Trypanosomosis risk factors and impact assessment of a tsetse and trypanosomosis eradication campaign in Burkina Faso

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I give thanks to Allah, the Almighty Lord for granting me the strength and knowledge and the help I needed for the compliment of this PhD work. May this thesis be to Your Glory.

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Last but not least, I dedicate this thesis to my children Mariam, Aïda and Mohamed Lassié, I hope you will do much better than your father has done.

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List of abbreviations

AAT:	African Animal Trypanosomiasis
ADP:	Apparent density per trap
AFLP:	Amplified Fragment Length Polymorphism
AU:	African Unity
AW-IPM:	Area-wide integrated pest management
bw:	body weight
BCS:	Body Condition Score
C.I.:	Confidence Interval
CIRDES:	Centre International de Recherche-développement en zone Subhumide
DA:	Diminazene aceturate
DDT:	Dichlorodiphényltrichloroéthane
DNA:	Deoxyribonucleic Acid
ELAT :	Ecole de Lutte anti Tsé-tsé
ELISA:	Enzyme Linked Immuno-Sorbent Assay
FITCA:	Farming in Tsetse Controlled Areas
GDP:	Gross Domestic Product
HAT:	Human African Trypanosomosis
IAA:	Index of Apparent Abundance
ISM :	Isometamidium
MEP:	Mitochondrial Electrical Potential
MRA:	Ministère des Ressources Animales
OCP:	Onchocerchiasis Control Programme
PATTEC:	Pan African Tsetse and Trypanosomiasis Eradication Campaign
PCR:	Polymerase Chain Reaction
PCV:	Packed Cell Volume
PLTA :	Programme de Lutte contre la Trypanosomose
RTTCP:	Regional Tsetse Fly and Trypanosomosis Control Programme
SAT:	Sequential Aerial Treatment
SIT:	Sterile Insect Technique
SSA:	Sub Saharan African
T&T:	Tsetse & Trypanosomiasis
TWB:	Tsetse Without Border
ULV:	Ultra Low Volume

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General introduction

General introduction

Sub Saharan African (SSA) countries are the less developed countries in the world and hunger and poverty are widespread, especially amongst the rural populations. Rural populations live upon agriculture, farming and livestock rearing. Livestock is a source of meat, milk, traction, and manure for improving crop production. In addition to the global climatic change which compromises crop production in the SSA by the irregularity of the rain falls (drought, flood), the presence of the tsetse fly and trypanosomosis in one third of the African continent (38 countries included South Sudan) constitutes the major constraint to the development of productive livestock (Shaw, 2004). Thirty five million doses of trypanocidal drugs are used yearly to maintain susceptible livestock in tsetse infested areas (Geerts *et al*., 2001) and farmers lose 3 million cattle every year because of trypanosomosis (Vreysen, 2006). Furthermore, Human African Trypanosomosis (HAT) caused by *Trypanosoma brucei rhodesiensis* and *T. brucei gambiensis* and transmitted cyclically by *Glossina* spp, affects more than half a million people per year, especially in war-torn zones such as, Angola, Sudan, the Democratic Republic of Congo and Côte d'Ivoire (WHO, 1998; Kabayo, 2002; Courtin *et al*., 2008). However, HAT is under control in many endemic countries thanks to continuous efforts of surveillance and the availability of drugs and the commitment of the international community (WHO, 2006; Courtin *et al*., 2008).

Common control methods of trypanosomosis in Africa are based on chemoprophylaxis, chemotherapy with trypanocide compounds and the elimination of vectors by various techniques, including the use of insecticides, bush clearing, shooting of game and the use of the sterile male insect technique (Du Toit, 1954; Cuisance *et al*., 1984; Vreysen *et al.*, 2000; Allsopp, 1984). An alternative to the latter methods is the breeding of trypanotolerant livestock (cattle and small ruminants). African traditional trypanotolerant livestock are very rustic and more resistant to many parasitic diseases. However, their milk productivity is lower than European breeds and in intensive production systems, they need to be improved genetically by crossbreeding with improved dairy cattle (d'Ieteren *et al*., 1998).

A century ago, a huge number of tsetse elimination campaigns were implemented in Africa. Hitherto, the most successful large-scale method for the elimination of tsetse has been the use of insecticides applied either on the ground or from the air (Ormerod, 1986). Tsetse biology and behavior make them readily susceptible to most of the available insecticides (Grant, 2001). In the past, the use of highly remanent insecticides at a large scale was affecting non-

target species. For example, helicopter applications of insecticide aerosols to riparian woodland and forest in West Africa, in order to alleviate sleeping sickness, affected canopy and ground-dwelling groups of insects for a few days and caused considerable mortalities of freshwater Crustacea (Takken *et al*., 1978; Everts *et al.*, 1983). Hence the environmental impact of the spray of insecticide has led to the development of tsetse selective techniques like impregnated screens, traps (fig. 1) and live baits (insecticide treated cattle) (Gouteux *et al.*, 1982; Laveissière and Couret, 1981; Laveissière *et al*., 2000; Bauer 1995, 1999).

Figure 1. Display of trap in tsetse control

In location with poor access, aerial spraying was reintroduced in a way that it can be considered as ecologically acceptable. The use of insecticides with short remanence for the control of tsetse (*Glossina* spp.) is now a widely adopted approach and known as Sequential Aerial Treatment (SAT). Briefly, the SAT consists in the spraying of reduced volumes of insecticide at low concentration that are applied strategically according to the life cycle of the tsetse fly. This allows a high efficiency against the *Glossina* group with a minimum of side effects for other species.

However, targets, traps, livestock insecticide treatment and SAT are not fullly effective to eradicate flies from an infested area, but they can reduce tsetse fly populations to levels that reduce the risks to humans and animals, and can also be used as barriers to prevent re-invasion of flies into a previously cleared area (Grant, 2001; Warnes *et al.,* 1999). The application of these techniques is necessary prior to the implementation of the Sterile Insect Technique (SIT) because the tsetse population has to be drastically reduced before the release of irradiated sterile males (Vreysen *et al*., 2000). The SIT was successful in the eradication of tsetse flies (*G. austeni*) on the Island of Unguja, Zanzibar in the 1990's (Vreysen *et al.,* 2000) and allowed elimination of tsetse flies in the agro-pastoral zone of Sideradougou in Burkina Faso and in the agro-pastoral land in central Nigeria (Politzar and Cuisance, 1984; Oladunmade *et al*., 1990).

Vector control is a long-term fight and therefore requires the involvement of decision makers, researchers and farmers. The lack of commitment of all the actors, especially of the beneficiary communities and the public national veterinary services, explains the failure of many tsetse control campaigns. Until now, the use of trypanocidal drugs to treat or to prevent susceptible livestock against trypanosomosis remains the only control measure for most of the African farmers.

Very limited trypanocidal compounds are available and they have been used for more than fifty years. The long-term use of the same molecules selected drug resistant strains of trypanosomes in many African countries (fig 2) (Geerts *et al*., 2001, Delespaux *et al.*, 2008, Authié, 1984). To date, international pharmaceutical companies have shown little interest in developing and licensing new trypanocidal drugs either for animals or for humans (Geerts *et al.*, 2001). Therefore, there is a need of a good management of the few available molecules to delay as long as possible the selection of multi-resistant strains of trypanosomes

Figure 2. African countries with reported resistance to trypanocide drug. A star indicates that resistance to trypanocidal drugs has been reported in animal trypanosomes in that country (Delespaux *et al*., 2008)

Regarding the magnitude of the socio-economical importance of tsetse and trypanosomosis (T&T) in SSA, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was created to fight this disease.. PATTEC was derived from a decision made by the African Heads of State and Government at the 36th summit of the African Unity (AU), held in Lomé (Togo) in July 2000, to act collectively and respond to the challenge of eradicating tsetse flies from the African continent. The PATTEC plan of action was subsequently endorsed by the African Heads of State at the 37th AU summit. Thereafter, the Governments of Ethiopia, Mali, Burkina Faso, Botswana, Kenya, Uganda, and Tanzania have begun to implement the plan in selected areas of their countries. In Burkina Faso, the T&T control and elimination plan is 1) to reduce the tsetse population to the lowest density using insecticide, 2) to release sterile irradiated males, and 3) to conduct some mass treatment of livestock with trypanocidal drugs.

The next chapter will highlight the epidemiology of African animal trypanosomoses and its control. This chapter emphasizes the drug resistance and vector control, especially the use of the sterile insect technique to eliminate tsetse flies in Africa.

References

Allsopp, R., 1984. Control of tsetse flies (Diptera: Glossinidae) using insecticides: a review and future prospects. Bull. Entomol. Res. 74, l–23.

Authié, E., 1984. Mise en évidence d'une résistance aux trypanocides parmi des souches de *Trypanosoma congolense* récemment isolées au Burkina. Rev. Elev. Méd. vét. Pays trop. 37, 219–235.

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

Bauer, B., Amsler–Delafosse, S., Kaboré, I., Kamuanga, M., 1999. Improvement of cattle productivity through rapid alleviation of African animal trypanosomosis by integrated disease management practices in the Agro–pastoral zone of Yale, Burkina Faso. Trop. Anim. Health Prod. 31, 89–102.

Budd, L, 1999. DFID–funded tsetse and trypanosome research and development since 1980. Volume 2: Economic Analysis, Livestock Production Programme. Chatham Maritime, UK: NRInternational. 123 p.

Courtin, F., Jamonneau, V., Duvallet, G., Garcia, A., Coulibaly, B., Doumenge, J. P., Cuny, G., Solano, P., 2008. Sleeping sickness in West Africa (1906–2006): changes in spatial repartition and lessons from the past. Trop. Med. Int. Health 13, 334–344.

Cuisance, D., Politzar, H., Mérot, P., Tamboura, I., 1984. Les lâchers de mâles irradiés dans la Campagne de lutte intégrée contre les glossines dans la zone pastorale de Sidéradougou (Burkina Faso). Rev. Elev. Méd. vét. Pays trop. 37, 449–467.

d'Ieteren, G.D.M., Authié, E., Wissocq, N., Murray, M., 1998. Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. Rev. Sci. Tech. Off. Int. Epizooties. 17, 154–175.

Delespaux, V., Geysen, D., Van Den Bossche, P., Geerts, S., 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 24, 236–242.

Du Toit, R., 1954. Trypanosomiasis in Zululand and the control of tsetse flies by chemical means. Onderstepoort J. Vet. Res. 26, 317–387.

Everts, J.W., Van Frankenhuyzen, K., Román, B., Cullen, J., Copplestone, J., Koeman, J.H., 1983. Observations on side effects of endosulfan used to control tsetse in a settlement area in connection with a campaign against human sleeping sickness in Ivory Coast. Trop. Pest Management 29, 177–182

FAO, 1999. Impacts of Trypanosomiasis on African Agriculture. PAAT tec sci ser 2: 46.

Geerts, S., Holmes, P.H., Diall, O., Eisler, M.C., 2001. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol. 17, 25–28.

Gouteux, J.-P., Challier, A., Laveissière, C., Couret D., 1982. L'utilisation des écrans dans la lutte anti–tsétsé en zone forestière. Tropenmedizin und Parasitologie 33, 163–168

Grant, I.F., 2001. Insecticides for tsetse and trypanosomiasis control: is the environmental risk acceptable? Trends Parasitol. 17, 10–14.

Kabayo, J. P., 2002. Aiming to eliminate tsetse from Africa. Trends Parasitol. 18, 473–475

Laveissière, C., Grébaut, P., Herder, S., Penchenier, L., 2000. Les glossines vectrices de la Trypanosomiase humaine africaine; IRD, editor. Yaoundé, Cameroun: IRD and OCEAC. 246p.

Laveissière, C., Couret, D., 1982. Essai de lutte contre les glossines riveraines à l'aide d'écrans imprégnés d'insecticide. Cah. ORSTOM, sér: Ent.méd. Parasitol. 19, 271–283.

Oladunmade, M.A., Feldmann, U., Takken, W., Tenabe, S.O., Hamann, H.J., Onah, J., Dengwat, L., Van der Vloedt, A.M.V., Gingrich, R.E., 1990. Eradication of *Glossina palpalis palpalis* (Robineau–Desvoidy) (Diptera: Glossinidae) from agro–pastoral land in central Nigeria by means of the sterile insect technique, pp. 5–23. *In* Sterile Insect Technique for Tsetse Control and Eradication. Proceedings Final Research Co–ordination Meeting, Vom, Nigeria, 6–10 June 1988. IAEA/RC/319.3/1.

Ormerod, W.E., 1986. A critical study of the policy of tsetse eradication. Land use policy 3, 85–99.

Politzar, H., Cuisance, D., 1984. Anintegrated campaign against riverine tsetse, *Glossina palpalis gambiensis* and *Glossina tachinoides,* by trapping and the release of sterile males. Insect Sci. Applic. 5, 439–442.

Shaw, A.P.M., 2004. Economics of African trypanosomosis. In: Maudlin I, Holmes PH, Miles MA, editors. The trypanosomosis. Wallingford, UK: CABI Publishing. pp. 369–402.

Takken, W., Balk, F., Jansen, R.C., Koeman, J.H., 1978. The experimental application of insecticides from a helicopter for the control of riverine populations of *Glossina tachinoides* in West Africa. VI. Observations on side effects. Trop. Pest Management 24, 455–466.

Vreysen, M.J.B., Saleh, K.M., Ali, M.Y., Abdulla, A.M., Zhu, Z.–R., Juma, K.G., Dyck, V.A., Msangi, A.R., Mkonyi, P.A., Feldmann, H.U., 2000. *Glossina austeni* (Diptera: Glossinidae) Eradicated on the Island of Unguja, Zanzibar, Using the Sterile Insect Technique. J. Econ. Entomol. 93, 123–135.

Warnes, M.L., Van Den Bossche, P., Chihiya, J., Mudenge, D., Robinson, T.P., Shereni, W., Chadenga, V., 1999. Evaluation of insecticide–treated cattle as a barrier to re–invasion of tsetse to cleared areas in northeastern Zimbabwe. Med. Vet. Entomol. 13, 177–184.

WHO (World Health Organization), 1998. *African trypanosomiasis; Control and Surveillance.* Report of a WHO expert committee, WHO technical report series no. 881. WHO, Geneva.

WHO (World Health Organisation), 2006. Human African Trypanosomiasis (sleeping sickness): epidemiological update. Weekly Epidemiological Record 8, 69–80.

Chapter 1:
Tsetse flies and animal Trypanosomosis and their control methods: A literature review

1.1. Introduction

Trypanosomosis is a parasitic disease of both animals and humans, caused by protozoans of the genus *Trypanosoma* and mainly cyclically transmitted by tsetse flies (*Glossina* spp). *Trypanosoma brucei gambiense* and *T.b. rhodesiense* infect humans and cause human African trypanosomiasis (HAT), also known as sleeping sickness. HAT leads to a protracted, debilitating and finally fatal disease in untreated cases. The East African form of HAT, caused by *T. b. rhodensiense*, is an acute and fatal disease, whereas the West African form, caused by *T. b. gambiense*, is generally more chronic and debilitating. HAT is a re-emerging tropical disease and constitutes a threat for million people in SSA (Kuzoe and Schofield, 2004). African animal trypanosomosis also known as *nagana* is caused by various species of *Trypanosoma*. The most important trypanosomes infecting livestock, in terms of economic losses, are *T. congolense*, *T. vivax* and *T. b. brucei* (Mulligan, 1970). *T. simiae* and *T. godfreyi* cause porcine trypanosomosis.

T. vivax occurs on the island of Mauritius and in Central and South America; in none of these places tsetse flies are present(Molyneux and Ashford, 1983; Abebe and Yobre, 1996). It has been shown that *T. vivax* can be mechanically transmitted by hematophagous insects of the genus *Tabanus*, *Stomoxys* and hippoboscids and, accidentally, by the syringe during vaccination campaigns. *T. evansi* is also mechanically transmitted by these biting flies *T. equiperdum* occurs in equines and is sexually transmitted from host to host (Molyneux and Ashford, 1983).

1.2. Epidemiology of African trypanosomosis

The epidemiology of trypanosomosis is complex and depends on the interactions between the parasite, vector and the mammalian host.

1.2.1. Parasite

Trypanosomes belong either to the Salivaria or Stercoraria groups (fig. 1.1). The Stercoraria trypanosomes are excreted in the faeces of blood sucking insect vectors like triatome bugs. The African pathogenic trypanosomes affecting human or livestock belong to the Salivaria group. They are transmitted by the insect vector saliva during the blood feeding event . In livestock trypanosomes, *T. congolense* Savannah type is the most pathogenic and is

responsible for acute infection and death of diseased animal. However, *T. congolense* Forest and Kilifi types cause mild infections (Bengaly *et al*., 2002).

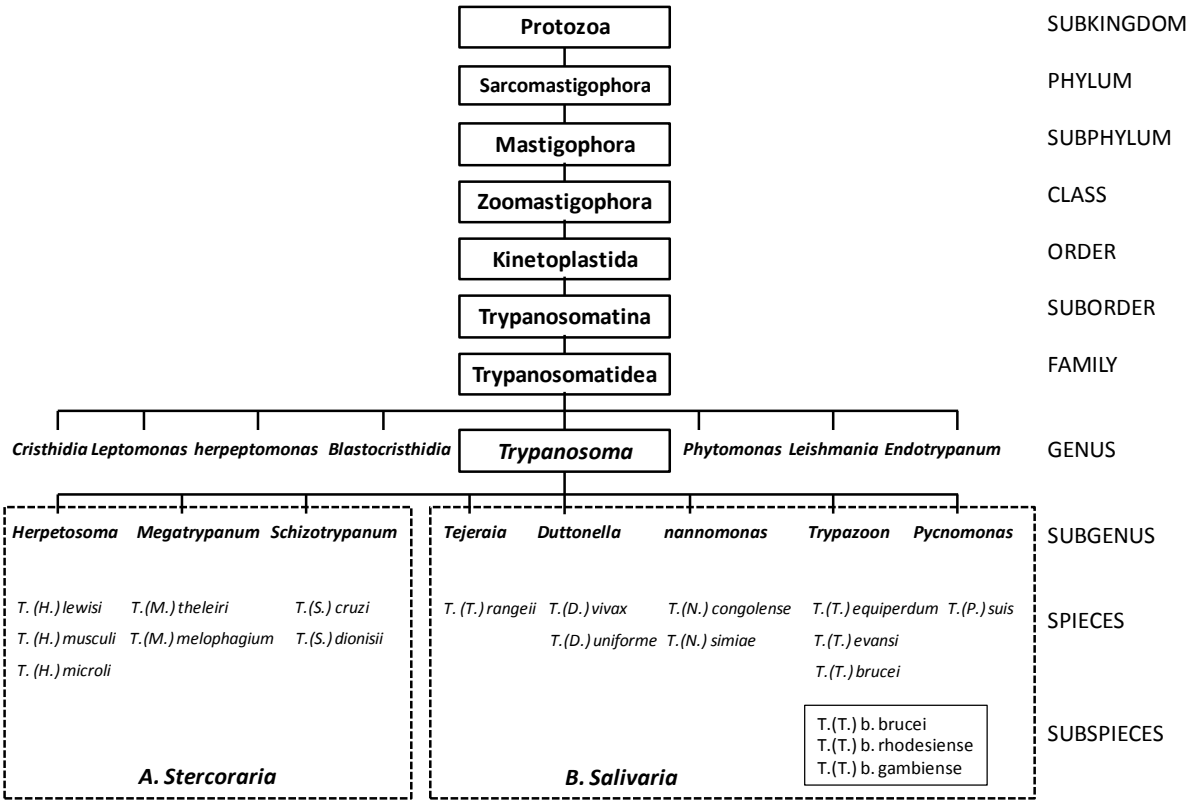

Figure 1.1. Classification of mammalian trypanosomes (Levine *et al.,* 1980)

1.2.1.1. Life cycle of trypanosomes

With the exception of *T. evansi* and *T. equiperdum* , all the pathogenic livestock trypanosomes are cyclically transmitted through the bite of tsetse flies (*Glossina* spp.), the biological vector (fig.1.2.). In addition, it has been reported that *T. vivax* as well as *T. congolense* can also be mechanically transmitted by various blood feeding dipters (Desquesnes and Dia, 2003; 2004; Desquesnes *et al*., 2009).

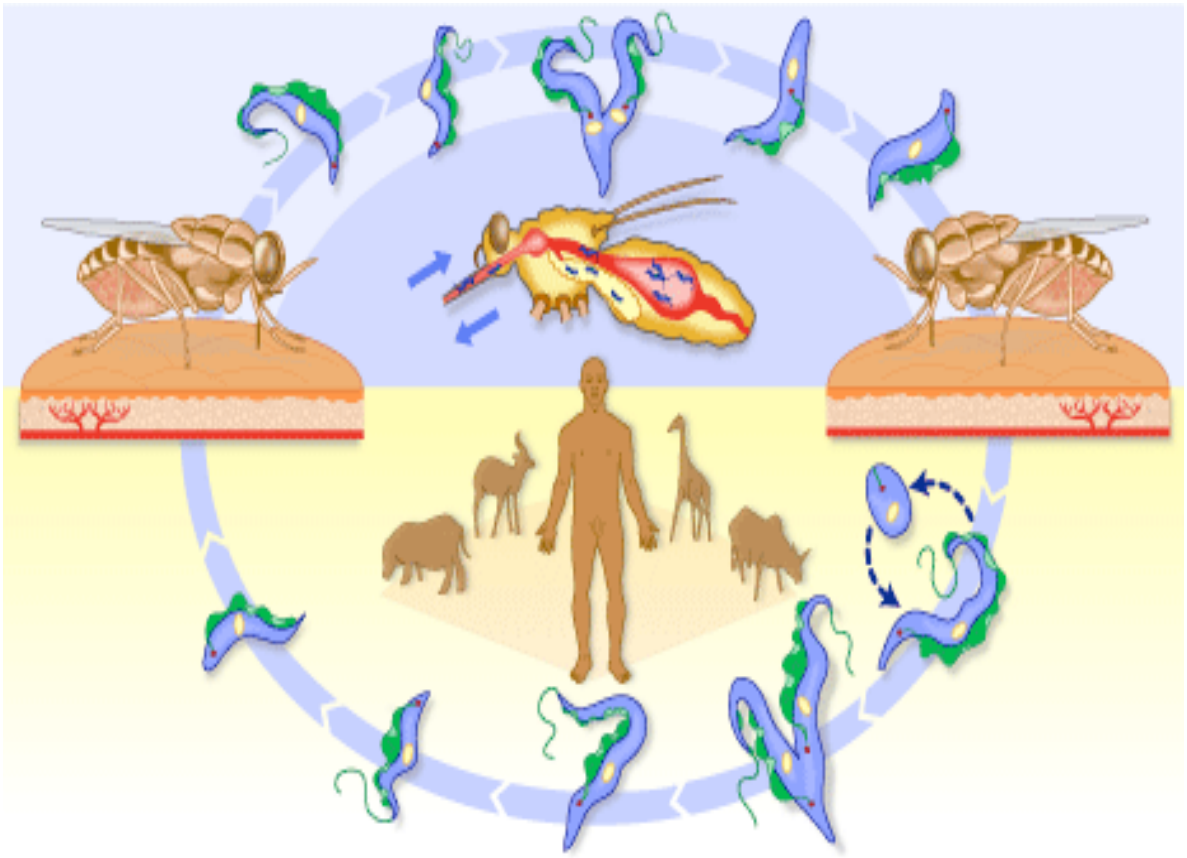

Figure 1.2. Life cycle of *Trypanosoma brucei* (http://www.who.int/tdr/diseases/tryp/lifecycle.gif)

1.2.1.2. Mammalian host-trypanosome relationship

During cyclical transmission, the trypanosome undergoes a developmental cycle within the tsetse fly involving substantial morphological, biochemical and physiological transformations to the final metacylic stage that is infective for a new mammalian host. Once inoculated in the mammalian host during the tsetse fly blood feeding event, these metacyclic forms undergo development and multiplication at the site of infection causing a swelling (chancre) and, eventually, trypomastigotes are released into the blood stream through the lymphatic system. During their development in the host, the parasites escape the host immune systemby changing their surface antigenic coat, the variable surface glycoprotein (VSG). During a trypanosome infection, the mammalian host physiology can be dramatically altered with changes in blood pH, hormones and nutrient concentration (Seed, 2001). Some reports suggest that the *Trypanosoma* infected mammalian hosts are bitten more frequently by tsetse flies (Baylis and Mbwabi, 1995). Here, there is a hypothesis that the trypanosome catabolites are excreted into the urine of the infected host and act like a chemoattractive to tsetse (El Sawalhy *et al*., 1995).

1.2.1.3. Tsetse-trypanosome relationship

The tsetse fly gets infected during a blood meal on a host infected by trypanosomes. Once in the insect body, the parasites undergo a cycle of development that takes from a few days to a few weeks depending on the trypanosome species. Trypanosomes of *Nannomonas* and *Trypanozoon* subgenus (*T. brucei, T. congolense*) change their morphology and metabolism to survive in the tsetse midgut. These changes and transformation to a specific midgut stage is essential for the parasite to adjust to a fluctuating temperature and to an oxygen-deficient environment where proline becomes the source of energy (Vickerman, 1985; Vickerman *et al*., 1988; Leak, 1999).

For successful transmission, the parasite undergoes two stages of differentiation in the fly: establishment in the midgut and subsequent maturation in the mouthparts (*T. congolense*) or salivary glands (*T. brucei*). In the midgut, the parasites rapidly differentiate to procyclic forms and begin to replicate. Once established in the midgut, trypanosomes migrate forwards to the proventriculus and the mouthparts, where they begin to differentiate into epimastigotes and colonize the proboscis or salivary glands, depending on the parasite species (Van Den Abbeele *et al*., 1999). They then differentiate into metacyclic forms, infective to mammals (Vickerman *et al*., 1988). It has been shown that infected tsetse flies feed more vigorously (Jenni *et al*., 1980). Therefore, it appears that an infected vector is modified to be a better transmitter of the infection.

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1.2.2. Vector

1.2.2.1. Geographic distribution

The distribution area extending over approximately 10 million km^2 is mainly in SSA. The upper limit of the geographic distribution area of the tsetse flies corresponds to the 15th northern parallel, the hydrometrical deficit of the sahelian zones (< 600 mm of annual rainfall), preventing the extension of tsetse in the north. The southernmost limit follows a line joining the Atlantic coast to the level of Angola (13th southern parallel) to Mozambique on the Eastern coast (27th southern parallel); below this limit, the low temperatures (<20°C) prevent their extension in the south. Tsetse flies are absent from islands close to the African continent, except the island of Zanzibar, where G. *austeni* was present but has been eliminated (Vreysen *et al*., 2000). About twenty specimens of *Glossina morsitans* and *G. fuscipes* were also recorded from southwestern Saudi Arabia (Solano *et al*., 2010).

G. palpalis gambiensis and *G. tachinoides*, and to some extent *G. morsitans submorsitans* are the main species of tsetse flies of epidemiological importance in Burkina Faso. The first two are riverine species occurring in narrow thickets along river banks (Sudanese ecotype), constituted by short *Mimosa pigra* and *Morelia senegalensis*, the swampy forests of *Mitragyna inermis,* and riparian vegetation (hydrographic networks of dry and wet savannahs (Guinean and Sudano-Guinean ecotypes) (Bouyer, 2006). *Glossina medicorum* was caught in some gallery forests of woodland savannahs in the South of Burkina Faso near the frontier with Côte d'Ivoire.

1.2.2.2. Taxonomy

Tsetse flies belong to the order of the Diptera, family Glossinidae. This family comprises a single genus, *Glossina,* in which thirty-one species and subspecies of *Glossina* have been described. The classification of the genus *Glossina* is based on the external morphological characteristics such as colour, shape of the antennae, presence of bristles on thoracic pleura, shape of the male and female external genitalia. and on the geographic distribution and certain bioecological features. The *Glossina* genus was divided (Newstead, 1911) into three species, which are now considered as subgenera (Fig. 1.3):

- Subgenus *Nemorhina* (Robineau-Desvoidy, 1830) (*Palpalis* group): Standard species: G. (N.) palpalis (R.D., 1830)
- Subgenus *Glossina s. str*. (*sensu stricto*) (Zumpt, 1935) (*Morsitans* group): Standard species: G. (G.) *longipalpis* (Wiedemann, 1830)

- Subgenus *Austenina* (Townsend, 1921) (*Fusca* group): Standard species: G. (A.) brevipalpis (Newstead, 1910). The last species, *Glossina frezili*, was described in 1987 (Gouteux, 1987).

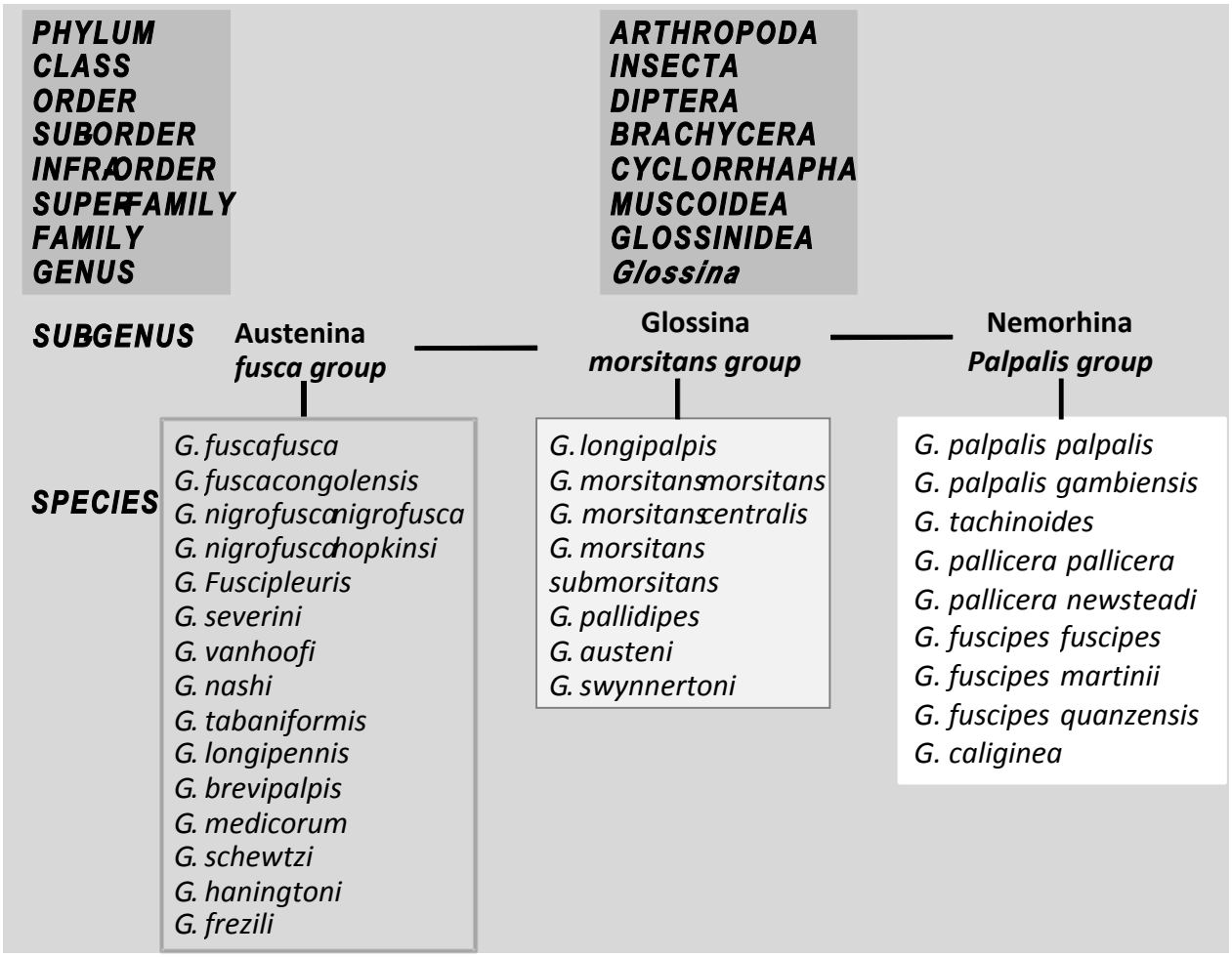


Figure 1.3. Glossinidae family comprising 3 sub-genera and 31 species (adapted from Bouyer, 2006)

1.2.2.3. Biology of the tsetse fly

Tsetse flies – both male and females - are obligatory blood feeders. Female tsetse flies are larviparous. They have a uterus in which the larva is kept until maturity. During its intra-uterine life, the larva is fed by secretions of lactiferous glands annexed to the uterus. Once the larva is expulsed from the uterus, it moves actively in the soil for pupation (Buxton, 1955).

Generally, the pupae are placed 2 to 8 cm deep into the soil. The exterior membrane of the pupa (or nymph), the puparium, carries the two black polypneustic lobes at its posterior end, enabling differentiation of tsetse pupae from other Diptera Muscoidae. The entire process of metamorphosis occurs within the puparium, leading to the adult form of the insect (figure 1.4). Duration of intrapupal development is variable, according to species, sex and climatic

conditions, in particular minimum and maximum ground temperatures. Pupae require sufficient ground moisture (over 60%) (Solano *et al*., 2010). Within the critical temperature limits compatible with life, the duration of pupation decreases as temperature rises (between 20 and 80 days) and females have shorter pupation duration than males, by approximately 2 to 3 days (Leak, 1999). During this period, the pupa lives only with the food stocks of the larva accumulated during its intra-uterine life. When metamorphosis is completed, the young fly leaves its puparium by breaking a circular slit at the anterior end. (Pollock, 1982).

When the tsetse fly emerges, it is sexually mature and mating can occur in the following hours. In natural conditions, almost all females are fertilized as soon as they exit from the puparium. Males can mate approximately ten times if mating is adequately spaced. Mating is long (30 min to 3h). The sperm migrates towards the spermathecae where it is stored. One mating event is generally sufficient for the female fly to produce larvae for several months; spermatozoids can survive for nearly 200 days in the spermathecas. Occasionally, a female can accept several matings to fill their spermathecas, but, in experimental conditions, beyond the tenth day, the proportion of females accepting more than one sexual union is only 0.7% (Solano *et al*., 2010).

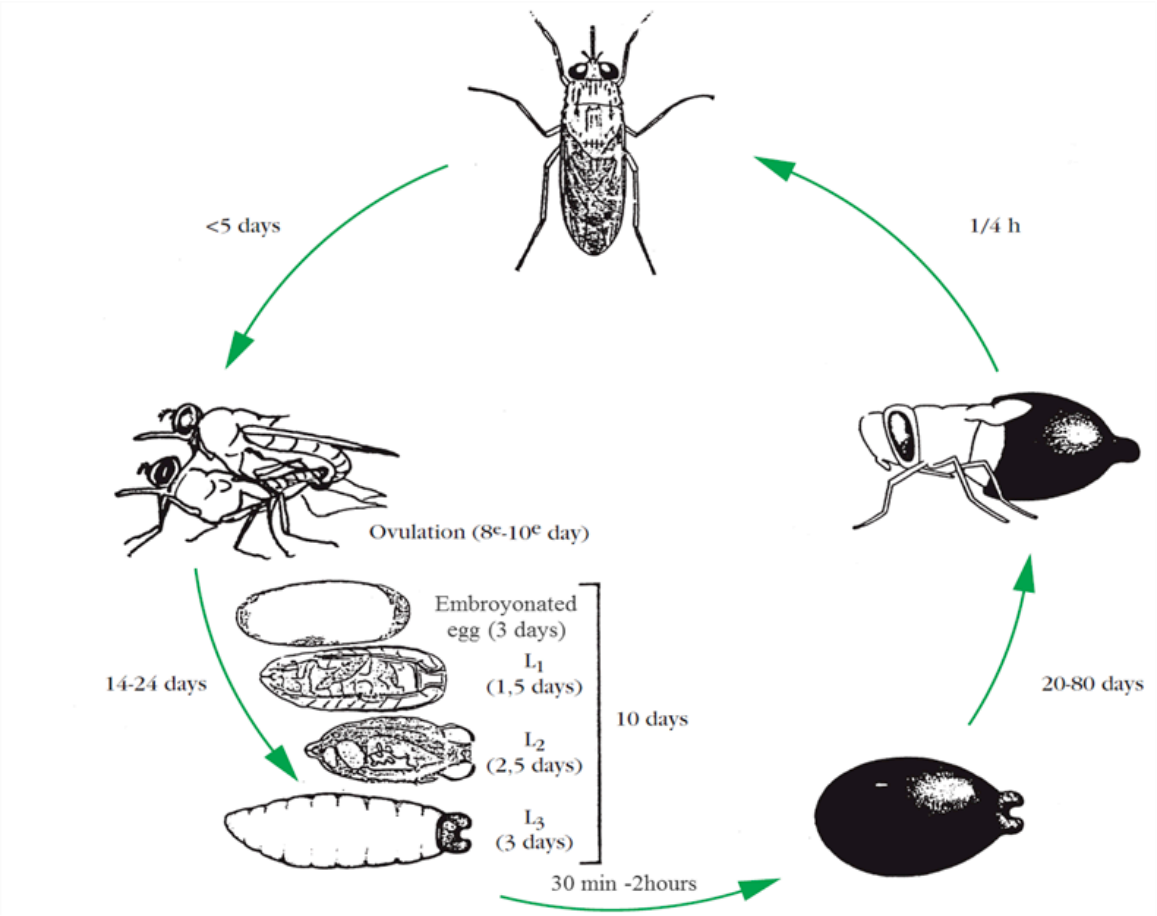


Figure 1.4. Reproductive cycle of a tsetse fly (Cuisance, 1989).

1.2.2.4. Ecology of the tsetse species in Burkina Faso

Tsetse flies need specific conditions to live, in particular temperature and moisture, luminosity, ease of flight, as well as the presence of animals on which they can feed. Tsetse flies are thus closely related to vegetation, which forms a shield from sun rays and wind. The type and density of the canopy and vegetation (shrubs and herbs) influence the temperature and moisture as well as the host abundance, its availability and attractiveness. These factors will determine the maintenance and development of tsetse populations in a particular environment. The species of the palpalis and fusca group, need a high relative humidity and are dependent on the ligneous vegetation of gallery forests or large forests. Tsetse species of the *morsitans* group are more xerophilous and disperse largely in savannah woodlands during the rainy season but are restricted to vegetation neighbouring water in the dry season. Within these habitats, flies choose places where the microclimate is the most favourable. Cuisance (1989) summarized the behaviour and the ecology of tsetse flies (Fig. 1.5).

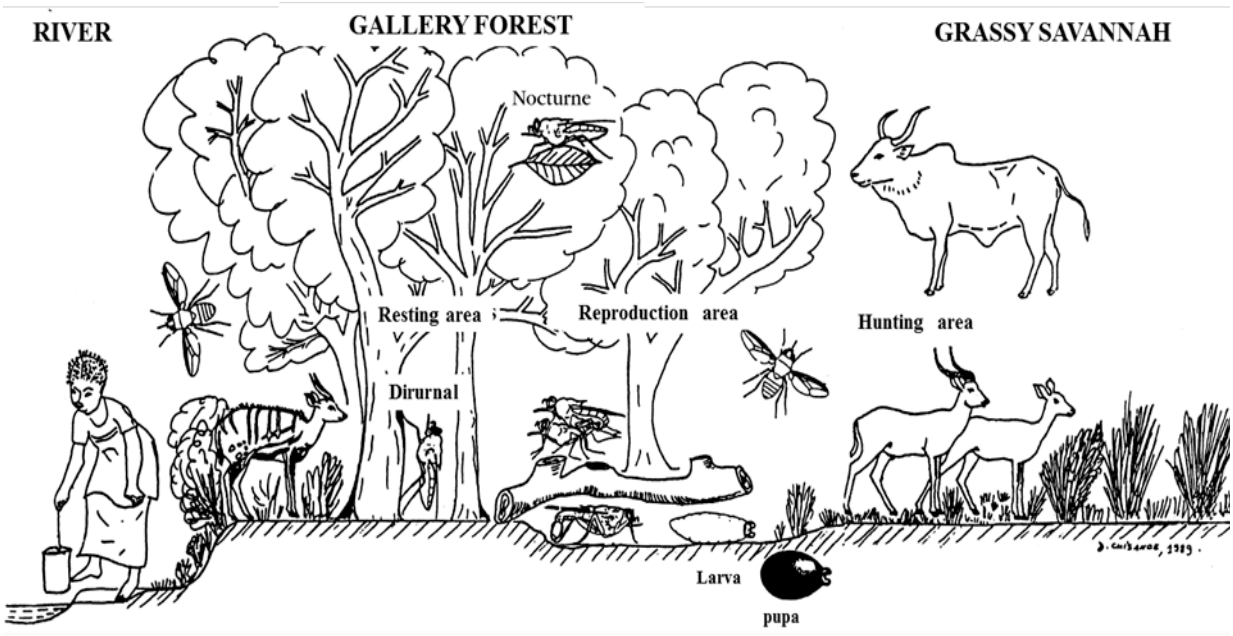


Figure1.5. Simplified diagram of a tsetse fly habitat (adapted from Cuisance, 1989)

Burkina Faso is mainly infested by two riverine species of tsetse i.e. *G. palpalis gambiensis* and *G. tachinoides,* and one savannah species, *G. morsitans submorsitans*. Hitherto, these three species remain the most important key players in the epidemiology of livestock trypanosomosis in the country. Thirty years ago, two other species, *G. longipalpis* and *G. medicorum* were observed in the south of the country, along the border with Côte-d'Ivoire (Challier and Laveissiere, 1977). Recently, only *G. medicorum* was trapped in the forest galleries in the same location (Rayaissé *et al.,* 2009; Bouyer, personal communication).

For the two riverine species *G. p. gambiensis* and *G. tachinoides*, after the drop linked to the dry hot season, the populations increase at the beginning of the rainy season with a peak in July followed by a drop in the middle of the rainy season (flood of the tsetse habitats); a second peak is observed around September/October. This decrease in the apparent density in the middle of the rainy season could also be explained by the wide dispersion of the tsetse flies in surrounding savannahs due to the increase in moisture which results in a decrease of the apparent density at the trapping points (Bouyer, 2006). During the cold dry season, there is an important reduction of the population of tsetse because of the lengthening of the duration of pupation associated with a significant mortality of the teneral flies hatching with a low stock of fat. The drop of the population is even more significant during the hot dry season: high temperatures associated with higher mortality of the pupa and adults, dehydratation) (Cuisance, 2001). It is also likely that tsetse dispersion could play a major role

in the regulation of the tsetse apparent density (Gouteux *et al.,* 2001; Rogers and Randolph, 1984).The concept of dispersion should be differentiated from the migration.Dispersion concerns a huge number of individuals, moving in one direction to a specific destination and followed by “coming back home” as described for the Monarch butterflies (*Danaus plexipus*) in America and *Catopsilia florella* in Botswana (Larsen, 1992). Migration is the same phenomenom without “coming back home (Itard and Cuisance, 2003).

In landscapes subjected to climatic and human pressure, like the basin of the Mouhoun River, the spatial pattern of the favorable biotope is patchy (Bouyer, 2006). The riparian vegetation along the rivers is most generally located in protected forests or "sacred" forests where human activities are limited. Therefore, they constitute a suitable habitat for tsetse flies. Zones like degraded riparian vegetation, pasture for livestock and crop production fields are not suitable for tsetse reproduction. Nowadays, in these fragmented landscapes, tsetse flies consist of small localized subpopulations with relatively short dispersal (~1 km/generation), in contrast to the high dispersal capacities observed 20 years ago in similar river section in the absence of any fragmentation (Bouyer *et al*., 2009b; Cuisance *et al*., 1985). Population genetic studies using polymorphic microsatellite DNA markers carried out in fragmented pockets in the agro-pastoral zone of Sideradougou, showed an important intraspecific genetic variability of *G. p. gambiensis* (Solano *et al.* 2000).

1.3. Control of tsetse flies

The terminology associated with disease control, elimination and eradication needs to be clarified according to Molyneux *et al* (2004), before developing the vector management strategies. The **control** can be defined as operations that aim to lower tsetse densities until there are no tsetse left or until an acceptable level of transmission risk has been attained. Whereas the **elimination** of tsetse flies is the reduction to zero of their population in a defined geographical area as a result of deliberate efforts. Continued intervention measures are required. The **eradication** of the tsetse flies is the permanent reduction to zero of the tsetse population as a result of deliberate efforts. Intervention measures are no longer needed.

With many years of experience, the knowledge of the tsetse flies, their biology, ecology and population dynamics is sufficient enough to undertake elimination or even an eradication campaign against these pests. However, the existence of density-dependent processes in tsetse populations is one of the most important facts and it must be taken into consideration

when selecting the control method (Rogers and Randolph, 1984). Indeed density-dependent factors change with population (predation, migration, competition), which makes the population return to its equilibrium. For example in The Loos islands in the littoral Guinea, the National Control Program against HAT has decided to eradicate *G. p. gambiensis* in order to sustainably protect humans and economic activities. Several control methods were implemented as a combination of impregnated traps and targets, selective ground spraying, epicutaneous insecticide treatment of pigs, and impregnated fences around pig pens. Within four years of fight, tsetse density became very low, less than one fly/trap/day. But *G. p. gambiensis* still occurs at very low, undetectable, densities on the islands (Kagbadouno et al., 2011). The control with those methods is very effective at high density of tsetse flies but cannot bring the population to a zero level because the density of tsetse flies is too low.

The choice of the control method to be applied will depend on the targeted zone, the impact on the environment, the tsetse species, the possibility of isolation of treated areas, the economic impact, the possibilities of land use after control and the available funding at the moment of the operations and for the future. So far, insecticides remain the most frequently used method of control due to their efficacy and they constitute the step of tsetse control before any other method can be applied (Allsopp, 1984).

1.3.1. Ecological tsetse control methods

In the past, game shooting and bush clearing separately or in combination, have been the basis of tsetse flies control programmes (Ormerod, 1986), but nowadays, this is not ecologically nor politically acceptable. Environment modification aimed to create unfavourable conditions for tsetse breeding. Bush clearing and riparian forest destruction severely modified thermal and hygrometric conditions of tsetse resting and breeding places. Pupae died from heat and dehydration. The limitation of the hosts from which the tsetse fly obtained its blood meal, its sole source of food, was an efficient method of limiting the spread of fly (Ford *et al*., 1970). These environmental changes were efficient but nowadays not acceptable because of their negative impacts on biodiversity (Bouyer *et al*., 2010).

In the 1980's, an epidemic of rinderpest occurred in Southern Africa and particularly in the Zambezi basin where a large number of the wild fauna died. Only small pockets of fauna survived to the disease. It was only in these pockets, known as "Jack's residual fly areas", that the main species of tsetse fly, *G. morsitans,* survived and from them the Zambesi valley has been repopulated with tsetse flies (Ormerod, 1986).

In Burkina Faso, crop and cotton production led to the disappearance of *G. morsitans submorsitans* in the Mouhoun River Basin where twenty years ago this species was trapped (Bouyer *et al*., 2010). Anthropic activities on the edges of the Mouhoun River and its main tributaries led to spoiled landscapes causing the fragmentation of the riverine tsetse populations (Bouyer, 2006). It has been suggested that the removal of the vegetation at ground level without removing high trees (discriminative partial bush clearing) or by cutting only some of the tree or shrub species (partial selective bush clearing) could be very effective to decline the tsetse population (Bouyer *et al*., 2010).

1.3.2. Traps and targets

Trapping tsetse was originally used for sampling tsetse populations. Recently, conical and biconical traps have been used with great success and little expense in reducing dense populations of riparian tsetse (fig. 1.6) (Challier and Laveissière, 1973; Laveisière and Couret, 1981; Vale, 1982). Based on the better knowledge of visual and olfactory attractants, traps and targets have been used as a control method (Vale, 1974; Politzar and Cuisance, 1984; Laveissière *et al*., 2003). Unfortunately, not all species are attracted by these traps and targets (Vale, 1982). Therefore, new forms of traps with new synthetic "ox odours" like octenol, metacresol, acetone and phenol, were designed to increase their effectiveness. Studies carried out in Burkina Faso showed that biconical traps baited with synthetic 'ox odour' capture up to 2.5 times and 7 times more *G. tachinoides* and *G. m. submorsitans* than unbaited traps, respectively (Filledier *et al.,* 1989; Politzar and Mérot, 1984; Vale *et al*., 1985). In spite of these promising results, odour attractants were never adopted during large-scale tsetse control operations. Actually, the efficiency of the use of odours attractants varies according to the tsetse species and their habitats. In addition, the cost of these molecules is comparable to ground insecticide spraying (Bouyer *et al*., 2010).

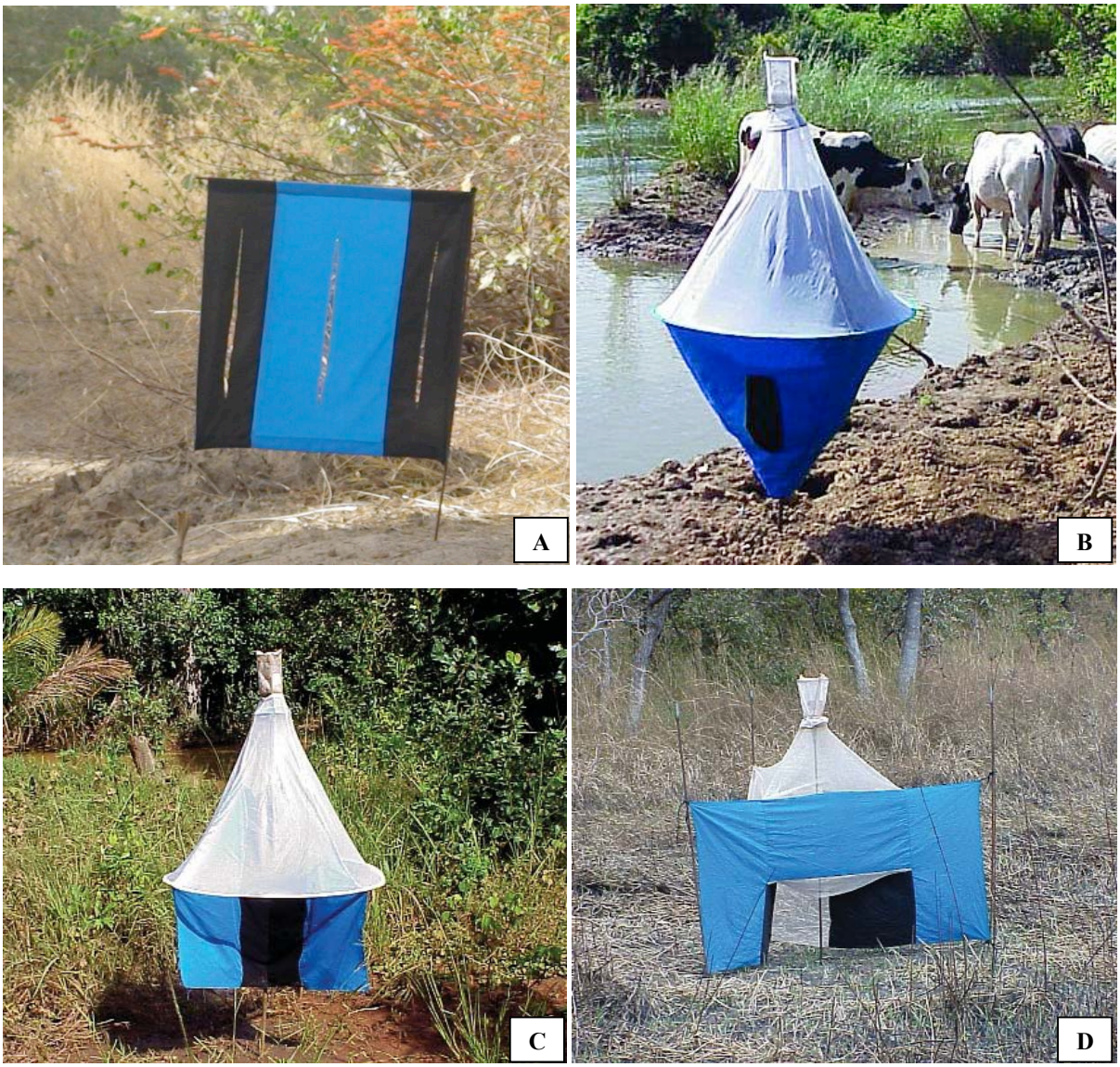


Figure 1.6. Screens and targets in tsetse control. A: impregnated screen or target, B: Biconical trap, C: Monoconical trap, D: Nzi trap (PATTEC-Burkina Faso, CIRDES)

Traps and targets used for tsetse control are usually impregnated with pyrethroids that instantaneously kill the flies or paralyse them. The use of impregnated traps and targets has significantly reinforced the efficacy of this tsetse control method. Although trapping on its own cannot always replace the other control methods, it is a very good complement for surveillance or when used in an integrated control package. In the 1980's, integrated tsetse control was carried out in the agro-pastoral zone of Sidéradougou, Burkina Faso. In this campaign, insecticide-treated screens were deployed during the dry season and sterile irradiated male tsetse flies were released during the rainy season. The campaign was very successful (Cuisance *et al*., 1984).

1.3.3. Insecticide use in tsetse control

The most successful tsetse control programmes carried in Africa were based on the insecticides either by ground or aerial spraying. In the colonial periods, tsetse control aimed at eradicating human trypanosomosis (Jamot, 1936). The insecticides that have most frequently been used were the organochlorines (DDT and dieldrin). Endosulphan was also used extensively (Ormerod, 1986). Many efforts have been deployed to improve the spraying techniques. Ground spray as fogging with apparatus like Swingfog® (Laveissière *et al*., 2000), residual application on tree branches were possible because of the remanence of this family of insecticides. The intensive use of organochlorines combined with bush clearing and game shooting allowed successful elimination of *G. pallidipes* from Zululand in South Africa in the 1940's (Du Toit, 1954).

However, organochlorines are slowly degraded and thus ecologically dangerous. Actually, their use has been forbidden or seriously restricted in several countries (Müller *et al.*, 1981; Phelps *et al.*, 1986; Douthwaite & Tingle, 1994; Grant, 2001). Indeed, because of their long remanence and their accumulation in the food chains, animals at the top of these chains (notably the predatory birds) are likely to be most affected. Novel insecticides such as, the synthetic pyrethroids have been used mainly in aerial spraying and they are of value due to their strong action at low concentrations with a rapid degradation in the environement.

1.3.3.1. Live bait technique

Insecticide treated livestock in *pour on* or spray formulations can be very effective in tsetse control (Hargrove *et al*., 2000). It exploits the blood sucking behaviour of both sexes of tsetse. The tsetse attempting to feed on treated livestock are killed by picking up a lethal deposit of insecticide (Leak, 1999). The application of insecticide on livestock has shown a great success in the control of tsetse flies. In Zimbabwe, it has a suppressive effect on *G. pallides* (Thomson and Wilson, 1992). In Burkina Faso, this technique was used successfully against *G. m. submorsitans* and *G. p. gambiensis* in the zones of Satiri and Bekuy and the pastoral zone of Yalé (Bauer *et al.,* 1995, 1999). Similarly, the method was very successful in Tanzania. The continuous use of insecticide-treated livestock led almost to the elimination of tsetse and trypanosomosis to an extent that prophylaxis measures against the disease was no longer needed (Hargrove *et al*., 2000). In the Eastern Province of Zambia, the insecticide-treated cattle method (cyfluthrin in *pour on*) resulted in a drastic decline in the incidence of trypanosomosis (Van den Bossche *et al*., 2004).

It has been suggested that the local application of insecticide in footbath could effectively contribute to tsetse fly control (Torr *et al*., 2007; Bouyer *et al*., 2009a). The insecticide treatment of livestock also contributes to the control of ticks and tick-borne diseases.

1.3.3.2. Sequential aerosol technique or Sequential aerial treatments (SAT)

Sequential aerosol technique is more environmental friendly than ground spraying or conventional aerial spraying. It needs planes that fly at an approximate speed of 250-300 km/h and the flight should be 10 to 15m above the canopy. The SAT involves the ultra-low volume spraying of non-residual insecticides. Tsetse flies are killed by direct contact with the insecticide micro-droplets; there is no residual effect and treatments must be repeated until all newly emerging adults are affected. The SAT was used successfully in Botswana to eliminate tsetse from the Okavango Delta (Kgori *et al*., 2006) and in Zimbabwe, where 48,000 km2 of savannah area were cleared in the 1980's (Shereni, 1990). The SAT application in tsetse control campaigns was very successful, but it needs institutional support and national commitment aimed at eradication because of the high cost of this technique. Multiple (5 to 6) applications are required, one every 16 to 18 days, to succeed in eradicating a tsetse population (Vreysen *et al*., 2006).

However, the SAT is a delicate technique: the insecticides should be applied during periods of cold temperature (night time) and does not tolerate any delays in the timing of the cycles because of the tsetse reproduction system. The SAT remains a perfect strategy for effective area-wide tsetse elimination in zones that are not accessible by ground spraying, especially dense humid forest, or for even eradication in open savannah (Allsopp and Hursey, 2004).

1.3.4. Sterile insect technique (SIT)

In 1937, U.S. scientist E.F. Knipling had the idea of using sterile insects to manipulate the reproduction rate of a natural insect population. It was not until the 1950's that a method was found to sterilize insects and that the idea could be realized (Knipling, 1955, 1959; Klassen, 2003). It was for the first time implemented in 1954 to eradicate the New World screwworm fly *Cochliomyia hominivorax* (Coquerel) from the Island of Curaçao, Netherlands Antilles (Baumhover *et al*., 1955). This successful trial was followed by the eradication of the pest from the southern USA, Mexico, Central America (1950–2000) (Vargas-Terán, 1991; Novy, 1991), and from Libya in 1990–1991 (Vargas-Terán *et al*., 1994). Since then, the SIT as part of area-wide integrated pest management (AW-IPM) approaches, has been

successfully used to suppress or eradicate several lepidopteran and dipteran pests, including fruit and tsetse flies (Dyck *et al*., 2005; Dagnachew *et al*., 2005).

The principle of SIT for tsetse consists of the mass production of the target *Glossina* in specialised production centres (like CIRDES, Bobo-Dioulasso, Burkina), the sterilization of the males by gamma irradiation and the sustained and systematic release of the sterile males over the target area in large number in relation to the wild male population to outcompete them for wild teneral females (Vreysen, 2006). Mating of the sterile males with virgin, teneral females will result in no offspring. Indeed, female tsetse flies usually mate only once during their lifetime . Gradually, over the generations, the ratio of sterile males to wild ones will increase (Vreysen *et al*., 2000). The technique becomes even more efficient with lower population densities. This is the reason why populations are decreased by conventional techniques before the release of the sterilized males. The SIT is nonintrusive to the environment, has no adverse effects on non-target organisms, is species specific and can easily be integrated with other biological control methods (Vreysen, 2006). However, the release of sterile insects is only effective when the target population density is low, it requires detailed knowledge on the biology and ecology of the target pest, and the insect should be amenable to mass rearing. In addition, the SIT necessitates efficient release and monitoring methods, which must be applied on an area-wide basis (Vreysen, 2005). However, to ensure the success of these control methods, factory-reared tsetse flies must be competitive with their wild counterparts and must exhibit a similar behavior in a natural environment (Vreysen *et al.*, 2011).

The SIT was tested for the first time against *G. m. morsitans* in Tanzania (Williamson *et al*., 1983) in the 1970's. The release of sterile males at a 12:1 ratio managed to maintain the population suppressed for 15 months at a 80–95% reduction level. The initial reduction was obtained by aerial spraying.

The release of sterile males was also successfully integrated with the deployment of insecticide impregnated targets to eradicate *G. p. gambiensis* Vanderplank, *G. tachinoides* Westwood and *G. m. submorsitans* Newstead from an agro pastoral zone of Sidéradougou in Burkina Faso (3,000 km^2, 1983–1985) and *G. p. palpalis* Rob. Desv. from a pastoral area in Nigeria (1,500 km^2, 1982–1985) (Takken *et al.*, 1986; Cuisance *et al*., 1984 ; Politzar and Cuisance., 1984).

The Area-Wide Integrated Pest Management (AW-IPM) approach was introduced into the area of tsetse control on the Island of Unguja, Zanzibar, where a population of *G. austeni* Newstead was eradicated using the SIT combined with pour-on treatment of cattle and insecticide impregnated targets/screens (Vreysen *et al*., 2000). The table 1.1 summarise the use of SIT in the control of tsetse flies in Africa.

Table 1.1. Use of sterile insect technique in the control of trypanosomosis in Africa

Country	**Tsetse species**	**Ratio* (IM/WM)**	**Suppression (%)**	**references**
Tanzania	*G. m. morsitans*	1.12:1	80–95	Williamson *et al*., 1983
Burkina Faso	*G. p. gambiensis* *G. tachinoides*	45:1	100	Cuisance *et al*., 1984
Nigeria	*G. p. palpalis*	10:1	100	Takken *et al.*, 1986
Zanzibar	*G. austeni*	1:1 - 10:1	100	Vreysen *et al*., 2000

* IM: irradiated males, WM: wild males

The success of this area-wide campaign was a strong argument for the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), to promote the use of SIT for the eradication of tsetse populations from selected areas in Africa after pre-release reduction of tsetse populations to 90-99% using other conventional techniques. Indeed, to be successful SIT needs to be applied directly after the conventional control which will reduce the tsetse population to a low density before to perform the release of sterile males as explained in the figure (fig. 1.6).

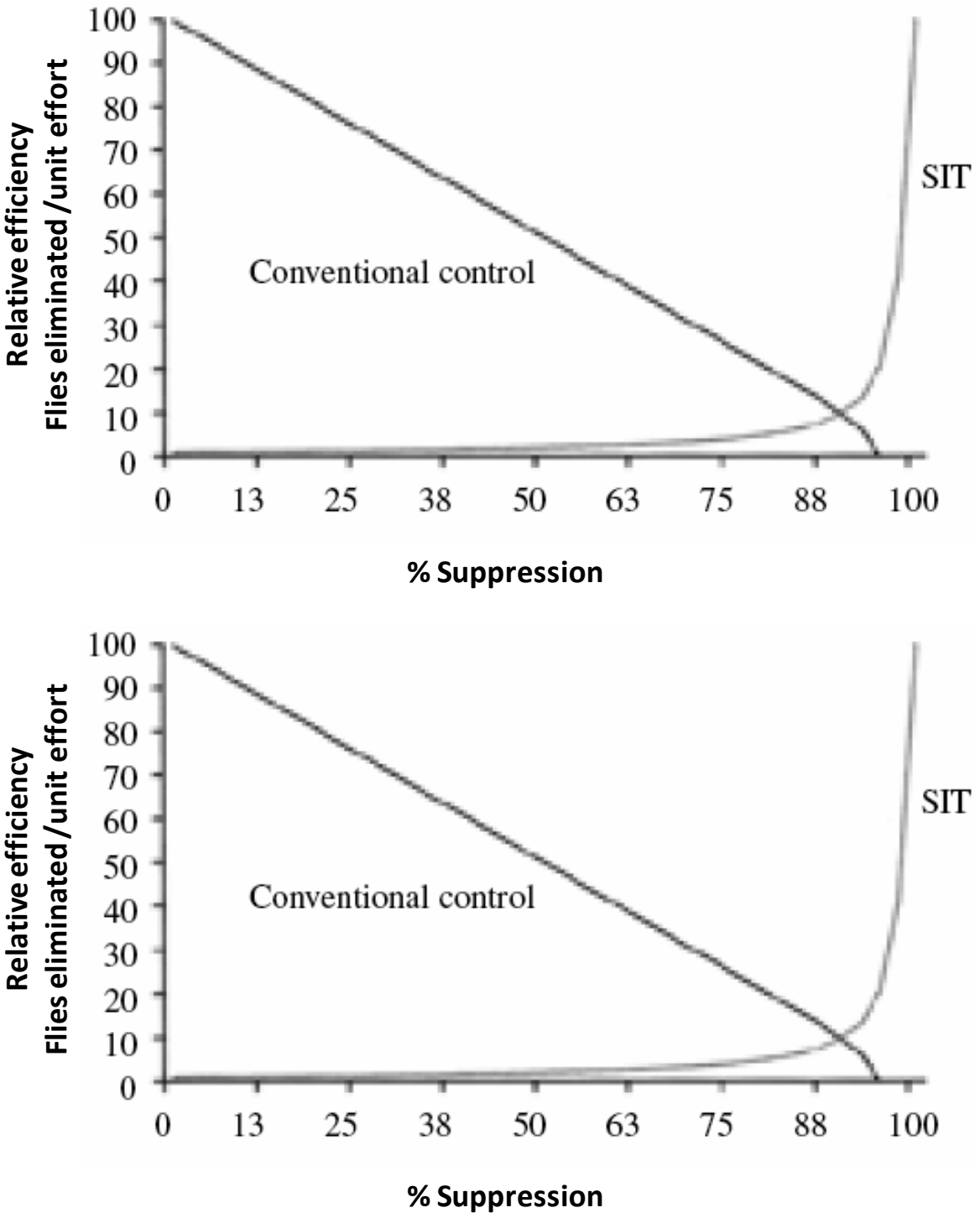


Figure 1.7. The efficiency of conventional tsetse control methods (screens, targets, traps, insecticides) and the Sterile Insect Technique (SIT) used in area-wide integrated pest management programmes (Vreysen, 2001).

1.4. Control of parasites

Curative or preventive trypanocidal drugs are used to maintain susceptible livestock in trypanosomosis enzootic area. So far, the protection of livestock by immunisation is not possible. The use of preventive drugs can help to prevent susceptible animals from contracting the disease for a period of two to four months. There are very few trypanocidal

drugs available for livestock treatment. Drug control of animal trypanosomosis relies essentially on three trypanocidal drugs, namely: homidium, diminazene aceturate (DA) and isometamidium (ISM). In West Africa, only two molecules are used to tackle the disease, namely DA and ISM.

1.4.1. Chemotherapy and chemoprophylaxis of African Animal Trypanosomosis

1.4.1.1. Chemoprophylaxis

- *Isometamidium*

ISM was discovered by Berg *et al*. (1961). It is a phenanthridine-aromatic diamidine marketed as both therapeutic and prophylactic drug. At the dose of 0.25-0.5mg/kg, this drug has a curative effect against *T. vivax* and *T. congolense* in ruminants. The dose of 1mg/kg is needed to be effective against *T. brucei* and *T. evansi* in domestic buffalo (Bouyer *et al*., 2010). For prophylactic purpose, the recommended dose is 0.5-1.0mg/kg in cattle. The preventive effect varies from 2 to 4 months, depending on the intensity of the risk of infection and the susceptibility of the trypanosome strains. Moreover, studies have shown that ISM can kill the trypanosomes developing in tsetse flies (Bouyer, 2008; Van den Bossche *et al*., 2006). During SIT campaigns, the incorporation of ISM in the first blood meal of sterile males will significantly reduce the ability of the released males to transmit trypanosomes (Bouyer *et al*., 2010). Intramuscular injection can cause transitory local reactions with persistent induration that are generally invisible from the outside. Actually, the preventive effect of ISM is conferred by the slow diffusion of the drug owing to the tissue binding at the intramuscular injection site (Eisler, 1996). ISM can be used for preventive and curative purposes in horses and donkeys at the dose of 0.5mg/kg in a slow intravenous injection (curative amounts: 0.5mg/kg to 1 mg/kg against *T. evansi*, 1mg/kg against *T. brucei* and *T. congolense*).

- The prophylactic effect of ISM increases significantly when, instead of an intramuscular injection, the same quantity is managed, in subcutaneous implants, in biodegradable polyesters with progressive release (Lemmouchi *et al*., 1998). However, this form of use of ISM and the mass treatment exert a strong selection pressure on the trypanosome population (Geerts and Holmes, 1998). As a result, multiple drug resistance has developed (Peregrine, 1994; Geerts and Holmes, 1998, Geerts *et al*., 2001). Therefore, the reduction of the number of trypanocidal treatments by integrating drug treatment with other control measures and the

alternative use of a curative compound may help to alleviate the problem (Geerts and Holmes, 1998).

- *Homidium*

Homidium is a phenanthridium whose antitrypanosomal activity was demonstrated more than 60 years ago by Browning *et al*. (1938). Homidium bromide and Homidium chloride, marketed under the name Ethidium® and Novidium®, respectively are used in cattle at a dose of 1mg/kg body weight in deep intramuscular injection, in a 1% or 2.5% solution. It is very effective against *T. vivax* but less against *T. congolense* because of the early appearance of resistance against these drugs (Scott and Pegram, 1974). Although it is mainly used as a curative drug, Ethidium® does have some preventive properties but they do not exceed six weeks in low risk conditions. Because of its mutagenic and carcinogenic effect, Homidium has been abandoned. To our knowledge, this drug is no longer marketed in West Africa.

1.4.1.2. Chemotherapy

- *Diminazene aceturate*

DA is manufactured by various pharmaceutical companies under different names. The first market name of DA was Berenil® (Hoechst, Germany). Currently, in Burkina Faso, a dozen of marketed names of DA are registered and sold. DA has remarkable curative properties against *T. vivax* and *T. congolense* as well as *Babesia* spp. DA offers many advantages namely the significant activity against most of the trypanosome species, stability, ease of use and the very low toxicity. DA is a yellow powder that dissolves easily in water at a rate of 7%. It is used in bovines in intramuscular injection at a dose of 3.5 and 7mg /kg bw, in the treatment of *T. vivax* and *T. congolense*, and *T. brucei* and *T. evansi*, respectively. However, DA is not recommended in equines and dromedaries (Taj Eldinl *et al*., 2005; Tuntasuvan *et al.*, 2003). The use of DA is contra-indicated in dogs, which can develop severe side effects (risk of appearance of oedematous or haemorrhagic encephalitis, hepatic and renal impairments) (Farwell *et al.*, 1982; Moore, 1979; Naudé, 1970).

- *Melarsomine*

Melarsomine is an arsenical compound. In veterinary medicine only Cymelarsan® is manufactured by Merial (Lyon, France). Cymelarsan® is the newest trypanocidal drug marketed and it has proved to be effective in mice against *T. evansi* strains resistant to the older trypanocidal drugs (suramin or quinapyramin sulfate). Cymelarsan® is currently used against *T. evansi* infection in camels, cattle, and horses (Musa *et al*., 1994; Payne *et al*., 1994;

Murilla *et al*., 2005). Cymelarsan is recommended in camels and horses to treat *T. evansi* infection at 0.25mg/kg bw.

1.4.2. Trypanocidal treatment strategies in cattle

The strategies of the use of trypanocidal drugs are defined according of the magnitude and the seasonality of the tsetse challenge, trypanosomosis risk, and the degree of livestock susceptibility (Dia and Desquesnes, 2005).

The use of a chemoprophylactic drug is not needed in case of low risk over the year; the few cases of trypanosomosis are treated with a curative trypanocide (DA). In many countries, the infection risk is high in a given period of the year; this period corresponds to the rainy season. It is recommended that all cattle undergo mass treatment with DA and then ISM treatment within 3 weeks at the beginning of the rainy season. The new infection cases are treated with diminazene (Chartier *et al.*, 2000; Diall, 1997).

In zones of a permanent risk over the year, an annual preventive programme is applied to all or part of the herd. The animals must receive a quasi continuous protection, either by repeated treatment at the interval of one month with DA (3.5mg/kg bw) in trypanotolerant cattle, either by permanent protection with ISM every 3 months at a dose of 0.5-1mg/kg bw in susceptible subjects. In any case, ISM should be alternated to diminazene at least once in the year to eliminate the resistant strains to the product used iteratively. For that purpose, animals undergo diminazene treatment at the dose of 7mg/kg bw, and then 15 days later, they are treated with ISM at the dose of 1mg/kg bw. This protocol is then repeated twice every year (Bouyer *et al.*, 2010; Chartier *et al.*, 2000; Diall, 1997).

1.4.3. Drug resistance

The two drugs used for treatment (DA) and for prevention (ISM) were marketed more than half a century ago and it is thus not astonishing that cases of drug resistance were reported in 18 countries of SSA (Delespaux *et al*., 2008a) and more recently in Benin, Ghana and Togo (Réseau d'épidémiosurveillance de la résistance aux trypanocides et aux acaricides en Afrique de l'Ouest – RESCAO, unpublished data). The first case of drug resistance in trypanosome was reported in 1967 in northern Nigeria (Na'isa, 1967). Thereafter, the appearance of drug-resistant trypanosomes has been reported elsewhere in tsetse infected SSA (Schönefeld *et al*., 1987; Ainanshe *et al*., 1992; Codjia *et al*., 1993; Mulugeta *et al*., 1997).

In Burkina Faso, chemoresistance was reported for the first time at the beginning of the 1980's in the province of Kénédougou (Authie, 1994; Pinder and Authie, 1984). The resistance to trypanocides was since reported in the other tsetse-infested areas of Burkina Faso and especially in the important cotton production zone (Clausen *et al*., 2010, 1992; Grace *et al*., 2009; McDermott *et al*., 2003).

All trypanocidal drugs can cause the appearance of chemoresistant trypanosome strains. Trypanosomes that have become resistant to one drug can acquire resistance to another trypanocidal compound. The cross-resistance generally occurs between chemically related drugs but it can also occur between very different drugs (Delespaux and Koning, 2007). Resistance appears to be stable with the trypanosomes remaining resistant for several years, even when transmitted between animals or cultivated over a long period of time in the absence of trypanocidal drugs (Chitanga *et al*., 2011). Strains of chemoresistant trypanosomes can be transmitted by tsetse, without losing their resistance over a long period (Peregrine *et al*., 1997; Scott *et al*., 1996).

1.4.3.1. Resistance mechanisms

Cells under long term exposure to a drug or a toxic tend to develop different strategies to survive (Gottesman, 2002). Mechanisms of resistance, such as loss of surface specific receptors or transporters for a drug, specific metabolism of a drug, or alteration by mutation of the specific target of a drug, result in resistance to only a small number of related drugs (Gottesman, 2002; Whiteside, 1962). More often, cells express mechanisms of resistance that confer simultaneous resistance to many different structurally and functionally unrelated drugs (Delespaux, 2004).

The mechanisms of resistance and cross-resistance to trypanocides in African trypanosomes are not well understood. It has been proven that genetic mutations may be associated with ISM resistance in livestock-infective trypanosomes. Progress is being made in elucidating the role of nucleoside transporters in resistance to trypanocidal drugs (Barrett and Fairlamb, 1999). Furthermore, changes in the mitochondrial electrical potential (MEP) have been demonstrated in ISM-resistant trypanosomes (Wilkes *et al.,* 1997). The genetic base of drug resistance in trypanosome is well documented in *T. congolense* and *T. brucei* (Delespaux *et al.*, 2008b, 2006). In a study using amplified fragment length polymorphism (AFLP) to compare two isogenic clones of *T. congolense*, the resistant clone withstood ISM doses 94-fold higher than the sensitive parent clone from which it was derived, and showed the

presence of a conserved GAA codon insertion (coding for an extra lysine) in a gene coding for a putative ABC (ATP-binding cassette) transporter (Delespaux *et al*., 2005). In the same study, some trypanosome strains characterized as resistant in mouse tests did not show the GAA insertion, which indicates that more than one resistance mechanism could be involved. An alternative mechanism of ISM resistance could include the alteration or modification of the targeting site of the drug in the trypanosome. Indeed, evidence for modification of topoisomerase genes as a drug resistance mechanism is reported in *Leishmania donovani* (Marquis *et al*., 2005). The modification of the topoisomerase gene as possible mechanism of resistance to ISM was explored in *T. congolense* (Delespaux *et al*., 2007). Topoisomerases of sensitive and resistant strains were compared and no conserved mutation was observed.

Similarly, genetic mutation occurs in trypanosome DA-resistant strains. The mechanism of resistance to diminazene was also explained by the alteration of a membrane transporter namely, a P2-type purine transporter (Mäser *et al*., 2003).

1.4.3.2. Detection of chemoresistance

In practice, drug resistance is suspected when a treatment, which has previously been satisfactory, becomes inefficient. However, all therapeutic failures are not associated with chemoresistance. Before confirming that a strain is resistant, it is important to elucidate all misuse of the trypanocidal drug (adequate dose, injection, storage of the drug etc.). Then a detailed investigation is needed. Laboratory tools and field tests are conventionally used for the detection of trypanocidal drug resistance.

Peregrine (1994) has described several methods to identify drug resistance in African trypanosomes. The common *in vivo* tests used to identify drug resistance are tests in ruminants and tests in mice. A standardized protocol for the assessment of resistance to trypanocidal drugs in mice and cattle has been described by Eisler *et al*. (2001). The inconvenience of these assays is their long duration: 60 days in mice and up to 100 days in cattle. Tests in mice are less expensive than tests in ruminants and may be useful as a general guide to resistance in an area. Although there is a good correlation between the tests in mice and in ruminants, the curative dose that must be used in ruminants cannot be extrapolated from the results in mice (Geerts *et al*., 2000). Another disadvantage of the mouse test is the fact that *T. vivax* and also some *T. congolense* isolates are poorly infective for mice (Holmes *et al*., 2004).

- *Test in mice*

Experimental mice are inoculated with the trypanosome isolate to be tested and treated with a trypanocidal drug. The parasitemia in the mice is checked 2-3 times per week by blood wet smears for a period of up to 60 days. The effective dose that gives temporary clearance of the parasite in 50% (ED50) and 95% (ED95) can be calculated as can the curative dose that gives complete cure in 50% (CD50) and 95% (CD95) of the animals (Eisler *et al*., 2001).

- *Test in ruminants*

The test in ruminants is mainly used to determine whether or not drugs are principally efficacious at the recommended curative doses in subjects infected with a particular trypanosome strain. This test may be used for the assessment of drug resistance in *T. vivax*, which is usually not infective for mice. The experimental cattle or ruminants should be a breed native to the area and without prior exposure to tsetse or trypanosomosis and therefore, negative for anti-trypanosomal antibodies. A minimum of 3 and preferably 6 animals are inoculated with the same trypanosome isolate. The experimental animals should be kept in a fly-proof stable to eliminate the risk of reinfection during the study. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose, i.e. the dose that clears the parasites from the circulation, and the curative dose, i.e. the dose that provides a permanent cure (Sones *et al*., 1988).

To decrease the costs, isolates can be pooled by 5. A relapsing infection is then indicative that one or more of the inoculated trypanosome populations was drug-resistant. 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 relapsing populations of trypanosomes. The shorter the period, the greater the level of resistance is (Ainanshe *et al*., 1992).

- *In vitro tests*

In vitro assays use bloodstream or metacyclic forms instead of procyclic forms. This test has been used to detect resistance in *T. brucei* and *T. congolense* (Gray *et al*., 1993; Hirumi *et al*., 1993; Clausen *et al*., 2000). The advantage of this technique is that large numbers of isolates can be examined; tests with metacyclic trypanosomes correlate well with field observations. A major disadvantage of these tests is the slow adaptation of the trypanosomes to the culture conditions. Indeed, it takes up to 40 to 50 days of *in vitro* incubation to generate metacyclic *T. congolense* and is not possible with *T. brucei* (Gray *et al.,* 1993). Moreover, *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). Furthermore, it is difficult to maintain *T. congolense in vitro* (Clausen *et al*., 2000). An approach for *T. congolense* is the drug incubation *Glossina* infectivity test (DIGIT) described by Clausen *et al*. (1999). Another approach is the drug incubation infectivity test (DIIT), in which mice are infected with trypanosomes after drug incubation (Knoppe *et al*., 2006).

- *Other laboratory tests*

Other tests that are the Tests based on the mitochondrial electrical potential (MEP) and the ISM-ELISA technique are still in the experimental stage or are not frequently used. It has been suggested that variation of the MEP might be the primary factor determining the rate of ISM accumulation in the trypanosome kinetoplast (Wilkes *et al*., 1997). Initial studies using a limited number of *T. congolense* populations have shown that an increased or decreased MEP might be a candidate quantitative marker for respectively ISM susceptibility and ISM resistance (Wilkes *et al*., 1997). Although not frequently used, enzyme linked immuno-sorbent assay (ELISA) has been used in the investigation on ISM resistance (Eisler *et al*., 1996; Murilla *et al*., 2002).The presence of trypanosomes in animals with an ISM serum concentration >0.4 ng ml/1 suggests that parasites are resistant (Eisler *et al*., 1997).

- *Field detection of trypanocidal drug resistance*

Field detection of resistance to ISM was described by Eisler *et al*. (2000). The approach is the "block treatment" under natural trypanosome challenge in field conditions. Briefly, at each field study location, approximately hundred cattle are randomised into treatment and sentinel groups. The treatment group is given ISM chloride at 1.0 mg/kg bw. All treatment and sentinel cattle are then monitored for clinical disease and sampled every 2 weeks for the presence of trypanosomes using the buffycoat technique (Murray *et al*., 1977) over a 2-month or 3-month period.

If more than 25% of ISM-treated cattle become infected within eight weeks of exposure, drug resistance is strongly suspected. This approach can also be used for assessing whether there is suspected resistance to DA by treating the control group at the start of the experiment, and all animals that become infected during the trial, with DA and checking for the presence of parasites two weeks after treatment (McDermott *et al*., 2003). Furthermore, longitudinal parasitological field data can be suitably analysed using appropriate statistical techniques to detect problems of resistance to DA (Rowlands *et al.*, 1993).

- *Molecular tools for the rapid detection of drug resistance*

The methods currently available for the detection of drug resistance are laborious, expensive and time consuming. Molecular methods for the diagnosis of ISM resistance were recently developed (Delespaux *et al*., 2005, 2006, 2008a; Afework *et al*., 2006). The first method enables discrimination between ISM sensitive and ISM-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. This method is not reliable for all regions of Africa and the correlation is sometimes very poor with a lot of false negative (Delespaux et al., 2008).

The second method has been developed to distinguish ISM-resistant from ISM-sensitive strains of *T. brucei* (Afework *et al*., 2006). This S*fa*NIPCR- 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 ISM (diamidine compound) and the detection of one of these six mutations could enable reliable identification of sensitivity or resistance to ISM (Mäser *et al.*, 2003).

1.4.3.3. Management of trypanocidal drug resistance

When chemoresistance is suspected, the quantity of drug should not be increased beyond the maximum amount recommended by the manufacturer and the number of treatments should not be increased. The product should be changed and a drug known for its activity against trypanosomes resistant to the first one should be used ("sanative drug"). The resistance to homidium bromide or ISM can be managed by diminazene in cattle and the resistance to diminazene should be treated by ISM.

1.5. Socio-economical importance of African Animal trypanosomoses

The problem of trypanosomosis "lies at the heart of Africa's struggle against poverty" and dealing with this disease has the potential to impact on all eight Millennium Development Goals of the 38 countries with tsetse infestations (Shaw, 2009). Indeed, African animal trypanosomosis (AAT) constitutes a major constraint to livestock production in SSA. The disease is enzootic in an area covering ca. 10 million km^2 and threatens nearly 50 million cattle (Kristjanson *et al.*, 1999). The disease causes many direct losses due to lower production, mortality and treatment costs, as well as indirect losses such as the opportunity of genetic improvement, and intensification of livestock production (Shaw, 2004). Attempts to

quantify the economical impact of trypanosomosis have taken into account the direct (mortality, fertility, milk production, animal traction and weight) and indirect effects on key productivity measures (Shaw, 2004). Livestock kept under trypanosomosis challenge have a 6-20 % higher annual calf mortality, a 6-19 % lower calving rate and a 20% decrease in milk yield (Shaw, 2004). Up to 38% weight loss and a reduction in work efficiency of oxen used to cultivate the land are additional direct effects of the disease (Shaw, 2004).

Direct losses and cost of AAT control is estimated to range between USD 600 and 1200 million per year for SSA (Swallow, 2000). According to the dynamic herd model of Kristjanson *et al*. (1999), the benefits of enhanced trypanosomosis control, alone in terms of increased meat and milk production would be US$ 700 million per year, and the eradication of trypanosomosis would increase agricultural production in Africa with a value of USD 4.5 billion/year (Budd, 1999). In addition, tsetse are the vectors of human sleeping sickness, a major neglected disease (Simarro *et al*., 2010).

Moreover, tsetse and trypanosomosis prevent the integration of crop farming and livestock keeping, which is crucial to the development of sustainable agricultural systems and therefore, affect human settlements in fertile zones (Feldmann *et al*., 2005; Feldmann and Hendrichs, 1999). Trypanosomosis among other debilitating diseases compromises the improvement of the local cattle breeds by exotic genetically improved animals. The lack of productive livestock in the tsetse infested area, is a key barrier in Africa to significantly improve agriculture and therefore the achievement of sustainable development. The removal of tsetse and trypanosomosis would be essential to the alleviation of hunger, food insecurity and poverty in SSA. This is exemplified by the remarkable correlation and overlap between the 38 tsetse-infested countries and the 34 heavily indebted poor countries in Africa (Feldmann *et al*., 2005).

1.6. References

Afework, Y., Mäser, P., Etschmann, B., von Samson-Himmelstjerna, G., Zessin, K.-H, Clausen, P.-H., 2006. Rapid identification of isometamidium-resistant stocks of *Trypanosoma b. brucei* by PCR-RFLP. Parasitol. Res. 99, 253-261.

Ainanshe, O.A., Jennings, F.W., Holmes, P.H., 1992. Isolation of drug-resistant strains of *Trypanosoma congolense* from the lower Shabelle region of southern Somalia. Trop. Anim. Health Prod. 24, 65–73.

Allsopp, R., Hursey, B.H., 2004. Insecticidal control of tsetse. *In*: Maudlin, I., Holmes P. H. & Miles M. A. (eds.), *The Trypanosomiasis*, Cabi publishing, Oxfordshire, UK, pp. 491–507.

Authie, E., 1994. Mise en évidence d'une résistance aux trypanocides parmi les souches de *Trypanosoma congolense* récemment isolées au Burkina. Rev. Elev. Méd. vét. Pays trop. 37, 219–235.

Barrett, M.P., Fairlamb, A.H., 1999. The biochemical basis of arsenical-diamidine crossresistance in African trypanosomes. Parasitol. Today 15, 136–140

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

Bauer, B., Amsler-Delafosse, S., Kaboré, I., Kamuanga, M., 1999. Improvement of cattleproductivity through rapid alleviation of African Trypanosomosis by integrated disease management practices in the Agropastoral zone of Yalé, Burkina Faso. Trop. Anim. Health Prod. 31, 89–102.

Baumhover, A.H., Graham, A.J., Bitter, B.A., Hopkins, D.E., New W.D., Dudley, F.H., Bushland, R.C., 1955. Screwworm control through release of sterile flies. J. Econ. Entomol. 48, 462–466.

Baylis, M., Mbwabi, A.L., 1995. Feeding behaviour of tsetse flies (*Glossina pallidipas* Austen) on Trypanosoma-infected oxen in Kenya. Parasitol. 110, 297–305.

Bengaly, Z., Sidibe, I., Ganaba, R., Desquesnes, M., Boly, H., Sawadogo, L., 2002. Comparative pathogenicity of three genetically distinct types of *Trypanosoma congolense* in cattle: clinical observations and haematological changes. Vet. Parasitol. 108, 1–19

Berg, S.S., Brown, K.N., Hill, J., Wragg., W.R., 1961. A new prophylactic trypanocidal drug, 2, 7-bis (M-amidino-pheny ldiazoamino)-10-ethyl-9-phenylphenanthri-dinium chloride di-hydrochloride (M&B 4596). Nature 192, 365–368.

Bouyer, J., 2006. Ecologie des glossines du Mouhoun au Burkina Faso : intérêt pour l'épidémiologie et le contrôle des trypanosomoses africaines. *Thèse de Doctorat* d'Université Montpellier II.

Bouyer, J., 2008. Does isometamidium chloride treatment protect tsetse flies from trypanosome infections during SIT campaigns? Med. Vet. Entomol. 22, 140–144.

Bouyer, J., Balenghien, T. Ravel, S. Vial, L., Sidibé, I., Thévenon, S., Solano, P., De Meeûs, T., 2009a. Population sizes and dispersal pattern of tsetse flies: rolling on the river? Mol. Ecol. 18, 2787–2797.

Bouyer, J., Solano P., Cuisance D., Itard, J., Frézil, J.-L., Authié E. 2010. Trypanosomosis: Control methods., pp. 1936-1943. In: P.-C. Lefèvre, J. Blancou, R. Chermette and G. Uilenberg [eds.], Infectious and parasitic diseases of livestock (vol. 2). Lavoisier (Tech & Doc), Paris.

Bouyer, J., Stachurski, F., Gouro, A., Lancelot, R., 2009b. Control of bovine trypanosomosis by restricted application of insecticides to cattle using footbaths. Vet. Parasitol. 161, 187–193.

Browning, C.H., Morgan, G.T., Robb, J.V.M., Walls, L.P., 1938. The trypanocidal action of certain phenanthridinium compounds. The Journal of Pathology and Bacteriology, 46, 203–204

Budd, L., 1999. DFID-funded tsetse and trypanosome research and development since 1980. Volume 2: Economic Analysis, Livestock Production Programme. Chatham Maritime: NRInternational. 123p.

Buxton, P.A., 1955. The natural history of tsetse flies. An account of the biology of the genus Glossina (Diptera). London School of Hygiene and Tropical Medicine. Memoir No. 10. H.K. Lewis and Co. Ltd, London, 816 pp.

Challier, A., Laveissière, C., 1973. Un nouveau piège pour la capture des glossines (*Glossina:* Diptera, Muscidae): description et essais sur le terrain. Cah ORSTOM, sér Ent Méd et Parasitol 10, 251–262.

Challier, A., Laveissiere, C., 1977. La répartition des glossines en Haute-Volta.1 carte couleur 1/2000000 et notice explicative. Paris, ORSTOM 39p.

Chartier, C., Itard, J., Morel, P., Troncy, P., 2000. Précis de parasitologie vétérinaire tropicale. Ed. Tec et Doc & Ed. Médicales Internationales Universités Francophones (AUPELF UREF), 774p.

Chitanga, S., Marcotty, T., Namangala, B., Van den Bossche, P., Van Den Abbeele, J., Delespaux, V., 2011. High Prevalence of Drug Resistance in Animal Trypanosomes without a History of Drug Exposure. PLoS Negl. Trop. Dis. 5(12), e1454. doi:10.1371/journal.pntd.0001454

Clausen, P.H ., Leendertz, F.H., Blankenburg, A., Tietjen, U., Mehlitz, D., Sidibe, I., Bauer, B., 1999. A drug incubation *Glossina* infectivity test (DIGIT) to assess the susceptibility of *Trypanosoma congolense* bloodstream forms to trypanocidal drugs (Xenodiagnosis). Acta Trop. 72, 111–117

Clausen, P.H., Bauer, B., Zessin, K.H., Diall, O., Bocoum, Z., Sidibe, I., Affognon, H., Waibel, H., Grace, D., Randolph, T., 2010. Preventing and containing trypanocide resistance in the cotton zone of West Africa. Transboundary and Emerging Diseases 57, 28–32.

Clausen, P.H., Pellman, C., Scheer, A., Tietjen, U., Schares, G., Bauer, B., Peregrine, A.S., Mehlitz, D., 2000. Application of *in vitro* methods for the detection of drug resistance in trypanosome field isolates. ICPTV Newsletter 2, 9–12

Clausen, P.H., Sidibe, I., Kabore, I., Bauer, B., 1992. Development of multiple drug resistance of *Trypanosoma congolense* in zebu cattle under high natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. Acta Trop. 51, 229–236.

Codjia, V., Mulatu, W., Majiwa, P.A.O., Leak, S.G.A., Rowlands, G.J., Authié, E., d'Ieteren, G.D.M., Peregrine, A.S., 1993. Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 3. Occurrence of populations of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium. Acta Trop. 53, 151–163.

Cuisance, D., 1989. Glossines et trypanosomes. Master international Erasmus/Comett/Alfa sur les Maladies parasitaires tropicales. Valençia (Spain). 115 p. and appendices.

Cuisance, D., 2001. Cours de certificat d'études approffondies véterinaires en Pathologies tropicales sur les glossines et les trypanosomoses. CIRAD-EMVT, Montpellier, France. 102p.

Cuisance, D., Février, J., Dejardin, J., Filledier, J. 1985. Dispersion linéaire de *Glossina palpalis gambiensis* et *G. tachinoides* dans une galerie forestière en zone soudano-guinéenne (Burkina Faso). Rev. Elev. Méd. vét. Pays trop. 38, 153–172.

Cuisance, D., Politzar, H., Mérot, P., Tamboura, I., 1984. Les lâchers de mâles irradiés dans la Campagne de lutte intégrée contre les glossines dans la zone pastorale de Sidéradougou (Burkina Faso). Rev. Elev. Méd. vét. Pays trop., 37, 449–467.

Dagnachew, S., Sangwan, A.K., Abebe, G., 2005. Epidemiology of Bovine Trypanosomosis in the Abay (Blue Nile) Basin Areas of Northwest Ethiopia. Rev. Elev. Méd. vét. Pays trop. 58, 151–157.

Delespaux, V. 2004. Improved diagnostic of trypanosome infection and drug resistant *Trypanosoma congolense* in livestock. PdD thesis, Université Libre de Bruxelles, 135p.

Delespaux, V., Chitanga, S., Geysen, D., Goethals, A., Van den Bossche, P., Geerts, S., 2006. SSCP analysis of the P2 purine transporter TcoAT1 gene of *Trypanosoma congolense* leads to a simple PCR-RFLP test allowing the rapid identification of diminazene resistant stocks. Acta Trop. 100, 6–102.

Delespaux, V., de Koning, H.P., 2007. Drugs and drug resistance in African trypanosomiasis. Drug Resist. Update 10, 30–50.

Delespaux, V., Dinka H., Masumub, J., Van den Bossche, P., Geerts, S., 2008b. Five-fold increase in *Trypanosoma congolense* isolates resistant to diminazene aceturate over a seven-year period in Eastern Zambia. Drug Resist. Updat. 11, 205–209

Delespaux, V., Geysen, D., Geerts, A.S., 2007. Point mutations in mitochondrial topoisomerase enzymes of *Trypanosoma congolense* are not involved in isometamidium resistance. Mol. Biochem. Parasitol. 151, 137–140

Delespaux, V., Geysen, D., Majiwa, P.A.O., Geerts, S., 2005. Identification of a genetic marker for isometamidium chloride resistance in *Trypanosoma congolense*. . Int. J. Parasitol. 35, 235–243

Delespaux, V., Geysen, D., Van den Bossche, P., Geerts, S., 2008a. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 24, 236–242.

Desquesnes, M., Biteau-Coroller, F., Bouyer, J., Dia, M.L., Foil, L.D., 2009. Development of a mathematical model for mechanical transmission of trypanosomes and other pathogens of cattle transmitted by tabanids. Int. J. Parasitol. 39, 333–346

Desquesnes, M., Dia M. L. (2004). Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanid *Atylotus fuscipes*. Vet. Parasitol. 119, 9–19

Desquesnes, M., Dia, M. L. (2003). *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. Exp. Parasitol. 103, 35–43.

Dia, M.L., Desquesnes, M., 2005. Les trypanosomoses animales : Utilisation rationnelle des trypanocides Recommandations Techniques, CIRDES/CIRAD No.3

Diall, O., 1997. Manuel technique sur l'utilisation des produits trypanocides, Laboratoire Central Vétérinaire/Bamako, Mali, 32 p.

Douthwaite, R.J., Tingle, C.C.D., 1994. DDT in the tropics: the impacts on wildlife in Zimbabwe of ground-spraying for tsetse fly control. Natural Resources Institute, Chatham.

Du Toit, R., 1954. Trypanosomiasis in Zululand and the control of tsetse flies by chemical means. Onderstepoort J. Vet. Res. 26, 317–387.

Dyck VA, Hendrichs J, Robinson AS (2005) Sterile insect technique. Dordrecht, The Netherlands: Springer. 787 p.

Eisler, M.C., 1996. Pharmacocinetics of the chemoprophylactic and chemotherapeutic trypanocidal drug isometamidium chloride (samorin®) in cattle. Drug Metab. Dispos. 24, 1355–1361.

Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.-H., Delespaux, V., Holmes, P.H., Ilemobade, A., Machila, N., Mbwambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung'u, J.M., Peregrine, A.S., Sidibé, I., Sinyangwe, L., Geerts, S., 2001. Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. Vet. Parasitol. 97, 171–182

Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.H., Delespaux, V., Holmes, P.H., Ilemobade, A., Machila, N., Mbwambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung'u, J.M., Peregrine, A.S., Sidibe, I., Sinyangwe, L., Geerts, S., 2001. Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. Vet. Parasitol. 97, 171–182.

Eisler, M.C., Elliott, C.T., Holmes, P.H., 1996. A simple competitive enzyme immunoassay for the detection of the trypanocidal drug isometamidium. Therapeut. Drug Monitor. 18, 73–79

Eisler, M.C., Gault, E.A., Moloo, S.K., Holmes. P.H., Peregrine, A.S., 1997. Concentrations of isometamidium in the sera of cattle challenged with drug-resistant *Trypanosoma congolense*. Acta Trop. 63, 89–100

Eisler, M.C., McDermott, J.J., Mdachi,R., Murilla, G.A., Sinyangwe, L., Mubanga J., Machila, N., Mbwambo, H., Coleman, P.G., Clausen, P.-H., Bauer, B., Sidibé, I., Geerts, S., Holmes, P.H., Peregrine, A.S., 2000. Rapid method for the assessment of trypanocidal drug resistance in the field. Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics, Colorado, USA. http://www.sciquest.org.nz/node/71200

El Sawalhy, A., Seed, J.R., El Attar, H., Hall, J.E., 1995. Catabolisrn of tryptophan by *Trypanosoma evansi*. J. Eukaryot. Microbiol., 42, 684–690.

Farwell, G.E., LeGrand, E.K., Cobb, C.C., 1982 Clinical observations on *Babesia gibsoni* and *Babesia canis* infections in dogs. J. Am. Vet. Med. Ass. 180: 507–511

Feldmann, U., Dyck, V. A., Mattioli R. C., Jannin, J., 2005. Potential impact of tsetse fly control involving the sterile insect technique. In : Dyck, V. A., Hendrichs J. & Robinson A. S. (eds.), Sterile insect technique. Principles and practice in area-wide integrated pest management . Springer, Dordrecht, The Netherlands, pp. 701–726.

Feldmann, U., Hendrichs, J., 1999. The concept for integration of the sterile insect technique as a key component of future subregional area-wide tsetse and trypanosomosis management operations. In: Animal Trypanosomosis: Vector and Disease Control using Nuclear Techniques, Backhuys Publishers, Leiden, The Netherlands, pp. 191–214.

Filledier, J., Duvallet, G., Merot, P., 1988. Comparaison du pouvoir attractif des bovins Zébu et Baoulé pour *Glossina tachinoides* Westwood, 1850 et *Glossina morsitans submorsitans* Newstead, 1910 en savane soudanoguinéenne, Burkina Faso. Rev. Elev. Med. Vet. Pays Trop. 41, 191–196.

Ford, J., Nash, T.A.M., Welch, J.R., 1970. Control by clearing of vegetation. In: H. W. Mulligan and W. H. Potts, eds, The African Trypanosomiases, George Allen and Unwin, London, pp 543–556.

Geerts, S., Holmes, P.H., 1998. Drug management and parasite resistance in bovine trypanosomosis in Africa. PAAT Tech. Sci. Series No. 1. FAO, Rome.

Geerts, S., Holmes, P.H., Diall, O., Eisler, M.C., 2001. African bovine trypanosomosis: the problem of drug resistance, Trends Parasitol. 17, 25–28.

Geerts, S., Ndung'u, J. M., Murilla, G.A., Mbwambo, H., Sinyangwe, L., Machila, N., Delespaux, V., Brandt, J., Peregrine, A.S., McDermott, J.J., Holmes, P.H., Eisler, M.C., 2000. In vivo tests for the detection of resistance to trypanocidal drugs: tests in mice and in ruminants. ICPTV Newsletter 2, 6–7

Gottesman, M.M., 2002. Mechanisms of cancer drug resistance. Annual Rev. Med. 53, 615-627,

Gouteux, J. P., Artzrouni M., Jarry M., 2001. A density-dependant model with reinvasion for estimating tsetse fly populations (Diptera: Glossinidae) through trapping. Bull. Entomol. Res., 91, 177–183.

Gouteux, J.P., 1987. Une nouvelle glossine du congo : *Glossina* (*Austenina*) *frezili* sp. nov. (Diptera : Glossinidae). Trop. Med Parasitol. 38, l–4.

Grace, D., Randolph, T., Affognon, H., Dramane, D., Diall, O., Clausen, P.H., 2009. Characterisation and validation of farmers' knowledge and practice of cattle trypanosomosis management in the cotton zone of West Africa. Acta Trop. 111, 137–143.

Grant, I.F., 2001. Insecticides for tsetse and trypanosomiasis control: is the environmental risk acceptable? Trends Parasitol. 17, 10–14.

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

Hargrove, J.W., Omolo, S., Msalilwa, J.S.I., Fox, B., 2000. Insecticide-treated cattle for tsetse control: the power and the problems. Med. Vet. Entomol. 14, 123–130

Hirumi, H., Hirumi, K., Peregine, A.S., 1993. Axenic culture of *Trypanosoma congolense* - application to the detection of sensitivity levels of blood-stream trypomastigotes to diminazene aceturate, homidium chloride, isometamidium chloride and quinapyramine sulfate. J. Protozool. Res. 3, 52–63

Holmes, P.H., Eisler, M.C., Geerts, S., 2004. Current chemotherapy of animal trypanosomiasis. *In* The trypanosomiases. Maulidn, I., Holmes, P.H., Miles M.A. (eds.). CABI International Wallingford, UK. pp 431–444.

Itard, J. and D. Cuisance. 2003. Vecteurs cycliques des trypanosomoses. pp. 139-165. In Editions Tec et Doc and Editions Médicales internationales. Principales maladies infectieuses et parasitaires du bétail. Europe et Régions chaudes. Lavoisier, Paris, France.

Jenni, L., Molyneux, D., Livesey, J.L., Galun, R., 1980. Feeding behavior of tsetse flies infected with salivarian trypanosomes. Nature, 283, 383–385.

Kagbadouno, M.S., Camara, M., Bouyer, J., Courtin, F., Morifaso, O., Solano, P., 2011 Tsetse control in Loos islands, Guinea. Parasites & Vectors 4, 18.

Kgori, P.M., Modo, S., Torr, S.J., 2006. The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. Acta Trop. 99, 184–199.

Klassen, W., 2003. Edward F. Knipling: Titan and driving force in ecologically selective area-wide pest management. J. Am. Mosq. Control. Assoc. 19, 94–103.

Knipling, E.F., 1955. Possibilities of insect population control through the use of sexually sterile males. J. Econ. Entomol. 48, 443–448.

Knipling, E.F., 1959. Sterile-Male Method of Population Control: Successful with some insects, the method may also be effective when applied to other noxious animals. Science 9, 902–904.

Knoppe, T.N., Bauer, B., McDermott, J.J., Peregrine, A.S., Mehlitz, D., Clausen, P.-H., 2006. Isometamidium sensitivity of *Trypanosoma congolense* stocks from cattle in West Africa tested in mice and the drug incubation infectivity test. Acta Trop. 97, 108–116

Kristjanson, P.M., Swallow, B.M., Rawlands, G.J., Kruska, R.L., Leeuw, P.N., 1999. Measuring the cost of African Animal Trypanosomiasis, the potential benefits of control and returns to research. Agr. syst. 59, 79–98.

Kuzoe, F.A.S., Schofield, C.J., 2004. Strategic review of traps and targets for tsetse and African trypanosomiasis control. World Health Organization, Special Programme for Research and Training in Tropical Diseases, Geneva, Vol. TDR/IDE/TRY/05.1, 58 pp. (www.who.int/tdr/publications/publications/pdf/tsetse_traps.pdf)

Larsen, T.B., 1992. Migration of *Catopsilia florella* in Botswana (Lepidoptera: Pieridae). Tropical Lepidoptera, 3, 2–11.

Laveissiere, C., Couret, D., 1981. Le piegéage, une méthode de lutte contre les vecteurs de trypanosomes humains, 17th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, Arusha, Tanzania, October 1981, OAU/ISTRC No 112, pp 609–617.

Laveissière, C., Garcia, B. Sané, B., 2003. Lutte contre la maladie du sommeil et soins de santé primaire. IRD Editions, Paris.

Laveissière, C., Grébaut, P., Herder, S., Penchenier, L., 2000. Les glossines vectrices de la Trypanosomiase humaine africaine; IRD, editor. Yaoundé, Cameroun: IRD and OCEAC. 246p.

Leak, S.G.A., 1999. Tsetse biology and ecology: their role in the epidemiology and control of trypanosomosis. Cabi publishing, Wallingford, UK, 529 pp

Lemmouchi, Y., Schacht, E., Kageruka, P., De Deken, R., Diarra; B., Diall, O., Geerts S., 1998. Biodegradable polyesters for controlled release of trypanocidal drugs: In vitro and in vivo studies, Biomaterials, 19, 1827–1837

Levine, N.D., Corliss, J.O., Cox, F.E.G., Deroux, G., Grain, J., Honigberg, B.M, Leedale, G.F., Loeblich, A.R., Lom, III.J., Lynn, D., Merinfeld, E.G., Page, F.C., Poljansky, G., Sprague V.,

Vavra, J., Wallace F.G., 1980. A Newly Revised Classification of the Protozoa. J. profozool. 27, 37–58

Luckins, A.G., 1988. Trypanosoma evansi in Asia. Parasitol. Today 4, 137–42.

Marquis, J.F., Hardy, I., Olivier, M., 2005. Topoisomerase I amino acid substitutions, Gly185Arg and Asp325Glu, confer camptothecin resistance in *Leishmania donovani*. Antimicrob. Agents Chemother. 49, 1441–1446

Mäser, P., Lüscher, A., Kaminsky, R., 2003. Drug transport and drug resistance in African trypanosomes. Drug Resist. Updat. 6, 281–290.

McDermott, J.J., Woitag, T., Sidibe, I., Bauer, B., Diarra, B., Ouedraogo, D., Kamuanga, M., Peregrine, A.S., Eisler, M.C., Zessin, K.H., Mehlitz, D., Clausen, P.H., 2003. Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. Acta Trop. 86, 93–103.

Molyneux, D.H., Ashford, R.W., 1983. The biology of *Trypanosoma* and *Leishmania* parasites of man and domestic animals. Taylor and Francis, Ed (London).

Molyneux, D.H., Hopkins, D.R., Zagaria, N., 2004, Disease eradication, elimination and control: the need for accurate and consistent usage. Trends Parasitol. 20, 347–351

Moore, D.J., 1979. Therapeutic implications of *Babesia canis* infection in dogs. J. South Afr. Vet. Ass. 50, 346– 352

Müller, P., Nagel, P., Flacke, W., 1981. Ecological side effects of dieldrin application against tsetse flies in Adamaoua, Cameroon. Oecologia 50, 194–198.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P.A.O., Masake, R., Peregrine, A.S., 1997. Long-term occurrence of *Trypanosoma congolense* resistant to diminazene, isometamidium nd homidium in cattle at Ghibe, Ethiopia. Acta Trop. 64, 205–217.

Murilla, G.A., Peregrine, A.S., Ndung'u, J.M., Holmes, P., Eisler, M., 2002. The effects of drug-sensitive and drug-resistant *Trypanosoma congolense* infections on the pharmacokinetics of homidium in Boran cattle. Acta Trop. 81, 185–195.

Murilla,G.A., R.E. Mdachi&J.K.Kagira. 2005. Evaluation on the local tolerance and efficacy of melarsan oxide cysteamine (Melcy, Cymelarsan) in horses.Minutes of the 26th Annual Meeting of the OIE ad hoc group on Non Tsetse Transmitted Animal Trypanosomosis, Paris, 22 May 2005.

Musa, M.M., Abdoon, A.M.O., Nasir, B.T., Salim, Y.I., Abdel-Rahman, A.Y., Shommein, A.M. 1994. Efficacy of Cymelarsan in the treatment of natural chronic *Trypanosoma evansi* infection in camels in the Sudan. Rev. Elev. Med. Vet. Pays Trop. 47, 397–400.

Na'isa, B.K., 1967. Follow-up of a survey on the prevalence of homidium resistant strains of trypanosomes in cattle in Northern Nigeria and drug cross-resistance tests on the strains with Samorin and Berenil. Bull. Epizoot. Dis. Afr. 15, 231–241.

Naudé, T.W., Basson, P.A., Pienaar, J.G., 1970. Experimental diamidine poisoning due to commonly used babecides. Onderstepoort J. Vet. Res. 37, 173–184

Novy, J.E., 1991. Screwworm control and eradication in the southern United States of America. World Anim. Rev. 10, 18–27.

Ormerod, W.E. (1986). A critical study of the policy of tsetse eradication. Land use policy. 3, 85–99.

Payne, R.C., Sukanto, I.P., Partoutomo, S., Jones, T.W. Luckins, A. G., Boid R., 1994. Efficacy of cymelarsan in Friesian Holstein calves infected with *Trypanosoma evansi*. *Trop*. Anim. Health Prod. 26, 219–226.

Peregrine, A.S., 1994. Chemotherapy and delivery systems - haemoparasites. Vet. Parasitol. 54, 223–248

Peregrine, A.S., Gray, M.A., Moloo, S.K., 1997. Cross-resistance associated with development of resistance to isometamidium in a clone of *Trypanosoma congolense*. Antimicrob. Agents Chemother. 41, 1604-1606.

Phelps, R.J., Focardi, S., Fossi, C., Leonzio, C., and Renzoni, A., 1986. Chlorinated hydrocarbons and heavy metals in crocodile eggs from Zimbabwe. Trans. Zimb. Sci. Assoc. 63, 8–15.

Pinder, M., Authie, E., 1984. The appearance of ISM resistant *Trypanosoma congolense* in West Africa. Acta Trop. 41, 247–252.

Politzar, H., Cuisance, D., 1984. An integrated campaign against riverine tsetse flies *Glossina palpalis gambiensis* and *Glossina tachinoides* by trapping and the release of sterile males. Insect Sci. Applic. 5, 439–442.

Politzar, H., Mérot, P., 1984. Attraction of the tsetse fly *Glossina morsitans submorsitans* to acetone, 1-octen-3-01, and the combination of these compounds in West Africa. Rev. Elev. Med. Vet. Pays Trop., 37, 468–473.

Pollock, J.N., 1982. Training Manual for Tsetse Control Personnel. Vol. 1: Tsetse biology, systematics and distribution techniques. Rome: FAO. 243 p.

Rayaissé, J.B., Courtin, F., Akoudjim, M., Cesar, J., Solano, P., 2009. Influence de l'anthropisation sur la végétation et l'abondance des tsé-tsé au sud du Burkina-Faso. Parasite, 16, 21–28.

Rogers, D. J., Randolph, S. E. 1984. A review of density-dependant processes in tsetse population. Insect Science and its Application 5, 397–402.

Rowlands, G.J., Mulatu, W., Authie, E., d'Ieteren, G.D., Leak, S.G., Nagda, S.M., Peregrine, A.S., 1993. Epidemiology of bovine trypanosomiasis in the Ghibe Valley, Southwest Ethiopia: factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence of recurrent infections. Acta Trop. 53, 135–150

Schönefeld, A.R., Röttcher, D., Moloo, S.K., 1987. The sensitivity to trypanocidal drugs of *Trypanosoma vivax* isolated in Kenya and Somalia. Trop. Med. Parasitol. 38, 177–180.

Scott, A.G., Tait, A., Turner, C.M.R., 1996. Characterisation of cloned lines of Trypanosoma brucei expressing stable resistance to MelCy and suramin. Acta Trop. 60, 251–262.

Scott, J.M., Pegram, R.G., 1974. A high incidence of *T.congolense* strains resistant to homidium bromide in Ethiopia. Trop. Anim. Health Prod. 6, 215–221.

Seed, J.R., 2001. African trypanosomosis research: 100 years of progress, but questions and problems still remain. International Journal for Parasitology, 31, 434–442.

Shaw, A.P.M., 2004. Economics of African trypanosomosis. In, Maudlin I, Holmes PH, Miles MA, editors. The trypanosomosis. Wallingford: CABI Publishing. pp. 369–402.

Shaw, A.P.M., 2009. Assessing the economics of animal trypanosomosis in Africa-history and current perspectives. Onderstepoort J. Vet. Res. 76, 27–32

Shereni, W., 1990. Strategic and tactical developments in tsetse control in Zimbabwe (1981-1989). Ins. Sc. Appl. 11, 399–409.

Simarro, P.P., Cecchi, G., Paone, M., Franco, J.R., Diarra, A., Ruiz, J.A., Fèvre, E.M., Courtin, F., Mattioli, R.C., Jannin, J.G., 2010. The Atlas of human African trypanosomiasis: a contribution to global mapping of neglected tropical diseases. Int. J. Health Geogr. 9, e57. doi:10.1186/1476-072X-9-57

Solano, P., Bouyer, J., Itard, J. Cuisance, D., 2010 Cyclical vectors of trypanosomosis, pp. 153-181. In: Infectious and parasitic diseases of livestock (vol. 1), Chapter 012, P.-C. Lefèvre, J. Blancou, R. Chermette and G. Uilenberg Editors. Lavoisier (Tec & Doc), Paris.

Solano, P., de La Rocque, S., de Meeûs, T., Cuny, G., Duvallet, G., Cuisance, D., 2000. Microsatellite DNA markers reveal genetic differentiation among populations of *Glossina palpalis gambiensis* in the agropastoral zone of Sideradougou, Burkina Faso. Insect Molecular Biology, 9, 433–439.

Sones, K.R., Njogu, A.R., Holmes, P.H., 1988. Assessment of sensitivity of *T. congolense* to ismetamidium chloride: a comparison of tests using cattle and mice. Acta Trop. 45, 153–164.

Swallow, B.M., 2000. Impacts of Trypanosomiasis on African Agriculture. Rome: FAO. 46 p.

Taj Eldinl, M., Mohammed, O.Y., Mohammed, A.H and Ahmed, E.M, 2005. Mechanism of some toxic effects of trypanocidal agents; Diminazene and ISM in camels. Omdurman J. Pharma. Sci. 1, 1–12.

Takken, V., Oladunmade, M.A., Dengwat, L., Feldmann H.U., Onah, J.A., Tenabe, S.O., Hamann, H.J., 1986. The eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) using traps, insecticide-impregnated targets and the sterile insect technique in central Nigeria. Bull. Entomol. Res. 76, 275–286.

Thomson, J.W., Wilson, A., 1992. The control of tsetse flies and trypanosomiasis by the application of deltamethrin to cattle. Bull. Anim. Health Prod. Afr. 40, 5–8.

Torr, S.J., Maudlin, I., Vale, G.A., 2007. Less is more: restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control. Med. Vet. Entomol. 21, 53–64.

Tuntasuvan, D., Jarabrum, W., Viseshakul, N., Mohkaew, K., Borisutsuwan, S., Theeraphan, A., Kongkanjana, N., 2003. Chemotherapy of surra in horses and mules with diminazene aceturate. Vet. Parasitol. 110, 227–233.

Vale, G.A., 1974. Responses of tsetse flies (Diptera, Glossinidae) to mobile and stationary baits, Bull. Entomol. Res. 64, 545–588.

Vale, G.A., 1982. The improvement of traps for tsetse flies (Diptera, Glossinidae), Bull. Entomol. Res. 72, 95–106.

Vale, G.A., Bursell, E., Hargrove J.W., 1985. Catching-out the Tsetse Fly. Parasitology Today, 1, 106–110

Van Den Abbeele, J., Claes, Y., van Bockstaele, D., Le Ray, D., Coosemans, M., 1999. *Trypanosoma brucei* spp. development in the tsetse fly: characterization of the postmesocyclic stages in the foregut and proboscis. Parasitol. 118, 469–478

Van den Bossche, P., Akoda, K., Djagmah, B., Marcotty, T., De Deken, R., Kubi, C., Parker, A., Van Den Abbeele, J., 2006. Effect of ISM Chloride Treatment on Susceptibility of Tsetse Flies (Diptera: Glossinidae) to Trypanosome Infections. J. Med. Entomol. 43, 564–567.

Van den Bossche, P., Munsimbwe, L., Mubanga, J., Jooste, R., Lumamaba, D., 2004. A large-scale trial to evaluate the effec- tiveness of a 1% cyfluthrin pour-on (Cylence1, Bayer) to control bovine trypanosomosis in Eastern Zambia. Trop. Anim. Health Prod. 36, 33–43.

Vargas-Terán, M., 1991. The New World Screwworm in Mexico and Central America. World Anim. Rev. Special Issue 28–35.

Vargas-Terán, M., Hursey, B.S., Cunningham, E.P., 1994. Eradication of the screwworm from Libya using the sterile insect technique. Parasitol. Today 10, 119–122.

Vickerman, K., 1985. Developmental cycles and biology of pathogenic trypanosomes. Br Med. J. 41,105–114

Vickerman, K., Tetley, L., Hendry, K.A., Turner, C.M., 1988. Biology of African trypanosomes in the tsetse fly. Biol. Cell 64, 109–119

Vreysen, M. J. B., Saleh, K. M., Lancelot, R., Bouyer, J., 2011. Factory tsetse flies must behave like wild flies: a prerequisite for the sterile insect technique. Plos Negl. Trop. Dis. 5(2), e907.

Vreysen, M.J.B., 2001. Principles of area-wide integrated tsetse fly control using the sterile insect technique. Med. Trop. 61, 397–411

Vreysen, M.J.B., 2005. Monitoring sterile and wild insects in area-wide integrated pest management programmes. *In*: Dyck, V.A., J. Hendrichs & A. S. Robinson (eds.), *Sterile insect technique. Principles and practice in area-wide integrated pest management*. Springer, Dordrecht, The Netherlands. pp. 39–68.

Vreysen, M.J.B., 2006. Prospects for area-wide integrated control of tsetse flies (Diptera: Glossinidae) and trypanosomosis in sub-Saharan Africa. Rev. Soc. Entomol. Argent. 65, 1–21

Vreysen, M.J.B., Saleh, K.M., Ali M.Y., Abdulla, A.M., Zhu, Z-R., Juma, K.G., Dyck ,V.A., Msangi, A.R., Mkonyi, P.A., Feldmann, H.U., 2000. *Glossina austeni* (Diptera: Glossinidae) Eradicated on the Island of Unguja, Zanzibar, Using the Sterile Insect Technique. J. Econ. Entomol. 93, 123–135.

Whiteside, E.F. 1962. Interactions between drugs, trypanosomes and cattle in the field. In: Drugs, parasites and hosts. Goodwin & Nimmo-Smith, eds., pp. 116-141.

Wilkes, J.M., Mulugeta, W., Wells, C., Peregrine, A.S., 1997. Modulation of mitochondrial electrical potential: a candidate mechanism for drug resistance in African trypanosomes. Biochem. J. 326, 755–761

Williamson, D. L., Dame D. A., Gates D. B., Cobb P. E., Bakuli B., Warner P. V., 1983. Integration of insect sterility and insecticides for control of *Glossina morsitans morsitans* (Diptera: Glossinidae) in Tanzania: V. The impact of sequential releases of sterilized tsetse flies. Bull. Entomol. Res. 73, 391–404.

Objectives of the thesis

Global objective

PATTEC is the most important T&T elimination campaign ever implanted in Burkina Faso. The campaign concerns 90 000 km^2 where live one half of the cattle population of the country. The project costed 6 billion Francs CFA (~12 million $US) and is financed by the Government of Burkina Faso and international donors. The global objective of this PhD study is to contribute to the development of rational intervention strategies for the tsetse and trypanosomiasis eradication campaign in Burkina Faso.

Specific objectives

In order to achieve this global objective, the study comprised five specific objectives:

- To make a review on the 50 years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso;
- To evaluate the parasitological and baseline situation in the PATTEC intervention area;
- To evaluate drug resistance in the Region of the Boucle du Mouhoun, PATTEC intervention area;
- To assess the competitiveness of irradiated male tsetse from a 40-year-old colony which will be used in preparation of an eradication campaign;
- To assess the impact of the campaign of elimination of T&T in the PATTEC intervention area.

Chapter 2:

Fifty years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso. An overview

Adapted from:

Sow A., Sidibé I., Bengaly, Z., Bouyer J., Bauer B., Van den Bossche P. (2010). Fifty years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso. An overview. *Bulletin of Animal Health and Production in Africa,* 58 (2), 95-118.

2.1. Introduction

Tsetse-transmitted trypanosomosis is the main constraint to livestock development in Burkina Faso. More than 11 million head of livestock are raised within the trypanosomosis endemic areas, requiring about 2.6 million doses of trypanocidal drugs to manage the disease (MRA, 2006). Moreover, Burkina Faso has been the West African epicenter of Human African Trypanosomosis between 1930-1940 but its prevalence seems to have decreased now. Nevertheless, many areas remain under surveillance (Courtin *et al.,* 2008).

During the last 50 years, efforts have been made by the government of Burkina Faso and supported by international organizations, to minimize the impact of the disease. Several projects such as the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), the Farming in Tsetse Controlled Areas (FITCA), the Regional Tsetse Fly and Trypanosomosis Control Programme (RTTCP) and the Programme de Lutte contre la Trypanosomose (PLTA/FAO) have been implemented to control tsetse flies in Burkina Faso and other sub-Saharan African tsetse infested countries. Because of the disease's regional importance, the Centre de Recherche sur les Trypanosomoses Animales (CRTA now CIRDES) and the Ecole de Lutte anti Tsé-tsé (ELAT) with regional objectives and based in Bobo-Dioulasso were created in the 1970s.

In Burkina Faso, a lot of research has been conducted in the field of tsetse and trypanosomosis (T&T). Nearly all the non-polluting vector control tools have been tested successfully. The first integrated control strategies were tested by the end of the 70s in the regions of Bobo-Dioulasso, Orodara and along the Guénako River at the sources of Mouhoun River (Politzar *et al.,* 1976, 1979; Taze *et al.,* 1977; Cuisance *et al.,* 1978a). These integrated strategies made use of a combination of insecticide-treated traps during the dry season and the release of sterile males during the rainy season. To prevent reinvasion of tsetse into the cleared areas, non-impregnated or impregnated traps or screens were placed at 100 m intervals to act as a barrier. In the 1980s, similar integrated strategies were used in the agro pastoral zone of Sideradougou (Cuisance *et al.,* 1990).

Many entomological surveys have been implemented in the southern and western regions of Burkina Faso to identify potential habitat for tsetse flies (Challier and Laveissière, 1977, Küpper, 1980, Cuisance *et al.,* 1985a, 1984a,b; de La Rocque *et al.,* 2001; Bauer *et al.,* 1988; Bouyer, 2006). In the same areas, the epidemiology of animal trypanosomosis was studied (Desquesnes *et al.,* 1999; Bengaly *et al.,* 2001; Michel *et al.,* 2002). Nevertheless, recent

entomological and parasitological studies indicated that livestock trypanosomosis still is of great importance in the southern region of Burkina Faso. Trypanocidal drug treatments are required to maintain trypanosusceptible animals within these infested zones. Unfortunately, trypanosome strains resistant to the most commonly used trypanocidal drugs (i.e. DA, ISM chloride, ethidium bromide) are present (Talaki *et al.,* 2006; McDermott *et al.,* 2003; Clausen *et al.,* 1992; Diarra, 2001; Pinder and Authié, 1984; Authié, 1984).

The purpose of this article is to present an overview of different programmes against T&T carried out in Burkina Faso. Based on the findings of the past, suggestions are made to improve the future activities against T&T in the country.

2.2. Historical background of tsetse and trypanosomosis in Burkina Faso

Human African Trypanosomosis (HAT) and African Animal Trypanosomosis (AAT) transmitted by tsetse flies have always been a major problem for public and animal health in Burkina Faso. During the colonial period, all the subhumid regions of West-Africa were enzootic for both diseases. By the end of the 1920s and the beginning of the 1930s, the colonial administration developed a plan for the control of HAT based on the detection and treatment of cases (Jamot, 1930, 1926). The programme was so effective that after 30 years, only a few HAT foci remained (Challier, 1986; Laveissière, 1976a). At the moment, the HAT situation seems to be under control, although surveillance is still needed mainly because of the massive movements of people from HAT endemic areas in Côte d'Ivoire (Courtin *et al.,* 2008).

During the post-colonial period, as HAT was under control, onchocerchiasis drew the attention of the public health administration in Burkina Faso. In the 1970s, a huge programme known as Onchocerchiasis Control Programme (OCP) was initiated in several West African countries including Burkina Faso (Prescott *et al.,* 1984; Le Berre, 1967a,b). The programme led to the eradication of this disease in Burkina Faso and other countries (Hougard *et al.,* 1994).

The zones freed from onchocerchiasis were very fertile and suitable for agriculture and livestock development. Therefore, a center for intensification of traditional livestock was established (known as CEZIET: Centre d'Encadrement des Zones d'Intensification de l'Elevage Traditionnel). The government of Burkina Faso encouraged farmers to settle in these regions to increase agriculture production and increase food self-sufficiency. However,

settlers were soon confronted with trypanosomosis threatening livestock and agricultural production.

During the 1980s, CRTA (Centre de Recherche sur les Trypanosomoses Animales) and ELAT (Ecole de Lutte Anti-Tsé-tsé) played an important role in research, training and the fight against tsetse-transmitted trypanosomosis. The Sideradougou pastoral zone was the site of many research experiments. This area was ideal to test the integrated tsetse control techniques, the sterile insect technique and to evaluate the efficacy of barriers to prevent tsetse invasion (Cuisance and Politzar, 1983; Politzar and Cuisance, 1983a,b, 1982, 1981; Cuisance *et al,* 1980; Politzar *et al.,* 1979). At a later stage, the pastoral zones of Samoroguan, Yalé, Békuy and Satiri were used by CRTA to test the insecticide-treated target and trap technology, insecticide-treatment of livestock and chemotherapy with participation of beneficiaries (Bauer *et al.,* 1999; 1995, 1992; CIRDES, 1996).

2.3. Tsetse and animal trypanosomosis situation in Burkina Faso

In 1949, Vilain (1949) produced a map fixing the northern limit of *Glossina tachinoides* on the 13th parallel up to the longitude of Kaya, declining towards 60 or 70 km north of Fada N'Gourma. By 1953, a map was issued, based on fly captures made between 1945 and 1952 (Laveissière, 1976b). According to this map, the distribution of G. tachinoides was limited to the Nazinon River and the border between Ghana and Burkina Faso. According to Rickenbach (1961), tsetse flies were present in the vicinity of Ouahigouya, Kaya, Pissila and Fada N'Gourma. The northern limit of the flies was not precisely defined.

After the big drought of 1972-1973, entomological surveys were carried out to update the distribution of the different tsetse species in Burkina Faso (Laveissière, 1976b). Two riverine species of tsetse i.e. *G. palpalis gambiensis* and *G. tachinoides* and one savannah species, *G. morsitans submorsitans,* were the most prevalent. Up to now, these three species remain the most important. Two other species, *G. longipalpis* and *G. medicorum* are present in the south of the country, along the border with Côte-d'Ivoire (Challier and Laveissiere, 1977). *Glossina Medicorum* is still present in the Diéfoula forest, in the forest galleries of *Guibourtia copalifera* (Rayaissé *et al.,* 2009; Bouyer, personal communication). The historical limit of the distribution of tsetse was situated along the 13th degree north in the western part of the country. The most northern place of tsetse capture was the village of Feremané located along the 13° 17' latitude (Challier and Laveissière, 1977).

During the last four decades, climate change, erosion and anthropogenic changes led to environmental degradation reducing forest galleries into thin patches of vegetation along the rivers. This degradation was enhanced by agricultural developments such as dams and irrigation schemes resulting in a substantial reduction in the vegetation coverage in the northern and central parts of the country. As a result of deforestation, the large-scale use of pesticides and disappearance of wild animal, *G. morsitans submorsitans* disappeared from the Mouhoun River basin and almost from the entire cotton zone in the southern part of the country (Bouyer, 2006; Tamboura *et al.,* 2000; Hendrickx, 1999). Currently, the limits of the tsetse distribution are situated south of the distribution limits identified in the 1970s. During the 1980s, entomological monitoring carried out by Küpper (1980) in the region of the Boucle du Mouhoun, showed that the northern limit of *G. palpalis gambiensis* and *G. tachinoides* was the edge of the curve of the Mouhoun River. Recent surveys carried out by CIRDES between 1999 and 2007 within the same area showed a northern limit similar to the one drawn by Küpper (Fig. 2.1) (Guerrini, 2009). The baseline entomological survey carried out by PATTEC in 2008 in the same area, supports these results. The most northern point where tsetse flies were captured is latitude 12°45’ (Sidibé, personal communication).

Figure 2.1. Limit of the distribution of tsetse flies in the area of the Boucle du Mouhoun according to Küper (1980) and adapted by Guerrini (2009)

Nota : *G p. gambiensis* in red and *G tachinoides* in orange

Notwithstanding the changes in the distribution of tsetse, trypanosomosis remains a major constraint to livestock production and intensification, especially so when improved livestock breeds are introduced in a tsetse infested zone. The distribution of trypanosomosis is very similar to the distribution of tsetse flies. However, due to cattle movements, animals outside the tsetse belt are also infected. Three major pathogenic trypanosome species that are transmitted by tsetse flies (i.e. *Trypanosoma vivax, T. congolense* type Savannah, and *T. brucei brucei*) are present in Burkina Faso. A survey to study the epidemiology of animal trypanosomosis was carried out in the region of Hauts Bassins. It revealed a parasitological prevalence in cattle of about 20% (Koné *et al.,* 1983). A more recent survey covering the entire tsetse-infested area was carried out by Bengaly *et al.* (2001a). It showed that the average prevalence of antitrypanosomal antibodies in cattle was 43% with prevalence rates reaching up to 71% in some zones such as the province of Comoé, neighboring Côte-d'Ivoire.

Recently Bouyer and Bengaly (2006), conducting a survey in the Mouhoun River basin, found serological prevalences of more than 80%. However, the parasitological prevalence was low due to frequent use of trypanocidal drugs in this trypanosomosis endemic zone. Similar studies in other endemic areas showed comparable results. For example, in the agro-pastoral zone of Sideradougou, the serological prevalence of trypanosomosis in cattle was about 82% with a low parasitological prevalence (Desquesnes *et al.,* 1999).

The outcomes of the surveys showed that irrespective of the season, the serological prevalence of trypanosome infections in susceptible cattle is high. During the rainy season, the tsetse population grows and its distribution increases due to the favourable environmental conditions, increasing the risk of trypanosome transmission. During the dry season, tsetse population density decreases and flies are concentrated in forest galleries were animals are challenged while drinking.

2.4. Development of tools to diagnose trypanosomosis

Since the 1990s, CIRDES has made significant progress in the development and evaluation of tools for the improved diagnosis of trypanosomosis. Such tools include the Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of anti-trypanosomal antibodies (Duvallet, 1984; Kanwé *et al.,* 1992; Bocquentin and Duvallet, 1990). In collaboration with the International Livestock Research Institute (ILRI, Nairobi, Kenya), studies conducted at CIRDES on the use of the Polymerase Chain Reaction (PCR) in the diagnosis of trypanosomosis significantly increased the sensitivity of the diagnosis of trypanosomal infections in animals and tsetse flies contributing to a better understanding of the

epidemiology of AAT in West Africa (Solano *et al.,* 1995, 1999; Reifenberg *et al.,* 1997; Bengaly *et al.,* 2001b; Lefrançois *et al.,* 1999). For the molecular diagnosis, Desquesnes *et al*. (2001) used primers designed by McLaughlin *et al.* (1996) in the Internal Transcribed Spacer (ITS1) region of the ribosomal DNA (rDNA). These primers can amplify in one PCR reaction the DNA sequence of all trypanosomes species located between 18S and 5.8S of the rRNA gene. The ITS1 region which is ribosomal DNA is common to all the Kinetoplastids, but its size varies from one species to another, hence its usefulness for PCR (Bachir, 2005; Gueye, 2004). Similarly, Geysen *et al*. (2003) used a single polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay to characterise all important bovine trypanosome species.

2.5. Trypanosomosis control programmes in Burkina Faso

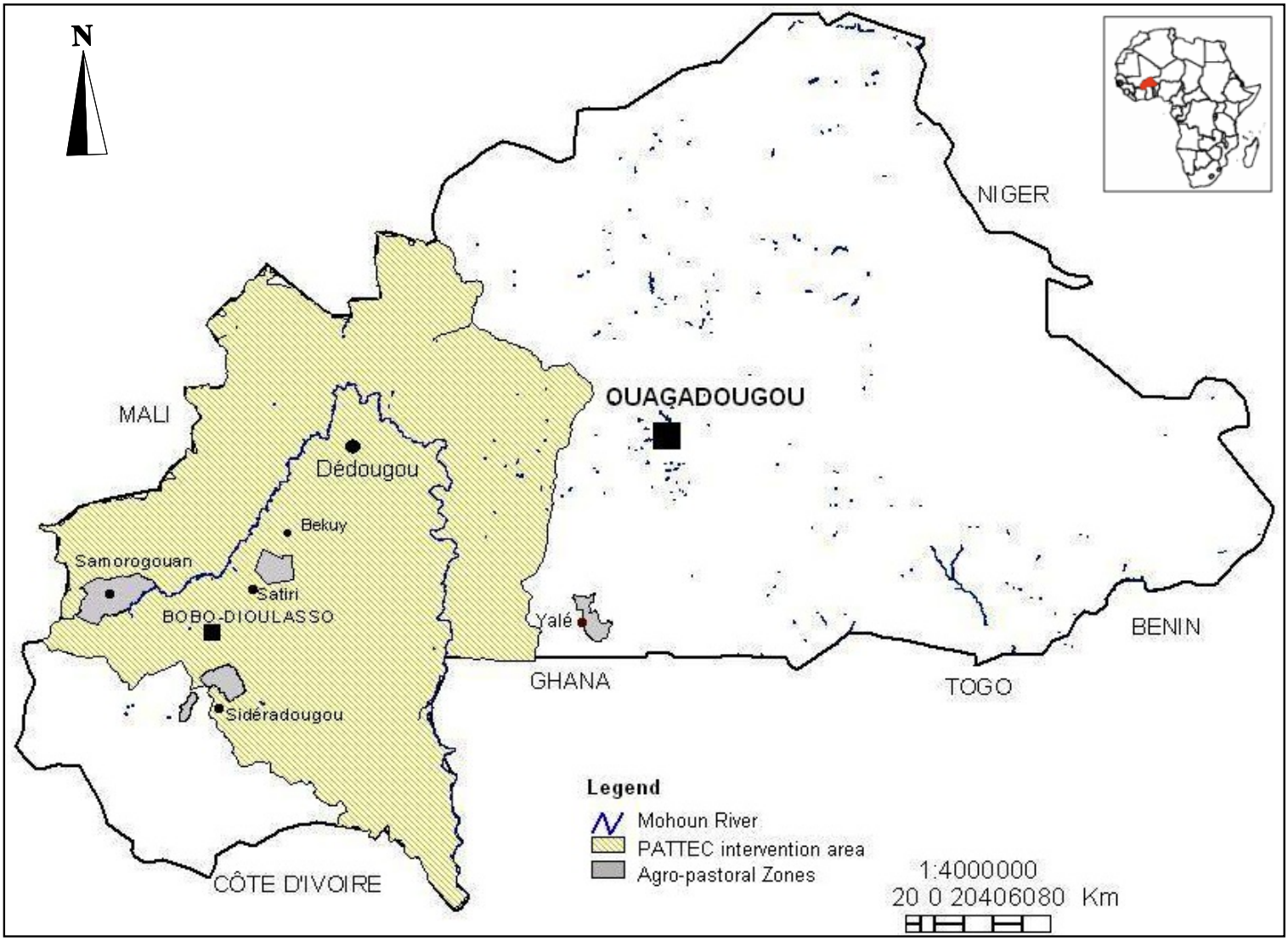
2.5.1. Vector control programmes

Several experiments using various tools to control tsetse have been conducted in Burkina Faso. During the 1970s, CRTA and ELAT based in Bobo-Dioulasso, played an important role in these experiments. The CRTA insectarium was established to mass-produce tsetse flies for experiments in the use of the Sterile Insect Technique (SIT). Between 1974 and 1979, the tsetse colony was maintained on rabbits (800 rabbits) and guinea-pigs (350 guinea-pigs) (Sellin *et al.,* 1980, 1977). Later on, flies were fed on blood through silicone membranes, allowing an increase in the colony size of female tsetse flies from 50,000 to 300,000 during the 1980s (Bauer *et al.,* 1984; Kaboré, 1982). Between 1974 to 1980, several experiments were conducted to refine the SIT and define operational parameters that were used in the Sideradougou pastoral zone and later campaigns (Cuisance *et al.,* 1979, 1978b; Clair *et al.,* 1978).

The Sideradougou tsetse control campaign covering an area of about 300,000 ha was an excellent example of an integrated approach to tsetse control. In this campaign combined use was made of insecticide-treated screens during the dry season and the release of sterile male flies, reared at CRTA, during the rainy season. The campaign targeted 3 tsetse species and was carried out over a 3 years period. Two trap barriers of 7 km wide and preventing the reinvasion of tsetse were deployed along the two main rivers. The campaign was technically a great success at acceptable costs (Politzar *e al.,* 1979; Cuisance *et al.,* 1984b; Brandl, 1988). Within 2 years the apparent density of tsetse dropped from about 65 (*G. tachinoides*) and 44 (*G. palpalis gambiensis*) flies/trap/day, to one or less than one (Cuisance *et al.,* 1985b, 1984c; Politzar *et al.,* 1985; Mérot *et al.,* 1984).

Despite the campaign's success, its achievements could not be sustained because no follow up was carried out after the control campaign. Hence, the re-invasion of tsetse flies in Sideradougou was reported in 1986 (Bauer *et al.,* 1988). Fifteen years later, the tsetse flies situation in Sideradougou became similar to the one before the control campaign (de La Rocque *et al.,* 2001).

Trypanosomosis control campaigns were also conducted in other zones of Burkina Faso (Fig. 2.2). Between 1993 and 1996, CIRDES carried out a trypanosomosis control operation in the agro pastoral zone of Yalé (Bauer *et al.,* 1999). This campaign used traps and screens impregnated with deltamethrin (1%) or cypermethrin (1%) during the dry season. During the rainy season, use was made of insecticide-treated cattle. A total of 1500 screens were deployed along the Sissili River, the main river of the zone. The deployment resulted in a dramatic reduction in the density of tsetse flies and the prevalence of trypanosomosis in livestock resulting in an increase in the farmers' income. Within 3 years, the apparent density of tsetse dropped from 60 to less than one fly/trap/day. The bovine trypanosomosis prevalence dropped from 30% to less than 5% (Bauer *et al.,* 1999). The control campaign resulted in a 25% increase in herd size and an increase in the number of oxen. Milk yield increased from 0.2 to 2.2 and 0.7 to 3.7 liters per cow per day in the dry season and rainy season, respectively (Kamuanga *et al.,* 2001a). Unfortunately, because of the high invasion pressure of tsetse from the adjacent Nazinga ranch, tsetse density increased sharply after the control campaign came to an end.



eas of tsetse and trypanosomoses control in Burkina Faso

Similar integrated tsetse control campaigns with participation of local communities were carried out in the Satiri and Bekuy zones (1986-1995), in Samorogouan (1989-1995) and in Padema between 1996 and 1998 (Bauer *et al.,* 1999, 1995, 1992). All campaigns used pour-on insecticide treatments of livestock and impregnated traps or screens. They resulted in a significant reduction in the tsetse population density and in the trypanosomosis prevalence in these areas. In Satiri, use was made of a flumethrin pour-on and the deployment of insecticide-treated targets in less accessible areas. Within 2 years, the trypanosomosis prevalence was less than 1.4%, and the apparent density of tsetse decreased more than 10 times (Bauer *et al.,* 1992). In Samorogouan too, the campaign was very effective. Within 11 months, the tsetse population was significantly reduced and the remaining flies were located in few zones where cattle didn't have access. Despite the impressive results and in the absence of effective barriers, tsetse flies reinvaded the control areas at the end of the campaign.

The insecticide-treatment of cattle has proven to be an effective tsetse control method and has the added advantage to also control other blood-sucking arthropods such as tabanids, Stomoxys and most importantly, ticks. An important constraint to this technique is the high cost of insecticides for farmers in Burkina Faso. Another problem is the farmers' illiteracy and the insufficient training in the correct use of the chemicals. Moreover, the method is only effective when carried out over a wide area and for a sufficiently long period with participation of farmers and public services in place. Based on observations on the preferred feeding sites of tsetse and to minimize the costs of insecticide treatments, the effectiveness of footbaths filled with an insecticide solution was investigated (Bouyer *et al.,* 2007a; Torr *et al.,* 2007). In the peri-urban areas, footbaths proved to be effective in reducing the incidence of animal trypanosomosis (Bouyer *et al.,* 2007a).

To improve the efficacy of targets and traps, different attractants such as cresol, octenol and acetone were tested and showed to be attractive for *G. tachinoides* and *G. morsitans submorsitans* (Amsler *et al.,* 1994). Biconical traps baited with cresol and octenol captured up to 2.5 times more *G. tachinoides* than unbaited traps (Filledier *et al.,* 1989). Politzar and Mérot (1984), using this combination captured up to 7 times more *G. m. submorsitans.* Animal odours, such as urine also showed to be attractive to G. tachinoides and *G. morsitans submorsitans* in both laboratory and field experiments (Mérot *et al.,* 1988; Filledier *et al.,* 1988). Notwithstanding these promising results, odour attractants were never adopted during control operations.

2.5.2. Trypanocides treatments

ISM, DA and ethidium bromide have been the trypanocides used most frequently in Burkina Faso. Nowadays, only the two first molecules are readily available. According to statistics of the Ministry of Animal Resources of Burkina Faso, 2.6 million doses of trypanocides are used every year (MRA, 2006). Because of the seasonal transhumance of cattle, trypanosomosis also affects animal arriving from the north and traversing the tsetse-infested zone. There is no approved protocol for the treatment or prevention of animal trypanosomosis in Burkina Faso. Every farmer has to make his/her own decisions based on the health of his/her cattle and his/her own financial means. However, all the farmers in trypanosomosis endemic areas apply at least one treatment per animal per year (Tamboura *et al.,* 2000). In Samorogouan, where the tsetse pressure is high, 6 treatments per year were necessary in 1990, 4 to cure sick animals and 2 to prevent infection (Clausen *et al.,* 1992). For transhumant cattle, one treatment at the beginning and a second treatment at the end of transhumance usually suffices (Tamboura *et al.,* 2000).

2.6. Chemoresistance in Burkina Faso

Despite the fact that 35 million doses of trypanocides are used annually throughout Africa (Geerts *et al.,* 2001), the trypanocidal drug industry doesn't seem to be profitable enough for manufacturers to invest into research to develop new compounds (Seed, 2001; Vreysen, 2006). As a result, the use of the same molecules for over 40 years has resulted in the selection of resistant strains in most of the tsetse-infested zones of Burkina Faso and elsewhere in Africa (Delespaux *et al.,* 2008). Trypanocidal drug resistance was described first in East Africa and is now widely spread over SSA (Geerts and Holmes, 1998). It was reported first in Burkina Faso in the early 1980s in Samorogoan, Kénédougou Province (Authié, 1984; Pinder and Authié, 1984). The *T. congolense* strain, Sam/CRTA/20, isolated in 1980 was partially resistant to ISM at the dose of 0.5 mg/kg in mice. *Trypanosoma congolense* Sam/CRTA/ 53, isolated in 1982, was highly resistant to ISM (Pinder and Authié, 1984). Both strains were also resistant toDA (Authié, 1984).

At a later stage, resistance to ISM was demonstrated in experimentally infected cattle (Clausen *et al.,* 1992). The same experiments showed that a *T. congolense* strain isolated in Samorogouan (Samorogouan/89/CRTA /26 7.1) was resistant to ethidium bromide and to all the other trypanocides available (Clausen *et al.,* 1992). McDermott *et al.* (2003) conducted a trypanocidal drug resistance survey in Kénédougou. The survey showed that resistant *T.*

congolense strains were distributed over the entire province. Recently, resistance was also demonstrated in the neighbouring Sikasso region of Mali and in Guinea (Talaki *et al.,* 2007).

In Burkina Faso, multiple resistance is suspected in other areas, such as the cotton belt and the South-West region neighbouring the border with Côte-d'Ivoire and Ghana. Additional studies are required to confirm this suspicion and to find out whether the resistant strains are similar to the ones isolated in Samorogouan. Chemoresistance is spreading fast in the subhumid zone of West Africa. By the end of the 1990s, it was reported in 13 sub-saharan African countries (Geerts and Holmes, 1998). Nowadays, it is reported in 18 countries where animal trypanosomosis is enzootic (Delespaux *et al.,* 2008).

The reasons for the development of chemoresistance include the long-term use of the same compounds and the mismanagement of veterinary drugs in sub-Saharan Africa, since the privatization of the veterinary profession in the 1990s and reduced control of the public sector over drug supply (Sen and Chander, 2003). The latter situation led to an increase in fraud and the production and sales of low quality trypanocides. Only 30% of the trypanocides used annually in Burkina Faso (over 2.6 million doses), are recorded officially as being imported by the veterinary services (MRA, 2006). In addition, quality control showed that most of the manufacturers don't comply with the standards. According to a FAO study, more than 65% of diminazene-based trypanocides sold in Sub-Saharan Africa don't meet the quality standards (Tettey *et al.,* 2002). Trypamidium® (Merial SAS, Lyon, France) is the only stable chloride-based preventive trypanocide sold in Burkina Faso (Schad *et al.,* 2008; Tettey *et al.,* 1999).

2.7. Trypanotolerant breed in Burkina Faso

Because the prevalence of resistant strains is increasing and tsetse control campaigns are difficult to sustain, some experts recommend the use of trypanotolerant cattle breeds in tsetse-infested areas (d'Ieteren *et al.,* 1998). Trypanotolerant livestock breeds are raised in the AAT endemic zones of the southern part of Burkina Faso. The most important trypanotolerant taurine cattle breeds in Burkina Faso are the baoule and the lobi. The trypanotolerant small ruminant breeds are the Djallonke and Dwarf goat. Baoule or lobi breeds are usually raised in traditional systems where there is no veterinary care (Sow, 2002), and fortunately, they are also resistant to parasitism such as helminthiasis (Mattioli *et al.,* 1992). For a few decades now, taurine breeds have come in competition with the bigger sahelian breeds that have a higher market value and more power. The preservation of the baoule breed is threatened by this infiltration of zebu cattle into the taurine cattle distribution area. Twenty years ago, the taurine

population represented more than 16% of the total bovine population (Hoste *et al.,* 1988). Nowadays, this ratio has decreased drastically, while the number of crossbred animal increased. This trend is favoured by the fact that crossbreds are more resistant to trypanosomosis than zebu cattle and crossbreeding is sometimes practiced by farmers. CIRDES is conducting research aiming at identifying genetic markers associated with trypanotolerance. The findings of this research could support better planning of selection and breeding programs for trypanotolerant cattle (CIRDES, 2006). Trypanotolerant cattle's farming allows reduction in the use of trypanocides and therefore, farm investments costs. However, to support extension programs economical analysis of its profitability needs to be carried out.

2.8. Social and economical impact of trypanosomosis

The economy of Burkina Faso relies mainly on agriculture and livestock. Because of the unreliability of the rains during the last years, many farmers have migrated towards southern regions of the country where rains are more frequent. Unfortunately, these regions are tsetse infested Shaw (2004) showed that the mortality rate of susceptible cattle living in such trypanosomosis endemic zones is 20% higher in calves. Trypanosomosis also results in a decrease in the calving rate, the milk production and the labor output (up to 38%).

Nevertheless, the exact economic impact of animal trypanosomosis is difficult to assess. Considering the fact that about 2.6 million doses of trypanocides are used annually, and at a cost of 1.5 US$ per dose, treatment alone already results in an annual expense of about 3.9 million US$.

Socio-economic surveys were carried out by Kamuanga and colleagues (Kamuanga *et al.,* 2001a; 2001b), in the Yalé pastoral zone and by Ouédraogo (2002) and Ouédraogo *et al*. (2004) in Kénédougou province. The Yalé survey was conducted during the trypanosomosis control campaign between 1993 and 1997 (Bauer *et al.,* 1999) and allowed to estimate the households' income. Thanks to the control campaigns, the subsequent reduction in the incidence of trypanosomosis led to a significant increase in the livestock population growth rate (up to 25%), milk production and the number of draft animals. At the same time, the rate of stillbirths decreased dramatically (Kamuanga *et al.,* 2001b). These results again show the negative impact of trypanosomosis on livestock in zones with high tsetse challenge.

The survey in the Kénédougou province was conducted without any ongoing control programmes. The study reported economic data on rural households' trypanocides expenses. The estimated cost to control trypanosomosis was 2,500 FCFA (5.25 US$) per animal per year

(Ouédraogo, 2002; Ouédraogo *et al.,* 2002). A herd simulation mode1 was applied to estimate direct economic benefits of a tsetse eradication campaign in the pastoral zone of Sideradougou. Three hypotheses for the impact of trypanosomosis on cattle productivity were tested in view of production increases after tsetse eradication. Value of additional production over a period of 10 years after tsetse eradication exceeds the value without eradication of 8,100 F CFA (16.2 US $) per hectare by 1000, 3,560 or 5,980 F CFA under the low, medium or high assumptions, respectively. Calf mortality was therefore set to 23, 19 and 15 % for hypotheses 1, 2 and 3, respectively. Milk production accounts for about 19 % of the total value of production in any of the scenarios (Brandl, 1985). There is no information available on the costs and benefits of the control campaigns in Samorogouan, Satiri or Békuy. However, socio-economic surveys showed real improvements in the farmers' income. The control campaigns improved the health status of the cattle, increased the herd size, the calving rate, milk production, the number of labor animals and lowered trypanocidal drug use (Kamuanga *et al.,* 2005).

2.9. Future prospects

2.9.1. Participation of beneficiary communities

According to Kamuanga (2003), community participation is a social process whereby specific groups with shared values, living in a defined geographical area actively pursue identification of their needs. Thus, where a programme is introduced from outside, efforts are made to sensitize the community to the issue to enable its members to understand the programme and make informed decisions. The notion reconciles outside objectives with local priorities, and provides an environment for community mobilization to enable active and sustained participation.

In 1990, community participation was tested by the PDRI/HKM project (Projet de Développement Rural Intégré/ Houet-Kossi-Mouhoun) financed by PTLA/FAO in 27 villages of 2 districts located along the Mouhoun River. Trypanosomosis control included a tsetse control component (target screens and insecticide treatments of cattle) and trypanocidal drug treatments of infected animals. Local trypanosomosis control committees were established at the village and district levels. Their role consisted of providing information, sensitization, and the collection of the contributions from the farmers, maintenance and re-impregnation of the screens every three months and their withdrawal during the rainy season. The farmers, from their part, have adhered to the initiative by accepting the insecticide treatment of all their cattle and the treatment of trypanosomosis cases. The chemotherapy and the insecticide treatment of

cattle were performed by private veterinarians. CIRDES was requested to evaluate the impact of the project on the animal health and the tsetse density. The PDRI/HKM project provided financial support in the form of equipment, the insecticides and 1500 screens to be displayed along the Mouhoun River edge. Moreover, 20% of the costs of the insecticide-treatment of cattle and 80% of the cost of a trypanocidal drug treatment were subsidized by the project. Spectacular results were obtained within 7 months of the control operation (Sigué, 1998). The decrease in the trypanosomosis incidence and the tsetse density significantly reduced the need for insecticide-treatments of cattle and, hence the participation of the livestock owners. One of the weaknesses of the experiment in participation was the low participation of a number of livestock owners and the transhumant herds in the project area (Bontoulougou *et al.,* 2000).

2.9.2. Remote sensing

In East and southern Africa, especially in Kenya, Tanzania, Zambia and Zimbabwe, remote sensing has been used to define priority zones for tsetse control (Robinson, 1998; Rogers and Randolph, 1993, 1991). In Togo, applying remote sensing allowed to identify tsetse habitat for the six tsetse species present in the country (Hendrickx *et al.,* 1999).

In Burkina Faso, remote sensing allowed to determine trypanosomosis high risk zones in Sideradougou for targeted control activities (de La Rocque, 1997; de La Rocque *et al.,* 2001). During a study carried out by de La Rocque *et al.* (2004) in the agro-pastoral of Sidéradougou, high spatial resolution SPOT (Satellite Pour l'Observation de la Terre) imagery has been used to identify the habitats of riparian tsetse flies with special emphasis on human impacts. By comparing field information and spectral values on the image, they carried out a supervised classification for the whole of the image and then focused analysis on the valleys. This analysis allowed drawing areas more or less favourable to tsetse along the hydrographical network (Michel *et al.,* 2002; de La Rocque *et al.,* 2001). In addition, detailed data on land-cover and herd management were combined, to evaluate host-vector interfaces. Results showed that although riparian tsetse flies are present all over the drainage system, distinct spatial patterns appear in the distribution of trypanosomosis. Some landscapes features, such as agriculture density, which can be remotely sensed, appeared to be good indicators for the characterization of local tsetse populations and their role in the transmission of trypanosomosis. Finally, spatial modeling tools were used to highlight epidemiological hot spots. These data allowed setting control strategies adapted to the local communities' capacities.

This approach was generalized later in the whole Mouhoun basin. Another study was carried out to assess the abundance of tsetse flies and AAT risk using remote sensing coupled to field environmental data, along a Mouhoun River section of 234 km long, harbouring an open riverine forest where *G. tachinoides* is the predominant tsetse species. The water course was classified into three different epidemiological landscapes, corresponding to a "disturbed", "natural" and finally "border" vegetal formation at the interface of the two former ones. Using the mean number of infected flies per trap per day as a risk indicator, the border landscape was found to be 5.4 and 15.8 times more risky than the natural and disturbed ones respectively. These results led to propose that a control campaign should be focussed on only 34% of the hydrographic network (Bouyer *et al.,* 2006; Guerrini and Bouyer, 2007).

2.9.3. Tsetse population genetics

Tsetse flies can either be controlled or eradicated. To eradicate, tsetse fly populations must be isolated. The degree of isolation of tsetse population can be determined by comparing the genetic composition of that specific populations with that of surrounding populations (Solano *et al.,* 2000; 1999).

Intraspecific genetic variability of *G. p. gambiensis* in the agro-pastoral zone of Sideradougou was determined using polymorphic microsatellite DNA markers. This genetic study was combined with other epidemiological information on the same tsetse i.e. blood meal identification, dissection of tsetse and molecular characterization of the trypanosomes detected. There was significant genetic differentiation among flies caught only a few kilometers apart, within the same riverine habitat. These distinct subpopulations also had different infection rates. In part of the study area, a Factorial Correspondence Analysis undertaken on the genotypes detected a Wahlund effect, suggesting the presence of tsetse originating from different source populations coming from two distinct drainage systems. The apparent structuring of populations of *G. p. gambiensis* is discussed relative to appropriate strategies to control African Trypanosomosis.

The structure of a population of *G. p. gambiensis* was analysed in the Mouhoun River basin (Bouyer *et al.,* 2007b). In this study, allele frequencies at five microsatellite loci, and metric properties based on 11 wing landmarks, were compared among four populations. The populations originated from the Mouhoun River and one of its tributaries. The among populations distances were 74, 61 and 81km upstream to downstream, totalizing 216km between the first and the fourth one, superimposed to an ecological cline. Both microsatellites

and wing geometry demonstrated a structuring between the populations, but no complete isolation. There was no clear relation between gene flow and geographic position whereas a positive correlation between metric distances and geographic distances was observed, and attributed mainly to the cline of environmental conditions. In the same area using *G. tachinoides,* Koné *et al.* (2010) showed that there was a complete panmixia and no genetic differentiation among population. This result was totally different from that obtained with *G. p. gambiensis* (Bouyer *et al.,* 2007b). In the absence of efficient and sustainable barriers, average recolonization rate for *G. p. gambiensis* would be of 7.5 km/year (Bouyer *et al.,* 2009a). It would be much higher for *G. tachinoides*. Control programs such as PATTEC acknowledge the importance of having such genetic information in designing more effective control strategies.

2.9.4. Importance of environmental factors

In Burkina Faso, game reserves and classified forest have been created for nature conservation or biodiversity programmes and cover about 38,150 km2 (14% of the total surface of the country) (MECV, 2004). In the PATTEC intervention area, classified forests represent 7% of the total area. Some of these reserves such as the classified forest of Kari are situated along of the Mouhoun River. Hence, such protected areas often are important reservoirs of tsetse and are real threats to tsetse cleared areas. This was the case during the control campaign carried out in the agro pastoral zone of Yalé. Indeed, the tsetse-cleared area was adjacent to the Safari and to the game ranch of Nazinga where high tsetse densities of more than 100 tsetse per trap per day had been recorded (Bauer *et al.,* 1999). After the campaign came to an end, tsetse re-invasion started.

In view of the above and notwithstanding the importance of conservation, there is a need for consultation between the ministries in charge of the management of the forest reserves, game ranches and livestock and T&T control initiatives to improve the sustainability of the achievement of control campaigns. The elimination of tsetse should be extended to protected areas to avoid the creation of reservoirs of tsetse. This implies that methods used to control or eradicate the fly should be environmentally friendly to reduce their impact on such fragile environments.

A good example of a successful vector elimination campaign was the Onchocerciasis Control Programme (OCP). This campaign was planned for a period of 20 years and conducted in 11 countries including Burkina Faso. It made use of pesticides targeting the aquatic larval population living in the fast flowing parts of the rivers without any measurable long-term

effects of the interventions on the fish population in term of abundance of species, trophic structure, community structure or health (Paugy *et al.,* 1999).

2.10. Conclusion

Trypanosomosis remains the main constraint to animal production and agricultural development in Burkina Faso. Most of the tsetse and trypanosomosis control campaigns have been very successful. Indeed, tsetse populations seem to be very vulnerable and can be controlled easily using the available arsenal of control tools (Bauer *et al.,* 1999, 1995, 1992; Cuisance *et al.*, 1990). However, previous experience has shown that such tsetse control operations are difficult to sustain. Moreover, sustainable use of trypanocidal drugs is threatened by the development and spread of trypanocidal drug resistance in trypanosomes (Talaki *et al.,* 2007; McDermott *et al.,* 2003; Clausen *et al.*, 1992). Nevertheless, trypanocidal drugs remain the main control tools used by livestock owners. Climate change and anthropogenic impacts on the ecosystems could lead to reduction of tsetse habitat (Rayaissé *et al.,* 2009; Reid *et al.,* 2000; Jordan, 1986), though natural extinction of flies is not expected because of their adaptive capacity.

It thus seems that tsetse eradication offers an attractive solution to the trypanosomosis problem in Burkina Faso. Such progressive tsetse control leading to eradication requires political will and combined actions between the different actors, at national and international level. In Burkina Faso, most of the failures in tsetse and trypanosomosis control campaign are attributed to the lack of commitment of the local beneficiaries and the limited geographic scope of the intervention. Through the PATTEC programme, an area-wide approach is currently adapted. It is hoped that this approach will ultimately lead to the eradication of tsetse from Burkina Faso and ultimately from the entire African continent.

2.11. References

Amsler, S., Filledier, J., Millogo, R., 1994. Attractifs olfactifs pour la capture de *Glossina tachinoides* et *Glossina morsitans submorsitans* (Diptera , Glossinidae) au Burkina Faso. Effet de la position du sachet diffuseur dans le piège biconique Challier-Laveissière. Rev. Elev. Méd. Vét. Pays trop. 47, 301–311.

Authié, E., 1984. Mise en évidence d'une résistance aux trypanocides parmi des souches de *Trypanosoma congolense* récemment isolées au Burkina. Rev. Elev. Méd. Vét. Pays trop. 37, 219–235.

Bachir, S.K., 2005. Evaluation du diagnostic par directe et PCR-ELISA sur les ITS des trypanosomes pathogènes du bétail, *Thèse de Doctorat Vétérinaire*, Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV), Dakar, Sénégal.

Bauer, B., Petrich-Bauer, J., Kaboré, I., Kourouma, B., Mattausch, M., Some, J., Tamboura ,I., 1988. Epidemiological survey in the pastoral zone of Sidéradougou, Burkina Faso. pp. 139–149 in Modern insect control, nuclear techniques and biotechnology. IAEASM-301/44, Vienna.

Bauer, B., Amsler-Delafosse, S., Clausen, P.-H., Kaboré, I., Petrich-Bauer, J., 1995. Successful application of deltamethrin pour-on to cattle in a campaign against tsetse flies (Glossina spp.) in the pastoral zone of Samorogouan, Burkina Faso. Trop. Med. Parasitol. 46, 183–189.

Bauer, B., Amsler-Delafosse, S., Kaboré, I., Kamuanga, M., 1999. Improvement of cattle productivity through rapid alleviation of African animal trypanosomosis by integrated disease management practices in the Agro-pastoral zone of Yale, Burkina Faso. Trop. Anim. Health Prod. 31, 89–102.

Bauer, B., Filledier, J., Kabore, I., 1984. Large scale rearing of tsetse flies (Diptera, Glossinidae) in the C.R.T.A. Bobo-Dioulasso, Burkina based on in vitro feeding techniques. Rev. Elev. Méd. Vét. Pays trop. 37, 9–17.

Bauer, B., Kaboré, I., Liebisch, A., Meyer, F., Petrich-Bauer, J., 1992. Simultaneous control of ticks and tsetse flies in Satiri, Burkina Faso, by the use of flumethrin pour-on for cattle. Trop. Med. Parasitol. 43, 41–46.

Bengaly, Z., Ganaba, R., Sidibe, I., Desquesnes, M., 2001a. Trypanosomose animale chez les bovins dans la zone Sud-soudanienne du Burkina Faso. Résultats d'une enquête sérologique. Rev. Elev. Méd. Vét. Pays trop. 54, 221–224.

Bengaly, Z., Kasbari, M., Desquesnes, M., Sidibé, I., 2001b. Validation of a polymerase chain reaction assay for monitoring the therapeuthic efficacy of diminazene aceturate in trypanosome-infected sheep. Vet. Parasitolol. 96, 101–113.

Bocquentin, R., Duvallet, G., 1990. Amélioration de la reproductibilité du test ELISA adapté à la détection d'anticorps anti-*Trypanosoma congolense* chez les bovins. Rev. Elev. Méd. Vét. Pays trop. 43, 179–186.

Bontoulougou, J., Oulé, J.M., Pellissier, J.P., Tallet, B., 2000. La participation des acteurs, un exercice difficile. Leçons de l'expérience d'un plan de lutte contre la trypanosomose animale africaine dans la vallée du Mouhoun (Burkina Faso). Revue Natures, Sciences, Sociétés 8, 33–43.

Bouyer, J., 2006. Ecologie des glossines du Mouhoun au Burkina Faso , intérêt pour l'épidémiologie et le contrôle des trypanosomoses africaines. *Thèse de Doctorat* d'Université Montpellier II.

Bouyer, J., Balenghien ,T., Ravel, S., Vial, L., Sidibé, I., Thévenon, S., Solano, P., De Meeûs, T., 2009. Population sizes and dispersal pattern of tsetse flies, rolling on the river? Mol. Ecol. 18, 2787–2797.

Bouyer, J., Bengaly, Z., 2006. Evaluation de la situation entomologique et épidémiologique en vue de l'élaboration d'un plan de lutte contre les trypanosomoses animales et leur vecteur dans la zone d'intervention du PAEOB. Rapport d'expertise, Bobo-Dioulasso, Burkina Faso.

Bouyer, J., Guerrini, L., Desquesnes, M., de La Rocque, S., Cuisance, D., 2006. Mapping African Animal Trypanosomosis risk from the sky. Vet. Res. 37, 633–645.

Bouyer, J., Ravel, S., Dujardin, J. P., De Meeûs, T., Vial, L., Thévenon, S., Guerrini, L., Sidibé, I., Solano, P., 2007b. Population structuring of *Glossina palpalis gambiensis* (Diptera, Glossinidae) according to landscape fragmentation in the Mouhoun River, Burkina Faso. J. Med. Entomol. 44, 788–795.

Bouyer, J., Stachurski, F., Kaboré, I., Bauer, B., Lancelot, R., 2007a. Tsetse control in cattle from pyrethroid footbaths. Prev. Vet. Med. 78, 223–238.

Brandl, F.E., 1985. The use of a herd simulation model for the estimation of direct economic benefits of tsetse control. Application to the pastoral zone of Sideradougou, Burkina Faso. Rev. Elev. Méd. Vét. Pays trop. 38, 364–370.

Brandl, F.E., 1988. Economies of trypanosomosis control in cattle. Farming systems and resource economics in Lie tropics. vol. 1. Germany, 7-8 Mars 1988. Kiel, Germany, Wissenschaftsverlag.

Challier, A., 1986. La lutte contre les glossines dans les foyers de maladie du sommeil, Tendances actuelles. IVè Congres sur la Protection de la Sante Humaine et des Cultures en Milieu Tropical, Marseille 2-3-4 juillet 1986.

Challier, A., Laveissiere, C., 1977. La répartition des glossines en Haute-Volta.1 carte couleur 1/2000000 et notice explicative. Paris, ORSTOM 39p.

CIRDES, 1996. Rapport scientifique et technique de recherche développement. Année 1995. Bobo-Dioulasso. Burkina Faso, 82p.

CIRDES, 2006. Rapport scientifique et technique de recherche développement. Année 2005. Bobo-Dioulasso. Burkina Faso.

Clair, M., Cuisance, D., Politzar, H., Sellin, E., Taze, Y., 1978. Synthèse des recherches effectuées en 1975 - 1976 et 1977 sur la lutte génétique contre *Glossina palpalis gambiensis* Vanderplank, 1949, par lâchers de mâles irradiés. Rapport IEMVT-GTZ.

Clair, M., Politzar, H., Cuisance, D., Lafaye, A., 1976. Observations sur un essai préliminaire de lâchers de mâles stériles de *Glossina palpalis gambiensis* (Haute-Volta). Rev. Elev. Méd. Vét. Pays trop. 29, 341–351.

Clausen, P.-H., Sidibé, I., Kaboré, I., Bauer, B., 1992. Development of multiple drug resistance of *Trypanosoma congolense* in Zebu cattle under natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. Acta Trop. 51, 229–236.

Courtin, F., Jamonneau, V., Duvallet, G., Garcia, A., Coulibaly, B. Doumenge, J. P., Cuny, G., Solano, P., 2008. Sleeping sickness in West Africa (1906–2006), changes in spatial repartition and lessons from the past. Trop. Med.Int. Health 13, 334–344.

Cuisance, D., Fevrier, J., Dejardin, J., Filledier, J., 1985a. Dispersion linéaire de *Glossina palpalis gambiensis* et *G. tachinoides* dans une galerie forestière en zone soudano-guinéenne (Burkina Faso). Rev. Elev. Méd. vét. Pays trop. 47, 69–75.

Cuisance, D., Merot, P., Politzar, H., Tamboura, I., 1984b. Coût de l'emploi d'écrans insecticides dans la lutte contre les glossines dans la zone pastorale de Sidéradougou (Burkina-Faso). Rev. Elev. Méd. vét. Pays trop. 37, 84–98.

Cuisance, D., Politzar, H, 1983. Etude sur l'efficacité contre *Glossina palpalis gambiensis* et *Glossina tachinoides* de barrières constituées d'écrans ou de pièges biconiques imprégnés de D.D.T., de deltaméthrine ou de dieldrine. Rev. Elev. Méd. vét. Pays trop. 36, 159–168.

Cuisance, D., Politzar, H., Clair, M., Sellin, E., Taze, Y., 1978a. Impact des lâchers de mâles stériles sur les niveaux de deux populations sauvages de *Glossina palpalis gambiensis* en Haute-Volta (sources de la Volta Noire). Rev. Elev. Méd. Vét. Pays trop. 31, 315–328.

Cuisance, D., Politzar, H., Clair, M., Sellin, E., Taze, Y., Bourdoiseau, G., Fevrier, J., 1979. La lutte contre *Glossina palpalis gambiensis* Vanderplank par lâchers de mâles irradiés en Haute-Volta , étude de paramètres opérationnels. Colloque international sur l'emploi des isotopes dans l'étude et la destruction des vecteurs de maladies animales, les rapports hôtes-pathogènes et les effets des méthodes de lutte sur l'environnement. Vienne (Autriche) 7-11 mai 1979.

Cuisance, D., Politzar, H., Clair, M., Taze, Y., Sellin, E., 1978b. Opération de lâchers de mâles stériles de *Glossina palpalis gambiensis* à Bobo-Dioulasso (Haute-Volta), efficacité de la lutte autocide. Joint FAO/IAEA. Research Coordination meeting on the use of the sterile insect technique for tsetse fly eradication or control. Anvers (Belgique), 18 -22 septembre 1978.

Cuisance, D., Politzar, H., Février J., Bourdoiseau G., Sellin E., 1980. Association d'un traitement insecticide avec la méthode du mâle stérile contre *Glossina palpalis gambiensis* , intérêt de la mise en œuvre de plusieurs méthodes. Rev. Elev. Méd. Vét. Pays trop. 33, 127–133.

Cuisance, D., Politzar, H., Mérot, P., Tamboura, I., 1984c. Les lâchers de mâles irradiés dans la Campagne de lutte intégrée contre les glossines dans la zone pastorale de Sidéradougou (Burkina Faso). Rev. Elev. Méd. Vét. Pays trop. 37, 449–467.

Cuisance, D., Politzar, H., Mérot, P., Tamboura, I., Bauer, B., Kabore, I., Filledier, J., 1985b. La campagne de lutte intégrée contre les glossines dans la zone pastorale d'accueil de Sidéradougou (Burkina Faso). 18ème Réunion du Conseil Scientifique International de Recherches sur les Trypanosomiases et leur contrôle (CSIRTC/OUA/CSTR.), Harare (Zimbabwe), 4-9 mars 1985.

Cuisance, D., Politzar, H., Tamboura, I., Mérot, P., 1990. Coût de l'emploi de barrières de pièges et d'écrans insecticides pour la protection de la zone pastorale d'accueil de Sidéradougou, Burkina Faso. Rev. Elev. Méd. Vét. Pays trop. 43, 207–217.

Cuisance, D., Politzar, H., Tamboura, I., Mérot, P., Lamarque G., 1984a. Répartition des glossines dans la zone pastorale d'accueil de Sidéradougou (Haute-Volta). Rev. Elev. Méd. Vét. Pays trop. 37, 99–113.

d'Ieteren, G.D.M., Authié, E., Wissocq, N., Murray, M., 1998. Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. Rev. Sci. Tech. Off. Int. Epizooties, 17, 154–175.

de La Rocque, S., 1997. Identification des facteurs discriminants de la présence de glossines dans une zone agro-pastorale du Burkina Faso. Intérêt pour l'évaluation du risque trypanosomien. *Thèse de doctorat* d'Université Montpellier II.

de La Rocque, S., Augusseau, X., Guillobez, S., Michel, V., De Wispelaere, G., Bauer, B., Cuisance, D., 2001. The changing distribution of two riverine tsetse flies over 15 years in an increasingly cultivated area of Burkina Faso. Bull. Entomol. Res. 91, 157–166.

de La Rocque, S., Michel, J. F., Cuisance, D., de Wispeleare, G., Solano, P., Augusseau, X., Arnaud, M., Guillobez, S., 2001. Du satellite au microsatellite. Le risque trypanosomien. Une approche globale pour une décision locale. CIRAD, Montpellier.

de La Rocque, S., Michel, V., Plazanet, D., Pin, R., 2004. Remote sensing and epidemiology, examples of applications for two vector-borne diseases. Comparative Immunology. Microbiol. Infect. Dis. 27, 331–341

Delespaux, V., Geysen, D., Van Den Bossche, P., Geerts, S., 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 24, 236–242.

Desquesnes, M., McLaughlin, G., Zoungrana, A., Dávila, A.M.R., 2001. Detection and identification of *Trypanosoma* of African livestock through a single PCR based on internal transcribed spacer 1 of rDNA. Int. J. Parasitol. 31, 610–614.

Desquesnes, M., Michel, J.F., de La Rocque, S., Solano, P., Millogo, L., Bengaly, Z., Sidibé, I., 1999. Enquête parasitologique et sérologique (ELISA-indirect) sur les trypanosomoses des bovins dans la zone de Sidéradougou, Burkina Faso. Rev. Elev. Méd. Vét. Pays trop. 52, 223–232.

Diarra, B., 2001. Caractérisation de la sensibilité à l'isométamidium et au diminazène des phénotypes de trypanosomes isolés dans la Province du Kénédougou au Burkina Faso. *Thèse Docteur 3e cycle*, Université Cheikh Anta Diop de Dakar, Sénégal.

Duvallet, G., 1984. Variants antigéniques détectés après transmission cyclique d'un clone de *Trypanosoma brucei brucei* chez des bovins trypanosensibles et résistants. Rev. Elev. Méd. Vét. Pays trop. 37, 277–287.

Filledier, J., Duvallet, G. Merot, P., 1988. Comparaison du pouvoir attractif des bovins Zébu et Baoulé pour *Glossina tachinoides* Westwood, 1850 et *Glossina morsitans submorsitans* Newstead, 1910 en savane soudanoguinéenne, Burkina Faso. Rev. Elev. Méd. Vét. Pays trop. 41, 191–196.

Filledier, J., Mérot, P., 1989. Etude de l'attractivité de solutions isolées par fractionnement de l'urine de bovin Baoulé pour *Glossina tachinoides* (Westwood, 1850) au Burkina Faso. Rev. Elev. Méd. Vét. Pays trop. 42, 453–455.

Geerts, S., Holmes, P.H., 1998. Drug management and parasite resistance in bovine trypanosomosis in Africa. PAAT Tech. Sci. Series No. 1. FAO, Rome.

Geerts, S., Holmes, P.H., Diall, O., Eisler, M.C., 2001. African bovine trypanosomosis, the problem of drug resistance, Trends Parasitol. 17, 25–28.

Geysen, D., Delespaux, V., Geerts, S., 2003. PCR–RFLP using Ssu-rDNA amplification as an easy method for species-specific diagnosis of Trypanosoma species in cattle. Vet. Parasitol. 110, 171–180

Guerrini, L., 2009. Le risque trypanosomien dans le bassin du Mouhoun au Burkina Faso , approches paysagères. Télédétection - Biogéographie des glossines - Spatialisation du risque. *Thèse de doctorat* de l'Université Montpellier III, France.

Guerrini, L., Bouyer, J., 2007. Mapping African Animal Trypanosomosis risk, the landscape approach. Veterinaria Italiana 43, 643–654.

Gueye Dramé, A., 2004. Mise au point d'amorces polyspécifiques amplifiant l'ITS1 de l'ADN ribosomal pour une PCR nested unique pour la détection des principaux trypanosomes africains pathogènes du bétail. Mémoire de DEA, Université Cheikh Anta Diop, Dakar, Sénégal.

Hendrickx, G., 1999. Georeferenced decision support methodology towards trypanosomosis management in West Africa. *Ph.D. thesis,* State University of Ghent, Veterinary Faculty, Belgium.

Hendrickx, G., Napala, A., Dao, B., Batawui, D., De Deken, R., Vermeilen, A., Slingenbergh, J.H.W., 1999. A systematic approach to area-wide tsetse distribution and abundance maps. Bull. Entomol. Res. 89, 231–44.

Hoste, C.H., Chalon, E., d'Ieteren, G., Trail, J.C.M., 1988. Le bétail trypanotolérant en Afrique occidentale et centrale Vol. 3 - Bilan d'une décennie ; Etude FAO Production et Santé Animales, FAO Rome, 217p.

Hougard, J.-M., Poudiougo, P., Zerbo, G., Meyer, R., Guillet, P., Agoua, H., Seketeli, A., Akpoboua, A., Sowah, S., Samba, E. M., Quillévéré, D,. 1994. La lutte contre les vecteurs de l'onchocercose en Afrique de l'Ouest, description d'une logistique adaptée à un programme de santé publique de grande envergure. Cahiers Santé 4, 389–398.

Jamot, E., 1926. Au sujet du traitement prophylactique de la maladie du sommeil. Bulletin de la Société de Pathologies Exotiques 19, 463–472.

Jamot, E., 1930. La prophylaxie de la maladie du sommeil In "les grandes endémies tropicales" Tomes I et II ; Vigot Frères Edit. Paris.

Jordan, A.M., 1986. Trypanosomosis control and African rural development. Longman Edit. London, 357p.

Kaboré, I., 1982. Rationalisation des techniques d'élevage de *Glossina palpalis gambiensi*s (Vanderplank, 1949) (Diptera , Muscidea) à Bobo Dioulasso , Possibilité d'utilisation de sang lyophilisé dans l'alimentation in vitro Nouvelle modalité de stockage des glossines en grande cages. Mémoire de Institut supérieur Polytechnique, Université de Ouagadougou, Burkina Faso.

Kamuanga, M., 2003. Socio-economic and cultural factor in the research and control of trypanosomiasis. PAAT Technical and Scientific series n°4. FAO, Rome.

Kamuanga M., Hamadou S., Bouyer, J., Yao Y., Sidibé, I., Kaboré I., 2005. Stratégies collectives de lutte contre la trypanosomose animale , Comment pérenniser les acquis de la lutte antivectorielle?, Fiche technique N°14, Santé animale en Afrique de l'Ouest, CIRDES/CIRAD.

Kamuanga, M., Sigué, H., Swallow, B., Bauer, B., d'Ieteren, G., 2001b. Farmers' perceptions of the impacts of tsetse and trypanosomosis control on livestock production, evidence from southern Burkina Faso. Trop. Anim. Health Prod. 33, 141–153.

Kamuanga, M., Swallow, B., Sigué, H., Bauer, B., 2001a. Evaluating contingent and actual contributions to a local public good, Tsetse control in the Yale agro-pastoral zone, Burkina Faso. Ecol. Econ. 39, 115–130.

Kanwe, A.B., Bengaly, Z., Saulnier, D.; Duvallet, G., 1992. Evaluation du test de détection des antigènes circulants des trypanosomes à l'aide d'anticorps monoclonaux. Infections expérimentales et naturelles. Rev. Elev. Méd. Vét. Pays trop. 45, 265–271.

Koné, N., De Meeûs, T., Bouyer, J., Ravel, S., Guerrini, L., N'Goran, E.K., Vial, L., 2010. Population structuring of testse *Glossina tachinoides* resulting from landscape fragmentation in the Mouhoun River Basin, Burkina Faso. Med. Vet. Entomol. 24, 162–168

Koné, P., Duavllet, G., Pinder, M., 1983. Diffusion de la trypanosomose dans la région de Bobo-Dioulasso , Etudes épidémiologiques, in Rapport d'activités CRTA, pp 43–56.

Küpper, W., 1980. Yahres und AbschluSbericht. Tsetse prospection OberVolta.1978-1980. Bobo Dioulasso.

Laveissière, C., 1976a. Un "foyer" de trypanosomiase humaine sans glossines , Ouahigouya (République de Haute-Volta). Cah. ORSTOM, sér. Entomol. méd. et Parasitol. 14, 359–367.

Laveissière, C., 1976b. Répartition des glossines en Haute Volta , effets de la grande sécheresse de 1972-1973. Cah. ORSTOM, sér. Entomol. méd. et Parasitol. 14, 293–299.

Le Berre, R., 1967a. Possibilité et modalités de réalisation d'une campagne régionale de lutte contre le vecteur de l'onchocercose. Mission ORSTOM auprès de l'OCCGE, Bobo-Dioulasso, n°316, 9p.

Le Berre, R., 1967b. Campagne OCCGE - FED. de lutte contre le vecteur de l'onchocercose. Bilan sommaire pour l'année 1967. Mission ORSTOM auprès de l'OCCGE, Bobo-Dioulasso, n°319, 5p.

Lefrançois, T., Solano, P., Bauer, B., Kaboré, I., Touré, S.M., Cuny, G., Duvallet, G., 1999. Polymerase chain reaction characterization of trypanosomes in *Glossina morsitans submorsitans* and *G. tachninoides* collected on the game ranch of Nazinga, Burkina Faso. Acta Trop. 72, 65–77.

Mattioli, R.C., Cassama, M., Kora, S., 1992. A comparative study of gastrointestinal nematode egg output in N'Dama, Zebu and N'Dama x Zebu crossbred cattle. Parasitología, 34, 109–113.

McDermott, J., Woitag, T., Sidibé, I., Bauer, B., Diarra, B., Ouédraogo, D., Kamuanga, M., Peregrine, A, Eisler, M., Zessin, K.-H., Mehlitz, D., Clausen, P.-H., 2003. Field studies of drug-resistant cattle trypanosomes in Kénédougou Province, Burkina Faso. Acta Trop. 86, 93–103.

McLaughlin, G.L., Ssenyonga ,S.S., Nanteza, E., Rubaire-Akiki, W.O., Hansen, R.D., Vodkin, M.H., Novak, R.J., Gordon, V.R., Montenegro-James, S., James, M., Aviles, H., Armijos, R., Santrich, C., Weigle, K., Saravia, N., Wozniak, E., Gaye, O., Mdachi, R., Shapiro, S.Z., Chang, K.-P. Kakoma, I., 1996. PCR-based detection and typing of parasites. In, Azcel, M.A. and Alkan, M.Z., Editors, 1996. Parasitology for the 21st Century, Chapter 25, CAB International, Wallingford Oxon, pp. 261–287.

MECV (Ministere de l'Environnement et du Cadre de Vie), 2004. Rapport national sur la gestion durable des forets au Burkina Faso. Ouagadougou.

Mérot, P., Filledier J., Mulato C., 1988. Pouvoir attractif pour *Glossina tachinoides*, de produits chimiques isolés des odeurs animales. Rev. Elev. Méd. Vét. Pays trop. 41, 79–85.

Mérot, P., Politzar, H., Tamboura, I., Cuisance, D., 1984. Résultats d'une campagne de lutte contre les glossines riveraines en Haute-Volta par l'emploi d'écrans imprégnés de deltaméthrine. Rev. Elev. Méd. Vét. Pays trop. 37, 175–184.

Michel, J.-F., Dray, S., de La Rocque, S., Desquesnes, M., Solano, P., De Wispelaere, G., Cuisance, D. 2002. Modelling bovine trypanosomosis spacial distribution by GIS in an agro-pastoral zone of Burkina Faso. Prev. Vet. Med. 56, 5–18.

MRA (Ministère des Ressources Animales du Burkina Faso), 2006. Les statistiques du Secteur de l'Elevage au Burkina Faso. Rapport 76p.

Ouédraogo, D., 2002. Analyse socio-économique des pratiques de gestion de la trypanosomose animale et les facteurs associés au développement de la chimiorésistance dans la Province du Kénédougou (Burkina Faso). *Thèse de Doctorat Unique*, (UFR/SEG) Université de Ougadougou.

Ouédraogo, D., Kamuanga, M., Savadogo, K., Mcdermott, J., Randolph, T., Diarra, B., Woitag, T., Clausen, P-H., 2004. Facteurs institutionnels et développement de la résistance aux trypanocides dans la zone cotonnière en Afrique de l'Ouest, Evidence empirique dans la province du Kénédougou au Burkina Faso. Sustainable crop–livestock production in West Africa 312–331.

Paugy, D., Fermon, Y., Abban, K.E., Diop, M.E., Traoré, K., 1999. Onchocerciasis Control Programme in West Africa, a 20-year monitoring of fish assemblages. Aquatic Living Resources 12, 363–378.

Pinder, M., Authié, E., 1984. The appearance of isométamidium resistant *Trypanosoma congolense* in West Africa. Acta Trop. 41, 247–252.

Politzar, H., Cuisance, D., 1981. The S.I.T. in the control and eradication of *Glossina palpalis gambiensis*. Colloque international sur les techniques du lâcher de mâles stérilisés et l'emploi des rayonnements comme moyen génétique de lutte contre les insectes. Neuherberg, République Fédérale d'Allemagne, 29 juin - 3 juillet 1981.

Politzar, H., Cuisance, D., 1982. Blocking of a river system against reinvasion by a serie Challier,-Laveissiere traps. I.A.E.A. Research Coordination meeting, Vienna, 10-14 mai 1982.

Politzar, H., Cuisance, D., 1983a. A trap-barrier to block reinvasion of a river system by riverine tsetse species. Rev. Elev. Méd. Vét. Pays trop. 36, 364–370.

Politzar, H., Cuisance, D., 1983b. An integrated approach against riverine tsetse flies (*Glossina p. gambiensis* and *G. tachinoides*) by a combination of trapping, followed by the release of sterile males of three species. International Study Workshop on Tsetse Behaviour and Population Ecology. I.C.I.P.E., Nairobi (Kenya), 24-29 octobre 1983.

Politzar, H., Cuisance, D., Itard, J., Bauer, B., Tamboura, I., Mérot, P., Kaboré, I., 1985. Les activités du C.R.T.A. dans la lutte contre les glossines. Joint Research Co-ordination Meeting. Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development. Co-ordinated Research Programme on Development of Methodologies for the Application of the SIT for Tsetse Eradication or Control. Nairobi (Kenya), 6-10 mai 1985.

Politzar, H., Cuisance, D., Lafaye, A., Clair, M., Taze, Y., Sellin, E., 1979. Expérimentation sur le terrain de la lutte génétique par le lâcher de mâles stériles , longévité et dispersion des mâles irradiés de *Glossina palpalis gambiensis* (Haute Volta). Annales de la Société Belge de Médecine 59–59.

Politzar, H., Mérot, P., 1984. Attraction of the tsetse fly *Glossina morsitans submorsitans* to acetone, 1-octen-3-01, and the combination of these compounds in West Africa. Rev. Elev. Méd. Vét. Pays trop. 37, 468–473.

Prescott, N., Prost, A., Le Berre, R., 1984. The economics of blindness prevention in Upper Volta under the onchocerciasis control program. Social Science and Medicine 19, 1051–1055.

Rayaissé, J.B., Courtin, F., Akoudjim, M., Cesar, J., Solano, P., 2009. Influence de l'anthropisation sur la végétation et l'abondance des tsé-tsé au sud du Burkina-Fazo. Parasite 16, 21–28.

Reid, R., Kruska, R.L., Deitchmann, U., Thorthon, P.K., Leak, S.G.A., 2000. Human population growth and the extinction of the tsetse fly. Agriculture, Ecosystems and Environment 77, 227–236.

Reifenberg, J.M., Solano, P., Duvallet, G., Cuisance, D., Simporé, J., Cuny, G., 1997. Molecular characterization of trypanosomes isolates from naturally infected animals in Burkina Faso. Vet. Parasitol. 71, 251–262.

Rickenbach, A., 1961. Carte de répartition des glossines en Afrique occidentale d'expression française. ORSTOM, Paris, 1/ 10 000 000.

Robinson, T.P., 1998. Geographic Information Systems and the selection of Priority Areas for Control of Tsetse-transmitted Trypanosomosis in Africa. Parasitol. Today, 14, 457–461.

Rogers, D.J., Randolph, S.E., 1991. Mortality rate and population density of tsetse flies correlated with satellite imagery. Nature 351, 739–41.

Rogers, D.J., Randolph, S.E., 1993. Distribution of tsetse and ticks in Africa, past, present and future. Parasitol. Today 9, 226–71.

Schad, G.J., Allanson, A., Mackay, S.P., Cannavan, A., Tettey, J.N.A., 2008. Development and validation of an improved HPLC method for the control of potentially counterfeit isométamidium products. J. Pharm. Biomed. Analysis 46, 45–51.

Seed, J.R., 2001. African trypanosomosis research, 100 years of progress, but questions and problems still remain. Int.J. Parasitol. 31, 434–442.

Sellin, E., Février, J., Politzar, H., Cuisance, D., 1980. Principales anomalies ovaro-utérines rencontrées chez les femelles de *Glossina palpalis gambiensis* (Vanderplank, 1949) dans les élevages de Bobo-Dioulasso (Haute-Volta). Rev. Elev. Méd. Vét. Pays trop. 33, 295–302.

Sellin, E., Politzar, H., Cuisance, D., Clair, M., 1977. L'élevage de *Glossina palpalis gambiensis* Vanderplank, 1949 (Diptera-Muscidae) à Bobo-Dioulasso (Haute-Volta). Rev. Elev. Méd. Vét. Pays trop. 30, 41–49.

Sen, A., Chander, M., 2003. Privatization of veterinary services in developing countries, a review. Trop. Anim. Health Prod. 35, 223–236.

Shaw, A.P.M., 2004. Economics of African trypanosomosis. In, I. Maudlin, P. H. Holmes & M. A. Miles (eds.), The trypanosomosis, CABI Publishing, Wallingford, UK, pp 369–402.

Sigué, H., 1998. Etude de l'impact socio-économique du Plan de Lutte contre la Trypanosomose Animale (PLTA) dans les départements de Padéma et de Solenzo. Rapport PDRI/ HKM, Burkina Faso.

Solano, P., Argiro, L., Yao, Y., Reifenberg, J. M., Duvallet, G., 1995. Field application of the Polymerase Chain Reaction to the detection and characterization of trypanosomes in *Glossina longipalpis* (Diptera, Glossinidae) in Côte d'Ivoire. Mol. Ecol. 4, 781–785

Solano, P., de La Rocque S., Cuisance, D., Geoffroy B., de Meeus T., Cuny G., Duvallet, G., 1999. Intraspecific variability in natural populations of *Glossina palpalis gambiensis* from West Africa, revealed by genetic and morphometric analyses. Med.Vet. Entomol. 13, 401–407.

Solano, P., de La Rocque, S., de Meeûs T., Cuny, G., Duvallet, G., Cuisance, D., 2000. Microsatellite DNA markers reveal genetic differentiation among populations of *Glossina palpalis gambiensis* collected in the agropastoral zone of Sideradougou, Burkina Faso. Insect Mol. Biol. 9, 433–439.

Sow, A., 2002. Epidémiologie et importance socio-économique de la dermatophilose dans les élevages bovins au sud-ouest du Burkina Faso (Pays Lobi). *Thèse de Doctorat vétérinaire* EISMV de Dakar, Sénégal.

Talaki, E., Diall, O., Sidibé, I., Belem, A.M.G., Pangui, L.J., 2007. Méthode de diagnostic rapide de la chimiorésistance des trypanocides à l'isométamidium et au diminazène sur le terrain. RASPA 5, 29–36.

Talaki, E., Sidibé, I., Diall, O., Grace, D., Barry, A.M., Djiteye, A., Bocoum, Z., Clausen, P.-H., Randolph, T., Affognon, H., Hendrickx, G., Pangui, L.J., Belem, A.M.G., 2006. Répartition spatiale des trypanosomoses animales en relation avec la chimiorésistance dans la zone cotonnière de l'Afrique de l'Ouest (Mali et Guinée). RASPA 4, 45–50.

Tamboura, I., Béré, A., Hendrickx G., 2000. Elements d'élaboration d'un plan de contrôle de la trypanosomose dans les zones libérées de l'onchocercose. Les techniques de l'information spatiale et de l'épidémiologie , des outils utiles pour planifier un développement intégré de l'élevage en Afrique Occidentale, Bobo-Dioulasso, IMT Anvers.

Taze, Y., Cuisance, D., Politzar, H., Clair, M., Sellin, E., 1977. Essais de détermination de la dose optimale d'irradiation des mâles de *Glossina palpalis gambiensis* (Vanderplank 1949) en vue de la lutte biologique par lâchers de mâles stériles dans la région de Bobo-Dioulasso (Haute-Volta). Rev. Elev. Méd. Vét. Pays trop. 30, 269–279.

Tettey, J., Astriku, C., Chizyuka, G., Slingenbergh, J., 2002. Non conformance of diminazene preparations to manufacturers' label claims; an extra factor in the development of chemo-resistance? ICPTV Newsl. Integr. 5, 24–26.

Tettey J.N.A., Skellern G.G., Grant M.H., Midgley J.M., 1999. Investigation of the chemical equivalence of the trypanocidal products, Samorin® and Veridium®. J. Pharm. Biomed. Analysis 21, 1–7.

Torr, S.J., Maudlin, I., Vale, G.A,. 2007. Less is more, restricted application of insecticide to cattle to improve the cost and efficacy of tsetse control. Med. Vet. Entomol. 21, 53–64.

Vilain, P., 1949. Répartition des glossines en Afrique occidentale française, 2 cartes. Service géographique de l'A.0.F. 1/ 3 000 000.

Vreysen, M.J.B., 2006. Prospects for area-wide integrated control of tsetse flies (Diptera, Glossinidae) and trypanosomosis in sub-Saharan Africa. Rev. Soc. Entomol. Argent. 65, 1–21

Chapter 3:

Baseline survey of animal trypanosomosis in the Region of the Boucle du Mouhoun, Burkina Faso.

Adapted from:

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3.1. Introduction

The economy of Burkina Faso is largely dependent on pastoral agriculture which represents approximately 86% of the total 15 million inhabitants and contributes to about 40% to the 7.9 billion US$ Gross Domestic Product (GDP) (MEDEV, 2002; World bank, 2009). The contribution of livestock to the gross domestic product ranges from 10 to 14% and is the second provider of foreign currency after the agricultural sector and before the mining sector (MEDEV, 2002; MEDEV, 2004).The livestock includes cattle (8 million), small ruminants (18 million), pigs (2 million) and equids (1 million) (MRA ,2006). Ruminants are either raised in sedentary or transhumant animal husbandry systems mostly on communal natural pastures and with limited access to adequate veterinary health care delivery. This system of livestock management highly exposes animals to the risk of disease outbreaks which are indeed often reported by the veterinary services (MRA, 2006). Among all the diseases, trypanosomosis is the most frequent one and greatly hampers livestock productivity in Burkina Faso. One third of the total surface of the country is at risk of the disease. More than 63% of the country's cattle population is raised in zones with high trypanosomosis risk (Kamuanga *et al*., 2001). Those zones are partially preserved from human activities offering good grazing capacities for the cattle but, at the same time, providing suitable refuges for the tsetse flies.

In Burkina Faso direct losses due to the purchase of trypanocidal compounds have been estimated to be 3.9 million US$ per annum (Sow *et al.,* 2010). Treatments with trypanocidal drugs constitute the most frequently used method for controlling trypanosomosis and maintaining susceptible breeds in tsetse infested areas. However, extended use of these drugs led to the development of chemoresistance in all trypanosomosis enzootic areas of Burkina Faso (Clausen *et al.*, 1992; McDermott *et al.,* 2003; Talaki *et al.*, 2007).

The Government of Burkina Faso tried to mitigate the impact of human and animal trypanosomosis with the support of international partners. All previous efforts to eliminate tsetse and trypanosomosis in the country failed because of the lack of a concerted international approach in dealing with this trans-boundary animal disease (Adam *et al.,* 2012).

The Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) is the most recent programme that was launched to eliminate tsetse and trypanosomosis. Thirty seven African countries endemic for human and animal trypanosomosis are participating to the

campaign. In Burkina Faso, the Region of the Boucle du Mouhoun was selected as the initial location for the implementation of the PATTEC initiative. Indeed, this region is located at the Northern limit of the tsetse distribution (Laveissière, 1976; Hendrickx *et al.,* 1999) and the subsequent elimination campaign will be carried out following the "rolling carpet" strategy from North to South. Preventing reinvasion of flies living in the adjacent river basins i.e. the Comoé, The Niger and the Sissili River Basins would be indicated since the population of flies originating from the different water basins are genetically interconnected (Solano *et al.,* 2010; Kone *et al.,* 2011). The present survey was conducted before implementing the eradication campaign with the aim of determining the prevalence of trypanosome infections and the health status of farm animals by using the PCV and Body Condition Score (BCS) as indicators. A cross-sectional survey was conducted from September to December 2007, during the dry season (i) to identify hot spots to conduct block treatment for trypanocidal drug resistance testing, (ii) to get an estimate of the disease prevalence, (iii) to assess the impact of the disease on the health status of the animals and indirectly (iv) to estimate the drug resistance situation in the area.

3.2. Material and methods

3.2.1. Study area

The Region of the *Boucle du Mouhoun*, is located at the North-West of Burkina Faso between 2°4' - 4.6° W and 11°23' - 13°7' N, and its surface is about 34,000 km² (12.6% of the national territory). The region is subdivided in 6 provinces namely Balé, Banwa, Kossi, Mouhoun, Nayala and Sourou. These provinces are divided in a total of 47 departments, themselves composed of 1061 villages (Ministère de l'Administration Territoriale et de la Décentralisation - Burkina Faso (MATD) ,2007). The climate of the region is of soudano-sahelian type with the annual rainfall ranging between 500 to 1400 mm (MEDEV ,2005). The region is drained by the Mouhoun River which is 280 km long. A dense hydrographic network made by permanent and temporary tributaries is woven around the Mouhoun River (fig. 3.1). The area has a dense hydrographic network linked to the Mouhoun River. The main socio-economic activities in the area are agriculture and livestock breeding. The Region hosts about 660.000 cattle and 1.450.000 small ruminants (MRA, 2006). The livestock breeding is extensive and making use of the natural pastures along the river edge and its basin. The cattle raised in the area are mainly zebus (*Bos indicus*), but taurines (*Bos taurus*) and cross-bred animals are also present. The area is infested with *G. palpalis gambiensis* and *G. tachinoides*, two riverine tsetse species, distributed unequally depending on the vegetation type and its

degree of degradation by human activities (Guerrini *et al.*, 2008). Their Index of Apparent Abundance (IAA) varies between 1 and 9.5 tsetse per trap and per day (Bouyer and Bengaly, 2006). The most frequent infection for both species is *T. vivax* (1.4%), followed by *T. congolense* (0.3%) and *T. brucei* (0.05%) (Kone *et al.*, 2011). The parasitological and serological prevalence's of bovine trypanosomosis are 7% and 83% respectively (Bouyer and Bengaly, 2006).

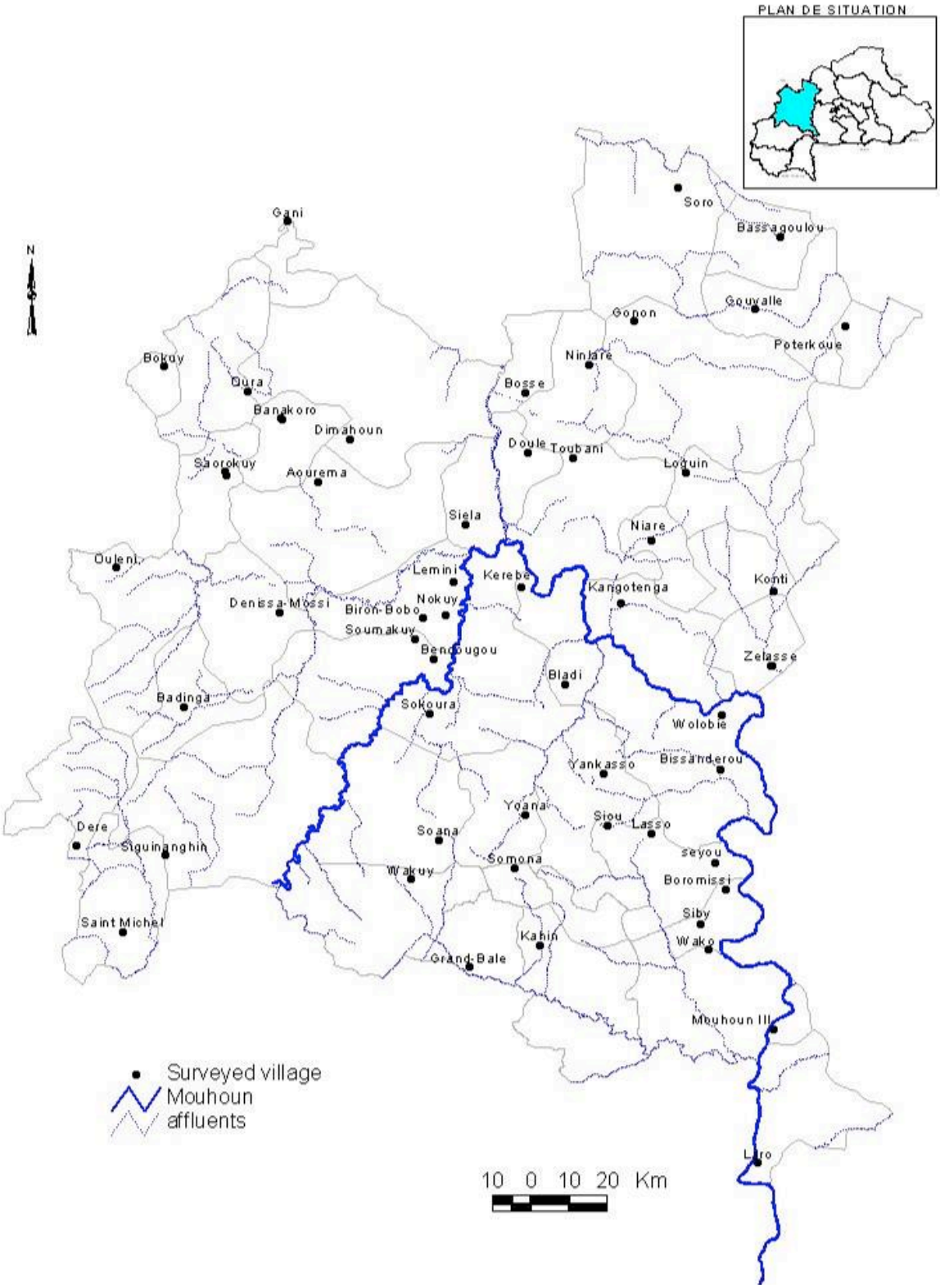

Figure 3.1. Region of Boucle du Mouhoun: location of the 53 surveyed villages and hydrographical network

3.2.2. Parasitological survey

3.2.2.1. Sampling

Among the 1061 villages of the 47 departments, one village was randomly selected from each district. To increase the chances to get a suitable environment for the block treatments for drug resistance testing i.e. where the risk of trypanosome infection is high enough, six additional villages were randomly selected from a list of 41 being located less than 5 km from the river or its main tributaries. Further random sampling of 50 cattle, 30 small ruminants and 10 donkeys. In total 2,650 cattle, 1,590 small ruminants and 530 donkeys were selected for sampling. For each sampled animal, information related to species, sex, age, breed, body condition score (BCS), livestock management system and history of trypanocidal treatments were recorded. The sampling took place in September and November 2007, i.e. in the beginning of the dry season.

3.2.2.2. Parasitological and serological analysis

A blood sample was collected in heparinised Vacutainer® tubes from each animal by vein puncture of the jugular vein for the determination of the PCV and the detection of trypanosomes using the buffy-coat technique (Murray *et al.*, 1977). The serum collected on dry Vacutainer® tubes was used for the detection of the anti-trypanosome antibodies. The dry tubes containing the blood of each animal were centrifuged (430 g, 10 min) and sera were collected into Eppendorf® tubes that were kept at cool temperature on ice during the field operations and then at -20°C before ELISA processing. The serological analyses were carried out using an indirect ELISA according to the protocol described by Desquesnes *et al.* (1999). Briefly, microplates were sensitized with soluble antigens (5µg / ml) of *T. vivax* (IL1392), *T. brucei* (ILTat1.2), *T. congolense* savannah type (IL1180) or *T. evansi*. The results of the ELISA analyses were expressed as relative percentage positivity (RPP) compared to positive and negative reference samples. The positivity thresholds (PT) were fixed at 20% (Desquesnes *et al.*, 1999).

3.2.3. Statistical analysis

Data from the different animal species were analysed separately in robust generalized linear models, using the villages as primary sampling units. Overall parasitological and serological prevalences were estimated from the 47 randomly selected villages (excluding the 6 additional villages closer to rivers) using logistic regressions without explanatory variables. All other analyses were performed on data originating from all 53 villages (including the 6

additional villages closer to rivers), considering the distance to main rivers (> or <= 5 km) as strata. Prevalences less than 5 km from main rivers and beyond were estimated using the distance to the rivers (> or <= 5 km) as only binary explanatory variable. The significance of the distance to rivers and the animals' age on their serological status was evaluated in a multivariate model using these two parameters as continuous explanatory variables. Finally, the effect of trypanosome infections (observation of trypanosomes and seropositivity; explanatory variables) on the PCV and the BCS (responses) was evaluated in ordered multinomial logistic regressions. Body condition was classified as good, medium and bad whereas 3 PCV classes were created using <20 and <26 as cut-offs. The proportion of animals and confidence interval were calculated for each class using a non-linear combination of estimators. A significance threshold of 5% was used in all statistical tests.

3.3. Results

3.3.1. Sampled animals

A total of 2,002 cattle, 1,466 small ruminants, 481 donkeys were sampled from the 53 selected villages participating to the survey (table 3.1). The sampled animals were mainly sedentary (98.6%), with 64.3% of males, because of the presence of draught oxen. In the herds, 72.4% were Zebus (*Bos indicus*), 3.9 % were taurines (*Bos taurus*) and 23.7% were crossbred. In sheep, the breed repartition was 80.2%, 5.4% and 14.4% for Djallonke, Bali-Bali and crossbreds respectively. Similarly, in goats, the proportion of Djallonke, Sahel and crossbred was 77.6%, 6.9% and 15.5% respectively.

Table 3.1. Number of sampled animals per province in the Region of the *Boucle du Mouhoun*

Povince	Number of villages	Number of sampled animals					
		Cattle	Sheep	Goats	Donkeys	Horses	Camel
Balé	11	428	268	45	101	0	0
Banwa	7	245	114	36	59	1	0
Kossi	12	508	226	126	224	3	0
Mouhoun	9	315	172	73	72	0	0
Nayala	6	279	88	88	60	0	0
Sourou	8	227	141	89	75	0	1
Total	53	2002	1009	457	481	4	1

The study showed that 11.7% and 12.9 % of the cattle, for DA and ISM respectively, had received a treatment less than three months before the sampling; for sheep, those proportions were 5.25% and 0.2% for DA and ISM respectively. When considering goats and donkeys, the proportion of animals treated with DA was of 1.5% and 0% respectively, none was treated with ISM.

ISM and DA were thus used in equal proportion in cattle whereas in small ruminants DA was used nearly exclusively. This result indicates that the use of trypanocides in small ruminants was much more curative than preventive, corroborating the low impact of trypanosomosis in these animals.

3.3.2. Prevalence

3.3.2.1. Parasitological Prevalence (buffy coat)

For the whole Region of the *Boucle du Mouhoun*, the parasitological prevalence of trypanosomosis was low. Indeed, in cattle, the most affected species, the mean prevalence was 0.77% (95%C.I. 0.30-1.95%). In goats no case of infection was observed. Detailed results per species are provided in table 3.2.

Table 3.2. Parasitological prevalence of trypanosomosis during the dry season

Province	Cattle (n=2002)			Sheep (n=1009)			Donkeys (n=481)		
	P(%)	<	>	P(%)	<	>	P(%)	<	>
Balé	0.7	0.4	1.2	0.4	0.2	1.1	2.0	1.1	3.7
Banwa	2.0	1.5	2.7	0.9	0.5	1.7	1.7	0.9	3.3
Kossi	0.8	0.5	1.3	0.9	0.5	1.7	0	nd	nd
Mouhoun	1.9	1.4	2.6	0	nd	nd	1.4	0.7	2.9
Nayala	2.2	1.6	2.9	1.1	0.6	2.0	0	nd	nd
Sourou	0.9	0.6	1.4	0	nd	nd	0	nd	nd

With P as prevalence; < lower limit confidence interval; > upper limit confidence interval

Trypanosome infections were mainly due to *T. vivax* (75%) with some cases of *T. congolense* infections in cattle (25%). The distance of the village from the river (more or less than 5 km) had no significant effect on the parasitological status of the animals (P=0.654). The BCS were independent from the parasitological status of the animals (P=0.642). However, the PCV values of parasitologically positive animals were significantly lower than the negative (P <

0.001). The proportion of cattle in the three PCV categories with their 95% C.I. are shown in table 3.3.

Table 3.3. Proportion of cattle in the 3 PCV categories

	PCV>26%	20%<PCV<26%	PCV<20%
Seronegative	86.0% (83.0-89.1)	12.7% (9.9-15.6)	1.2% (0.7-1.7)
Seropositive	58.5% (40.3-76.6)	36.5% (21.4-51.5)	12.7% (1.4-8.8)

Between brachets the 95% C.I.

3.3.2.2. Serological prevalence

In cattle, the serological prevalence of trypanosomosis, for the entire Region of the Boucle du Mouhoun, was 34.2% (95%C.I. 26.1-43.4%). For sheep, goats and donkeys, the prevalence were of 20.9% (95%C.I. 12.2-33.5%), 8.5% (95%C.I. 5.7-12.5%) and 5.8% (95%C.I. 3.9-8.6%) respectively. Detailed serological prevalences per species and per province are shown in table 3.4. The ten villages with the highest prevalence were selected for further block treatment for drug resistance testing (Débé, Laro, Déré, Bendougou, Nokuy, Kangotenga, Mou and Boromissi). The distance of the village to the river had a significant effect ($P < 0.001$) on the serological prevalence of cattle with a 3% (95%C.I. 1.6-4.6%) reduction of the Odd Ratio per additional kilometre from the river. The same trend was observed for the small ruminants but not for the donkeys. As expected, the age of the cattle also influenced the serological status with a 10% (95%C.I. 5.4-15.7%) increase of the Odd Ratio per additional year of age. The same trend was observed for the other species. The BCS of the seropositives were not significantly different from the seronegatives (P=0.066). The same trend was observed for the other species. The serological status was not correlated with the PCV values (P=0.567). The same trend was observed in the other species.

Table 3.4. Serological prevalence of trypanosomosis in the Region of Boucle du Mouhoun

Province	Cattle (n=2002)			Sheep (n=1009)			Goats (n=457)			Donkeys (n=481)		
	P(%)	<	>	P(%)	<	>	P(%)	<	>	P(%)	<	>
Balé	68.9	66.84	70.9	51.1	48.0	54.2	26.7	22.8	30.9	5.9	4.1	8.4
Banwa	37.1	35.0	39.2	15.8	13.7	18.2	11.1	8.5	14.3	17.0	13.9	20.6
Kossi	19.3	17.6	21.1	6.2	4.9	7.9	10.3	7.8	13.4	10.5	8.1	13.6
Mouhoun	45.7	43.5	47.9	13.4	11.4	15.6	5.5	3.7	8.0	2.8	1.6	4.7
Nayala	27.2	25.3	29.2	3.4	2.4	4.7	1.1	0.5	2.6	15.0	12.1	18.5
Sourou	19.8	18.1	21.6	5.0	3.8	6.5	10.1	7.7	13.2	6.7	4.8	9.3

With P as prevalence; < lower limit confidence interval; > upper limit confidence interval

3.4. Discussion

This study highlighted (i) a low parasitological prevalence in all species, (ii) a high serological prevalence in cattle, (iii) a moderate serological prevalence in the other species, (iv) the efficient use of prophylactic and curative trypanocidal drugs in cattle and (v) a parsimonious use of curative treatments in the other animal species.

About 25% of the cattle was treated (12.9% with ISM and 11.7% with DA) against trypanosomosis less than 3 months before the survey while less than 3% and 0.5 % of small ruminants and donkeys respectively had received a trypanocide treatment. The intensive use of trypanocidal drugs in cattle might be explained by the fact that herds reared in the Region of the *Boucle du Mouhoun* were composed from 96% of trypanosomosis susceptible breed, mainly zebu and crossbred zebu / taurine. This is a common situation as the susceptible breeds are intuitively considered as generating more profit by the farmers because of their larger format despite the fact that they are equally productive (Maichomo *et al.*, 2009). The restricted use of trypanocidal drugs in sheep might find its roots in the fact that the Djallonke is the most popular breed in the area as it presents a higher level of trypanotolerance compared to the local Bali-Bali breed and crossbreds (Agyemang, 2005; Bengaly *et al.*, 2001; Geerts *et al.*, 2009). Goats were even less affected by the disease than sheep, which was expected, as this species is known to show an active defence against the tsetse flies by body movements and skin thrilling (Simukoko *et al.*, 2007). The low rate of treatments in donkeys could be explained by the fact that ISM induces a local inflammatory reaction at the injection place (Eisler *et al.*, 1996) and that the usual site of deep intramuscular injection corresponds

to the place of harnessing which causes wounds handicapping draught donkeys for several days or weeks. Lastly, trypanosomosis prevalence in the various animal species seemed to reflect their systems of husbandry practice. Cattle and to a lesser extent sheep, which are generally led away from the village usually on the river edges in search of pasture, had the highest prevalence of trypanosomosis. On the contrary, goats and donkeys are generally kept around the village, away from the tsetse habitats e.g. in the dry season, donkeys are used for house works such as the transportation of water and building materials. Therefore, these two species presented less positive cases of trypanosomosis.

The study showed that *T. vivax* was the most prevalent trypanosome species (75%, n=21) followed by *T. congolense*. This finding corroborates reports from previous surveys conducted in the Region of the Boucle du Mouhoun and other tsetse infested area in Burkina (Bengaly *et al.*, 2001; Bouyer and Bengaly, 2006; Desquesnes *et al.*, 1999). The predominance of *T. vivax* might be explained by the exclusive presence in the study area of tsetse flies belonging to the palpalis group which is known as the effective vectors of *this trypanosome species* (Moloo and Kutuza ,1988) and also by the abundance of mechanical vectors also known to be effective transmitters of *T. vivax* (Desquesnes and Dia, 2003; Desquesnes and Dia, 2004).

As expected, haematocrits were consistently lower in seropositive animals (table 3.3). Nearly 60% of those seropositives had a PCV higher than 26 and only around 5% with PCV values lower than 20. This value of 26% constitutes a threshold that is a valuable indicator of trypanosomal infections when associated with the parasitological results (Marcotty *et al.*, 2008). The low proportion of anaemic animals is a strong signal that treatments are effective in this area and that the health condition of the animals can be maintained even in the presence of the parasites and their vectors.

The low parasitological prevalences associated to high serological prevalences in cattle indicates that animals are frequently in contact with the parasites but are effectively cured by the trypanocidal drugs. The term of “cured” should be here considered as a persisting negative microscopical diagnosis after treatment. Diagnosis by PCR could certainly detect more positive animals but the question is then raised about the usefulness of diagnosing very low parasitaemia in healthy animals. In an experimental model, it was shown that some goats inoculated with *T. vivax* and treated either with DA or ISM developed very low and intermittent parasitaemia that were not affecting the weight and PCV’s. All the goats presenting clinical signs were microscopically positive (Vitouley *et al.*, 2012).

Interestingly, there was no correlation between the parasitological or serological status and the BCS. This brings even more weight on the efficacy of the drug treatment that are administered by the farmers.

This study showed a strong correlation between the proximity to the river or main tributaries and the serological status of the cattle. In the dry season, moment of our survey, the river edges still provide some pasture for livestock even though the risk of trypanosome infection is high. Because of regular trypanocidal treatments, the parasitological prevalence remains low. In this study, prevalences were 0.77% and 34.2% for parasitology and serology respectively in cattle.

The parasitological prevalence (all animal species considered) in this study was far lower than what was found in previous surveys carried out in the same Region of the Boucle du Mouhoun. Indeed, Bouyer and Bengaly (2006) and Bengaly *et al.* (2001) found parasitological prevalence of 3.2% and 7.7% respectively as against 0.77% prevalence in this study. Those two previous studies were limited to the sole province of Mouhoun (not extended to the whole Region of the Boucle du Mouhoun), and were both carried out in the rainy season.

3.5. References

Adam, Y., Marcotty, T., Cecchi, G., Mahama, C.I., Solano, P., Bengaly, Z., Van den Bossche, P., 2012. Bovine trypanosomosis in the Upper West Region of Ghana: Entomological, parasitological and serological cross-sectional surveys. Res. Vet. Sci. 92, 462−468.

Agyemang, K., 2005. Trypanotolerant livestock in the context of trypanosomiasis intervention strategies. PAAT Technical Scientific Series 7, 1−66.

Bengaly, Z., Ganaba, R., Sidibe, I., Desquesnes, M., 2001. Trypanosomose animale chez les bovins dans la zone Sud-soudanienne du Burkina Faso. Revue d'Elevage et de Médecine vétérinaire des Pays tropicaux 54, 221−224.

Bouyer, J., Bengaly, Z., 2006. Evaluation de la situation entomologique et épidémiologique en vue de l'élaboration d'un plan de lutte contre les trypanosomoses animales et leur vecteur dans la zone d'intervention du PAEOB. Rapport d'expertise du Projet d'Appui aux Organisations d'Eleveurs de l'Ouest (PAEOB) - Bobo-Dioulasso 1−30.

Clausen, P.H., Sidibe, I., Kabore, I., Bauer, B., 1992. Development of multiple drug resistance of *Trypanosoma congolense* in zebu cattle under high natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. Acta Trop. 51, 229−236.

Desquesnes, M., Dia, M.L., 2003. *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. Exp. Parasitol. 103, 35−43.

Desquesnes, M., Dia, M.L., 2004. Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanid *Atylotus fuscipes*. Vet. Parasitol. 119, 9−19.

Desquesnes, M., Michel, J.F., de la Rocque, S., Solano, P., Millogo, L., Bengaly, Z., Sidibe, I., 1999. Enquête parasitologique et sérologique (Elisa-indirect) sur les trypanosomoses des bovins dans la zone de Sidéradougou, Burkina Faso. Rev. Elev. Méd. vét. . Pays trop. 52, 223−232.

Eisler, M.C., Elliott, C.T., Holmes, P.H., 1996. A simple competitive enzyme immunoassay for the detection of the trypanocidal drug isometamidium. Therapeutic Drug Monitoring 18, 73−79.

Ganaba, R., Bengaly, Z., Ouattara, L., 2002. Calf morbidity, mortality and parasite prevalences in the cotton zone of Burkina Faso. Prev. Vet. Med. 55, 209−216.

Geerts, S., Osaer, S., Goossens, B., Faye, D., 2009. Trypanotolerance in small ruminants of sub-Saharan Africa. Trends Parasitol. 25, 132−138.

Guerrini, L., Bord, J.P., Ducheyne, E., Bouyer, J., 2008. Fragmentation Analysis for Prediction of Suitable Habitat for Vectors: Example of Riverine Tsetse Flies in Burkina Faso. J. Med. Entomol. 45, 1180–1186.

Hendrickx, G., Napala, A., Dao, B., Batawui, K., Bastiaensen, P., De Deken, R., Vermeilen, A., Vercruysse, J., Slingenbergh, J.H.W., 1999. The area-wide epidemiology of bovine trypanosomosis and its impact on mixed farming in subhumid West Africa; a case study in Togo. Vet. Parasitol. 84, 13−31.

Kamuanga, M., Swallow, B.M., Sigue, H., Bauer, B., 2001. Evaluating contingent and actual contributions to a local public good: Tsetse control in the Yale agro-pastoral zone, Burkina Faso. Ecol. Econ. 39, 115−130.

Kone, N., N'Goran, E.K., Sidibe, I., Kombassere, A.W., Bouyer, J., 2011. Spatio-temporal distribution of tsetse and other biting flies in the Mouhoun River basin, Burkina Faso. Med. Vet. Entomol. 25, 156−168.

Laveissière, C., 1976. Répartition des glossines en Haute Volta : effets de la grande sécheresse de 1972-1973. Cahiers ORSTOM, série Entomologie médicale et Parasitologie 14, 293−299.

Maichomo, M.W., Kosura, W.O., Gathuma, J.M., Gitau, G.K., Ndung'u, J.M., Nyamwaro, S.O., 2009. Economic assessment of the performance of trypanotolerant cattle breeds in a pastoral production system in Kenya. Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging 80, 157−162.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N., Van den Bossche, P., 2008. Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia 2. Prev. Vet. Med. 87, 288−300.

McDermott, J.J., Woitag, T., Sidibe, I., Bauer, B., Diarra, B., Ouedraogo, D., Kamuanga, M., Peregrine, A.S., Eisler, M.C., Zessin, K.H., Mehlitz, D., Clausen, P.H., 2003. Field studies of drug-resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. Acta Trop. 86, 93−103.

MEDEV, 2002. Lettre de Politique de Développement Rural Décentralisé. MEDEV (Ministère de l'Economie et du Développement du Burkina Faso) http://www.dgatdlr.bf/download/lpdrd.pdf.,

MEDEV, 2004. Etude nationale prospective "Burkina 2025" : Rapport général. MEDEV (Ministère de l'Economie et du Développement du Burkina Faso) http://www.capdev.org/index.php?option=com_phocadownload&view=category&download=4:pp-l-2025&id=2&Itemid=192,

MEDEV, 2005. Région de la Boucle du Mouhoun; Cadre stratégique Régional de Lutte contre la pauvreté, Ouagadougou, Burkina Faso. Rapport du Ministère de l'Economie et du Développement du Burkina Faso (MEDEV) 1−106.

Ministère de l'Administration Territoriale et de la Décentralisation - Burkina Faso (MATD), 2007. Liste des villages du Burkina Faso. Villages de la boucle du Mouhoun. http://www.matd.gov.bf/SiteMatd/documents/villagesbf1.pdf.

Moloo, S.K., Kutuza, S.B., 1988. Comparative study on the infection rates of different laboratory strains of Glossina species by *Trypanosoma congolense*. Med. Vet. Entomol. 2, 253−257.

MRA, 2006. Les statistiques du Secteur de l'Elevage au Burkina Faso. Rapport du Ministère des Ressources Animales du Burkina Faso 1−76.

Murray, M., Murray, P.K., Mcintyre, W.I.M., 1977. Improved parasitological technique for diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg. 71, 325−326.

PATTEC, 2009. Rapport annuel de projet - Exercice 2008 - Bobo Dioulasso. PATTEC reports 1−47.

Simukoko, H., Marcotty, T., Phiri, I., Geysen, D., Vercruysse, J., Van den Bossche, P., 2007. The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. Vet. Parasitol. 147, 231−238.

Solano, P., Ravel, S., de, M.T., 2010. How can tsetse population genetics contribute to African trypanosomiasis control? Trends Parasitol. 26, 255−263.

Sow, A., Bengaly, Z., Bouyer, J., Bauer, B., Van den Bossche, P., 2010. Fifty years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso. An overview. Bull. .Anim. Health Prod. Africa 58, 95−118.

Talaki, E., Diall, O., Sidibe, I., Belem, A.M.G., Pangui, L.J., 2007. Méthode de diagnostic rapide de la chimiorésistance des trypanocides à l'isométamidium et au diminazène sur le terrain. RASPA 5, 29−36.

Vitouley, H.S., Sidibe, I., Bengaly, Z., Marcotty, T., Van den Abbeele, J., Delespaux, V., 2012. Is trypanocidal drug resistance a threat for livestock health and production in endemic areas? Food for thoughts from Sahelian goats infected by *Trypanosoma vivax* in Bobo Dioulasso (Burkina Faso). Vet. Parasitol. 187, 105−111.

World bank, 2009. Burkina Faso at a glance. In. World bank – http://devdata.worldbank.org/AAG/bfa_aag.pdf.

Chapter 4:

Field detection of resistance to isometamidium chloride and diminazene aceturate in *Trypanosoma vivax* from the Region of the Boucle du Mouhoun in Burkina Faso

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Adapted from:

Sow A., Sidibé I., Bengaly Z., Marcotty T., Séré M., Diallo A., Vitouley H. S., Nebié R. L., Ouédraogo M., Akoda G. K., Van den Bossche P., Van Den Abbeele J., De Deken R., Delespaux V. (2012). Field detection of resistance to isometamidium chloride and diminazene aceturate in *Trypanosoma vivax* from the Region of the Boucle du Mouhoun in Burkina Faso. *Veterinary Parasitology*, 187, 105–111.

4.1. Introduction

The Western and South-western parts of the Burkina Faso have a great potential for agricultural and livestock productions because of the relatively wet climate compared to the Northern part of the country. Unfortunately, the development of the livestock sector is there hindered by trypanosomosis.

Because of the problems associated with protecting tsetse cleared areas from re-invasion (Bauer *et al.*, 1992; Bauer *et al.*, 1995; Bauer *et al.*, 1999; Cuisance *et al.*, 1990), the use of trypanocidal drugs remains the main tool used by the livestock keepers to control the disease. Two drugs are mainly used: DA for treatment and ISM for prevention. Those drugs were marketed more than half a century ago and it is thus not astonishing that cases of drug resistance were reported in 18 countries of sub-Saharan Africa (Delespaux *et al.*, 2008). In Burkina Faso, chemoresistance was reported for the first time at the beginning of the 1980's in the province of Kénédougou (Authie, 1994; Pinder and Authie, 1984). The resistance to trypanocides was since reported in the other tsetse infested areas of Burkina Faso and especially the important cotton production zone (Clausen *et al.*, 2010; Grace *et al.*, 2009; McDermott *et al.*, 2003). To date, no reliable or consistent data on trypanosome drug susceptibility is available for the Region of the Boucle du Mouhoun which is another important region for cattle breeding in Burkina Faso. The purpose of this study was thus (i) to evaluate the prevalence of the animal trypanosomosis in the area by means of a cross-sectional survey, (ii) to evaluate the level of the resistance to ISM in trypanosome by comparing the incidence of trypanosomal infections in treated and untreated herds during a period of 8 weeks (longitudinal survey), (iii) to evaluate the effect of the treatment against the infection by measure of the PCV in both groups and finally (iv) to determine the frequency of relapses after treatment with DA.

4.2. Material and methods

4.2.1. Study area

The fig 41a and 4.1b show localization of the study area and le the villages where the block treatment was conducted. Based on the outcome of the baseline survey, the 10 villages with the highest parasitological prevalence of trypanosomosis were selected.

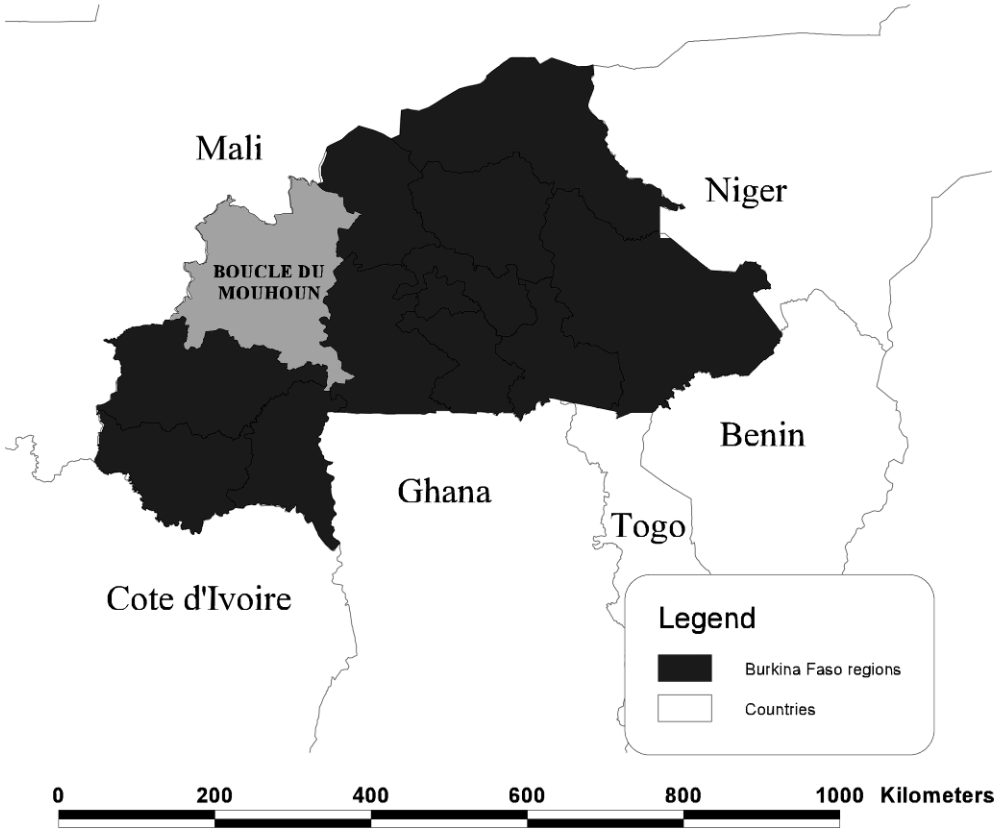

Figure 4.1. Localization of the study area

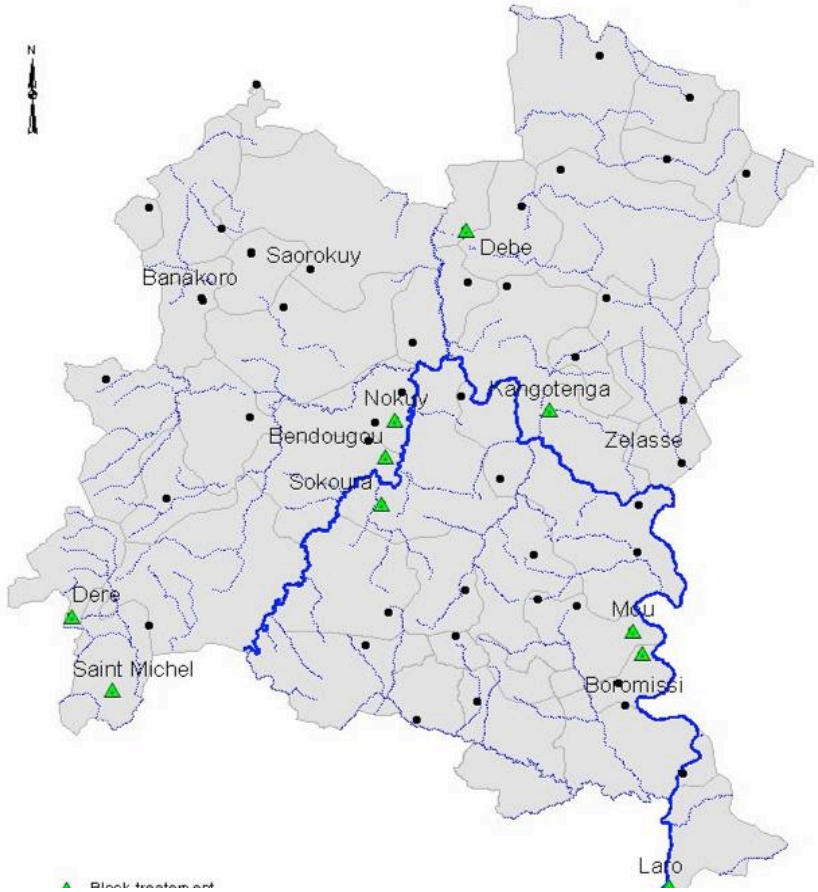

Figure 4 1b. Localization of villages where block treatment was conducted.

4.2.2. Longitudinal survey

The longitudinal survey was conducted between February and May 2009 i.e. at the end of the dry season. Based on the outcome of the cross-sectional survey, the ten villages with the highest parasitological prevalence were selected. In each of the 10 villages, 74 to 100 cattle aged more than 1 year were identified and ear tagged. 96.2% were zebu cattle (*Bos indicus*), 3.6% crossbred and 0.2% taurine cattle (*Bos taurus*). The breed composition between treated and control groups was similar. The average age of the animals was 4.8 years with a standard deviation of 2.9 years.

Trypanocidal drug resistance was evaluated by means of treated and control sentinel herds as described by Eisler *et al.* (2000). Briefly, the selected cattle in each village (about hundred) were randomly divided in 2 equal groups, one being treated with ISM (Trypamidium® MERIAL) at a dose of 1mg/kg bw while the other remained untreated as control. The animals were examined on day 0 (day of treatment with ISM), 14, 28, 42 and 56 for the presence of trypanosomes and PCV measurement to assess the efficacy of the treatment against the infection. Any positive animal was treated with DA (Trypadim® MERIAL) at 3,5mg/kg bw. The level of resistance to ISM was estimated by comparing the incidence of trypanosomal infections in the two groups (calculation of the relative risk, which is the ratio of the incidence in the control and the treated groups).

4.2.3. Statistical analysis

The overall trypanosomal incidence data was analysed separately for *T. congolense* and *T. vivax* in Stata 10. Animals that were positive on the first day of observation were discarded from the analysis. Treatment was used as only explanatory variable to calculate the relative risk of infection in control animals compared to treated cattle. The hazard was assumed to be constant (exponential distribution) in all survival analysis for ease of interpretation.

Variations of treatment efficiency in the different study sites were further evaluated for *T. vivax* infections. Here, site, treatment and the interaction between the two were used as explanatory variables. Sites with low number of events were excluded from this part of the analysis. For each site, the logarithm of the relative risk of treated animals and its 95% confidence interval were calculated by summing the model coefficients of the treatments and site/treatment interaction explanatory variables (linear combination of estimators in Stata 10). Actual relative risks and 95% confidence intervals were derived by calculating the

exponential of these values. The monthly hazard and its 95% confidence interval were calculated using the exponential of the linear predictions and considering that a month is made of 28 days.

PCV values of *T. vivax* infected animals recorded 8 to 56 days after treatment in the ISM and control groups were compared in a linear regression using the treatment as only explanatory variable. PCV values were arcsine transformed to ensure normality of the distribution of the response variable (Osborne, 2002). The homoscedasticity of the model and the normality of the residuals' distribution were checked. The final model used the individual animals as random effects to account for repeated measures. A possible seasonal effect was not considered as the longitudinal survey was completely conducted within the 4 last months of the dry season.

4.3. Results

4.3.1Cross sectional survey

The results are summarized in table 4.1. Trypanosomosis was observed in 13 villages. Infections were mainly (88.5%) caused by *T. vivax* whereas *T. congolense* was found in only 2 villages. No mixed infections were recorded. The prevalence reached 7.4%, 9.6% and 16.1% at Débé, Kangotenga and Soukoura respectively.

Table 4.1. Prevalence of bovine trypanosomosis in various villages located in the Boucle du Mouhoun Region of Burkina Faso

Village	Number sampled	Number positive	Prevalence (%)	*Trypanosoma* spp.	
				T. vivax	*T. congolense*
Laro	47	1	2.1	1	0
Mou	23	1	4.3	1	0
Boromissi	25	1	4.0	1	0
St Michel	44	2	4.6	2	0
Déré	51	2	3.9	2	0
Bendougou	21	1	4.8	1	0
Bamakoro	46	1	2.2	1	0
Nokuy	36	2	5.6	2	0
Saorokuy	50	1	2	2	0
Soukoura	31	5	16.1	3	2
Zelassé	44	1	2.3	1	0
Kangotenga	52	5	9.6	4	1
Débé	27	2	7.4	2	0

4.3.2. Longitudinal survey

The longitudinal study was conducted in the 10 villages with the highest prevalence (ranging from 2.1 to 16.1%). Among the 978 sentinel cattle selected from 70 different herds, 492 were treated with ISM and 486 served as a control. During the block treatment study a total of 250 new trypanosomal infections were detected. 83.6% (n=209) and 16.4% (n=41) of the infections were due to *T. vivax* and *T. congolense* respectively. One animal only presented a mixed infection.

Considering the infections with *T. congolense*, the monthly (28 days) hazard of *T. congolense* infections in control cattle was 3.2% (95% CI: 2.2-4.5%). The overall relative risk (control/treated hazard ratios) of *T. congolense* infections was 4.3 (95% CI: 1.9-10). The analysis of the relative risk at site level was not performed in regard of the low number of new infections with *T. congolense*.

The monthly hazard of *T. vivax* infections in control cattle was 12% (95% CI 10-14%) and the overall relative risk of *T. vivax* infections for the 10 villages was 1.8 (95% CI: 1.3-2.5). Because the number of new infections was low in cattle sampled at St Michel and Soukoura with one and no new infection in the ISM-treated groups and 4 and 2 new infections in control groups respectively, these two villages were excluded from the analysis of the relative risk at site level.

The total number of new *T. vivax* infections in each of the 8 remaining villages and in each of the experimental groups during the 8 weeks observation period is presented in table 2. The monthly hazard of new infections during the 8 weeks observation period in the control groups varied between 24.3% and 59.5%. The relative risk ranged from 0.9 in Laro to 3 in Déré and varied significantly between sites. However, the interactions between sites and treatment were not significant (i.e. the effect of treatment did not significantly vary in the different sites; p=0.47) (Table 2). Five villages (Débé, Bendougou, Kangotenga, Laro and Mou) presented a relative risk lower than two strongly suggesting ISM resistance (Figure 4.2 and **Error! Reference source not found.**4.2).

Table 4.2. Predicted monthly risk of *T. vivax* infection in ISM-treated and control groups and control/treated hazard ratios for each of the study sites.

Villages	Group	Number of animals	Number of *T. vivax* infections	Monthly hazard (%)	Relative risk	95% C.I.
Débé	Treated	50	10	9.8	1.09	0.43-2.74
	Control	49	12	10.7		
Laro	Treated	37	8	7.6	0.89	0.30-2.65
	Control	37	9	6.8		
Déré	Treated	43	8	9.1	3.04	1.33-6.97
	Control	39	17	25.3		
Bendougou	Treated	50	9	5.1	1.75	0.57-5.37
	Control	50	21	8.7		
Nokuy	Treated	45	9	7.1	2.17	0.80-5.86
	Control	43	21	14.9		
Kangotenga	Treated	45	11	8.5	1.15	0.44-2.98
	Control	44	11	9.7		
Mou	Treated	40	15	16.4	1.67	0.84-3.34
	Control	42	25	26.0		
Boromissi	Treated	48	7	5.2	2.70	0.95-7.68
	Control	49	16	13.6		

C.I. : confidence interval

The PCV values of cattle infected with *T. congolense* were not further analysed because of the low number of observations. Interestingly, ISM-treated animals developing a parasitaemia (*T. vivax*) presented a mean PCV of 0.30 (95% CI 0.28-0.31) significantly higher than the infected animals from the control group with a value of 0.26 (95% CI 0.25-0.28).

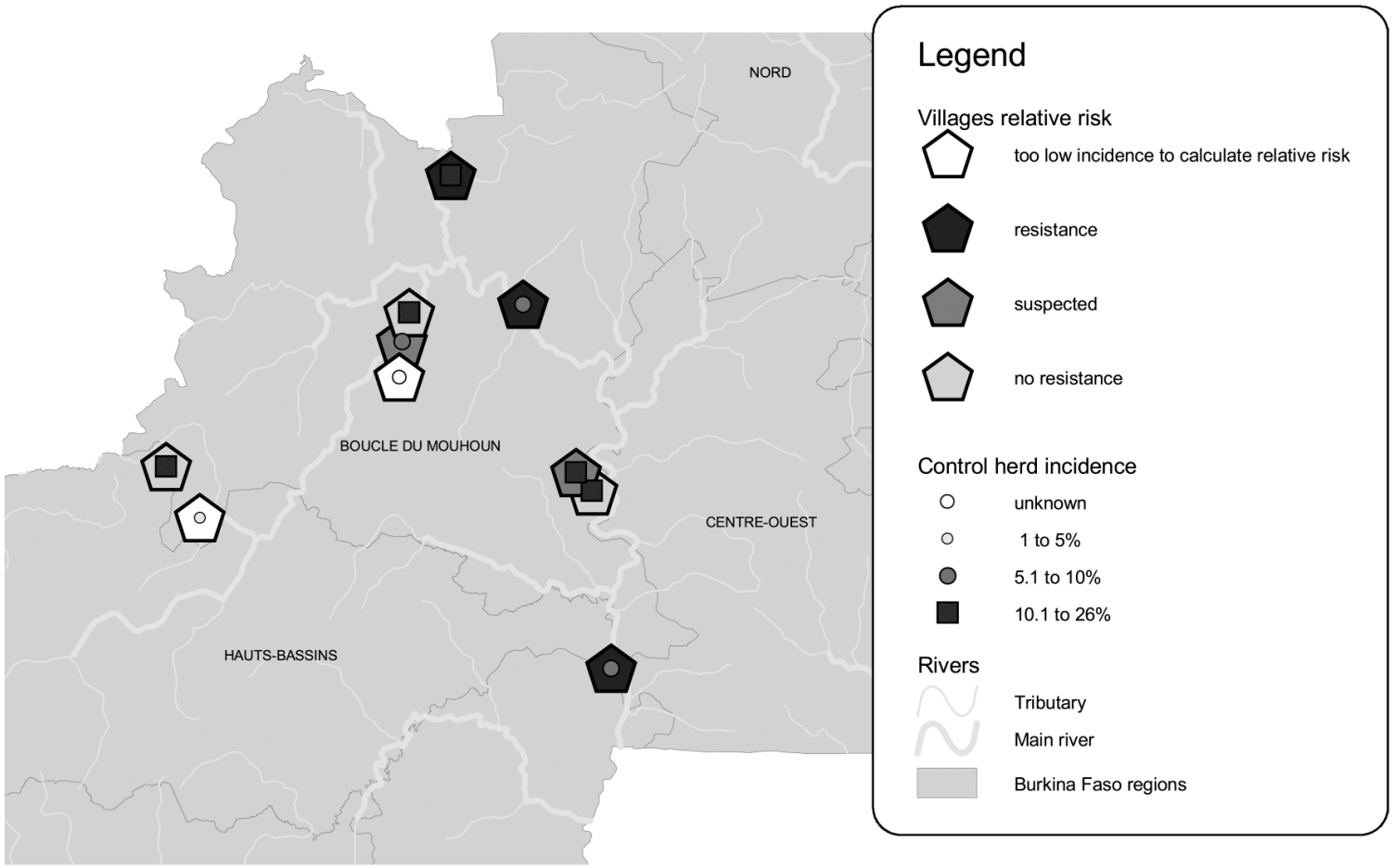

Figure 4.2 Villages relative risk and control herd incidence

During the follow-up, 93 animals of the control group needed to be treated with DA. Within 14 days post treatment, 8.6% only of the treated animals relapsed (Table 4.3) suggesting no acute problem of resistance to DA.

Table 4.3. Number of animals belonging to the control group relapsing after treatment with DA (3.5mg/kg bw) within 14 days post treatment.

Village	Number treated	Number relapsed (%)
Déré	23	2 (8.7)
Nokuy	34	2 (5.9)
kangotenga	19	3 (15.8)
Laro	17	1 (.9)

4.4. Discussion

The first reports of trypanocidal drug resistance in Burkina Faso date from the early 1980's in the province of Kénédougou (Authie, 1994; Pinder and Authie, 1984) and were focused on *T. congolense*. Worrying multi-drug resistance to ISM, DA and ethidium bromide was then observed by Clausen *et al.* (1992). Further studies confirmed those observations (Clausen *et al.*, 2010; McDermott *et al.*, 2003; Talaki *et al.*, 2007). When considering *T. vivax*, albeit case reports of resistance to ISM or DA has been described in East and West Africa (Gray and Roberts, 1971; Kupper and Wolters, 1983; Mwambu and Mayende, 1971; Schönefeld *et al.*, 1987), the information is scarcer compared to the data available for *T. congolense*. The reason for the delay in the reporting of trypanocidal drug resistance in *T. vivax* is largely due to (i) the preponderance of *T. congolense* infections in large parts of tsetse-infested Africa, (ii) the difficulties associated with the isolation of *T. vivax* in laboratory animals and (iii) the absence of any molecular diagnostic tool for this species.

Our observations confirm thus the strong suspicion of resistance to ISM in the Region of the Boucle du Mouhoun. Indeed, based on the criteria used by Eisler *et al.* (2000), a relative risk lower than two strongly suggest resistance to ISM. No resistance was observed in three villages (Déré, Nokuy and Boromissi). This cannot be attributed to a poor challenge since the incidence of the control herds in these villages were among the highest (figure 2). Resistance was observed in 5 out of the 8 villages included in the study (i.e. Laro, Bendougou, Débé, Kangotenga and Mou), though the upper limit of the 95% confidence interval was always

>2.5. Nevertheless, no difference was observed in the level of resistance between the different sites, i.e. the relative risks between the 5 sites where ISM resistance was detected were not statistically different. It should here be emphasized that ISM was used as recommended by Eisler *et al.* (2000) at a dose of 1mg/kg bw rather than the dosage that is commonly used in the field by veterinary technicians, i.e. 0.5mg/kg bw. The low overall relative risk of *T. vivax* infections (1.8 with 95% CI: 1.3-2.5) with this dosage of ISM twice higher than commonly used is a supplementary argument in favour of the existence of ISM resistance. Reasons for this widespread distribution of resistance are attributed to a range of factors such as the long-term use of the same molecules, the misuse of the drugs and the often low quality of drugs available on the local markets (Geerts *et al.*, 2001). Over the 2.8 million doses of trypanocides are used annually in Burkina Faso (MRA, 2006) of which only 23% are officially imported. Recent studies on the quality of the trypanocides DA and ISM sold in sub-Saharan Africa, showed that a great majority of these products do not respect the standards established by the original producers (Schad *et al.*, 2008; Tettey *et al.*, 2002). Recently, the Ministry of Animal Resources of Burkina Faso in collaboration with FAO funded an investigation to determine the quality of the veterinary drugs on the local markets. The study showed that about half of the trypanocides were not in conformity with the quality requirements (Têko, personal communication). According to the official report of the MRA (2006), the annual use of trypanocides in the area of the Boucle du Mouhoun decreased from 1,054,004 doses to 77,910 doses between 2003 and 2006. This drastic drop in the official sales figures reflects the existence of parallel providers of veterinary drugs including trypanocides.

Notwithstanding the observed resistance in *T. vivax* and the resulting relapses in treated animals, treatment with ISM still seems to have a beneficial effect on the condition of the animals. This is reflected by the higher PCV values of cattle infected by ISM resistant trypanosomes and treated with ISM compared to untreated animals of the control herds. The fact that animals from the control herds were followed up every two weeks and treated with DA when positive obviously decreased the difference between ISM-treated and non-treated cattle. In uncontrolled field conditions, the beneficial effects of treating with ISM would thus be even more apparent. This phenomenon was already observed for *T. congolense* in an experimental model where cattle were inoculated with ISM-resistant trypanosomes. The impact of the infection on the PCV was not very pronounced with an average PCV reduction 8 to 14 weeks after treatment of only 5.9% (95% CI: 4.5–7.3) (Delespaux *et al.*, 2010).

4.5. Conclusion

Chemoresistance in the region of the Boucle du Mouhoun seems to remain manageable as no evidence of multi-resistance was found. A rational use of the sanative pair technique is certainly recommended to maintain the situation at controllable level. The concept of the sanative pair recommends the use of two trypanocides (e.g. DA and ISM) which are chemically unrelated and, therefore, are unlikely to induce cross-resistance. The first pair is used until resistant strains of trypanosomes appear and then the second is substituted and used until the resistant strains have vanished from cattle and tsetse (Whiteside, 1962). However, in field conditions the choice of the drug is rather determined by the availability and price than by strategic options. Furthermore, as demonstrated in this study, animals infected with drug resistant trypanosomes and treated with ISM will have a better health condition and productivity which will encourage farmers to continue treating. A tsetse eradication program was initiated by the PATTEC in the area that will probably improve the livelihood of the farmers in the near future. However, as *T. vivax* can be mechanically transmitted, trypanocidal drugs have still to be used strategically till complete eradication of the disease.

4.6. References

Authie, E., 1994. Mise en évidence d'une résistance aux trypanocides parmi les souches de *Trypanosoma congolense* récemment isolées au Burkina. Rev Elev Méd vét Pays trop 37, 219–235.

Bauer, B., Amsler-Delafosse, S., Clausen, P.H., Kabore, I., Petrichbauer, J., 1995. Successful application of deltamethrin pour on to cattle in a campaign against tsetse flies (*Glossina spp*) in the pastoral zone of Samorogouan, Burkina Faso. Trop. Med. Parasitol. 46, 183–189.

Bauer, B., Kabore, I., Liebisch, A., Meyer, F., Petrichbauer, J., 1992. Simultaneous control of ticks and tsetse flies in Satiri, Burkina Faso, by the use of flumethrin pour on for cattle. Trop. Med. Parasitol. 43, 41–46.

Bauer, B., Slingenbergh, J., Hendrickx, G., 1999. A trial to control tsetse at Galana Ranch: Examining the issues. Parasitol Today 15, 82.

Bouyer, J., Bengaly, Z., 2006. Evaluation de la situation entomologique et épidémiologique en vue de l'élaboration d'un plan de lutte contre les trypanosomoses animales et leur vecteur dans la zone d'intervention du PAEOB. Rapport d'expertise du Projet d'Appui aux Organisations d'Eleveurs de l'Ouest (PAEOB) – Bobo–Dioulasso, 1–30.

Clausen, P.H., Bauer, B., Zessin, K.H., Diall, O., Bocoum, Z., Sidibe, I., Affognon, H., Waibel, H., Grace, D., Randolph, T., 2010. Preventing and containing trypanocide resistance in the cotton zone of West Africa. Transboundary and Emerging Diseases 57, 28–32.

Clausen, P.-H., Sidibe, I., Kabore, I., Bauer, B., 1992. Development of multiple drug resistance of *Trypanosoma congolense* in zebu cattle under high natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. Acta Trop. 51, 229–236.

Cuisance, D., Politzar, H., Tamboura, I., Mérot, P., 1990. Coût de l'emploi de barrières de pièges et d'écrans insecticides pour la protection de la zone pastorale d'accueil de Sidéradougou. Rev Elev Méd vét Pays trop, 207–217.

Delespaux, V., Geysen, D., Van den Bossche, P., Geerts, S., 2008. Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol 24, 236–242.

Delespaux, V., Vitouley, H.S., Marcotty, T., Speybroeck, N., Berkvens, D., Roy, K., Geerts, S., Van den Bossche, P., 2010. Chemosensitization of *Trypanosoma congolense* strains resistant to isometamidium chloride by tetracycline and fluoroquinolone. PLoS Neglect Trop D 4, e828. doi:10.1371/journal.pntd.0000828.

Eisler, M.C., McDermott, J.J., Mdachi, R.E., Murilla, G.A., Sinyangwe, L., Mubanga, J., Machila, N., Mbwambo, H., Coleman, P., Clausen, P.H., Bauer, B., Sidibe, I., Geerts, S., Holmes, P.H., Peregrine, A.S., 2000. Rapid method for the assessment of trypanocidal drug resistance in the field. Proceedings of the 9th Symposium of the International Society for veterinary epidemiology and economics, Colorado, USA, 1–3. http://www.sciquest.org.nz/node/71200.

Geerts, S., Holmes, P.H., Diall, O., Eisler, M.C., 2001. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol. 17, 25–28.

Grace, D., Randolph, T., Affognon, H., Dramane, D., Diall, O., Clausen, P.H., 2009. Characterisation and validation of farmers' knowledge and practice of cattle trypanosomosis management in the cotton zone of West Africa. Acta Trop. 111, 137–143.

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

Kone, N., N'Goran, E.K., Sidibe, I., Kombassere, A.W., Bouyer, J., 2011. Spatio–temporal distribution of tsetse and other biting flies in the Mouhoun River basin, Burkina Faso. Med. Vet. Entomol. 25, 156–168.

Kupper, W., Wolters, M., 1983. Observation on drug resistance of *Trypanosoma (Nannomonas) congolense* and *Trypanosoma (Duttonella) vivax* in cattle at a feedlot in the Northern Ivory Coast. Tropenmed. Parasitol. 34, 203–205.

McDermott, J.J., Woitag, T., Sidibe, I., Bauer, B., Diarra, B., Ouedraogo, D., Kamuanga, M., Peregrine, A.S., Eisler, M.C., Zessin, K.H., Mehlitz, D., Clausen, P.H., 2003. Field studies of drug–resistant cattle trypanosomes in Kenedougou Province, Burkina Faso. Acta Trop. 86, 93–103.

MRA, 2006. Les statistiques du Secteur de l'Elevage au Burkina Faso. Rapport du Ministère des Ressources Animales du Burkina Faso, 1–76.

Murray, M., Murray, P.K., Mcintyre, W.I.M., 1977. Improved parasitological technique for diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg. 71, 325–326.

Mwambu, P.M., Mayende, J.S.P., 1971. Berenil resistant Trypanosoma vivax isolated from naturally infected cattle in Teso District, eastern Uganda. Proceedings of the 13th meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Lagos, Nigeria, September 1971. OAU/STRC Publication, 133–138.

Osborne, J.W., 2002. Notes on the use of data transformations. Practical Assessment, Research & Evaluation 8, 6.

Pinder, M., Authie, E., 1984. The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. Acta Trop. 41, 247–252.

Schad, G.J., Allanson, A., Mackay, S.P., Cannavan, A., Tettey, J.N.A., 2008. Development and validation of an improved HPLC method for the control of potentially counterfeit isometamidium products. J. Pharm. Biomed. Anal. 46, 45–51.

Schönefeld, A., Rottcher, D., Moloo, S.K., 1987. The sensitivity to trypanocidal drugs of *Trypanosoma vivax* isolated in Kenya and Somalia. Trop. Med. Parasitol. 38, 177–180.

Talaki, E., Diall, O., Sidibe, I., Belem, A.M.G., Pangui, L.J., 2007. Méthode de diagnostic rapide de la chimiorésistance des trypanocides à l'isométamidium et au diminazène sur le terrain. Revue Africaine de Santé et de Productions Animales (RASPA), 29–36.

Tettey, J.N.A., Astriku, C., Chizyuka, G., Slingenbergh, J., 2002. Non conformance of diminazene preparations to manufacturers' label claims: an extra factor in the development of chemoresistance? Newsletter Integrated Control Pathogenic Trypanosomes Vectors (ICPTV), 24–26.

Whiteside, E.F., 1962. The control of cattle trypanosomiasis with drugs in Kenya: Methods and costs. East Afr. Agr. J., 67–73.

Chapter 5:

Irradiated male tsetse from a 40-year-old colony are still competitive in a riparian forest in Burkina Faso

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Adapted from:

Sow A., Sidibé I., Bengaly Z., Augustin B., Sawadogo G. J., Solano, P., Vreysen M.J.B., Lancelot R., Bouyer, J. (2012). Irradiated Male Tsetse from a 40-Year-Old Colony Are Still Competitive in a Riparian Forest in Burkina Faso. *PLoS ONE*, 7(5), e37124. doi:10.1371/journal.pone.0037124.g001,

5.1. Introduction

Tsetse flies are the sole cyclical vectors of trypanosomes, the causative agents of AAT and HAT. The maintenance of non-trypanotolerant cattle in tsetse-infested areas is often only feasible through continuous prophylactic and curative treatment with trypanocidal drugs and as a result, more than 35 million doses are being administered annually (Geerts *et al*., 2001). Chemoresistance against these drugs is however, becoming more and more widespread (Geerts *et al*., 2001; Delespaux *et al*., 2008) making tsetse control the only way to sustainably manage trypanosomosis.

Most control tactics against tsetse flies are effective and allow quick reduction of their abundance. The use of insecticide impregnated targets and the application of insecticide pour-ons on cattle reduced tsetse populations in Burkina Faso and in some East African countries drastically (Bauer *et al*., 1999; Bauer *et al*., 1995; Hargrove *et al*., 2003; Kagbadouno *et al*., 2011). However, these control methods generally do not eradicate the tsetse population because their efficiency is density dependent (Bouyer *et al*., 2010a). Other vector control methods, such as the Sequential Aerosol Technique (SAT), are not tsetse density dependent and can be used to manage tsetse populations in open savannah areas such as *Glossina morsitans centralis* in the Okavango Delta of Botswana (Kgori *et al*., 2006). The SAT relies on a high percentage of adult mortality (>99%) during each spraying cycle, which can rarely be attained in the humid or sub-humid areas of West Africa, in view of the dense gallery forests. Finally, the SIT has “negative” density dependent properties, i.e. its efficiency is inversely proportional to the density of the target population because of an increase of the ratio of sterile to wild males at each generation (Dyck *et al*., 2005). Therefore, the combination of the SIT (effective at low population densities) with other control techniques that are effective at high population densities is an optimal strategy to achieve eradication of riverine tsetse fly populations in West Africa (Van der Vloedt *et al*., 1980; Takken *et al*., 1986). However, to warrant a sustainable impact, these tsetse control tactics must be applied area-wide, i.e directed against an entire tsetse population within a delimited area, especially if eradication is the strategy of choice. For example in West-Africa, riverine tsetse species occur in large distribution belts, with established gene flows between the various river basins (Bouyer *et al*., 2010b; Koné *et al*., 2011): their eradication would thus require a sequential approach, including the implementation of barriers between eradication blocks.

Although initially successful, the previous campaigns in Burkina Faso and Nigeria were not sustainable as the approach was not area-wide i.e. it did not target the entire tsetse population in a circumscribed geographical area (Vreysen *et al*., 2007) and the local beneficiary communities and authorities failed to create or maintain adequate buffer areas to prevent re-invasion of the cleared areas (Sow *et al*., 2010).

In addition to the mass-rearing of male flies to be sterilized by ionizing irradiation, the SIT can only be successful if these sterile males (i) can locate the wild virgin females and successfully transfer their sterile sperm, and (ii) disperse and aggregate in a similar pattern as their wild counterparts (Vreysen *et al.*, 2011). The Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo Dioulasso, Burkina Faso has been maintaining a *G. p. gambiensis* colony since 1972, with introduction of wild pupae from time to time. The colony however, has, since the eradication campaign in Sidéradougou in the early 1980's not been used for any operational releases (Cuisance *et al*., 1984). It is intended to use the sterile flies from this colony for AW-IPM programmes in Burkina Faso and Senegal and it was therefore deemed necessary to re-confirm its field competitiveness (Bouyer *et al*., 2010c).

In this study, field releases of sterilized male *G. p. gambiensis* were carried out in riverine gallery forest habitat in Burkina Faso to study their survival, dispersal and aggregation pattern, as well as their mating frequencies with wild female tsetse flies.

5.2. Materials and methods

5.2.1. Study area

The study area was situated close to the village of Kadomba (11°53' North; 3°97' West), 70 km north of Bobo-Dioulasso (fig. 5.1) and contained guinean riverine forest (Bouyer *et al*., 2005) along the Leyessa River (a tributary of the Mouhoun River) which has its origin in the protected forest of Maro. Previous entomological surveys showed that almost all caught tsetse were *G. p. gambiensis*, with an average apparent density of 10 flies per trap per day (Koné *et al*., 2011). Laboratory-reared *G. p. gambiensis* were released over 3 km along the river in geo-referenced release sites. One km upstream of the release area, a 1-km barrier was established with 20 biconical traps impregnated with deltamethrin (800 mg/m^2) and deployed every ~50 meters during the whole study. Other studies had revealed that the tsetse population of the

study area was genetically differentiated (and thus partially isolated) from that of the Mouhoun River (Esnault, 2007).

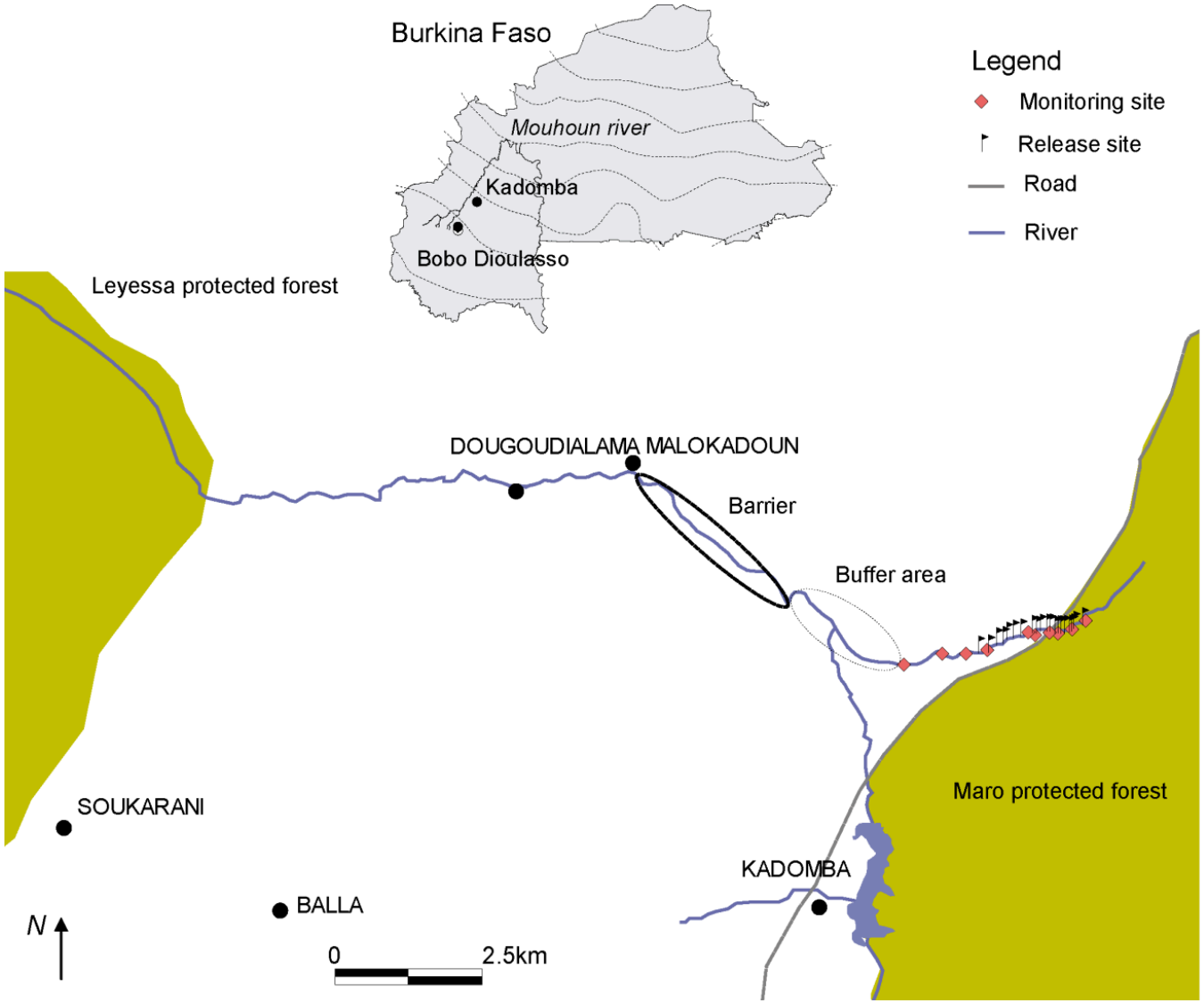


Figure 5.1. Location of the study area in Burkina Faso The release and monitoring sites along the Leyessa River are displayed. The 1-km barrier was established with 20 biconical traps impregnated with deltamethrin (see material and methods for details).

5.2.2. Tsetse sterile males

Laboratory-reared tsetse flies used for this study were produced at the CIRDES in Bobo Dioulasso, Burkina Faso. Newly emerged adult male flies were irradiated in a Cs^{137} GAAA® irradiator with a dose of 110 Gy (dose rate of 4.49 Gy/min), routinely used at CIRDES since the Sideradougou eradication campaign (Taze *et al*., 1977). Irradiated males were marked with acrylic paint on the thorax using different colours to differentiate between series of released insects. Before release, flies were offered twice a blood meal containing ISM (Trypamidium® MERIAL SAS, Lyon, France batch nDG/20058) at a concentration of 10 mg/L to prevent the development of trypanosomes in the released flies (Van den Bossche *et al*., 2006; Bouyer *et al*., 2008). Batches of 50 4-day-old male flies were transferred to Roubaud cages (4.5×13×8 cm) that were covered with a net of mesh 1×1 mm. Cages were then put in a humidified container and transported to the release sites.

5.2.3. Preliminary entomological data collection

Before initiating the field releases, two entomological sampling efforts were carried out during 5 consecutive days, at 10-day intervals. Tsetse flies were sampled with 20 biconical traps (Challier and Laveissière, 1973) deployed at 150 m intervals along the river. All trapping sites were georeferenced. Caught female flies that were still alive were dissected. The same person assessed the percentage of pregnant females and the spermathecal fill during these preliminary sampling periods and during intervention period.

5.2.4. Release of sterile males

Seven releases were carried out at weekly intervals in January–February 2010. A thousand sterile males were released during each of the first 2 releases, and the number of males released was increased to 2,000 and 4,000 sterile males for the 3 following releases and the last two releases, respectively. A total of 16,000 sterile males were thus released over the 7-week interval, to obtain a ratio of irradiated to wild males upon 1:1. Releases were made along the river between 4h30 and 6h30 p.m. at equal proportions in 10 different sites interspaced at approximately 300 m. During the releases, dead flies and non-flyers were recorded after opening the Roubaud cages.

5.2.5. Sterility levels of irradiated male *Glossina palpalis gambiensis*

Twenty newly emerged virgin *G. p. gambiensis* females from the CIRDES colony were mated with 10 newly emerged irradiated males and maintained under normal insectary conditions

(both males and females were 4 days old). Produced pupae were regularly collected, weighed and stored. Females were dissected after 4 weeks to assess spermathecal fill and their reproductive status. The results were compared to those of a control group of 2000 females of the main colony, maintained in the same conditions.

5.2.6. Dissection of sampled wild female *Glossina palpalis gambiensis*

All trapped live female flies were dissected for ovarian ageing to determine the physiological age of the population (Challier, 1965). Proportions of nulliparous, young (less than 4 ovulations) and old (4 ovulations or more) parous females were determined (Laveissière *et al*., 2000). Pregnant females were classified as having a larva or a developing egg *in utero*, and non-pregnant females as having an empty uterus. The rate of sterility (natural abortion and induced sterility) was determined taking into consideration the status of the uterus and the follicle next in ovulation sequence: i.e, those females that had recently aborted an egg in embryonic arrest or still had the degenerated egg *in utero*.

The competitiveness of the irradiated males was assessed using the Fried index (Fried, 1971) by comparing the abortion rates obtained during the entomological surveys carried out before, during and after the releases of sterile males. After dissection, spermathecae were placed in a droplet of normal saline solution and were observed under the microscope at 40× magnitude. Spermathecal fill was scored as empty (0) quarter-full (0.25), half-full (0.5), three quarter-full (0.75) and full (1.0) (Pollock, 1982; Abila *et al*., 2003).

5.2.7. Dispersal and population dynamics of the irradiated males

Trapping surveys were implemented weekly after each sterile male release session using 10 biconical traps set along the release area during 2 to 5 days to assess the relative abundance of wild and irradiated males. The colour of the acrylic spot on the thorax indicated the date of release, and thus the age of the trapped flies. The ratio of irradiated to wild male flies in the samples was also calculated.

Recapture of released irradiated males provided an estimate of the number of alive, marked flies for the different series of releases. After the last release, entomological monitoring was continued every week, up to one month after the last release.

5.2.8. Statistical analyses

Mortality rate and survival of the released male flies were estimated using the temporal relative abundance data, assuming a constant daily mortality rate within each released batch.

The linear evolution of the captures of the sterile males released on 06/02/10 is presented in fig. 5.2: this is illustrative of what was observed with other batches. The daily survival rate was thus estimated as the exponential of the slope of the natural logarithm (ln) of total captures for each batch against time.

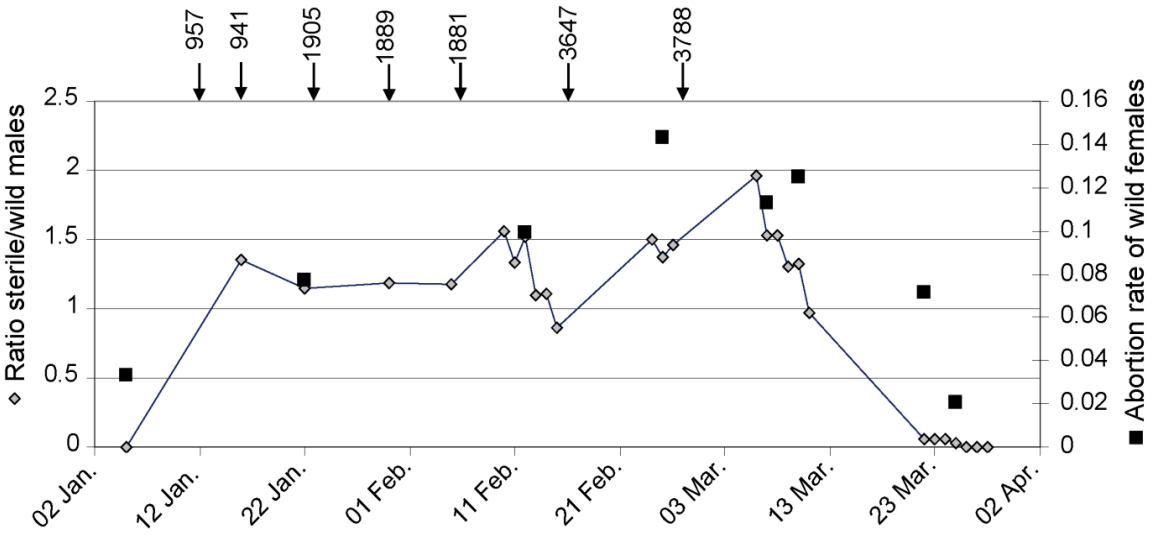


Figure 5.2. Temporal pattern of the ratio of sterile to wild males and the abortion rates of wild females during the study period (Jan.-Apr. 2010). The number and dates of sterile flies released are represented by the black arrows at the top.

To assess similarities or differences in the spatial pattern of apparent densities of wild and sterile male flies using trap records, the existence of a spatial trend in log (wild male counts) was tested (Vreysen *et al*., 2011). This trend was subtracted from log-counts before assessing the independence of trap locations and wild male fly abundance. This was achieved with a Monte Carlo test for marked point processes (Schlather *et al*., 2004): the point process being the set of trap locations and the marks being the wild male fly counts. Secondly, we used a χ^2 test to assess the spatial heterogeneity in wild male fly abundance, and correlation tests to assess the independence of wild males and females, and sterile males.

To plot the data, we transformed fly counts into standardized contributions. For each fly category i (wild male or female, sterile male) and trap j (j= 1,… J), each observed trap count $n_{i,j}$ was divided by the total observed count N_i for this fly category to give the observed relative contribution of each trap $o_{i,j} = n_{i,j}/N_i$. The expected relative contribution of trap j under the assumption of homogeneous spatial distribution ($e_{i,j} = 1/J$) was then subtracted to $o_{i,j}$ and the result was divided by $e_{i,j}$, thus providing the standardized contribution $c_{i,j} = (o_{i,j} - e_{i,j})/e_{i,j} = J\, n_{i,j}/N_i - 1$.

The proportion of flies having aborted and the spermathecal fill were compared using χ^2 tests. A Pearson's correlation test was used to assess the correlation between the ratio of sterile to wild male flies and the abortion rate of wild females, and between the mortality of the sterile males and the number of flies released.

5.3. Results

5.3.1. Baseline situation

During the two pre-release entomological sampling events, 1950 wild *G. p. gambiensis* were caught giving a relative abundance of 12.2 tsetse flies/trap/day. The sample contained 53.4% female flies of which 166 were dissected. Of these dissected females, 25.9% were nulliparous, 36.1% young and 38% old parous flies. The natural abortion rate was 3.3%, an additional 4.9% of the female flies had an empty uterus (post larviposition) while 39.8% of the female flies contained an egg and 52% a larva.

5.3.2. Sterile male fly losses during transport

Of the 16,000 irradiated males shipped to the release points, 15,008 (93.8%) were actually released (Table 5.1), i.e. mortality rate of the male flies at the release sites was 1.9% due to handling, marking, transport or irradiation, and 4.3% were non-flyers and either too weak to take off or had non-functional wings due to acrylic painting.

Table 5.1. Characteristics of the batches of irradiated male *G. p. gambiensis* released in Kadomba

Date	Released flies	Flyers (%)	Daily mortality rate (%)	Mean lifespan (days)	Recapture rate (%)
12 Jan	1,000	95.7	10.0	6.60	3.6
16 Jan	1,000	94.1	11.9	5.45	5.6
23 Jan	2,000	95.3	16.8	3.76	4.7
30 Jan	2,000	94.4	16.1	3.95	5.0
6 Feb	2,000	94.0	14.7	4.34	15.0
16 Feb	4,000	91.2	12.5	5.19	4.4
26 Feb	4,000	94.7	22.0	2.79	9.4
Total	16,000	93.8 ±1.5	14.9 ± 4.0	4.6 ± 1.3	7.2 ±0.1

5.3.3. Population dynamics of the irradiated males

During the monitoring of the dispersal of the irradiated males, 1,068 wild females, 1,048 wild males and 1,142 released males (i.e. 7.6% of the released flies) were trapped. The mean daily mortality rate of the released sterile males was 14.9±4.0%, corresponding to a mean lifespan of 4.6±1.3 days (fig. 5.3 and table 5.1). There was no significant correlation between the mortality rate and the number of released flies (p = 0.15). The population of irradiated flies decreased quickly and, one month after the last release no more marked flies were trapped during 3 consecutive days of trap deployment (fig. 5.3).

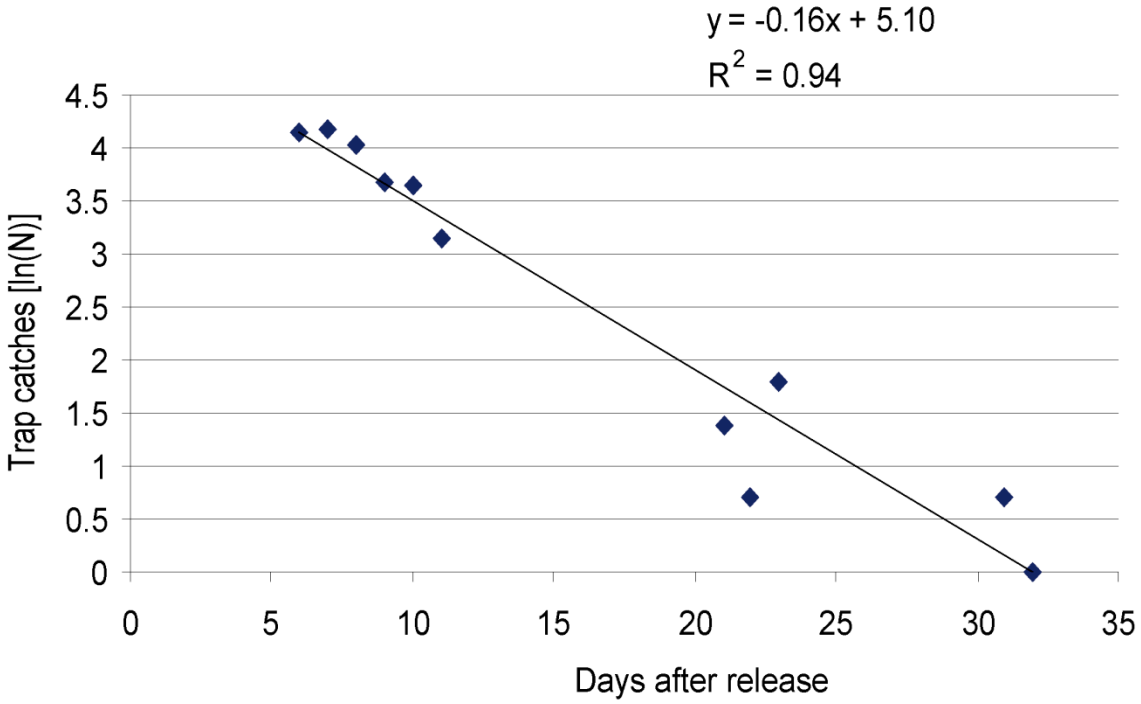


Figure 5.3 Dynamics of the number of sterile males recaptured (batch released the 06/02/2010 in Kadomba).

5.3.4. Mating of virgin females with irradiated males

In the laboratory, 48.9% of the colony *G. p. gambiensis* females that had mated with 110 Gy irradiated males had an empty uterus due to abortion, and 6.7% and 44.4% of the female flies had a larva and a degenerating egg *in utero* respectively. The abortion rate was thus higher ($p<0.001$) than in the control group, where only 1.1%±0.7% of the females aborted. The weight of pupae collected from the different experimental batches was significantly lower ($p<0.001$) than that of pupae produced by the untreated control group i.e. a mean weight of 20.7±2.3 mg vs. 26.8±0.3 mg. Moreover, no adult flies emerged from these pupae. The abortion rate and the spermathecal fill did not vary significantly from one experimental group to another ($F = 0.40$; $df = 6$; $p = 0.75$). However, less than one third of females mated with the

irradiated males had full spermathecae (31.1%) while 20% had empty spermathecae, which was significantly lower than the mean spermathecal fill of the wild females dissected during the pre-release entomological sampling ($\chi^2 = 5.90$; $p = 0.015$).

5.3.5. Competitiveness of irradiated males as compared to wild males

From the second week of sterile male fly releases, the rate of induced sterility increased from 3.3% to 7.7% (s.e. 7.7%) reaching 14% at the end of the releases (fig. 5.2). It dropped again to 2.1% (s.e. 1.5%) one month after the last release. During the release period, the abortion rate was significantly higher than the natural abortion rate before (n = 338, $\chi^2 = 4.932$, $p = 0.026$) and after (n = 311, $\chi^2 = 5.548$, $p = 0.019$) the release period. There was no significant difference between the natural abortion rates recorded before and after the releases (n = 219, $\chi^2 = 0.012$, $p = 0.914$).

During the entire experiment, the ratio of irradiated to wild males fluctuated between 1 and 2 (fig. 5.2), and was on average 1.16 (s.d. 0.38). There was a strong positive correlation ($r = 0.95$, $p<0.001$) between the ratio of sterile to wild males and the abortion rate measured during the same week. The competitiveness of the sterile males (Fried index) was 0.07 (s.d. 0.02), corresponding to a sterile to wild male ratio of 14.4 required to obtain 100% induced sterility in female flies.

5.3.6. Spermathecal fill

During the preliminary fly sampling there was no significant difference between spermathecal fill of the 3 age groups, i.e. teneral/nulliparous, young and old parous wild flies (F = 0.13; df = 2, $p = 0.88$). During the release period, no significant difference was observed between the spermathecal fill of the various age groups (F = 2.05; df = 2, $p = 0.130$). More than 50% of the flies had spermathecae completely filled with sperm and less than 5% had empty spermathecae. Average spermathecal fill of the wild female flies before and after the releases was similar ($p = 0.149$).

5.3.7. Spatial distribution of the sterile males

Significant spatial trends were observed in the count data: a non-linear (quadratic-like) trend ($p<10^{-4}$) was observed for longitude with a maximum close to the eastern region of the study area, and a linear trend ($p<10^{-4}$) was observed in latitude, with a maximum in the northern region of the study area.

Similar trends were observed for wild female and sterile male flies, with significant and very similar *p*-values. These spatial trends were removed from the data sets for further analyses. The point marked process analysis showed that wild male fly counts were independent from trap locations (Monte Carlo test, $p>0.05$), i.e., no interaction was detected between trap locations and fly counts.

Although sterile male flies were released rather homogeneously along the river (fig. 5.1, mean distance between release sites = 102 m, s.d. = 49 m) their spatial distribution of recapture was highly heterogeneous, as evidenced by trapping counts with spatial trend removed ($\chi^2 = 34$, df = 9, $p = 10^{-4}$). The distributions of male and female wild fly catches were also heterogeneous ($\chi^2 = 133$, df = 9, $p<10^{-4}$; $\chi^2 = 25$, df = 9, $p = 0.003$). The joint distribution of these de-trended counts is shown in Figure 5.4. The spatial distribution of sterile and wild male fly catches was similar ($p = 0.94$).

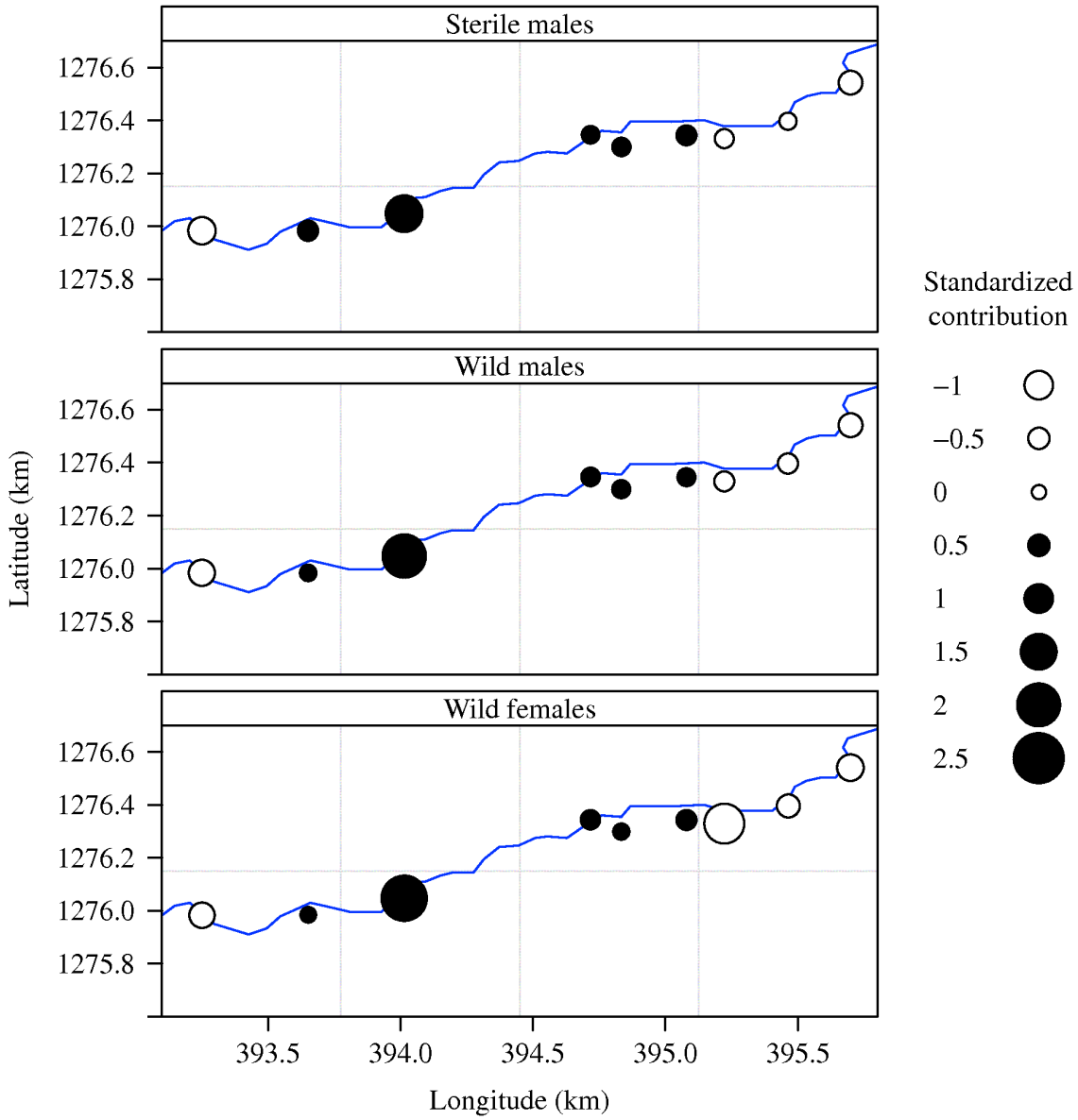


Figure 5.4. Spatial distribution (standardized abundance) of wild and sterile *Glossina palpalis gambiensis* sampled with 10 biconical traps along the Leyessa River. See text for explanations on standardized abundance. Longitude and latitude are expressed according to the coordinate reference system UTM 30N, Clarke 1880 ellipsoid.

5.4. Discussion

The use of sterile insects as part of AW-IPM can only be successful if the male flies released are of the highest biological quality. They should have adequate survival, intermingle with the wild insect population and be capable of transferring their sterile sperm to virgin females preferably in the same frequency as their natural male counterparts. The competitiveness of sterile insects becomes the more questionable when they have been colonised for multiple generations, as is the case with the *G. p. gambiensis* colony maintained at the CIRDES in Burkina Faso. Recently, it was demonstrated that this strain was still competitive with two

field strains originating from Mali and Senegal in experimental conditions (Mutika *et al*., 2012). The results of these experimental releases clearly indicate that the competitiveness and behaviour of irradiated male *G. p. gambiensis* derived from the CIRDES colony under field conditions was comparable with data obtained 30 years ago.

The percentage of flies actually released (94%) as a proportion of total flies transported, was satisfactory and similar to that obtained by Clair *et al.* (1976) in Burkina Faso. Mortality rates before release and the proportion of non-flying alive flies in our experiment were very close to their observations, indicating adequate handling, irradiation, transport, and release procedures. Although the average daily mortality rate after release can be considered as high i.e. 14.9% and corresponding to a mean lifespan of 4.6 days, these data are in line with results obtained from January to March 1984 during the Sidéradougou eradication campaign using flies derived from the same colony (Cuisance *et al*., 1984; Politzar and Cuisance, 1984). A re-analysis of the raw data available during this period (Bouyer J., unpublished) revealed a daily mortality of 13.6%, s.d. 6%. However, during the rainy season of the same year (August-October 1984) a much lower daily mortality rate of 9% (s.d. 3%) was observed. The high mortality rates in our experiment could therefore be attributed to the hot dry season in the area when temperatures were high and the stress on the sterile flies considerable. It would be useful to expand this study and assess performance of the sterile males during the rainy season. The mean lifespan obtained during our study is also close to the one obtained with irradiated males of *G. tachinoides* released around N'Djamena (Chad) in 1973 (4.8 days) (Cuisance and Itard, 1973). Generally, the longevity of irradiated male tsetse flies is reduced as compared with wild flies due to (i) successive anaesthesia using cold temperatures, (ii) handling in the insectary for sorting and marking, and (iii) irradiation using ionizing radiation that inflicts somatic cell damage (Vreysen *et al*., 2001). Former studies in the laboratory showed that mean longevity was considerably reduced in irradiated *G. tachinoides*, *G. fuscipes fuscipes* Newstead, and *G. brevipalpis* (Vreysen *et al.*, 1996). These shorter lifespans must be compensated for by regular (twice weekly) releases during an eradication campaign to maintain critical sterile to wild male overflooding ratios (Hendrichs *et al.*, 2005). In the case of tsetse, the shorter lifespan presents however an advantage as it reduces the risk of sterile males transmitting the trypanosomoses (Van den Bossche *et al*., 2006; Bouyer, 2008).

The spatial analysis showed that the observed heterogeneous fly distribution among traps was not related to differences in trap efficiency, but to a fly aggregation in preferred sites (and fly emigration from other sites) as was observed on Unguja Island (Zanzibar) during the

eradication campaign of *G. austeni* (Vreysen *et al.*, 2011). Barclay (1992) has shown the importance of insect aggregation in pest control, especially when using the SIT or any other genetic control method. The weaker correlation between wild males and females, and absence of correlation between sterile males and wild females might be related to different, sex-specific preferences in fly habitat, as observed on Unguja Island (Vreysen *et al.*, 2011). In addition, the present data confirm that tsetse fly dispersal cannot be solely considered as a homogeneous diffusion process, as often assumed (Bouyer *et al*., 2009b; Hargrove, 2000). It also confirms that mass-reared and gamma-sterilized male tsetse flies were able to respond to environmental cues and to aggregate in the preferred sites of the wild males, even after being colonised for about 40 years. Their aggregation behaviour was therefore similar to that of wild males, confirming that sterile male flies derived from the CIRDES colony are still very good candidates for genetic control.

The competitiveness of irradiated males was assessed through the comparison of the abortion rate and spermathecal fill before, during and after the releases of irradiated males, and the analysis of their spatial distribution, as compared with their wild counterparts. During the collection of initial baseline data, the observed natural abortion rate of 3.3% was close to the rate of natural abortion noted in *G. austeni* (Vreysen *et al*., 2000). Under adverse climatic conditions, the abortion rate in female tsetse can reach 9%, as observed in *G. m. morsitans* in Zambia (Challier, 1982). However, there was no climatic accident during the study, but instead a progressive increase of the mean temperature from 27.6°c to 32.6°c and of the mean relative hygrometry from 16.6% to 35.8% (data not shown), whereas the abortion rate dropped again after the release period. The low spermathecal fill observed in laboratory cage conditions in comparison to the wild females is probably related to the use of 4 day-old males, which are not as competitive as older males. During 11 weeks of monitoring, the ratio of irradiated/wild males was on average 1.16 (s.d. 0.38). These results confirmed that 110 Gy-irradiated, previously fed male *G. p. gambiensis* disperse well and have a significant impact on the reproduction of wild females. Obviously, such a low release rate would neither allow a significant induction of sterility in the wild female population or/and a significant reduction of the wild tsetse population in the release area. In our experiment, the wild *G. p. gambiensis* population was deliberately not suppressed before release of sterile male flies because we needed an adequate number of wild female flies for dissection to assess the impact of sterile males on their sterility. The data of the experiment clearly indicated that irradiated male *G. p. gambiensis* reared at CIRDES are still compatible with the wild females

and competitive with the wild males in the Mouhoun River basin. This is probably due to the slow reproductive cycle of tsetse (one offspring every 10 days resulting in a mean number of only 4.7 pupae by female in the insectarium of CIRDES), which imposes relatively low selection pressures on flies maintained in a colony. This makes it necessary to keep a large colony to produce the sterile males, as it was done in CIRDES (between 50 000 and 100 000 females during the last 20 years), thus allowing to maintain the genetic diversity.

Within the framework of the eradication project in Burkina Faso, it will however be necessary to overflood the wild male population with a higher ratio (>14.4). That is why pre-release suppression by other effective techniques (target, pour on treatment of cattle, SAT and ground fogging) will be implemented in the Mouhoun River basin before using the SIT as was done in the 1980's in the Sidéradougou area (Cuisance *et al*., 1984; Politzar and Cuisance., 1984).

5.5. References

Abila, P.P., Kiendrebeogo, M., Mutika, G.N., Parker, A.G., Robinson, A.S., 2003. The effect of age on the mating competitiveness of male *Glossina fuscipes fuscipes* and *G. palpalis palpalis*. J. Insect Sci. 3, 13–19

Barclay, H.J., 1992. Modelling the effects of population aggregation on the efficiency of insect pest control. Res. Pop. Ecol. 34, 131–141.

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

Bauer, B., Amsler-Delafosse, S., Kaboré, I., Kamuanga, M., 1999. Improvement of cattle productivity through rapid alleviation of African Trypanosomosis by integrated disease management practices in the Agropastoral zone of Yalé, Burkina Faso. Trop. Anim. Health Prod. 31, 89–102.

Bouyer, J., 2008. Does isomethamidium chloride treatment protect tsetse flies from trypanosome infections during SIT campaigns? Med. Vet. Entomol. 22, 140–144.

Bouyer, J., Balenghien, T., Ravel, S., Vial, L., Sidibé, I., Thévenon S., Solano P., De Meeûs, T., 2009. Population sizes and dispersal pattern of tsetse flies: rolling on the river? Mol. Ecol. 18, 2787–2797.

Bouyer, J., Guerrini, L., César, J., de la Rocque, S., Cuisance, D., 2005. A phyto-sociological analysis of the distribution of riverine tsetse flies in Burkina Faso. Med. Vet. Entomol. 19, 372–378.

Bouyer, J., Ravel, S., Guerrini, L., Dujardin, J.P., Sidibé, I., Vreysen, M.J., Solano, P., de Meeûs, T., 2010b. Population structure of *Glossina palpalis gambiensis* (Diptera: Glossinidae) between river basins in Burkina-Faso: consequences for area-wide integrated pest management. Inf. Gen. Evol. 10, 321–328.

Bouyer, J., Seck, M.T., Sall, B., Guerrini, L., Vreysen, M.J.B., 2010c. Stratified entomological sampling in preparation of an area-wide integrated pest management programme: the example of *Glossina palpalis gambiensis* in the Niayes of Senegal. J. Med. Entomol. 47, 543–552.

Bouyer, J., Solano P., Cuisance D., Itard, J., Frézil, J.-L., Authié E. 2010a. Trypanosomosis: Control methods., pp. 1936-1943. In: P.-C. Lefèvre, J. Blancou, R. Chermette and G. Uilenberg [eds.], Infectious and parasitic diseases of livestock (vol. 2). Lavoisier (Tech & Doc), Paris.

Bouyer, J., Stachurski, F., Gouro, A., Lancelot, R., 2009. Control of bovine trypanosomosis by restricted application of insecticides to cattle using footbaths. Vet. Parasitol 161, 187–193.

Challier, A., 1965. Amélioration de la méthode de détermination de l'âge physiologique des glossines. Bull. Soc. Path. Ex. 58, 250–259.

Challier, A., 1982. The ecology of tsetse (*Glossina* spp.) (Diptera, Glossinidae): a review. Insect Sci. Applic. 3, 97–143.

Challier, A., Laveissière, C., 1973. Un nouveau piège pour la capture des glossines (*Glossina:*Diptera, Muscidae): description et essais sur le terrain. Cah ORSTOM, sér. Ent. Méd. et Parasitol. 10, 251–262.

Clair, M., Politzar, H., Cuisance, D., Lafaye, A., 1976. Observations sur un essai préliminaire de lâchers de mâles stériles de *Glossina palpalis gambiensis* (Haute-Volta). Rev. Elev. Méd. vét. Pays Trop. 29, 341–351.

Cuisance, D., Itard, J., 1973. Comportement de mâles stériles de Glossina tachinoides West. lâchés dans les conditions naturelles – environs de Fort-Lamy (Tchad). II. Longévité et dispersion. Rev. Elev. Méd. vét. Pays Trop. 26, 169–186.

Cuisance, D., Politzar, H., Merot, P., Tamboura, I., 1984. Les lâchers de mâles irradiés dans la campagne de lutte intégrée contre les glossines dans la zone pastorale de Sidéradougou, Burkina Faso. Rev. Elev. Méd. vét. Pays Trop. 37, 449–468.

Delespaux, V., Geysen, D., Van Den Bossche, P., Geerts, S. (2008) Molecular tools for the rapid detection of drug resistance in animal trypanosomes. Trends Parasitol. 24, 236–242.

Dyck, V.A., Hendrichs, J., Robinson, A.S., 2005. Sterile insect technique. Dordrecht: Springer. 787 p.

Esnault, O., 2007. Etude de la structuration de deux sous-populations de Glossina palpalis gambiensis Vanderplank sur la Leyessa, un affluent du Mouhoun au Burkina Faso. Bobo: CIRDES. 37 p.

Fried, M., 1971. Determination of Sterile-Insect Competitiveness. J. Econ. Entomol. 64, 869–872.

Geerts, S., Holmes, P.H., Diall, O., Eisler, M.C., 2001. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol. 17, 25–28.

Hargrove, J.W., 2000. A theoretical study of the invasion of cleared areas by tsetse flies (Diptera: Glossinidae). Bull. Entomol. Res. 90, 201–209.

Hargrove, J.W., Torr, S.J., Kindness, H.M., 2003. Insecticide-treated cattle against tsetse (Diptera: Glossinidae): what governs success? Bull. Entomol. Res. 93, 203–217.

Hendrichs, J., Vreysen, M.J.B., Enkerlin, W.R., Cayol, J.P., 2005. Strategic Options in Using Sterile Insects for Area-Wide Integrated Pest Management. In: Dyck VA, Hendrichs J, Robinson AS, editors. Sterile insect technique. Dordrecht: Springer. pp. 563–600.

Kagbadouno, M.S., Camara, M., Bouyer, J., Courtin, F., Morifaso, O., Solano, P., 2011 Tsetse control in Loos islands, Guinea. Parasites & Vectors 4, 18.

Kgori, P.M., Modo. S., Torr, S.J. 2006. The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. Acta Trop. 99, 184–199.

Koné, N., Bouyer, J., Ravel, S., Vreysen, M.J.B., Domagni, K.T., Causse, S., Solano, P., de Meeûs T., 2011. Contrasting Population Structures of Two Vectors of African Trypanosomoses in Burkina Faso: Consequences for Control. PLoS Negl Trop. Dis. 5, e1217.

Koné, N., N'Goran, E.K., Sidibé, I., Kombassere, A.W., Bouyer, J., 2011. Spatio-temporal distribution of tsetse (Diptera: Glossinidae) and other biting flies (Diptera: Tabanidae and Stomoxinae) in the Mouhoun River Basin, Burkina Faso. Med. Vet. Entomol. 25, 156–168.

Laveissière, C., Grébaut, P., Herder, S., Penchenier, L., 2000. Les glossines vectrices de la Trypanosomiase humaine africaine; IRD, editor. Yaoundé: IRD and OCEAC. 246 p.

Mutika, G.N., Kabore, I., Seck, M.T., Sall, B., Bouyer, J., Parker, A.G., Vreysen, M.J.B., 2012. Mating performance of *Glossina palpalis gambiensis* strains from Burkina Faso, Mali and Senegal. Entomol. Exp Appl. (in press).

Novy, J.E., 1991. Screwworm control and eradication in the southern United States of America. World Anim Rev. pp. 18–27.

Politzar, H., Cuisance, D., 1984. An integrated campaign against riverine tsetse flies *Glossina palpalis gambiensis* and *Glossina tachinoides* by trapping and the release of sterile males. Insect Sci. Applic. 5, 439–442.

Pollock, J.N., 1982. Training Manual for Tsetse Control Personnel. Vol. 1: Tsetse biology, systematics and distribution, techniques. Rome: FAO. 243 p.

Schlather, M., Ribeiro, P.J., Diggle, P.J., 2004. Detecting dependence between marks and locations of marked point processes. J. Roy. Stat. Soc. Ser. B (Stat Method) 66, 79–93.

Sow, A., Sidibé, I., Bengaly, Z., Bouyer, J., Bauer, B., Van den Bossche, P., 2010. Fifty years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso. An overview. Bull. Anim. Health Prod. 58, 95–118.

Takken, V., Oladunmade, M.A., Dengwat, L., Feldmann H.U., Onah, J.A., Tenabe, S.O., Hamann, H.J., 1986. The eradication of *Glossina palpalis palpalis* (Robineau-Desvoidy) (Diptera: Glossinidae) using traps, insecticide-impregnated targets and the sterile insect technique in central Nigeria. Bull. Entomol. Res. 76, 275–286.

Taze, Y., Cuisance, D., Politzar, H., Clair, M., Sellin, E., 1977. Essais de détermination de la dose optimale d'irradiation des mâles de *Glossina palpalis gambiensis* (Vanderplank, 1949) en vue de la lutte biologique par lâchers de mâles stériles dans la région de Bobo-Dioulasso (Haute Volta). Rev. Elev. Méd. vét. Pays Trop. 30, 269–279.

Van den Bossche, P., Akoda, K., Djagmah, B., Marcotty, T., De Deken, R., Kubi, C., Parker, A., Van Den Abbeele, J., 2006. Effect of Isometamidium Chloride Treatment on Susceptibility of Tsetse Flies (Diptera: Glossinidae) to Trypanosome Infections. J. Med. Entomol. 43, 564–567.

Van der Vloedt, A.M.V., Baldry, D.A.T., Politzar, H., Kulzer, H., Cuisance, D. 1980. Experimental helicopter applications of decamethrin followed by release of sterile males for the control of riverine vectors of trypanosomiasis in Upper Volta. Insect. Sci. Applic. 1, 105–112.

Vreysen, M., Robinson, A.S., Hendrichs, J., 2007. Area-Wide Control of Insect Pests, from research to field implementation. Dordrecht: Springer. 789 p.

Vreysen, M.J.B., 2001. Principles of area-wide integrated tsetse fly control using the Sterile Insect Technique. Med. Trop. 61, 397–411.

Vreysen, M.J.B., Saleh, K.M., Ali, M.Y., Abdulla, A.M., Zhu, Z.-R., Juma, K.G., Dyck ,V.A., Msangi, A.R., Mkonyi, P.A., Feldmann, H.U., 2000. *Glossina austeni* (Diptera: Glossinidae) Eradicated on the Island of Unguja, Zanzibar, Using the Sterile Insect Technique. J. Econ. Entomol. 93, 123–135.

Vreysen, M.J.B., Saleh, K.M., Lancelot, R, Bouyer, J., 2011. Factory tsetse flies must behave like wild flies: a prerequisite for the sterile insect technique. PLoS Negl. Trop. Dis. 5(2), e907.

Vreysen, M.J.B., Van der Vloedt, A.M.V., Barnor, H., 1996. Comparative gamma radiation sensitivity of *G. tachinoides* Westw., *G. f. fuscipes* Newst., and *G. brevipalpis* Newst. Int. J. Radiat. Biol. 69, 67–74.

Chapter 6:

Longitudinal survey of tsetse flies and trypanosomosis in the PATTEC intervention area in Burkina Faso

6.1. Introduction

The PanAfrican Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) is the most important tsetse control programme implemented in Burkina Faso. The current programme learnt from previous experiences and has taken into account many aspects of trypanosomosis control. Vector control was done by using standard control measures such as insecticide impregnated screens/traps, live baits (insecticide treated cattle), ground spraying, SAT and the use of barriers to prevent reinvasion of the cleared areas. The campaign financed the mass treatment of livestock against trypanosomosis. The particularity of the PATTEC campaign is the intensive involvment of the local communities benefiting from the control of the disease. The main failure in the previous campaigns in Burkina Faso was the sustainability of the achievements on a long term perspective. In the 1980's and 1990's tsetse control projects were implemented namely in the agro-pastoral zone of Sidéradougou (Cuisance *et al*., 1984) and the pastoral zone of Yalé (Bauer *et al*., 1999, Kamuanga *et al.*, 2001). In these campaigns, beneficiary communities, the farmers and the government did not continue the efforts after the projects ended. With the end of the control, the tsetse-cleared areas were quickly re-invaded by tsetse and trypanosomosis incidence raised rapidly to the level observed before the project implementation. Tsetse control campaigns carried out in Burkina Faso were described in the first chapter of this thesis. The success and the failure of all these projects were discussed (Sow *et al*., 2010).

The aims of this longitudinal survey were: to assess the effectiveness of the tsetse control by i) the entomological monitoring of the operations with biconical traps and ii) the measure of the bimonthly trypanosomosis incidence in cattle sentinel herds in the intervention area.

6.2. Material and methods

6.2.1. Study area

The study was carried out in the Region de la Boucle du Mouhoun, the PATTEC intervention area in Burkina Faso. The region is mainly a rural area where most of the population earn their living by crop production and livestock husbandry (MEDEV, 2005). The region hosts almost 700,000 head of cattle, 1,400,000 small ruminants and around 50,000 pigs, horses and donkeys (MRA, 2008). The region has the highest prevalence of trypanosomosis of the country. The Mouhoun River crosses the region on nearly 280 km, describing a loop. Permanent secondary tributaries flood in the main river. This topology explains the abundance

of the riverine tsetse species *G. p. gambiensis* and *G. tachinoides*. The recent surveys financed by the PATTEC, before the intervention measures, showed that the apparent density per trap was high; especially along the edge of the river and permanent tributaries with more than 70 tsetse caught per trap per day (PATTEC, 2008). The region benefited from the PATTEC tsetse elimination campaign since November 2009.

6.2.2. Trypanosomosis and Tsetse control strategies

6.2.2.1. Tsetse control

- ***Communities' awareness***

The tsetse control activities were intensively involving the local communities. Indeed, previous experiences showed that the involvement of beneficiary communities is an important element for achieving sustainability of a control campaign (Bauer *et al*., 1995, Sow *et al*., 2010). In this particular PATTEC campaign, the communities were informed, trained and made responsible for large panels of the field work i.e. the display of insecticide impregnated screens during the dry season and their withdrawal or moving in the rainy season. They were technically and logistically assisted by the PATTEC team and field extension agents. In each village, at least 5 people were chosen as auxiliaries to help the PATTEC field teams for the deployment and the surveillance of the impregnated screens.

- ***Impregnated screens***

The screens used in this control campaign were manufactured by VESTERGAARD (http://www.vestergaard-frandsen.com/). They were made from polyester material. The screens were made from a blue rectangular piece (50cm×100cm) with a black strip (25cm×100cm) on each side. The total surface of a screen was $1m^2$. According to the manufacturer, impregnated screens are effective at killing tsetse for at least 2 years (http://vestergaard-frandsen.com/public-health/sleeping-sickness/downloads).

More than forty thousand impregnated screens were displayed along the banks of the Mouhoun and its main tributaries from November 2009 to April 2010 (fig. 6.1). The screens were displayed alternatively on the left and right banks of the river with an average distance of 100 meters between screens. Sometimes, bush clearing was necessary to increase the screen visibility. Screens were fixed either on a metallic stake or hung on tree branches over the river. Missing screens were systematically replaced by new ones (fig. 6.2). Vandalism and thefts were restricted to a minimum because of the involvement of the local communities.

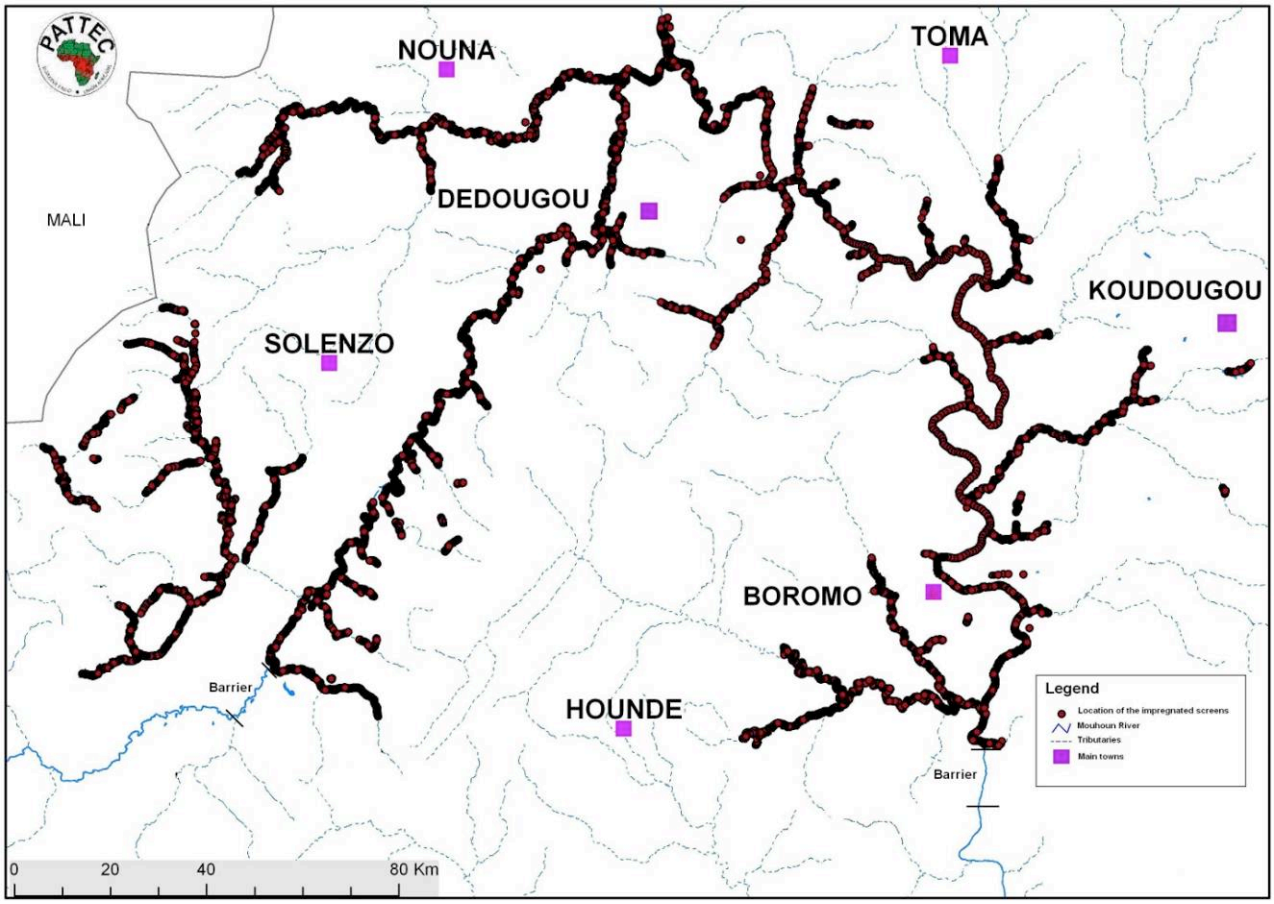


Figure 6.1. Display of the impregnated screens on the Mouhoun River and its main tributaries

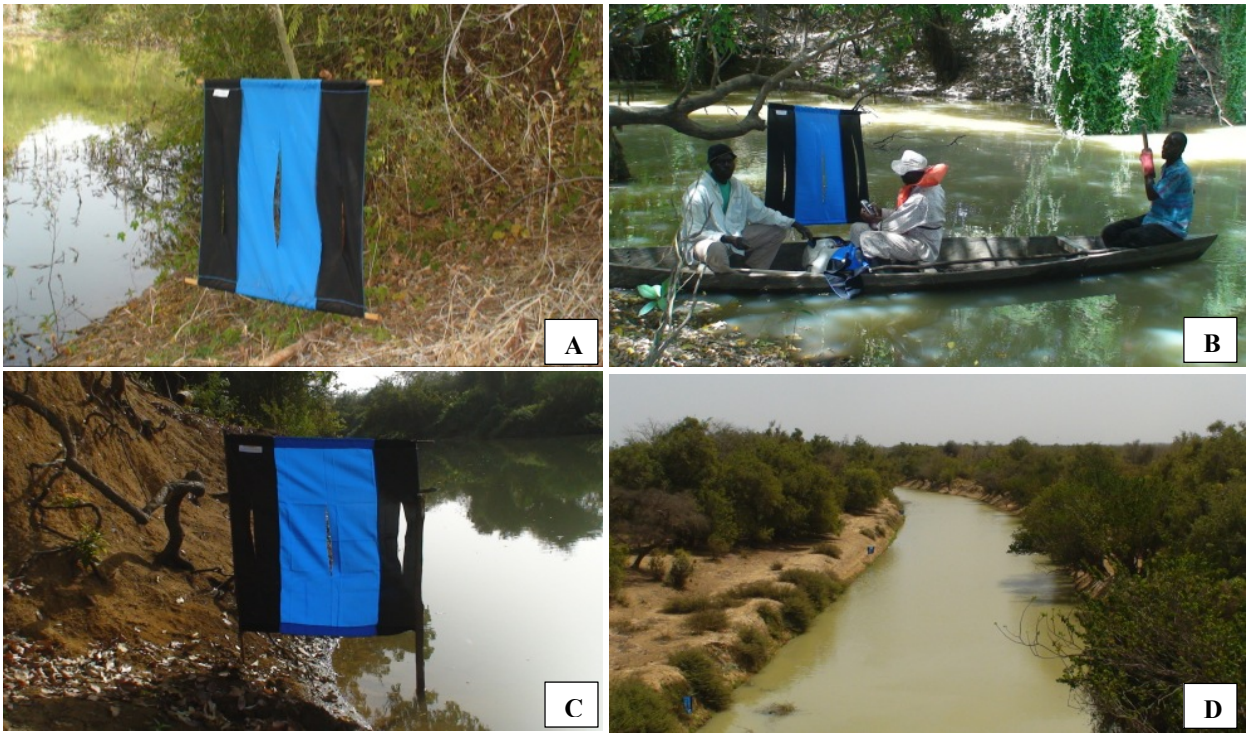


Figure 6.2. Display of impregnated screen along the banks of the Mouhoun River and its main tributaries. A&B: display of screens on three branches, C: Screen fixed on a metallic stake, D: deployment of screens on the edges of the River.

- ***Sequential aerial treatment***

SAT was carried out jointly with the counterparts in Ghana. It targeted the descendant part of the Mouhoun River. Specifically equipped airplanes were therefore hired from ORSMOND Aviation, South Africa. The operation was implemented from April to June 2010 (beginning of rainy season). The airplanes sprayed at a dose of 0.33g deltamethrin/ha, fortnightly at low altitude and by night.

- ***Ground spraying***

Ground spraying was used in the barrier areas to render them more effective. It was also applied in very bushy zones. The ground spraying was carried out with Swingfog® foggers (SN 50 and SN 101 models). Insecticide used was deltamethrin (Aqua-k-othrin® at the concentration of 2%). Fogging was applied in the early morning and late in the evening in ultra low volume (ULV). The amount of insecticide with the solution of 0.15% (1.50g per hectare) was enough for tsetse control (Laveissière *et al*., 2000); it was calculated by taking into account the surface covered and the speed of the fogger on the ground or in the boat (fig. 6.3).

Figure 6. 3. Use of fogger (Swingfog®) in a boat on the Mouhoun River during PATTEC campaign

- ***Barriers***

The tsetse control zone was isolated by 2 barriers of 10 km set at both sides of the Mouhoun River, as recommended by Laveissière *et al*. (2000). Barriers were constituted by impregnated biconical traps and screens displayed on each side of the river at 50 meters intervals. Most of the screens were tightly fixed in tree's branches. The barriers prevent the reinvasion of the cleared area by tsetse. In wooded areas, two or three rows of traps were needed to render the barriers more effective. The barriers were maintained regularly by bush clearing. Destroyed screens or traps were systematically replaced (strong winds, floods, …).

- ***Epicutaneous mass treatment***

Epicutaneous treatment (cypermethrin *pour on* and spray) was carried while implementing trypanocide mass treatment. Treated animals constitute live bait for tsetse and contributed to the drastic reduction of their population.

6.2.2.2. Trypanosomosis control

Mass treatment was carried out in almost all the villages located in the AAT distribution area in the Region de la Boucle du Mouhoun. The treatment concerned cattle, small ruminants and donkeys. The trypanocide treatment was prioritized in villages located at less than 10 km of the Mouhoun River or its permanent tributaries. This task was devoted to private veterinarians. A treatment protocol was provided by the PATTEC. It consisted of curative treatment with DA followed by a preventive treatment within three weeks period with ISM. The cost of trypanocide treatment was fully supported by the PATTEC.

6.2.3. Longitudinal entomological survey

About 400 sentinel biconical traps (Challier and Laveissière, 1973) were set along the Mouhoun River and tributaries (fig. 6.4). The monitoring points were selected based on the entomological baseline survey carried out from December 2007 to June 2008 (PATTEC, 2008). Sentinel traps were displayed in tsetse habitats where the apparent densities per trap (ADP) were the highest. All trapping sites were georeferenced. To increase the attractiveness of the traps, olfactory bait (3n propyl-phenol, octenol and parametacresol) were used. The survey was carried out every two months. Traps were displayed for 3 days before collection. Trapped insects were counted, identified by species and sex and recorded in a data sheet.

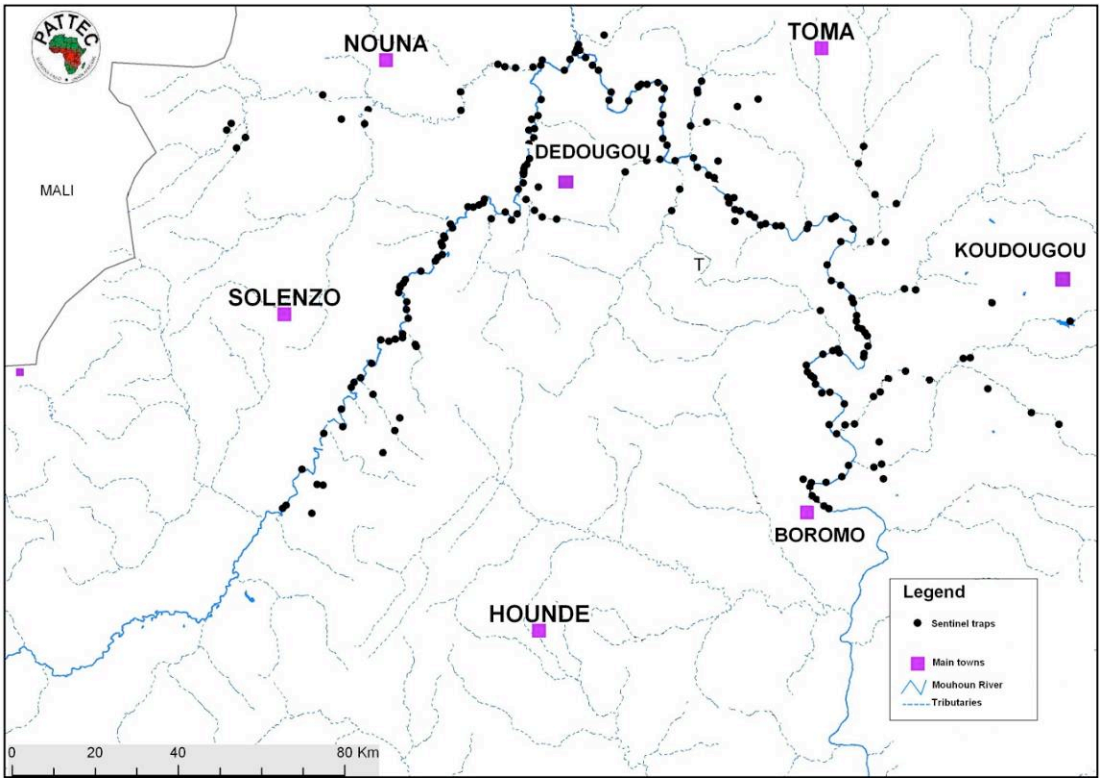


Figure 6.4. Location of the sentinel traps in the PATTEC intervention area in Burkina Faso

6.2.4. Longitudinal parasitological survey

This study concerned sedentary cattle only. Based on the cross sectional study in the PATTEC intervention area (Sow *et al*., 2013), 11 villages where the prevalence of the disease was the highest, were selected for the longitudinal survey (fig. 6.5). In each village, a sentinel herd of 50 cattle was selected. All sentinel animals were ear tagged. At the beginning of the survey sampled cattle were treated with DA (Trypadim®, MERIAL) at the dose of 3.5mg/ kg b.w., dewormed with albendazole (Vermitan®, CEVA) (7.5 mg per kg b.w.) and received every two months cypermethrin-amitraz in *pour-on* formulation (Cypertraz®, CEVA). Before receiving any treatment, blood samples were collected from each animal by jugular vein puncture using Vacutainer® EDTA tubes for the detection of motile trypanosome by the buffy coat technique as described by Murray *et al*. (1977).

Bimonthly follow up was carried out in all villages during a year to detect trypanosomose infections in sentinel herds. Each positive animal was treated with diminazene at the dose of 3.5mg/ kg b.w. To avoid self medication by farmers, it was required from them to contact a field veterinary technician who was available for any health care. Animals which missed one of the bimonthly follow-up were discarded from the study. Parasitological results were recorded as well as any other notable events, which occurred during the two months interval.

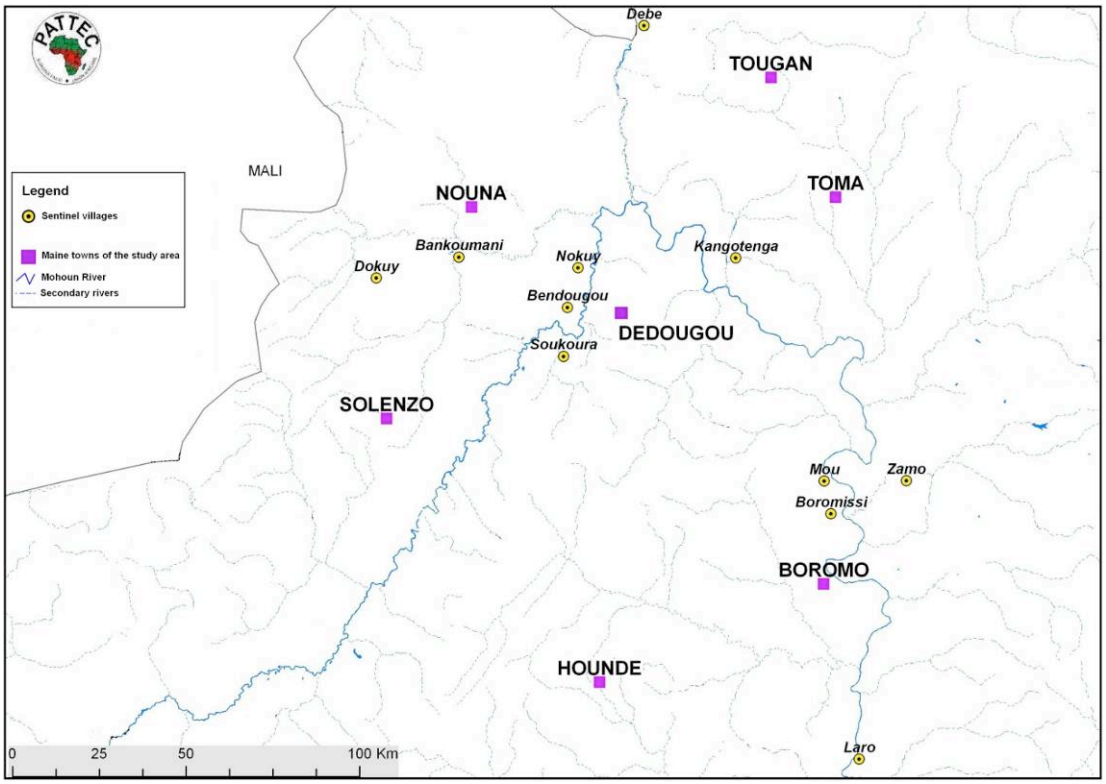


Figure 6.5. Location of the sentinel cattle herds in the PATTEC intervention area

6.2.5. Statistical analysis

Data were processed with Excel and statistical analysis was carried out with STATA® 9.2. The incidence of bovine trypanosomosis was calculated by dividing the number of new cases within the two months interval by the total number of cattle at risk in the same period.

The rate of reduction of the tsetse population was calculated as follows (Gouteux *et al*., 1982):

$$\frac{To - Tn}{To} \times 100$$

To: ADP before the control, Tn : ADP at a given period of the follow up.

6.3. Results

6.3.1. Trypanosomosis prevalence at the beginning of the survey

The longitudinal survey started in April 2010 and ended in May 2011. In total, 11 villages agreed to undergo the longitudinal survey. In total, 547 cattle were selected. The number of 50 animals was reached in all villages except in Bendougou where 47 animals only could be selected. At the beginning of the survey, trypanosomosis infection rate was 3.28% (95% CI: 1.79 - 4.79) in the sentinel herds. Two third of the infections were due to *T. vivax* and one

third was *T. congolense*. Meanwhile, this infection rate varied between 0 and 10% from one village to another. The mean PCV value was 30.7% (95% CI: 30.28-31.12) (table 6.1).

Table 6.2. Prevalence of bovine trypanosomosis and PCV values in the sentinel herds in the PATTEC intervention area before the control implementation

Village	Number of sentinel cattle	PCV (%)	Prevalence (%)
Bankoumani	50	32.0±4.9	0
Bendougou	47	32.6±6.4	2.13
Boromissi	50	28.2±4.2	2
Debe	50	30.2±4.6	0
Dokuy	50	28.8±3.7	4
Kangotenga	50	29.6±5.6	8
Laro	50	32.5±4.7	6
Mou	50	31.1±3.8	0
Nokuy	50	30.9±4.7	10
Soukoura	50	33.6±4.1	0
Zamo	50	28.4±5.0	4
Total	**547**	**30.7±5.0**	**3.28**

6.3.2. Incidence of bovine trypanosomosis

Amongst the 11 villages, ten of them were correctly followed up during the 12 months of the survey. In the village of Bankoumani no data was collected for the third bimonthly follow-up and therefore was excluded from the study. More than half of the sentinel cattle (59.60%) was correctly followed up during this longitudinal study. During the longitudinal survey, 55 cases of trypanosomosis were recorded, 43 (78.18%) were *T. vivax*, 11 (20%) were *T. congolense* and 1 case of mix infection (both species).

The bimonthly incidence in the whole sentinel villages from June 2010 was 1.97% in July 2010, and it increased to 2.85% in September then it reached a peak of 4.42% in November (fig. 6.6). Except for the peak of November 2010, the disease incidence became very low in May 2011.

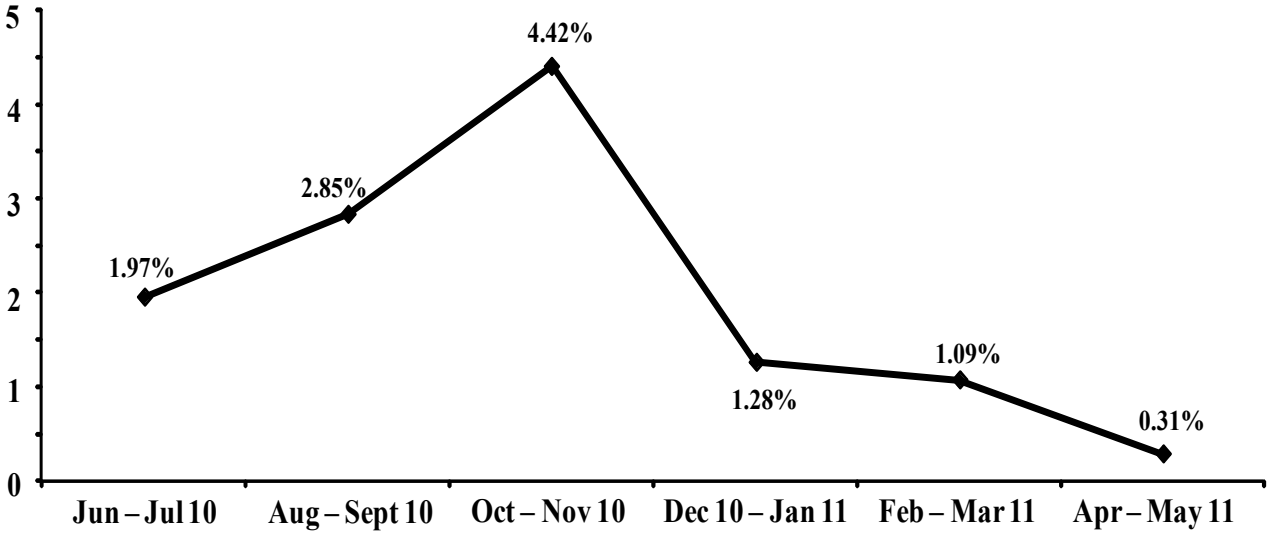


Figure 6.6. Evolution of trypanosomosis incidence in the sentinel herd in the PATTEC intervention area

However, this trend was not consistant in all sentinel villages (fig. 6.7). The highest incidences were recorded in September and October with 10.9% and 10% in Zamo and Soukoura respectively. In January 2011, the incidence of bovine trypanosomosis dropped to 0% in all villages except in Mou, Laro and Bendougou where the incidences were 2.5%, 2.7% and 8.7%, respectively but afterwards the incidence drops whereas for Nokuy it rises again.

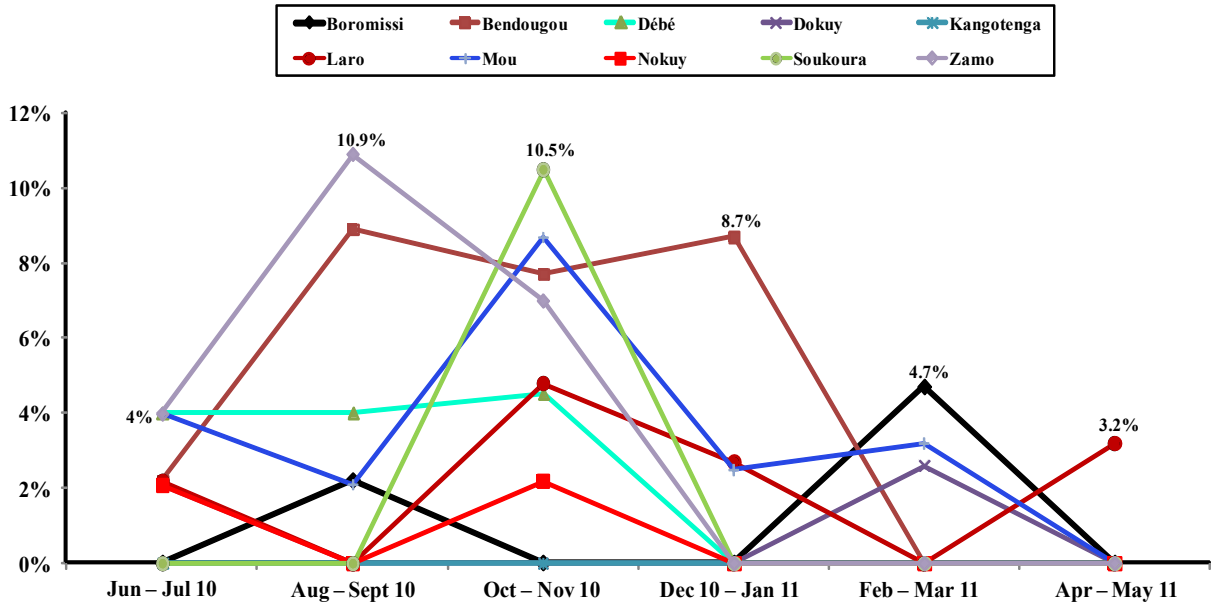
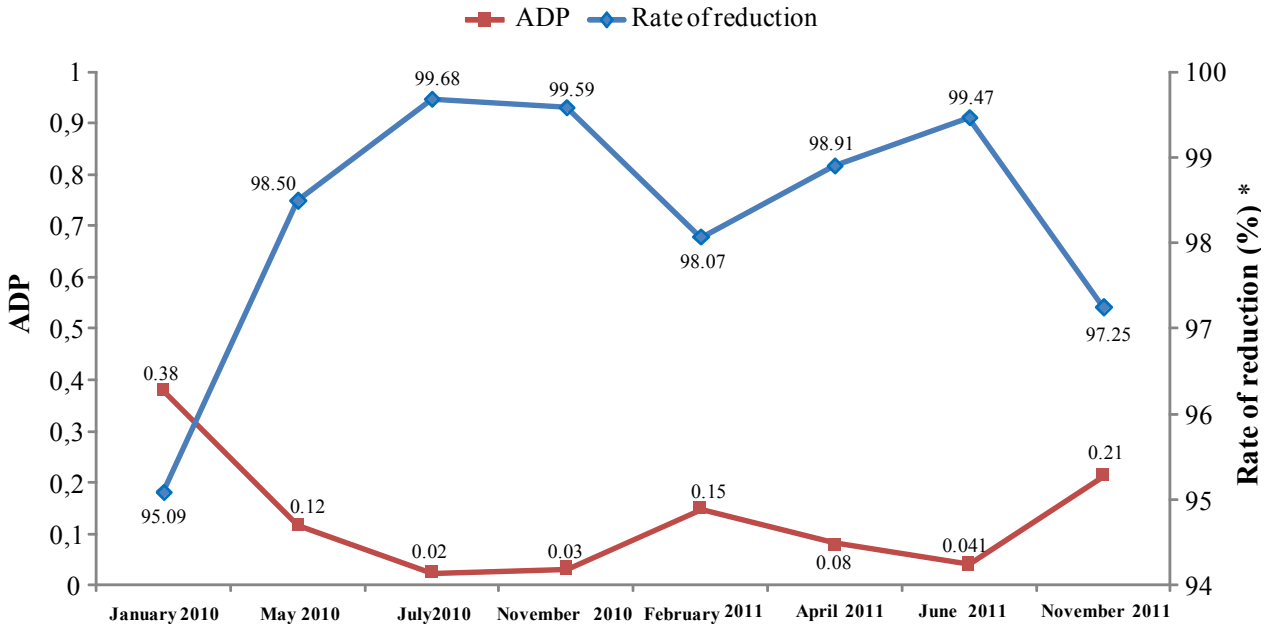


Figure 6.7. Evolution of cattle trypanosomosis in the sentinel herds living in the PATTEC intervention area

6.3.3. Tsetse capture results

The results of the tsetse capture and the reduction rate are shown in the figure 6.8. The evolution of the ADP confirmed the decline of the tsetse population during the control. One month after the deployment of the impregnated screens, the tsetse ADP dropped from 7.73 glossines/trap/day end November 2009 to 0.38 glossines/trap/day begin January 2010. With

the persistence of the control measures (deployment of impregnated screens and insecticide treated animals), the ADP remained lower than the value obtained in January 2010. Even during the rainy season when most of the screens were removed or flooded by the rivers, the ADP remained very low i.e. 0.02. The figure 6.8 showed a slight inscrease of the ADP in the beginning of the dry season i.e. 0.15 glossine/trap/per day (November 2010- February 2011) and a return to 0.041 glossine/trap/per day in June 2011 but in November 2011 it rises again. The control measures decreased quickly the tsetse population and the rate of reduction reached 95% within only 3 months of control and a peak of reduction of 99.68% was reached in July 2010.



* The rate of reduction was calculated base on the baseline ADP of November 2009 (7.73 glossines/trap/day).

Figure 6.8. Evolution of the tsetse apparent density of tsetse per trap per day and the trend of the rate of reduction

6.4. Discussion

The current PATTEC strategy for tsetse control is environmental friendly. The impregnated screens kill tsetse flies specifically and the insecticide treated cattle are live baits for tsetse, biting flies and ticks. The SAT uses non-residual ultra-low volumes of insecticides, strategically applied, which decreases the side effects on other species to a minimum. Our data showed the effectiveness and adequacy of the PATTEC strategy.

Like in the baseline survey (Sow et al., 2013), almost 80% of the trypanosomosis infections were due to *T. vivax*. About 40% of the cattle could not be followed up during the entire

observation period. Some of the animals were found dead or were sold by the owners. Some others went in transhumance and were thus excluded from the study.

There were two riverine species of tsetse flies in the PATTEC intervention area. The deployment of impregnated screens was so effective that within 3 months of control, the tsetse population decreased significantly. The restricted dispersal of the riverine species of tsetse in the PATTEC intervention area is an advantage in the success of the control. Indeed, the edges of the Mouhoun River and its tributaries are the only areas to be covered by the impregnated screens.

The evolution of the tsetse abundance (fig. 6.6 & fig. 6.8) showed that the control measures of PATTEC were successful. The percentage of suppression reached above 99% and the apparent density was less than 0.1 fly/trap/day. The complete elimination can now be achieved by the release of sterile males (Sow *et al*., 2012).

The trypanosomosis incidence decreased quickly within the 12 months of tsetse control in the PATTEC intervention area. The incidence at the end of our survey was very low (0.31%). This confirms the success of the control campaign. The slight increase in incidence of cattle trypanosomosis in the rainy season (June-September 2011) might be worrying as at the same period, the tsetse ADP remained very low (less than 0.1 tsetse/trap/day). The higher incidence might then have been caused by a mechanical transmission. Indeed, *T. vivax* can be mechanically transmitted (Desquesnes and Dia, 2003; 2004) and was the predominant species in our study (78.18%). What will be the evolution of this mechanical cycle on a long term perspective remains an open question. Some perspectives are proposed in the general discussion.

6.5. Conclusion

PATTEC could reduce drastically the tsetse population in its intervention area very quickly and maintain the tsetse population to a very low level during 3 years of control. The use of SIT will allow the complete elimination of tsetse flies in the intervention areas. The main challenge will be sustainability. The identification of the exact borders of the zones to be cleaned from vectors will be one of the greatest challenges in the future. Moleculars tools like microsatellite markers are now available to identify isolated pockets (big or small) where trypanosome populations are circulating. Eradicating the tsetse flies from one “island” before

shifting to the next one by coordinating efficiently all the efforts will allow avoiding endless control operations.

6.6. References

Challier, A, Laveissière, C., 1973. Un nouveau piège pour la capture des glossines (*Glossina:* Diptera, Muscidae): description et essais sur le terrain. Cah. ORSTOM, sér. Ent. Méd. Parasitol. 10, 251–262.

Desquesnes, M., Dia, M.L., 2003. *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. Exp. Parasitol. 103, 35–43.

Desquesnes, M., Dia, M.L., 2004. Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanid *Atylotus fuscipes*. Vet. Parasitol. 119, 9–19

Kamuanga, M., Swallow, B., Sigué, H., Bauer, B., 2001. Evaluating contingent and actual contributions to a local public good: Tsetse control in the Yale agro-pastoral zone, Burkina Faso, Ecol. Econ. 39, 115–130.

Laveissière, C., Grébaut, P., Herder, S., Penchenier, L., 2000. Les glossines vectrices de la Trypanosomiase humaine africaine; IRD, editor. Yaoundé, Cameroun: IRD and OCEAC. 246 p.

MEDEV, 2005. Région de la Boucle du Mouhoun; Cadre stratégique Régional de Lutte contre la pauvreté, Ouagadougou, Burkina Faso. Rapport du Ministère de l'Economie et du Développement du Burkina Faso 1–106.

MRA (Ministère des Ressources Animales du Burkina Faso), 2008. Les Statistiques du Secteur de l'Elevage au Burkina Faso Ouagadougou, 124 p.

Murray, M., Murray, P.K., Mcintyre, W.I.M., 1977. Improved parasitological technique for diagnosis of African trypanosomiasis. Trans. R. Soc. Trop. Med. Hyg. 71, 325–326.

PATTEC, 2008. Resultats entomologiques de base dans le bassin du mouhoun. Rapport final. Bobo Faso, Burkina Faso 57p.

Sow, A., Ganaba, R., Percoma, L., Sidibé, I., Bengaly, Z., Adam, Y., Koné, P., Sawadogo, G.J., Marcotty, T., Van Den Abbelee, J., Delespaux V., 2013. Baseline survey of animal trypanosomosis in the Region of the Boucle du Mouhoun, Burkina Faso. *Res. Vet. Sci.* http://dx.doi.org/10.1016/j.rvsc.2012.12.011.

Sow, A., Sidibé, I., Bengaly, Z., Bancé, Z., Sawadogo, G.J., Solano, P., Vreysen, M.J.B., Lancelot, R., Bouyer, J., 2012. Irradiated male *Glossina palpalis gambiensis* (Diptera: Glossinidae) from a 40-years old colony are still competitive in a riparian forest in Burkina Faso. PLos 7(5) e37124

Sow, A., Sidibé, I., Bengaly, Z., Bouyer J., Bauer, B., Van den Bossche, P., 2010. Fifty years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso. An overview. Bull. Anim. Health Prod. Afr. 58, 95–118.

Chapter 7: General discussion: Trypanosomosis risk factors and impact assessment of a tsetse and trypanosomosis eradication campaign in Burkina Faso

7.1. Introduction

Nearly 80% of the 15 million Burkinabese live upon rural sector mainly crop production and livestock production. The livestock contribution to the GDP ranges from 10 to 14% (MEDEV, 2004). It has been demonstrated that T&T hinders sustainable agriculture integration. Furthermore, T&T prevent the introduction in the infested area of genetically improved animal (Swallow, 1999, Feldmann *et al*., 2005; Feldmann and Hendrichs, 1999). Therefore, it is clear that the eradication of tsetse and trypanosomosis from Burkina Faso will improve the livelihood of the rural population, the most underprivileged people of the country. So, there is clearly a need for Burkina Faso to have a well-thought tsetse control policy in order to achieve sustainable development.

Exact losses due to trypanosomosis have not been estimated in Burkina Faso at the country level but 3.9 million US$ are spent per year for the prevention and treatment against the disease (Sow *et al*., 2010). The case study of the pastoral zone of Yalé is very informative on the magnitude of trypanosomosis in Burkina Faso (Kamuanga *et al*., 2001). This study showed that subsequent reduction in the incidence of trypanosomosis leads to a significant increase in the livestock population growth rate (up to 25%), milk production and the number of draft animals. At the same time, the rate of stillbirths decreased drastically.

Hitherto, many efforts have been deployed to tackle T&T in Burkina Faso. The previous T&T control campaigns were implemented in specific targeted zones. The most important programme ever implanted in Burkina Faso is the one driven by PATTEC. This campaign has adopted strategies inspired from lessons from the past, from successful tsetse control elsewhere, and benefits from the research results carried out locally, namely by the CIRDES. This campaign aims at the complete elimination of tsetse and trypanosomosis in Burkina Faso and from possible reinvasion sources. The campaign should focus in shifting from national perspectives to “trypanosome territories”, “trypanosome islands” or “trypanosomes without borders (TWB)” i.e. eradicating the tsetse flies from one “pochet/island” before dealing with the next one.

7.2. Baseline situation of animal trypanosomosis and PATTEC intervention strategies

In the Region of the Boucle du Mouhoun, the PATTEC intervention area, two riverine species of tsetse (*G. p. gambiensis* and *G. tachinoides*) are the sole cyclical vectors of the two species of pathogenic trypanosomes to livestock (*T. vivax* and *T. congolense*). Therefore, the Mouhoun River and its tributaries play the key role in the epidemiology of the disease in the region. Indeed, the Mouhoun River crosses the region on 280 km and constitutes with its tributaries a dense hydrographic network. The rivers edges are the main areas where PATTEC tsetse control is focusing because these are the ecological habitats of the riverine tsetse fly species.

The works of Bouyer (2006) and the entomological baseline data showed that *G. palpalis gambiensis* is mainly distributed along the ascendant portion of de Mouhoun River whereas the descendant portion of the river is infested by *G. tachinoides*. This is an important observation that should be taken into account during the final assault against the tsetse flies i.e. the release of sterile males. Moreover, there is a natural limit of the tsetse distribution in the region. The recent entomological survey carried out by the PATTEC showed that the most northern point where tsetse flies were captured is latitude 12°45' (Sidibé, personal communication). The elimination of tsetse could begin from the natural limit i.e. from North towards the South following the "rolling carpet" strategy.

The gallery forests along the Mouhoun River constitute an excellent habitat for the tsetse. In this ecosystem, tsetse can feed upon monitor lizards, snakes and small mammals that dwell under the vegetation.The use of SAT only will never allow the elimination of riverine tsetse of the Region of the Boucle du Mouhoun like that was done in the Okavango Delta of Botswana (Kgori *et al*., 2006). However, the SAT was jointly applied by PATTECs Burkina and Ghana on the descendant branch of the Mouhoun River with success. This operation helped to reinforce the barrier set to prevent reinvasion of tsetse in the current suppression area. The most effective strategy in this kind of landscape will be the ground spraying. In the very dense *Mitragyna inermis*, *Mimosa pigra* and *Acacia seyal* forest along the edge of the river, fogging with deltamethrine (aqua-K-Othin) was applied to suppress the tsetse population. The Swingfog® fogging is well adapted to tsetse control and was used since the 1970's for tsetse and HAT control in Africa (Laveissière *et al*., 2000).

The deployment of impregnated screens and traps, along the rivers' course (Mouhoun River and its main tributaries), allowed a percentage of suppression varying from 95-99.6% within only one year. Unfortunately, the percentage of suppression decreased once the screens and targets were removed or destroyed during the rainy season. Normally, live insecticide-treated cattle and ground spraying should take over the deployment/maintenance of impregnated target in the rainy season. Unfortunatly, because of the delay in the release of funds and organisational matters, ground spraying was not carried out at the convenient time.

The prevalence of trypanosomosis in cattle is higher in the rainy season than in the dry season. In the dry season when the baseline parasitological survey was carried out, the parasitological prevalence of trypanosomosis in cattle was less than 1% (0.77% (95%C.I. 0.30-1.95%)) and the seroprevalence was 34.2% (95%C.I. 26.1-43.4%). This information is very important in the control of the disease as control measures will differ according to the prevalence. In the PATTEC intervention strategies, mass treatment of livestock with DA was done in the low prevalent zone and continuous surveillance was carried out in the view of early detection and treatment of new trypanosomosis cases. In the zone where the prevalence was higher, like in the herds living on the edge of the Mouhoun River and its main tributaries, all animals were treated with DA followed by preventive treatment with ISM within 2 to 3 weeks thereafter. The application of this control protocol in addition to the tsetse suppression measures yielded good results concerning trypanosomosis incidence in the intervention area. The incidence of trypanosomosis was very low (0.31%) after a year of implementation of the campaign. Although significant progress was made in the control of the disease, the use of trypanocidal drugs is confronted with the appearance of drug resistant strains of trypanosomes.

7.3. Drug resistance in the PATTEC intervention area

During the baseline survey 80% of the infections were due to *T. vivax*. This is of course questioning the efficiency of the vector control strategy in the area. Two crucial parameters will have to be further explored as: (i) in the presence of tsetse flies, what is the importance of the mechanical transmission, (ii) if the mechanical transmission is low in the presence of tsetse flies, how fast does it develop in the absence of the cyclical vector.

In the presence of a wild reservoir, the improvment of the recently designed traps/targets against hematophagous insects could be a solution (Krcmar et al., 2007; Hogsette et al.,

2008). Additionally, modelisation based on quantified (host parasitaemia, mean individual insect burden, initial prevalence of infection) or estimated parameters (not measured like frequency of interruption of the meals, distance between animals and movement of hematophagous insects between hosts) allows the the fine tuning of control methods in a mechanical context (Desquesnes et al., 2009).

If the main source for feeding is cattle than the use of insecticide treated cattle combined to the use of trypanocidal drugs constitutes an efficient option. The insecticide treatment targets both cyclical and mechanical vectors. The use of trypanocides depends on the drug resistance situation prevailing in the control area. Drug resistance was demonstrated for the first time in *T. vivax* in field and experimental conditions (Sow *et al*., 2012). Drug resistant strains of *T. vivax* were detected in 5 out of 10 villages that were surveyed. However, DA is still efficient. The careful use of the sanative pair is thus strongly advised complemented by pour on or spraying of insecticide.

7.4. Competiveness of irradiated males in the preparation of the PATTEC campaign

The release of sterile male tsetse will be used to eliminate tsetse flies in the PATTEC intervention area. The common conventional methods like the insecticide ground spraying, the deployment of impregnated traps/targets and the live baits are density dependent (Bouyer *et al*., 2010). Therefore, they are less effective when the tsetse populations are very low. However, these techniques could keep the tsetse population at acceptable levels and are suitable for the control of the disease but not for the elimination of the tsetse flies. Even the use of SAT, which is not density dependent, could hardly eliminate the total tsetse populations of the dense gallery forests.

The release of the irradiated sterile males should occur when the suppression of the tsetse population reaches the threshold of 99%. The PATTEC campaign allows a significant suppression of the tsetse population in the intervention area. In June 2011, the suppression was 99.48% and the ADP was 0.04 tsetse/trap/day for the whole region of the Boucle du Mouhoun. That was a suitable period to implement the release of the irradiated sterile males.

The insectarium of PATTEC should rear in mass the two riverine species of tsetse namely *G. p. gambiensis* and *G. tachinoides*. These two species are reared at the insectarium of CIRDES since 40 years. Experimental release of *G. p. gambiensis* showed that they are competitive and

can be used for the PATTEC eradication campaign. Within the framework of the eradication project, it will however be necessary to overflood the wild male population with a higher ratio (>14.4) (Sow *et al*., 2012).

The main challenge for PATTEC lies in the capacity of its new insectarium to provide enough and good quality irradiated males for sustained systematic weekly or fortnightly release on a wide area (90.000 km^2) over a time span that is sufficient to cover several generations of the target population. Regarding the width of the PATTEC intervention area, the sterile males should be released using aircraft. In rainy season, the aerial release optimizes the distribution of the sterile insects (Feldmann and Hendrichs, 2001). Strategic release of the irradiated males could be done using ground release along the rivers in the gallery forests in the dry season. The experimental release of irradiated *G. p. gambiensis* carried out in the dry season (January-April), showed a good dispersal and competitiveness. In spite of the season (high temperature and the dryness of the weather) the life span of the released flies was acceptable (Sow *et al*., 2012). In the dry season, the riverine tsetse flies, both *G. p. gambiensis* and *G. tachinoides*, have a linear dispersal along the gallery forests (Cuisance *et al*., 1985).

7.5. PATTEC intervention strategies

PATTEC Burkina has adopted thoroughly elaborated strategies for the elimination of tsetse flies in the country. The use of the "rolling carpet" strategy has an important advantage. The barriers are not needed in the north, which constitutes a natural barrier. The strategies includes almost all components of an integrated tsetse and trypanosomosis control: the use of impregnated traps/targets, the living baits, the mass treatment with trypanocidal drugs and the involvement of the beneficiary communities (including farmers and political decision makers). Tsetse control is very effective, and the setting of the barriers to prevent reinvasion of the cleared area is also efficient.

The elimination of tsetse flies from Burkina Faso is definitely achievable. However, until the complete elimination of tsetse flies from Burkina Faso and neighbouring zones is achieved, the rural communities will have to live together with both the trypanosomosis and tsetse flies and to formulate a suitable control method which fits best of their fragile economies.

It is finally essential to keep in mind the "tsetse without border" concept: eradicating the tsetse flies from one "pochet/island" before dealing with the next one by coordinating efficiently all the efforts. This will allow avoiding the endless control operations. This recommendation will be proposed for discussion to the technical committee of the PATTEC.

7.6. References

Bouyer, J., 2006. Ecologie des glossines du Mouhoun au Burkina Faso: intérêt pour l'épidémiologie et le contrôle des trypanosomoses africaines. *Thèse de Doctorat* d'Université Montpellier II.

Bouyer, J., Solano P., Cuisance D., Itard, J., Frézil, J.-L., Authié E. 2010. Trypanosomosis: Control methods., pp. 1936-1943. In: P.-C. Lefèvre, J. Blancou, R. Chermette and G. Uilenberg [eds.], Infectious and parasitic diseases of livestock (vol. 2). Lavoisier (Tech & Doc), Paris.

Challier, A., Laveissière, C., 1973. Un nouveau piège pour la capture des glossines (*Glossina:* Diptera, Muscidae): description et essais sur le terrain. Cah ORSTOM, sér Ent Méd et Parasitol 10, 251–262.

Cuisance, D., Politzar, H., Merot, P., Tamboura, I., 1984. Les lâchers de mâles irradiés dans la campagne de lutte intégrée contre les glossines dans la zone pastorale de Sidéradougou, Burkina Faso. Rev. Elev. Méd. vét. Pays Trop. 37, 449–468.

Desquesnes, M., Dia, M.L., 2003. *Trypanosoma vivax*: mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. Exp. Parasitol. 103, 35−43.

Desquesnes, M., Dia, M.L., 2004. Mechanical transmission of *Trypanosoma vivax* in cattle by the African tabanid *Atylotus fuscipes*. Vet. Parasitol. 119, 9−19.

Desquesnes, M., Biteau-Coroller, F., Bouyer, J., Dia, M.L., Foil, L., 2009. Development of a mathematical model for mechanical transmission of trypanosomes and other pathogens of cattle transmitted by tabanids. International Journal for Parasitology 39, 333-346.

Feldmann, U., Dyck, V. A., Mattioli R. C., Jannin, J., 2005. Potential impact of tsetse fly control involving the sterile insect technique. In : Dyck, V. A., Hendrichs J. & Robinson A. S. (eds.), Sterile insect technique. Principles and practice in area-wide integrated pest management . Springer, Dordrecht, The Netherlands, pp. 701–726.

Feldmann, U., Hendrichs, J., 1999. The concept for integration of the sterile insect technique as a key component of future subregional area-wide tsetse and trypanosomosis management operations. In: Animal Trypanosomosis: Vector and Disease Control using Nuclear Techniques, Backhuys Publishers, Leiden, The Netherlands, pp. 191–214.

Feldmann, U., Hendrichs, J., 2001. Integrating the sterile insect technique as a key component of area-wide tsetse and trypanosomiasis intervention; PAAT technical and scientific series No. 3, FAO, Rome, pp.1–66

Geerts, S., Holmes, P.H., Diall, O., Eisler, M.C., 2001. African bovine trypanosomiasis: the problem of drug resistance. Trends Parasitol. 17, 25–28.

Hogsette, J.A., Nalli, A., Foil, L.D., 2008. Evaluation of different insecticides and fabric types for development of treated targets for stable fly (Diptera : Muscidae) control. Journal of Economic Entomology 101, 1034-1038.

Kamuanga, M., Swallow, B., Sigué, H., Bauer, B., 2001. Evaluating contingent and actual contributions to a local public good: Tsetse control in the Yale agro-pastoral zone, Burkina Faso. Ecol. Econ. 39, 115–130.

Kgori, P.M., Modo. S., Torr, S.J. 2006. The use of aerial spraying to eliminate tsetse from the Okavango Delta of Botswana. Acta Trop. 99, 184–199.

Krcmar, S., 2007. Responses of Tabanidae (Diptera) to canopy traps baited with 4-methylphenol, 3-isopropylphenol, and naphthalene. Journal of Vector Ecology 32, 188-192.

Laveissière, C., Grébaut, P., Herder, S., Penchenier, L., 2000. Les glossines vectrices de la Trypanosomiase humaine africaine; IRD, editor. Yaoundé, Cameroun: IRD and OCEAC. 246p.

MEDEV (Ministère de l'Economie et du Développement du Burkina Faso), 2004. Etude nationale prospective "Burkina 2025" : Rapport de diagnostic stratégique du Burkina Faso. (http://www.medev.gov.bf/SiteMedev/burkina25/enp/rapport-diagnostic-strategique.pdf)

Mortelmans, J., Socio-economic problems related to animal trypanosomiasis in Africa. 1984. Soc. Sci. Med. 19, 1105–1107.

Okoth, J.O., 1986. Community participation in tsetse control. Parasitol. Today 2, 8.

Sow, A., Sidibé, I., Bengaly, Z., Augustin, B., Sawadogo, G. J., Solano, P., Vreysen, M.J.B., Lancelot, R., Bouyer, J., 2012. Irradiated Male Tsetse from a 40-Year-Old Colony Are Still Competitive in a Riparian Forest in Burkina Faso. Plos One 7(5), e37124.

Sow, A., Sidibé, I., Bengaly, Z., Bouyer, J., Bauer, B., Van den Bossche, P., 2010. Fifty years of research and fight against tsetse flies and animal trypanosomosis in Burkina Faso. An overview. Bull. Anim. Health Prod. 58, 95–118.

Sow, A., Sidibé, I., Bengaly, Z., Marcotty T., Séré M., Diallo A., Vitouley H. S., Nebié R. L., Ouédraogo M., Akoda, G. K., Van den Bossche, P., Van Den Abbeele, J., De Deken, R., Delespaux, V., 2012a. Field detection of resistance to isometamidium chloride and diminazene aceturate in *Trypanosoma vivax* from the Region of the Boucle du Mouhoun in Burkina Faso. Vet. Parasitol. 187, 105–111.

Summary/Samenvatting

Summary

Tsetse and animal trypanosomosis (T&T) are the main constraints to animal production in Africa, especially in Burkina Faso where the economy relies mainly on the rural sector. To eliminate this scourge in Africa, the Heads of States and Governments have decided in July 2000, during the summit of the African Union in Lomé, Togo, to create the Pan African Tsetse and Trypanosomiaisis Eradication Campaign (PATTEC). Burkina Faso was one of the six pilot countries to implement the campaign. The experimental work that is presented in this thesis aimed at i) evaluating the parasitological and baseline situation in the Region of the Boucle du Mouhoun, the PATTEC intervention area, ii) evaluating drug resistance in the area, iii) assessing the competitiveness of irradiated male tsetse from a 40-year-old colony, which will be used in preparation of an eradication campaign and finally iv) assessing the impact of the campaign of elimination of T&T.

The first chapter of this thesis reviews the control of tsetse flies and animal trypanosomoses in Africa. In this literature review, the epidemiology and the biology of both parasite and vector are described as well as the socio-economical importance of African Animal Trypanosomoses (AAT) was highlighted.

In the second chapter, a review is made of the different campaigns against the T&T implemented in Burkina Faso. The successes and failures of these campaigns are discussed. The present situation of T&T is explored, with a particular focus on the epidemiology of AAT, the trypanotolerance and the chemoresistance against trypanocides that appeared during the 1980s in the province of Kénédougou. The research on trypanosomosis in Burkina Faso is discussed, especially the innovations in diagnosis. Finally, suggestions are made for the PATTEC in order to improve their future actions.

In the third chapter, the baseline situation of AAT before the implementation of the PATTEC campaign is presented. This cross sectional survey showed that cattle had been mostly treated with trypanocidal drugs compared to small ruminants and donkeys. Parasitological prevalence in all species was low, but cattle were the most infected. Infections were mainly due to *Trypanosoma vivax* (75.0%), with fewer cases of *Trypanosoma congolense* infection (25.0%). The age and distance to the river were the two main risk factors associated with the infection status.

Chapter 4 investigates drug resistance in the region of the Boucle du Mouhoun. In this study, a longitudinal study assessed the chemoresistance to Isometamidium chloride (ISM) and Diminazene aceturate (DA) in the 10 villages with the highest parasitological prevalences after the baseline survey. Field detection of drug resistance based on the "block treatment"

method was carried out. In total, 20 herds comprising 978 heads of cattle were followed up. Resistance to ISM was observed in 5 of the 10 villages. In contrast, this study did not show evidence of resistance to DA. Our results suggest that because of the low prevalence of multiple resistances, a meticulous use of the sanative pair system would constitute the best option to delay as much as possible the spread of chemoresistance till complete eradication of the disease by vector control operations.

In chapter 5, experimental work was carried out to assess the competitiveness of the sterile males originating from the 40-year-old colony from the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES). Since the government of Burkina Faso embarked on a tsetse eradication program targeting the Mouhoun River and including a SIT component that plans to use a *G. p. gambiensis* colony that has been maintained for about 40 years, it was necessary to test its competitiveness. We thus released 16,000 marked sterile males in 2010 along a tributary of the Mouhoun River and estimated their competitiveness by assessing the abortion rate of wild females, which increased significantly due to the releases. We estimated that a sterile to wild male ratio of 14.4 would be necessary to obtain nearly complete induced sterility in the female population. We concluded that gamma sterilised male *G. p. gambiensis* derived from the CIRDES colony have a competitiveness that is comparable to their competitiveness obtained 35 years ago and can still be used for an area-wide integrated pest management campaign with a sterile insect component in Burkina Faso.

Chapter 6 presents the impact of the PATTEC campaign on the tsetse population and the incidence of animal trypanosomosis in the campaign intervention area. In the prospect to eliminate T&T, the PATTEC implemented a number of actions, amongst which (i) the deployment of impregnated screens and traps, (ii) the mass treatment of livestock against trypanosomosis, (iii) the treatment of cattle by the application of insecticide and (iv) the partial use of sequential aerial treatment (SAT). This study was carried out to assess the incidence of trypanosomosis and the apparent density per trap (ADP) of the tsetse. For the disease incidence, cattle sentinel herds were selected in the 11 most prevalent villages through the PATTEC intervention area. To evaluate the effectiveness of the tsetse elimination, four hundred sentinel biconical traps were deployed along the Mouhoun River and its main tributaries. Within one year of tsetse elimination plan, the campaign was very effective. The ADP of the tsetse declined to 0.38 fly/trap/day after only 3 months of intervention and it remained low. Similarly, the prevalence of the disease dropped at 0.35% after 1 year of intervention.

In the final general discussion (chapter 7), the major findings of the thesis are discussed. The control strategies of PATTEC were effective. The sustainability of the achievements of the campaign remains a big challenge for the farmers and the local authorities.

Samenvatting

Tseetseevlieg en dierlijke trypanosomose (T & T) zijn de belangrijkste belemmering voor de dierlijke productie in Afrika en zeker in in Burkina Faso waar de economie voornamelijk van de landbouw afhankelijk is. Tijdens de vergadering van de Afrikaanse Unie in Lomé (Togo) in juli 2000, hebben de Staatshoofden en Regeringsleiders besloten om de Pan African Tsetse and Trypanosomiaisis Eradication Campaign (PATTEC) te beginnen met als doel tseetseevliegen in Afrika te elimineren,. Burkina Faso was één van de zes geselecteerde pilootlanden die de campagne mocht uitvoeren. Het studie werk dat in dit proefschrift wordt voorgesteld is gericht op i) de evaluatie van de parasitologische en basislijn situatie van T&T in de regio van de Boucle du Mouhoun, ii) de evaluatie van drug resistentie in dit gebied, iii) de evaluatie van de competentie van bestraalde mannelijke tseetseevliegen uit een 40-jaar oude kolonie die zullen gebruikt worden in het kader van het tseetsee-uitroeiingsprogramma en ten slotte iv) de evaluatie van de impact van deze T & T uitroeingscampagne .

Het eerste hoofdstuk van dit proefschrift geeft een overzicht van de controle van de tseetseevliegen en dierlijke trypanosomoses in Afrika. In deze literatuurstudie worden de epidemiologie en de biologie van zowel de parasiet als de vector beschreven. De socio-economische aspecten van de Afrikaanse dierlijke Trypanosomoses (AAT) worden ook besproken.

In het tweede hoofdstuk wordt een overzicht gemaakt van de verschillende campagnes tegen T & T die in Burkina Faso geïmplementeerd werden. De successen en mislukkingen van deze campagnes worden besproken. De huidige situatie van T & T wordt onderzocht, met bijzondere nadruk op de epidemiologie van AAT, de trypanotolerantie en de chemoresistentie tegen trypanociden die gedurende de jaren ’80 voor het eerste werd vastgesteld in de provincie Kenedougou. Het onderzoek rond trypanosomose in Burkina Faso wordt besproken met een speciale focus op de innovaties in de diagnose. Tenslotte worden suggesties gedaan voor de PATTEC om hun toekomstige acties beter te maken.

In het derde hoofdstuk wordt de situatie van AAT voor de uitvoering van de PATTEC campagne gepresenteerd. Uit dit onderzoek blijkt dat runderen meer behandeld werden met trypanociden in vergelijking met kleine herkauwers en ezels. Parasitologisch prevalentie in alle diersoorten was laag waarbij de hoogste besmettingsgraad bij runderen werd

vastgesteld. Infecties waren voornamelijk te wijten aan *Trypanosoma vivax* (75,0%) en soms aan *Trypanosoma congolense* (25,0%). De leeftijd van de dieren en de afstand tot de rivier waren de twee voornaamste risicofactoren in verband met de besmetting status.
In hoofdstuk 4 wordt de resistentie tegen trypanociden in het gebied van de Boucle du Mouhoun besproken. In een longitudinale studie werd de chemoresistentie tegen ISM en DA geëvalueerd in de 10 dorpen met de hoogste parasitologische prevalenties in de transversale onderzoek. De detectie van resistentie werd op basis van de "block-treatment" methode uitgevoerd. In totaal werden 20 kuddes van 978 runderen opgevolgd. Resistentie tegen ISM was aanwezig in 5 van de 10 dorpen. Daarentegen heeft deze studie geen bewijs van resistentie tegen DA gevonden. Onze resultaten suggereren dat vanwege de lage prevalentie van multiresistentie, een zorgvuldig gebruik van het “sanative pair” de beste optie is om zoveel mogelijk de verspreiding van chemoresistentie te vertragen tot de volledige uitroeiing van de ziekte.
In het hoofdstuk 5 werd een experimenteel werk verricht om de competentie te evalueren van de steriele mannetjes afkomstig van de 40-jaar oude kolonie van het Centre International de Recherche-Développement sur l’Elevage en zone Subhumide (CIRDES). De regering van Burkina Faso is met een tseetsee uitroeiingsprogramma begonnen gericht op de Mouhoun rivier. Dit programma heeft een SIT component waarvoor een 40-jaar oude *G. p. gambiensis* kweek zal gebruikt worden. Het was dus noodzakelijk om in eerste instantie de competentie van deze vliegen te testen in de natuurlijke situatie. Zestienduizend steriele mannetjes werden in 2010 vrijgelaten in een een zijriviergebied van de rivier Mouhoun. De competentie van de vrijgelaten mannetjes werd beoordeeld aan de hand van de abortuscijfers die bij de wilde wijfjes werden geobserveerd. Er werd geobserveerd dat deze aanzienlijk was toegenomen als gevolg van de vrijlating van de steriele mannetjes in dit gebied. Op basis van deze studie schatten we dat een steriele/wilde mannejes ratio van 14,4 noodzakelijk is om bijna volledige steriliteit bij de tseetseevlieg wijfjes te veroorzaken. We concluderen dat gamma-gesteriliseerde mannelijke *G. p. gambiensis* uit de CIRDES kolonie een competentie hebben die vergelijkbaar is met hun competentie van 40 jaar geleden. Bijgevolg kunnen ze nog in een eradicatie operatie gebruikt worden.
Hoofdstuk 6 presenteert de impact van de PATTEC campagne op de tseetseevliegpopulatie en de incidentie van dierlijke trypanosomose. Om de situatie van T & T te verbeteren heeft de PATTEC een aantal acties uitgevoerd gebaseerd op o.a (i) het gebruik van geïmpregneerde schermen en vallen, (ii) de massa behandeling van runderen tegen trypanosomosis, (iii) de behandeling van runderen door toepassing van externe insecticiden

en (iv) het gedeeltelijk gebruik van ‘Sequential Aerial Treatment’(SAT). Onze studie werd uitgevoerd om de incidentie van trypanosomose en de densiteit (‘apparant density’) per val van de tseetseevlieg in te schatten. Om de incidentie van de ziekte te kunnen schatten, werden t kuddes van runderen geselecteerd in de 11 dorpen met de hoogste prevalentie. Om de effectiviteit van de tseetseevlieg eliminatie te evalueren, werden vierhonderd biconische vallen ingezet langs de Mouhoun rivier en langs de belangrijkste zijrivieren. Uit onze observaties blijkt dat de controle actie zeer effectief is. Na 3 maanden van interventie is de densiteit per val van de tsetse tot 0,38 vlieg/val/ dag gedaald en bleef laag. Ook de prevalentie van de ziekte daalde tot 0,35% na 1 jaar interventie.
In de laatste algemene discussie (hoofdstuk 7) worden de belangrijkste bevindingen van onze thesis besproken. De controle strategieën van de PATTEC zijn effectief. De duurzaamheid van de resultaten van de campagne blijft de grootste uitdaging voor de boeren en de lokale overheden.