and targets)

Stationary attractive devices (SADs) attract and either kill the flies through tarsal contact with insecticides embedded in the fabric or the flies are guided and trapped in a non-return cage (Reichard, 2002). The method exerts an additional daily mortality of 2-3% to the female segment of the fly population (Leak, 1999). Several technical aspects are essential for the efficient application of this technology such as appropriate trap/target site selection, adequate maintenance, periodic replacement and replenishment of the odours, appropriate reflectivity pattern of the used cloth, degradation of the insecticide deposits by UV light, among others (Vreysen, 2001). The technique is suitable for deployment by local farmer communities to treat small areas, but the high target densities required against certain species and in certain dense habitats make the use of these devices over large areas uneconomical (FAO, 1992;

Kappmeier et al., 2007). Polyester/cotton fabrics are highly efficient if used as screens/targets (Laveissière et al., 1987). Closely woven fabric with thin thread allows for good insecticide fixation, although this prevents tsetse flies from receiving a lethal dose (Leak, 1999).

The major disadvantage of SADs is that the active ingredient gets washed off by rain water, hence compromising its efficacy (Torr et al., 1992). Increasing the concentration of insecticide to 0.6-0.8% allows the SADs to remain deployed even during the wet season, retaining tsetse mortality rates of > 90% for about 300 days (Torr et al., 1992). Oil formulations of lambda-cyhalothrin have been reported to result in a 100% knock-down effect for up to 10 months and 100% mortality after 24h for 8 months compared with periods of 4 months and 3 months, respectively, for wettable powder formulations (Langley et al., 1992). Blue fabrics lose colour and become inefficient after a short time, depending on the dye used and method of fixation. Theft of the targets, bush fires and maintenance problems are some further problems associated with use of SADs (Leak, 1999).

3.1.4.2.3 Live bait technique

This method involves the application of insecticide onto cattle as pour-ons, sprays or dips so that tsetse flies attempting to feed on the treated cattle get killed on picking up a lethal deposit of the insecticide through their tarsi and pre-tarsi. Live baits were used in Zimbabwe against *Glossina pallidipes* Austen (Hagrove et al., 2000), in Burkina Faso against *G. morsitans submorsitans* Newstead and *G. palpalis gambiensis* Vanderplank (Bauer et al*.,* 1992, 1995, 1999), against *G. fuscipes fuscipes* Newstead and *G. pallidipes* in Ethiopia (Leak et al., 1995) and against *G. m. morsitans* in Zambia (Van den Bosche et al., 2004). Unlike SADs, the live bait technique is less prone to theft and does not suffer from maintenance problems. Because of its added advantage of also controlling ticks, the use of live baits is appreciated as a private good and can easily be adopted by the rural farming community (Bauer et al., 1995; Torr et al., 2001; 2002).

The required cattle density for effective tsetse control, the proportion of a herd that requires treatment, host preference of different tsetse species, among other factors, still require further research (Bourn et al., 2005). Other disadvantages with the live bait treatment schemes are the high treatment frequency, the high cost of the insecticides, insecticide residues in cattle dung, motivation and participation of farmers and the potential development of resistance to the

insecticides in ticks and insects with a high reproductive rate (Wardhaugh et al., 1998; Vale and Grant, 2002). It is also thought that the use of insecticides on live animals has the profound effect of interfering with the enzootic stability that is manifested in young indigenous cattle when exposed to tick challenge (Bourn et al., 2005). Since the attachment sites of ticks and the feeding sites of tsetse differ, treating only legs and bellies of older cattle will help, as >95% of tsetse feed come from on adult cattle (Torr et al., 2001). This approach will more effectively control tsetse without threatening the enzootic stability in calves.

3.1.4.2.4 Sterile insect technique (SIT)

The SIT is used if the objective is tsetse eradication. As was the case in the island of Zanzibar, the introduction of the SIT helped eradicate the fly from this island in 1996 in a campaign that had been commenced two years earlier (Reichard, 2002). As a prerequisite, tsetse density has to be suppressed through the widespread application of insecticide treated SADs, live baits or fly trapping to a point where the SIT is considered feasible. In Zanzibar, a sterile insect plant producing 70,000 irradiated pupae weekly was constructed that made the release of over 7.8 million sterile male flies possible. Dispersal of the irradiated males over time was done to achieve an estimated ratio of 50 sterile males for every 1 wild male in order to overwhelm the residual wild tsetse population (Reichard, 2002). The released sterile males in the target area do out-compete the wild male population for wild females (Vreysen, 2005). Mating of the sterile males with virgin, native females results in no offspring. With each generation, the ratio of sterile to wild insects will increase, making this technique more and more efficient with lower wild female population densities (inversely-density dependent).

The SIT is non-intrusive to the environment, has no adverse effects on non-target organisms, is species-specific and can easily be integrated with biological control methods such as parasitoids, predators and pathogens (Leak, 1999). There is no threat of resistance development to the effects of sterile males, provided that adequate quality assurance is assured during the production process and that the sterile insects cannot get established in released areas as is the case with other biological control programmes (Vreysen, 2001). In addition, the SIT necessitates efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen, 2005).

Since the irradiated tsetse are fully capable of developing and transmitting mature trypanosomes of all three main species pathogenic to cattle (Moloo and Kutuza, 1984), the sterilized tsetse are fed on either uninfected blood meals or blood-meals are medicated with trypanocidal drugs before the sterilized insects are fed. Sterilized tsetse are less likely to become infected (at least with *Nannomonas* and *Trypanozoon* parasites) after they have taken an uninfected blood meal and trypanocidal drugs in medicated meals helps reduce the establishment of infections in subsequent meals.

3.1.4.3 Trypanotolerant breeds

Trypanotolerance, the ability of some species and breeds of livestock to survive, reproduce and remain productive under trypanosomosis risk with minimal trypanocidal drug treatment requirements, was recognized and exploited by farmers long before research on trypanotolerance began (d'Ieteren, 2001). Trypanotolerant breeds control the development of parasitaemia and limit the pathological effects, the most important of which is anaemia (d'Ieteren et al., 1998). N'Dama cattle acquire significant control of *T. vivax* infections, but apparently not against *T. congolense* (Trail et al., 1994). Due to the uncertain genetic make-up of animals within these so called trypanotolerant breeds, the level of tolerance may also vary between individual animals within a breed and it can be overcome by heavy tsetse challenge, malnutrition, or other stress factors (pregnancy, lactation, draught power, effect of other infections and diseases) (Murray et al., 1982). Some indigenous breeds of small ruminants, notably the West African Dwarf sheep and goats and the East African goats also exhibit some degree of trypanotolerance (Murray et al., 1982).

3.1.5 Trypanocidal drug resistance

3.1.5.1 Definition

Drug resistance is the heritable loss of sensitivity of a micro-organism to a drug to which it was sensitive to before. Trypanocidal drug resistance is caused by the exposure of trypanosomes to sub-therapeutic drug concentrations, resulting from under-dosing and the irrational use of drugs and the lack of proper diagnosis (Whiteside, 1962). The prolonged and frequent use of trypanocides in high tsetse challenge areas, even when used at the right doses, is also likely to cause resistance (Clausen et al., 1992; Geerts and Holmes, 1998). Two types

of resistance against trypanocidal drugs are recognized: single drug resistance and multiple drug resistance. In single drug resistance, trypanosomosis control still could be achieved by using one of the drug pairs in which resistance has not developed through the application of the sanative pair principle (Geerts and Holmes, 1998). However, the second drug should be used with caution in order to avoid resistance development against it as well. Multiple drug resistance is resistance concurrently to two or more drugs, making sanative drug pairs ineffective. Multiple drug resistance can only be counteracted by intervening at the level of the vector (Fox et al., 1993; Peregrine et al., 1994; Geerts and Holmes, 1998).

3.1.5.2 Current situation of resistance

Currently, there are close to 20 African countries in which resistance has already been reported (Delespaux et al., 2008). In addition to the 13 countries mentioned by Geerts and Holmes, (1998), resistance has been reported in Mozambique (Jamal et al., 2005), Mali, Guinea (Diall et al., 2003; Grace, 2005), Cameroon (Mamoudou et al., 2008) and recently in Ghana and in Benin (Allegye-Cudjoe, 2009). It is suspected that in several other African countries, resistance is present but is yet to be demonstrated (Delespaux et al., 2008). Large-scale surveys have been conducted in 13 African countries including Kenya (Mdachi, 1999; Murilla et al., 2002), in Uganda and Tanzania (Eisler et al., 2000), in Ethiopia (Afework et al., 2000; Tewelde et al., 2004), in Zambia (Shinyangwe et al., 2004), in Zimbabwe (Joshua et al., 1995), in Cameroon (Mamodou et al., 2008), in Nigeria (Geerts et al, 2001), in Burkina Faso (McDermott et al, 2003), in Mali and Guinea (Diall et al., 2003; Grace, 2005) and in Ghana and Benin (Allegye-Cudjoe, 2009) demonstrating area-wide resistance in at least one region of these countries.

Confirmed reports about resistance in the cotton zone of West Africa were first made particularly in Burkina Faso in the early 1980s (Pinder and Authie, 1984; Authie, 1984). These authors described stocks of *T. congolense* isolated from cattle in the Samorogouan area in 1982 which were resistant to isometamidium. Later, tests in mice showed that certain *T. congolense* strains from the same area were also resistant to diminazene aceturate indicating existence of multiple drug resistant *T. congolense* (Authie, 1984). Clausen et al. (1992) working in the same area, confirmed the existence of multiple drug resistance. Recent studies on resistance in the same area of Burkina Faso (McDemott et al., 2003; Gall et al., 2004;

Knoppe et al., 2006) and in other areas of West Africa underline that the problem is present and expanding (Diall et al., 2003; Allegye-Cudjoe, 2009; Grace 2009).

3.1.5.3 Mechanisms involved in resistance to ISMM and DIM

ISMM is known to accumulate in two compartments of trypanosomes, the cytoplasm and the kinetoplast (Wilkes et al., 1997). Decreased levels of ISMM accumulation have been observed in drug-resistant populations of *T. congolense* (Sutherland et al., 1991) and later work found indirect evidence of an increased efflux of this drug from resistant trypanosomes (Sutherland and Holmes, 1993). Mulugeta et al. (1997) showed that maximal uptake rates (Vmax) of ISMM in resistant *T. congolense* were significantly lower than in sensitive populations. The role of nucleoside transporters in resistance to ISMM by *T. congolense* remains to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* (Carter and Fairlamb, 1993; Carter et al., 1995; Ross and Barns, 1996). Changes in the mitochondrial electrical potential (MEP) have been demonstrated in isometamidium-resistant trypanosomes (Wilkes et al., 1997).

The accumulation of DIM has been shown to be markedly reduced in arsenical-resistant *T. brucei*, *T. evansi* and *T. equiperdum* due to alterations in the P2-type purine transport system (de Konig and Jarvis, 1999). In addition to this resistance mechanism, a novel gene, T*e*DR40, might be a factor contributing to high DIM-resistance in *T. evansi* (Witola et al., 2004). It is suspected that DIM resistance is multi-factorial and that the T*e*DR40 gene might be a contributing factor to resistance linked to the alteration of the gene coding for the P2-type purine transporters. A putative P2-type purine transporter T*co*AT1 in *T. congolense* was identified by reciprocal blasting of the T*b*AT1 gene of *T. brucei* and a conserved Val306 to ile306 permutation in this gene was observed in *T. congolense* strains that show resistance to DIM (Delespaux et al., 2006).

3.1.5.4 Detection of drug resistance

3.1.5.4.1 Field methods

Eisler et al. (2000) proposed a method for the assessment of prevalence of resistance to isometamidium chloride by monitoring cattle populations under natural challenge in the field. Briefly, two groups consisting of 30 to 80 cattle each are used. One group is treated with 1 mg/kg bw ISMM and the other is used as untreated control. The two groups then are exposed to natural challenge and tested for trypanosomes using the phase contrast buffy coat technique (BCT) (Murray et al., 1977) every two weeks for two to three months. A comparison through survival analysis curves is made on the data of new trypanosome infections between the group of cattle treated with ISMM and the untreated control group (Eisler et al., 2000; Tewelde et al., 2004). If >25% of the ISMM treated cattle become infected within 8 weeks of exposure, drug resistance is strongly suspected (Mdachi, 1999; Eisler et al., 2000; Tewelde et al., 2004).

Several epidemiological studies to map field trypanocidal drug resistance, based on the protocol by Eisler et al. (2000), have been documented. McDermott et al. (2003) working in the Kénédougou Province of Burkina Faso, Shinyangwe et al. (2004) working in Eastern Zambia, Tewelde et al. (2004) in Ethiopia, Grace (2005) in Guinea and south-eastern Mali and Allegye-Cudjoe (2009) in Ghana and in Benin are some examples.

An abbreviated version of the original 8-12 week-protocol by Eisler and colleagues was validated in the cotton zone of West Africa and found effective and reliable (Diall et al., 2005) for use not by researchers but by the national veterinary services. This involves a 4-week long follow-up (Diall, 2005) period in order to reduce costs and still generate data within a very short time. The abbreviated protocol is effective in areas where trypanomosis risk is high (prevalence is >10%) as has been demonstrated in the cotton zone of West Africa (Diall et al., 2003; Grace 2005). Rowlands et al. (1993) developed a model to distinguish new and recurrent infections to determine if the high infection rates observed in cattle in the Ghibe valley, south-west Ethiopia, following treatment of *T. congolense* infections with diminazene aceturate were due to the tsetse challenge or if they rather were a relapse of infections following treatment. An infection was defined as new if it was preceded by two previous months in which monthly collected samples had packed cell volumes (PCV) of >= 26% and in which trypanosomes were not detected.

3.1.5.4.2 Drug sensitivity studies in experimental animals

Tests in ruminants (Eisler et al., 2001)

Neither the single-dose nor the multiple-dose tests in mice are able to predict accurately the curative doses of trypanocidal drugs needed to clear trypanosome populations from infected cattle (Eisler et al., 2001). The test in ruminants should hence be used to just determine whether or not drugs are principally efficacious at recommended curative doses in cattle infected with a particular trypanosome populations. The test in calves may further be used for investigations on drug resistance in *T. vivax*, which is usually not infective for mice.

A group of cattle or small ruminants, preferably of a breed native to the area and without prior exposure to tsetse or trypanosomosis are used (Eisler et al., 2001). They should also be negative for anti-trypanosomal antibodies as determined by the indirect fluorescent antibody test or ELISA (Luckins and Mehlitz, 1978) if these tests are available. Specific detailed protocols on this are as contained in Eisler et al. (2001). Due to individual variation in the response to trypanocidal drug treatment among ruminants inoculated with the same *T. congolense* isolate (Peregrine et al., 1991; Ndoutamia et al., 1993; Kone, 1999), it is advisable to use a minimum of three and preferably six animals. However, economic considerations may often preclude the use of more than a single animal per stabilate for drug-sensitivity testing.

The experimental animals must be kept in a fly-proof stable or in a non-tsetse infested area to eliminate the risk of reinfection during the study. A breakthrough infection, indicative that one of the inoculated trypanosome populations was drug-resistant can be inoculated into a group of calves and mice to determine the level of drug resistance. A variation of this method also exists whereby blood from a group of infected cattle is pooled and inoculated into a single recipient calf which is monitored and later, if parasitaemic, treated with trypanocide at the recommended dose. This technique is appropriate where laboratory facilities are limited but only allows for a qualitative assessment of resistance. Further constraints of the technique are that not all trypanosome populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together (Sones et al., 1989); this however, is not a consistent observation (Burudi et al., 1994).

A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and detection of breakthrough populations of trypanosomes. The shorter the period, the greater the level of resistance (Ainanshe et al., 1992).

Tests in mice

Either single-dose or multi-dose tests are conducted in mice to provide information on resistant trypanosome isolates from a given area, as described in the protocol by Eisler et al. (2001). After expansion of an isolate in a donor mouse, experimental mice are inoculated with the test trypanosome isolate and treated with a trypanocidal drug. Tail blood wet smears are checked 2-3 times per week for parasites for a period of up to 60 days. The ED50 and ED95 (effective dose that gives temporary clearance of the parasite in 50% or 95% of the animals, respectively) can be calculated as can the CD50 and CD95 (curative dose that gives complete cure in 50 and 95% of the animals, respectively). Sones et al. (1988) used a group of five mice, which allowed an easy calculation of ED80 and CD80 values (one out of five mice not cleared or cured).

Knoppe et al. (2006), using the standard mouse test (SMT), screened a number of *T. congolense* isolates collected in the Kénédougou Province of Burkina Faso against isometamidium chloride at a dose of 0, 0.25, 1.0, 5.0, 10, 15 or 20 mg/kg bw and found the method very sensitive but labour intensive.

There are however several disadvantages with this method. Firstly, most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice (Holmes et al., 2004). Secondly, although there is a reasonable correlation between drug sensitivity between mice and cattle, higher doses of drugs must be used in mice (normally ten times higher) in order to obtain results comparable to those from cattle, because of the vast different metabolic size (Sones et al., 1988). Thus, the curative dose for ruminants cannot be extrapolated from the assay results in mice (Sones et al., 1988). Thirdly, a danger further exists of selecting against particular trypanosome species, particularly in mixed infections. Fourthly, precise assessment of resistance requires a large number of mice per isolate. Finally, it takes as long as 60 days to evaluate the drug sensitivity of an isolate.

3.1.5.4.3 In vitro assays

Since the review of Kaminsky and Brun (1993) further progress has been made in the field of in vitro assays to determine the drug sensitivity of trypanosomes. In vitro assays use blood stream or metacyclic forms instead of procyclic forms. This technique has been used to detect resistance in *T. brucei* and *T. congolense* (Gray et al., 1993, Clausen et al., 2000). It takes up to 40 to 50 days of in vitro incubation to generate metacyclic trypanosomes (Gray et al., 1993). The advantage with this technique is that large numbers of isolates can be examined and tests with metacyclic trypanosomes correlate well with field observations. However, in vitro cultivation of bloodstream forms is only possible using pre-adapted lines and not isolates directly from naturally infected animals (Hirumi et al., 1993). A simplified axenic culture system has been developed by these authors, but further research is still necessary to study the correlation with field data. A potential problem associated with this lengthy time adaptation is the possible selection against trypanosomes that possess the phenotype of the original population. Further, in vitro assays are quite expensive and require good laboratory facilities and well-trained staff.

The drug incubation infectivity test (DIIT) combining both in vivo and in vitro techniques is suitable for drug sensitivity testing of *T. b. brucei*, *T. evansi* and *T. vivax* (Kaminsky et al., 1990). It was modified by Sutherland et al. (1991) and it proved suitable for *T. congolense.*

3.1.5.4.4 Xenodiagnosis

Xenodiagnosis is the feeding of a clean susceptible vector species on a suspected case of trypanosomosis, after which it is either dissected and examined for the presence of infection, or allowed to feed on a clean animal which then is itself examined for the development of infection. A modification of this approach, the drug incubation *Glossina* infectivity test (DIGIT), in which trypanosomes are exposed to the trypanocidal drugs in vitro for a short time and thereafter are fed to tsetse flies to check whether or not they develop into metacyclic forms was successfully validated and proved sensitive for detecting drug resistance (Clausen et al., 1999). This technique distinguishes resistant from sensitive isolates and does not require experimental animals. However, it does require a ready supply of teneral tsetse flies from an artificially reared colony.

3.1.5.4.5 Serological techniques

Although not frequently used, enzyme linked immuno-sorbent assay (ELISA) has proved valuable in diagnosing isometamidium resistance (Eisler et al., 1996; Geerts et al., 1999; Murilla et al., 2002). The use of ELISA in the detection of ISMM in the serum of cattle can be combined with field block treatment studies or for individual treatment of ruminants to detect resistant trypanosomes (Eisler et al., 1996). The presence of trypanosomes in animals with an ISMM serum concentration > 0.4ng/ml suggests that parasites are resistant (Eisler et al., 1997). Similar drug-ELISAs have been developed for the detection of sub-nanogramme amounts of homidium and diminazene (Holmes et al., 2004). A closely related technique to drug-ELISAs is the mitochondrial electrical potential (MEP) which determines the rate of ISMM accumulation in the trypanosome kinetoplast (Wilkes et al., 1997).

3.1.5.4.6 Molecular techniques

Polymerase chain reaction (PCR)

Because of the problems associated with the low sensitivity of the parasitological techniques (Paris et al., 1982) and the long follow-up time of study animals (Eisler et al., 2001), PCR with high sensitivity and specificity is a good solution to these problems. Gall et al. (2004) used this method in Burkina Faso and found it four times more sensitive compared to the field parasitological techniques.

PCR-RFLP

Molecular methods for the diagnosis of ISMM resistance were recently developed (Delespaux et al., 2005, Afework et al., 2006). The first method enables discrimination between ISMM-sensitive and ISMM-resistant strains of *T. congolense* by M*bo*II-PCR-RFLP (Delespaux et al., 2005). This test is based on the polymorphism observed in the 381 bp fragment (in sensitive strains) or the 384 bp fragment (in resistant strains) of a putative gene presenting some homologies with an ABC transporter. The second method has been developed to distinguish ISMM-resistant from ISMM-sensitive strains of *T. brucei* (Afework et al., 2006). This S*fa*NI-PCR-RFLP test is based on the polymorphism of the 677 bp fragment of the T*b*AT1 gene. The same set of six point mutations could confer resistance to the melarsenoxide cysteamine cymelarsan (an arsenical diamidine) and to ISMM (diamidine compound) and the detection of

one of these six mutations could enable reliable identification of sensitivity or resistance to ISMM (Mäser et al., 2003).

3.2 Helminths in cattle

3.2.1 Relevance of helminth infections

The effects of gastrointestinal parasitism in ruminants are impaired production and even death. Where estimates of economic losses on a large-scale have been attempted, it is evident that more precise information on the various features of production losses are needed before accurate fiscal losses may be calculated (Parkins and Holmes, 1989). Heavy untreated helminth infections may be fatal in calves. In adult cattle, helminth infection is sub-clinical and causes poor productivity, is reason for premature culling and makes animals unsuitable as replacement breeding stock (Murphy et al., 2006). In sub-Saharan Africa (SSA), the impact of gastro-intestinal helminths could be higher compared to other zones due to the wider range of agro-ecological factors suitable for very diversified hosts and parasite species (Sissay et al., 2006).

3.2.2 Trematoda

3.2.2.1 Family Fasciolidae

These are large leaf-shaped flukes that are hermaphroditic, with potential for cross-and self-fertilization (Urquhart et al., 1996). They occur in the liver and bile ducts of ruminants. There are 3 important genera: *Fasciola*, *Fascioloides* and *Fasciolopsis*. Of the three, the genus *Fasciola* consisting of *Fasciola hepatica* and *F. gigantica* is of veterinary importance (Urquhart et al., 1996). *Fasciola* species have zoonotic importance (Mas-Coma 2005).

Fasciola hepatica whose intermediate host is the *Galba truncatula* snail occurs in the mild cold climates typical of temperate climates (Mas-Coma and Bargues, 1997). In sheep, acquired immunity against *Fasciola* species is poorly developed leading to severe pathology upon infection with these parasites (Mas-Coma and Bargues, 1997). In sheep, *Fasciola* species can live for as long as 11 years. In cattle, *F. hepatica* causes a self-limiting disease,

since infected cattle acquire immunity causing most flukes to be eliminated within 9-12 months (Mas-Coma and Bargues, 1997; Spithill et al., 1999). Unlike in sheep, the duration of egg output in cattle lasts only a few weeks.

Fasciola gigantica is an important parasite for the tropics and occurs throughout western, sub-Saharan and eastern Africa (Hansen and Perry, 1994; Wamae et al., 1998; Keyyu et al., 2005). Its preferred intermediate host is the Lymnaeid snail *Radix natalensis*. Other intermediate hosts for *F. gigantica* in eastern and southern Africa include *Galba truncatula* and *Pseudosuccionea columella* (Brown, 1994).

Fasciola species have an indirect life cycle with the snail as the intermediate host (Urquhart et al., 1996). Eggs are passed out in ruminant faeces before they hatch into a ciliated motile miracidia in the environment. Soon after hatching, miracidia enter snails to develop into redia and then cercariae. Motile cercariae are shed by the snails and attach on to grass blades to encyst, forming metacercariae, the infective stage. This process takes a minimum of 6-7 weeks for completion from miracidium to metacercaria, although under unfavourable circumstances a period of several months is required. An infection of a snail with one miracidium can produce over 600 metacercariae. Definitive hosts get infected by ingesting the metacercariae together with grass. *Fasciola* species have a long a pre-patent period of up to 10-16 weeks (Urquhart et al., 1996).

Fasciolosis causes high mortalities especially in small ruminants and calves (Hansen and Perry, 1994; Maingi et al., 1997; Mas-Coma and Bargues, 1997; Wamae et al., 1998). Other losses associated with fasciolosis include total condemnation of infected livers or partial condemnation after trimming off affected parts of the liver, which reduces the volume sold (Kithuka et al., 2004; Mungube et al., 2006). Other indirect losses include reduced weight gains, poor feed utilization, poor quality of meat and milk products, some of which are difficult to quantify.

3.2.2.2 Family Paramphistomatidae

Paramphistomum species occur in the forestomachs of ruminants, although a few can also be found in the intestines of ruminants, pigs and horses (Urquhart et al., 1996). They are conical

in shape and use water snails as intermediate hosts. Several genera of paramphistomatidae exist but the *Paramphistomum* species is the most common and wide spread.

The development in the snail intermediate host is similar to that of *Fasciola* species and the life cycle is completed in four weeks. The parasite causes diarrhoea accompanied by anorexia and intense thirst in heavy infestations (Urquhart et al., 1996). There may also be rectal haemorrhage with mortality of up to 90% in acute outbreaks.

3.2.2.3 Family Dicrocoelidae

The lancet liver fluke *Dicrocoelium* species lives in the bile ducts of many mammals during its adult stage in bovidae, particularly cattle, goats, sheep and deer. The lancet liver fluke is widely present in backward areas of Europe and Asia, and is sparsely distributed in Africa, the New World and Australia (Smyth, 1994; Otranto and Traversa, 2002; 2003). Actual cases in humans are exceptional because of the biology of the parasite. The discovery of *Dicrocoelium* species eggs in human faecal samples is often a sign of pseudo-parasitosis, resulting from the consumption of animal livers infected by the lancet liver fluke (Otranto and Traversa, 2002; 2003).

The entire life cycle of the lancet liver fluke was elucidated in 1952–1953 by Krull and Mapes who discovered that two intermediate hosts were necessary for complete parasite development: first a land snail (*Zebrina* species, *Helicella* species, *Cionella* species), then an ant (*Formica* species, *Lasius* species). Final host infection occurs by ingesting ants infected with the parasite at the larval stage. Normally such ants have abnormal behaviour and are found biting and sticking onto grass blades (Smyth, 1994). Severe dicrocoeliosis is not necessarily accompanied by clinical symptoms but may cause weight loss, anaemia and cirrhosis, which can result in important economic losses in herds in terms of milk and meat production (Kaufmann, 1996).

3.2.3 Cestoda

3.2.3.1 Family Anoplocephalidae

These are tapeworms of herbivores. They have a scolex with neither rostellum nor hooks. The gravid segments are wider than they are long. In ruminants, *Moniezia* species found in the small intestine of sheep, goats and cattle are the most important. The life cycle is indirect and involves mites of the orbatidae family which ingest eggs that develop into a cysticercoid within 2-4 months (Urquhart et al., 1996). The definitive hosts get infected by ingesting infected mites with the herbage. While a variety of clinical signs, including unthriftiness, diarrhoea, respiratory signs and even convulsions may occur, *Moniezia* species infection is generally symptomless.

3.2.4 Nematoda

3.2.4.1 Order Rhabditida

This consists of a primitive group of small intestinal nematodes which are mostly free living or are parasitic in lower vertebrates and invertebrates (Dorris et al., 2002). *Micronema, Rhabditis* and *Strongyloides* are the most important species, with the latter being an important genus from the veterinary point of view (Eberhardt et al., 2008).

Strongyloides infections found in the small intestines of very young animals are common in most domestic ruminants (Pienaar et al., 1999; Wymann et al., 2007; Eberhardt et al., 2008). Although these infections often proceed without clinical symptoms (Pienaar et al., 1999), they may also cause severe enteritis which even can be fatal (Taira and Ura, 1991; Pienaar et al., 1999).

The life cycle is unique, as it has alternate free-living and parasitic generations. The eggs may hatch into larvae that develop into free-living adult male and female worms. Parasitic males do not exist and parasitic females do not contain male gonads. The filariform parasitic female produces eggs by mitotic parthenogenesis (not from fertilized eggs) and the larvae that hatch from these eggs are termed homogonic rhabditiform larvae to distinguish them from the heterogonic offspring of the free-living, sexual generation (Bowman, 1999).Larval stage 3 is

the infective stage and infects the definitive host through skin penetration or via ingestion and migrates via the venous system, lungs and trachea to develop into female adult worms in the small intestines.

3.2.4.2 Order Strongylida

This consists of nematodes that cause trichostrongylidosis in ruminants, depending on the quantity and species of worms present, the general health, the nutritional and immunological status and the age of the animal (Waller, 2006). These nematode species occur mostly as mixed infections of different GIN species. Adult worms live in the abomasums, the small intestine, large intestines. Emaciation, persistent diarrhoea, respiratory symptoms and weight loss are usually the main symptoms. Villous atrophy results in impaired digestion and malabsorption of nutrients. This leads to decreased live-weight gain, fibre and milk production and reproductive performance and therefore has serious impact on animal health and productivity (Perry and Randolph, 1999). Among the prominent members in this order strongylida are trichostrongylidae (abomasums and small intestines) that include *Haemonchus* species, *Trichostrongylus* species, *Teladorsagia* (*Ostertagia*) species, *Cooperia* species and the family is Molineidae in which *Nematodirus* species belong. *Bunostomum* species, with prominent leaf crowns surrounding the mouth opening and a large buccal capsule containing a pair of cutting plates also reside in the small intestines (Urquhart et al., 1996). These are hookworm of ruminants. Chabertiidae (*Chabertia* and *Oesophagostomum* species) are also members of the trichostrongyloids found in the large intestines. *Dictyocaulus viviparous*, a respiratory system nematode also belongs to the order strongylida although some taxonomists have classified it under metastrongyloidea (Bowman, 1999).

Strongylida have a direct life cycle which is similar in all species and enable the worms to be readily transmissible in livestock (Urquhart et al., 1996). The prepatent period is approximately 20 days. The female worms lay eggs, which are excreted into the environment with the faeces. Under appropriate conditions, the eggs develop into first stage larvae (L1), second stage larvae (L2) and finally into infective third stage larvae (L3) that are ingested by the host during grazing. These larvae exsheath and migrate to their final location in the host's gastrointestinal system, where they develop into L4 and then into adult female or male worms. Under appropriate conditions of humidity and temperature the developmental stages, eggs and L1-L3 can survive several months on the pasture.

Adult *Haemonchus* species are attached to the abomasal mucosa and feed on blood, causing anaemia and even death, making *H. contortus* one of the most pathogenic nematodes of ruminants (Urquhart et al., 1996). Another reason that makes *H. contortus* dangerous is its ability to rapidly develop resistance against anthelmintics (Coles et al., 2005) and the severe anaemia it causes can excerbate the pathological effects in animals infected with *Trypanosoma congolense* (Kaufmann et al., 1992). *Teladorsagia* (*Ostertagia*) causes gastritis in cattle and has also been associated with anthelmintic resistance (Kaplan, 2004; Coles et al., 2005; Demeler et al., 2009). Adult *Bunostomum* species are blood suckers and 100-500 worms will produce anaemia, hypoalbuminaemia, weight loss and occasionally diarrhoea. In calves, skin penetration is accompanied by foot-stamping and signs of itching (Urquhart et al., 1996; Radostits et al., 2002).

3.2.4.3 Order Ascaridida

Toxocara vitulorum, a large nematode infecting various bovinae species (Urquhart et al., 1996) is the classical example of ascaridida in cattle. Adult nematodes develop sometimes up to 40cm in length in the small intestines of young calves. They are thick worms with pinkish colour, when fresh with a transparent cuticular that allows a clear view of the internal organs. Female worms can lay up to 100,000 eggs per day (Roberts, 1990a). Calves over 6 months get infected through ingestion of larvated eggs in contaminated water, soil and pasture, while calves < 6 months get infected from the milk of the dam in which larvae might persist up to 30 days post-parturition (Roberts, 1989a; Urquhart et al., 1996). Infective eggs hatch and larvae migrate through different organs, most of them getting stored in the liver. In pregnant cows, resumption of larvae development occurs late in pregnancy and movement to the udder around calving time. The larvae pass out in milk into the newborn suckling calves (Roberts, 1990b). The half-life of larvae in the tissues of the cows is estimated to be about one to two years, so that cows may infect several successive calves even if they are placed in *T. vitulorum*-free environment (Roberts, 1993).

The main clinical signs are due to the adult worms in the intestines of calves up to six months of age. Heavy infestations are associated with poor thriving and intermittment diarrhoea, and in buffalo calves may cause fatalities.

3.2.5 Diagnosis of helminth infections

3.2.5.1 Qualitative coprological techniques

3.2.5.1.1 Sampling method

Faecal samples for diagnosis of helminths in cattle should be collected from the rectum or just after being passed to avoid contamination from free living nematodes or other elements in the environment, which might skew the diagnosis (Kaufmann, 1996). Soon after collection of a sufficient amount of faeces, each sample should be well labelled with animal identity, breed, sex, date of collection and, if possible, herd identity. The samples ought to be delivered to the laboratory for processing in an ice-packed cool box and where examination is to be delayed, stored refrigerated at 4° C to slow down development of the parasites. Some formal saline (8% formolized water) could also be mixed in a faecal sample for preservation, although this method is not advisable for larval culture faecal samples.

3.2.5.1.2 Sedimentation

The principle of this method is to dilute the faecal sample in a low-density watery solution so as to concentrate parasite eggs, which are of a higher density, in the beaker or test tube pellet. The collected faeces are evaluated for trematode eggs using the sedimentation method as described in Urquhart et al. (1996). Briefly, 5-10g of freshly collected faeces is mixed in a 100 ml beaker with the help of a spatula. The suspension is then strained through a tea strainer before thoroughly washing the material retained on the strainer using a fine water jet. The filtrate is transferred into a conical flask and allowed to stand for 2-3 minutes before the supernatant is decanted carefully and the remaining sediments transferred into a flat-bottomed tube. After sedimentation for a further 2-3 minutes, the supernatant is again drawn off, a few drops of 5% methylene blue solution are added and the sediment screened at low power of a stereo-microscope. The dye and faecal suspension are mixed and spread over the object-glass. No cover slip is required. Methylene blue stains the faecal matter bluish whereas the *Fasciola* species eggs remain yellowish brown and are easily recognized.

3.2.5.1.3 Flotation

The principle of this procedure is to dilute the faecal sample in a high-density solution (the flotation liquid-saturated sodium chloride solution (NaCl) so as to concentrate nematode eggs which are lower in density on the surface of the liquid (Urquhart et al., 1996). Briefly, 5g of freshly collected faecal samples are put into the flotation cups and some saturated salt solution (NaCl) is added before thoroughly being homogenized using two wooden spatulas. Some more saturated NaCl solution is added to fill the cup until a meniscus forms. A clean cover slip is placed on top of the filled cup to ensure contact with the solution. The cover slip will be left in place for 10-20 minutes after which it is removed vertically and placed on a microscope slide for microscopic examination using a magnification of 100 diameters.

3.2.5.1.4 Baermann technique

This method is used in the diagnosis of lungworm larvae or 3rd stage larvae of gastrointestinal nematodes (Kaufmann, 1996). The principle is that the larvae migrate actively from faeces into the aqueous phase of the Baermann apparatus consisting of a glass/plastic funnel held in a retort stand. A rubber tube is attached to the funnel, its bottom constricted by a clip. A sieve (aperture 250μm) is placed on top of the funnel, which has been partially filled with water. A double layer of gauze is then placed on top of the sieve and a faecal sample placed on it before slowly filling the funnel with water to immerse the faecal sample. The sample stays overnight at room temperature during which time the larvae migrate out of the faeces through the sieve to the sediment at the neck of the funnel. The following morning the clip is released and water together with larvae in the neck of the funnel are collected into a Petri dish for microscopic examination.

A simple adaptation of the above technique is to suspend faeces enclosed in gauze in a glass filled with water and allowed to stay over night. The larvae leave the faeces, migrate through the gauze and settle at the bottom of the glass. This is collected in a Petri dish and examined under a low power microscope as above.

3.2.5.2 Quantitative McMaster Technique

The modified McMaster technique is used for establishing nematode faecal egg counts (FECs). Briefly, 4 g faeces are put into a Petri dish and some saturated NaCl is added (Kaufmann, 1996). Using 2 wooden spatulas, the faecal sample is thoroughly stirred to mix and homogenise it. The mixture is transferred by pouring through a sieve into a measuring cylinder and topped up to 60 ml with saturated NaCl solution. The suspension is then transferred into a sealable container or bottle and thoroughly mixed by gently shaking it. The McMaster slide chambers are carefully loaded with the suspension to avoid transferring air bubbles into the counting chambers. Thereafter, the number of eggs within the two grids of the McMaster slide is counted and the FEC established by multiplying the number of eggs counted by a correction factor of 50 to give the eggs per gram faeces (EPG) by application of the formula:

EPG (FEC) = n x d /4g faeces x v

n= number of eggs counted in both chambers

d= volume of saturated salt solution (60ml) used to mix faecal sample

v= volume of the 2 McMaster chambers which was 1 cm by 1 cm with a depth of 0.15 cm

[v = 2 x 1x 1x 0.15=0.3 cm^3=0.3 ml]

3.2.5.3 Larval culture

This method is for diagnosing GIN species prevalent in a given area (Kaufmann, 1996). It can hence be used to establish the gastrointestinal nematode genus and in some instances the species by counting the intestinal cells of third stage larvae and also through examination of their morphology. Briefly, pooled faecal samples are made from which about 50g is taken, combining similar sized samples from each animal. The faecal sample is then broken into fine particles using a wooden spatula and mixed with vermiculite (Rajapack®, Birkenfeld, Germany) and tap water, ensuring they remain moist and crumbly but not really wet. The pooled faecal material is filled into glass or plastic culture dishes before partially covering and incubating at 27ºC for 7 days or at 20ºC for 10-20 days. Larvae 3 are recovered as described in the Baermann technique (3.2.5.1.4).

3.2.6 Management of helminth infections of cattle

3.2.6.1 Trematoda

Effective control of fasciolosis has to have two components: measures targeting the parasite and those aimed at controlling the intermediate host (snails) to reduce the chances of contact between intermediate and final hosts. Anthelmintic drugs, such as salicylanilides closantel (10mg/kg bw), oxyclozanide (15 mg/kg bw), Nitroxynil, Rafoxanide, Clorsulon plus (2 mg/kg bw) and triclabendazole are effective against *Fasciola* species (Fairweather and Boray, 1999; Fairweather, 2005). Triclabendazole (12 mg/kg bw in cattle) is effective against both immature and mature flukes. However, the long withdrawal period associated with triclabendazole makes its use inappropriate in lactating animals. Nonetheless, with the widespread use of triclabendazole comes a potential risk of *F. hepatica* populations developing genetic resistance against this drug, if used excessively. Indeed, some reports (Moll et al., 2000; Thomas et al., 2000; Gaasenbeek et al., 2001) indicate the emergence of such anti-triclabendazole resistance in *F. hepatica*. Other drugs, including albendazole (10mg/kg bw) and Netobimin (20 mg/kg bw) are effective, but on mature flukes only. With no new drugs in the offing in the near future, combinations of some older drugs appear to have a high efficacy against mature and immature flukes (Boray, 1994).

In developed countries, treatment should be supported by an analysis of costs and benefits; for subsistence farming families in developing countries there are higher priorities to utilize the limited reserves of cash, and treatment of animals grazing on common lands is inefficient, unless a high proportion of livestock owners do treat. A shotgun approach of treating 4-times a year with an anthelmintic effective against young parasites will control the parasite (Fairweather and Boray, 1999; Fairweather, 2005), but there are few market places in which the anthelmintics are affordable.

In addition to the use of anthelmintic compounds in strategic treatment regimens for ruminants there has also been a major focus on the development of a vaccine against fasciolosis over many years (reviewed by Spithill and Dalton, 1998; Dalton and Mulcahy, 2001). A range of molecules, including cysteine peptidases (cathepsins B and L), glutathione S-transferases, fatty acid-binding proteins and leucine aminopeptidase are being assessed as potential immunogens or vaccines for future use (Spithill and Dalton, 1998; Acosta et al., 2008; Jayaraj et al., 2009).

The use of molluscicides in the control of snail intermediate hosts is the second tool in the control of fasciolosis. Chemical molluscides, including copper sulphate and N-trityl morpholine, are effective in the control of snails (Urquhart et al., 1996). Their application though is restricted owing to the associated environmental implications and their effect on non-target acquatic organisms. Beyond known chemical molluscicides, trials in Kenya have shown that also leaves of *Eucalyptus globosus* are effective in the control of snail intermediate hosts (Cheruiyot and Wamae, 1988). However, due to the hydrological effect of *Eucalyptus* tree species, their use as molluscicides is of limited practical importance.

Biological control of snails using ducks to directly feed on the snails and or the use of the non-parasitic trematode (*Echinostoma revolutum*) in duck faeces in fasciolosis control was found effective in Asia (Suhardono et al., 2006). Since *E. revolutum* use the snail hosts like *Fasciola* species, miracidium are out-competed by the larvae of *E. revolutum* for snail hosts hence reducing chances of cercariae developing in snails. This strategy requires that ducks are reared in close proximity of cattle stables so that duck faecal material and cattle dung does mix or keeping them separately but mixing duck droppings with cattle manure before using it on for example rice farms, from where rice husks are later fed to cattle.

Perhaps the long-term and best method for controlling fasciolosis is the permanent destruction of snail habitats by drainage of snail habitats, as suggested by Urquhart et al. (1996). However, farmers are often hesitant to undertake expensive drainage schemes because of the associated costs and labour inputs. Fencing off snail habitats would help animals to avoid contact with infected snails. Although these methods, together with the intensification of livestock production systems, are likely to have an impact on the fasciolosis infection pressure, their success in extensive livestock production systems which are predominant across many African countries is of less practical relevance.

3.2.6.2 Cestoda

Chemotherapy is the principal method of managing cestodes (monieziosis) in cattle (Urquhart et al., 1996). A number of drugs, including niclosamide, praziquantel, bunamidine and a number of broad spectrum benzimidazole compounds, which have the advantage to also act against nematodes are available for *Moniezia* infection treatment. Treating calves does reduce the chance of mites getting infected on pastures. When anthelmintic treatments are used,

changing the type of anthelmintic used each year does help, athough necessary experiments to prove that annual rotation of anthelmintic types prevent resistance development have not yet been undertaken; it is generally assumed that changing anthelmintic types may be of benefit (Coles, 2002).

3.2.6.3 Nematoda

Nematodes can be controlled through anthelmintic treatments and pasture management, including rotational grazing and separate herding in time and in space (calves grazing in front or animals of similar age herd together) as described by Urquhart et al. (1996). Michel, (1985) classified the grazing management strategies aimed at controlling nematode parasite infections in ruminant livestock as follows:

• *Preventive*: These are strategies that rely on putting worm-free animals on to a clean pasture, or by suppressing worm egg output by anthelmintic treatment in the early part of the grazing season, until the initial population of infective larvae on pasture has declined to safe levels.

• *Evasive*: These strategies do not attempt to restrict contamination of the pasture with parasite eggs, but rely on movement of livestock to another pasture just before the larvae resulting from this contamination are likely to appear in significant numbers on the original pasture.

• *Diluting*: Strategies that exploit the concurrent grazing of susceptible animals together with a greater population of animals with acquired natural resistance to parasites of the same livestock species (generally dry adult stock), or of different livestock species, in order to reduce herbage infestation resulting from their combined faecal outputs of worm eggs.

Up until relatively recently, the combination of anthelmintic treatment with all of these grazing management strategies was highly recommended. This was based on the sensible interpretation of drug efficacy *vis a vis* parasite epidemiology, whereby “clean animals go onto clean pasture”. By doing so, re-infection rates are extremely low and the suppressive effect of anthelmintic treatment on nematode egg output is prolonged for several months, rather than for a few weeks as seen on contaminated pastures (Waller et al., 1995). Unfortunately, although such combination strategies were very highly effective in controlling parasite infections, they have also proved (at least in the sheep industry in Australia) to select potently for anthelmintic resistance (Waller, 1997; Van Wyn et al., 1999). This is due to the fact that any parasites that survive anthelmintic treatment – although initially few in the first instance – do carry resistance genes. They have an enormous survival advantage, given that,

following a move to parasite-free pastures, there will be only restricted pick up of nematode larvae that escaped anthelmintic selection; thus, the resident worm populations will make a disproportionate contribution to the anthelmintic resistance status of forthcoming parasite generations (Barnes et al., 1995).

Benzimidazoles, imidozothiazoles and macrocyclic lactones are used in the control of nematodes. Treatment approaches include:

3.2.6.3.1 Selective treatment approach

The objective of this method is to minimize selection pressure and slow down the rate of resistance development. One option is to use selective treatments of individual animals instead of systematic treatment of the whole flock, i.e. to treat only the most infected animals within the flock. In this way, a reservoir of susceptible worms remains within the population and the resistant alleles are diluted (Barnes et al., 1995; Hoste et al., 2002a). However, the success of this method largely relies on the correct identification of infected animals or those most susceptible to the harmful effects of infection. Towards this end, several methods or criteria for targeting infected animals or those most in need of treatment have been used, such as clinical signs of anaemia, the so called FAMACHA® method (vanWyk and Bath, 2002), milk yield (Hoste et al., 2002a) or body condition score (BCS) (van Wyk et al., 2006). When introducing cattle from other properties, ‘cleaning them out’ with‘quarantine’ drench, at least for roundworms and possibly also for liver flukes, should also be considered as they could serve as source of infection for the herd (Hutchinson, 2003). The treatment could also target the most productive animals like draught oxen and lactating cows in order to mimimize production losses. Highly producing dairy animals and especially those in their first lactation period are often less resistant to GIN infections (Hoste and Chartier, 1993; Chartier and Hoste, 1994). Therefore, the animals with the highest milk yield of a herd should be part of the treated proportion. If applied optimally, selective treatment does not result in any significant production losses to farmers. Since the amount of anthelmintics used is minimised, expenses for drugs are reduced.

3.2.6.3.2 Strategic helminth treatment approach

Strategic deworming programs are designed to prevent the accumulation of large numbers of infective larvae on pasture and to reduce the acquisition of infection by grazing animals (Williams et al., 1986). All animals in a herd should be drenched during periods when helminth egg output is deemed high. In temperate countries, strategic drenching should be done at the start of spring when animals are turned out to pasture and then repeated after 3, 6 and 9 weeks (Williams et al., 1986). The frequency of treatment depends on the helminth ecology and the persistence of the drench. In the tropics, strategic helminth treatment is timed to occur shortly after the on-set of the rainy season and may be repeated in the middle and end of the rainy season, depending on how long the wet season lasts.

In West Africa, strategic anthelmintic treatments should be done shortly before the onset of the rainy season and be repeated towards the end of the rainy season, since it is reported that shedding of helminth eggs increases during the rainy season (Kaufmann and Pfister, 1990; Ndao et al., 1995; Zinsstag et al., 2000). Young cattle are most susceptible to worms, but usually develop useful immunity by around 20–24 months (Hutchinson, 2003). They should hence as much as possible be given the strategic treatments to protect them during the period when their immunity is still weak.

3.2.7 Anthelmintic resistance

3.2.7.1 Definition

Anthelmintic resistance (AR) has been defined by Conder and Campbell (1995) as a problem that arises when naturally occurring helminths, containing genes permitting survival of anthelmintic treatment, are given an opportunity to increase as a percentage of the total worm population. The intensive and repeated use of anthelmintics selects for nematodes that can survive the treatment, these being nematodes that are genetically and physiologically resistant to the anthelmintic treatment (Prichard, 1994). These resistant individuals will reproduce and transmit the resistant alleles to their offsprings. Treatment becomes ineffective when the proportion of resistant genes increases and thereby dilutes the susceptible genes (Sangster, 1999). Point mutations have also been known to result in a change of the structure of proteins

that may lead to a decrease in susceptibility to a pharmaceutical compound (Tiwari et al., 2006; Walsh et al., 2006; Prichard and Roulet, 2007).

3.2.7.2 Current situation

Since initial reports of resistance to the first broad-spectrum anthelmintics in the 1960s, as reviewed by Kaplan (2004), AR has been reported for most important nematodes of sheep (and goats), usually within a few years after the introduction of each drug group (Mckellar and Jackson, 2004; Waller, 2006). Multiple drug resistance has been reported in virtually all major sheep-and goat-producing regions and for all major nematode species, in particular *Haemonchus contortus*, *Teladorsagia* (*Ostertagia*) *circumcincta* and *Trichostrongylus* species (Kaplan, 2004).

Anthelmintic resistance is not as widely documented in cattle as it is in sheep or goat production systems. In cattle nematodes, AR does not appear to be a big problem for bovine health management, with the exception of New Zealand where some but few cases were cited in the past (Coles, 2005). Recently, more reports of bovine nematode AR, mostly from regions where production systems are based on grazing management, like New Zealand, Brazil, Argentina and the UK, have been made (Coles, 2002). Reports on AR of cattle nematodes in Europe (Coles et al., 2001; Coles et al., 2005; Stafford et al., 2007; Demeler et al., 2009), South America (Fiel et al., 2001; Anziani et al., 2004; Soutello et al., 2007; Suarez and Cristel, 2007) and New Zealand (West et al., 1994; Vermunt et al., 1996; Coles, 2005) are cumulating. In an earlier world-wide review of AR in cattle, Hutchison (2003) states that: although evidence of resistance in cattle worms is only slowly coming to light and has so far been restricted to the less-pathogenic species of *Trichstrongylus axei* and Cooperia species, it should be expected that resistance to macrocyclic lactones is likely to become established in Australia. Already reports on multiple drug resistance in cattle have been made in Europe (Coles, 2002; Demeler et al., 2009), in Brazil (Soutello et al., 2007) and in Argentina ((Mejıa et al., 2003; Anziani et al., 2004).

In sub-Saharan Africa, the development of AR is likely to be slow because of the limited availability and the infrequent and haphazard use of anthelmintics, the common practice of communal grazing and the lack of a coordinated approach to anthelmintic treatment whenever mostly by small-scale subsistence farmers use these drugs (Sissay et al., 2006). The latter

system implies that it has little to gain from parasite control as treated stock would only have few helminth-free days before they immediately are re-infected, but it assures that a substantial proportion of the parasite population remains unselected for AR-remaining *in refugia*.

Although therefore reports on AR in cattle are scarce for Africa, a number of studies for small ruminants in Kenya (Wanyangu et al., 1996; Maingi et al., 1998, Wairuiru et al., 1998; Gatongi et al., 2003), in Tanzania (Bjorn et al., 1990; Ngomuo et al., 1990; Keyyu et al., 2002), in Zimbabwe (Mukaratirwa et al., 1997), Zambia (Gabrie et al., 2001), Cameroon (Ndamukong and Sewell, 1992), in South Africa (Van Wyk et al., 1999; Vatta et al., 2001; Bakunzi et al., 2003), in Ethiopia (Sissay et al., 2006) and in Nigeria (Mbah et al., 1992) among others have been carried out. In majority of these reports, *Haemonchus contortus* was demonstrated to be resistant to benzimidazoles, levamisole and in some instances ivermectin. Other worm species resistant to the above anthelmintic drugs include *Trichostrongylus* species and *Oesophagostomum* species.

3.2.7.3 Diagnosis of anthelminthic resistance

3.2.7.3.1 Faecal Egg Count Reduction Test

This is an in vivo diagnostic test used for detecting AR in the field as described by Coles et al. (1992). The test provides an estimate of anthelmintic efficacy by comparing worm egg counts from animals pre- and post-treatment. Counts from a group of treated animals provide a measure of change which can occur during a test period. Experimental animals for treatment should be young or, if adults their FEC should be > 150. In addition, animals should not have been treated with anthelmintics in the previous 8-12 weeks because the test may target on 'pre-selected' worms and not represent the normal population (Coles et al., 1992). One limitation of this method is that the number of GIN eggs excreted usually does not correlate with the actual worm burden. There is no correlation between FECs and worm counts for for example *Trichostrongylus colubriformis.*

3.2.7.3.2 In vitro tests

These tests, as described by Lacey et al. (1990), are cost-effective and take less time than in vivo tests. Briefly, free living stages of helminth parasites are incubated in a range of anthelmintic drug concentrations, followed by measurements of their vitality in form of development, motility or migration pattern. The larval development test is the most widely used in vitro system for the detection of resistance against benzimidazoles, imidazothiozoles and macrocyclic lactones (Coles et al., 1988; Taylor, 1990; Gill and Lacey, 1998). The egg hatch test (Coles et al., 1992) is a modification of this. In this test the ability of thiabendazole to inhibit the embryonation and hatching of freshly collected nematode eggs is used to calculate the 50% lethal dose (LD_{50}) of the drug. This test has only been shown to work on nematode species in which eggs hatch rapidly. Another in vitro test system is the larval migration and motility test that measures motility of third stage larvae incubated in a range of drug concentrations to observe for decreased motility as described by Geerts et al. (1989) and Gill et al. (1991). Some work on nematodes of sheep has been conducted in various parts of the world (Kimambo and MacRae, 1988; Wagland et al., 1992; Rabel et al., 1994; Gatongi et al., 2003) using in vitro tests.

3.2.7.3.3 Molecular techniques

The available molecular techniques, encompassing both conventional and real time polymerase chain reaction (PCR), for diagnosing benzimidazole resistance of trichostrongyles are extensively reviewed and outlined in von Samson-Himmelstjerna (2006). These tests utilize the resistance-related single nucleotide polymorphism (SNP) in codon 200 of the beta-tubulin isotype 1 and were initially developed for the analysis of benzimidazole-resistance in small ruminant trichostrongyles (Humbert et al., 2001). Allele-specific PCR-based tests were described to determine the genotype of *H. contortus* (Kwa et al., 1994) and of *Telodorsagia circumcincta* (Elard et al., 1999) adult worms. Silvestre and Humbert (2000) combined the allele-specific beta-tubulin codon 200 PCR with a PCR-restriction fragment length polymorphism (PCR-RFLP) to genotype and identify single *H. contortus*, *T. colubriformis* and *Telodorsagia circumcincta* third stage larvae. More recently, a similar approach was followed to establish an allele-specific beta-tubulin codon 200 PCR for several cyathostomin species (von Samson-Himmelstjerna et al., 2002b). Real time PCR has also been described and found relevant in the analysis of AR (Alvarez-Sanchez et al., 2005; von Samson-Himmelstjerna et al., 2003; 2009). Recently, a molecular diagnostic technique for the

microcyclic lactones (ivermectin) resistance has been developed and validated (Demeler et al., 2009).

4 Materials and Methods

4.1 Study area description

The study was carried out in the administrative district of Sikasso, southeastern Mali. Sikasso lies on longitude 11° 19' N, latitude 5° 40' W at an altitude of 410m above sea level. The district is bordered to the east by Burkina Faso, Guinea Conakry to the west, to the south by Côte d'Ivoire and by Koulikoro and Segou regions to the north and northeast. The region has a sub-humid climate typical of the Guinean savannah zone (McDermott et al., 2003) with 3 seasons: cold dry season (November to January), hot dry season (February to May) and rainy season (June to end of October). The single rainy season in Sikasso registers ~1000-1200 mm of rainfall annually with peaks in July and August (Meterological Service, 2009), making it one of the highest agricultural potential areas in Mali. Mixed crop-livestock farming is the main source of livelihoods where maize, sorghum, millet and rice are the main subsistence crops, and cotton and groundnuts the main cash crops. Other crops include root crops (yams, sweet and Irish potatoes), legumes and fruit trees, and small-scale horticulture (vegetables). Cotton ginning is the main industrial activity in Sikasso, although the majority of the farming communities engage as well in localised cottage industries, such as the extraction of cooking fat from Shea nuts.

There are 2 cattle-keeping systems in Sikasso: pastoral and agro-pastoral. Cattle-keeping is the principal source of livelihoods for the pastoralists whereas the agro-pastoralists mainly use cattle for draught power needs, provision of manure, savings as "living banks" and social obligations (dowry and rituals) and only secondarily for their milk and meat (Grace et al., 2009). During the dry season, when fodder and water become scarce in the north, and when at the same time tsetse challenge is reduced in the more humid areas in the south, Peuhl pastoralists bring transhumant herds (movement of animals from one place to the other in search of pastures and water during the dry season) to Sikasso, creating seasonal or permanent complementary and competitive relationships between the settled agro-pastoralist communities and the immigrant pastoralist groups, sometimes leading to conflicts.

Several seasonal rivers drain across Sikasso, and these systems are dominated by riparian vegetation types that are still largely undisturbed due to limited human activity, providing

favourable habitats for the *palpalis* (Nemorhina) group of tsetse flies. Savannah flies, formerly present in the area, have since disappeared due to the degradation of their habitat following increased human population and expanded crop fields (Djiteye et al., 1997).

4.2 Study implementation

The study was implemented in three phases: the pre-intervention phase, the intervention phase and post-intervention from November 2007 to December 2009. A summary of the activities undertaken in each phase is presented in Table 1.

Table 1: Summary of activities and their timing, Sikasso, south-east Mali (November 2007 to December 2009)

	2007		2008												2009											
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Pre-intervention phase																										
Tsetse and trypanosomosis survey	X																									
Drug sensitivity testing	X	X																								
Mobilization and logistics			X	X																						
Intervention phase																										
Stationary attractive devices					X	X	X	X						X	X	X	X	X	X	X						
Cattle spraying									X	X	X	X	X								X	X	X	X	X	
Strategic helminth control								X		X			X							X		X			X	
Risk cattle recruitment						X	X			X		X			X				X			X				
Tsetse and trypanosomosis survey								X					X			X				X					X	
Diminazene treatments								X					X			X				X					X	
Trypanocide use practices								X					X			X				X					X	
Post-intervention phase																										
Tsetse and trypanosomosis survey																									X	
Drug sensitivity testing																									X	X
Anthelmintic resistance testing																										X

X=Activity implemented at the date in question

4.3 Pre-intervention phase

4.3.1 Study design

A cross-sectional and a longitudinal survey were both undertaken during the pre-intervention phase. The cross-sectional survey helped to establish the pre-intervention levels of tsetse, trypanosome prevalence, trypanocide use practices while the longitudinal survey established the pre-intervention trypanocidal drug resistance levels.

4.3.2 Study sites

The study area was divided into two sectors: a eastern sector located along the road from Sikasso to Burkina Faso, and a western sector along the road from Sikasso to Côte d'Ivoire (Figure 1).

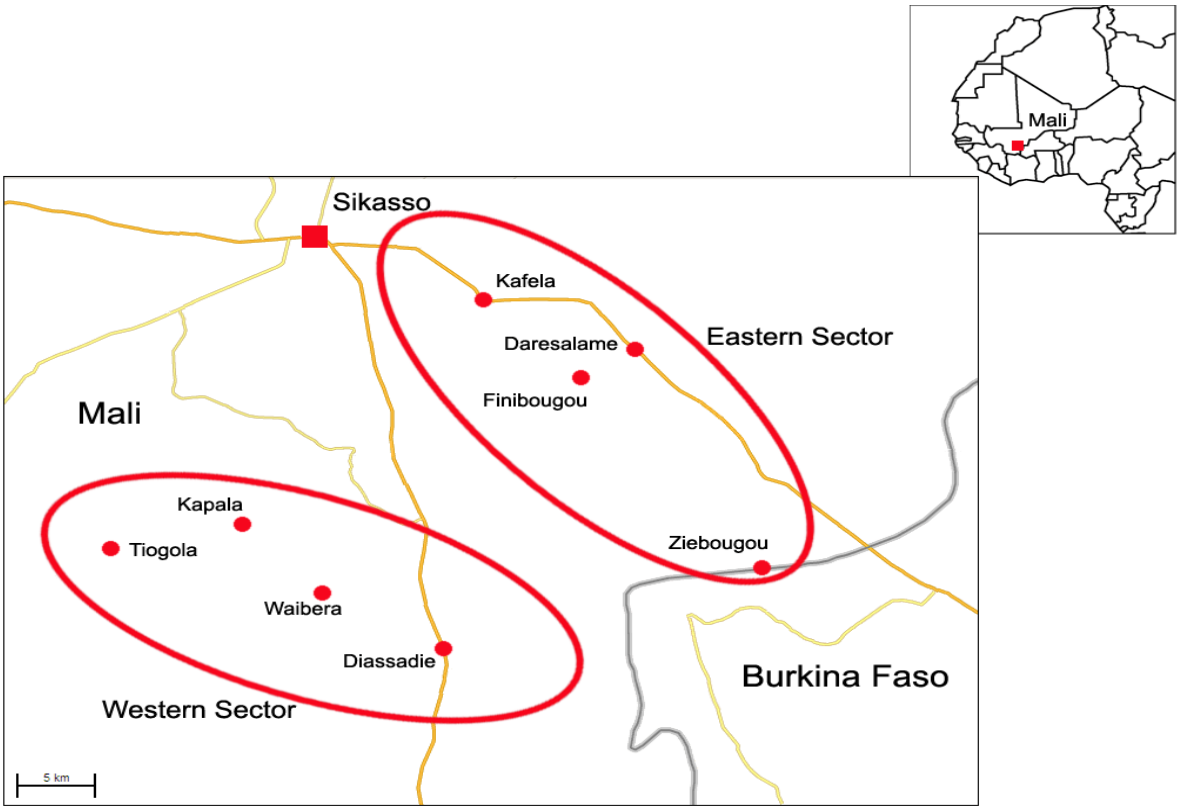

Figure 1: Map showing the study sites (circled) within the administrative district of Sikasso, south-eastern Mali (November to December 2007)

The two sectors had comparable agricultural production systems, vegetation type and drainage system. A total of 8 study villages (4 each from the eastern and western sectors) were purposively selected from the 25 villages surveyed in 2003-2004 and in which high

trypanosome prevalence (>10%) had been detected (Grace, 2005). Since it was assumed that only riverine tsetse species would be present, eight tsetse trapping points were selected after identifying epidemiologically risk points (the points of most frequent vector/host contacts) along the rivers and streams draining across each village.

4.3.3 Study animals

A mixture of both trypanosusceptible zebu and their crosses (*métis*) with the indigenous trypanotolerant cattle breeds, the Méré and N'Dama were used as study animals. Cattle in the two sectors were generally herded under the extensive grazing systems on communal pastures with minimal and occasional supplements (discarded mango fruits, maize and millet bran). Source herds (cows, bulls and yearlings) from which draught oxen were derived are grazed far away from the homesteads (farms) during the cropping season (rainy season) but allowed to graze on crop residues after harvesting, with their dung directly fertilizing the farms in readiness for the next cropping season. Oxen (highly valued animals) are grazed and watered close to the homesteads throughout the year. Cattle are watered in rivers and at community bore holes after the seasonal rivers dry out. Herds were composed of between as few as 2 cattle to 70 cattle.

4.3.4 Tsetse survey

A cross-sectional entomological survey was conducted in November 2007 to establish pre-intervention tsetse fly densities. Eight unbaited and untreated bi-conical traps (Challier Laveissière, 1973) per village were deployed at an interval of about 100-200 m in the forest galleries along the drainage lines. The traps were emptied 24 hours later and flies counted, identified, and separated into their respective species and sex.

4.3.5 Trypanosome prevalence survey

4.3.5.1 Cattle sampling

From each of the 2 study sectors, study cattle were selected assuming an estimated trypanosomosis prevalence of 20% (Grace, 2005) and a desired accuracy level of 5% at the 95% confidence level. Following Dohoo et al. (2003), the formula below was used to determine the sample size at sector level:

$$n = \frac{Z_{\alpha}^{2} \cdot p \cdot (1-p)}{L^{2}}$$

where $Z_{\alpha} = Z_{0.05} = 1.96$ (the value of Z_{α} required for confidence=95%); L = the precision of the estimate (allowable error or margin of error) equal to ½ the normal approximation of sample size for the binomial distribution confidence interval. L^2 =0.0025 or 1/400, p= 20%, the *priori* trypanosome prevalence estimate. Applying this formula, 246 animals would have constituted the optimal estimated sample size for each sector (four villages). However, to improve precision of the trypanosomosis prevalence estimates and to get reliable results for the sub-groups, the sample size was increased to 400 cattle, that is 100 cattle/village. Cattle aged at least 12 months were considered for the survey. Since the cattle population per village was not known, a systematic sampling approach as described in Dohoo et al. (2003) was adopted.

4.3.5.2 Parasitological examination

A jugular vein blood sample was collected in November 2007 from the selected cattle into vacutainer tubes containing di-sodium salt of ethylene diamine tetra-acetate (EDTA) for parasitological and haematological analysis. The vacutainer tubes were labelled and stored in an ice-packed cool box awaiting examination within 2 hours of collection. Micro-haematocrit capillary tubes were filled with blood samples and centrifuged at 8000g for 5 minutes. The packed cell volume was estimated soon after centrifugation using the Hawksley microhaematocrit reader (Hawksley, Lancing, United Kingdom). Trypanosome species were identified based on their movement and morphology using x 40 objective lens (Paris et al., 1982). Next, the tubes were cut 1 mm below the buffy-coat and the buffy-coat zone expressed onto a slide, covered with a cover slip and examined for trypanosomes using dark-ground phase microscopy (Murray et al., 1977).

4.3.6 Block treatment study

The block treatment study that lasted 4 weeks from November to December 2007 was initiated on the same day the trypanosome prevalence survey was conducted. All village trypanosome positive cattle were randomly allocated into two groups soon after the trypanosome prevalence survey. Briefly, the cattle were assigned numbers 1, 2, 3, .., n that were transferred on to small pieces of paper. These were rolled into small balls placed in a bowl and randomly drawn one at a time without replacement. One group of animals was assigned to treatment with diminazene aceturate (DIM) (Veriben®, Ceva Animal Health INC., France) and the other to isometamidium chloride (ISMM) (Trypamidium®, Merial, France). The cattle were then ear-tagged and their weights estimated using a weighing band and the conversion tables of Bosma (1992), developed for south Mali, to convert centimetre girth to kilogram body weight. The drugs were reconstituted in distilled water as per the manufacturers' recommendation, and the DIM group treated with a curative dose (3.5 mg/kg body weight (b.w.) of 7% DIM by deep intramuscular injection (i.m.) and the ISMM group with 0.5 mg/kg b.w. of 2% ISMM i.m. by an animal health assistant under supervision of a team of veterinarians. Treatment day for every village was considered day 0. In total, there were 125 cattle trypanosome positive (62 on DIM and 63 on ISMM) that were monitored for trypanosomes and PCV on days 14 and 28 post-treatment. Cattle with persisting trypanosomes in both groups at days 14 and 28 were treated with 7 mg/kg bw DIM.

4.3.7 Trypanocidal drug use practices

A rapid appraisal into the usage of trypanocidal drugs in the study villages was conducted in which participating herd owners were asked to state their preference for curative and prophylactic treatments, source of drugs used, and who administered treatments. Prophylactic treatments were those that affected the entire herds or the majority of the risk cattle in herds, whereas curative treatments were those affecting only a small number of sick animals at a given point in time. A targeted questionnaire administered to herd owners was used during the collection of these data (Annex 1).

4.4 Intervention phase

4.4.1 Study design

This was implemented as a longitudinal field study conducted from March 2008 to November 2009. The study involved application of tsetse control, diminazene treatments and strategic helminth control as best-bet techniques for containing trypanocidal drug resistance.

4.4.2 Study sites

The same study area used during the pre-intervention phase was retained with the eastern sector serving as the intervention area and the western sector as the control area.

4.4.3 Study animals

4.4.3.1 Reference population

This consisted of the entire cattle herds in both the intervention and control area which were at risk for trypanosome and helminth infections and on which the results of the longitudinal phase could be extrapolated. These were cattle of all breeds, sexes and ages. Feeding and husbandry practices of the reference population were as described in 4.3.3. Only cattle in herds situated within the study area and permanently exposed to tsetse challenge and helminth infections were qualified to serve as the reference population. Preference was given to herds whose animals had participated in the pre-intervention survey.

4.4.3.2 Risk group cattle

The risk group cattle were firstly recruited from among the reference cattle population through a cattle census conducted between April and May 2008 in the 8 study villages (4 from the intervention and 4 from the control area). All cattle aged 3-12 months at the time of census constituted the risk group animals. Ageing of animals was done through dentition. The purpose and duration of the study was explained to the herd owners in participating villages in

a bid to secure their consent as participants. Thereafter, herds with risk group cattle were identified on the willingness of their owners to participate. All the risk cattle from the recruited herds were ear-tagged for ease of identification during monitoring visits. A list of the village herds was generated by aggregating all selected herds in each village. The village lists were then consolidated into the intervention and control area herd lists. Once recruited, the risk group cattle were retained as study subjects throughout the study period. The risk cattle were continuously replenished by identifying and recruiting new born calves upon attainment of 3 months.

Data on changes in the number of the risk cattle that participated since the start of the monitoring in June 2008 to the end of the monitoring in November 2009 was collected to track changes and reasons for these.

4.4.4 Intervention packages

4.4.4.1 Tsetse control

Tsetse control intervention was conducted in the intervention area between March 2008 and November 2009. The intervention area study villages (Kafela, Finibougou, Daresalame and Ziébougou) and an additional twelve other villages namely; Bougoula Hameau, Karamokhobougou, Finkolo, Farako, Mamouroubougou, N'Golokounadougou, Sanoukoro, Sibiribougou, Tagabougou, Tienfinibougou, Ziékoroudougou and Hérémakono in an area approximately 500 km^2 participated in tsetse control. Deltamethrin treated stationary attractive devices (SADs) used during the dry season and the targeted treatment of cattle with deltamethrin during the rainy season were the two tsetse control techniques used. The SADs were either targets or monoconical vavoua traps. A total of 957 targets ($1m^2$ piece of cloth made of 35% cotton and 65% polyester with black sandwiched with blue on both edges), locally made in Mali by tailors, were impregnated in 0.4% deltamethrin (DECIS®, Roussel-Uclaf, France). Additionally, 401 monoconical Vavoua traps ($3m^2$ of white mosquito net with 3 vertical panels of black and blue targets), also locally made, were impregnated in 0.5% deltamethrin (Glossinex®, AgrEvo, Zimbabwe). The impregnated SADs were deployed along drainage lines 100-300 m apart except in human or animal watering points (points of frequent contact between tsetse-hosts) where the distance was reduced to 30 m.

The SADs were withdrawn at the start of the rainy season (June 2008) and fortnight targeted treatment of cattle with deltamethrin commenced. Cattle were sprayed on the limbs, lower abdominal area, thoracic and brisket region using 0.05% deltamethrin (Butox®, Intervet International, the Netherlands). As many cattle as possible in each of the 16 villages participating in the tsetse control exercise were sprayed as required. To facilitate the spraying process and to ensure spraying was appropriately done, the project provided a 20-litre knapsack sprayer and enough insecticide to each village free of charge through an auxiliary, nominated and seconded to the project by the villagers themselves. In December 2008, at the start of the dry season, the same number of SADs was reimpregnated and redeployed. These were again withdrawn in June 2009 at the onset of the rains and another cattle spraying regimen commenced.

4.4.4.2 Trypanocidal drug treatments

This intervention package was used on trypanosome positive risk group cattle and those with PCV<=20% (life threatening PCV levels). They were treated with 3.5 mg/kg bw diminazene aceturate (DIM) (Veriben®, Ceva Animal Health INC., France) by the project staff during monitoring. Because trypanosome infected animals were being treated, farmers in the intervention area were prevailed upon not to administer any trypanocides to their animals except when clearly justified.

4.4.4.3 Strategic helminth control

Kafela, Finibougou, Daresalame and Ziebougou from the intervention area and Diassadie and Waibera from the control area participated on the strategic helminth control trial. Prior to the commencement of this trial, all the identified risk group cattle were randomly allocated into an albendazole treatment and control groups. The random allocation of the risk group cattle was done at area level shortly before the first treatment was conducted. The random allocation was done as described under 4.3.7.

Risk group cattle on albendazole treatment were drenched *per os* using 10% albendazole (Albenzole®, 10 mg/kg b.w., Kela Laboratories, Belgium) in June and November 2008 and in June and November 2009. The treatments were timed to coincide with the season when egg shedding increased (Kaufmann et al., 1990). Therefore, in accordance with Kaufmann and

colleagues, albendazole treatments were conducted at the beginning and end of the rainy season. To blind the farmers, control group subjects were treated with a placebo every time albendazole treatments were done. The placebo was prepared by mixing several table spoonfuls of milk powder in lukewarm water and stirring to homogeneity. This was transported to the study villages packed in empty albendazole containers.

4.4.5 Monitoring

4.4.5.1 Tsetse surveys

Five tsetse monitoring surveys were conducted in June and November 2008, February, June and November 2009 to measure tsetse abundance in the intervention and control areas. Trapping sites and methodology were as described in 4.3.4. Only points where humans and livestock had access to water (frequent vector/host contact points) were retained as tsetse trapping points. Consequently, in every village tsetse trapping points were reduced to only 5 from the 8 used during the pre-intervention phase.

4.4.5.2 Trypanosome infections

Five monitoring surveys undertaken simultaneously with tsetse surveys were conducted to measure trypanosome infections and PCV %. Parasitological and haematological examination procedure was done as described in 4.3.5.2. All trypanosome positive and those with PCV =< 20% were treated as described in 4.4.4.2 above. Stabilates were made by inoculating 0.2 ml of *T. congolense* positive blood samples into mice for the in vivo (mice) resistance characterization studies. Likewise, filter paper blood spots were collected using blood of all trypanosome positive cases for the genetic characterization of resistance.

4.4.5.3 Trypanocidal drug use practices

A targeted questionnaire was administered to herd owners in both the intervention and control area to collect information on the prophylactic and curative trypanocidal drug treatments during the intervention phase, as was described in 4.3.7. This information was collected simultaneously with tsetse and trypanosome infection surveys. Each participating herd owner

was provided with a book and pen for recording trypanocidal treatment events and also other health occurrences. They were required to also note treating personnel. This information was retrieved during the monitoring visits (annex 2).

4.4.5.4 Faecal helminth egg counts (FECs)

Faecal samples from the risk group cattle participating on the strategic helminth control were analysed for helminth eggs in November 2008, February, June and November 2009. The faeces were collected per rectum using an arm long sleeve that was turned inside out and labelled with the animal tag number, breed, sex, date of collection and herd identity. The samples were delivered to the laboratory at the Institut Economique de Recherche (IER), Sikasso for processing in an ice-packed cool box. The modified McMaster technique (Kaufmann, 1996) was used for quantifying the egg load in the faecal samples. Briefly, 4 g of faeces were put into a petri dish before some saturated sodium chloride solution was added. This was thoroughly mixed and stirred using two wooden spatulas. The solution was transferred into a measuring cylinder by pouring through a sieve and then topping the measuring cylinder up to 60 ml with more saturated salt solution. The suspension was then transferred into a sealable container and properly mixed by gentle shaking. Both chambers of the McMaster slide (Chalex, Wallowa, USA) were carefully loaded with the suspension to avoid transferring air bubbles in the counting chambers, before eggs were counted using a compound microscope at × 40 magnification. The faecal egg count (eggs per gram faeces (EPG)) was calculated as the number of eggs counted multiplied by a correction factor of 50.

4.4.5.5 Larval cultures

The diagnosis of the nematode genus and species in the study area was done through examination of third-stage larvae. Two faecal cultures done in November 2008 and in February 2009 were used. Faecal samples from the albendazole treated group and the placebo treatment group were separately investigated. Briefly, about 50 g of faecal sample were pooled by taking a small amount of faeces from all animals sampled before vermiculite (Rajapack®, Birkenfeld, Germany) was added. The moist and crumbly samples were transferred into plastic culture dishes that were partially sealed and incubated for 7 days at room temperature (22-27°C) to allow for the development of larvae. The larvae were collected in a Baermann apparatus or suspended in water in muslin bags. To immobilize

larvae, a few drops of Lugol's iodine were added to the petridish or by slightly heating the bottom of a glass slide on to which a drop of the solution containing larvae had been dropped before examination under a compound microscope using × 40 magnification. Larvae specimens were identified by counting the intestinal cells.

4.4.5.6 Body weight development

Risk group cattle's body weights were estimated at every monitoring point using a weighing band and the conversion tables of (Bosma, 1992), developed for south Mali, to convert centimetre girth to kilogram body weight. For consistency and uniformity, only zebu cattle that participated in all the 5 monitoring points with effect from June 2008 to November 2009 were considered for weight development estimation.

4.4.5.7 Herd dynamics

During the monitoring period, changes in herds in form of births and deaths were noted and compared with the cattle population established during the census of April to May 2008. Information on other movements out of herds like payments in kind (herdsmen salary) and sales of animals was collected. Similarly, information on purchased animals entering herds was also collected.

4.5 Post-intervention phase

4.5.1 Study design

This was conducted as both a cross-sectional and a longitudinal study to measure the impact of the intervention on trypanosome infections and the evolution of the trypanocidal drug resistance levels post-intervention. Comparisons were then made between the pre-intervention (November to December 2007) and post-intervention (November to December 2009) phases.

4.5.2 Study sites

The same sites used during both the pre-intervention and the intervention phases were used.

4.5.3 Study animals

Adult cattle that were present in the study villages of the intervention and control areas throughout the intervention phase were used for this purpose. Sampling was systematic as was the case during the pre-intervention phase (4.3.5.1).

4.5.4 Trypanosome prevalence survey

In each of the 8 villages, 100 cattle were selected and examined for trypanosomes and PCV. During the survey, the same methodological approach as described under section 4.3.5.2 was applied.

4.5.5 Block treatment study

As was the case for the pre-intervention phase (4.3.6), only trypanosome positive cattle identified during the post-intervetion cross-sectional survey were considered for the drug sensitivity study. The trypanosome positive cattle were treated with 0.5mg/kg bw isometamidium chloride (ISMM) in both areas. The cattle were monitored for trypanosomes and PCV at day 14 and at day 28 post treatment. Parasitaemic cattle at day 14 were treated with 3.5 mg/kg bw DIM.

4.5.6 Faecal egg count reduction test (FECRT)

This procedure was undertaken in November of 2009 to assess the efficacy of albendazole against the gastrointestinal (GI) nematodes found in Sikasso. The method as described by Coles et al. (1992) was used. The albendazole (Albenzole® 10% suspension, Kela Laboratories, Belgium) sourced locally on Malian markets and used throughout the intervention period was the test drug. However, since there was no report on anthelmintic resistance in Sikasso, it was decided that it should be evaluated alongside a positive control, albendazole (Albendazol® 10% suspension, aniMedica, Südfeld, Germany). Briefly, the albendazole treated risk cattle cohort was randomly divided into two groups through the toss of a coin. One group was treated with 10mg/kg bw of the positive control (Germany albendazole) and another group with the test albendazole (locally available in Mali) at the same dose on day 0. The group risk cattle on placebo were simultaneously treated with

reconstituted milk. Faecal samples were collected and examined at day 0 and day 14 post-treatment.

4.6 Weather data

Data on weather was collected to triangulate the entomological and parasitological data. The collected weather data included rainfall and relative humidity. Secondary data for the period January 2006-October 2007 were collected from the Sikasso regional Metereological Department annual reports. Thereafter, quarterly weather reports with effect from November 2007 to October 2009 were used.

4.7 Data analysis

4.7.1 Pre-intervention phase

Tsetse catches were summarized for species at sector level as the number of tsetse caught per trap per day. Selected cattle were characterised by sex and breed. Trypanosome prevalence was estimated at sector, village and species levels as the proportion of trypanosome positive cattle in the population at risk. Univariate analysis through Chi square (χ^2) tests determined associations between trypanosome infection and animals' sex, breed, and age and where justified, multivariate analysis was done. Packed cell volume (PCV) was summarized by sector and cattle trypanosome infection status. Differences in mean PCV were tested between sectors and infection status using t-tests at significance levels of 0.1% and 5%. Data on drug handling and use was summarized by drugs and function. The block treatment data were summarized by drug and sector with DIM response considered only 14 days post-treatment. ISMM treatment response considered both day 14 and day 28 checks. Persisting trypanosomes in DIM treated cattle were interpreted as indicative of resistance to the used drug. A treatment failure rate of 25% of the cattle in the ISMM treated group at days 14 and 28 was indicative of resistance based on the evaluation done for chloroquine efficacy (Laufer et al., 2006). Pearson χ^2 and Fischers exact test were used to establish the difference in treatment response between villages and sectors.

4.7.2 Intervention phase

Tsetse fly catches data were analyzed as was done for the pre-intervention phase. Trypanosome prevalence and incidence density for the period June 2008 to June 2009 were estimated. Trypanocidal drug use practices were estimated by monitoring point and comparisons were done at area level. Trypanosome prevalence was estimated as trypanosome infections in the population at risk and presented at herd, village and area level. A herd was deemed infected if at least one risk group cattle had been detected with a trypanosome infection and vice versa. Incidence density rates (IDR) according to Dohoo et al. (2003) were calculated as the number of new trypanosome infections over the cattle-time at risk. Individual risk group cattle's risk time was estimated by subtracting the calendar date for the last and next survey before summing them up for herds, villages and aggregated to area level. Because trypanosome infected cattle were treated with a curative dose of diminazene, they were deemed to be at risk again 7 days post-treatment (Holmes et al., 2004). Thus, for such animals, the calculated risk period was 7 days shorter than for non-treated animals.

Incidence density rate comparisons were also done at village and area levels through estimation of rate ratios (RRs). The effect of the risk factors age and season were assessed by estimating the cumulative incidence among cohorts of animals, stratified by time when recruited and by monitoring visit. Due to non-normality of helminth FECs, median FECs by area and animal treatment were summarized and significance differences derived through the Mann-Whitney test. Faecal egg shedding was calculated by stratifying egg shedding by breed and monitoring time. Three strata were derived: Low helminth burden (0-500), moderate level (500-1000) and high level (>1000) as described by Urquhart et al. (1996). Incidence density rates for the albendazole and placebo treated cohorts were calculated and RRs used to compare the groups. The PCV was evaluated as described under the pre-intervention phase. Weight development was assessed by tracing weight changes only in the risk group cattle recruited in June 2008 in both areas. Herd dynamics including calving and mortality rate estimates during the study period were summarized for both areas.

4.7.3 Post-intervention phase

Trypanosome prevalence and drug sensitivity data were analyzed as described under section 4.5.1. FECRT result comparisons for risk cattle treated with albendazole and placebo was done as described by Coles et al. (1992). The percentage faecal egg count reduction was

calculated as 100 * (1 – Mean eggs/gramme faeces (EPG) of treated group/mean EPG of the control group) after 14 days. Resistance was declared when the FECRT was less than 95% and or the lower limit of the 95% confidence level less than 90%.

4.7.4 Weather situation

Rainfall data were summarized by year before illustrating the relationship between rainfall and relative humidity with tsetse abundance, trypanosome infections and heliminth FECs through time series analysis for the period January 2006 to October 2009.

Data were stored in MS Excel and analysis conducted by the Statistical Package for Social Sciences (SPSS) version 18. The accessible online program OpenEpi (http://www.openepi.com) was used to generate confidence intervals of proportions.

5 Results

5.1 Pre-intervention phase

5.1.1 Tsetse catches

Two riverine tsetse species, *Glossina palpalis gambiensis* and *G. tachinoides*, were encountered in the study area during the pre-intervention phase. A total of 580 tsetse flies (348 from the eastern sector and 232 from the western sector) were caught (Table 2).

Table 2: Number of *Glossina* caught by species and village (8 traps/village) in Sikasso, south-east Mali (November to December 2007)

Village	***Glossina p. gambiensis***		***G. tachinoides***		**Total fly catch**	
	No. flies	Flies/trap/day	No. flies	Flies/trap/ day	No. flies	Flies/trap/ day
Eastern sector						
Kafela	27	3.4	26	3.3	53	6.6
Finibougou	94	11.8	64	8.0	158	19.8
Daresalame	63	7.9	37	4.6	100	12.5
Ziébougou	35	4.4	2	0.3	37	4.6
Sector total	**219**	**6.8[a]**	**129**	**4.0[b]**	**348**	**10.9**
Western sector						
Diassadié	43	5.4	22	2.8	65	8.1
Waibera	72	9.0	47	5.9	119	14.9
Kapala	15	1.9	1	0.1	16	2.0
Tiogola	10	1.3	22	2.8	32	4.0
Sector total	**140**	**4.4[a]**	**92**	**2.9[b]**	**232**	**7.3**

[a, b] No significant (Kruskal Wallis test, $p > 0.05$) differences in the flies/trap/day for *G. p. gambiensis* and *G. tachinoides* between in the two sectors.

Glossina p. gambiensis was the dominant tsetse species trapped, with 6.8 flies/trap per day in the eastern sector and 4.4 flies/trap per day in the western sector. For *G. tachinoides,* 4.0

flies/trap per day were trapped in the eastern sector and 2.9 flies/trap per day in the western sector.

5.1.2 Cattle characterization

A total of 396 cattle from the eastern sector and 400 from the western sector were examined for trypanosomes during the pre-intervention phase. Three hundred and seventy-seven female cattle and 418 male cattle participated in the survey. One animal's sex failed to be recorded.

Overall, the zebu breed dominated, accounting for 560 (70.4%) study subjects, followed by cross-bred cattle with 236 (29.6%) subjects. Out of the 796 cattle examined, 124 (15.6%) were draught oxen (Table 3). The eastern sector had 44 draught oxen from 17 herds, an average of about 3 oxen/herd and 80 oxen in 27 herds in the western sector, a mean of about 3 oxen/herd. The preference for zebu cattle increased from east (less preference in the eastern sector) to west (more preference in the western sector).

Table 3: The distribution of draught oxen by breed and sector in Sikasso, south-east Mali (November to December 2007)

Village	**Draught oxen by breed and village**			
	No. herds	Zebu	Cross breed	Total
Eastern sector				
Kafela	6	5	4	9
Finibougou	4	5	4	9
Daresalame	4	3	10	13
Ziébougou	3	11	2	13
Sector total	**17**	**24**	**20**	**44**
Western sector				
Diassadie	10	23	3	26
Waibera	4	23	5	28
Kapala	7	9	2	11
Tiogola	6	12	3	15
Sector total	**27**	**67**	**13**	**80**

5.1.3 Trypanosome prevalence

Pre-intervention trypanosome prevalences were recorded at three levels: herd, village and sector level, respectively.

5.1.3.1 Herd level trypanosome prevalence

Table 4 presents herd-level preintervention point prevalences for the eastern sector villages. A herd was positive when one or more risk cattle was detected with trypanosomes. All herds in Daresalame and Ziébougou had trypanosome positive cattle as opposed to those in Kafela and Finibougou. Limited inter-herd variations were observed across the villages in the eastern sector. Ziébougou had the lowest herd prevalence.

Table 4: Pre-intervention phase trypanosome point prevalences by herd and village in the eastern sector in Sikasso, south-east Mali (November to December 2007)

Herd No.	**Village**	**Trypanosome positive cattle**			**No. cattle**	**Prevalence %**	**95 % CI**
		T.c.	T.v.	Total			
1	Kafela	2	1	3	17	17.6	4.7-40.9
2	Kafela	1	1	2	14	14.3	2.5-39.7
3	Kafela	0	0	0	17	0	0
4	Kafela	2	1	3	15	20.0	5.4-45.4
5	Kafela	2	0	2	15	13.3	2.3-37.5
6	Kafela	3	1	4	22	18.2	6.1-38.2
Total Kafela		**10**	**4**	**14**	**100**	**14.0**	**8.2-21.9**
1	Finibougou	0	0	0	7	0	0
2	Finibougou	3	0	3	7	42.9	12.3-78.4
3	Finibougou	2	1	3	31	9.7	2.5-24.1
4	Finibougou	0	0	0	7	0	0
5	Finibougou	2	0	2	2	100	-
6	Finibougou	2	0	2	6	33.3	6.0-73.8
7	Finibougou	5	1	6	40	15.0	6.3-28.6
Total Finibougou		**14**	**2**	**16**	**100**	**16.0**	**9.8-24.2**
1	Daresalame	2	1	3	31	9.7	2.5-24.1
2	Daresalame	1	0	1	12	8.3	0.4-34.0

3	Daresalame	0	1	1	30	3.3	0.2-15.4
4	Daresalame	9	0	9	23	39.1	21.1-59.8
Total Daresalame		**12**	**2**	**14**	**96**	**14.6**	**8.5-22.7**
1	Ziébougou	1	2	3	18	16.7	4.4-39.0
2	Ziébougou	4	0	4	54	7.4	2.4-16.9
3	Ziébougou	0	4	4	28	14.3	4.7-31.0
Total Ziébougou		**5**	**6**	**11**	**100**	**11.0**	**5.9-18.3**

T.c.= *Trypanosoma congolense*; T.v. = *T. vivax*
95% CI=95 % confidence interval

In the western sector, all villages had trypanosome negative herds unlike the situation in the eastern sector (Table 5). Overall, inter-herd prevalence variations were also observed like was the case in the eastern sector. Waibera had the lowest variability.

Table 5: Pre-intervention phase trypanosome point prevalence by herd and village in the western sector in Sikasso, south-east Mali (November to December 2007)

Herd No.	**Village**	**Trypanosome positive cattle**			**No. cattle**	**Prevalence %**	**95% CI**
		T.c.	T.v.	Total			
1	Diassadié	0	0	0	3	0	0
2	Diassadié	2	0	2	8	25	4.4-61.2
3	Diassadié	1	0	1	1	100	-
4	Diassadié	1	0	1	1	100	-
5	Diassadié	0	0	0	3	0	0
6	Diassadié	1	0	1	7	14.3	0.7-53.0
7	Diassadié	1	0	1	5	20	1-66.6
8	Diassadié	7	0	7	35	20	9.2-35.6
9	Diassadié	0	0	0	2	0	0
10	Diassadié	4	0	4	31	12.9	4.2-28.3
11	Diassadié	0	2	2	4	50.0	9.4-90.6
Total Diassadié		**17**	**2**	**19**	**100**	**19.0**	**12.2-27.6**
1	Waibera	8	2	10	48	20.8	11.1-34.0
2	Waibera	6	2	8	30	26.7	13.2-44.4
3	Waibera	0	0	0	12	0	0
4	Waibera	0	0	0	10	0	0

Total Waibera		**14**	**4**	**18**	**100**	**18.0**	**11.4-26.5**
1	Kapala	1	0	1	6	16.7	0.8-59.1
2	Kapala	2	0	2	5	40.0	7.3-81.7
3	Kapala	0	3	3	11	27.3	7.5-57.8
4	Kapala	1	0	1	10	10.0	0.5-40.4
5	Kapala	0	0	0	5	0	0
6	Kapala	0	0	0	5	0	0
7	Kapala	1	0	1	3	33.3	1.7-86.8
8	Kapala	5	1	6	28	21.4	9.2-39.3
9	Kapala	0	0	0	16	0	0
10	Kapala	0	0	0	5	0	0
11	Kapala	0	0	0	6	0	0
Total Kapala		**10**	**4**	**14**	**100**	**14.0**	**8.2-21.9**
1	Tiogola	4	1	5	17	29.4	11.7-53.7
2	Tiogola	0	0	0	10	0	0
3	Tiogola	2	2	4	22	18.2	6.1-38.2
4	Tiogola	2	3	5	14	35.7	14.4-62.4
5	Tiogola	0	2	2	20	10.0	1.7-29.3
6	Tiogola	1	2	3	17	17.6	4.7-40.9
Total Tiogola		**9**	**10**	**19**	**100**	**19.0**	**12.2-27.6**

T.c.= *Trypanosoma congolense*; T.v. = *T. vivax*
95% CI=95 % confidence interval

5.1.3.2 Village level trypanosome prevalence

Pre-intervention phase village trypanosome prevalence was derived by aggregating all trypanosome positive herds in a given village over the monitoring period. Cattle in the study villages of the eastern sector had prevalences that ranged between 11% and 16% (Table 6). Finibougou with 16% (95% CI: 9.8-24.2) had the highest prevalence, followed by Daresalame with 14.6% (95% CI: 8.5-22.7). Ziébougou with 11% (95% CI: 5.9-18.3) had the lowest village prevalence in the eastern sector.

In the western sector villages, prevalences ranged between 14% and 19%. Diassadié and Tiogola with 19% (95% CI: 12.2-27.6) had the highest prevalences and Kapala with 14%

(95% CI: 8.2-21.9) the lowest. Village level prevalence estimates between the eastern and western sectors were not significantly (p>0.05) different.

5.1.3.3 Sector level trypanosome prevalence

The eastern sector had an aggregated trypanosome prevalence of 13.9% (95% CI: 10.9-17.4) and the western sector 17.5% (95% CI: 14.1-21.3) (Table 6). There was no significant (p>0.161) difference in trypanosome prevalence between the two sectors.

Table 6: Prevalences of trypanosome infections in cattle in the eastern and western sectors of Sikasso, south-east Mali, by village and by species (November to December 2007)

Village	Trypanosome positive cattle			No. cattle	Prevalence %	95% confidence interval
	T.c.	T.v.	Total			
Eastern sector						
Kafela	10	4	14	100	14.0	8.2-21.9
Finibougou	14	2	16	100	16.0	9.8-24.2
Daresalame	12	2	14	96	14.6	8.5-22.7
Ziébougou	5	6	11	100	11.0	5.9-18.3
Sector total	**41**	**14**	**55**	**396**	**13.9** [a]	**10.7-17.6**
Western sector						
Diassadié	17	2	19	100	19.0	12.2-27.6
Waibera	14	4	18	100	18.0	11.4-26.5
Kapala	10	4	14	100	14.0	8.2-21.9
Tiogola	9	10	19	100	19.0	12.2-27.6
Sector total	**50**	**20**	**70**	**400**	**17.5** [a]	**14.0-21.5**

T.c.= *Trypanosoma congolense*; T.v. = *T. vivax*

[a] No statistical significant (χ^2 test, $p > 0.05$) difference in trypanosome prevalence between the eastern and western sector

Only *Trypanosoma congolense* and *T. vivax* were encountered during the survey, with the former dominating (Table 6). *Trypanosoma congolense* accounted for 74.6% (41/55) of all trypanosome infections in the eastern sector and for 71.4% (50/70) of all trypanosome infections in the western sector. No *T. brucei* or mixed trypanosome infections were diagnosed.

Univariate analysis showed that breed, sex, age, village and sector had no statistical (Pearson χ^2 test, $p > 0.05$) association with trypanosome prevalence. For this reason, no further multivariate logistic regression analysis of risk factors was performed.

Bivariate correlation revealed a weak positive correlation (Pearson correlation squared (R^2) = 0.061, $p>0.05$) between village flies/trap per day and village trypanosome prevalence.

Other haemo-parasites encountered during this survey included microfilariae 46/796 (5.8%) and *Trypanosoma theileri* 13/796 (1.6%).

5.1.4 Trypanocidal drug use practices

Farmers reported using two trypanocidal drugs to manage trypanosomosis: isometamidium chloride (ISMM), colloquially known as the 'red' product and diminazene aceturate (DIM), known as the 'yellow' product. ISMM was used once annually for prophylaxis when trypanosomosis risk was perceived to be highest (during the rainy season), either at the onset or in the middle of the rainy season, typically in August or September. Quantative data on the number of treatments were not collected as the majority respondents had not made records of treatments.

The majority (90% and 100%) of ISMM all-herd treatments were administered by private veterinarians. Besides being used for clinical cases, DIM was strategically used either at the end of the dry season (May or June), shortly before the ISMM treatments, or at the start of the dry season (between October and December). The majority (80% and 100%) of the DIM treatments were performed by farmers or their herdsmen. Trypanocides were bought from private *agrovet* shops located in Sikasso town or in Hérémakono on the Mali-Burkina Faso border.

5.1.5 Packed cell volume (PCV) profiles

Packed cell volumes (PCV) ranged between 11% and 44% with a mean of 26.4 ± 5.0% (mean ± standard deviation) across the study villages. The mean PCV for cattle from the eastern sector was 27.3 ± 5.1 which was statistically ($p<0.001$) higher than the 25.5 ± 4.9 for the

cattle from the western sector. The mean PCV for crossbred cattle was 26.9 ± 5.2% and for zebu 26.2 ± 5.0%. These estimates were not significantly ($p>0.05$) different.

The mean PCV for trypanosome-positive cattle was 23.2 ± 5.5% which was significantly ($p<0.001$) lower than the 27.0 ± 4.8% for the trypanosome-negative cattle. *Trypanosoma congolense* positive cattle had a mean PCV of 22.7 ± 4.9% which was lower than the 26.5 ± 5.1% for *T. vivax* positive cattle, although this difference was not statistically significant ($p>0.05$). Trypanosome positive female cattle had mean PCV of 23.7 ± 5.9 that was significantly ($p<0.05$) higher than the 22.8 ± 5.2% of infected male cattle.

Stratifying study animals into two groups according to PCV < 25% and PCV => 25% with the animals of the former group considered anaemic and the latter normal, showed that 24.7% (41/166) of the cross breed cattle in the eastern sector had PCVs of < 25% as compared to 33% (76/230) zebu cattle in the same sector (Table 7). The difference between the two breeds was not significant ($p>0.05$).

Table 7: Number of cattle by PCV score, breed and village in Sikasso, south-east Mali November to December 2007

Village	**PCV cross breed cattle**			**PCV zebu cattle**		
	<25%	=>25%	Total	<25%	=>25%	Total
Eastern sector						
Kafela	7	23	30	19	51	70
Finibougou	23	42	65	10	25	35
Daresalame	10	56	66	5	25	30
Ziébougou	1	4	5	42	53	95
Sector total	**41**	**125**	**166**	**76**	**154**	**230**
Western sector						
Diassadié	3	9	12	32	56	88
Waibera	16	16	32	34	34	68
Kapala	3	6	9	31	60	91
Tiogola	4	13	17	24	59	83
Sector total	**26**	**44**	**70**	**121**	**209**	**330**

In the western sector, the proportion of cross breed cattle with PCVs < 25% was 37.1% (26/70) and 36.7% (121/209) for zebus. This also was not significantly different (p>0.05). Overall, 57.6% (72/125) of trypanosome-positive cattle had PCVs < 25% while 28.6% (192/671) of the trypanosome-negative cattle had PCVs < 25%.

5.1.6 Trypanocidal drug resistance

This was estimated through the results of the block treatment study at village and sector levels (Table 8). Persistent trypanosomes in the treated trypanosome-positive cattle during the parasitological controls was indicative of a failed treatment and hence resistance. Diminazene treatment failure was observed in all study villages. In the eastern sector villages, DIM treatment failures ranged between 20% and 71.4% being highest in Kafela (71.4%) and lowest in Ziébougou (20%). The village level DIM failure rates in the eastern sector were not significantly (p>0.05) different.

In the western sector villages, DIM treatment failure ranged between 20% and 42.9% with Kapala having the highest (42.9%) treatment failure rate and Tiogola the lowest (20%). There was also no difference (p>0.05) between village DIM failures in the western sector.

At the sector level, DIM failure was 37.0% (10/27) in the eastern sector and 25.7% (9/35) in the western sector (Table 8). This was not significantly (p>0.338) different. In both sectors, *T. congolense* was responsible for all the DIM treatment failures as *T. vivax* strains were apparently sensitive to DIM and were cleared by this drug (Table 8).

Table 8: Treatment failure rates in trypanosome positive cattle treated with 3.5 mg/kg b.w. diminazene (DIM) or 0.5 mg/kg b.w. isometamidium (ISMM) chloride by village and sector in Sikasso, south-east Mali (November to December 2007)

Village	Response 14 days post-treatment with								Response 28 days post-treatment with			
	DIM (n=62 cattle)				ISMM (n=63 cattle)				ISMM			
	T.c.	T.v.	Total	%	T.c.	T.v.	Total	%	T.c.	T.v.	Total	%
Eastern sector												
Kafela	5/6[a]	0/1	5/7	71.4	1/4	0/3	1/7	14.3	1/3	2/3	3/6	50
Finibougou	2/7	0/1	2/8	28.6	4/7	0/1	4/8	50.0	1/3	0/1	1/4	25
Daresalame	2/6	0/1	2/7	28.6	4/6	0/1	4/7	57.1	0/2	1/1	1/3	33.3
Ziébougou	1/2	0/3	1/5	20.0	3/3	0/3	3/6	50.0	-	0/3	0/3	0
Sector total	**10/21**	**0/6**	**10/27**	**37.0**	**12/20**	**0/8**	**12/28**	**42.9**	**2/8**	**3/8**	**5/16**	**31.3**
Western sector												
Diassadié	2/8	0/1	2/9	22.2	3/9	0/1	3/10	30.0	2/6	0/1	2/7	28.6
Waibera	2/7	0/2	2/9	22.2	0/7	0/2	0/9	0	1/7	1/2	2/9	22.2
Kapala	3/5	0/2	3/7	42.9	2/5	1/2	3/7	42.9	0/3	0/1	0/4	0
Tiogola	2/4	0/6	2/10	20.0	1/5	1/4	2/9	22.2	1/4	1/3	2/7	28.6
Sector total	**9/24**	**0/11**	**9/35**	**25.7**	**6/26**	**2/9**	**8/35**	**22.9**	**4/20**	**2/7**	**6/27**	**22.2**

[a] Relapsed/treated
T.c= *Trypanosoma congolense*
T.v= *Trypanosoma vivax*
%=Proportion of treatment failure

ISMM treatment failures at day 14 ranged between 14.3% and 57.1% in the eastern sector villages, with Daresalame (57.1%) having the highest failure, followed closely by Finibougou and Ziébougou, both villages with a failure rate of 50%. There was no difference ($p>0.05$) in the village ISMM failure rates. In the western sector villages, ISMM failure ranged between 0% and 42.9%. Kapala (42.9%) had the highest ISMM treatment failure rate, followed by Diassadié (30%) and Waibera had the lowest (0%). This was also not significantly ($p>0.05$) different. There was variability in treatment failures in both sectors, although it was highest in the eastern sector.

At day 14, the cumulative ISMM failure in the eastern sector was 42.9% and 22.9% for the western sector (Table 8). These were not significantly ($p>0.05$) different. In both sectors, *T. congolense* was responsible for the highest number of ISMM treatment failures. All *T. vivax* apparently were sensitive to ISMM in the eastern sector (Table 8).

Aparasitaemic cattle on ISMM at day 14 were followed to day 28 and had failure rates between 0% and 50% in the eastern sector villages and 0% and 28.6% in the western sector villages. Higher variablility was observed among the villages in the eastern sector than those in the western sector. At day 28, *T. vivax* accounted for more treatment failures than *T. congolense.*

Table 9 summarizes the treatment responses of cattle with relapsed trypanosomes at day 14 that were retreated with 7mg/kg bw DIM. The DIM treatment failure ranged between 40 and 50% in the eastern sector villages and between 0 and 50% in the western sector villages. High variablility was observed among the villages in the western sector as compared to those from the eastern sector. Overall treatment failure in the DIM cohort was 45.5% (5/11) in the eastern and 22.2% (2/9) in the western sector (Table 9). There was no significant ($p>0.05$) difference in these treatment failures between the sectors.

On the other hand, the majority of relapsed trypanosomes after ISMM treatment were cleared after retreatment with 7 mg/kg bw DIM, as was shown by a failure rate of only 8.3% (1/12) in the eastern sector and 25% (2/8) in the western sector. The persistent *T. vivax* strains that persisted after ISMM treatment in the western sector were all cleared by 7mg/kg bw DIM (Table 9).

Table 9: Treatment failure rates in cattle with relapsed trypanosomes re-treated with 7mg/kg b.w. DIM at day 14 in Sikasso, south-east Mali (November to December 2007)

Village	**Response 14 days post-treatment with 7mg/kg bw DIM**					
	Day 14 DIM failures		**Day 14 ISMM failures**			
	T.c.	%	T.c.	T.v.	Total	%
Eastern sector						
Kafela	2/5	40	0/1	0	0/1	0
Finibougou	1/2	50	0/4	0	0/4	0
Daresalame	1/2	50	1/4	0	1/4	25
Ziébougou	1/2	50	0/3	0	0/3	0
Sector total	**5/11**	**45.5**	**1/12**	**0**	**1/12**	**8.3**
Western sector						
Diassadié	0/2	0	2/3	0	2/3	66.7
Waibera	1/2	50	0	0	0	0
Kapala	1/3	33.3	0/2	0/1	0/3	0
Tiogola	0/2	0	0/1	0/1	0/2	0
Sector total	**2/9**	**22.2**	**2/6**	**0/2**	**2/8**	**25**

T.c.= *Trypanosoma congolense;* T.v.= *Trypanosoma vivax*

Treatment of the trypanosome-positive cattle with DIM or ISMM significantly ($p<0.001$) increased the PCV % from 23.2 ± 5.5% at day 0 to 27.3 ± 6.3% at day 14 and eventually to 29.5 ± 6.0 at day 28 (Figure 2).

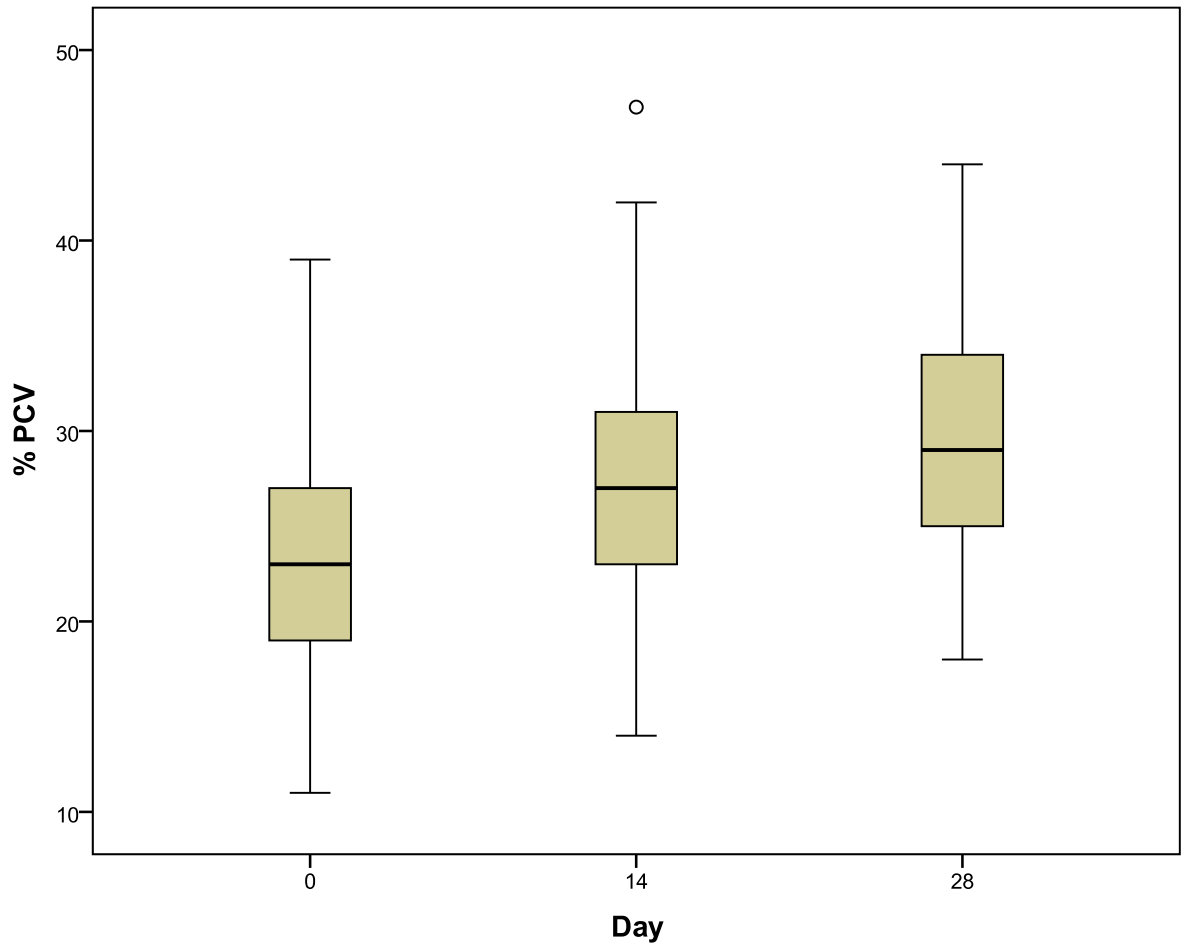

Figure 2: Mean PCVs of trypanosome-positive cattle at day 0 and days 14 and 28 post treatment with 3.5mg/kg b.w. diminazene or 0.5 mg/kg b.w. isometamidium in Sikasso, south-east Mali (November to December 2007)

5.2 Intervention phase

5.2.1 Tsetse catches

Tsetse catch data were summarized at village level first, before being aggregated at area level.

5.2.1.1 Village level tsetse catches

Tsetse catches for the intervention area villages are presented in Figures 3 to 6. *Glossina p. gambiensis* was dominant over *G. tachinoides* in all villages of the intervention area over the monitoring period. Inter-village tsetse catch variations were observed during the intervention phase.

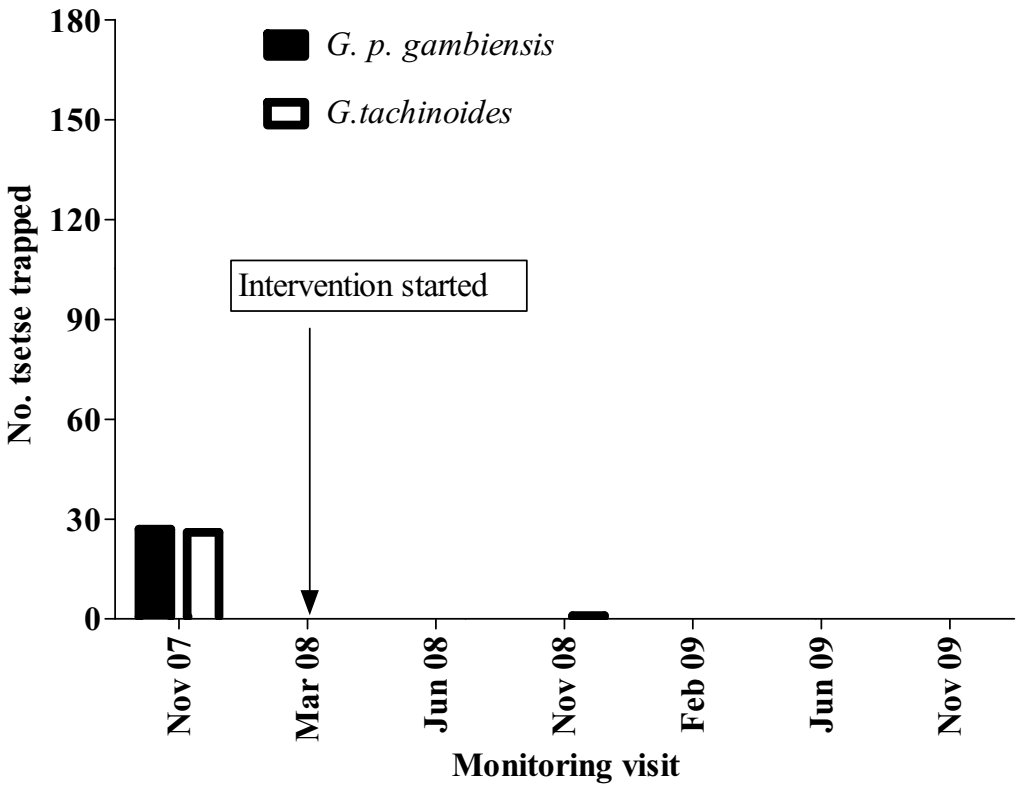

Figure 3: Tsetse catches by species in Kafela village within the intervention area by monitoring visit, Sikasso south-east Mali (June 2008 to November 2009)

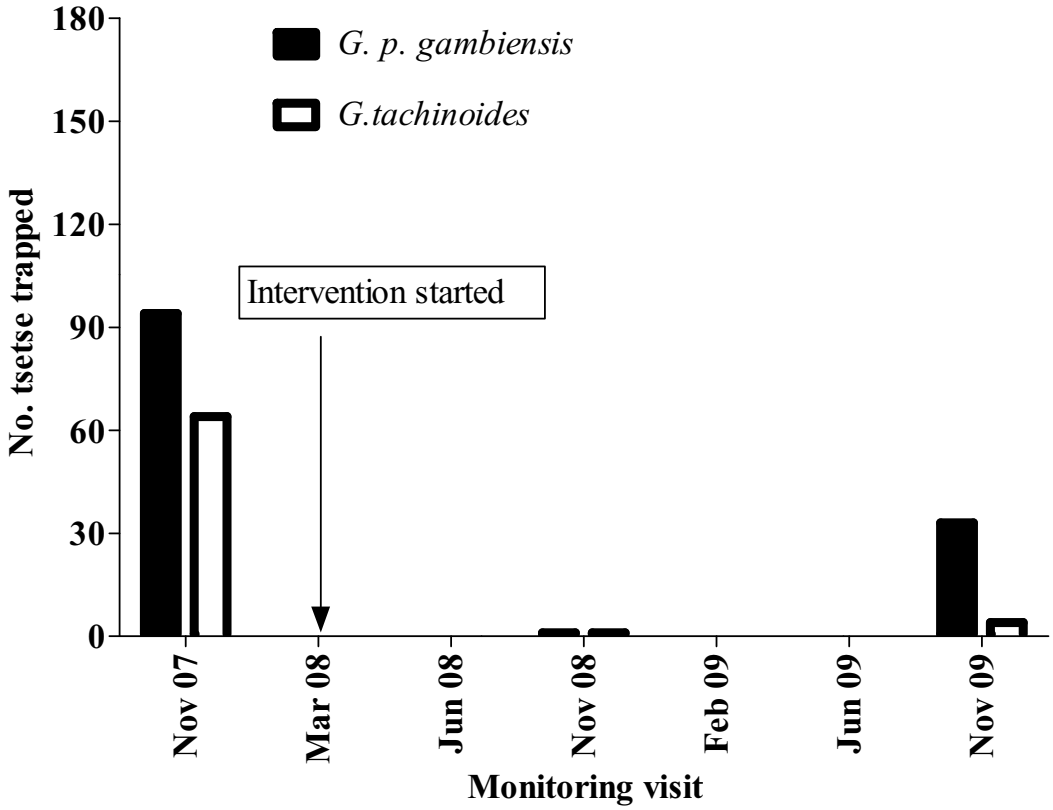

Figure 4: Tsetse catches by species in Finibougou village within the intervention area by monitoring visit, Sikasso south-east Mali (June 2008 to November 2009)

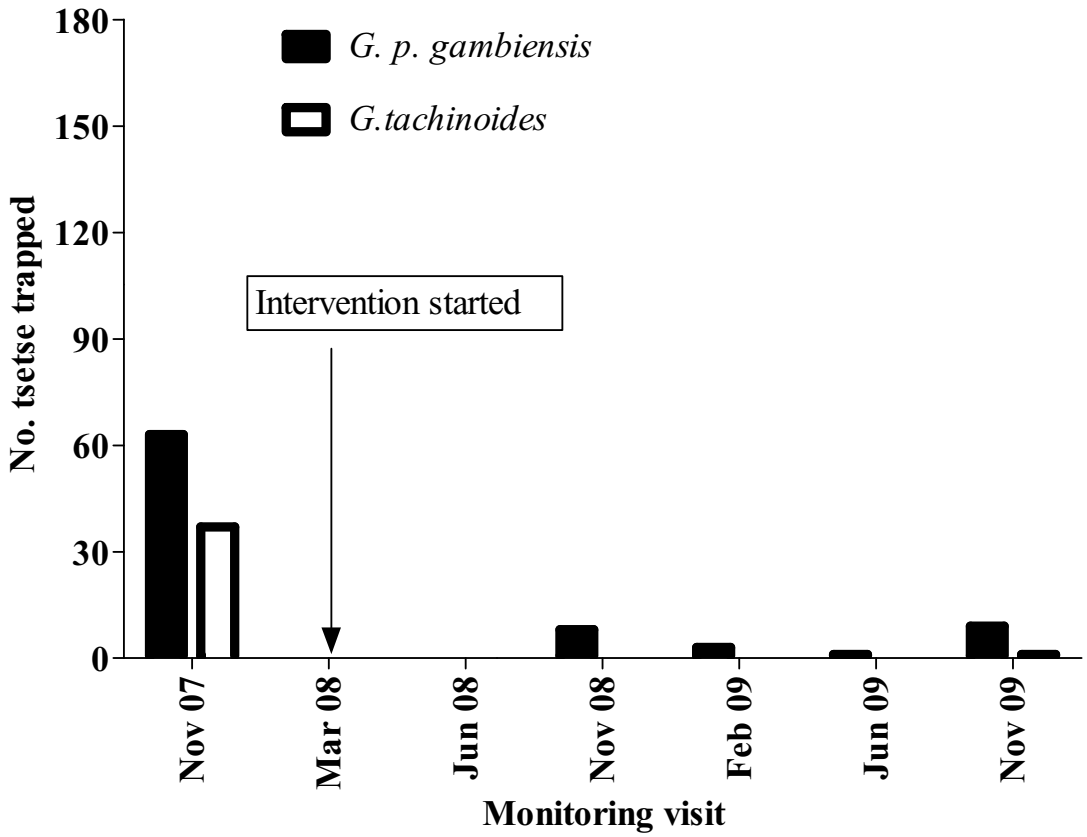

Figure 5: Tsetse catches by species in Daresalame village within the intervention area by monitoring visit, Sikasso south-east Mali (June 2008 to November 2009)

Finibougou had the highest tsetse catch followed by Daresalame, Ziébougou and Kafela in that order.

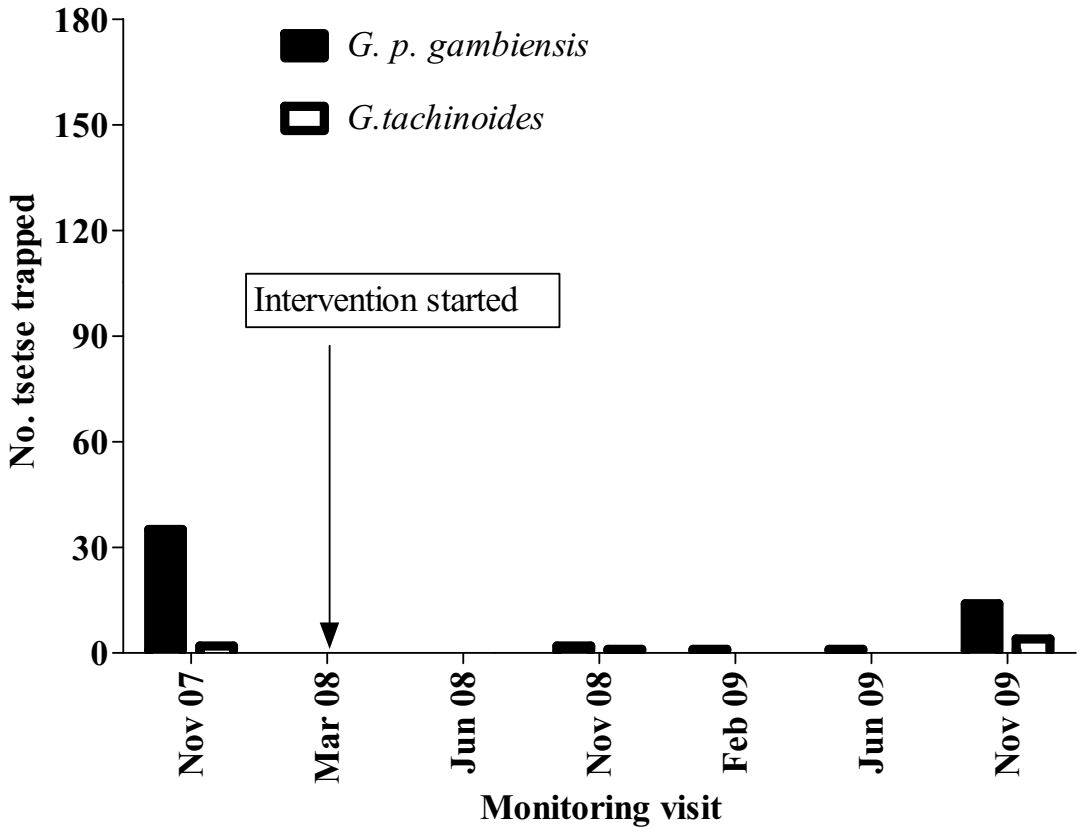

Figure 6: Tsetse catches by species in Ziébougou village within the intervention area by monitoring visit, Sikasso, south-east Mali (June 2008 to November 2009)

In June 2008, the first monitoring was conducted exactly three months after the commencement of the tsetse control intervention, all the four villages in the intervention area reported zero fly catches. Overall, a slight recovery in tsetse catches in all intervention area villages was observed during the November 2009 monitoring visit except in Kafela.

In the control area study villages, tsetse catch levels during the intervention study period fluctuated by monitoring visit but did not show much variation from the pre-intervention level (Figures 7 to10).

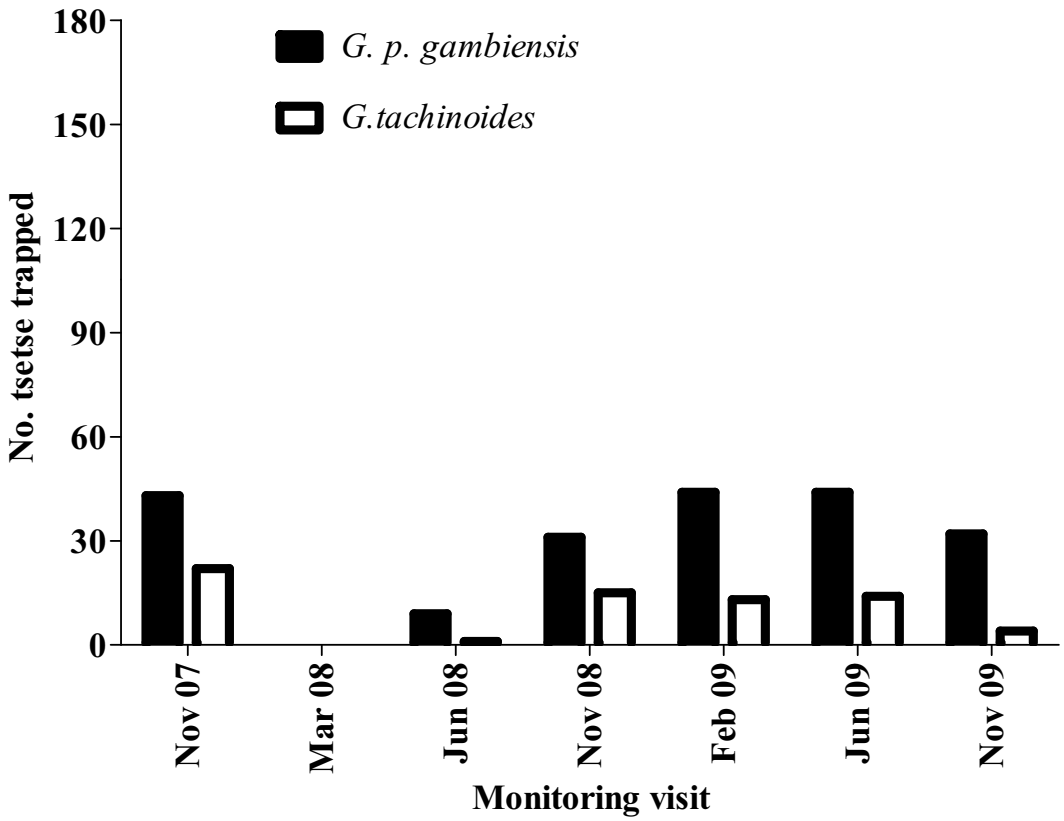

Figure 7: Tsetse catches by species in Diassadié village within the control area by monitoring visit, Sikasso, south-east Mali (June 2008 to November 2009)

Glossina p. gambiensis was dominant in two villages, Diassadié and Waibera, while *G. tachinoides* dominated catches in Kapala and Tiogola. Waibera had the highest tsetse catch throughout the monitoring period, followed by Diassadié. Kapala had the least fly catch. In November 2009, the *G. p. gambiensis* catch in Waibera was about 5-fold higher than its catch over the other 4 monitoring visits (Figure 8).

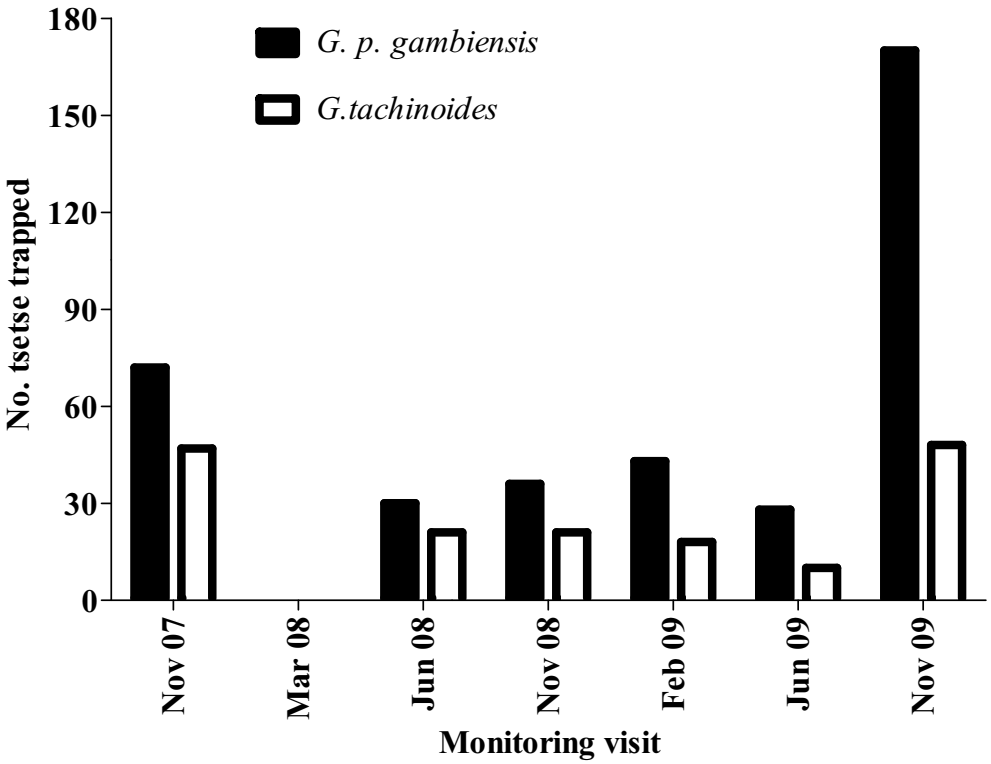

Figure 8: Tsetse catches by species in Waibera village within the control area by monitoring visit, Sikasso, south-east Mali (June 2008 to November 2009)

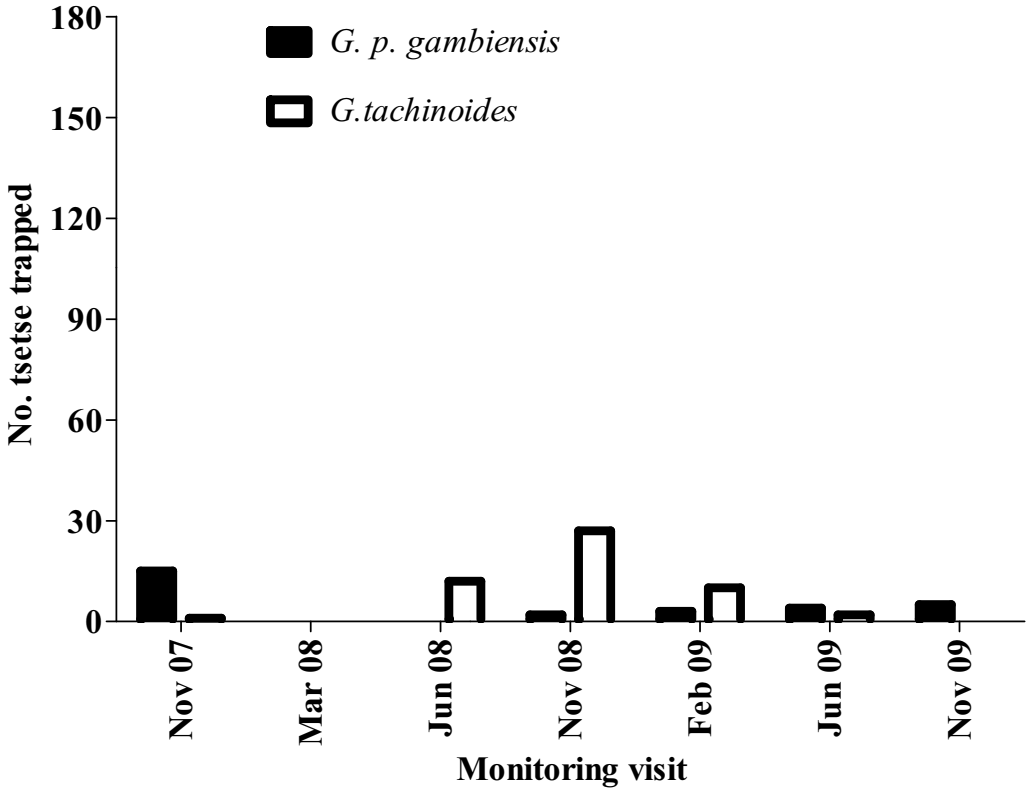

Figure 9: Tsetse catches by species in Kapala village within the control area by monitoring visit, Sikasso, south-east Mali (June 2008 to November 2009)

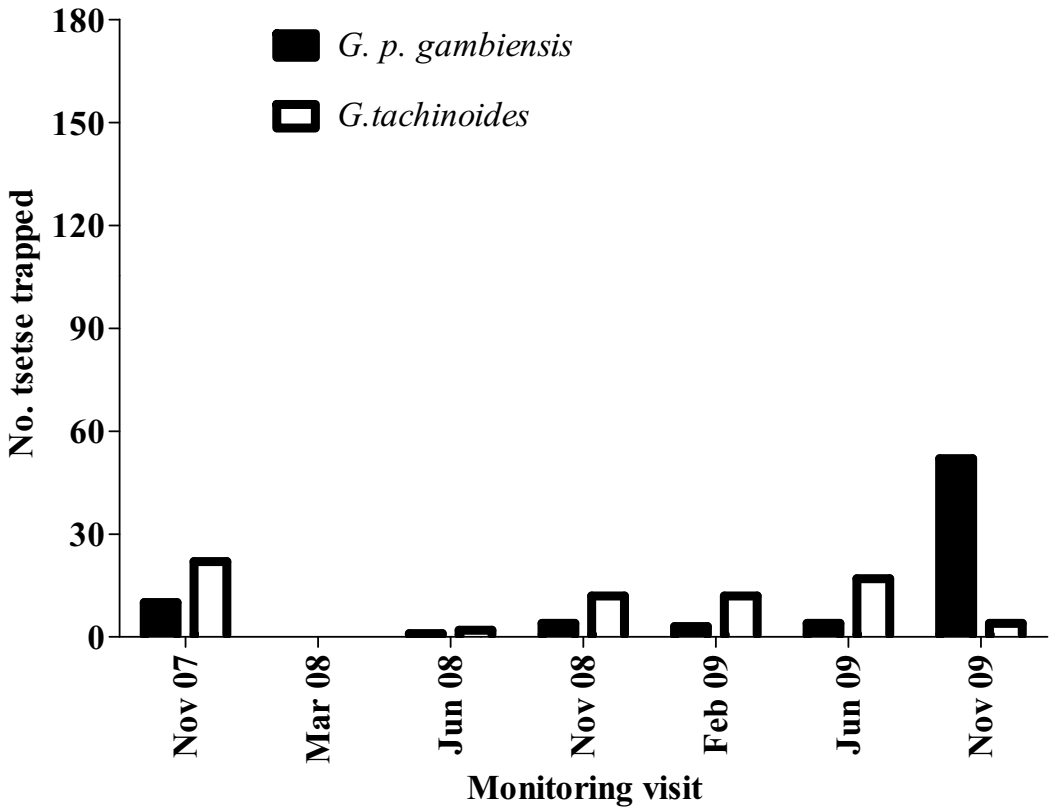

Figure 10: Tsetse catches by species in Tiogola village within the control area by monitoring visit, Sikasso southeast Mali (June 2008-November 2009)

In the control area, there was an increase in tsetse catches from east to west, with Waibera (Figure 8) and Diassadié (Figure 7) located to the east of the control area having the highest catches compared to Kapala (Figure 9) and Tiogola (Figure 10) situated to the west of the control area. Similarly, *G. p. gambiensis* was dominant in the eastern portion with *G. tachinoides* catches dominating in the western portion of this area.

5.2.1.2 Area level tsetse catches

Tsetse catch data in the intervention and the control areas were aggregated from the respective village catches per monitoring visit and differentiated for fly species (Table 10). Following the start of the tsetse control intervention, there was a sharp decline in tsetse catches in the intervention area. In the control area, fly catches remained constantly high with only minimal fluctuations over the entire monitoring period. However, in this area, the *G. p. gambiensis* catches in November 2009 almost doubled the number trapped during the pre-intervention phase (Table 10).

Table 10: Number of *Glossina* caught by species, area (20 traps/area) and time in Sikasso, south-east Mali (June 2008 to November 2009)

Monitoring visit	*G. p. gambiensis*		*G. tachinoides*		Total fly catch	
	No. flies	FTD	No. flies	FTD	No. flies	FTD
Intervention area						
November 2007[a]	**167**[b]	**8.35**	**91**[b]	**4.5**	**258**	**12.9**
June 2008	0	0	0	0	0	0
November 2008	11	0.55	3	0.15	14	0.7
February 2009	4	0.2	0	0	4	0.2
June 2009	2	0.1	0	0	2	0.1
November 2009	56	2.8	9	0.45	65	3.25
Area total	**73**	**0.73**	**12**	**0.12**	**85**	**0.85**
Control area						
November 2007[a]	**129**[b]	**6.45**	**84**[b]	**4.2**	**213**	**10.65**
June 2008	40	2.0	25	1.25	65	3.25
November 2008	73	3.63	60	3.0	133	6.65
February 2009	93	4.65	70	3.5	163	8.15
June 2009	80	4.0	51	2.55	131	6.55
November 2009	259	12.95	58	2.9	317	15.85
Area total	**545**	**5.45**	**264**	**2.64**	**809**	**8.09**

[a] Pre-intervention tsetse catches used as a basis for comparison and not part of the totals
[b] Tsetse catches of the pre-intervention phase in the 5 tsetse trapping points used throughout the monitoring period
FTD = flies/trap/day

Figure 11 shows percent reduction in tsetse catches in the two areas. In the intervention area, *G. p. gambiensis* catches overall reduced by 91.3% (from FTD of 8.35 in November 2007 to 0.73 in November 2009) while *G. tachinoides* catches reduced by 97.3% (from FTD of 4.5 in November 2007 to 0.12 in November 2009). Lowest reduction in the *G. p. gambiensis* and *G. tachinoides* catches in the intervention area was observed in November 2009. In the control area, the respective reduction was 18.3% (from FTD of 6.45 in November 2007 to 5.45 in November 2009) for *G. p. gambiensis* catches and 37.1% (from FTD 4.2 in November 2007 to 2.64 in November 2009) for *G. tachinoides*. There was, however, an increase of 85% in *G. p. gambiensis* catches in the control area in November 2009.

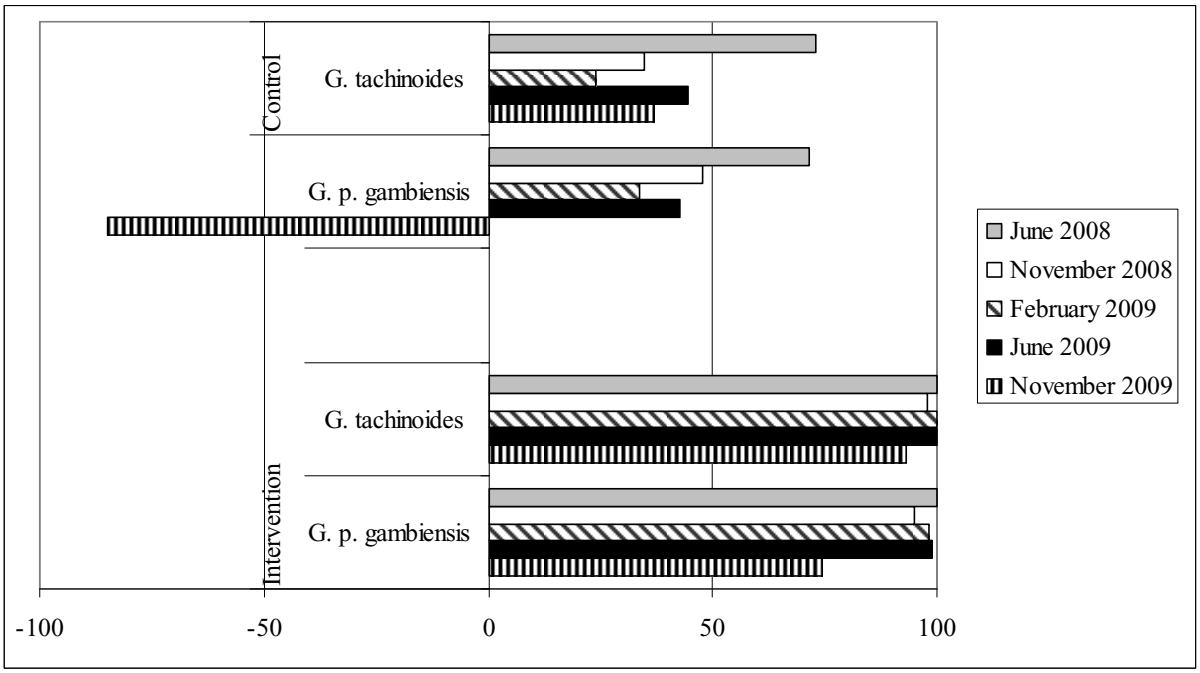

Figure 11: Percent reduction in tsetse catches in the intervention and control area during the study period by species in Sikasso, south-east Mali (June 2008 to November 2009)

June 2008 and 2009 (start of the rainy season) and February 2009 (dry season) recorded higher percent reductions in tsetse catches, while November 2008 and 2009 (end of rainy season) had the lowest reduction rates (Figure 11). *Glossina palpalis gambiensis* dominated all fly catches.

5.2.2 Risk group cattle characterization

The number and proportion of risk group cattle for each monitoring visit is presented in Table 11. In the intervention area, 71 risk group cattle were examined in June 2008 (monitoring visit 1) but increased by almost 200% to 212 in November 2009 (monitoring visit 5). In this area, among the crossbred cattle, females accounted for 63.6% (199/313). Male cattle were the majority within the zebu breed, accounting for 58.6% (232/396) with female cattle accounting for 41.4% (160/396).

Table 11: Risk group cattle examined by monitoring visit, sex and breed in the intervention and control areas of Sikasso, south-east Mali (June 2008 to November 2009)

Monitoring visit	No. crossbred cattle				No. Zebu cattle				Grand Total
	Female	Male	Total	%	Female	Male	Total	%	
Intervention area									
June 2008	20	12	32	**45.1**	13	26	39	**54.9**	**71**
November 2008	36	20	56	**44.4**	29	41	70	**55.6**	**126**
February 2009	41	21	62	**44.6**	34	43	77	**54.4**	**139**
June 2009	48	24	72	**44.7**	39	50	89	**55.3**	**161**
November 2009	54	37	91	**42.9**	49	72	121	**57.1**	**212**
Control area									
June 2008	19	6	25	**29.8**	33	26	59	**70.2**	**84**
November 2008	22	12	34	**21.7**	59	64	123	**78.3**	**157**
February 2009	25	13	38	**23.3**	58	67	125	**76.7**	**163**
June 2009	22	13	35	**20.0**	63	77	140	**80.0**	**175**
November 2009	27	12	39	**20.7**	63	87	150	**79.3**	**188**

In the control area, a 123% increase in the number of risk group cattle was recorded over the study period. The number rose from 84 (21 crossbred and 63 zebu) in June 2008 to 188 (37 crossbred cattle and 151 zebu) in November 2009. In this area, the proportion of female animals for cross-bred cattle was 67.3% (115/171) with the 32.7% (56/171) being male cattle. Males were the majority among zebu cattle accounting for 53.2% (322/605) while females accounted for 46.3% (276/596).

As new born animals joined the study group, so were withdrawals witnessed. In both study areas, the number of risk cattle that participated in the June 2008 monitoring was high but some of these animals were progressively lost for follow-up in the succeeding monitoring visits. In the control area, of the initial 84 risk cattle examined in June 2008, 20 of them were lost to follow-up, hence a 23.8% (20/84) loss compared to 14.1% (from 71 to 61) in the intervention area. Reasons for losses of animals were varied but were mainly due to mortalities (particularly in the control area) and due to non-compliance of animal keepers witnessed in both areas.

5.2.3 Trypanosome infections

5.2.3.1 Trypanosome prevalence

As done for the pre-intervention phase, trypanosome prevalences for the intervention phase are presented at the level of herd, village and area for all monitoring visits.

5.2.3.1.1 Herd level trypanosome prevalence

A total of 20 herds were monitored in the intervention area for trypanosome prevalence. There was clustering of trypanosome infections within individual herds in study villages of the intervention area. In Kafela for instance, of the 7 herds monitored, one herd had the highest trypanosome prevalence throughout the monitoring period except in November 2009 (Table 12). Two other herds in Kafela were positive for trypanosomes only once (November 2008 visit). In Finibougou and Daresalame, in contrast, only one single positive herd was present from which one animal was affected, respectively (Table 12).

Inter-herd prevalence variability was particularly evident in Kafela, with herd prevalences ranging between 0% and 20%. This was followed by Finibougou with herd prevalences between 0% and 3.8% (Table 12). Ziébougou's two herds were negative for trypanosomes throughout all monitoring visits. Seasons did affect affect herd prevalence as the majority of herds were affected in November 2008 (end of the rainy season) and in June of 2008 and 2009 (start of the rainy season). In the monitoring visit February 2009 (dry season), in contrast, the lowest herd prevalences in the intervention area were recorded.

Table 12: Herd trypanosome prevalences by village and monitoring point in the intervention area of Sikasso, south-east Mali (June 2008 to November 2009)

Herd No.	Village	June 2008	November 2008	February 2009	June 2009	November 2009	Herd Total	95% CI
		Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	
1	Kafela	0 (0/5)	14.3 (1/7)	0 (0/8)	0 (0/7)	0 (0/13)	2.5 (1/40)	0.1-11.7
2	Kafela	0 (0/1)	0 (0/2)	0 (0/2)	-	0 (0/6)	0 (0/11)	0
3	Kafela	0 (0/1)	100 (1/1)	0 (0/1)	0 (0/1)	0 (0/1)	20.0 (1/5)	1-66.6
4	Kafela	0 (0/2)	0 (0/10)	0 (0/8)	0 (0/6)	0 (0/16)	0 (0/42)	0
5	Kafela	-	14.3 (1/7)	0 (0/7)	0 (0/6)	0 (0/10)	3.3 (1/30)	0.2-15.4
6	Kafela	28.6 (4/14)	33.3 (5/15)	6.3 (1/16)	5 (1/20)	0 (0/21)	12.8 (11/86)	6.9-21.1
7	Kafela	-	0 (0/2)	0 (0/2)	0 (0/2)	0 (0/3)	0 (0/15)	0
Kafela total		**17.4 (4/23)**	**18.2 (8/44)**	**2.3 (1/44)**	**2.4 (1/42)**	**0 (0/70)**	**6.3 (14/223)**	**3.6-10.1**
1	Finibougou	0 (0/2)	0 (0/2)	-	0 (0/5)	0 (0/5)	0 (0/14)	0
2	Finibougou	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/1)	0 (0/5)	0
3	Finibougou	0 (0/5)	0 (0/12)	0 (0/18)	0 (0/19)	0 (0/23)	0 (0/77)	0
4	Finibougou	0 (0/9)	0 (0/10)	0 (0/13)	0 (0/22)	0 (0/19)	0 (0/73)	0
5	Finibougou	0 (0/1)	0 (0/1)	-	0 (0/3)	0 (0/4)	0 (0/8)	0
6	Finibougou	0 (0/2)	20 (1/5)	0 (0/6)	0 (0/5)	0 (0/7)	4 (1/25)	0.2-18.2
7	Finibougou	0 (0/3)	0 (0/3)	0 (0/4)	0 (0/5)	0 (0/5)	0 (0/20)	0

Finibougou total		**0 (0/23)**	**2.9 (1/34)**	**0 (0/42)**	**0 (0/60)**	**0 (0/64)**	**0.5 (1/222)**	**0.0-2.2**
1	Daresalame	0 (0/5)	0 (0/9)	0 (0/10)	0 (0/10)	0 (0/14)	0 (0/48)	0
2	Daresalame	0 (0/4)	0 (0/4)	0 (0/7)	14.3 (1/7)	0 (0/7)	3.4 (1/29)	0.2-15.9
3	Daresalame	0 0/5)	0 (0/12)	0 (0/11)	0 (0/12)	0 (0/18)	0 (0/58)	0
4	Daresalame	0 (0/2)	0 (0/3)	0 (0/3)	0 (0/3)	0 (0/7)	0 (0/18)	0
Daresalame total		**0 (0/16)**	**0 (0/28)**	**0 (0/31)**	**3.1 (1/32)**	**0 (0/46)**	**0.7 (1/153)**	**0.0-3.2**
1	Ziébougou	-	0 (0/5)	0 (0/7)	0 (0/8)	0 (0/8)	0 (0/8)	0
2	Ziébougou	0 (0/9)	0 (0/15)	0 (0/15)	0 (0/19)	0 (0/24)	0 (0/24)	0
Ziébougou total		**0 (0/9)**	**0 (0/20)**	**0 (0/22)**	**0 (0/27)**	**0 (0/32)**	**0 (0/110)**	**0**
Intervention area total		**5.6 (4/71)**	**7.1 (9/126)**	**0.7 (1/139)**	**1.2 (2/161)**	**0 (0/212)**	**2.3 (16/709)**	**1.3-3.6**

Prev %=Prevalence in %
Parentheses (x/n) represents number of trypanosome positive cattle over the population at risk
95% CI = 95% confidence interval
- = failure of herd to participate

In the control area, 21 herds were monitored for trypanosome prevalence over the study period. Both herds in Waibera had the highest prevalence ranging between 50% and 58.3% over the monitoring period (Table 13). This was followed by herds in Kapala where prevalence ranged between 0.1% and 50%. Diassadié herds had the lowest prevalences that ranged between 10.6% and 14.3% over the monitoring period.

As was the case with the intervention area, inter-herd variation was observed also in the control area. Generally, trypanosome infections were stabile endemic in most herds, as opposed to the sporadic pattern observed in the intervention area. The seasonal effect was also clearly manifest, with herds having highest prevalences in November 2008 and November 2009 followed by June 2008 and June 2009 (Table 13). Herds had the lowest prevalences in November 2009.

Table 13: Herd trypanosome prevalences by village and monitoring point in the control area of Sikasso, south-east Mali (June 2008 to November 2009)

Herd No.	Village	June 2008	November 2008	February 2009	June 2009	November 2009	Herd Total	95% CI
		Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	
1	Diassadié	0 (0/4)	0 (0/9)	11.1 (1/9)	30.0 (3/10)	0 (0/15)	8.5 (4/47)	2.8-19.3
2	Diassadié	0 (0/2)	8.3 (1/12)	6.7 (1/15)	42.9 (6/14)	5 (1/20)	14.3 (9/63)	7.2-24.6
Diassadié total		**0 (0/6)**	**4.8 (1/21)**	**8.3 (2/24)**	**37.5 (9/24)**	**2.9 (1/35)**	**11.8 (13/110)**	**6.7-18.9**
1	Waibera	100 (4/4)	66.7 (2/3)	0 (0/4)	25.0 (1/4)	66.7 (2/3)	50.0(9/18)	27.8-72.2
2	Waibera	66.7 (4/6)	100 (4/4)	42.9 (3/7)	66.7 (2/3)	50.0 (1/2)	63.6 (14/22)	42.4-81.5
Waibera total		**80 (8/10)**	**85.7 (6/7)**	**27.3 (3/11)**	**42.9 (3/7)**	**60 (3/5)**	**54.8 (23/42)**	**39.6-69.2**
1	Kapala	100 (2/2)	25.0 (1/4)	-	-	-	50.0 (3/6)	14.7-85.3
2	Kapala	100 (2/2)	44.4 (4/9)	22.2 (2/9)	22.2 (2/9)	-	34.5 (10/29)	19.0-52.9
3	Kapala	0 (0/3)	-	60.0 (3/5)	60.0 (3/5)	0 (0/4)	35.3 (6/17)	15.7-59.5
4	Kapala	0 (0/5)	30.0 (3/10)	0 (0/10)	0 (0/11)	23.1 (3/13)	12.2 (6/49)	5.1-23.7
5	Kapala	25.0 (1/4)	0 (0/10)	0 (0/10)	20.0 (2/10)	44.4 (4/9)	16.3 (7/43)	7.4-29.6
6	Kapala	0 (0/1)	40.0 (0/5)	0 (0/5)	20.0 (1/5)	0 (0/5)	4.8 (1/21)	0.2-21.3
7	Kapala	0 (0/10)	0 (2/14)	14.3 (2/14)	0 (0/14)	31.3 (5/16)	13.2 (9/68)	6.7-22.9
8	Kapala	50.0 (1/2)	25.0 (1/4)	0 (0/4)	33.3 (1/3)	0 (0/3)	18.8 (2/16)	2.2-35.5
9	Kapala	25.0 (1/4)	-	-	-	-	25.0 (1/4)	1.3-75.8
10	Kapala	0 (0/5)	8.3 (1/12)	16.7 (2/12)	0 (0/11)	54.5 (6/11)	22.0 (9/41)	11.3-36.5
11	Kapala	0 (0/15)	7.4 (2/27)	13.0 (3/23)	8.7 (2/23)	34.8 (8/23)	13.5 (15/111)	8.1-20.9
12	Kapala	0 (0/4)	33.3 (2/6)	13.2 (2/11)	18.2 (2/11)	11.1 (2/18)	16.0 (8/50)	7.7-28.1

Kapala total		**12.3 (7/57)**	**15.7 (16/102)**	**13.6 (14/103)**	**12.0 (13/108)**	**27.5 (28/102)**	**19.0 (77/406)**	**15.4-23.0**
1	Tiogola	0 (0/1)	25.0 (1/4)	-	0 (0/5)	0 (0/6)	6.3 (1/16)	0.3-27.2
2	Tiogola	25 (1/4)	45.5 (5/11)	0 (0/10)	0 (0/4)	5.9 (1/17)	15.2 (7/46)	7.0-27.8
3	Tiogola	0 (0/1)	50.0 (2/4)	0 (0/3)	0 (0/3)	50.0 (2/4)	26.7 (4/15)	9.1-52.5
4	Tiogola	0 (0/4)	25.0 (1/4)	40 (2/5)	0 (0/5)	20.0 (2/10)	17.9 (5/28)	6.9-35.2
5	Tiogola	0 (0/1)	0 (0/4)	14.3 (1/7)	12.5 (1/8)	0 (0/9)	6.9 (2/29)	1.2-21.0
Tiogola total		**9.1 (1/11)**	**33.3 (9/27)**	**12 (3/25)**	**2.8 (1/36)**	**10.9 (5/46)**	**14.2 (19/134)**	**9.0-20.9**
Control area total		**19 (16/84)**	**20.4 (32/157)**	**14.1 (22/163)**	**16 (26/175)**	**20.2 (37/188)**	**17.3 (133/767)**	**14.8-20.1**

Prev %=Prevalence in %
Parentheses (x/n) represent number of trypanosome positive cattle over the population at risk
95% CI = 95% confidence interval
- = failure of herd to participate

5.2.3.1.2 Village level trypanosome prevalence

Information on village level prevalences was derived by aggregating all trypanosome-positive herds in each village. Overall, the study villages within the intervention area had lower prevalences throughout the monitoring period when compared to those in the control area (Table 14). In the intervention area, Kafela village with prevalence ranging from 0% and 18.2% and a mean of 6.3% had the highest prevalence. It was followed by Daresalame with 0.7% and then Finibougou with 0.4% (Table 14). Ziébougou was the least affected village, with no trypanosome infections throughout the monitoring period. Inter-village prevalences ranged between 0% and 6.3%, with trypanosome prevalence being significantly (non-overlapping 95% CI) higher in Kafela compared to other villages of the intervention area.

Table 14: Village level trypanosome prevalences by area and monitoring visit of Sikasso, south-east Mali (June 2008 to November 2009)

Village	June 2008	November 2008	February 2009	June 2009	November 2009	Total period	95% CI
	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	Prev % (x/n)	**Prev % (x/n)**	
Intervention area							
Kafela	17.4 (4/23)	18.2 (8/44)	2.3 (1/44)	2.4 (1/42)	0 (0/70)	**6.3 (14/223)**	**3.6-10.1**
Finibougou	0 (0/23)	2.9 (1/34)	0 (0/42)	0 (0/60)	0 (0/64)	**0.4 (1/222)**	**0.02-2.2**
Daresalame	0 (0/16)	0 (0/28)	0 (0/31)	3.1 (1/32)	0 (0/46)	**0.7 (1/153)**	**0.03-3.2**
Ziébougou	0 (0/9)	0 (0/20)	0 (0/22)	0 (0/27)	0 (0/32	**0 (0/110)**	**0**
Area total	**5.6 (4/71)**	**7.1 (9/126)**	**0.7 (1/139)**	**1.2 (2/161)**	**0 (0/212)**	**2.3 (16/709)**	**1.3-3.6**
Control area							
Diassadié	0 (0/6)	4.8 (1/21)	8.3 (2/24)	41.7 (9/24)	2.9 (1/35)	**11.8 (13/110)**	**6.7-18.9**
Waibera	80 (8/10)	85.7 (6/7)	27.3 (3/11)	42.9 (3/7)	60 (3/5)	**57.5 (23/40)**	**41.9-72.0**
Kapala	12.3 (7/57)	15.7 (16/102)	13.6 (14/103)	12.0 (14/108)	27.5 (28/102)	**19.0 (77/406)**	**15.4-23.0**
Tiogola	9.1 (1/11)	33.3 (9/27)	12.0 (3/25)	2.8 (1/36)	10.9 (5/46)	**14.2 (19/134)**	**9.0-20.9**
Area total	**19.0 (16/84)**	**20.4 (32/157)**	**14.1 (22/163)**	**16.0 (26/175)**	**20.2 (37/188)**	**17.3 (133/769)**	**14.8-20.1**

95% CI=95% confidence interval
Parentheses () contains number of trypanosome positive cattle over the population at risk

Waibera village in the control area had the highest trypanosome prevalences, ranging between 27.3% and 85.7% with a mean of 57.5% (Table 14). Kapala with trypanosome prevalences ranging between 12% and 27.5% and a mean of 19.0% had the second highest prevalence in the control area while Diassadié had the lowest trypanosome prevalence among the control area villages (Table 14). Again, inter-village variation (ranging between 11.8% and 57.5%) was evidently clear within the control area, with trypanosome prevalence in Waibera village being significantly (non-overlapping 95% CI) higher than that of other villages in this area. Seasonal effects again were clearly manifest.

5.2.3.1.3 Area level prevalence

Village trypanosome prevalences were aggregated into area prevalences. At the area level, prevalence was generally higher in the control area as compared to the intervention area. Over the monitoring period, trypanosome prevalences ranged between 0 and 7.1% with a cumulative mean of 2.3% in the intervention area and 14.1% and 20.4% with a mean of 17.3% in the control area (Table 15). November 2008 showed the highest prevalence in both areas and February 2009 the lowest in the control area. The overall prevalence for the intervention area was significantly ($p<0.001$) lower than that of the control area. The estimated odds ratio between the two areas was 7.68 (95% CI: 4.621-12.78). Except for the June 2008 monitoring visit, prevalence was significantly (non-overlapping 95% confidence intervals) higher in the control area than in the intervention area during every visit.

Table 15: Trypanosome prevalences by area and trypanosome species by evaluation time of Sikasso, south-east Mali (June 2008 to November 2009)

Monitoring visit	Trypanosome positive cattle				No. risk cattle	Prevalence (%)	95% CI
	T. c.	T. v.	Mixed[a]	Total			
Intervention area							
June 2008	1	3	0	4	71	5.6	1.8 - 13.0
November 2008	2	7	0	9	126	7.1	3.5 -12.7
February 2009	0	1	0	1	139	0.7	0.0-3.5
June 2009	1	1	0	2	161	1.2	0.2-4.0
November 2009	0	0	0	0	212	0	0
Area total	**4**	**12**	**0**	**16**	**709**	**2.3**	**1.3-3.6**
Control area							
June 2008	2	14	0	16	84	19.0	11.7-28.5
November 2008	13	17	2	32	157	20.4	14.6-27.2
February 2009	11	10	1	22	163	14.1	9.4-20.1
June 2009	11	13	2	26	175	16	10.2-20.7
November 2009	21	15	1	37	188	20.2	14.9-26.4
Area total	**58**	**69**	**6**	**133**	**767**	**17.3**	**14.8-20.1**

[a] Mixed infection = *T. congolense* detected in the same animal together with *T. vivax*
T.c.= *Trypanosoma congolense*; T.v. = *T. vivax*
95% CI= 95% confidence interval

Trypanosoma congolense and *T. vivax* were the two trypanosome species encountered during the monitoring visits. *Trypanosoma vivax* was the dominant species in both areas, accounting for 75% (12/16) of all trypanosome infections within the intervention area and for 51.9% (69/133) within the control area. *Trypanosoma congolense* accounted for just 25% (4/16) in the intervention area and for 43.6% (58/133) in the control area. Mixed trypanosome infections (*T. congolense* and *T. vivax*), totalling 4.5% (6/133), were detected only in the control area (Table 15). No *T. brucei* were detected in both areas.

In the intervention area, there was weak correlation (R^2 = 0.186) between the monitoring visits flies/trap/day and the trypanosome point prevalence. In the control area, this correlation was equally weak (R^2 = 0.0947).

Other haemo-parasites encountered during the monitoring visits included microfilariae 14/1476 (1.0%) and *Trypanosoma theileri* 6/1476 (0.4%).

5.2.3.2 Trypanosome incidence density rate (IDR)

In June 2008, when the first monitoring visit was conducted, the risk group cattle started with zero risk time into the calculations; hence no IDR is presented for this visit.

5.2.3.2.1 Herd level IDR

Sporadic clustering of trypanosome infections in the herds of the intervention area was observed. Kafela had the highest herd IDRs within the intervention area (Figure 12). In this village, the highest risk-period was June-November 2008.

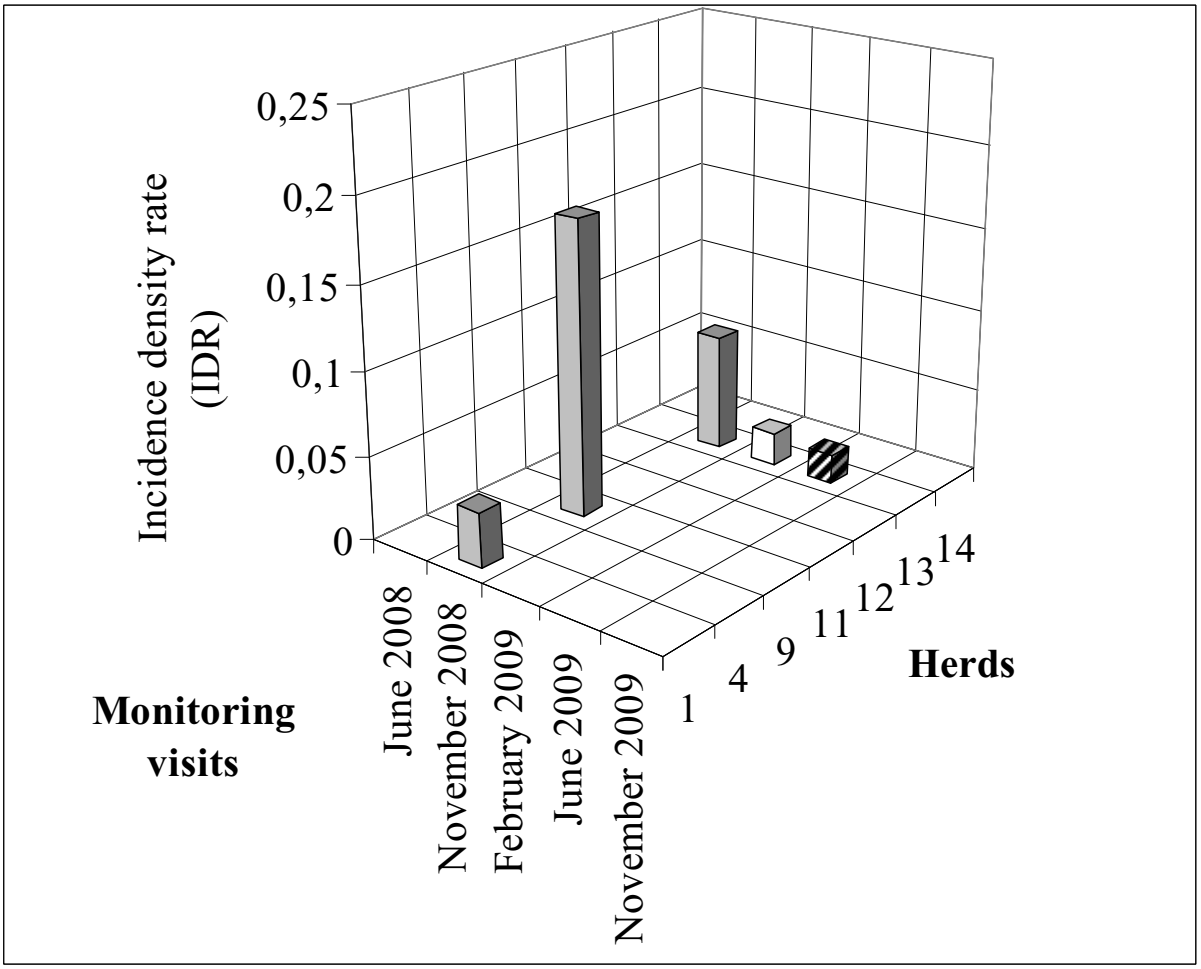

Figure 12: Monitoring period trypanosome herd incidence density rates for the risk group cattle in Kafela village of the intervention area, Sikasso, south-east Mali (June 2008 to November 2009)

In Finibougou and Daresalame only a single animal in a herd was affected once. Ziébougou had no trypanosome infections throughout the monitoring period. Generally, herds within the control area had higher IDR compared to those in the intervention area. Inter-herd variations were evident in the control area.

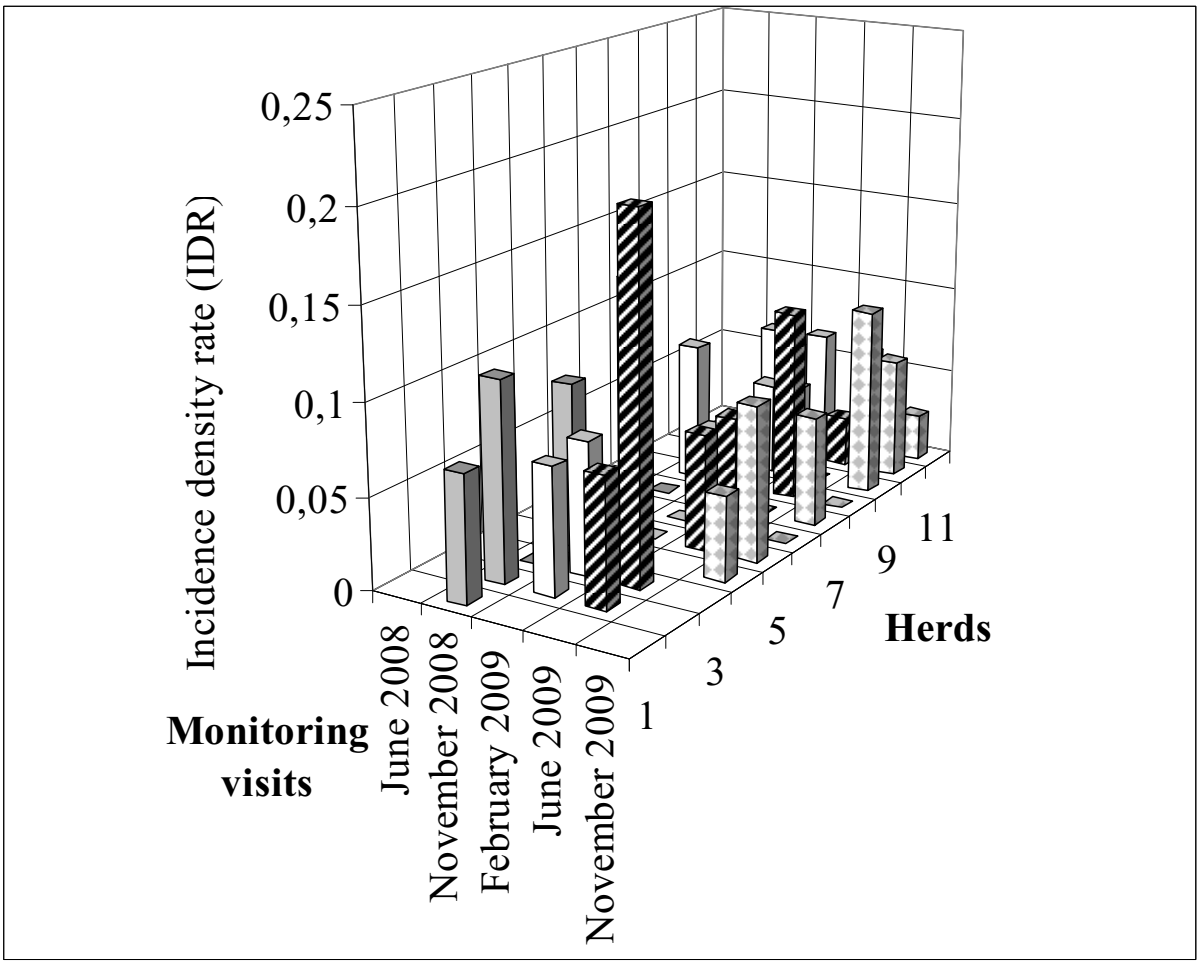

Figure 13: Monitoring period trypanosome herd incidence density rates for the risk group cattle in Kapala village of the control area, Sikasso, south-east Mali (June 2008 to November 2009)

The disease showed an endemic trend in herds in Kapala as (Figure 13) as opposed to the other villages in the control area. Despite this, herds in Waibera had the highest trypanosome incidence (Figure 14).

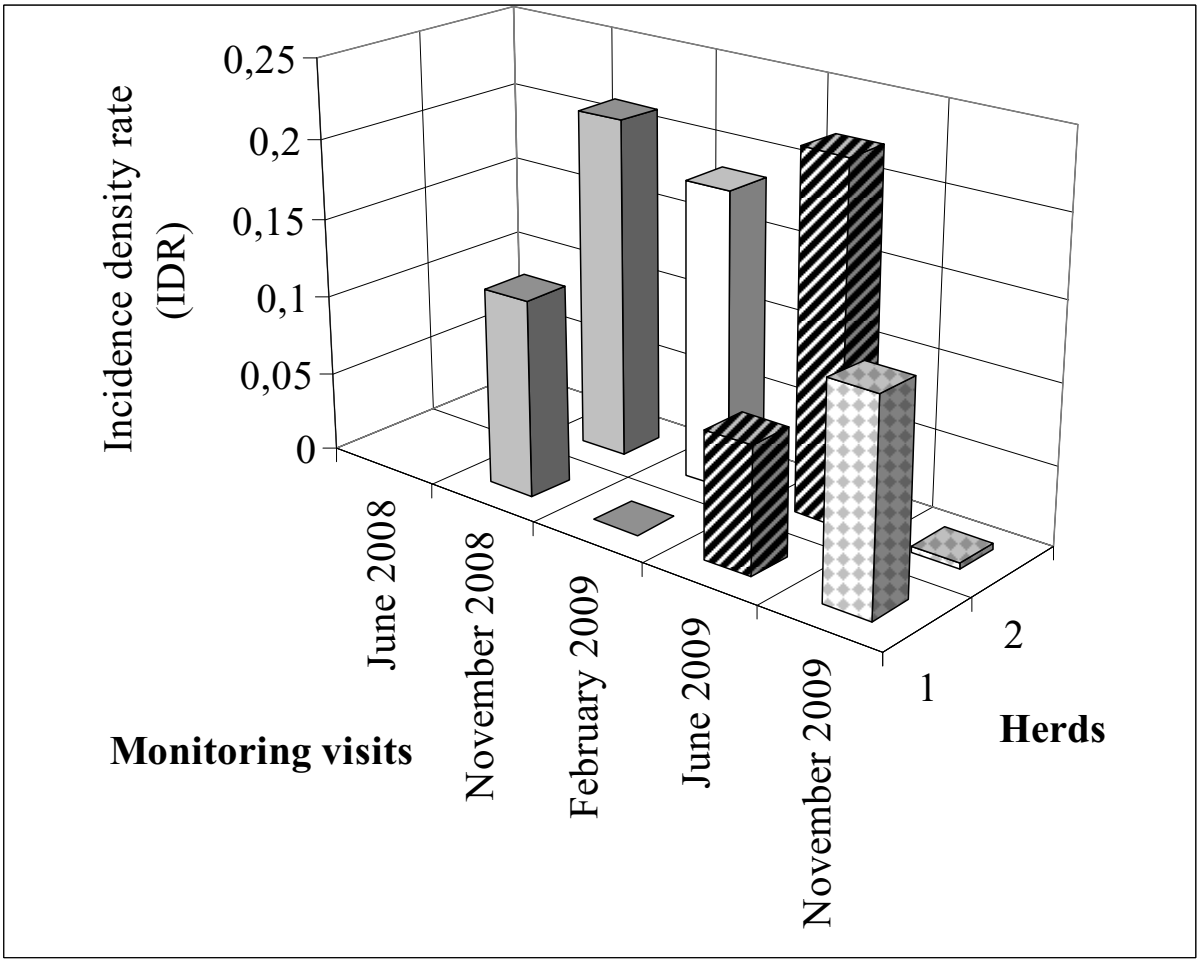

Figure 14: Monitoring period trypanosome herd incidence density rates for the risk group cattle in Waibera village of the control area, Sikasso, south-east Mali (June 2008 to November 2009)

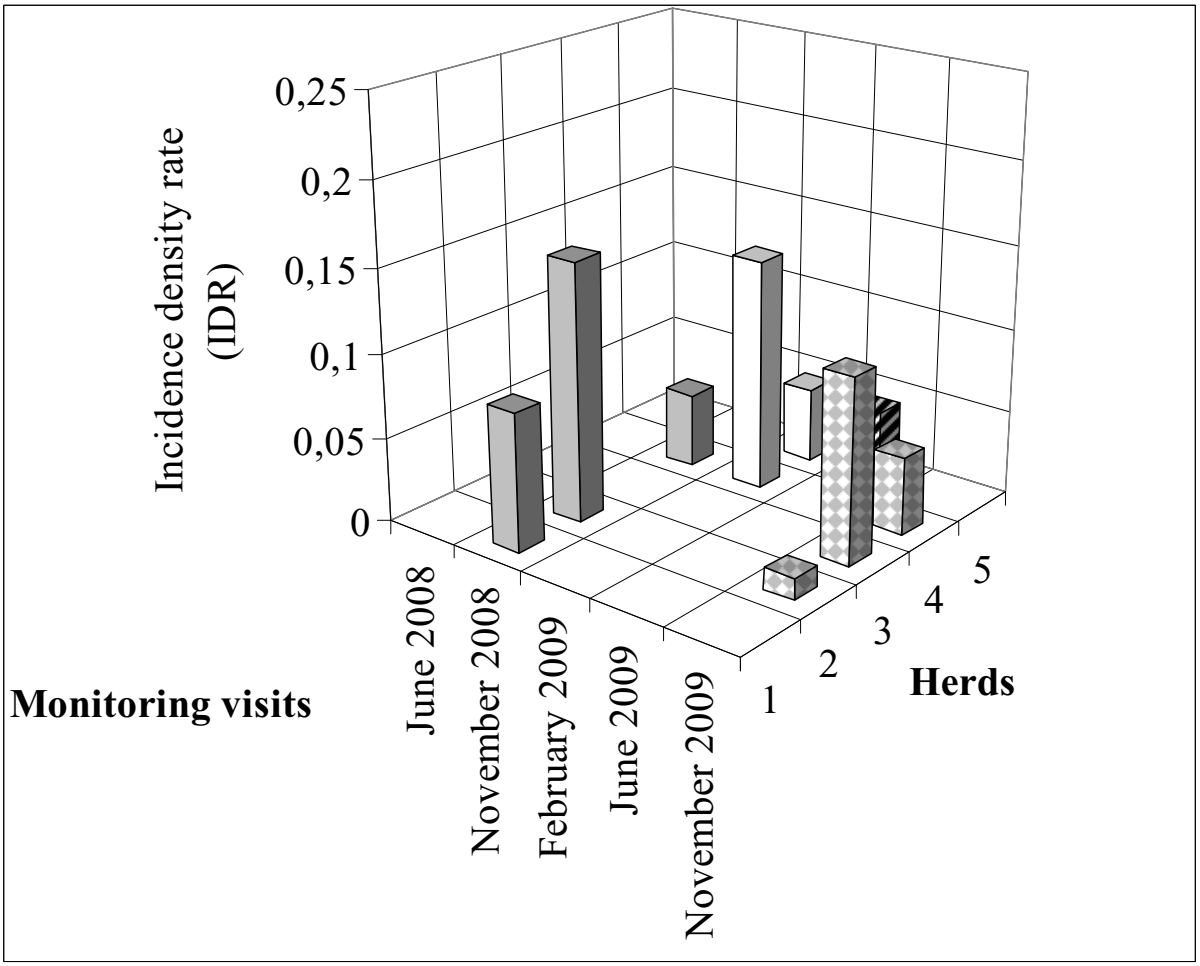

Figure 15: Monitoring period trypanosome herd incidence density rates for the risk group cattle in Tiogola village of the control area, Sikasso, south-east Mali (June 2008 to November 2009)

Tiogola (Figure 15) had the third highest herd IDRs and Dissadié village (Figure 16) the lowest herd IDR in the control area.

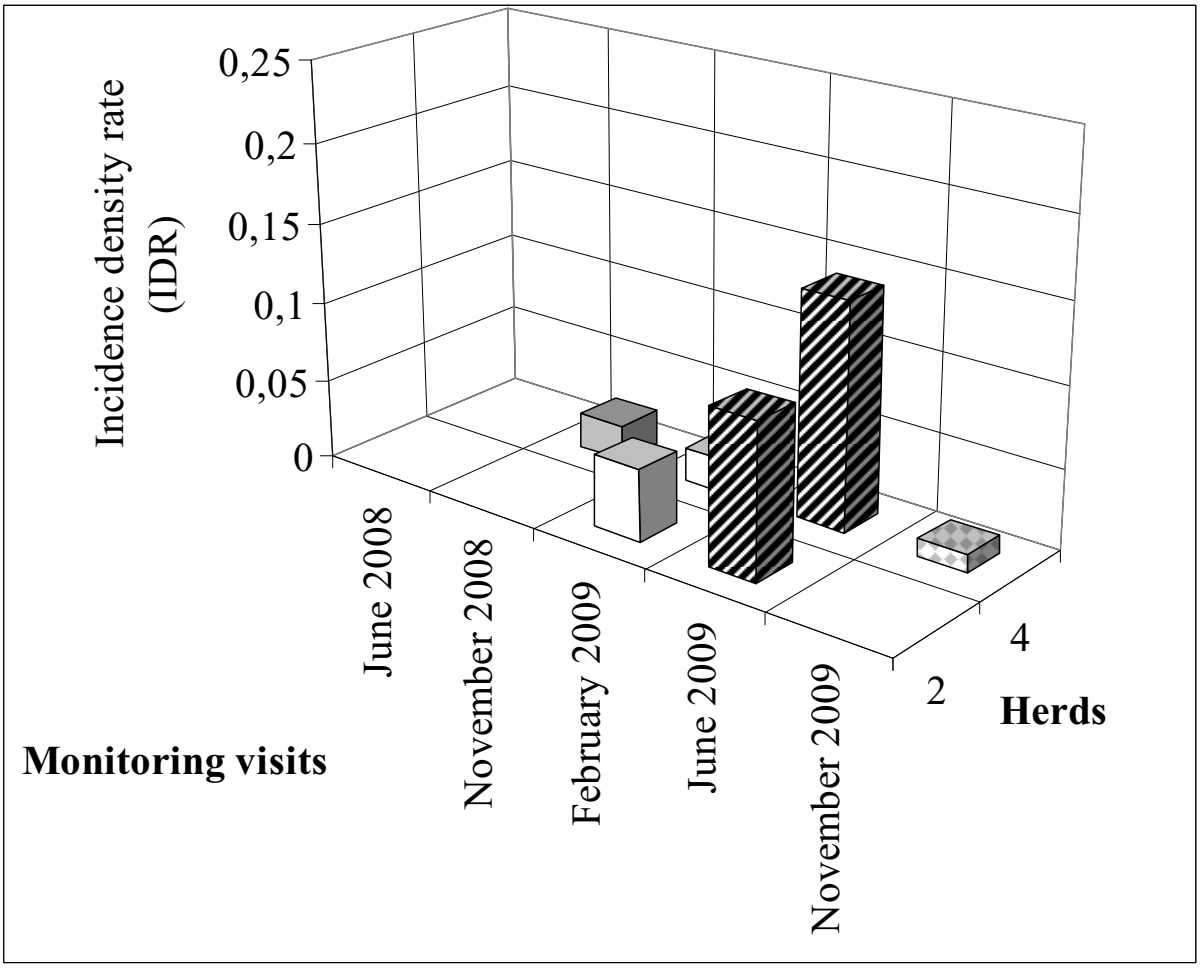

Figure 16: Monitoring period trypanosome herd incidence density rates for the risk group cattle in Diassadié village of the control area, Sikasso, south-east Mali (June 2008 to November 2009)

5.2.3.2.2 Village level IDR

In the intervention area, Kafela with an aggregated IDR of 0.014 (95% CI: 0.007-0.025) trypanosome cases/cattle-month at risk had the marked highest IDR followed by Daresalame with 0.002 (95% CI: 0.000-0.01) and Finibougou with a considerably lower IDR of 0.001 (95% CI: 0.000-0.007) trypanosome cases/cattle-month at risk (Figure 17). Ziébougou recorded zero trypanosome cases throughout the monitoring period.

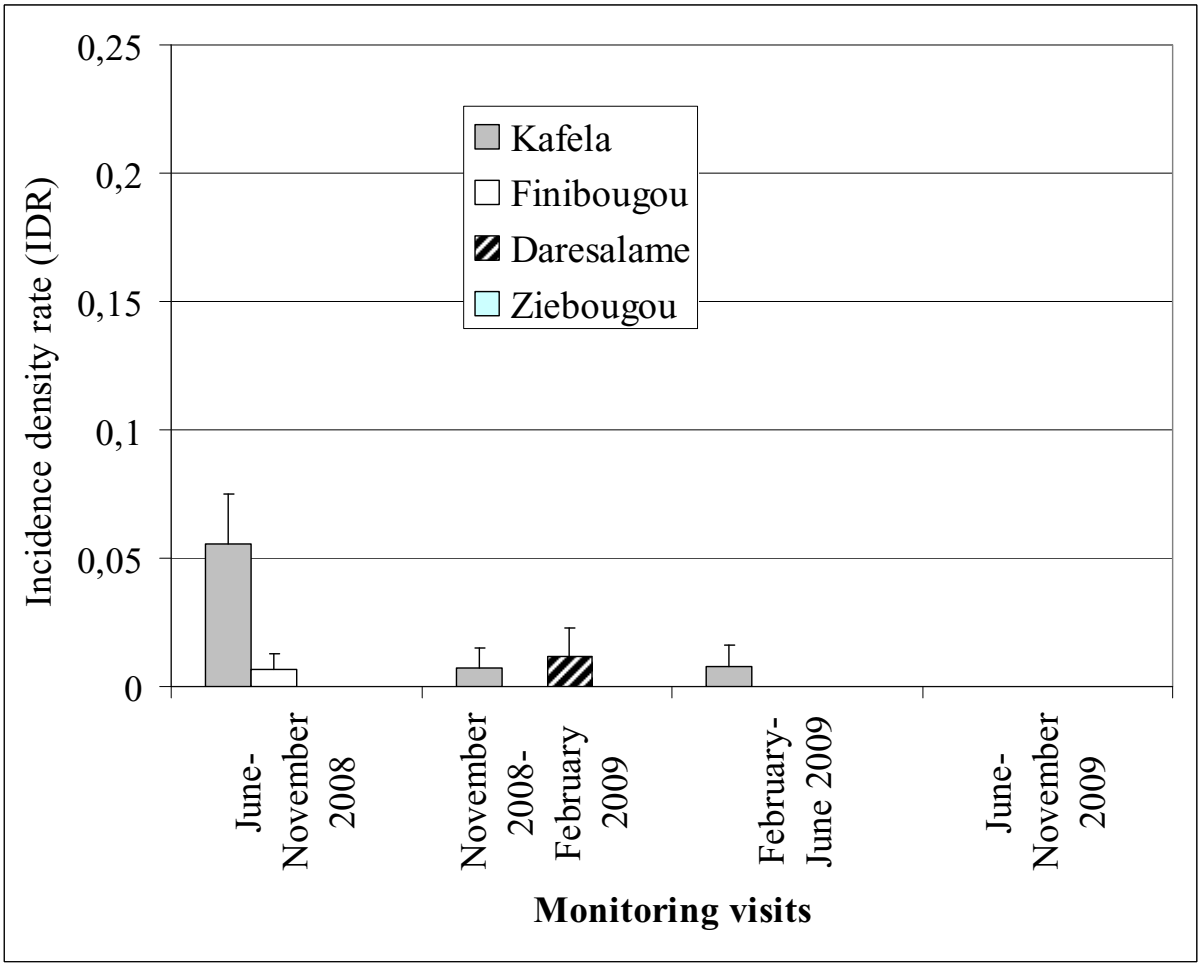

Figure 17: Monitoring period village level trypanosome incidence density rates for the risk group cattle in intervention area Sikasso, south-east Mali (June 2008 to November 2009)

In the control area, Waibera village with 0.140 (95% CI: 0.08-0.226) trypanosome cases/cattle-month at risk had the highest IDR, followed in descending order by Kapala village with 0.045 (95% CI: 0.035-0.056), Tiogola village with 0.038 (95% CI: 0.023-0.059) and Diassadié village with 0.033 (95% CI: 0.018-0.055) (Figure 18). There was a significant ($p<0.05$) difference between the IDR of Waibera and that of the other 3 villages of the control area.

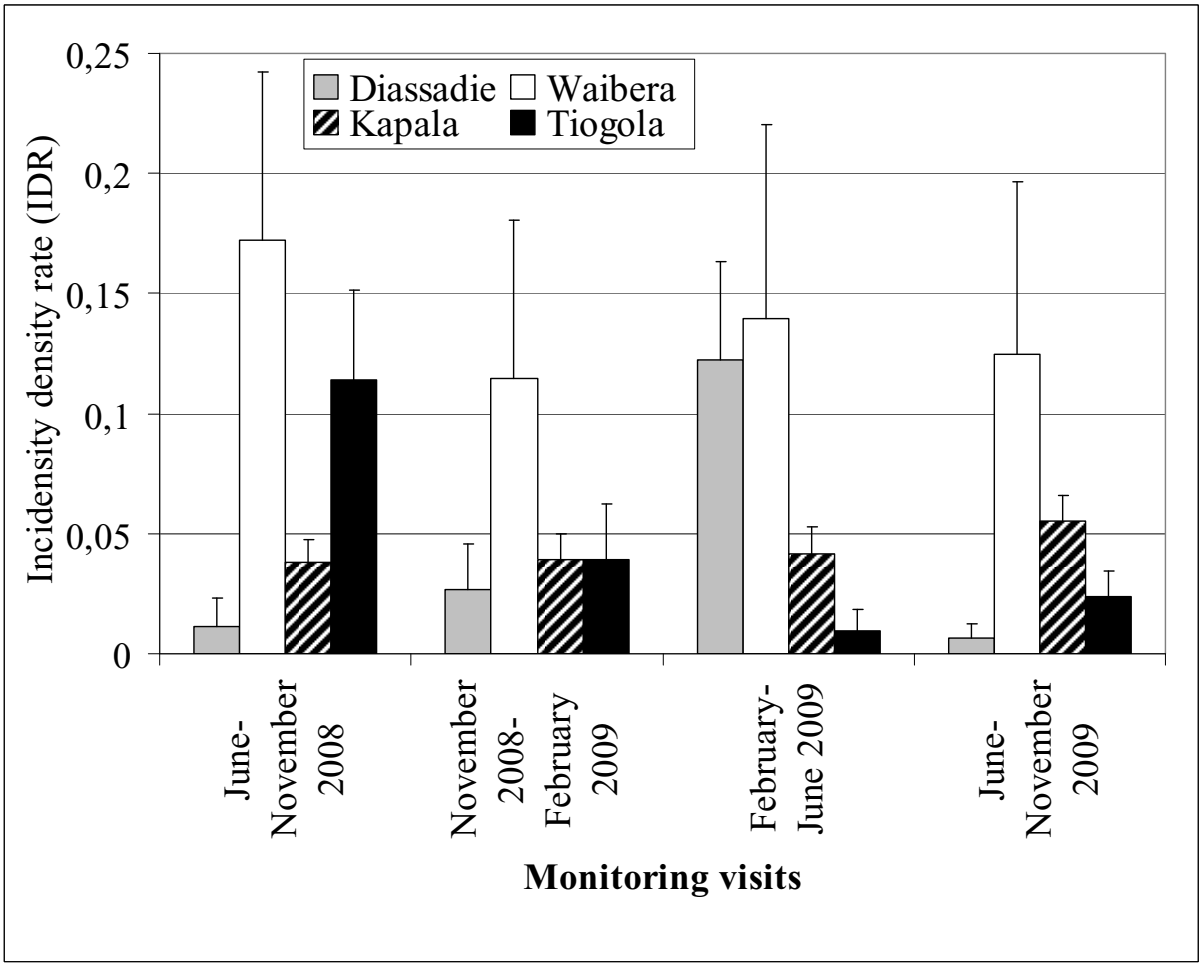

Figure 18: Monitoring period village level trypanosome incidence density rates for the risk group cattle in control area Sikasso, south-east Mali (June 2008 to November 2009)

The period June-November 2008 (rainy season) showed the highest IDR at village level as opposed to the risk period November 2008-February 2009 (dry season) in the control area (Figure 18).

5.2.3.2.3 Area level IDR

The area was the highest level of aggregation for estimation of IDRs for the risk group cattle. Table 16 summarizes the IDRs for the intervention and control areas by monitoring visit. In the intervention area, the overall IDR of the risk group cattle was 0.005 (95% CI: 0.0027-0.0085), translating into 5 new trypanosome infections in every 1000 cattle at risk per month. In the control area, it was 0.046 (95% CI: 0.038-0.054), translating into 46 new trypanosome cases in every 1000 cattle at risk per month (Table 16).

The rate ratio (RR) showed that the control area had a 9.056 (95% CI: 4.999–16.4) greater risk than the intervention area. This difference was highly significant (lower bound of the 95% confidence interval of RR > 1).

Table 16: Trypanosome incidence density rates (IDRs) for the risk group cattle in the study areas of Sikasso, south-east Mali (June 2008 to November 2009)

Monitoring visits	Trypanosome cases	Cattle-months	IDR	95% confidence interval
Intervention area				
June – Nov 2008	9	488	0.018	0.009-0.034
Nov 2008 - Feb 2009	1	424	0.002	0.000-0.012
Feb - Jun 2009	2	480	0.004	0.001-0.014
Jun – Nov 2009	0	995	0	0
Total	**12**	**2387**	**0.005**	**0.003-0.009**
Control area				
Jun – Nov 2008	32	621	0.052	0.036-0.072
Nov 2008 - Feb 2009	22	534	0.041	0.025-0.061
Feb - Jun 2009	26	515	0.052	0.037-0.073
Jun – Nov2009	37	900	0.041	0.029-0.056
Total	**117**	**2570**	**0.046**	**0.038-0.054**

Trypanosusceptible zebu cattle were most sensitive in terms of acquisition of new trypanosome infections in both the intervention and control area throughout the study period. Of the 12 risk group cattle that did acquire trypanosome infections in the intervention area, 9 (75%) were zebu while only 3 (25%) were cross breed cattle (Table 16). In the control area, 79.5% (93/117) of trypanosome-positive cattle were zebu and the remaining 20.5% (24/117) were cross-bred cattle. The age of the animal, defined by the length of time the risk group subjects stayed in the study, also had an effect on trypanosome infection. Risk of infection increased proportionally with increasing age (Table 17).

Table 17: Temporal distribution of trypanosome infections by cohort, risk periods and study area of Sikasso, south-east Mali (June 2008 to November 2009)

Cohorts/ area	Risk period 1 (Jun - Nov 2008			Risk period 2 (Nov 2008 - Feb 2009			Risk period 3 (Feb - Jun 2009			Risk period 4 (Jun - Nov 2009			Total risk period and CI		
	cases	%	95% CI	cases	%	95% CI	cases	%	95% CI	cases	%	95% CI	Total cases	%	95% CI
Risk cattle cohort with 4 risk periods															
Intervention (n=50)	7	14	6.3-25.7	0	0	0	0	0	0	0	0	0	**7**	**14**	**6.3-25.7**
Control (n=47)	7	14.9	6.8-27.3	5	10.6	4.0-22.0	2	4.3	0.7-13.4	8	17	8.2-29.8	**22**	**46.8**	**33-61.1**
Risk cattle cohort with 3 risk periods															
Intervention (n=44)				0	0	0	2	4.5	0.8-14.2	0	0	0	**2**	**4.5**	**0.8-14.2**
Control (n=55)				5	9.1	3.4-19	6	10.9	4.5-21.3	9	16.4	8.3-27.9	**20**	**36.4**	**24.5-49.6**
Risk cattle cohort with 2 risk periods															
Intervention (n=21)							0	0	0	0	0	0	**0**	**0**	**0**
Control (n=9)							2	22.2	3.9-56.2	0	0	0	**2**	**22.2**	**3.9-56.2**

Feb=February; Jun=June; Nov=November
%=cumulative incidence
95% CI= 95% Confidence interval

5.2.4 Trypanocidal drugs use practices

Treatment with DIM was carried out either for curative purposes where only the sick animals were targeted or was massively done when farmers perceived the risk of trypanosomosis to be high. In both areas, DIM treatments were done irrespective of time in the intervention area but mostly at the start of the rainy season (June). In the control area, it was done in November 2008 (end of the rainy season), in the middle of the dry season (February 2009) and in June 2009 (start of the rainy season). Between 2 and 3 DIM treatments per animal per year were applied on average in both areas.

Table 18: Number of cattle treated with DIM by herd owners and by project in the intervention and control areas in Sikasso, south-east Mali (June 2008 to November 2009)

Monitoring visits	**No. cattle in intervention area treated with DIM by**				**No. cattle in control area treated with DIM by**			
	Owner[a]	Project [b]			Owner[a]	Project [b]		
		PCV <=20%	Tryps +ve	Total		PCV <=20%	Tryps +ve	Total
June 2008	402	0	4	4	279	6	16	22
November 2008	131	7	9	16	304	13	32	45
February 2009	137	1	1	2	489	12	22	34
June 2009	261	1	2	3	336	1	26	27
November 2009	204	1	0	1	120	14	37	51
Total	**1135**	**10**	**16**	**26**	**1568**	**46**	**133**	**179**

[a]Herd owners' treatments targeted cattle of all ages
[b]Project treatments were restricted to the risk group cattle

Since at the start of the epidemiological monitoring visits, the ratio of risk group cattle to adults was approximately 1: 5 in the intervention area and 1: 6 in the control area; it is estimated that of the 1135 DIM treatments administered in the intervention area by herd owners over the entire monitoring period, 227 treatments were given to the risk group cattle. Likewise, in the control area, out of the 1568 DIM treatments done, 261 were given to the risk group cattle (Table 18).

A comparison with the project DIM treatments by area shows that in the intervention area herd owner treatments were approximately 9 times more frequent than those carried out by the project (Table 18). In the control area, the project applied 179 DIM treatments over the monitoring period compared to the 261 administered by herd owners. Herd owner treatments here were only 1.5 times more frequent than those administered by the project (Table 18).

During the study period, 312 and 457 cattle received ISMM prophylactic block treatment within the intervention and control areas, respectively. These prophylactic treatments were done during the rainy season, mostly by private veterinarians as opposed to DIM that was administered by herd owners themselves.

In the study villages, vaccinations against cattle epizootics were also commonly done by the private veterinarians. Cattle were vaccinated against contagious bovine pleuropneumonia (CBPP), blackquarter, haemorragic septicaemia and pasteurellosis. Vaccinations for all disease except CBPP were done shortly before the onset of the rainy season and towards the end of the rainy season. Use of oxytetracycline 5% and 10% was also commonly reported.

5.2.5 Helminth infections

5.2.5.1 Strongyle faecal egg counts (FECs)

Strongyle FECs for the albendazole treated risk group cattle within the intervention area ranged between 0-2500, while that for the placebo treated cattle ranged between 0 and 4700. In the control area, FECs for animals on albendazole treatment ranged between 0-900 and those on placebo between 0 and 1100. Placebo treated animals had slightly higher FECs than the albendazole treated ones. Table 19 summarizes the descriptive statistics of the village and area level trichostrongyloid FECs. Overall, intervention area villages had higher FECs as

compared to villages in the control area. In the intervention area villages, albendazole treated risk cattle had lower FEC than the placebo treated ones (Table 19). In the control area villages there was no clear trend in FECs for the albendazole and placebo treated cattle, as was the case in the intervention area villages.

Inter-village variations in FECs were encountered. For instance in the intervention area, Ziébougou had the highest FECs for both albendazole and placebo treated cohorts throughout the monitoring period. Kafela, Finibougou and Daresalame followed in this order. Within the control area villages, the sample sizes were too small for meaningful statistical comparisons, although generally Diassadié had higher FECs than Waibera (Table 19).

At area level, the overall strongyle median FECs were statistically ($p<0.001$) higher in the intervention area (median FECs=150) than in the control area (median FECs=50). However, comparing the median FECs of the albendazole and placebo treated cattle in both areas resulted in no principal statistical ($p>0.05$) significant difference between the placebo and the albendazole treated, except for the period February 2009 (Table 19).

Table 19: Median faecal egg counts (FECs) for strongyles in the risk group cattle in Sikasso, south-east Mali (June 2008 to November 2009)

Village	November 2008		February 2009		June 2009		November 2009	
	Albendazole	Placebo	Albendazole	Placebo	Albendazole	Placebo	Albendazole	Placebo
	n=50	n=61	n=61	N=66	n=66	n=75	n=84	n=82
Intervention area								
Kafela	100	300	0	50	50	200	625	750
Finibougou	100	200	0	0	100	100	400	850
Daresalame	100	100	0	25	200	150	450	375
Ziébougou	225	175	0	50	150	150	650	700
Area total	**100**	**200**	**0**	**50**	**100**	**150**	**600**	**500**
Control area								
Diassadié	100	100	0	50	0	0	250	250
Waibera	25	0	0	0	0	0	0	150
Area total	**50**	**100**	**0**	**0**	**0**	**0**	**250**	**225**

n= Number of risk cattle that were faecal sampled

Stratifying risk cattle into 3 strata based on egg shedding: low helminth burden (0-500), moderate helminth burden (500-1000) and high helminth burden (>1000) as described by Urquhart et al. (1996) showed that majority of the risk cattle did belong to the low egg shedding category (Table 20). Zebu cattle in both areas constituted the majority of the heavily infected group.

Table 20: Stratification of risk cattle into strongyle egg shedding categories by monitoring visit in Sikasso, south-east Mali (November 2008 to November 2009)

Area	No. cross breed in the category of			No. zebu in the category of		
	Low	Moderate	Heavy	Low	Moderate	Heavy
Intervention area						
November 2008	49 (90.7)	7 (7.4)	1 (1.9)	48 (84.2)	7 (12.7)	2 (3.5)
February 2009	57 (100)	0	0	70 (100)	0	0
June 2009	55 (87.3)	8 (12.7)	0	71 (91.0)	5 (6.4)	2 (2.6)
November 2009	42 (56.8)	24 (32.4)	8 (10.8)	37 (40.2)	26 (28.3)	29 (31.5)
Total	**203 (81.9)**	**36 (14.5)**	**9 (3.6)**	**226 (76.1)**	**38 (12.8)**	**33 (11.1)**
Control area						
November 2008	11 (100)	0	0	14 (100)	0	0
February 2009	16 (100)	0	0	18 (100)	0	0
June 2009	10 (100)	0	0	14 (93.3)	1 (6.7)	0
November 2009	5 (50)	5 (50)	0	17 (89.5)	0	2 (10.5)
Sector total	**42 (89.4)**	**5 (10.6)**	**0**	**63 (95.5)**	**1 (1.5)**	**2 (3.0)**

Parentheses () are percentages

Shedding of strongyle eggs by the risk group cattle was heavily dependent on season (Tables 19 and 20). November (end of the rainy season) had the highest FECs and February 2009 (dry season) the lowest. Strongyle egg shedding increased again in June 2009 (start of the rainy season).

In both helminth treatment categories, FECs were heavily skewed to the right since the majority of animals had FECs of just between 0-500, while only a few of them had FECs >500 (Figure 19).

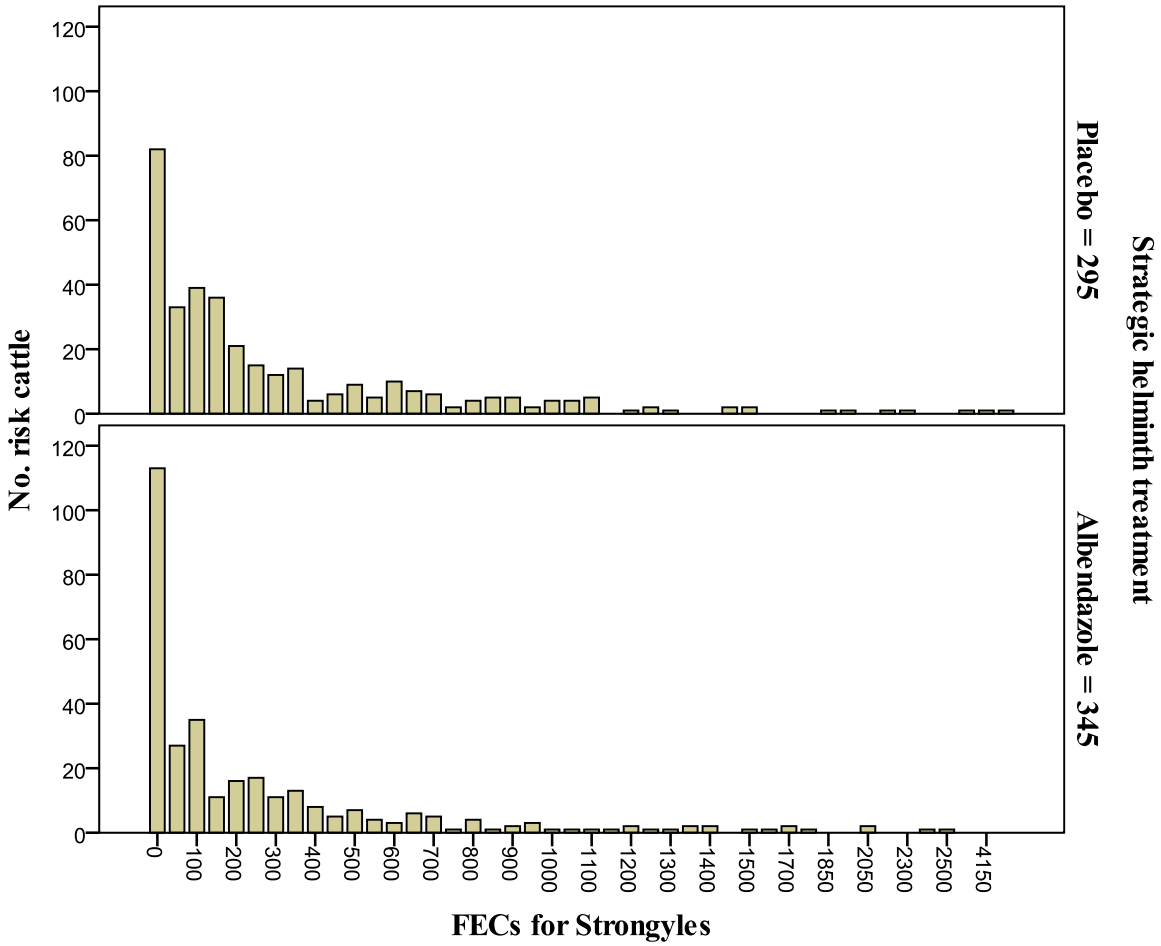

Figure 19: Strongyle FECs of risk group cattle by helminth treatment in Sikasso, south-east Mali (June 2008 to November 2009)

Higher FEC were observed within the Zebu breed (median FEC=150) as compared to the cross-bred cattle (median FEC=100), although the difference was non-significant ($p>0.05$) as shown in Figure 20. The FEC distributions were heavily skewed to the right for both breeds.

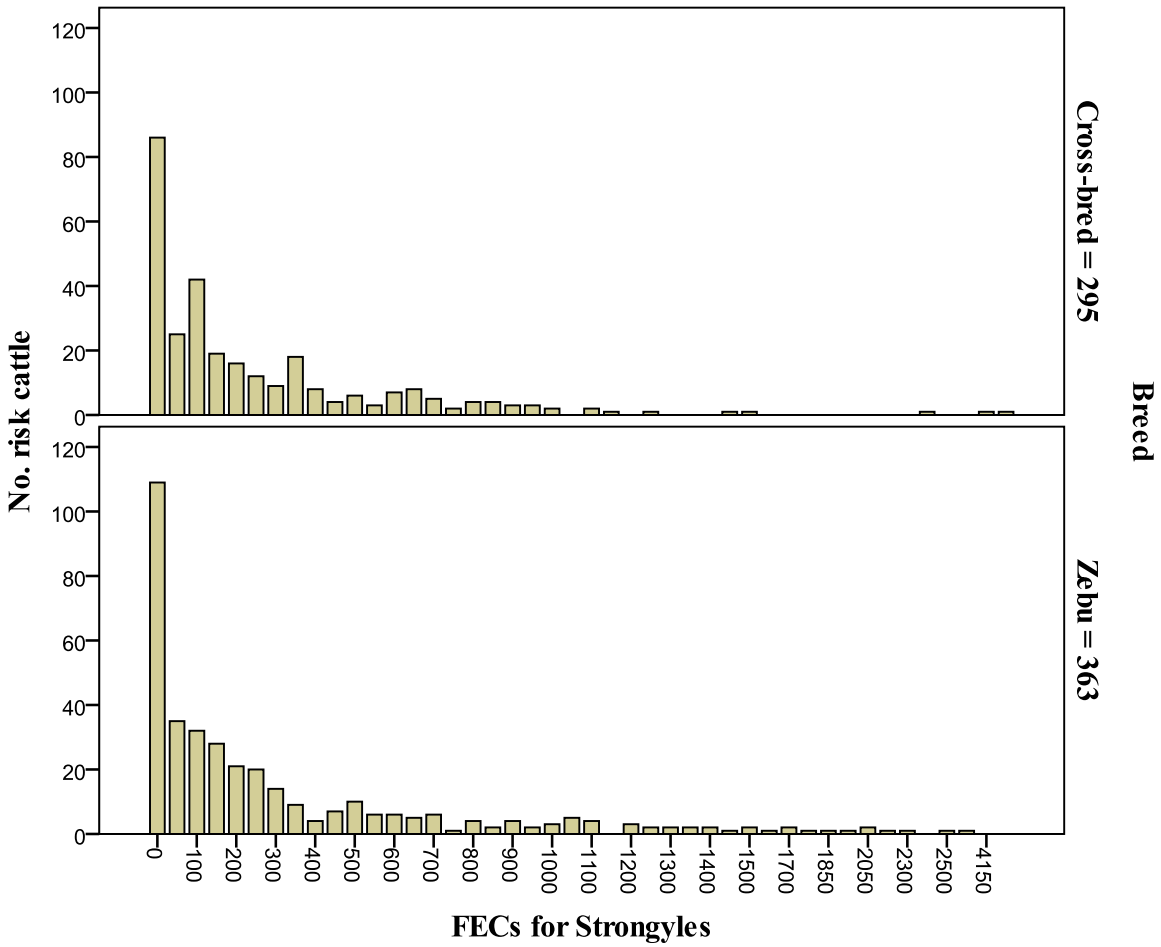

Figure 20: Strongyle FECs by breeds of the risk group cattle in Sikasso, south-east Mali (June 2008 to November 2009)

Female cattle had slightly lower median FECs (median=100) than males (median FECs=150), although this difference was statistically not significant ($p>0.05$) (Figure 21).

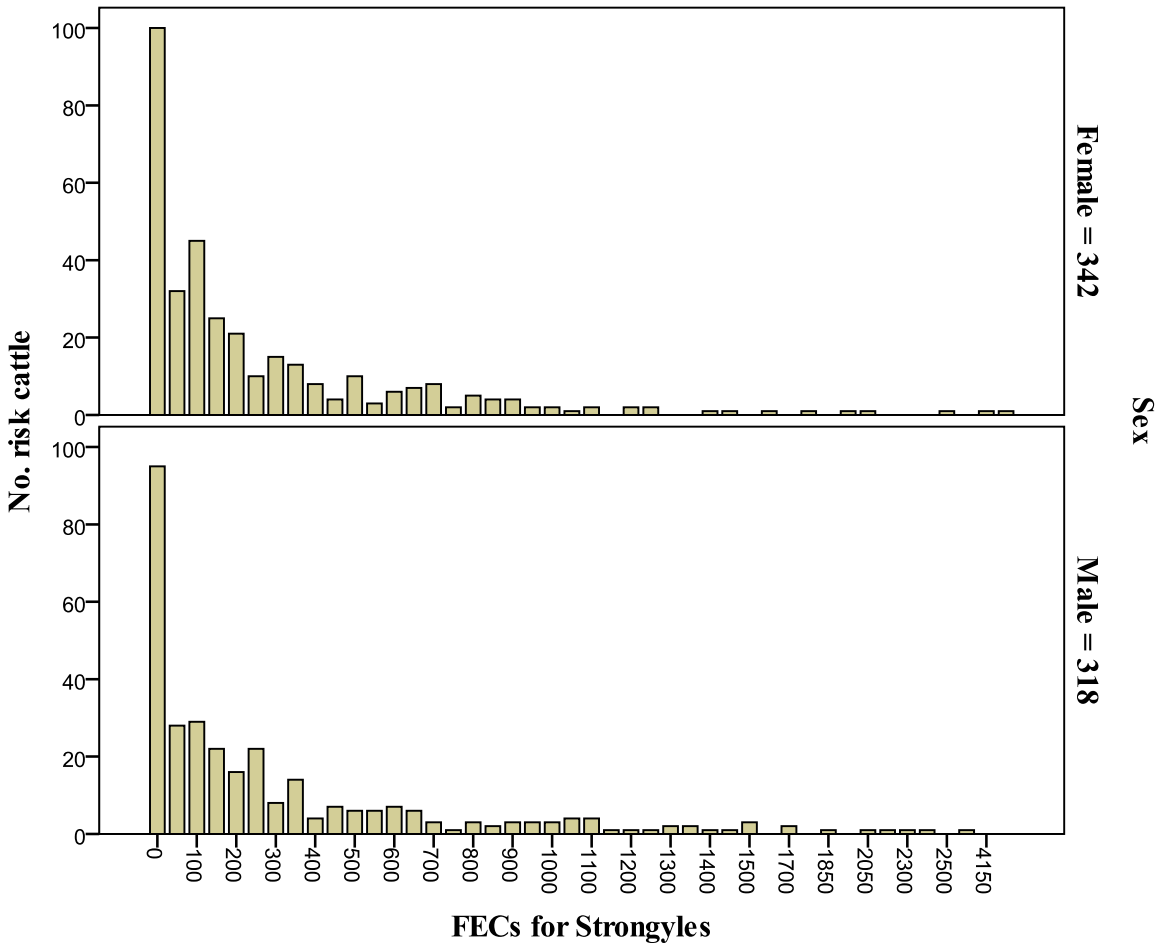

Figure 21: Strongyle FECs by sex of the risk group cattle in Sikasso, south-east Mali (June 2008 to November 2009)

When risk cattle were stratified by age into those aged between 3-12 months, 13-24 months and above 24 months, strongyle egg shedding was higher in the first group (3-12 months) and lower in those aged > 24 months (Figure 22).

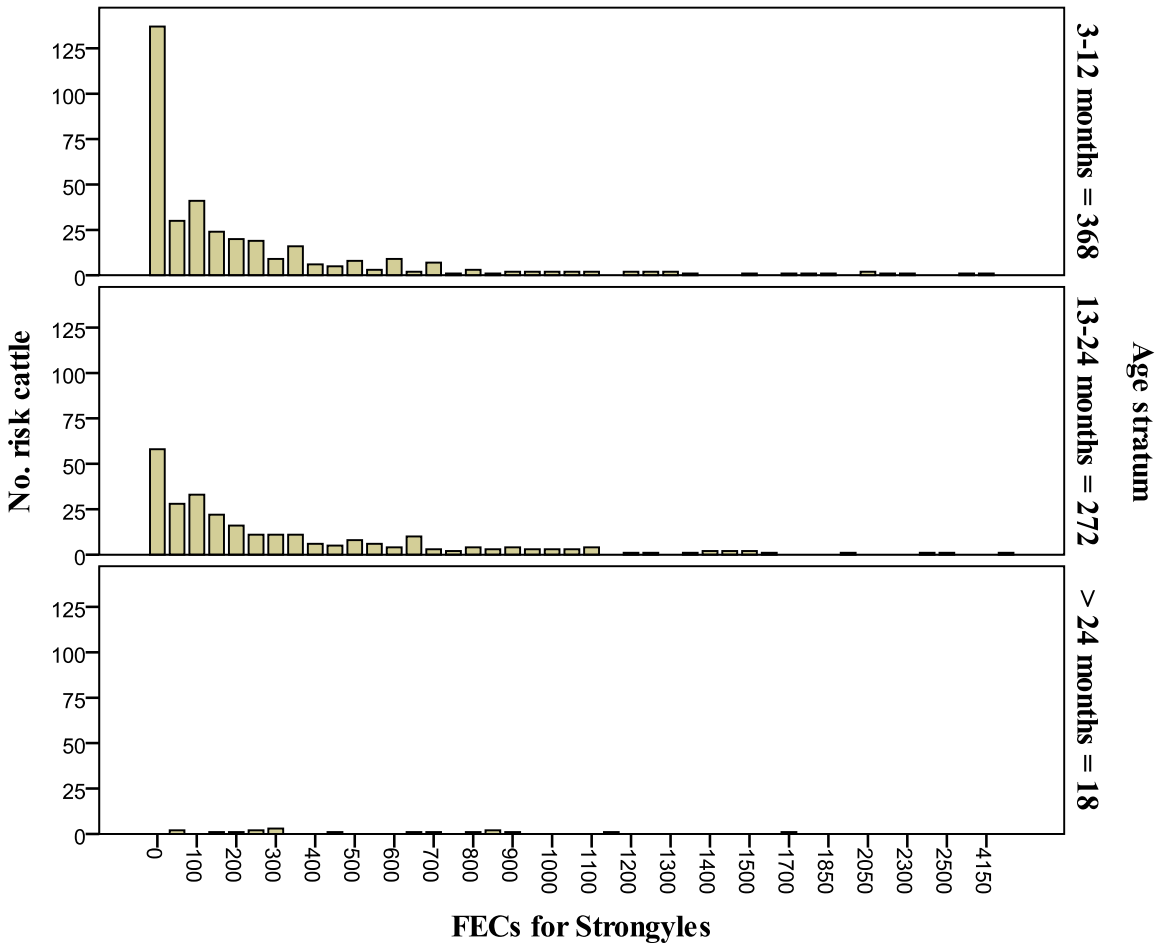

Figure 22: Strongyle FECs by age stratum of the risk group cattle in Sikasso, south-east Mali (June 2008 to November 2009)

5.2.5.2 Other helminths

Other helminths whose eggs were occasionally encountered but in too low frequencies for meaningful analysis, included *Strongyloides* species, ascarids (*Toxocara* species) *Moniezia* species, *Capillaria* species and *Trichuris* species. *Strongyloides* species and *Toxocara* species eggs rarely occurred particularly in risk cattle aged <12 months. Despite conducting larval migration tests for lung worms and sedimentation for trematode eggs, neither *Dictyocaulus* species nor *Fasciola* species were detected in the study area. In contrast, *Paramphistomum* species eggs were occasionally detected from some risk group cattle in the study area, although in too low numbers to permit statistical analysis.

5.2.5.3 Larval cultures

Results of larval cultures revealed the presence of *Bunostomum* species, *Strongyloides* species, *Oesophagostomum* species and those with 16 intestinal cells like *Cooperia* species**,** *Ostertagia* species**,** *Trichostrongylus* species and *Haemochus* species, among other GINs detected in Sikasso.

5.2.6 Effect of albendazole treatment on trypanosome IDR

5.2.6.1 Village level effect of albendazole treatment on IDR

Herds in Kafela were the most affected with trypanosome infections among those conducting strategic helminth control in the intervention area (Table 21). In Kafela village, during the June-November 2008 risk period, the albendazole treatment significantly (χ^2 test; $p<0.05$) reduced the IDR (0.016) when compared to the placebo group (0.086). Due to sporadic trypanosome infections in the other villages of the intervention area, this effect could not be verified.

In the control area villages, the effect of albendazole treatment on trypanosome incidence seemed to be dependent on trypanosome infection pressure. There was a weak positive effect of albendazole treatment in Waibera village, where the trypanosome infection risk was very high (Table 21). Risk group cattle treated with albendazole in this village had slightly lower IDR than the placebo group although this difference was not significant (χ^2 test; $p>0.05$). This reduced IDR was apparent both in the rainy season (June-November 2008) and in the dry season (November 2008-February 2009).

Table 21: Effect of albendazole treatment on trypanosome IDR for the risk group cattle in the intervention and control areas of Sikasso, south-east Mali (June 2008 to November 2009)

Village	June-November 2008		November 2008-February 2009		February – June 2009		June– November 2009	
	Albendazole	Placebo	Albendazole	Placebo	Albendazole	Placebo	Albendazole	Placebo
Intervention area								
Kafela	0.016	0.086	0.016	0	0	0.016	0	0
Finibougou	0	0.012	0	0	0	0	0	0
Daresalame	0	0	0	0	0.021	0	0	0
Ziébougou	0	0	0	0	0	0	0	0
Village Total	**0.004**	**0.031**	**0.005**	**0**	**0.004**	**0.004**	**0**	**0**
Control area								
Diassadié	0.025	0	0.052	0	0.152	0.098	0	0.011
Waibera	0.122	0.217	0.070	0.167	0.163	0.109	0.20	0.071
Village total	**0.053**	**0.061**	**0.057**	**0.041**	**0.155**	**0.1**	**0.027**	**0.002**

5.2.6.2 Area level effect of albendazole treatment on IDR

In the intervention area, risk group cattle treated with albendazole had an IDR of 0.003 (95% CI: 0.001-0.007) versus 0.007 (95% CI: 0.004-0.013) of the placebo group. This difference statistically was not significant (overlapping 95% CI) (Table 22). The rate ratio (RR) between these two treatment groups was 2.889 (95% CI: 0.782-10.67) suggesting that albendazole treated risk cattle had a 2.889 lower chance of contracting a new trypanosome infection as compared to those not treated with albendazole.

Within the control area, the IDR of albendazole treated risk group cattle was 0.066 compared to 0.048 for their counterparts treated with the placebo (Table 22). The estimated RR of the two cohorts of 0.7273 (95% CI: 0.3461-1.528) underlines that helminth treatment obviously did not add effect to lowering the number of new trypanosome infections.

Table 22: Trypanosome incidence density rates (IDR) for the risk group cattle treated with albendazole and placebo treatment within the intervention and control areas of Sikasso, south-east Mali (June 2008 to November 2009)

Monitoring visits	Albendazole treated risk cattle cohort				Placebo treated risk cattle cohort			
	Trypanosome cases	Cattle months	IDR	95% CI	Trypanosome cases	Cattle months	IDR	95% CI
Intervention area								
Jun - Nov 2008	1	247.6	0.004	0.000-0.020	8	293.6	0.031	0.010-0.050
Nov 2008 – Feb 2009	1	192.8	0.005	0.000-0.026	0	209.2	0	0
Feb – Jun 2009	1	240.3	0.004	0.000-0.021	1	256.3	0.004	0.000-0.019
Jun – Nov 2009	0	517.5	0	0	0	493.1	0	0
Total	**3**[a]	**1198.2**	**0.003**	**0.001-0.007**	**9**[b]	**1252.2**	**0.007**	**0.004-0.013**
Control area								
Jun - Nov 2008	3	56.7	0.053	0.013-0.144	4	65.2	0.061	0.019-0.148
Nov 2008 – Feb 2009	3	52.8	0.057	0.014-0.155	2	48.6	0.041	0.007-0.136
Feb – Jun 2009	7	45.2	0.155	0.068-0.306	5	49.7	0.10	0.037-0.223
Jun – Nov 2009	2	73.8	0.027	0.005-0.090	2	108.2	0.002	0.003-0.061
Total	**15**[c]	**228.5**	**0.066**	**0.038-0.106**	**13**[d]	**272.3**	**0.048**	**0.027-0.080**

[a] Two *T. vivax* and one *T. congolense*
[b] Seven *T. vivax* and two *T. congolense*
[c] Six *T. vivax*, eight *T. congolense* and 1 mixed infection (*T. congolense* and *T. vivax*)
[d] Five *T. vivax,* six *T. congolense* and 2 mixed infections (*T. congolense* and *T. vivax*)
95% CI=95% confidence interval

5.2.7 Packed cell volume (PCV) development

Packed cell volumes (PCV) were measured for all risk cattle present and participating in every monitoring visit. The number of risk cattle progressively increased with the monitoring visits in both areas. This increase was slightly higher in the intervention area (Figure 23).

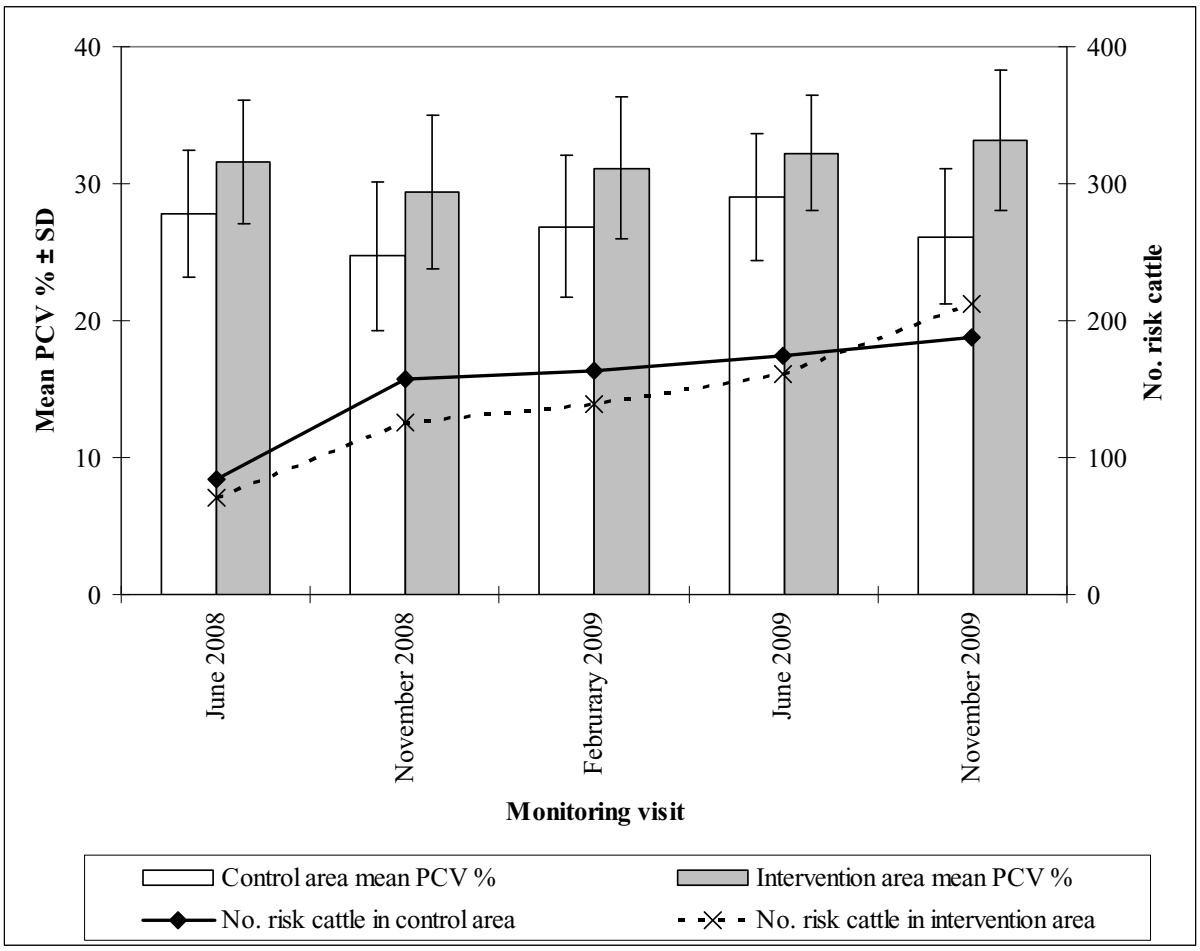

Figure 23: The packed cell volume (PCV) of the risk cattle in the intervention and control area by monitoring visits in Sikasso, south-east Mali (June 2008 to November 2009)

The PCVs for all 5 monitoring visits ranged between 13% and 46%, with a mean of 31.7 ± 5.1% in the intervention area and between 12% and 45%, with a mean of 26.8 ± 5.2% in the control area. This difference was statistically significant ($p<0.001$). Although the PCVs were principally higher in the intervention area than in the control area at each of the individual monitoring visits, these differences were not significant (overlapped 2 standard deviations around the mean). In the intervention area, the mean PCV for cross-bred cattle was 32.7 ± 4.9, which was significantly ($p<0.001$) higher than the 30.9 ± 5.2 of zebu cattle. Cross-bred cattle in the control area in contrast had mean PCV of 27.5 ± 5.0, which was significantly ($p<0.05$) lower than the 28.6 ± 5.2 for zebu cattle.

Within the intervention area, trypanosome-negative cattle had mean PCV of 31.8 ± 5.1% and positive cattle mean PCV of 29.3 ± 5.9%. In the control area, respective values were 27.6 ± 4.8 for trypanosome-negative and 23.1 ± 5.5 for trypanosome-positive cattle; this difference was not significant (p>0.001). *Trypanosome congolense* positive cattle had mean PCV of 30.8 ± 5.1% and 21.7 ± 5.1% in the intervention area and control area, respectively, while *T. vivax* positive cattle had mean PCV of 28.8 ± 5.1% in the intervention area and 24.0 ± 6.4% in the control area.

Stratifying study animals into two groups, PCVs < 25% and PCVs => 25%, with the former considered animals as anaemic and the latter normal showed that 5.8% (18/313) of the cross breed cattle in the intervention area had PCVs < 25% as compared to 13.9% (55/396) zebu cattle in the same area (Table 23).

Table 23: Proportions of cattle by PCV scores at monitoring points in Sikasso, south-east Mali (June 2008 to November 2009)

Area	**Cross breeds PCV**			**Zebu PCV**		
	<25%	=>25%	Total	<25%	=>25%	Total
Intervention area						
June 2008	0	33	33	7	31	38
November 2008	7	49	46	22	48	70
February 2009	5	56	51	14	64	78
June 2009	2	70	72	4	85	89
November 2009	4	87	91	8	113	121
Total	**18**	**285**	**313**	**55**	**341**	**396**
Control area						
June 2008	8	13	21	20	43	63
November 2008	15	17	32	73	52	125
February 2009	14	23	37	52	74	126
June 2009	4	31	35	33	107	140
November 2009	12	25	37	75	76	151
Total	**53**	**109**	**162**	**253**	**352**	**605**

The risk ratio between the crossbreed risk cattle and the zebu breed was 2.415 (95% CI: 1.449-4.026) indicating a significant (Pearson χ^2=12.54, p<0.001) anaemic status difference between the breeds. For the control area, the risk ratio was calculated as 1.478 (1.005-1.625). There was a significant (Pearson χ^2=4.415, p=0.036) intra-breed difference in regards to the anaemic status.

Of the 149 trypanosome-positive cattle, 65.8% had a PCV < 25%, with 97% of them coming from the control area.

5.2.8 Weight development

Only the zebu risk group cattle, that were the majority in both areas, were considered in the determination of weight development between the two areas (Figure 24). Similarly, only those weights of cattle that were present at all monitoring visits (between June 2008 and November 2009) were included in the analysis, after data were matched by season.

At the first monitoring visit in June 2008, 3 months after commencement of the intervention, zebu risk cattle had a mean weight ± SD of 90.4 ± 27.88 kg compared to a mean weight of 82.4 ± 22.83 kg for the zebu cattle in the control area (Figure 24). The slightly higher mean weight within the intervention area was sustained throughout the entire subsequent monitoring period. The final mean weight of these cattle in the intervention area was 155.1 ± 16.57 kg, 5.2 kg heavier than 149.9 ± 24.45 kg in the control area.

In both areas, the number of zebu cattle that participated in the June 2008 monitoring was higher but some of these animals were progressively lost to follow-up in the succeeding monitoring visits. 27.0% of the 63 zebu cattle were lost to follow-up in the control area over the 17 month study period. The figure was 15.8% in the intervention area. Reasons for loss of animals were varied, but were mainly due to mortalities (particularly in the control area) and sometimes to non-compliance of animal keepers witnessed in both areas.

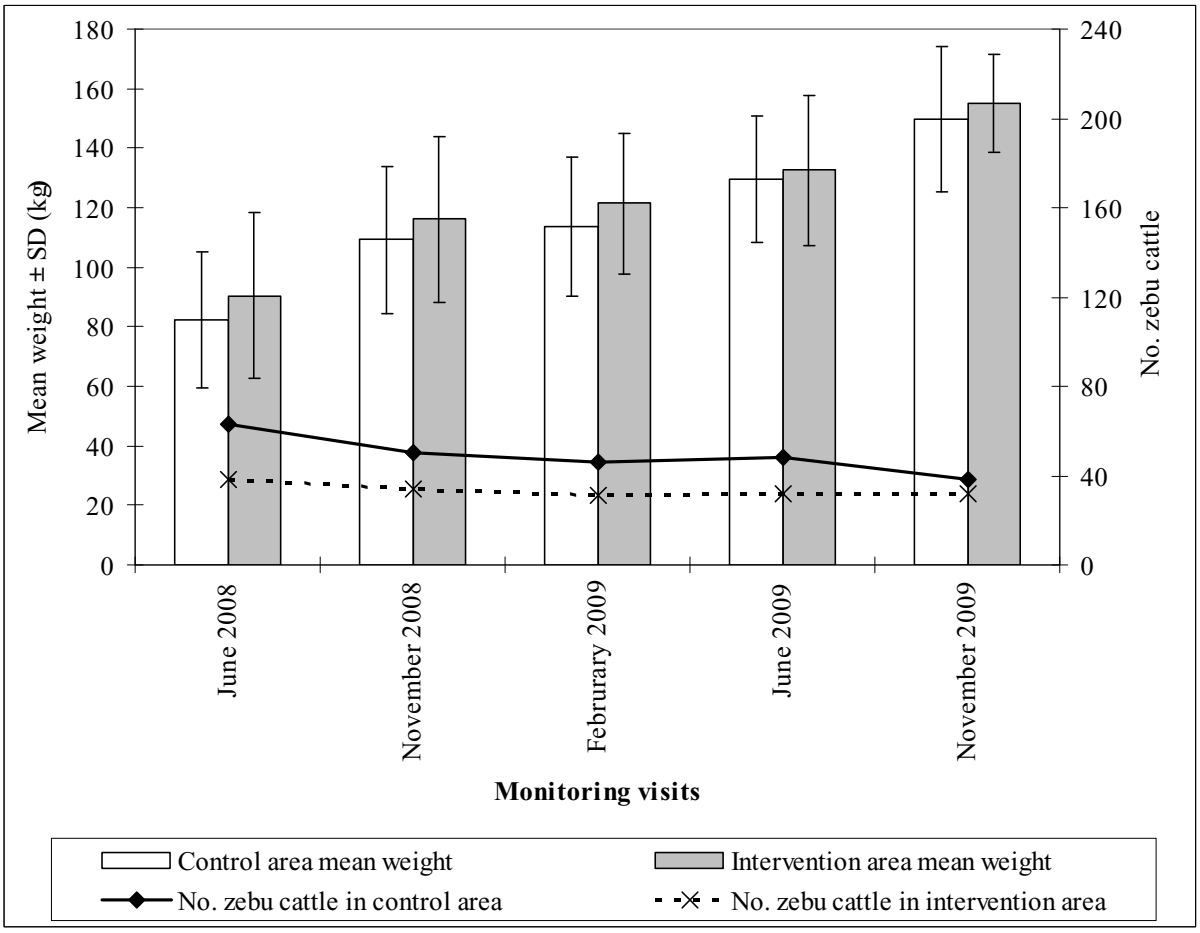

Figure 24: Weight development (Mean weight (kg) ± standard deviation) of zebu cattle from the start to end of study within the intervention and control areas of Sikasso, south-east Mali (June 2008 to November 2009)

5.2.9 Herd dynamics

Indicators of herd performance were estimated through crude birth rates and crude death rates. At November 2009, when the project ended, the estimated cattle population was 785, up from 608 (April/May 2008 census), in the intervention area and 970 up from 770 (April/May 2008 census), in the control area. Over the 17 month monitoring period, respective herd growth in the intervention area was 29.1% and 26.0% in the control area. Of the 29.1% herd growth in the intervention area, 77.4% was due to calves born. In the control area, of the 26% growth, calves born during the monitoring period contributed 66.5%. This translated into birth rates of 17.5% and 13.7%, respectively. Interestingly, Finibougou residents also reported a substantial increase in the number of foalings in donkeys during this period.

Deaths were also monitored in the two areas. For the intervention area, a crude death rate of 1.7% and 3.9% for the control area was calculated. It was not possible to determine the exact

cause of the deaths, as no post-mortem examinations could be conducted on the dead cattle. It was also not possible to estimate the proportion of risk cattle in the total of dead cattle.

Other forms of dynamism in herds involved cattle paid out to herdsmen as salary. In the intervention area, 11 cattle were paid out to Fulani herdsmen in exchange for their herding services. Normally, one animal was used to compensate 6 months herding services. Thus through this arrangement, a herdsman received 2 animals in exchange for a year's uninterrupted herding services. In addition, they also received free supply of food stuffs including grain and milk over and above free accomodation at the homestead of their employers. It was not possible to quantify these indirect benefits in monetary terms.

Some cattle were also sold or purchased. Selling was occasioned by family reasons or by disposal of sick or older animals in order to replace them with younger ones. In the intervention area, 22 cattle were sold while in the control area 38 were sold. Similarly, of the 785 cattle in the intervention area 69 were purchased over the study period and of the 970 in the control area 63 were similarly bought. In either area, one bovine each was sacrificed.

5.3 Post-intervention phase

5.3.1 Cattle population characterization

A total of 393 (229 females and 164 males) cattle from the intervention area and 363 (186 females and 177 males) from the control area participated in the post-intervention trypanosome prevalence survey. Cross-bred cattle were the majority accounting for 54.1% (409/756) of study subjects and zebu cattle for 45.9% (347/756). Among the zebu, males were the majority (215/347) and among the cross-bred cattle, females were the majority (283/409).

5.3.2 Trypanosome prevalence

5.3.2.1 Herd level prevalence

A total of 28 herds participated in the trypanosome prevalence survey in the intervention area from which only 3 herds in two villages were found positive for trypanosomes (Table 24).

The other two villages, Kafela and Finibougou, with a combined total of 20 herds (200 cattle; 100 from each village) were negative for trypanosomes and are excluded from Table 24. Overall, risk cattle within the herds of the intervention area only showed sporadic trypanosome infection pattern.

Table 24: Post-intervention phase trypanosome herd point prevalence in Daresalame and Ziébougou villages in Sikasso, south-east Mali (November to December 2009)

Herd No.	**Village**	**Trypanosome positive cattle**			**Total cattle**	**Prevalence %**	**95 % CI**
		T.c.	T.v.	Total			
1	Daresalame	0	1	1	29	3.4	0.2-15.9
2	Daresalame	0	0	0	16	0	0
3	Daresalame	0	0	0	30	0	0
4	Daresalame	0	0	0	18	0	0
Total Daresalame		**0**	**1**	**1**	**93**	**1.1**	**0.1-5.2**
1	Ziébougou	0	0	0	8	0	0
2	Ziébougou	0	1	0	24	4.2	0.2-18.9
3	Ziébougou	0	1	0	43	2.3	0.1-10.9
4	Ziébougou	0	0	0	9	0	0
Total Ziébougou		**0**	**0**	**0**	**100**	**2**	**0.3-6.5**

All 3 positive herds each only did contain a single *T. vivax* positive animal. Herd prevalence in the two villages consequently was very low and ranged between 0% and 3.4% in Daresalame village and 0% and 4.2% in Ziébougou village.

Results of the herd level post-intervention trypanosome point prevalence for the control area villages are presented in Table 25. Although the number of trypanosome positive cattle in herds also in the control area was also low, an endemic pattern of trypanosome infections in the majority herds was exhibited except for herds in Diassadie.

Table 25: Post-intervention phase trypanosome herd point prevalence by village in the control area in Sikasso, south-east Mali (November to December 2009)

Herd No.	Village	Trypanosome positive cattle			Total cattle	Prevalence %	95% CI
		T.c.	T.v.	Total			
1	Diassadié	0	0	0	35	0	0
2	Diassadié	0	0	0	13	0	0
3	Diassadié	2	0	2	45	4.4	0.8-13.9
4	Diassadié	0	0	0	3	0	0
5	Diassadié	0	0	0	4	0	0
Total Diassadié		**2**	**0**	**2**	**100**	**2**	**0.3-6.5**
1	Waibera	3	0	3	17	17.6	4.7-40.9
2	Waibera	0	0	0	7	0	0
3	Waibera	1	0	1	5	20.0	1.0-66.6
4	Waibera	1	0	1	3	33.3	1.7-86.8
5	Waibera	1	0	1	11	9.1	0.5-37.3
6	Waibera	0	0	0	7	0	0
7	Waibera	2	0	2	12	16.7	2.9-45.1
Total Waibera		**8**	**0**	**8**	**62**	**12.9**	**6.2-23.0**
1	Kapala	1	0	1	5	20.0	1.0-66.6
2	Kapala	0	1	1	11	9.1	0.5-37.3
3	Kapala	1	0	1	16	6.3	0.3-27.2
4	Kapala	0	0	0	23	0	0
5	Kapala	2	0	2	25	8	1.4-24.0
6	Kapala	0	0	0	11	0	0
7	Kapala	0	0	0	9	0	0
Total Kapala		**4**	**1**	**5**	**100**	**5**	**1.9-10.7**
1	Tiogola	0	2	2	19	10.5	1.8-30.6
2	Tiogola	0	0	0	17	0	0
3	Tiogola	1	2	3	24	12.5	3.3-30.4
4	Tiogola	0	0	0	11	0	0
5	Tiogola	1	0	1	29	3.4	0.2-15.9
Total Tiogola		**2**	**4**	**6**	**100**	**6**	**2.5-12.1**

T.c. = *Trypanosoma congolense*; T.v. = *T. vivax;* 95% CI = 95% confidence interval

Waibera village had the highest number of trypanosome-positive herds, with prevalences ranging between 0% and 33.3% followed by Tiogola with herd trypanosome prevalences ranging from 0% and 12.5%. Diassadie had the lowest number of trypanosome-positive herds, prevalences ranged between 0% and 4.4%. Inter-village herd prevalence variability was clearly manifest in the control area.

5.3.2.2 Village level trypanosome prevalence

Post-intervention phase village trypanosome prevalences were estimated by aggregation of herd level trypanosome prevalences (Table 26). Generally, trypanosome prevalence was very low at village level in the intervention area. Ziébougou village only had prevalence of 2% (95% CI: 0.3-6.5), followed by Daresalame village, with 1.1% (95% CI: 0.1-5.2). Both Kafela and Finibougou villages had prevalences of 0%.

Table 26: Post-intervention trypanosome prevalences in cattle in the intervention and control areas by villages of Sikasso, south-east Mali (November to December 2009)

Village	Trypanosome positive cattle			Total cattle	Prevalence (%)	95% confidence interval
	T. c.	T. v.	Total			
Intervention area						
Kafela	0	0	0	100	0	0
Finibougou	0	0	0	100	0	0
Daresalame	0	1	1	93	1.1	0.1-5.2
Ziébougou	0	2	2	100	2.0	0.3-6.5
Area total	**0**	**3**	**3**	**393**	**0.8** [a]	**0.2-2.1**
Control area						
Diassadié	2	0	2	100	2.0	0.3-6.5
Waibera	8	0	8	62	12.9	6.2-23.0
Kapala	4	1	5	100	5.0	1.9-10.7
Tiogola	2	4	6	100	6	2.5-12.1
Area total	**16**	**5**	**21**	**362**	**5.8** [b]	**3.7-8.6**

T.c. = *Trypanosoma congolense*; T.v. = *T. vivax*
Different letter superscripts indicate statistical significant difference (χ^2 test, $p < 0.05$)

All the villages of the control area did contain trypanosome-positive cattle. Waibera village with 12.9% (95% CI: 6.2-23.0) had the highest prevalence, followed by Tiogola with 6% (95% CI: 2.5-12.1). Diassadié with 2 (95% CI: 0.3-6.5) had the lowest prevalence. The inter-village prevalence estimates in the control area were not significantly different (Table 26).

5.3.2.3 Area level trypanosome prevalance

For the intervention area, a mean prevalence of 0.8% (95% CI: 0.2-2.1) was calculated compared to the control area with 5.8% (95% CI: 3.7-8.6) (Table 26). These prevalences between the two areas were highly significantly (Pearson χ^2=15.54, 1 d.f., p<0.001) different, resulting in an estimated odds ratio of 8.0 (95% CI: 2.4-27.1); meaning a bovine in the control area was 8 times more likely to be trypanosome-positive.

All the 3 trypanosome positive cattle in the intervention area had *T. vivax,* while of the 21 trypanosome positive cattle in the control area, 76.2% had *T. congolense* and 23.8% *T. vivax*. In both areas, neither *T. brucei* nor mixed trypanosome infections were detected during the survey (Table 26). Like for the pre-intervention phase, breed, sex, area and village had no association with trypanosome prevalence.

Although the overall correlation between the village flies/trap/day and the trypanosome prevalence was strong (R^2 = 0.773, p = 0.004), this strength was particularly marked in the control area (R^2 = 0.8345) while weaker in the intervention area (R^2 = 0.0029). This was due to the very low trypanosome prevalence in the latter, compared to the moderately high prevalence in the control area.

Other haemo-parasites encountered during this survey included 2.9% microfilariae and 0.8% *Trypanosoma theileri*.

5.3.3 Post-intervention phase packed cell volumes (PCVs)

Packed cell volumes (PCVs) of cattle from the intervention area were generally higher than for cattle in the control area. In the intervention area, PCVs ranged between 20% and 55% with a mean of 31.6 ± 5.1% while they ranged between 10% and 41% with a mean of 27.4 ± 4.8% in the control area. These PCV measurements between the two areas were significantly

($p<0.001$) different. In the intervention area, cross-bred cattle had slightly higher mean PCVs (31.8 ± 5.2) as compared to the zebu cattle (31.4 ± 5.0). In the control area, zebu cattle in contrast, had slightly higher mean PCV (28.1 ± 5.1) than crosses (26.9 ±4.6%).

Trypanosome-negative cattle within the intervention area had a mean PCV of 31.6 ± 5.1% and positive cattle lower mean PCVs of 28.0 ± 6.4%. In the control area, mean PCVs among trypanosome-positive cattle (24.4 ± 4.4%) also were lower than for trypanosome-negative cattle (27.6 ± 4.8%). Among the trypanosome species, the mean PCV of cattle infected with *T. congolense* in the control area was 23.6 ± 4.3 lower than 27.4 ± 6.1 for *T. vivax* positive cattle. Packed cell volume comparisons were not possible for trypanosome species in the intervention area due to only 3 *T. vivax* cases left after intervention.

Stratification of study animals into two groups of PCVs < 25% and PCVs => 25%, showed that the intervention area had fewer anaemic cattle than the control area (Table 27). In this area, 12.4% of the cross-bred cattle had PCVs < 25% as compared to 13% zebu cattle (Table 33). In the control area, the proportions of cross-bred cattle and zebu cattle with < 25% were 38.6% and 32.1%, respectively.

Table 27: Proportions of cattle in PCV scores by village in Sikasso, south-east Mali (November to December 2009)

Sector	**No. cross breeds**			**No. zebu**		
Zebu	<25%	=>25%	Total	<25%	=>25%	Total
Intervention area						
Kafela	1	18	19	13	68	81
Finibougou	7	57	68	4	32	36
Daresalame	1	65	66	0	27	27
Ziébougou	15	29	44	9	47	56
Total	**24**	**169**	**193**	**26**	**174**	**200**
Control area						
Diassadié	23	38	61	8	31	39
Waibera	10	12	22	23	17	40
Kapala	32	41	73	6	21	27
Tiogola	18	41	59	7	34	41
Total	**83**	**132**	**215**	**44**	**93**	**137**

Of the 50 (24 cross and 26 zebu) anaemic (PCV < 25%) cattle in the intervention area, 2 (4%) were trypanosome-positive cattle. Both these animals were zebu. In the control area, of the 127 (83 crosses and 44 zebu) anaemic animals, 9.4% (12/127) were positive for trypanosomes.

5.3.4 Trypanocidal drug resistance

Isometamidium treatment failures were observed in Ziébougou, one village in the intervention area and in all villages of the control area (Table 28). In Ziébougou, a single relapse (*T. vivax*) was observed at day 14 (Table 28). In the control area, Diassadié village with a ISMM treatment failure rate of 50% had the highest treatment failure and Waibera village with 12.5% the lowest during day 14 monitoring (Table 28). At day 28 monitoring, the failure rate in the control area villages had decreased ranging between 0% and 25% with Kapala village having the highest failure rate. *Trypanosoma congolense* was responsible for all day 14 failures in the control area.

At area level, the intervention area had 33.3% treatment failure at day 14 and the control area 14.3%. There was no significant ($p>0.05$) difference between the treatment failures in the two areas. The day 28 failure rate in the control area was 16.7% with both *T. congolense* and *T. vivax* being involved.

The sole *T. vivax* that relapsed in the intervention area during day 14 and was retreated with 3.5mg/kg bw DIM resulted in successful treatment (trypanosomes cleared). In the control area, of the of the 3 relapsed *T. congolense* cases re-treated with 3.5mg/kg bw DIM, 1 had relapsed by day 28 in Kapala village while the rest were successfully cleared. Unlike during the pre-intervention phase where trypanocidal drug treatment led to a significant increase in PCVs, not much such improvement occurred after trypanocide treatment. At day 0, mean PCV was 23.3 ± 4.5, only slightly increased to 24.9 ± 4.5 at day 14 and was 24.9 ± 6.0 at day 28.

Table 28: Treatment failure rates by area of trypanosome-positive cattle treated with 0.5 mg/kg b.w. isometamidium (ISMM) in Sikasso, south-east Mali (November to December 2009)

Village	Response 14 days post-treatment				Response 28 days post-treatment			
	T.c.	T.v.	Total	%	T.c.	T.v.	Total	%
Intervention area								
Daresalame	0	0/1[a]	0/1	0	0	0/1	0/1	0
Ziébougou	0	1/2	1/2	50.0	0	0/1	0/1	0
Area total	**0**	**1/3**	**1/3**	**33.3**	**0**	**0/2**	**0/2**	**0**
Control area								
Diassadié	1/2[a]	0	1/2	50	0/1	0	0/1	0
Waibera	1/8	0	1/8	12.5	1/7	0	1/7	14.3
Kapala	1/4	0/1	1/5	20	1/3	0/1	1/4	25
Tiogola	0/3	0/3	0/6	0	0/3	1/3	1/6	16.7
Area total	**3/17**	**0/4**	**3/21**	**14.3**	**2/14**	**1/4**	**3/18**	**16.7**

[a] Relapsed/treated

T.c= *Trypanosoma congolense*

T.v= *Trypanosoma vivax*

%=Proportion of treatment failure

5.3.5 Faecal egg count reduction test (FECRT) results

5.3.5.1 Trichostrongyloids

The FECRT results are shown in Table 29. Albendazole, locally sourced from Malian markets, resulted in a faecal egg reduction (FECR) of only 55.6% (95% CI: 46.7-64.0) while the positive check (albendazole from Germany) reduced egg counts by 79.3% (95% CI: 71.9-85.7).

Table 29: Results of the FECRT of the risk group cattle treated with albendazole and placebo in Sikasso, south-east Mali (June 2008 to November 2009)

FECRT	Placebo	Treatment with	
		[a]Test albendazole	[b]Positive check
Number sampled	84	43	41
Mean pre-treatment EPG	773	685	651
Mean post-treatment EPG	493	219	102
% Reduction	36.2	55.6	79.3
Lower 95% CL of % FECRT	32.9	46.7	71.9
Upper 95% CL of % FECRT	39.7	64.0	85.7

[a]=Albendazole (Albenzole) used for carrying out the strategic helminth control scheme
[b]=Alendazole from Germany

5.3.5.2 Other helminths' FECRT

Strongyloides species occurred seldomly and had FECR of 85% (95% CI: 77.0 – 91.0%) for albendazole sourced from Malian markets and 100% FECR for the positive check. Other species like *Moniezia* species, *Capillaria* species and *Trichuris* species and *Toxocara* species occurred too rarely for statistical analysis.

5.4 Weather report

Total rainfall in the study area amounted to 1175mm, 1361.4 mm, 947 mm and 1178.4 mm per annum in 2006, 2007, 2008 and 2009, respectively (Figure 25). The year 2007 was the wettest with well distributed monthly rainfall followed by 2009. Although, it has been known

that the rainy season in Sikasso started in June, in 2008, it started as early as February in 2009 and March in 2006 and 2008. In 2007 and 2008, rainfall peaked in July/August while in 2006 and 2009; it peaked in August/September. The period October to February was dry season (0 wet days).

The relative humidity in the study area was directly dependent on precipitation, being low in the dry months and high in the wet months.

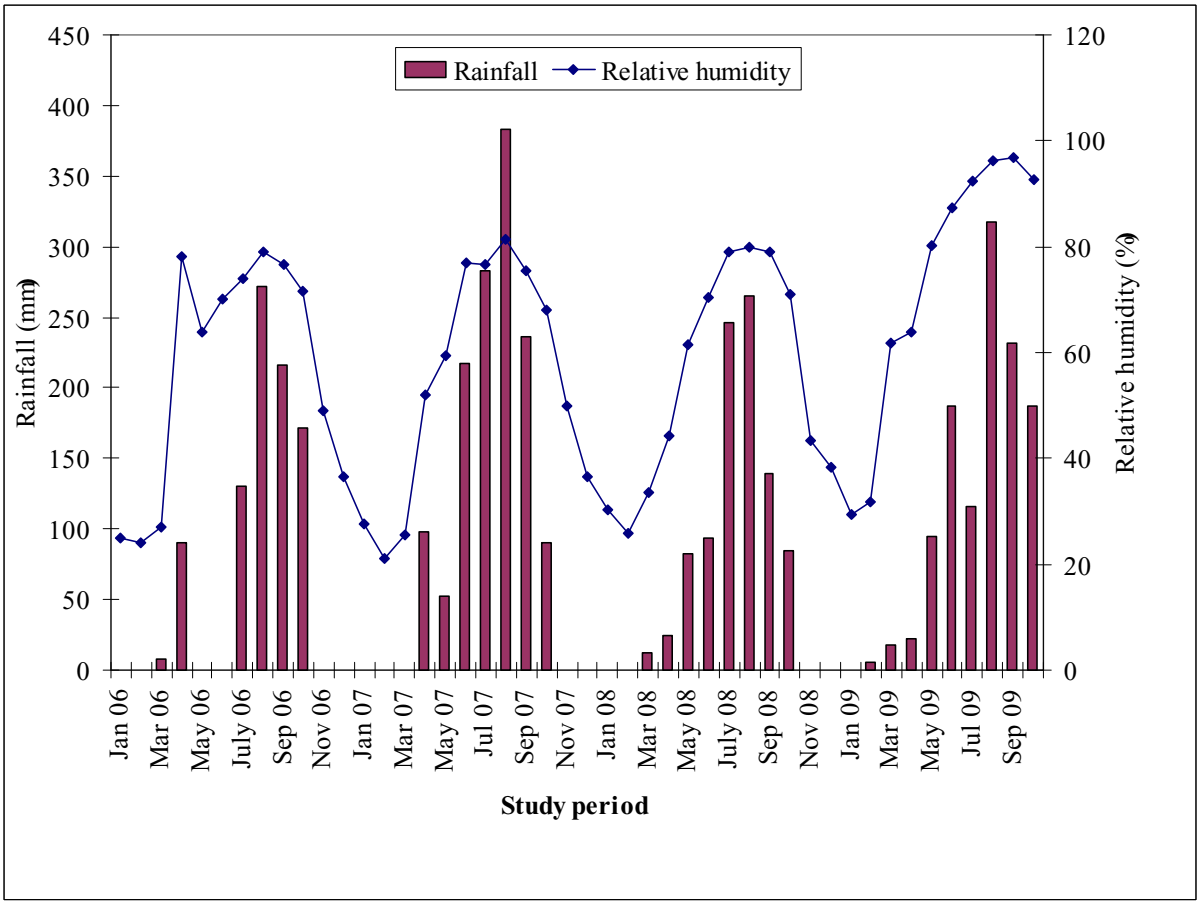

Figure 25: Rainfall and relative humidity data of Sikasso, south-east Mali (January 2006 to October 2009)

6 Discussion

6.1 Pre-intervention phase

6.1.1 Tsetse survey

During the pre-intervention survey phase *G. palpalis gambiensis* and *G. tachinoides* were the only tsetse species detected in the study area, with the former dominating. Savannah tsetse species were not detected during this survey. They are believed to have disappeared from the area following destruction of their habitat (Djiteye et al., 1997). The failure to catch such savannah flies in Sikasso agrees with the findings from south-west Burkina Faso by McDermott et al. (2003). The presence of only the riverine tsetse species has important implications for tsetse control planning, with emphasis to be targeted at the riparian habitats.

Variability in tsetse catches was observed across the study villages during this survey. Villages in the eastern sector generally had high fly catches with a mean flies/trap/day (FTD) of 6.8 for *G. p. gambiensis* and FTD of 4 for *G. tachinoides.* The reported FTD for *G. p. gambiensis* was however considerably lower than the 15-20 for the same species reported in the Samorogouan pastoral area of Burkina Faso (Bauer et al., 1995).

6.1.2 Cattle characterization

Cattle characterization showed that zebu dominated in Sikasso, perhaps an indication of their importance for draught power needs. This was confirmed by the high number of zebus (73.3%) among the draught oxen that participated in this study. This finding was in accordance with that by Kamuanga et al. (2001b) and Grace et al. (2009) who underlined that draught power was the most important consideration for rearing cattle in the cotton belt of West Africa. The composition of herds of zebu and of trypanotolerant cross-bred cattle was an indication that farmers recognized the value of the trypanotolerant cattle in trypanosomosis control. However, farmers'preference for trypanosusceptible breeds for reasons (draught power and big sale returns) unrelated to animal health, as reported by Kamuanga et al. (2001b) and Clausen et al. (2010), suggests that the continued intromission of the zebu genotype, if left unchecked, eventually may threaten the conservation of the trypanotolerant cattle breeds in Sikasso and in adjacent areas (Grace 2003).

6.1.3 Trypanosome prevalence

This study established that trypanosomosis was an endemic disease in Sikasso. Village trypanosome prevalences ranged between 11% and 16% in the eastern sector villages and between 14% and 19% in the western sector villages. Village trypanosome prevalence variability was apparent, similar to what was observed in the Kénédougou province of Burkina Faso (McDermott et al., 2003). The results of this study confirm findings by Diall et al. (2003) and Grace (2005) who also reported trypanosome prevalences of more than 10% in some villages in Sikasso. There was no significant difference in trypanosome prevalences between the eastern and the western sectors although cattle in the western sector were 1.3 times more likely to be infected with trypanosomes than those in the eastern sector. This was possibly because in the eastern sector slightly more trypanotolerant cattle breeds were used than in the western sector. Other cattle variables like age and sex had no effect on trypanosome prevalence.

Trypanosoma congolense dominated in both the eastern and western sectors followed by *T. vivax.* Earlier studies in Sikasso and in the neighbouring Kénédougou province in Burkina Faso also reported a dominance of *T. congolense* (Clausen et al., 1992; McDermott et al., 2003; Diall et al., 2003; Grace, 2005). No mixed trypanosome infections were observed during the present survey, probably due to the limited sensitivity of the parasitological method used (Reifenberg et al., 1997; Gall et al., 2004). *Trypanosoma brucei* was not detected in cattle in this area during this survey probably due to ecological reasons. This finding was consistent with reports that *T. brucei* is rare in Sikasso (Diall et al., 2003; Grace, 2005) and in neighbouring Burkina Faso (Bauer et al., 1992; McDermott et al., 2003). Also, the BCT is an inappropriate diagnostic method for *T. brucei* (Paris et al., 1982) and hence need exists to use more sensitive techniques like the HCT, in vitro culture systems, animal inoculation and or polymerase chain reaction (PCR) for this trypanosome species.

6.1.4 Trypanocidal drug use practices

In the absence of control activities against tsetse flies in Sikasso, trypanosomosis control is predominantly done through trypanocidal drugs (Clausen et al., 2010). This could be because the benefits of using trypanocidal drugs accrue largely to the individual user and their efficacy is not dependent on the participation of other livestock keepers (Shaw, 2004). Hence, many individual livestock owners choose the private, immediate and obvious benefits of using

trypanocides rather than the more expensive, public and long-term benefits of controlling tsetse.

Data on the number of trypanocidal drug treatments were not collected as farmers do not keep treatment records. Nonetheless, such data would have been useful in understanding the genesis of trypanocidal drug resistance in Sikasso. It has been reported that mass treatments done over a prolonged period of time exert a strong selection pressure on the trypanosome population circulating in a given area, leading to resistance development (Trail et al., 1985). The higher the proportion of trypanosome population exposed to the drug(s) is and the lower the population *in refugia* (trypanosomes in tsetse flies and other hosts), the higher is the selection pressure. Only few studies have been conducted on the frequency of drug use in various tsetse challenge situations. A survey on trypanocidal drug use in Zambia reported that on average about 1.5 treatments were given to each cattle annually, irrespective of the drug, age and sex of the concerned animal (Van den Bossche et al., 2000). In Ethiopia, about 6.1 treatments/year were administered to every cattle when trypanosomosis pressure was high, although this frequency was reduced to 1.4 following a tsetse control intervention (Leak et al., 1995). In another study in Burkina Faso, 5.1 trypanocidal treatments were given before intervention but the number was reduced to 1.8 treatments during intervention (Bauer et al., 1995). It essentially ought to be emphasized that the number of treatments is affected by such factors like the availability of resources which can be dedicated to enhance health of livestock, amongst many other competing livelihood needs of livestock keepers in a typical rural household setting.

Two trypanocidal drugs, ISMM and DIM, were in use in Sikasso. No other trypanocides are reportedly used in the study area. All trypanocides were sourced from privately owned agrovet shops where these drugs are dispensed without restrictions. This was contrary to restricted dispensation as reported for Zambia (Van den Bossche et al., 2000). In many areas of Africa, since the restructuring of veterinary services, many farmers are increasingly treating their own sick animals. This practice has serious drawbacks as most farmers do not have adequate knowledge on diagnosis and appropriate drug use, even in areas of high prevalence of trypanosomosis. A study in Sikasso confirms such misgivings where farmer trypanosomosis diagnosis only had sensitivity of 34.6% and a specificity of 77.9%, suggesting that a great number of clinically sick cattle are unnecessarily treated with trypanocidal drugs (Grace, 2005). A similar observation of lack of confirmatory diagnosis

was made in Zambia especially involving unjustified treatments that target productive cattle groups like cows and oxen (Van den Bossche et al., 2000).

6.1.5 Packed cell volumes

The PCV levels of the study animals ranged between 11% and 44%, with a mean of 26.4 ± 5.0%. This was lower than the 32.9 ± 5.0% recently reported in Burkina Faso (Dayo et al., 2010). One of the undeniable effects of trypanosome infections in cattle is the occurrence of anaemia, measured by a significant decline of PCV (Murray et al., 1984). Indeed this study established a significant ($p<0.001$) difference between trypanosome-infected cattle and non-infected ones and their PCVs.

Several studies have established that trypanosome-infected cattle show decreased PCVs (Trail et al., 1993; Van den Bosche and Rowlands, 2001; Cherenet et al., 2006; Dayo et al., 2010), also depending on the trypanosome species. *Trypanosoma congolense*-positive cattle in this study had lower PCVs than *T. vivax*-positive cattle, in accordance with other reports (Mattioli et al., 1999; Bengaly et al., 2002; Grace et al., 2008; Dayo et al., 2010). The study took place during the dry season when feed and water stress had started taking toll of cattle and it is likely that these factors further lowered the PCVs in accordance with Dwinger et al. (1992) and Fall et al. (1999). These authors observed that in the dry season, the frequency of trypanosomosis infections in cattle in West Africa increase with the affected cattle developing severe clinical disease.

6.1.6 Trypanocidal drug resistance

Standard laboratory methods to characterize drug resistance in trypanosomes, both *in vivo* and *in vitro* are expensive and time consuming (Eisler et al., 2000). In addition, the results of laboratory assays in rodents may only approximate the drug resistance status of *T. congolense;* they do not work for *T. vivax* and some *T. congolense* that do not readily grow in mice (Holmes et al., 2004). *In vitro* assays are time-consuming and are suitable mainly for *T. brucei.* Adapting bloodstream *T. congolense* from naturally infected animals to *in vitro* cultures continues to be difficult (Gray and Peregrine 1993). The drug incubation *Glossina* infectivity test (DIGIT) described by Clausen et al. (1999) and the drug incubation infectivity test (DIIT) by Kaminsky et al. (1990) used in characterizing *T. congolense* resistance

(Knoppe et al., 2006) are reliable screening methods but time-consuming. The DIGIT proved to be extremely sensitive in detecting drug-resistant *T. congolense* populations, but requires availability of tsetse flies. Genetic markers can be used to characterize trypanocidal drug resistance (Delespaux et al., 2005; Afework et al., 2006). The practical application of genetic markers is still limited in many sub-Saharan African countries (SSA) due to the lack of appropriately equipped laboratories and skilled personnel. Longitudinal studies (McDermott et al., 2003; Tewelde et al., 2004) are reliable but take long (sometimes up to 90 days) to generate results.

In the present study, an abbreviated 28-day field protocol was used to estimate resistance to 3.5 mg/kg and 7.0 mg/kg bw DIM and to 0.5 mg/kg bw ISMM in trypanosome-positive cattle (Diall et al., 2005). Although this method carries the risk of underestimating the resistance situation as observed by Gall et al. (2004) and Knoppe et al. (2006), the method was preferred because of other advantages. Firstly, it takes only one month to generate results at a reasonably low cost. The abbreviated protocol uses cattle of known trypanosome infection status in comparison to longitudinal studies that start off with susceptible animals to follow them for eventual trypanosome infections. Secondly, the efficacy of both DIM and ISMM is evaluated simultaneously, making it possible to estimate the resistance of trypanosomes to both drugs. In order to maximize the potential sample size and increase the precision of the resistance estimates, the study survey was timed to coincide with the period of highest tsetse and trypanosome risk (end of the rainy season).

With the abbreviated 28-day protocol, drug treatment failures were observed in all villages of both sectors. There was high variability (20% and 71.4%) for DIM failure rates for villages in the eastern as compared to the villages in the western sector where failures varied between 20% and 42.9%. Similar variability was reported for the Kénéndougou province in Burkina Faso (McDermott et al., 2003). Kafela village with the highest (71.4%) DIM failure rate is located closest to Sikasso, the administrative town of the region where the majority of the drug outlets are concentrated. Moreover, this village had the highest number of cattle traders who bought cattle from all over the country further increasing the likelihood of DIM usage and resistance development.

At sector level, treatment failure with 3.5 mg/kg bw DIM was lowest in the western sector (26.5%) and highest in the eastern sector (37.0%). The observed 3.5 mg/kg bw DIM

resistance is similar to what has been reported in the Kénéndougou province of Burkina Faso (Clausen et al., 1992; Bauer et al., 1995) and in Ethiopia (Afework et al., 2000).

Trypanosoma congolense exhibited resistance to 3.5 mg/kg bw DIM in both sectors as it accounted for the largest number of treatment failures. *Trypanosoma vivax* still was sensitive to 3.5 mg/kg bw DIM treatment throughout. These results are consistent with those of other authors (Clausen et al., 1992; Bauer et al., 1995; Afework et al., 2000; McDermott et al., 2003; Knoppe et al., 2006). Increasing the dose of DIM from 3.5 to 7 mg/kg bw DIM did not clear the *T. congolense* that had relapsed on 3.5 mg/kg bw DIM. This result agrees with that of Silayo et al. (1992) who reported that even using a double dose of DIM (two 3.5 mg/kg bw within 8-24 hours) did only slightly improve the therapeutic efficacy on resistant *T. congolense* strains at these doses. Clausen et al. (1992) who used up to 17.5 mg/kg bw DIM and Bauer et al., (1995) who used up to 14 m/kg bw DIM also reported relapsed *T. congolense*. It is of little value to increase the dose of DIM, once resistance in *T. congolense* against this drug has established.

As was the case with DIM, ISMM failures also occurred in the treated trypanosome-positive cattle in all the study villages at days 14 and 28. Variability in treatment failures was quite evident at village level, with villages in the eastern sector accounting for highest failures during days 14 and 28 monitoring. Similar village variability was also reported by McDermott and colleagues for Burkina Faso. This ISMM resistance in the present study also agrees with that reported earlier for Mali (Diall et al., 2003; Grace, 2005). Unlike 3.5 mg/kg bw DIM, against which only *T. congolense* was resistant, for ISMM, both *T. congolense* and *T. vivax* in both sectors at 0.5 mg/kg bw ISMM were associated with treatment failures. Resistance of *T. congolense* to ISMM has since long time been reported in neighbouring Burkina Faso (Authie, 1984; Pinder and Authie, 1984; Clausen et al., 1992; Bauer et al., 1995; McDermott et al., 2003; Knoppe et al., 2006), in Ethiopia (Peregrine et al., 1994; Afework et al., 2000; Tewelde et al., 2004) and in Tanzania (Fox et al., 1993).

The results of this study confirmed the presence of multiple drug-resistant *T. congolense* strains in Sikasso. Although the cause of this multiple drug-resistance as yet is not fully understood, interviews with farmers and veterinary staff revealed a high use of trypanocides in the area. This in all likelihood is related to resistance through a strong selection pressure on the trypanosome population in circulation in the area, as suggested by Trail et al. (1985). Cross-resistance associated with the past use of quinapyramine sulphate in cattle also hastens

the development of multiple drug resistance (in *T. congolense*) as reported by Ndoutomia et al. (1993). The multiple-drug resistance in the Samorogouan area in Burkina Faso (Clausen et al., 1992) and in the Ghibe valley of Ethiopia (Peregrine et al., 1994) was related to such past use of quinapyramine sulphate. The prolonged use of drugs without replacement, as is true for trypanocides principally encourages the development of resistance in accordance with Waller (1994). Cattle movements between Sikasso and Kénéndougou Province of Burkina Faso, the original foci of multiple-resistant *T. congolense* (Authie, 1984; Pinder and Authie, 1984; Clausen et al., 1992; Bauer et al., 1995; McDermott et al., 2003; Knoppe et al., 2006), can not be excluded as a possible cause.

Multiple-drug resistance can be contained by minimizing disease transmission, as was demonstrated in Ethiopia by Peregrine et al. (1994), Burkina Faso by Bauer et al. (1995), and in the Mkwaja ranch in Tanzania by Fox et al. (1993). Integrated health packages that target the vector (reduce transmission) and the parasites (self-cure through improved health) are required to clean-up all resistant trypanosomes to be able to avoid their spreading in case of tsetse reinvasion. Vector control helps minimize the transmission of trypanosomes amongst livestock but has no effect on the trypanosomes already in livestock. Sensitive trypanosomes get cleared when animals are treated with curative trypanocidal drugs, but the resistant trypanosome populations do persist. Unless animals infected with resistant trypanosomes are killed, the only feasible way out for them to overcome resistant trypanosome populations is through self-cure. Therefore, improved animal health through supplementary feeding and control of other diseases and infections may lead to better immunological competence and self-cure against trypanosomes. The demonstration of a strong pathological synergy between *T. congolense* and *Haemonchus* species (Kaufmann et al., 1992) further justifies the inclusion of strategic helminth control and feed supplementation package in any trypanosomosis control initiative. Moreover, studies on malaria have shown that malnourished children are at higher risk of treatment failure resulting from drug-resistant *Plasmodium falciparum* than the well nourished ones (Hess et al., 1997). Such findings underline the importance of immunological-competence in clearance of parasites.

The pre-intervention phase had the objective of establishing baseline data to assist in the selection of suitable sites for subsequent testing best-bet strategies to contain resistance. The pre-intervention phase survey established that the eastern and western sectors were similar from a disease ecology perspective and had high prevalence of trypanocidal drug resistance. They hence were found ideal for testing best-bet strategies.

6.2 Intervention phase

6.2.1 Tsetse catches

Tsetse densities in the intervention and control areas were not significantly different during the pre-intervention phase. However, following the tsetse control intervention using deltamethrin treated SADs and targeted treatment of livestock with deltamethrin that commenced in March 2008, differences in fly densities between the two areas started manifesting. By the time the first monitoring survey was conducted (June 2008), three months after commencement of the exercise, tsetse catches in all the villages of the intervention area were significantly lower compared to those in the control villages. These low tsetse densities in the intervention area were sustained throughout the monitoring period. There were, however, occasional small peaks in *G. p. gambiensis* catches, particularly in November 2008 and November 2009, with catches again reducing to negligible levels in June 2008 and June 2009. Cuisance et al. (1985) and Bouyer et al. (2006) reported that riverine tsetse species respond to changes in the relative humidity (RH) by dispersing away from drainage lines when RH rises (wet season) and concentrating along the drainage lines with a micro-climate to support their survival after the RH drops (dry season). With trapping exclusively undertaken along the drainage lines and owing to the behavioural changes occasioned by the RH gradient, fly catches in both areas followed this pattern; they increased during the dry season and decreased during the wet season.

Interestingly, in the November 2009 monitoring visit, there was a general increase in fly catches and particularly for *G. p. gambiensis.* In the control area, catches for this species increased by about 85%. It is suspected that the enhanced rainfall amounts in the period between August to October 2009 could have caused this build-up in tsetse density. Additionally, November 2009 was shortly after the rainy season when the gallery forests are still relatively intact, with good resting points for the flies as opposed to dry period months when bush fires destroy the gallery forests and fly resting areas. It ought to be remembered that the increased catches in the intervention area also could have been due to reinvasion, as this survey took place after the cessation of tsetse control activities.

A reduction of 91.3% (from FTD of 8.35 to 0.73) in *G. p. gambiensis* catches and of 97.3% (from FTD of 4.2 to 0.12) in *G. tachinoides* catches was recorded for the intervention area.

Reduction was highest in June 2008 and June 2009 (start of the rainy season) when the tsetse control technique switched from the use of SADs to treated live baits and vice versa. As has been noted earlier, the dispersal effect following the increase in RH resulted in an increased tsetse insecticide-treated cattle interface. On the whole, *G. tachinoides* percent catch reduction was higher than that for *G. p. gambiensis.* This was not surprising, since blood meal analysis showed that *G. tachinoides* did prefer cattle as opposed to *G. p. gambiensis* (Hoppenheit et al, unpublished) in agreement with findings of other tsetse host species identifications (Bauer et al., 1995; 1999; Clausen et al., 1998). It hence became easier to reduce the density of the former species using insecticide treated live baits than those of the latter. Additionally, research evidence shows that *G. tachinoides* are attracted to cattle odours (Rayaisse et al., 2010), making them more likely to be controlled through insecticide treated cattle than *G. p. gambiensis*. Bauer et al. (1995) conclusively demonstrated that *G. tachinoides* almost disappeared from the pastoral zone of Samorogouan (Burkina Faso) following such a successful application of deltamethrin on cattle.

Surprisingly, a reduction of 18.3% (from FTD of 6.45 to 5.45) in the catches of *G. p. gambiensis* and of 37.1% (from FTD 4.2 to 2.64) of *G. tachinoides* was observed also within the control area over the study period. This reduction in the control area was likely due to seasonal effects. The close proximity of the intervention area and the control area (35 km approximately apart) could have unreportedly resulted in a spill-over effect in terms of information exchange. Consequently, herd keepers in the control area reportedly also did commence some low-scale spraying of acaricides, indirectly affecting tsetse densities in this area.

This study is the first to report results of a tsetse control intervention in one area and a comparable area left as untreated control. The evidence that tsetse population reductions occurred also in the control area, even in the absence of tsetse control interventions, calls for caution in interpreting tsetse catch reduction results when no control area is included. This fact was highlighted as a weakness in a tsetse control campaign in Eastern Zambia (Van den Bosche et al., 2004) and in an impact study after a tsetse control exercise in Burkina Faso (Kamuanga et al., 2001a). It is for this reason that many of the past projects (Leak et al., 1995, Bauer, et al., 1999, Van den Bosche et al., 2004; Bauer et al., 2006) on tsetse control interventions may in fact have overestimated their catch reductions.

Although differences in the size of the tsetse control area, in trial execution methodology, in cattle management and in tsetse habitat aggrevates the difficulties to compare the results of this study directly with those from earlier tsetse control initiatives, the percent tsetse catch reductions (>90%) achieved after using deltamethrin treated SADs and live baits are in range of what has been reported before (Thompson et al., 1991; Fox et al., 1993, Peregrine, et al., 1994, Bauer et et al., 1995; 1999; 2006; Leak et al., 1995; Van den Bossche et al., 2004). Consistent with findings in the above studies, the continuous use of insecticide treated baits (SADs and cattle) over the 21 months did not result in a 100% elimination of tsetse flies from the intervention area. The residual tsetse population in the intervention area throughout the monitoring period possibly may represent tsetse re-invasion from the untreated surrounding areas. This problem of tsetse re-invasion also was underlined in a tsetse control trial in the Ghibe valley of Ethiopia (Leak et al., 1995).

The high mobility of tsetse means that to control them successfully, tsetse control must be applied over a large area approximately 1000 km^2 for at least a year. Smaller operations, conducted over approximately 500 km^2 as was the case in this study only can help reduce the incidence of trypanosomosis (Hargrove *et al.*, 2000) but will not eliminate a tsetse population requiring the intervention to be sustained indefinitely. However, it even seems advantageous that a certain minimum level of tsetse challenge be still maintained after tsetse control, so that the newly born animals get exposed to low-level trypanosome infections for them to develop acquired immunity against such locally circulating trypanosome strains. Some residual tsetse numbers thus foster enzootic stability (Holmes et al., 2004) in a given area. Similarly, the use of curative trypanocidal drugs should also be restricted to clinically sick animals, in order not to interfere with immune system development.

The riverine tsetse species are generally difficult to eliminate with treated live baits because of their wide host spectrum (Bauer et al., 1995; 1999; Clausen et al., 1998). In contrast, the savannah species are a lot easier to eliminate from an area due to a limited host range and the fact that they get attracted to cattle urine and other skin emissions making it easier to eliminate them with insecticides treated cattle (Moloo et al., 1979). In Zanzibar, where complete tsetse eradication was achieved, a combination of tsetse control techniques was used over a prolonged period of time. There was initial use of insecticides and tsetse trapping to suppress the tsetse population before the sterile insect technique (SIT) was used to finish off the job (Reichard, 2002). Importantly, however, was the requirement to safe-guard the tsetse cleared area by setting up and maintaining barriers at identified reinvasion fronts. In Sikasso,

barriers were not used and reinvasion occurred shortly after cessation of tsetse control activities. This was evidenced by the high tsetse catches in the intervention area during the November 2009 monitoring visit.

6.2.2 Trypanosome prevalence

During the pre-intervention phase, trypanosome prevalences between the eastern sector (intervention area) and the western sector (control area) were not significantly (χ^2 test, $p>0.05$) different. In contrast, after commencement of the intervention, trypanosome infection risk significantly ($p<0.001$) dropped about 8-fold as indicated by the odds ratio of 7.68 (95% CI: 4.621-12.78). Intervention notably led to significantly lower trypanosome infections. Other studies before also have reported a similar drop in trypanosome prevalence by use of a combination of tsetse control actvities and limited trypanocidal treatments was done (Leak et al., 1995, Bauer, et al., 1999, Bauer et al., 2006).

Three months after commencement of the interventions, trypanosome infections in the risk cattle herds in the intervention area became sporadic occurring further on only occasionally in a few herds. In contrast, herds in the control area continued to depict an endemic trypanosome infection pattern. There was a clear herd effect that exerted marked influence on village trypanosome prevalence, particularly in Kafela village. The high inter-herd variation demonstrates the variations in village level prevalence. Similar variability in village level trypanosome prevalence was also reported in Burkina Faso (McDermott et al., 2003). Trypanosome infection was season-dependent, with highest prevalence in November 2008 (end of the rainy season) and February 2009 (dry season) having the lowest prevalence. During the rainy season, the dispersal effect of flies (Cuisance et al., 1985, Bouyer et al., 2006) increases the interface between tsetse and cattle and hence the transmission risk. The reverse is true for the dry season when tsetse flies get concentrated along drainage lines, hence reducing the interface between tsetse and cattle. These dynamics are compounded by the fact that several seasonal streams and rivers in Sikasso dry out and that the majority of cattle are watered at community boreholes.

The results of the pre-intervention phase indicated that *T. congolense* was the dominant trypanosome species in the study area, accounting for over 70% of all infections in both the eastern and western sectors of the study area. Following intervention, there was a reversal of

roles, with eventually *T. vivax* accounting for 75% of all trypanosome infections in the intervention area and for 51.9% of the infections in the control area. A study in Ethiopia (Rowlands et al., 1993) established more *T. vivax* infections than other trypanosome species in young growing cattle as compared to adult cattle. This helps explain the dominance of *T. vivax* infections in this study since monitoring was conducted on the young growing cattle. Similarly, the reduced *T. congolense*/*T. vivax* ratio could also have been due to the reduced life span of the tsetse flies in the intervention area. *Trypanosoma vivax* has the shortest life cycle (as short as 5 days at 26º C or 12-13 days at 22º C) in tsetse compared to *T. congolense* that take long (15-20 days) to mature (Moloo and Gray, 1989). Such parasite biology most likely influenced trypanosome transmission patterns and particularly in the intervention area. The likelihood of mechanical transmission can not be ruled out, since the majority of trypanosomes were *T. vivax* in accordance with some field study reports from other African countries (Leak et al., 1995, Desquesnes and Dia, 2003a, Cherenet et al., 2006; Mamodou et al., 2006).

Although drug resistance has been confirmed in the study area (Diall et al., 2003; Grace, 2005) and from the block treatment study during the pre-intervention phase, DIM treatments done on the few trypanosome-positive and the risk cattle with PCV < 20% during the monitoring phase of this study were effective and particularly in the intervention area, since all the treated trypanosome positive cattle never relapsed during the subsequent monitoring visits. Rowlands et al. (1993) observed that despite the high level of trypanocidal drug resistance and the associated trypanosome relapse infections in the Ghibe valley, Ethiopia, the significant decrease in trypanosome prevalence indicated that drug treatment was associated with a better ability of animals to control existing trypanosome infections under reduced tsetse challenge. Additionally, improved livestock productivity was noted in the same area after trypanocidal drugs were use the constraint of trypanocidal drug resistance notwithstanding (Itty et al., 1995). Therefore, the DIM treatments administered by the project to all the parasitaemic cattle during the monitoring visits must have greatly influenced the trypanosome infection patterns in both areas. Furthermore, the pre-intervention phase had established that *T. vivax* was still sensitive to DIM, implying that the majority of these trypanosome species were cleared by these treatments.

6.2.3 Trypanosome incidence

The IDR was principally lower in the intervention area compared to the control area. Clustering of trypanosome cases at the level of the herd, village and area particularly in the intervention area was evident. In the control area, herd IDRs in contrast were fairly predictable with lower intra- and inter-herd variations.

On the area basis, reducing tsetse challenge in the intervention area was accompanied by a corresponding reduction in trypanosome transmission risk of about 9.1 times. This was a clear indication of how trypanosome infections were dependent on tsetse densities. The risk of trypanosome transmission though did not only depend on tsetse density but also on the tsetse species. Waibera village with the highest tsetse catch throughout the monitoring period had the highest IDR. In contrast, in Kapala village and in Tiogola village, with low fly densities dominated with *G. tachinoides,* risk of trypanosome infection was higher than in Diassadié, a village where the second highest tsetse density was noted. When a large proportion of *G. tachinoides* flies are infected with trypanosomes, transmission of trypanosomes to cattle is more likely to occur than for *G. p. gambiensis* which have a wide-spectrum of hosts (Bauer et al., 1995; Clausen et al., 1998). This preference for cattle by *G. tachinoides* was also reported in a recent study in Cote d'Ivoire and Burkina Faso (Rayaisse et al., 2010).

In a tsetse control trial in Zimbabwe, trypanosome incidence reached zero in 3 months when cattle were dipped in deltamethrin and in 6 months when cattle were treated with a pour-on preparation of the same acaricide (Thompson et al., 1991). Similarly, a combination of deltamethrin treated SADs and live baits used to control tsetse in Sikasso had the greatest impact on the incidence density rate in the first three months of the intervention. The incidence density significantly reduced in response to the decreased tsetse density from 0.018 (95% CI: 0.009-0.034) during the risk period June-November 2008 to 0.002 (95% CI: 0.0001-0.0012) during the period November 2008 – February 2009. It remained at that low level until it eventually dropped to zero during the risk period June-November 2009. The fact that incidence during the risk period June-November 2009 was zero in the intervention area may however, be misleading, since trypanosome transmission does not completely halt even after tsetse challenge is significantly reduced (Bauer et al., 1995; Leak, et at., 1995; Cherenet et al., 2006; Mamadou et al., 2006). The residual tsetse population assisted by mechanical transmitters sustain trypanosome transmission especially those of *T. vivax* (Desquesnes and

Dia, 2003a). Comparatively, trypanosome infection risk remained significantly ($p<0.001$) higher in the control area except for the seasonal fluctuations.

Univariate analysis conducted on cohorts of risk cattle stratified by time when they first entered the study showed that age was an important risk factor in terms of acquisition of a new trypanosome infection. The probability of acquiring a new infection increased with old age. This observation is consistent with that by Torr and Mangwiro (2000) and Vale and Torr, (2005). Breed effect was also important as trypanosusceptible zebu cattle are known to be more sensitive to trypanosome infections than the cross-bred "trypanotolerant" cattle (Murray et al., 1982; Clausen et al., 1993; d'Ieteren et al., 1998). However, for the "trypanotolerant" cattle breeds, previous exposure to challenge is of paramount importance in modulating pathogenesis and severity of the disease (Clausen et al., 1993).

6.2.4 Trypanocidal drug use practices

The results of the trypanocidal drug use practices show that DIM and ISMM were used in both areas throughout the monitoring phase. DIM was mostly used for curative purposes and ISMM exclusively for prophylactic purposes. The majority of curative treatments were administered in June (start of the rainy season) or November (end of the rainy season). As discussed before (6.2.1 and 6.2.2), the risk of trypanosome transmission does increase during the rainy season because of the dispersal effect which increases the interface between hosts and tsetse flies.

Although data on the number of risk cattle treated with trypanocidal drugs were not collected, estimates show that some of them might have been treated by the farmers throughout the monitoring period. Correspondingly, in the intervention area it is estimated that herd owners did administer an estimated 227 DIM treatments to the risk cattle, which was about 9 times higher than the 26 treatments administered by the project. Obviously, majority of these treatments were unwarranted and probably likely to interfere with acquired immunity or resistance against the locally circulating trypanosomes strains especially for cattle with sub-clinical trypanosome infections and the naïve ones as stated by Holmes et al. (2004). Other than wasting scarce resources on trypanocides, such unjustified treatments make the environment unsound and serve as a potential source of drug residues in meat and milk, if withdrawal periods are not observed. Other studies have also reported the high use of

trypanocidal drugs even where tsetse challenge does not justify this (Rowlands et al., 1994; Van den Bosche et al., 2000). Despite the low tsetse challenge in the intervention area, farmers continued using drugs. It will take quite some effort to reverse the life-long believes and practices that seem to be central in forming farmer trypanocidal drug use decisions in Sikasso.

In the control area, 261 DIM treatments by herd owners were administered to the risk cattle, compared to 179 treatments done by the project. The DIM usage in this area by the project and the herd owners was comparable. It appears that the use of DIM in the control area was well below the prevailing high tsetse challenge and trypanosome infection rates. This is despite the estimated actual costs of trypanosomosis that are on average 12% to 28% of the output derived from cattle production in the cotton belt of West Africa (Affognon, 2007). These costs could be reduced to 7% to 8% of the output (depending on the disease prevalence and drug resistance levels) if cattle farmers were to adopt economically optimal levels of ISMM and DIM use.

Although more cattle in the control area were treated with ISMM than in the intervention area, the ISMM use situation in the control area was still low, given the prevailing high trypanosome risk. The finding of the current study confirm reports from other African countries that only a small proportion of cattle at risk for trypanosomosis get treated with trypanocides (Geerts and Holmes, 1998; Van den Bossche et al., 2000; Holmes et al., 2004). The main reason for the lower use of ISMM was its high cost. However, interviews with farmers and local veterinarians also revealed that ISMM is perceived to be a strong drug only to be used during the rainy season (when animals are in good body condition) and on non-pregnant cows, as believe exists that it can cause abortion.

6.2.5 Helminth infections

From this study, it is apparent that trichostrongyloids are the predominant helminth species in Sikasso. This finding confirms reports of past studies in West Africa (Kaufmann and Pfister, 1990; Ndao et al., 1995; Zinsstag et al., 2000; Wynn et al., 2008). The majority of the risk group cattle belonged to the low to moderate levels of shedding of strongyle eggs. This could have possibly been due to the climatic influences (prolonged dry periods) that do not favour

survival of the infective stages in the environment for long (Kaufmann and Pfister, 1990; Zinsstag et al., 2000; Wynn et al., 2008). Another possible reason why FECs were low is the occasional use of ivermectin as reported for one herd in Diassadié village of the control area. It should be emphasized that the low to moderate FECs as reported by this study are not necessarily indicative of low strongyle loads in the study animals. Presence of a high proportion of hypobiosed helminth stages or the animals having acquired immunity against the helminth species (Urquhart et al., 1996; Fall et al., 1999) may have contributed to this low helminth burden.

Analysis of faecal egg counts in this study agrees with those reported earlier in other helminth surveys in West Africa (Kaufmann and Pfister, 1990, Ndao et al., 1995; Zinsstag et al., 2000; Wynn et al., 2008). Season was an important factor in determining GIN egg shedding potential. The lowest FECs (median =0) was observed in February 2009 that is in the middle of the dry season while the highest (median=500) egg shedding rates were recorded in November 2009, end of the rainy season. From June, the start of the rainy season or the end of the dry season, a remarkable increase in egg shedding could be observed. These seasonal FEC shedding patterns compare well with those reported by others in the West African region (Kaufmann and Pfister, 1990; Ndao et al., 1995; Zinsstag et al., 2000; Wynn et al., 2008). Various reasons are listed for seasonal fluctuations in egg shedding in most strongyle helminth parasites. In the dry season, suppression of egg output can be due to the different survival strategies of helminth species. During the dry season some nematode species (*Cooperia* species, *Bunostomum* species and *Oesophagostomum* species) survive as adults while some others like *Haemonchus* species survive as inhibited larvae in the mucosa of the gastrointestinal tract (Kaufmann and Pfister, 1990). Conversely, during the rainy season, the inhibited larvae resume development and become adults increasing faecal egg output. Pasture contamination with infective larvae is also highest during the rainy season which increases the worm load and hence faecal egg out (Kaufmann and Pfister, 1990).

Animal risk factors also influenced the strongyle egg shedding patterns. Cross-bred animals (zebu x trypanotolerant breeds) had low FECs compared to the zebu cattle. Mattioli et al. (1992) observed that N'Dama, a trypanotolerant cattle breed had low prevalence of strongyle infections and produced lower egg outputs than the Gobra, a trypano-susceptible zebu cattle breed. Further, strongyle egg shedding was inversely proportional to age. The risk cattle aged between 3 and 12 months had the highest strongyle egg shedding, followed by cattle in the

age stratum between 12 and 24 months and the lowest shedders of age above 24 months. This points to to the eventual build-up of immunity with increase in the age of animals. A similar observation was made by Fall et al. (1999) in Senegal.

Other helmith eggs encountered, but rarely during the study, included those of *Strongyloides* species, *Toxacara* species, *Moniezia* species, *Trichuris* species and *Capillaria* species. *Strongyloides* and *Toxacara* species eggs were commonly detected in the young animals (<12 months old). Owing to the mode of transmission of *Toxacara* species (via milk), this condition is self-limiting in mature animals, after acquired immunity develops in accordance with Wynn et al. (2008).

Fasciola species eggs were completely not detected in the risk cattle during the monitoring phase. This could be attributed to the soil type (a mixture of loam and sandy) and possibly water pH in Sikasso which are unfavourable for the survival of the lymnaeid snails, the intermediate hosts of *Fasciola* species. However, interviews with the the personnel involved in meat inspection at the Sikasso regional slaughter house revealed that this parasite was only occasionally encountered during post-mortem meat inspection. This was because cattle for slaughter were sourced from different areas within Mali some of which were endemic for fasciolosis. In Mali, fasciolosis is reported to be endemic in the Segou region where the extensive rice pads (Traore, 1989) and in the Mopti region prone to flooding from river Niger (Tembley et al., 1995) favour the survival of the lymnaeid snails.

6.2.6 Effect of albendazole treatment on trypanosome IDR

This study pioneered the investigation on the use of an integrated health package under a chemoresistance hot-spot environment. Although reports on the interactions between helminths and trypanosomes in West Africa did exist before (Kaufmann et al., 1992; Dwinger et al., 1994; Faye et al., 2002), none of them attempted to establish the impact of controlling helminthosis on trypanosomosis management in cattle especially in trypanocidal drug resistance hot spots.

Although the sample size used during this study may not have been large enough to make generalizations about the effect of strategic helminth control in a trypanosomosis control package, albendazole treatment had some positive protective effect on trypanosome infections

in cattle. This was clearly manifested in the intervention area, where the albendazole treated risk cattle had a 2.9 times reduced risk of acquiring a new trypanosome infection compared to the control (placebo) animals. Although this difference was not significant ($p>0.05$), the inter-cohort risk difference underlines that albendazole treatment was probably responsible for this calculated risk difference.

The failure to observe an outright effect of drenching on trypanosome infections could have been due to a number of reasons. Firstly, the anthelmintic drug used was not fully efficacious in the control of helminths as had been anticipated. Secondly, the influence of *refugia* (worms in cattle not treated with albendazole) could have diluted the effect of the albendazole treatments through the continued re-infection (Boa et al., 2001; Pomproy, 2006; Waghorn et al., 2008). Finally, blinding was restricted to farmers and not to investigators, so it was possible that information on treatments did leak to the farmers. Through this, the placebo treated risk cattle could have been secretly treated with albendazole by the farmers themselves.

It is an established fact that trypanosome infections cause immuno-suppression (Nantulya et al., 1982). It is also established that some helminth species including *Ostertagia* species in cattle and *Haemochus* species in sheep also cause immuno-suppression (Gasbarre, 1997). This immuno-suppressive effect was clearly excerbated when sheep experimentally infected with *Haemonchus contortus* were also infected with *T. congolense* that resulted in a severe pathological outcome as both parasites' immuno-suppressive effect weakened the immune system of the animal (Kaufmann et al., 1992; Goossens et al., 1997). In areas where both gastro-intestinal nematodes and trypanosomes are prevalent, controlling either or both of these parasites in cattle is likely to result in improved health of such animals. The effect is likely to be bigger if supplementary feeding is included following Katunguka-Rwakishaya et al. (1993).

As discussed under trypanosome infections in 6.2.2 and 6.2.3, season was crucial in determining trypanosome infection pressure. The effect of the drench in influencing trypanomosis risk seemed to be dependent on season as well. The risk period June-November 2008 had the highest number of trypanosome infections within the intervention area. During the same period, IDR of albendazole treated risk group cattle was 0.004 (95% CI: 0.0002-0.02) and 8 times lower than the IDR of placebo group of 0.031 (95% CI: 0.01-0.05) in Kafela village of the intervention area. The same observation was replicated in Waibera

village in the control area where the albendazole treated risk cattle had a slightly lower IDR (0.132) compared to (0.148) of the placebo group.

Although these results show that integrating strategic helminth control in a tsetse control initiative could be beneficial, further investigations, using sufficiently large sample sizes, are required to ultimately elucidate the supporting effect of strategic helminth control on trypanosomosis control.

6.2.7 Packed cell volume (PCV) development

Despite the fact that the risk cattle in both the intervention and the control areas were exposed to similar management and to comparable climatic conditions, three months after the start of intervention the study animals in the intervention area had significantly ($p<0.001$) higher mean PCVs than those from the control area. During the preceding pre-intervention phase, PCVs in both areas had averaged 26%. The mean PCVs rose to > 30% in the intervention area during the intervention phase while it remained < 30% in the control area during the central intervention phase. This difference in animal PCVs is attributed to the effect of the intervention in full concordance with many observations by other investigators (Bauer et al., 1992; Bauer et al., 1995; Leak, et al., 1995; Van den Bosche et al., 2004).

Due consideration ought to be given to other parasitic conditions such as anaplasmosis, babesiosis, haemonchosis, in certain instances fasciolosis, acute bunostomosis, acute paramphistomosis and external parasitism, particularly tick infestation that equally can cause anaemia in cattle (Leak et al., 1995; Urquhart et al., 1996; Radostitis et al., 2002). The severity of some of the tick-borne conditions in this study may have concomitantly been reduced through tick control following commencement of the (restricted) deltamethrin spraying of cattle in the intervention area. Fox et al. (1993) used deltamethrin in a plunge dip at Mkwaja ranch in Tanzania and Bauer et al. (1992; 1995) in Burkina Faso used the same acaricide as a pour-on and observed supporting distinct impact on reducing tick population within the participating cattle herds.

6.2.8 Weight development

It should be noted that weight development in animals is influenced by age, nutrition, health status, breed and sex. Other than health that was influenced by the intervention, all other factors were uniform across cattle of both the intervention and control areas. The effect of the intervention on growing cattle (not of total herds) was not significantly different although risk animals in the intervention area started off heavier than those in the control area. A study in Burkina Faso did demonstrate that cattle did gain weight after commencement of a tsetse control initiative (Bauer et al., 1995).

It should also be recalled that the project did administer DIM treatments to all trypanosome-positive cattle and those with PCVs < 20% across the two areas. Since majority of the DIM treatments were done on animals in the control area, this may have influenced weight development since DIM, other than controlling trypanosomosis, also has an effect on babesiosis (Radostits et al., 2002). Some control measures on other diseases particularly the tick borne and other parasitic infections may have also influenced the levelling out of weights on herd level.

6.2.9 Herd dynamic indicators

At the start of the intervention, the control area had a larger cattle population than the intervention area. However, following the commencement of interventions, the cattle population in the intervention area grew faster than in the control area. Cattle population growth was accounted for through births (the principal way) and through purchases. The crude calving rates were 17.5% in the intervention area, compared to 13.7% in the control area. Since the pre-intervention phase survey showed that the two areas were comparable in terms of trypanosomosis infection pressure, ecology/climate and husbandry practices, the extra 4% calving rates for the intervention area may be attributed to direct effects of the intervention. It has been reported that among the largest and most consistent impacts of trypanosomosis control is their effect on birth rates and mortalities in young animals (Swallow, 2000). Trypanosomosis may reduce calving rates by between 1 to 12% in trypanotolerant and by 11 to 20% in trypanosusceptible cattle. Similarly, the same author estimated a calf mortality of between 0 to 10% in trypanotolerant and 10 to 20% in susceptible breeds.

In another study that measured impacts of trypanosomosis on livestock production, a 25% increase in herd sizes, mostly through births and transfers-in, was reported after a successful tsetse control campaign in the agro-pastoral zone of Yalé in Burkina Faso (Kamuanga et al., 2001a). The same study noted a drop of mortality on herd level from 63.1% to 7.1%. In this current study, mortality in the intervention area was much lower, estimated at 1.7% and at 3.9% in the control area. The high mortality in the agro-pastoral zone of Yalé was because the area was newly settled by Fulani herders who kept zebu cattle predominantly. These cattle might have been naïve to trypanosomosis challenge since the Fulani herders home area is semi-arid sahelian zone that is outside the tsetse belt. In our case, the herd owners have stayed in the study area for long and cattle without doubt have established some form of tolerance to the circulating trypanosome populations. Additionally, trypanocidal drug use is rampant in the study area.

It would also have helped to calculate herd performance indices including proportional mortality rates and trypanosomosis case fatality rates if data on these aspects was collected.

6.3 Post-intervention phase

6.3.1 Trypanosome prevalence

During the pre-intervention trypanosome prevalence results for four villages of the intervention area ranged between 11% and 16%; the aggregated sector level prevalence was 13.9% (95% CI: 10.9-17.4). *Trypanosoma congolense* was the dominant trypanosome species accounting for 75% of all infections and *T. vivax* only 25%. After conducting intervention, trypanosome prevalence dropped to just 0.8% (95% CI: 0.2-2.1) with only 3 *T. vivax* detected in the entire intervention area. The percent reduction in trypanosome prevalence in this area was 94.2% (from the pre-intervention level of 13.9% to the post-intervention level of 0.8%). Leak et al. (1995) observed that trypanosome infection can not be completely halted after tsetse control interventions, since the tsetse population normally gets essentially suppressed but not totally eliminated. It should also be noted that despite the reported decrease in trypanosome prevalence, longitudinal monitoring over several years is required to generate sufficient evidence about the evolution of the new trypanosome infection patterns.

In other similar tsetse control intervention, trypanosome prevalence at the end of the exercise ranged between 5% and 10% (Bauer et al., 1992; Bauer et al., 1995; Bauer et al., 1999; Leak et al., 1995). In this study, a very low trypanosome prevalence at the end of the intervention was realized which may possibly be an under-estimation of the real situation since herd owners had just carried out both ISMM prophylactic and DIM strategic treatments in both study areas before this survey.

Despite the fact that herd owners had massively treated their cattle with trypanocides shortly before this survey, inter-village variability of trypanosome prevalence between 2% and 12.9% in the control area villages was observed. The mean prevalence for the control area was 5.8% (95% CI: 3.7-8.6), which was significantly ($p<0.001$) higher than the 0.8% (95% CI: 0.2-2.1) of the intervention area. The estimated odds ratio was 8.006 (95% CI: 2.368-27.07) suggesting, that the intervention helped reduce the risk of trypanosome infections in the intervention area 8-fold. As was the case during the pre-intervention phase, *T. congolense* still dominated in the control area, with 76.2% of all the trypanosome infections, the rest being *T. vivax.*

In the intervention area, there was a significant ($p<0.001$) improvement in the PCVs from 27.3 ± 5.1% before intervention to 31.6 ± 5.1% post-intervention, representing an increase of 15.8%. Other studies also reported PCV improvement after tsetse control intervention (Bauer et al., 1992; 1995; 1999; Leak et al., 1995; Van den Bosche et al., 2004). Surprisingly though, there was also a 7.5% increase in PCVs of the cattle within the control area from the pre-intervention mean PCVs of 25.5 ± 4.9% to 27.4 ± 4.8%. This was attributed to the observed drop in tsetse population in the control area discussed under 6.2.1.

6.3.2 Trypanocidal drug resistance

It is known that trypanosomes can not persist in the absence of tsetse and further vector eradication is an effective means of eliminating trypanocidal drug resistance (Fox et al., 1993; Peregrine et al., 1994; Bauer et al., 1995). However, for vector control to be able to achieve this objective, sustainability of the control activities with for example permanent barriers to ensure that cleared areas are protected from reinvasion is crucial. Moreover, to effectively be able to contain resistance, tsetse control and disease impact monitoring need to be undertaken over a long period of time to be able to confidently make conclusions about the impact of the

interventions. In absence of this, uncertainty surrounding the effectiveness and permanence of resistance containment can be somewhat reduced by complementing epidemiological field surveys with mathematical models (Austin and Anderson, 1999). The relationship between antibiotic use and resistance has been modelled in this way (Bonten et al., 2001) and this proved useful in designing more effective control programmes. Already, modelling plans are at an advanced stage to simulate the impact of tsetse control and trypanocidal drug resistance.

Generally, reduction of tsetse densities through vector control resulted in a reduction of trypanosomes circulating in the intervention area. Despite the existence of the multiple drug-resistant *T. congolense* populations in the study area, as earlier reported, the parasitological outcomes of this study indicate that the intervention package still managed to eliminate the majority of trypanosomes from the intervention area. During monitoring, only 4 residual *T. congolense* were encountered. These trypanosomes still seemed to be sensitive to 3.5 mg/kg bw DIM, as they all were cleared following treatment with this drug.

In the post-intervention prevalence survey, only 3 *T. vivax* had remained in the intervention area, of which only one failed to be cleared by ISMM by day 14. Subjecting this relapsed trypanosome to 3.5 mg/kg bw DIM, the cattle tested negative 2 weeks later. This suggests that the majority *T. vivax* strains are still sensitive to DIM, as was confirmed during the pre-intervention phase drug sensitivity testing.

6.3.3 Anthelmintic resistance

The results of the FECRT showed indications of the likely presence of albendazole resistant GINs in Sikasso. According to Coles et al. (1992), for the drug to be effective against nematodes, the FECRT in animals treated with an anthelmintic must be 95% and or the lower bound of the 95% confidence level must be at least 90%. In this study, neither of the two conditions was met, an indication of existence of gastro-intestinal nematodes resistant to albendazole. This was a very unexpected and surprising result as so far no report on anthelmintic resistance in the cotton belt of West Africa does exist.

Although, albendazole resistance is strongly suspected in the cattle nematodes in Sikasso, it is still not clear which nematodes are responsible for the resistance since larval cultures for the albendazole treated and control groups were not done when FECRT was conducted.

Consistent with the WAAVPs protocol (Coles et al., 1992) and from the numerous field studies that have been documented (Demeler et al., 2009, Soutello et al., 2007, Anziani et al., 2004; Gatongi et al., 2003; and Sissay et al., 2006) among others, differentiation of the nematode species into those still sensitive and those resistant to anthelmintic drugs is done through larval cultures before and after anthelmintic treatment. Culturing is necessary as it gives an indication of which nematodes species are resistant to the drugs information that is correlated with the pathogenicity of the worms in question. Additionally, it would have been interesting to test the efficacy of other commonly available anthelmintics like levamisole and macrocyclic lactones including ivermectin, doramectin and moxidectin to come up with a list of the anthelmintics that could still be relied upon in the control of helminths in the West African region. With such a list, it becomes possible to design a sustainable helminth control strategy using drugs in accordance with the recommendations by Waller (1997).

Further investigations on anthelmintic resistance ought to be conducted in Sikasso and adjacent areas to confirm and map out the extent of anthelmintic resistance. Investigations should use a variety of methods if possible some of the molecular techniques reviewed by Von Samson-Himmelstjerna (2006) and /or in vitro testing described in Coles et al. (1992) since they more sensitive than the FECRT and are likely to answer this anthelmintic resistance questions quickly. Should anthelmintic resistance be confirmed, this will be an additional constraint to the already stressed livestock development in the region.

Although anthelmintic resistance reports in West Africa have not been made, tricholostrongyle resistance against benzimidazoles and imidothiazoles has been reported in eastern and southern Africa particularly in sheep and goat farms (Waruiru et al., 1998; Gabrie et al., 2001; Keyyu et al., 2004; Sissay et al., 2006). Anthelmintic resistance, in contrast, has been rarely documented for cattle, most likely because of the lower anthelmintic use in cattle.

6.4 Weather influences

Rainfall and relative humidity are crucial in influencing the survival and perpetuation of pathogenic parasites of veterinary importance. The riverine tsetse species are known to be sensitive to the hygrometric gradient (Cuisance et al., 1985; Bouyer et al., 2006) consequently transitions from high to low gradient had a strong influence on the effect on the trypanosome transmission. As already discussed, trypanosome infection pressure seemed to be directly

proportional to the hygrometric gradient; increasing during the rainy season because of the increased interface between cattle and tsetse following fly dispersal away from their habitats. The rainy season is associated with increased fly population growth and the same declining during the dry season (Leak, 1999). Dry periods are associated with insufficient forage for cattle making majority of the animals to be very weak and most susceptible to trypanosomosis as was reported by Fall et al. (1999).

The influence of rainfall on helminth infection patterns was also clear. Faecal egg counts rose drastically at the start of the rain season and remained high until the start of the next dry season when the counts started dropping. This pattern was consistent with past reports (Kaufmann and Pfister, 1990; Zinsstag et al., 2000). It is known that most nematode eggs quickly hatch and develop into larvae that survive and transform themselves into infective stages in pastures when humidity is high (Urquhart et al., 1996). In Sikasso, due to the weather patterns, the period November to May (dry season) had the least pasture contamination (lowest FECs) because the chance that helminth eggs passed in faeces can hatch and moult into infective stages is very small as larvae get dessicated in the dry hot sun.

7 Conclusions

The following conclusions are drawn from this study:

- Trypanosomosis risk was moderately high in Sikasso
- Trypanocidal drugs are the main method used in the control of trypanosomosis
- Trypanocidal drug supply chain is fully in the hands of private operators with limited regulatory checks and balances
- Only two riverine tsetse species: *G. p. gambiensis* and *G. tachinoides* are prevalent in Sikasso
- Despite multiple drug resistance, trypanocidal drugs are still fairly effective against trypanosomes
- Multiple drug-resistant *T. congolense* strains were confirmed to be prevalent in Sikasso
- *Trypanosoma vivax* is still sensitive to DIM
- There was significant reduction in trypanosome infections commensurate to the tsetse density reduction
- Although tsetse control significantly did reduce tsetse densities, it did not result in the elimination of the flies
- Tsetse control control needs to be sustained to prevent re-invasion of tsetse cleared areas
- In the control area even without active tsetse control never took place, tsetse density reduction attributable to other factors occurred
- Trypanocidal drug use by herd owners in the intervention area remained unjustifiably high despite the noticeable reduced tsetse challenge
- Albendazole treatment helped to reduce risk of trypanosome infections in risk cattle in the intervention area
- There is suspected albendazole resistance against GINs in Sikasso
- The intervention led to improved animal health (packed cell volumes)

The results of this study point to a declining tsetse population in Sikasso area. Already, the savannah flies originally present here have disappeared thanks to the socio-demographic pressure that resulted in the destruction of their habitat. The same may happen to the riverine tsetse species after the riverine forests will have been destroyed. However, in the short to the medium term, there is need to gradually start substituting prophylactic isometamidium treatments with herd owner managed targeted treatment of cattle with insecticides during the rainy season. This approach is likely to save farmers some money, prevent the incidence of trypanocidal drug resistance and additionally help to control tick-borne diseases. The long term targets should be to intensify cattle production systems through the promotion of appropriate zebu genotypes and eventually cross-breeding those with relevant dairy cattle instead of promoting trypanotolerant cattle breeds which farmers in this area have less preference for.

At an efficacy of only 56%, the effect of albendazole treatments trypanosome on infection was not fully discernible. Nonetheless, it appears that combining tsetse control and strategic helminth control, particularly when a highly efficacious anthelmintic is used is likely to result in lower trypanosome infection risk although more investigations are still needed to prove this.

Finally, this study concentrated on the epidemiological and parasitological aspects of control activities. The economic viability of the integrated best-bet intervention packages on the livelihoods of the farmers in the cotton belt of West Africa was outside the scope of this thesis but it does merit further such analyses.

8 Summary

Management of trypanocidal drug resistance in cattle in identified chemoresistance hot spots in the administrative District of Sikasso, south-east Mali

Animal trypanosomosis still remains a major disease constraining livestock production across sub-Saharan Africa. Additionally, the development and spread of chemoresistance further severely threatens the cattle-based livelihoods of the rural poor in the cotton belt of West Africa. If not addressed, it will exacerbate rural poverty. Best-bet strategies, including tsetse control and strategic helminth control were tested for their efficacy to contain and or reverse trypanocide resistance in Sikasso, south-east Mali, where drug resistance had earlier been detected. The study was implemented in three phases: pre-intervention, intervention and post-intervention phase. Two areas of Sikasso, the eastern sector and the western sector, with comparable ecology and production systems were covered. The study was conducted in four villages from each sector.

The pre-intervention phase, conducted between November and December 2007, involved a cross-sectional (tsetse catches, trypanosome prevalence and drug use practices) and a longitudinal (drug sensitivity testing) survey. Two tsetse species, *Glossina palpalis gambiensis* and *G. tachinoides* occurred in the study area. The eastern sector had a mean trypanosome prevalence of 13.9% which was not significantly different ($p > 0.05$) from 17.5% in the western sector. Two trypanocidal drugs, isometamidium chloride (ISMM) for prophylaxis and diminazene aceturate (DIM) for therapy, were found to be commonly used. Multiple drug-resistant *Trypanosoma congolense* were prevalent in both areas while *T. vivax* were sensitive to 3.5 mg/Kg bw DIM.

Effects of tsetse control and de-worming to contain and reverse trypanocide resistance were assessed during in the intervention phase. In the intervention area (eastern sector), targets (N=957) impregnated in 0.4% deltamethrin (DECIS®, Roussel-Uclaf, France) (dry season) and targeted treatment of cattle (spraying on the limbs, lower abdominal area, brisket and perineal area) with 0.05% deltamethrin (Butox®, Intervet, the Netherlands) (rainy season) were used to control tsetse between March 2008 and November 2009. Additionally, strategic helminth treatment of risk group cattle (3-12 months) with 10mg/kg bw albendazole (10% Albenzole®, Kela, Belgium) was conducted in the intervention and control areas between June 2009 and November 2009. Albendazole was first used in June 2008 and repeated during the November 2008, June 2009 and November 2009 monitoring visits after randomly allocating the animals at risk into an albendazole treatment group and a control group. Five

epidemiological visits (June 2008, November 2008, February 2009, June 2009 and November 2009) were conducted to estimate the resultant dynamics in tsetse catches, trypanosome infections and helminth infections in the two areas. All trypanosome positive risk cattle and those with PCV ≤ 20% were treated with 3.5mg/kg bw DIM. Tsetse control resulted, respectively, in 91.3% and 97.3% reductions of *G. p. gambiensis* and *G. tachinoides* catches in the intervention area. A reduction of 18.3% for the former and of 37.1% for the latter fly species also occurred in the control area. Significantly ($p < 0.001$) lower trypanosome infections in the intervention area than in the control area were observed at herd, village and area levels. Strongyle eggs, *Strongyloides* species eggs, *Toxocara* species eggs and *Moniezia* species eggs, among others, were detected. No *Fasciola* species eggs were detected in cattle of both areas. In the intervention area, the probability of albendazole treated cattle to acquire new trypanosome infections was calculated as 0.003 which was not significantly ($p > 0.05$) different from 0.007 for the control group.

The post-intervention phase involved a cross-sectional and a longitudinal survey and was conducted from November to December 2009. As for the pre-intervention phase, trypanosome prevalence and drug sensitivity investigations were undertaken. There was a significant ($p < 0.05$) reduction in trypanosome prevalence in the intervention area from 13.9% during the pre-intervention phase to < 1% after intervention. In contrast, trypanosome prevalence remained unchanged in the control area. Only 3 *T. vivax* cases remained in the intervention area of which one was resistant to 0.5mg/kg bw ISMM but was cleared by 3.5 mg/kg bw DIM. Trypanocidal resistance patterns in the control area did not change. A faecal egg count reduction test (FECRT) to estimate the efficacy of albendazole revealed a 55.6% reduction of strongyle faecal egg counts. The low FECRT warrants further evaluation before concluding that resistance in strongyle helminths does exist.

This investigation has shown that in a region of high trypanosomosis risk and trypanocidal drug resistance, tsetse control undertaken simultaneously with strategic helminth control and targeted treatment with trypanocidal drugs is likely to reduce trypanosomosis risk to negligible levels.

9 Zusammenfassung

Management von Chemoresistenzen bei Trypanosomen-Infektionen von Rindern im Landkreis von Sikasso, Süd-Ost-Mali.

Die Trypanosomose der Rinder ist nach wie vor eine der bedeutendsten limitierenden Faktoren für die Tierproduktion in Afrika südlich der Sahara. Die Entwicklung und die Verbreitung von Chemoresistenzen bedroht zusätzlich ernsthaft die Lebensgrundlage der auf die Rinderhaltung angewiesenen ländlichen Bevölkerung im Baumwollgürtel Westafrikas. Wenn die negativen Auswirkungen der Chemoresistenz nicht reduziert werden, wird sich die ländliche Armut weiter verstärken.

Um eine weitere Verbreitung von chemoresistenten Trypanosomen zu verhindern bzw. die Resistenzlage in dem betroffenen Gebiet umzukehren, sollten erfolgversprechende Strategien, wie Tsetsefliegenbekämpfung bei gleichzeitigem strategischen Einsatz von Anthelminthika auf ihre Effizienz getestet werden.

Die Studie wurde in drei Phasen, in einer Präinterventions-, Interventions- und Postinterventionsphase durchgeführt. Es wurden zwei Versuchsgebiete mit vergleichbarer Ökologie und vergleichbaren Produktionssystemen identifiziert. In jedem Gebiet wurden vier Dörfer ausgewählt.

Die Präinterventionsphase umfasste den Zeitraum von November bis Dezember 2007 und beinhaltete eine Querschittsuntersuchung (Tsetsefliegenfänge, Bestimmung der Trypanosomen-prävalenz, bisheriger Arzneimitteleinsatz durch die Tierhalter) und eine Longitudinalstudie (Prüfung der Empfindlichkeit der Medikamente). Zwei Tsetsefliegenspezies, *Glossina palpalis gambiensis* und *G. tachinoides* traten in dem Studiengebiet auf. Das östliche Untersuchungsgebiet hatte eine durchschnittliche Trypanosomenprävalenz von 13,9%, die sich unwesentlich ($p > 0,05$) von der Prävalenz im westlichen Sektor unterschied (17,5%). Zwei Medikamente bzw. trypanozide Wirkstoffe, das Phenanthridinderivat Isometamidiumchlorid (ISMM) zur Chemoprophylaxe und das Diminazenaceturat (DIM) zur Chemotherapie, wurden im Untersuchungsgebiet von den Tierhaltern eingesetzt. Bei *Trypanosoma congolense* wurden in beiden Gebieten Resistenzen gegen beide Wirkstoffe nachgewiesen. *Trypansoma vivax*- Infektionen konnten noch erfolgreich mit 3,5 mg/kg KGW DIM behandelt werden.

Die östliche Untersuchungsregion wurde als Interventionsgebiet erklärt, die westliche Region diente als Kontrollgebiet. Im Interventionsgebiet wurde eine Tsetsefliegenbekämpfung von März 2008 bis November 2009 durchgeführt. In der Trockenzeit wurden stationäre, mit

Deltamethrin (0,4%, DECIS®, Roussel-Uclaf, Frankreich) behandelte Tücher (N = 957) aufgestellt. Während der Regenzeit erfolgte eine Sprühbehandlung der distalen Körperregionen (Gliedmaßen, Unterbrust und Unterbauch, Perianalregion) der Rinder mit 0.05% Butox® (5% Deltamethrin, Intervet, Holland). Zusätzlich wurde in beiden Gebieten zwischen Juni 2008 und November 2009 bei 3-12 Monate alten Kälbern (= Risikogruppe für Wurminfektionen) eine anthelminthische Behandlung mit Albendazol (10mg/kg KGW; Albenzole®, Kela Laboratories, Belgien) vorgenommen. Albendazol wurde erstmalig zu Beginn der Regenzeit im Juni 2008 eingesetzt. Wiederholte Behandlungen fanden während der Kontrollbesuche im November 2008 (am Ende der Regenzeit), Juni 2009 und November 2009 statt. Trypanosomen-positive Kälber der Risikogruppe und diejenigen mit einem Hämatokrit von ≤ 20% wurden mit 3,5mg/kg KGW DIM behandelt.

Die Tsetsefliegenbekämpfung führte im Interventionsgebiet zu einer Reduktion der Überträger um 91,3% (*G. p. gambiensis)* bzw. 97,3% (*G. tachinoides)*. Auch im Kontrollgebiet wurde eine Reduktion um 18,3%, bzw. um 37,1% für die genannten Glossinenarten beobachtet. Im Interventionsgebiet wurden auf Herden-, Dorf- und Gebietsebene bedeutend weniger Infektionen mit Trypanosomen ($p < 0,001$) als im Kontrollgebiet beobachtet. Es wurden unter anderem Strongylideneier, Eier von *Strongyloides* spp., Eier von *Toxocara* spp. und Eier von *Moniezia* spp. gefunden. In beiden Gebieten konnten keine Eier von *Fasciola* spp. bei den Rindern nachgewiesen werden.

Die Postinterventionsphase wurde von November bis Dezember 2009 durchgeführt. Es wurde ein signifikanter Rückgang ($p < 0.05$) der Trypanosomenprävalenz von 13,9% vor der Intervention (November 2007) auf unter 1% nach der Intervention (November 2009) beobachtet. Im Gegensatz dazu blieb die Trypanosomenprävalenz im Kontrollgebiet unverändert.

Nur 3 *T. vivax*- Infektionen wurden nach der Intervention diagnositziert, von denen eine resistent gegenüber 0,5mg/kg KGW ISMM, aber empfindlich gegenüber 3,5mg/kg KGW DIM war. Die Resistenzsituation im Kontrollgebiet hatte sich nicht verändert. Der Eizahlreduktionstest zur Abschätzung der Wirksamkeit von Albendazol ergab eine Reduktion der Anzahl der im Kot detektierten Magendarm-Strongyliden (MDS) Eier um nur 55,6%. Weitere Untersuchungen sind erforderlich, um schlüssige Aussagen über das Vorliegen einer Resistenz gegenüber Albendazol zu machen.

Die Untersuchungen haben gezeigt, dass es möglich ist, in einem Gebiet mit hohem Trypanosomose- und Resistenz-Risiko durch eine Tsetsefliegenbekämpfung, bei

gleichzeitigem gezielten Einsatz von Trypanoziden und strategischer anthelminthischer Intervention, das Trypanosomose-Risiko auf ein vernachlässigbares Niveau zu drücken.

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11 Annexes

Annex 1: Questionnaire sheet for the trypanocidal drug use practices before and after intervention, Sikasso south-east Mali (November 2007 and November 2009)

1. Which category of animals do you give priority in trypanocidal drug treatment?

i). Oxen

ii). Milking cows

iii). Calves

iv). Others

v). None

2. What is the treatment strategy?

i). Block treatment using ISMM/year plus DIM for clinical case curative treatments

3. Source of the trypanocidal drugs?

i). Government veterinary services

ii). Private practitioners

iii). Privately run drug outlets

iv). All of the above

4. Who administers trypanocidal drug treatments?

i). Government veterinarians

ii). Private veterinarians

iii). Farmers/herdsmen

iv). All of the above

5. Dosage knowledge: How much do you give an adult cattle cow?

Please state……..

Which type of water used?

i). Boiled water

ii). Distilled water

iii). Water meant for home use

iv). Any other (please specify)…..

In how much water do you dissolve a small sachet of diminazene?

Please state……

Annex 2: Questionnaire sheet for the trypanocidal drug treatments, births, purchases, deaths and sales in herds during the intervention phase, Sikasso south-east Mali (June 2008-November 2009)

1. Health record

Herd owner…….Village……..Visit date…….

Date of event	Disease D, Re, R, L	Treatment			Treating personnel	Signature
		Drug	Dose	No. days		

Diagnosis/affected system: D=diarrhoea; Re=reproductive, R=respiratory, LN=swollen Lymph nodes

2. Entries into herds

Date of entry	Identification	Age (optional)	Origin (birth/purchased)	Remarks

3. Out of herds' movements

Date of exit	Identification	Destination	Exit reason(death/sold)	Remarks

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SELBSTSTÄNDIGKEITSERKLÄRUNG

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angerfertigt und nur die erwähnten Quellen und Hilfen verwendet habe.
Die Arbeit ist erstmalig und nur an der Freien Universität Berlin eingereicht worden.

Berlin, den 10 August 2010

Erick Mungube Ouma