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EVALUATION OF RISK FACTORS OF ANIMAL TRYPANOSOMOSIS IN GHANA, AND THE MONITORING OF THE IMPACT OF DISEASE AND VECTOR ERADICATION INTERVENTION IN THE UPPER WEST REGION OF GHANA

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THÈSE

Pour obtenir le grade de
Docteur

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des maladies animales exotiques et émergentes

Spécialité: Entomologie Médicale

Présentée par **Yahaya ADAM**

**Evaluation of Risk Factors of Animal
Trypanosomosis in Ghana, and the Monitoring of
the Impact of Disease and Vector Eradication
Intervention in the Upper West Region of Ghana**

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Abstract : Evaluation of Risk Factors of Animal Trypanosomosis in Ghana, and the Monitoring of the Impact of Disease and Vector Eradication Intervention in the Upper West Region of Ghana

African animal trypanosomosis (AAT) is a major constraint to viable and sustainable livestock production systems in Ghana. Under the umbrella of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), Ghana is collaborating with Burkina Faso in a sub-regional initiative aiming at creating tsetse-free areas across their common borders. The objective of this thesis was to conduct research to guide project implementation and specifically seeks to i) determine the pre-intervention vector and disease situation of the intervention area, ii) determine tsetse population structuring and the consequences on sustainable tsetse control efforts, iii) evaluate SAT for the control of riverine tsetse species in Ghana and iv) evaluate the environmental risk of the intervention programmes.

Results of a baseline survey conducted in the Upper West Region (study area) indicated a wide-spread prevalence of *Glossina tachinoides* but *Glossina palpalis gambiensis* was limited to the southern edge of the study area. Average parasitological prevalence in cattle was estimated at 2.5% (95% CI: 1.06–5.77) and serological prevalence measured at 19% (95% CI: 14.03–25.35). The mean Index of Apparent Abundance (IAA) of tsetse was 8.7, 1.9 and 1.3 for samples taken along the Black Volta, Kulpawn and Sissili Rivers, respectively.

Investigations of the *G. tachinoides* populations confirmed significant structuring within and between the three main river-basins of the study area, and indicated a local density of 0.048-0.061 flies/m² and dispersal distance that approximated 11 m per generation [CI 9 - 17]. No significant sex-biased dispersal was detected. However, the observed dispersal was deemed sufficient for a *G. tachinoides*-cleared area to be reinvaded from neighbouring populations in adjacent river basins.

The potential of Sequential Aerosol Technique (SAT) to eliminate riverine tsetse species in a challenging subsection (dense tree canopy and high tsetse densities) and the subsequent efficacy of an integrated strategy, one year after the SAT operations, were also investigated. Results indicated failure to achieve elimination, largely attributed to insufficient penetration of insecticide aerosols in thick riverine forest galleries amongst other factors. However the overall reduction rate due to SAT was important (98%) and the subsequent integrated strategy maintained high levels of tsetse suppression. Finally an environmental impact assessment revealed no significant impact of deltamethrin aerosols on non-targeted aquatic and terrestrial arthropods.

Key words: Trypanosomosis, Glossina, Sequential Aerosol Technique (SAT), Deltamethrin, PATTEC, Riverine tsetse flies.

Résumé

Les Trypanosomoses Animales Africaines (TAA) sont une contrainte majeure à la viabilité et à la durabilité des systèmes de production de bétail au Ghana. Sous la tutelle de la Campagne Pan-Africaine d'Eradication des Tsé-tsé et des Trypanosomoses (PATTEC), le Ghana collabore avec le Burkina Faso au sein d'un projet sous régional pour créer une zone libérée de glossines à leur frontière commune.

Les objectifs de cette thèse sont de i) déterminer la situation initiale avant intervention en ce qui concerne le vecteur et la prévalence de la maladie dans la zone de lutte, ii) déterminer la structure des populations de glossines et ses conséquences sur la durabilité des efforts de lutte anti-vectorielle, iii) évaluer l'efficacité de la Pulvérisation Séquentielle d'Aérosols insecticides (SAT) pour contrôler les glossines riveraines et iv) évaluer les risques environnementaux associés à cette stratégie de lutte.

Les résultats de l'enquête de base conduite dans la région nord-ouest du Ghana (zone d'étude) ont montré une large distribution de *Glossina tachinoides* alors que *Glossina palpalis gambiensis* était limitée à la limite sud de la zone d'étude. La prévalence parasitaire moyenne chez les bovins était de 2.5% (IC 95%: 1.06-5.77) et la prévalence sérologique de 19% (IC: 14.03-25.35). La densité apparente par piège et par jour (DAP) des glossines était de 8.7, 1.9 et 1.3 respectivement le long des rivières Volta noire, Kulpawn et Sissili.

Une structuration génétique importante des populations de *G. tachinoides* a été observée entre sites d'étude d'un même bassin versant et entre bassins versants. Une densité locale de 0.048-0.061 glossines/m² a été inférée, ainsi qu'une distance de dispersion d'environ 11m par génération [IC 9 - 17]. Aucun biais de dispersion sexe-spécifique n'a été détecté. La dispersion observée était suffisante pour qu'une zone libérée de *G. tachinoides* puisse être ré-ennahie par les populations mitoyennes des bassins versants adjacents.

L'efficacité de la SAT à éliminer les espèces de glossines riveraines dans une section particulièrement difficile (canopée très dense et fortes densités de glossines) et l'efficacité ultérieure, un an après la SAT, d'une stratégie de lutte intégrée, ont également été testées. Les résultats montrent l'échec de l'éradication, attribué à une pénétration insuffisante des aérosols insecticides dans les galeries forestières denses entre autres facteurs. Toutefois, le taux de réduction global obtenu par la SAT fut important (98%) et la stratégie intégrée parvint à maintenir un niveau important de suppression des glossines.

Enfin, une mesure de l'impact environnemental du projet a montré un impact non significatif de la deltaméthrine en aérosols sur les arthropodes aquatiques et terrestres non-ciblés.

Mots-clés: Trypanosomose, Glossina, Pulvérisation Séquentielle d'Aérosols insecticides (SAT), Deltaméthrine, PATTEC, riverine tsetse species

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List of Abbreviations

AAT :	Animal African Trypanosomiasis
BCS :	Body Condition Score
BHC :	benzene hexachloride (Gammallin)
DA :	Diminazene Aceturate
DDT :	Dichlorodiphényltrichloroéthane
<i>F</i> -statistics :	Fixation indices (Expected level of heterozygosity in a population)
GPS:	Global Positioning System
HAT :	Human African Trypanosomosis
IAA :	Index of Apparent Abundance
IPM:	Integrated Pest Management
ISM :	Isometamidium
ml:	Milliliter
µl:	Microliter
MSGs	Subgroups
MtDNA :	Mitochondrial DNA
PATTEC :	Pan African Tsetse and Trypanosomosis Eradication Campaign.
PBS :	Phosphate buffered saline
PAAT:	Program against African Trypanosomosis
PCR:	Polymerase Chain Reaction
PCV:	Packed Cell Volume
SAT :	Sequential Aerosol Technique
SIT :	Sterile Insect Technology
UWR :	Upper West Region
VMD:	Volume Median Diameter

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List of Articles

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Adam, Y., Marcotty, T., Cecchi, G., Mahama, C., Solano, P., Bengaly, Z. and 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.

Article 2 :

Adam, Y., et al. Genetic comparison of *Glossina tachinoides* populations in three river basins of the Upper West Region of Ghana and implications for tsetse control. *Infect. Genet. Evol.* (2014), <http://dx.doi.org/10.1016/j.meegid.2014.03.023>

Article 3 :

Adam Y., Cecchi G., Kgori PM, Marcotty T., Mahama CI., Abavana, M., Anderson, B., Paone M., Mattioli, R., Bouyer, J. (2013) The Sequential Aerosol Technique: A Major Component in an Integrated Strategy of Intervention against Riverine Tsetse in Ghana. **PLoS Negl Trop Dis** 7(3): e2135. [oi:10.1371/journal.pntd.0002135](https://doi.org/10.1371/journal.pntd.0002135)

Article 4 :

Adam Y., Cecchi G., Bouyer, J. Considerations for the selection of Monitoring Sites in a post SAT tsetse control programme

Article 5:

Adam Y., Bouyer J., Marcotty T., Mahama C.I., Grant I.F. Assessment of the impacts of Sequential Aerosols Technique of Deltamethrin application for the suppression of Tsetse fly populations on aquatic and terrestrial invertebrates in the Upper West Region of Ghana.

Scientific Presentations

Oral Presentation

Adam Y., Cecchi G., Mahama CI., Bouyer J. An Integrated Strategy of Intervention against Riverine Tsetse in the Upper West Region of Ghana in the framework of PATTEC. *32nd Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC)* 8-12 September 2013, Khartoum, Sudan

Adam Y. An Integrated Strategy of Intervention against Riverine Tsetse in the Upper West Region of Ghana in the framework of PATTEC. *10th PATTEC coordinators' meeting*, 13-15 June 2012, Accra, Ghana.

Poster presentation

Adam, Y., Marcotty, T., Cecchi, G., Mahama, C., Solano, P., Bengaly, Z. and Van den Bossche, P. Bovine trypanosomosis in the Upper West Region of Ghana: entomological, parasitological, and serological cross-sectional surveys. *31st meeting of the ISCTRC, organized under the auspices of the AU/DREA*, 12-16 September 2011, Bamako, Mali.

General Introduction

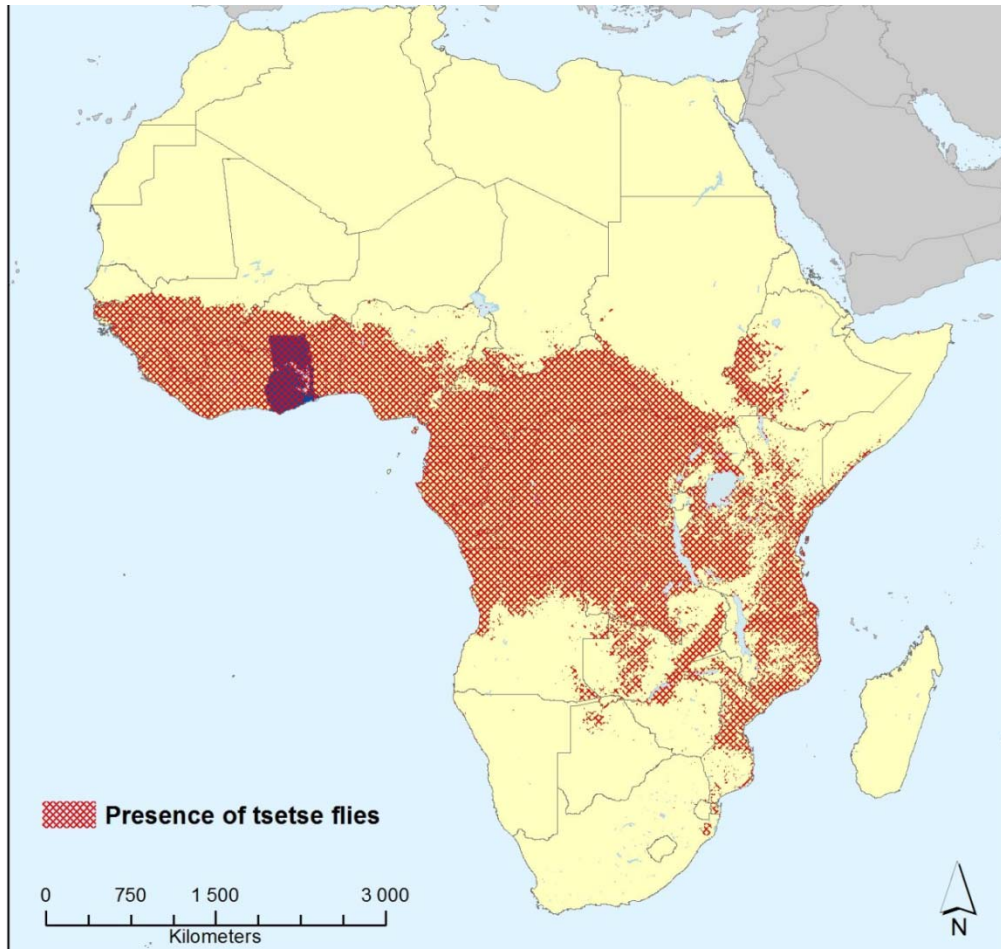
General Introduction

The problem of tsetse-transmitted trypanosomosis is typically African and peculiar to Sub-Saharan Africa. These cyclical vectors of trypanosomosis, affecting both human beings and livestock, are present in 38 Countries of sub-Saharan Africa (Hargrove et al., 2000). Animal trypanosomosis is a major constraint to agricultural production in over more than 10 million square kilometers of sub-Saharan Africa. The disease is a serious constraint to livestock health and production, resulting in annual losses estimated at ~US\$4.5 billion (Budd, 1999). This figure is underestimation, considering a more recent analysis by Bouyer, J., et al., (2013). Rural populations, which depend on agriculture, fishing, animal husbandry or hunting, are the most exposed to Human African Trypanosomosis (HAT) or Sleeping sickness. The Food and Agriculture Organization of the United Nations (FAO) estimates that trypanosomosis of livestock, nagana, is a major cause of rural poverty, severely limiting meat and dairy production, and preventing the use of draught oxen for ploughing. African Animal Trypanosomosis (AAT) in sub-Saharan Africa constitutes the major constraint to the development of productive livestock (Shaw, 2004). In the tsetse-infested areas of Africa, maintaining livestock requires more frequent treatment with preventive and curative doses of trypanocidal drugs. 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). Consequently, sub-saharan African countries are the least developed in the world with widespread hunger and poverty amongst the rural populations (Feldmann *et al.*, 2005).

The presence of tsetse flies in Ghana has been reported as far back as in the Colonial era of Ghana (Simpson J., 1914). This early publication showed the presence of the following economically important species : *Glossina palpalis* s.l., *G. tachinoides*, *G. morsitans* s.l, and

G. longipalpis. The occurrence of tsetse species in Ghana according to vegetation cover types and climate has been published by Nash T.A.M., 1948 and Mahama *et al.*, 2004. In the drier parts of the country, *G. palpalis gambiensis* (Vanderplank, 1911) co-exists with *G. tachinoides* (Westwood, 1850) which is predominant in the open woodland pasture areas, whereas *G. morsitans* s.l. (Westwood, 1850) is found to be associated with game animals in open woodland savannah. In the forest zone of the country, Ofori E.D. (1964), reported the presence of the following tsetse species : *G. fusca*, *G. nigrofusca*, *G. tabaniformis* and *G. medicorum* whereas in the transitional forest *G. longipalpis* was reported. Indeed the entire country lies within the tsetse-infested belt of Africa (lat. 15° N to 20° S) with tsetse density varying according to land cover types (Fig. 1). An updated narrative of the distribution of tsetse flies in the Northern and Upper West Regions of Ghana by Mahama *et al.*, (2003) and Adam *et al.*, (2012) respectively, show the importance of riverine tsetse species in the prevalence of AAT. All past reports confirmed the presence of *G. tachinoides* and *G. palpalis gambiensis* with varying species distribution and densities from North Southward. Whereas *G. tachinoides* was found to be distributed all over the study areas, *G. palpalis gambiensis* was restricted to the southern limits (Below Latitude 9.88°N) with ample riverine forest galleries (Mahama *et al.* 2012).

A major epidemiological feature of AAT in Ghana as reported by Mahama *et al.*, (2004) is the wide variation in trypanosomosis prevalence in cattle, sheep and horses across the country. Studies conducted in the Northern and Upper West Regions (Mahama *et al.*, 2004), found *T. vivax* as the most prevalent trypanosome infection, followed by *T. congolense* (savannah type) and then *T. brucei*.

Figure 1. Ghana within the Tsetse Infested belt of Africa.

Trypanosomosis control efforts since pre-independent Ghana have largely been directed against tsetse flies. Persistence of the trypanosomosis problem despite the use of vector control as the most sustainable control method was due to the lack of appreciation of the transboundary nature of the vector resulting in controlled areas being easily invaded. Prophylactic and therapeutic treatment against trypanosomosis is however routine cattle health maintenance practice amongst livestock farmers to maintain viable production in tsetse endemic areas albeit at a high cost. Hence, for economic reasons, Ghana despite the

abundance of vast forage resources is unable to increase livestock production to meet local demand due to high tsetse challenge (Mahama *et al.*, 2004).

Early researchers involved in tsetse control and livestock health management had considered tsetse habitat alteration as a means to controlling the main vector for trypanosomosis. In pre-independent Ghana, bush clearings were carried out on a massive scale in the Nabogu Valley of the Northern region (Stewart, 1937.), and in Lawra, along the river Kamba (Morris, 1947). There were also reports of limited game destruction as means of controlling tsetse flies in the early 1950s (Mahama *et al.*, 2003). According to Ford *et al.* (1970) ‘Total, sheer or ruthless clearing means the destruction of all trees and shrubs in the area treated. It is completely an effective method of eliminating *Glossina*, and the oldest’. By removing all of the vegetation, one is removing not only the supposed ‘home’ of the tsetse fly, but also the habitat of game, essential for the fly’s survival (Hargrove, 2003). Human intervention into riparian forest for settlement and crop production often result in the loss of habitat for tsetse: in Burkina Faso, crop and cotton production led to the disappearance of *G. morsitans submorsitans* in the Black Volta River Basin where twenty years ago this species was trapped (Bouyer *et al.*, 2010). Entropic activities on the edges of the Black Volta 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). For the sake of conservation of flora and fauna this method has been abandoned because of their negative impacts on biodiversity (Bouyer *et al.*, 2010).

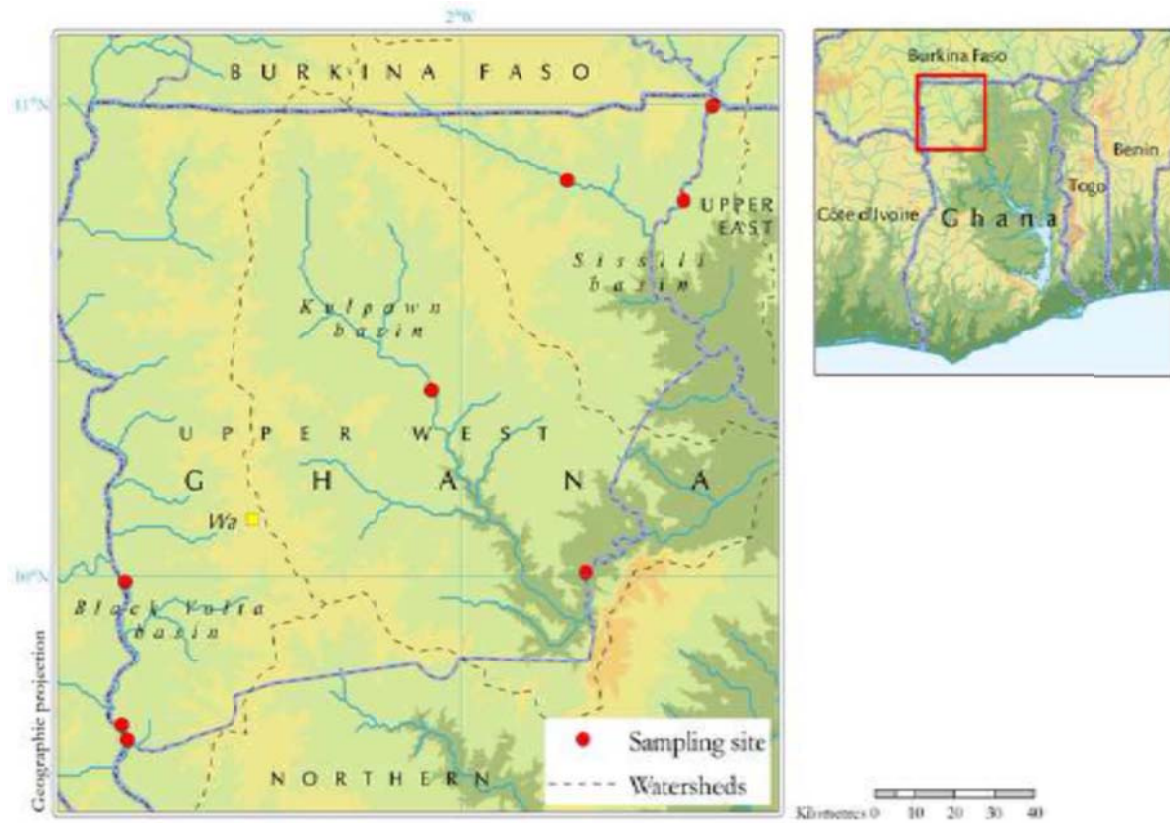
Trials on the possible control of tsetse flies using various trap designs were carried out in West Africa (Morris, 1949 ; Rayaisse, et al. 2011, 2012). The evolution of tsetse control methods in Ghana actually had a boost, when in 1996 a community-based tsetse control

project involving the use of Deltamethrin-impregnated blue screens was carried out in the coastal savannah. About 600 Deltamethrin-impregnated blue screens and 200 Deltamethrin-impregnated traps were deployed in the Lower Volta river basin drainage network (TTCU report, 1996). In the same exercise, about 500 cattle were topically treated with Deltamethrin “pour on”. Within three months of the application of these methods, tsetse population was reduced by 98%.

In an effort to have greater impact on tsetse control efforts and for better utilization of resources, African Heads of State and governments in Lome 2000 passed a declaration that essentially called for a concerted effort between countries in the fight against tsetse flies. The signing of the protocol saw the establishment of the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). Under the PATTEC initiative, Ghana, Burkina Faso and Mali in the West African sub-region initiated a project to create tsetse free areas across their common borders. A similar common border initiative was established between Kenya, Uganda and Ethiopia in the East African sub-region.

In Ghana, the Upper West Region was chosen as the target area for the implementation of the Multinational Tsetse Project from 2006 to 2011 (Fig. 2). The Upper West Region is bordered to the north and west by Burkina Faso, between latitude 8°N and 11°N and covers an area of about 18,000km² and is infested with riverine species of tsetse flies along the three main river basins (Black Volta, Kulpawn and Sissili Rivers).

Figure 2. PATTEC Project Area in Ghana (Adam Y., et al. 2012)



To give decision support to the campaign, there was the need to conduct operational research work before, during and after the intervention phases of the project. The design of this thesis was geared towards evaluating the frame work of the PATTEC Ghana strategies by focusing on baseline data collection, tsetse population structuring, and impact assessment of the SAT operation on tsetse densities as well as the environmental impact on vulnerable non-target arthropods. The overall objective of this thesis was to assist and optimize the implemented PATTEC strategies in Ghana, and evaluate the impact of the PATTEC intervention strategies in Ghana as captured in the under mentioned five thematic point specific objectives of the thesis:

1. To determine the pre-intervention vector and disease situation of the intervention area in Ghana.
2. To determine whether tsetse populations in the UWR of Ghana were able to disperse between the existing three river basins and the consequences on sustainable tsetse control effort.
3. To evaluate SAT for the control of riverine tsetse species.
4. To evaluate two comparative study designs for the selection of appropriate entomological monitoring sites.
5. To conduct an environmental risk assessment of the intervention programmes.

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Chapter1: A General Review

Bovine Trypanosomosis (Disease) and Vector Control :
A General Review

1.1. Introduction

African trypanosomosis is a haemo-protozoan disease complex caused by several species of trypanosomes, affecting both humans and animals. Trypanosomes (*Trypanosomatidae* : *Trypanosoma*) - in Africa are cyclically transmitted by tsetse flies (Diptera: Glossinidae). The disease in humans is termed Human African Trypanosomosis (HAT) or sleeping sickness and African Animal Trypanosomosis (AAT) or Nagana in animals. In humans, HAT can be categorized into two forms - chronic and acute- according to the causal trypanosome subspecies involved. The chronic form is due to *Trypanosoma brucei gambiense* (*T.b.g.*), found in West and Central Africa and the acute form which is due to *Trypanosoma brucei rhodesiense* (*T.b.r.*), is found in East Africa.

In livestock, AAT is a chronic debilitating condition caused by various trypanosome species including; *T. congolense*, *T. vivax*, *T. brucei brucei*, *T. simiae* and *T. godfrey*. The disease is characterised by a reduction in fertility (calving rate), weight lose, milk and meat off take by at least 50% (USD 2750 million per year) (Budd, 1999; Swallow, 1999; Shaw, 2004; Feldmann, 2005). It can also result in death of affected animals (especially calves) if left untreated (Hursey and Slingenbergh, 1995). The impacts of the disease on other sectors of rural economy can be viewed in terms of the potential reduction in opportunities for integrating livestock and crop production (mixed farming), through less drought animal power to cultivate land and less manure to fertilize soils for enhanced crop production and also affects human settlement as people tend to avoid areas with tsetse flies (Shaw, 2004; Feldmann, 2005).

1. 2. Epidemiology and Risk factors of Trypanosomosis

The epidemiology of trypanosomosis relates to the expressed disease patterns under field conditions and depends on the complex interactions between the parasite, vector and the

mammalian host. The risk factors on the other hand relate to predisposing and external factors that influence trypanosomosis infection on the field.

1.2.1. Patterns of trypanosomosis on the field

The occurrence of pathogenic trypanosomes (*Trypanosoma vivax*, *T. congolense* and *T. brucei*) and the wide variation in the prevalence of trypanosomosis is a major feature of the epidemiology of the disease in Africa and is consistent with the correlation between tsetse apparent density and the risk of trypanosomosis described by Rogers (1985), (Mahama, et al., 2003) The African pathogenic trypanosomes affecting human or livestock belong to the Salivaria group, 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 infected animal. Livestock trypanosomosis mostly occurs in areas that border wildlife zones or those areas that were previously occupied by wildlife. On the other hand two subspecies of *Trypanosoma brucei* cause disease in humans (FAO, 1998). The two subspecies, *T. b. rhodesiense* and *T. b. gambiense* are predominantly found in Eastern and Central Africa respectively. *T. b. gambiense* is also found in West Africa and the disease is referred to as the West African sleeping sickness. At present, the two subspecies of *T. brucei* do not overlap in their geographic distribution. The inter-relationship between the tsetse fly, trypanosome species, and the mammalian host epitomises the epidemiological implications of African trypanosomosis.

1.2.2. Vector-Parasite-Host Relationship

Tsetse flies are obligate haematophagus cyclical vectors of trypanosomes, small-single-celled organisms, acquired and transmitted in the process of feeding. During a blood meal on the mammalian host, an infected tsetse fly (genus *Glossina*) injects metacyclic trypomastigotes into skin tissue (Vickerman *et al.*, 1988). The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes,

carried to other sites throughout the body, reach other blood fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission (Vickerman *et al.*, 1988). The entire life cycle of African Trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In the fly's mid-gut, the parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission (Van Den Abbeele *et al.*, 1999). This transformation is necessitated by drastic changes in the parasite's living conditions i.e. from a stable temperature and oxygen-rich environment in the vertebrate bloodstream where glucose is used for energy to a fluctuating temperature and oxygen-deficient environment in the tsetse where proline becomes their source of energy (Vickerman, 1985; Vickerman *et al.*, 1988; Leak, 1999).

Tsetse transmits trypanosomes in two ways, *mechanical* and *biological* transmission. Mechanical transmission involves the direct transmission of the same individual trypanosomes taken from an infected host into an uninfected host. This form of transmission requires that tsetse feed on an infected host and acquire trypanosomes in the blood-meal, and then, within a relatively short period, feed again on an uninfected host and regurgitate some of the infected blood from the first blood meal into the tissue of the uninfected animal. This type of transmission occurs most frequently when tsetse are interrupted during a blood meal and attempt to satiate themselves with another meal. Other biting flies, such as tabanids and horse-flies, can cause mechanical transmission of trypanosomes (Cherenet, T., *et al.*, 2004). On the other hand, biological transmission requires a period of incubation of the trypanosomes within the tsetse host. The term *biological* is used because trypanosomes must reproduce through several generations inside the tsetse host during the period of incubation, which requires adaptation of the trypanosomes to their tsetse host. In this mode of transmission,

trypanosomes reproduce through several generations, changing in morphology at certain periods. This mode of transmission also includes the sexual phase of the trypanosomes. Tsetse flies are more likely to become infected by trypanosomes during their first few blood meals. Tsetse infected by trypanosomes is thought to remain infected for the remainder of their lives. Cyclical development - the establishment and development of the trypanosomes in the tsetse vectors - is a complex process, involving a series of vector and parasite defence and counter-defence mechanisms (Wellburn & Maudlin, 1999), and there is evidence that tsetse become harder to infect as they mature. Because of the adaptations required for biological transmission, trypanosomes transmitted biologically by tsetse cannot be transmitted in this manner by other insects.

1.2.3. Risk factors of Trypanosomosis

Risk factors of trypanosomosis are largely attributed to tsetse habitat suitability (Vegetation cover and water sources) and the presence of animals as sources of blood meal for the tsetse fly. Animals are the primary reservoir of infection. Cattle form the main vehicle for spreading the disease to new areas and initiating local outbreaks. Wild animals serve as reservoir for most species of trypanosome and thought to be responsible for sporadic transmission to humans as they visit game and forest reserves.

The major epidemiological risk factor in African trypanosomosis is contact between humans and tsetse flies. This interaction is influenced by an increasing tsetse fly density, changing feeding habits and by expanding human development into tsetse fly-infested areas.

1.2.4. Trypanosomosis as a Trans-boundary Animal Disease

African animal trypanosomosis is a Trans-boundary Animal Disease (TADs) with the potential for spread along transhumance livestock rearing systems in sub-Saharan Africa. Indeed the practice of transhumance is frequently observed in Ghana where cattle from

Burkina Faso, Niger and from Northern Nigeria cross over into Ghana for grazing (Adam, Y., et al., 2012). In some areas like Southern Angola, ethnic groups from the Ovambo and part of the Nyaneka-Khumbi areas are entirely organized around the practice of transhumance (Eduardo Cruz de Carvalho et al., 1973).

Tsetse are opportunistic feeders, especially the riverine species present in our study area, but their preference or specific attraction can arise from learning mechanisms (Bouyer J., et al., 2007).

Nomadic pastoralists are often bitten by tsetse flies as they strive for their blood meal. This phenomena lead tsetse to new habitats and tsetse dispersal across regional boundaries. Studies have shown the potential of tsetse to disperse actively over a distance of approximately one kilometre per generation (Cuisance et al. 1985, Bouyer J., et al., 2007) and this distance is long enough to cross international boundaries in sub-Saharan African with the implication of trypanosomosis as a trans-boundary Animal disease.

1.3. Tsetse and Trypanosomosis Control

Tsetse and trypanosomosis control are a series of efforts employed to reduce or eliminate the incidence of African trypanosomosis, transmitted by tsetse flies. Two complementary strategies have been used in the attempts to reduce or eliminate the disease. The two strategies are chemotherapeutic, targeted at reducing the incidence of trypanosomosis in livestock and entomological, aimed at disrupting the cycle of transmission of the disease by reducing or eliminating the vector of trypanosomosis (Bouyer et al. 2010).

Until recently, tsetse control efforts have been undertaken as individual national projects in sub-Saharan Africa without recourse to the trans-boundary nature of the disease and the problem has always been a relapse after any control effort due to the complex mitigating factors such as rural poverty, civil strives and the lack of a concerted efforts to sustain the

individual national control efforts. Despite this situation in Africa, examples of successful tsetse elimination efforts can be sighted in Botswana Kgori et., al, 2006), Namibia and the Islands of Zanziba (Vreysen et al. 2000).

The potential benefits of trypanosomosis control thus appear to be highest in areas where there is good potential for integrating livestock into profitable and sustainable mixed crop-livestock farming systems (Swallow, 1999). This conclusion has clear implications for the development and implementation of the Action Plan for the Programme against African Trypanosomiasis (Swallow, 1999) and thus calls for careful considerations to be made prior to embarking on a control programme (Vreysen et al. 2013).

1.3.1. Considerations for Tsetse Control Programmes

To embark on a national tsetse intervention programme, a number of pre-control operations are undertaken to ensure a well monitored and successful control programme (Vreysen et al. 2013). These preconditions may include socio-economic baseline survey, entomological baseline survey and tsetse population structuring (population genetic profiling). Environmental impact assessment must be performed before, during and after the control intervention phase to evaluate the impact on non-targeted animal species.

1.3.1.1. Socio-economic Baseline Survey

A baseline socio-economic study is undertaken to quantify the economic impact of tsetse and trypanosomosis control programme, taking 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 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).

It has been estimated that both direct losses and cost of AAT control range between USD 600 and 1200 million per year for Sub-Saharan Africa (SSA) (Swallow, 2000). The benefits of enhanced trypanosomosis control, according to the dynamic herd model of Kristjanson *et al.* (1999), 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 per year.

1.3.1.2. Entomological and disease Baseline Survey

Control programmes must necessarily be monitored to assess the distribution and densities of tsetse species, as well as the prevalence of bovine trypanosomosis (Sow A., et al., 2013). In Burkina Faso for example, a cross-sectional survey was conducted before implementing the eradication campaign in the region of the Boucle du Mouhoun with the aim of determining the prevalence of trypanosome infections and the health status of farm animals by using the packed cell volume (PCV) and Body Condition Score (BCS) as indicators (Sow, A., et al., 2013).

1.3.1.3. Tsetse population structuring (Population Genetic profiling)

The application of population genetics techniques to quantify rates of gene flow between sub-populations (Solano, 2000; Gooding, 2005; Bouyer, 2007; Bouyer, 2009b; Solano, 2009b), is employed to guide decisions on the choice of tsetse control strategies (Kagbadouno, 2009). A sound decision whether to select an eradication or suppression strategy will be facilitated when the population structure within the target region, in particular the degree of genetic isolation of the target population from its adjacent populations is clearly understood (Bouyer, 2010). For isolated populations, eradication may be the most cost-effective strategy, as reported for *Glossina austeni* Newstead in Unguja Island, Zanzibar (Vreysen, 2000). But for

most mainland populations of tsetse, the geographical limits of target tsetse populations are less easily defined, and an autonomous integrated control of AAT by farmers should generally be preferred (Figure3) (Bouyer et al. 2013).

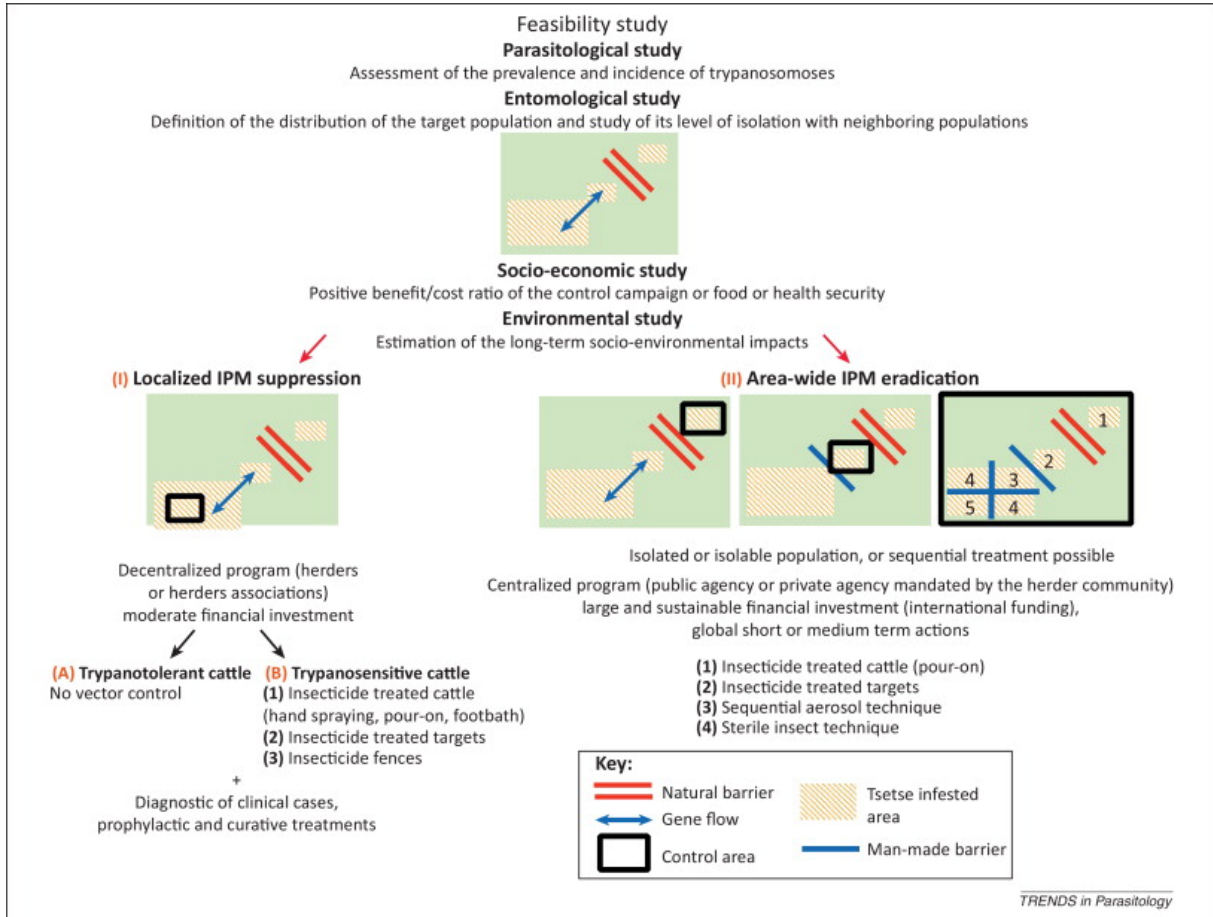


Fig3. Selection of the AAT control strategy depending on the environmental and socio-economic situation (source : (Bouyer et al. 2013)).

The level of isolation of the targeted tsetse populations was considered an important parameter to guide the Government of Senegal to select the most optimal control strategy. Solano et al., (2010) reported population genetic analyses of microsatellite and mtDNA markers combined with morphometrics of *G. p. gambiensis* populations sampled from the Niayes area and from the nearest population in the south-eastern part of the Senegal to assess their degree of isolation by measuring gene flow among the different populations. They confirmed complete isolation of the target population and thus advised an eradication strategy to the Government of Senegal.

1.3.2. Control of parasitic Trypanosomes

Trypanosomosis disease control is based on the use of trypanocidal drugs. Depending on the control strategy, drugs are used for curative or preventive purposes. 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 (Sow 2013). 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. In animal trypanosomosis, several compounds such as diminazene aceturate and isometamidium chloride have been used to combat the parasite. Isometamidium chloride has a prophylactic activity whilst diminazene aceturate has a short-term therapeutic activity (Diall, 1992; Chartier *et al.*, 2000). However, there has been widespread incorrect use of these drugs which has led to the development of drug resistance by the parasite (Geerts *et al.*, 2001). Vector control must be combined to cattle treatment to avoid the spread of resistance (Bouyer *et al.* 2013). In the case of sleeping sickness, the most widely used drugs to treat the patients are Suramin, Melarsoprol and Eflornithine (difluoromethylornithine) depending on the trypanosome species and the clinical stage of the disease. A new treatment using a combination of Nifurtimox-Eflornithine is available for sleeping sickness (Priotto and Sevcsik, 2008; WHO and DNDi, 2009). There is no vaccine available to control both human and animal trypanosomosis.

1.3.3. Entomological (Vector) Control Strategy

Vector control intends to disrupt the cycle of disease transmission by reducing or eliminating the fly population in a given geographic area with the objective to eliminating trypanosomosis related problems in both human and livestock. Tsetse control techniques have gone through evolutions, with earlier crude methods recently being replaced by methods that are economically cheaper, more directed, and ecologically sustainable. These techniques include : environmental management (bush clearing and game destruction), pesticide campaigns, traps/targets, animal baits and the use of Sterile Insect Technology (SIT).

The choice of the control method to be applied will depend on the target zone, the impact on the environment, the tsetse species, and the possibility of isolation of treated areas, the economic impact, the possibilities of post control land use and the available funding of the operations now and for the future (Sow, 2013). So far, insecticides remain the most frequently used method of control due to their efficacy and they constitute the first step of tsetse control before any other method can be applied (Allsopp, 1984).

1.3.3.1. Environmental management as a vector control strategy

Bush clearing and game destruction is targeted at disrupting the ecology of tsetse flies, thereby bringing down tsetse populations to sustainable levels for human habitation and livestock development. Initial attempts to control tsetse flies were based on tsetse habitat alteration and game removal to starve out tsetse flies whose source of livelihood depended on the existence of game as the main source of food. 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 adverse effect on biodiversity (Bouyer *et al.*, 2010).

1.3.3.2. Pesticide campaign in Vector Control

Current vector control interventions involve the use of insecticides either through aerial spraying; ground spraying; insecticide-treated targets or insecticide-treated animals – live baits and the use of other-baited traps or screens (Vale *et al.*, 1988; Bauer *et al.*, 1995; Hargrove *et al.*, 2000).

1.3.3.2.1. Live bait technique

Live bait technique involves treating cattle with appropriate insecticide formulations, usually by means of cattle dips, or as pour-on, spot-on, or spray-on veterinary formulations. These are highly effective against tsetse, and have the additional advantage of controlling other flies and cattle ticks. In the 1940's, following the advent of DDT, the use of cattle as live baits to achieve control by treating them with DDT was introduced but did not prove successful because the insecticide formulation used was not sufficiently persistent on cattle which, therefore, required spraying at frequent and impractical intervals (Vale, G.A. 1988). It was therefore replaced by pyrethroids. More recently, restricted application of insecticides has been promoted to reduce the cost and environmental impact of this strategy (Bouyer, J., *et al.*, 2007; Bouyer, J., *et al.*, 2009; Torr, *et al.*, 2007).

1.3.3.2.2. Ground spraying

Trials with insecticides against tsetse started in 1945, when DDT and BHC (HCH) were the only synthetic compounds available. The application of residual deposits of persistent insecticides to tsetse resting sites was very widely used, but is now discouraged due to concerns about effects on non-target organisms. Extensive insecticide ground spraying which was used to control tsetse in Zimbabwe and Nigeria is seldom used today because of

concerns over residual insecticides and because the operational demands are beyond the means of most African governments (WHO, 2013).

1.3.3.2.3. Aerial Spraying: The Sequential Aerosol Technique (SAT)

SAT is a technique used in tsetse fly control, sometimes called the sequential drift spraying technique. SAT uses small drops, which we call aerosols, applied several times (in a sequence) at intervals to kill eclosing flies before they breed, and hence before they can produce a new generation. Because the sequences are timed to coincide with the tsetse life cycle, based on daily temperature (which controls their rate of development), it is less damaging to other insect species (Cooper, J., and Dobson, H., 1993). An extensive area of tsetse control or elimination is achieved by the application of these insecticidal aerosols from aircrafts over large tracts of land (Kgori, 2006).

Eventough Deltamethrin insecticide was used in the current SAT operations in Ghana, it is worth noting that the pyrethroids are not the sole insecticides used in the past for SAT. Apart from the environmentally contaminating effect, organochlorines and organophosphorous insecticides have been used successfully during SAT campaigns : Endosulfan (a 20% or 30% E.C. formulation at a rate of 10 to 20 g active ingredient/ha) was used apart from deltamethrin (at 0.2 to 0.5 g/ha). When used at the concentrations as used for SAT, both deltamethrin and endosulfan are considered as having no serious environmental impact. A potential alternative insecticide for SAT could be spinosad, which is almost as toxic to tsetse as deltamethrin, but of which the toxicity does not differ significantly between teneral and gravid flies, contrary to deltamethrin (De Deken et, al., 2004)

SAT, which can effectively clear large areas of tsetse in a relatively short time, requires substantial economic and infrastructure support. Recent advances in aircraft guidance systems have considerably increased the accuracy and the efficiency of insecticide delivery as

shown in recent control operations in the Okavango delta in Botswana and Namibia (Kgori et al., 2006; WHO, 2013). SAT has recently been undertaken in Ghana and Burkina Faso under the respective country PATTEC projects (WHO, 2013). The main limitation of aerial spraying as with other methods is the re-invasion pressure if the area is not isolated, and the occurrence of dense vegetation cover lowering the penetration of insecticide droplets. Other limitations to the use of SAT are; the terrain must be flat, the timing of the flights and the prevailing meteorological conditions at the time those flights are scheduled, the short duration of the treatment effect of SAT necessitating a perfect isolation of the treatment area on beforehand (Alsopp, R., 1991).

The measurement of droplet penetration both under the canopy and in the open is a procedure that has to be carried out during each cycle especially the first ones and is essential to assess spraying efficacy! Slides are best collected 15 to 30 minutes after the aircraft has passed the monitoring site. Craters on the magnesiumoxyde covered slides caused by aerosol droplets can be distinguished from craters formed by dew droplets through the mixing of a fluorescent dye in the aerosol solution (De Deken, R., et al., 2004).

1.3.3.3. Traps and Targets

The use of highly efficient fly traps and screens impregnated with synthetic pyrethroids insecticides (Hargrove et al., 2003) are techniques currently employed to control tsetse flies.

As vector control tools, traps and targets function by removing individuals from the existing tsetse population. Their efficiency depend on the length of time the devices remain operational, and the likelihood that an individual fly will encounter the device and be killed by it. The length of time each device remains operational depends on a number of factors, including resistance to environmental damage (e.g. wind and/or damage by large animals), theft of all or part of the device, and component degradation (particularly colour fade,

depletion of odour baits, and loss of insecticidal activity in the case of targets). The likelihood that an individual fly will encounter and be killed or captured by the device depends on the number of traps or targets relative to the local abundance of tsetse, and on the particular foraging and dispersal behaviour of the target tsetse species. In the case of savannah species such as *G. morsitans*, each fly may disperse up to 500 metres in a single day, so that with an average trap density of just four traps per km², there is a high likelihood that each fly may disperse sufficiently to encounter at least one trap (Hargrove, 1993; 2003[a,b]). By contrast, forest species such as *G. fuscica* probably disperse little more than 5-10 metres per day, so that effective trap densities need to be much higher (see section 3.2.3).

In general therefore, it can be argued that deployment of traps and/or targets that destroy a proportion of the tsetse population will lead to a reduction in trypanosomosis transmission. Moreover, in the case of human-infective trypanosomes, where the infection rate in the flies tends to be very low, the infection will be unable to spread once the vector population is below a threshold level (Rogers, 1988; Atzrouni & Gouteux, 1996, 2001), so that an outbreak of human infection could be contained by a sustained trapping campaign that keeps the density of tsetse vectors below that threshold (Gouteux & Artzrouni, 1996).

1.3.3.3 The Sterile Insect Technique (SIT)

The SIT exploits the particular mating biology of tsetse, whereby female flies rarely mate more than once. Male flies are therefore mass reared in the laboratory, sterilized by irradiation, and released to mate with wild females. Females mated with sterile males are unable to produce offspring. Unlike all other tsetse control techniques, SIT has no effect on non-target organisms. Also unlike other techniques, SIT becomes more efficient at lower fly densities, and is ideally suited to the final phase of local tsetse eradication (F.A.S. Kuzoe and C.J. Schofield, 2004). Sterile Insect Technique (SIT) involves mass rearing of target insects in a laboratory and sterilizing the males by exposing them to low doses of radiation. These

sterile male flies are then released by air over infested areas, where they mate with wild females. If the sterile males vastly outnumber the fertile wild males, the wild fly population quickly dies out. The proportion of infertile males to fertile wild males must be at least 10:1. The most notable success of SIT against tsetse was in eradicating *G. austeni* Newstead on Unguja Island; Zanzibar with 50:1 infertile to fertile male proportion (Hargrove, 2003). The SIT is an area-wide method used to “mop up” residual tsetse populations when conventional techniques succeed in reducing populations to extremely low levels (Vreysen, et al., 2013). Currently in Ghana, the SIT is considered a future option when tsetse populations have been reduced to significantly low levels.

1.4. A Continental Approach to Tsetse Control/Eradication

African Heads of State and Government, having been under pressure from their communities to do something about the tsetse fly, and realizing that individual country solutions would not work, came to the conclusion that the tsetse problem was trans-boundary in nature and had to be tackled on a continent-wide basis (AU Commission Action Plan 2001). In an effort to have greater impact on tsetse control efforts and for better utilization of resources, African Heads of State and governments in Lome 2000 passed a declaration that essentially called for a concerted effort between countries in the fight against tsetse flies. The signing of the protocol saw the establishment of the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). Under the PATTEC initiative, Ghana, Burkina Faso and Mali in the West African sub-region initiated a project to create tsetse free areas across their common borders. A similar common border initiative was established between Kenya, Uganda and Ethiopia in the East African sub-region.

1.5. References

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Chapter2: Cross-sectional Surveys

Bovine trypanosomosis in the Upper West Region of Ghana: Entomological, parasitological and serological cross-sectional surveys

Adapted from:

Y. Adam, T. Marcotty, G. Cecchi, C.I. Mahama, P. Solano, Z. Bengaly, P. Van den Bossche (2012). Bovine trypanosomosis in the Upper West Region of Ghana: Entomological, parasitological and serological cross-sectional surveys. *Research in Veterinary Science*, 92, 462–468

Abstract

Baseline surveys were conducted in the Upper West Region of Ghana to assess the distribution and densities of tsetse species, as well as the prevalence of bovine trypanosomosis.

The entomological survey was designed to cover the suitable tsetse habitats along the three main rivers in the study area (i.e. Black Volta, Kulpawm and Sissili). Results indicated the presence of *Glossina tachinoides* in all three river basins, whilst *Glossina palpalis gambiensis* was only found close to the southern limit of the study area only.

A random sampling of 1800 cattle of the West African Short Horn, Sanga and Zebu breeds from 36 randomly selected grid cells covering the study area showed substantial differences between parasitological and serological prevalences. The average parasitological prevalence was estimated at 2.5% (95% CI: 1.06–5.77) with the majority of the infections due to *Trypanosoma vivax*. Most of the infected cattle were found close to the major river systems. The serological prevalence, measured using Enzyme Linked Immunosorbent Assay (ELISA) test was 19% (95% CI: 14.03–25.35). Cattle with anti-trypanosomal antibodies were also found throughout the study area.

2.1. Introduction

Trypanosomosis is a haemoprotozoan disease, mostly transmitted by the tsetse fly (*Glossina* spp.), which cause severe disease in humans and livestock in sub-Saharan Africa. The disease results in loss of livestock and agricultural productivity with serious socio-economic consequences (Swallow, 2000). The occurrence of tsetse species within different climatic zones and vegetation types in Ghana were studied by various researchers. In the drier, northern parts of the country *Glossina palpalis gambiensis* was found to coexist, at comparatively lower densities, with *Glossina tachinoides* (Nash, 1948). In these areas, suitable land cover for tsetse flies was represented by open woodland and rangeland, mostly clustered along the main rivers (Black Volta, Kulpawm and Sissili) and their tributaries. Both *G. palpalis gambiensis* and *G. tachinoides* belong to the *palpalis* group. They play a role in the transmission of both human and animal trypanosomosis and can adapt to high human densities (Solano et al., 2010; Courtin et al., 2009). By contrast, *Glossina morsitans submorsitans* and *Glossina longipalpis* (*morsitans* group) were found to be associated with game animals in open woodland savannah. In the transitional forest zone, *G. longipalpis* was said to be present whereas in the forested southern part of the country, tsetse species of the *fusca* group (*Glossina fusca*, *Glossina nigrofusca*, *Glossina tabaniformis* and *Glossina medicorum*) (Offori, 1964) were present.

In the past, the persistence of this trans-boundary disease has certainly been enhanced by the lack of a concerted, multinational approach. The ‘‘Pan-African Tsetse and Trypanosomosis Eradication Campaign’’ (PATTEC) was therefore established by the African Union in the year 2000 aiming at eradicating tsetse and trypanosomosis from Africa by bringing together the efforts of all affected countries. Under the PATTEC initiative, Ghana, Burkina Faso and Mali initiated a project to create tsetse-free areas within their common tsetse fly belt. Whilst the epidemiological situation of human African trypanosomosis in the region has recently

been reviewed (Cecchi et al., 2009; Simarro et al., 2010) there is a need to clarify the current prevalence of animal trypanosomosis and the distribution of tsetse flies, to facilitate the making of sound decisions in the campaign. The main aim of this field-based research work is therefore to provide information on the prevalence of trypanosomes and its vector to guide decision making in project implementation, as well as to generate sound baseline data for monitoring and evaluation.

The Upper West Region in Ghana was chosen as project area for the first phase of the Ghana component of the PATTEC initiative based on economic (i.e. potential for high economic return; Shaw et al., 2006), entomological (i.e. proximity to the northern tsetse distribution limits) and strategic criteria referring to the geographic location of the three national projects in West Africa. The study area is bordered to the North and West by Burkina Faso. It is sub-divided into 8 districts. The climate of the study area is tropical with a single rainy season from May to October, followed by a long dry season from November to April.

Three main breeds of cattle are found here: the West African Shorthorn (WASH), Zebu and Sanga. The WASH is the most common breed of cattle raised in the area because of its high trypano-tolerance as compared to the other two breeds (Mahama et al., 2003). Of the three breeds of cattle, the Zebu is the most susceptible to trypanosomosis and the least represented in the total cattle population of the area. The zebu cattle are found mainly in the North-eastern corridors of the Upper West Region (Sissala Districts), where the tsetse population is comparatively less dense (Mahama et al., 2003). Cattle in the Upper West Region are mostly herded by Fulani immigrants, acting as paid employees, from neighbouring countries north of Ghana. The husbandry system is sedentary with communal grazing along rivers, shuttling from settlements to the main peri-riparian grazing areas, usually located in a radius of 2–5 km (Mahama, 2005). The practice of transhumance is also occasionally encountered

in the area. Indeed, it is not infrequent to observe cattle from Burkina Faso cross the Black Volta River for grazing in and outside the study area.

2.2. Materials and methods

2.2.1. Study area

The study area, the Upper West Region, is located in the Volta basin, in the North-western corner of Ghana between latitude 9.62–11° N and longitude 1.40–2.76° W. The Black Volta River represents the natural border with Burkina Faso. The total surface of the Upper West Region is approximately 18,000 km².

2.2.2. Entomological surveys

A cross-sectional tsetse fly survey was conducted along the three main rivers of the Upper West Region (Black Volta, Kulpawn, and Sissili Rivers), during dry seasons (April–May 2008 and December 2008–March 2009). At this time of the year, and due mainly to annual bush fires, vegetation in the Upper West Region is reduced to patches of riparian vegetation along the main river banks. In view of this and because of logistical constraints associated with sampling the entire river basins, trapping was restricted to the main rivers where riparian vegetation was present based on satellite images and visual inspection in the field. Hence, trapping sites were interspaced with open and often cultivated river banks unsuitable for tsetse (de la Rocque et al., 2001).

Apart from the Black Volta River, which holds water throughout the year and provides ample habitat for tsetse, the other main rivers, Kulpawn and Sissili, are extensively silted and provide little potential habitat for tsetse flies. Biconical traps (Challier et al., 1977) were deployed in the suitable riparian vegetation and at cattle watering points. An average of 20 traps was deployed at each site at 200 m spacing. The location of each trap was recorded with a Global Positioning System (GPS). A total of 564 traps were deployed along the three

rivers. Traps deployed at 8 am were recovered the following day at the same time, having been inspected twice within that period. Thus, tsetse flies were collected twice daily and the number, species and sex of flies caught determined and recorded. An index of apparent abundance (IAA) of tsetse was calculated as the number of flies (males and females) caught per trap per trapping day.

2.2.3. Parasitological and serological survey of bovine trypanosomosis

2.2.3.1. Sampling framework

To determine the prevalence of livestock trypanosomosis, the study area was divided into 180 grid cells of 10 km x 10 km. Grid cells that on the basis of census figures appeared not to host enough cattle were left out. With the aid of a computer-generated random table, 36 out of the 148 grid cells were selected. These 36 grid cells had total cattle population of 11,531 animals, out of which 1800 cattle were selected (50 cattle from each of the grid cells). The 50-head sample represents the average size of a cattle herd in the area. Moreover, a sample size of 50 animals provides a 95% certainty of detecting at least one positive trypanosomosis case at a prevalence of 5% (Cannon and Roe, 1982). For the purpose of sample collection, and because of the communal grazing system practiced in Ghana, we considered all herds of cattle in a village as one herd. A village with cattle in each of the 36 grid cells was thus selected for sampling by a computer generated random table. Where the randomly selected herd could not give the required 50 cattle, the balance was obtained from a herd of another randomly selected village within the same grid cell. Transhumant cattle were not considered because their kraals and locations would not fit in the sampling framework.

2.2.3.2. Sampling and processing

The location of each sampling site, normally located in the vicinity of the herd's village,

was determined using a GPS, and the relevant coordinates were used to map the distribution of bovine trypanosomosis. At each sampling site, blood samples were obtained from the jugular vein into heparinised tubes. Each animal was classified by breed, sex and age, the latter based on two categories: adult (≥ 3 years), and young (<3 years). Two heparinized capillary tubes were filled for each sample and centrifuged for 5 min at 9000g. The packed cell volume (PCV) was measured and recorded. The Buffy coat and the uppermost layer of red blood cells of each sample were extruded onto a microscope slide and examined for trypanosome parasites under a compound microscope using the Buffy coat technique (Murray et al., 1977). The plasma was collected on a filter paper, (Whatman No. 4, Whatman Ltd.) for further serological examination.

2.2.3.3. Serological diagnosis

Anti-trypanosomal antibody detection in the plasma on the filter papers was performed using an Enzyme Linked Immunosorbent Assay (ELISA). Plasma spots on filter paper were eluted in 1.5 ml of phosphate buffered saline (PBS) containing 0.1% Tween 20 for 2 h on a rotation shaker. Three indirect antibody ELISAs were performed on each sample using antigens of *Trypanosoma vivax* (Zaria 81/699), *Trypanosoma congolense* (IL1180) and *Trypanosoma brucei* (Desquesnes et al., 2001). The sensitivities of these three tests, under controlled conditions, were above 90% in experimentally infected sheep and in non-infected cattle specificity was above 99% (Desquesnes et al., 2001). The absorbance values were determined by photometry on an ELISA plate reader (Labsystem Multiskan MCC/340) using a 405 nm filter and interfaced to a desktop computer. For each type of antibody ELISA (ELISA – *T. vivax*, ELISA – *T. brucei* and ELISA – *T. congolense*), results were expressed in the Relative Percentage Positivity (RPP). Samples with RPP value exceeding the mean RPP of negative samples plus three standard deviations were considered seropositive (Desquesnes et al., 2001).

2.2.4. Statistical analysis

For each grid cell, the average PCV of the sampled cattle and its standard deviation were calculated. In addition, the average and confidence intervals of the parasitological and serological prevalence were calculated for each grid cell using a binomial distribution or the exact method when the prevalence was null. The overall parasitological and serological prevalence parameters were estimated in a robust logistic regression in Stata 10 (Stata Corp., 2009). The grid cells were considered as primary sampling units and were given their cattle population as weight. The proportion of grid cells selected was used as a finite population correction factor. The data were further analysed in similar models using the animals' breed (WASH, Zebu or Sanga), sex and age classes, and their interactions between them as discrete explanatory variables. The relation between the PCV and the animals' breed, sex, age class, parasitological and serological status was first evaluated in a stepwise backward selection of estimators ($p < 0.1$). The distribution of the residuals of the retained model was assessed in a Q–Q plot. The data were then analysed in a robust linear regression, using the retained estimators and the interaction between them as discrete explanatory variables. Primary sampling units, weights and finite population correction factors were as above.

2.3. Results

2.3.1. Distribution of *Glossina* species in the study area

A total of 3561 tsetse flies were caught (Table 1). Flies caught on the first day of the survey (482 flies) were lost before their sex and species could be determined. The tsetse survey revealed the presence of two *Glossina* species i.e. *G. tachinoides* and *G. palpalis gambiensis*. *G. tachinoides* was caught adjacent to the three main rivers of the Upper West Region, and thus appears to be distributed throughout the study area (Fig. 1). The highest values of apparent abundance were found in the South-western tip of the Upper West

Region, along the Black Volta River (IAA > 100). By contrast, *G. palpalis gambiensis* was absent from most of the study area, and flies of this species were only found at its extreme South, along the Kulpawn and Black Volta River basins (Fig. 1). The mean IAA of tsetse was 8.7, 1.9 and 1.2 for samples taken along the Black Volta, Kulpawn and Sissili Rivers, respectively.

Table 1
Number of male and female *G. tachinoides* and *G. palpalis gambiensis* caught in traps deployed along the Black Volta, Kulpawn and Sissili river basins of the Upper West Region of Ghana and index of apparent abundance (IAA) of tsetse along each river basin.

River basin	Number of traps deployed	Number of traps with captures	Number of tsetse captured				Undetermined species and sex	IAA
			<i>G. tachinoides</i>		<i>G. palpalis gambiensis</i>			
			Male	Female	Male	Female		
Black Volta	375	220	1301	1415	13	36	482	8.7
Kulpawn	119	39	110	99	5	11	0	1.9
Sissili	70	22	37	52	0	0	0	1.2
Total	564	281	1448	1566	18	47	482	

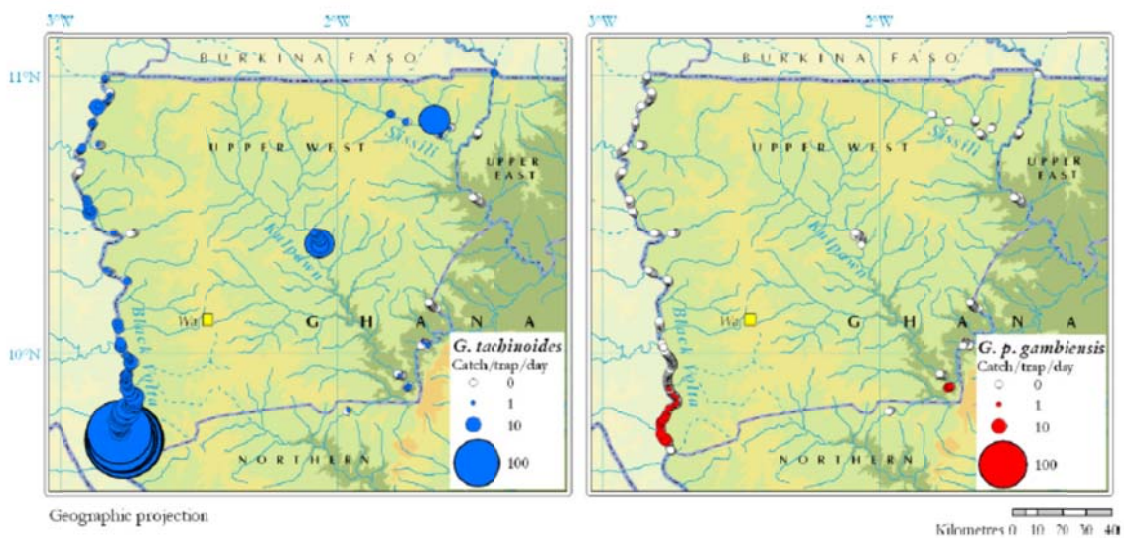


Fig. 1. Distribution of *G. tachinoides* and *G. palpalis gambiensis* in the Upper West Region of Ghana. The index of apparent abundance for each tsetse species is expressed as the number of flies captured per trap per day.

2.3.2. Prevalence of bovine trypanosomosis based on serological and parasitological examination

The parasitological and serological prevalence of bovine trypanosomosis in each of the grids of the Upper West Region are presented in Table 2 and Fig. 2. Trypanosomal infections were detected in cattle from 18 of the 36 selected grids. The average parasitological prevalence was 2.5% (95% CI: 1.1–5.8). Only infections with *T. vivax* (27)

and *T. brucei* (7) were detected parasitologically. The parasitological prevalence of trypanosomal infections was highest in the Sanga breed. Anti-trypanosomal antibodies were detected in cattle sampled in 29 of the 36 grids (Fig. 2). In all grids, the serological prevalence of trypanosomal infections was substantially higher compared to the parasitological prevalence. The average prevalence of anti-trypanosomal antibodies was 19% (95% CI: 14–25). The prevalence of anti-trypanosomal antibodies was highest in adult animals of the Zebu breed (Fig. 3). The average PCV of the WASH, the Sanga and Zebu cattle was 30%, 31% and 32%, respectively (Table 2). The PCV did not differ significantly between parasitologically positive and negative animals ($p = 0.4$ in stepwise backward selection of estimators). Little variations were observed between PCV values in seropositive and seronegative animals of the WASH and Zebu breeds (Fig. 3). In Sanga breed cattle, on the other hand, the average PCV of seropositive animals was lower ($p = 0.01$ in females and 0.1 in males; Fig. 3).

Table 2
Parasitological and serological prevalence (95% CI) of bovine trypanosomosis in each of the 36 randomly selected grid cells in the Upper West Region of Ghana (T.v. = *T. vivax*, T.c. = *T. congolense* and T.b. = *T. brucei*).

Grid ID	Village	Number sampled	Cattle breed			Average PCV (in%) \pm std	Number infected			Parasitological prevalence \times 100 (95% CI)	Number sero-positives	Serological prevalence \times 100 (95% CI)
			WASH	Sanga	Zebu		T.v.	T.b.	T.c.			
G1	Furo	50	50	0	0	33.6 \pm 4.7	0	0	0	0(<5.8)	3	6(1.9–17)
G2	Yipelle	50	49	1	0	29.7 \pm 5.0	0	1	0	2(0.3–12.9)	1	2(0.3–12.9)
G3	Tokali	50	28	22	0	30.1 \pm 6.8	3	0	0	6(1.9–17)	23	46(32.8–59.8)
G4	Nambeg	50	39	11	0	30.3 \pm 5.0	0	0	0	0(<5.8)	15	30(19–44)
G5	Kpesiin	50	37	5	8	29.6 \pm 5.8	0	0	0	0(<5.8)	8	16(8.2–28.9)
G6	Banwo	50	3	47	0	29.2 \pm 4.1	0	0	0	0(<5.8)	0	0(<5.8)
G7	Joripeti	50	50	0	0	30.1 \pm 4.1	0	0	0	0(<5.8)	0	0(<5.8)
G8	Eggu	50	24	26	0	32.9 \pm 4.9	1	0	0	2(0.3–12.9)	0	0(<5.8)
G9	Sawale	50	16	32	2	33.8 \pm 4.8	1	0	0	2(0.3–12.9)	0	0(<5.8)
G10	Gyanvu	50	36	12	2	32.9 \pm 5.4	0	0	0	0(<5.8)	0	0(<5.8)
G11	Konguo	50	19	30	1	29.3 \pm 4.5	0	0	0	0(<5.8)	9	18(9.6–31.1)
G12	Yaasoyi	50	23	27	0	29.0 \pm 6.4	0	0	0	0(<5.8)	7	14(6.8–26.6)
G13	Kpaguri	50	0	50	0	32.5 \pm 6.0	1	0	0	2(0.3–12.9)	9	18(9.6–31.1)
G14	Nyuglu	50	13	36	1	31.0 \pm 5.1	2	0	0	4(1–14.6)	0	0(<5.8)
G15	Kaa	50	17	31	2	30.6 \pm 6.1	0	0	0	0(<5.8)	5	10(4.2–21.9)
G16	Dinaso	50	0	50	0	33.9 \pm 4.4	2	0	0	4(1–14.6)	24	48(34.6–61.7)
G17	Jang	50	17	33	0	31.3 \pm 6.2	1	0	0	2(0.3–12.9)	18	36(24–50.1)
G18	Naro	50	0	46	4	30.0 \pm 5.8	1	0	0	2(0.3–12.9)	17	34(22.3–48)
G19	Guri	50	13	30	7	27.1 \pm 5.8	0	0	0	0(<5.8)	21	42(29.2–55.9)
G20	Bilingu	50	50	0	0	33.5 \pm 3.6	1	0	0	2(0.3–12.9)	9	18(9.6–31.1)
G21	Vieto	50	0	7	43	32.5 \pm 4.4	0	1	0	2(0.3–12.9)	22	44(32.8–59.8)
G22	Bullu	50	1	41	8	29.2 \pm 5.0	0	0	0	0(<5.8)	32	64(49.9–76)
G23	Joiyiri	50	15	35	0	35.3 \pm 5.7	3	0	0	6(1.9–17)	3	6(1.9–17)
G24	Chasia	50	6	44	0	30.5 \pm 4.7	0	0	0	0(<5.8)	2	4(1–14.6)
G25	Sagla	50	10	50	0	31.7 \pm 4.7	3	0	0	6(1.9–17)	10	20(11.1–33.3)
G26	Tiisa	50	18	32	0	30.8 \pm 6.2	0	0	0	0(<5.8)	15	30(19–44)
G27	Wahab	50	16	34	0	31.4 \pm 6.2	1	0	0	2(0.3–12.9)	14	28(17.3–41.9)
G28	Jijen	50	40	10	0	28.9 \pm 4.7	1	2	0	6(1.9–17)	8	16(8.2–28.9)
G29	Gorima	50	0	50	0	29.6 \pm 5.9	6	1	0	14(6.8–26.6)	19	38(25.7–52)
G30	Dolinda	50	34	16	0	27.4 \pm 4.7	0	0	0	0(<5.8)	19	38(25.7–52)
G32	Nabulu	50	28	22	0	29.9 \pm 4.5	0	0	0	0(<5.8)	0	0(<5.8)
G33	Dolibiz	50	24	24	2	31.6 \pm 5.0	0	0	0	0(<5.8)	2	4(1–14.6)
G34	Sombis	50	29	21	0	33.4 \pm 4.9	0	1	0	2(0.3–12.9)	1	2(0.3–12.9)
G35	Fachub	50	41	9	0	29.9 \pm 9.9	0	1	0	2(0.3–12.9)	8	16(8.2–28.9)
G36	Pina	50	32	17	1	30.0 \pm 5.2	0	0	0	0(<5.8)	11	22(12.6–35.5)
G37	Guosi	50	36	13	1	26.6 \pm 3.7	0	0	0	0(<5.8)	7	14(6.8–26.6)
Totals		1800	814	904	82		27	7	0		342	

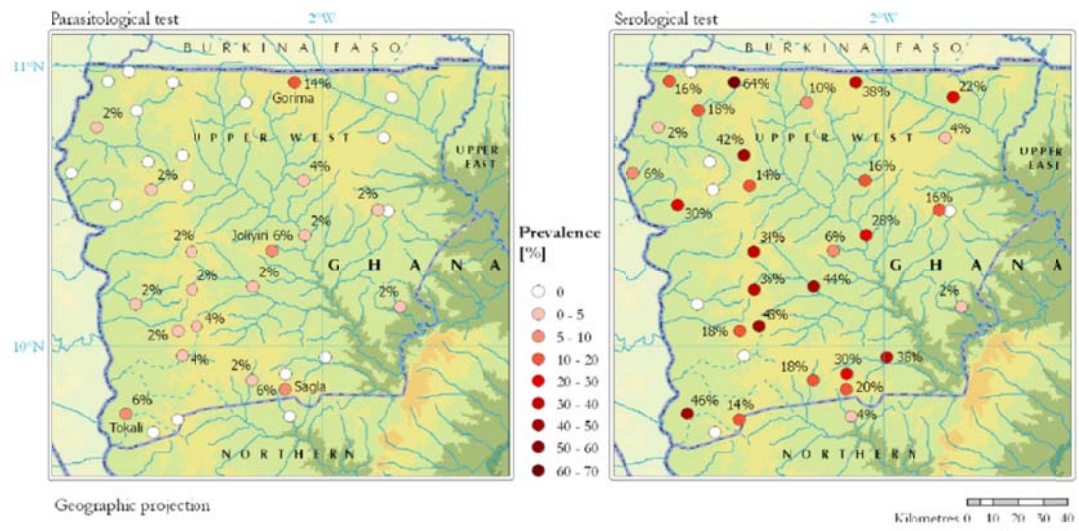


Fig. 2. Parasitological and serological prevalences of livestock trypanosomosis in 36 randomly selected sampling grids in the Upper West Region of Ghana.

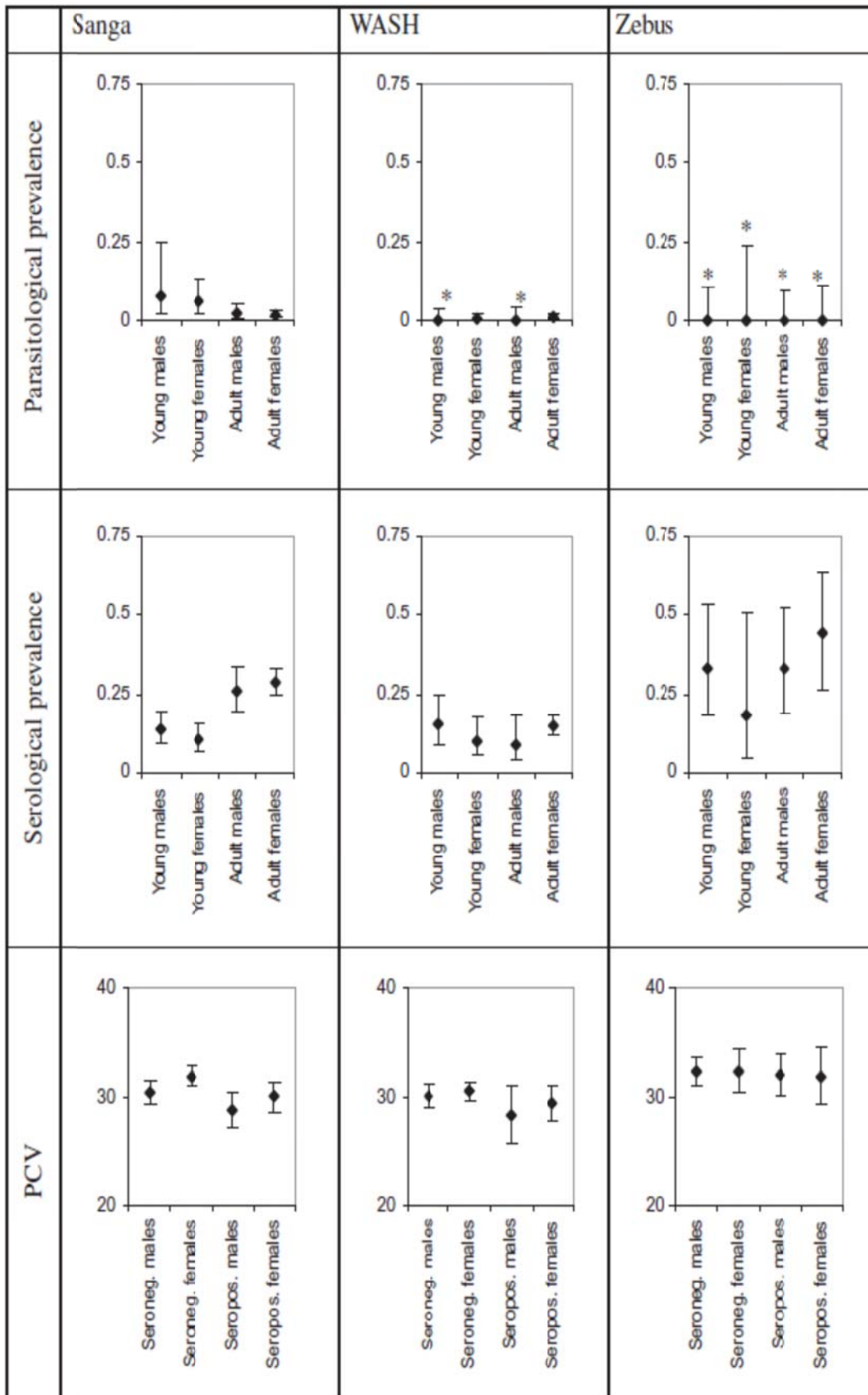


Fig. 3. Parasitological prevalence and serological prevalence of trypanosomosis and average PCV (95% CI) for the various age and sex categories of each of the cattle breeds in the Upper West Region of Ghana. /Estimated using an exact method (prevalence = 0).

2.4. Discussion

Three tsetse species (i.e. *G. palpalis*, *G. tachinoides* and *G. morsitans*) have been reported in the northern part of Ghana (Buxton, 1955; Ford, 1971). Results from the survey only revealed the presence of *G. palpalis* and *G. tachinoides* with *G. tachinoides* being present at the highest apparent density and distributed over a broader area.

The restricted distribution of *G. palpalis gambiensis* broadly follows the predictive distribution maps of this species in the region (Wint and Rogers, 2000; Wint, 2001). However, failure to detect *G. palpalis gambiensis* in the most northern section of the Upper West Region suggests a tendency towards a southerly retreat of this species.

Tsetse flies have specific ecological requirements that differ between species (Buxton, 1955). *G. palpalis gambiensis* lives in forests where optimal atmospheric conditions can be maintained (Challier, 1973). These forests include the gallery forests along the Black Volta River. Hence, the disturbance of the riverine forests along the Black Volta River and other rivers in the study area and beyond significantly reduce the suitable habitat of *G. palpalis gambiensis*, sometimes leading to the complete disappearance of the species (de La Rocque et al., 2001; Guerrini et al., 2008). According to the distribution models, the entire study area is predicted to be suitable for *G. tachinoides* (Wint and Rogers, 2000; Wint, 2001). Contrary to *G. palpalis gambiensis*, *G. tachinoides* can cope with more open vegetation (Laveissière, 1976) and is less affected by anthropogenic changes of the vegetation (de La Rocque et al., 2001; Van den Bossche et al., 2010). Nevertheless, the observed distribution of *G. tachinoides* is possibly more patchy and the apparent density highest close to the river basins.

Results of the parasitological and serological tests indicate that trypanosomosis is widespread in the study area. However, the parasitological prevalence of trypanosomal

infections is generally lower than the parasitological prevalence of trypanosomal infections in cattle in the Savelugu and West Maprusi Districts of Northern Ghana (Mahama et al., 2004). This is most likely due to low tsetse challenge because of the patchy distribution of tsetse although the regular use of trypanocidal drugs cannot be excluded. The dominance of *T. vivax* infections is in line with observations made in northern Ghana and elsewhere in the region (Mahama et al., 2004; Bengaly et al., 1998). With the exception of Sagla, all villages with a parasitological prevalence of bovine trypanosomosis >5% (Tokali, Joliyiri and Gorima) were located close to areas with relatively high apparent densities of tsetse flies and were located close to one of the three main rivers or an important tributary suggesting an important role of the remaining riverine vegetation in harbouring tsetse. This finding was considered and appropriated in the planning of the tsetse control programme. Despite the presence of trypanosomal infections in cattle sampled at 18 sampling sites, the average PCV values were normal. This is attributed to the large proportion of trypanotolerant cattle (WASH) and to some extent the Sanga breed in the sample.

The average prevalence of anti-trypanosomal antibodies in the study area was 19%. This is in line with the 24% observed in the Savelugu District in the Northern Region of Ghana (Mahama et al., 2004). It is not surprising that the prevalence of anti-trypanosomal antibodies is substantially higher than the parasitological prevalence. This is partly due to the low sensitivity of parasitological diagnostic methods for trypanosomosis (Marcotty et al., 2008) especially in trypanotolerant cattle where parasitaemia is usually very low (Naessens, 2006) and the persistence of anti-trypanosomal antibodies in the absence of infection (Bocquentin et al., 1990; Van den Bossche et al., 2000). Hence, some of the seropositive but parasitologically negative animals are likely to be infected with trypanosomes (false negatives) whereas others may have been infected but have, for example, been treated. It is difficult to allocate a seropositive animal to one of those two

categories.

However, on the basis of the PCV of an animal with anti-trypanosomal antibodies, assumptions can perhaps be made on its infection status. A typical sign of bovine trypanosomosis is anaemia which is best measured by determining the PCV (Murray and Dexter, 1988). The lower average PCV of seropositive Sanga breed suggests that a proportion of the seropositive animals of this breed is or has recently been infected with trypanosomes resulting in a reduced PCV. The absence of such a relationship in the WASH may not be surprising since they are trypanotolerant and thus support trypanosomal infections without impact on their health (Naessens, 2006). Surprisingly, no such relationship was observed in the Zebu cattle. This may be due to the frequent use of trypanocidal drugs in this very susceptible breed. These observations again demonstrate the value of indirect test such as the anti-trypanosomal antibody detection ELISA to improve the accuracy of trypanosomosis surveys especially in areas where trypanotolerant livestock is present.

2.5. Conclusion

G. tachinoides and *G. palpalis gambiensis* are the main vectors of trypanosomosis in the Upper West Region of Ghana. Entomological surveys conducted along the three main river systems suggest a possible southward retreat of the tsetse fly. However, trypanosomosis remains widespread in the Upper West Region, with the predominance of infections by *T. vivax* and, to a lesser extent, *T. brucei*.

The findings of the present study enabled an integrated tsetse elimination strategy to be developed, which combines aerial spraying (Sequential Aerosol Technique–SAT), ground spraying, insecticide treated targets and cattle pour-on. Implementation started in the period March–May 2010 with SAT operations, conducted along the three main rivers. SAT is being followed by targeted, selective deployment of the other control techniques.

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Chapter3: Tsetse Population Genetics

Genetic Comparison of *Glossina tachinoides* Populations in Three River Basins of the Upper West Region of Ghana and Implications for Tsetse Control

Adapted from:

Adam, Y., et al. (2014) Genetic comparison of *Glossina tachinoides* populations in three river basins of the Upper West Region of Ghana and implications for tsetse control. *Infect. Genet. Evol.* (2014), <http://dx.doi.org/10.1016/j.meegid.2014.03.023>

Abstract

Tsetse flies are the cyclical vectors of African animal trypanosomosis (AAT) and human African trypanosomosis (HAT). In March 2010, the Government of Ghana initiated a large scale integrated tsetse eradication campaign in the Upper West Region (UWR) ($\approx 18,000 \text{ km}^2$) under the umbrella of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC).

We investigated the structuring of *Glossina tachinoides* populations within and between the three main river basins of the target area in the UWR. Out of a total sample of 884 flies, a sub-sample of 266 was genotyped at nine microsatellite loci. The significance of the different hierarchical levels was tested using Yang's parameters estimated with Weir and Cockerham's method. A significant effect of traps within groups (pooling traps no more than 3 km distant from each other), of groups within river basins and of river basins within the whole target area was observed. Isolation by distance between traps was highly significant. A local density of 0.48-0.61 flies/m² was estimated and a dispersal distance that approximated 11 m per generation [CI 9, 17]. No significant sex-biased dispersal was detected.

Dispersal distances of *G. tachinoides* in the UWR were relatively low, possibly as a result of the fragmentation of the habitat and the seasonality of the Kulpawn and Sissili rivers. Moreover, very high fly population densities were observed in the sample sites, which potentially reduces dispersal at constant habitat saturation, because the probability that migrants can established is reduced (density dependent dispersal). However, the observed spatial dispersal was deemed sufficient for a *G. tachinoides*-cleared area to be reinvaded from neighboring populations in adjacent river basins. These data corroborate results from other population genetics studies in West Africa, which indicate that *G. tachinoides* populations from different river basins cannot be considered isolated.

3.1. Introduction

Tsetse flies are the cyclical vectors of African animal trypanosomosis (AAT) – nagana – and of human African trypanosomosis (HAT) – sleeping sickness. HAT is only reported sporadically from Ghana, the last case having been diagnosed in the year 2000 ([Simarro et al., 2010](#)). As a result, the risk of infection in the country is presently considered marginal ([Simarro et al., 2012](#)). By contrast, AAT is a major constraint to the development of more efficient and sustainable livestock production systems in Ghana, and the disease prevents utilization of abundant natural pastures across the country.

Increased parasite resistance to the commonly used trypanocidal drugs were observed in Ghana, due to their widespread and indiscriminate use by livestock keepers ([Turkson, 1993](#)). Over the years, the Veterinary Services Directorate of the Ministry of Food and Agriculture has thus been committed to controlling AAT. Despite past control efforts, AAT remains prevalent in Ghana, particularly in the Upper West Region (UWR) ([Adam et al., 2012](#)).

In 2001, the African Union launched the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), advocating the eradication of tsetse populations as the most sustainable way of controlling trypanosomosis in sub-Saharan Africa. Under the umbrella of the PATTEC, Ghana is collaborating with Burkina Faso in a sub-regional initiative aimed at creating tsetse-free areas across their common borders.

The UWR shares its northern and western border with Burkina Faso and it was selected as a target zone for the first phase of the PATTEC initiative in Ghana. In March 2010, the Government of Ghana initiated a large-scale integrated campaign for the eradication of tsetse, with the ultimate goal of removing riverine tsetse populations from $\approx 18,000 \text{ km}^2$ ([Adam et al., 2013](#)). The project integrated several tools such as the sequential aerosol technique (SAT),

insecticide-treated targets, insecticide-treated cattle and ground spraying. As a result of the inclusion of a SAT-component, it was estimated that the applied strategy reduced the target tsetse populations by 98% within one month of the start of the control operations. However, entomological monitoring conducted one year after the conclusion of the SAT operations indicated that whereas the applied strategy failed to eradicate the *Glossina palpalis gambiensis* and *Glossina tachinoides* populations in the target area, it succeeded in maintaining a high level of suppression ([Adam et al., 2013](#)). The failure to achieve eradication was attributed to an insufficient penetration of the insecticide droplets in the dense riparian vegetation during various SAT cycles, a patchy application of the other control techniques, and a strong re-invasion pressure. Within the framework of this campaign and accompanying field operations, we conducted a population genetic study in the intervention area to assess the dispersal capabilities and isolation status of the target populations.

Population genetic studies can be used to estimate dispersal between various populations of tsetse flies ([De Meeûs et al., 2007a](#) and [Gooding and Krafsur, 2005](#)), and as such, they provide a very useful tool to assist decision-making (e.g. to identify sites for the deployment of artificial barriers against re-infestation ([Politzar and Cuisance, 1983](#))) or to facilitate the selection of eradication or suppression strategies depending on the level of isolation of the target population ([Solano et al., 2010a](#); [Solano et al., 2009](#) and [Solano et al., 2010b](#)).

In the UWR of Ghana, the three main rivers (Black Volta, Kulpawn and Sissili) are characterized by varying but generally increasing density of riverine vegetation along a north-south, downstream gradient. Riverine tsetse populations inhabit these riparian forests and their abundance is modulated by the ecotype of vegetation and its degree of disturbance ([Bouyer et al., 2005](#) and [Cecchi et al., 2008](#)). The pre-intervention baseline data survey revealed the presence of two riverine *Glossina* species in the region: *G. tachinoides* and *G. p.*

gambiensis ([Adam et al., 2012](#)). *G. tachinoides* was predominant (98% of all captures) and widespread along all three main rivers, with population densities increasing from north to south. *G. p. gambiensis* was only detected at low densities in the southernmost end of the target area ([Adam et al., 2012](#)).

Previous population genetic studies of *G. tachinoides* in Burkina Faso, upstream on the Black Volta River, did not reveal strong structuring within or between populations from different river basins ([Koné et al., 2011](#) and [Koné et al., 2010](#)). Populations of *G. p. gambiensis* in Burkina Faso appeared more structured than those of *G. tachinoides* ([Bouyer et al., 2009](#) and [Bouyer et al., 2010a](#); [Bouyer et al., 2007](#) and [Koné et al., 2011](#)), but data on gene flow indicated that both species were able to disperse between river basins. The capability of riverine flies to cross watersheds was also demonstrated by a mark-release-recapture study with sterile *G. p. gambiensis* in Mali ([Vreysen et al., 2013](#)).

Genetic structuring of tsetse populations in the UWR of Ghana had not been studied before. The riparian vegetation along the three main rivers is heavily fragmented, and their tributaries become largely unsuitable for riverine tsetse during the dry season (December-May). Bush fires and the absence of rain during this season seriously limit the availability of favorable vegetation ([Adam et al., 2012](#)), so that tsetse flies retract from the tributaries to residual spots along the main rivers, where permanent water and vegetation mitigate the harsh ambient macroclimatic conditions (e.g. temperatures often rise above 40°C). Whilst the Black Volta River is a permanent water course, the Kulpawn and Sissili are seasonal. It was therefore hypothesized that it might be more challenging for tsetse to disperse between the main rivers in the UWR of Ghana than between the Mouhoun and adjacent river basins in Burkina Faso.

The objective of the present study was to determine whether tsetse populations in the UWR of Ghana were able to disperse between river basins, which were used as the basic operational

units for the SAT operations, and hence, whether the tsetse populations of the target area ([Adam et al., 2013](#)) could be considered as isolated from adjacent north-eastern populations. The study focused on *G. tachinoides*. The results of this study can be used by managers of the eradication campaign to inform decisions on the potential need to establish adequate barriers to prevent re-invasion of areas where tsetse populations were reduced to very low levels.

3.2. Materials and Methods

3.2.1. Study area

The Black Volta, Kulpawu and Sissili Rivers are the three main rivers in the UWR. Eight sites located along the three rivers were investigated ([Fig. 1](#)). The selected sites had been previously studied in terms of tsetse species composition and abundance ([Adam et al., 2012](#)).

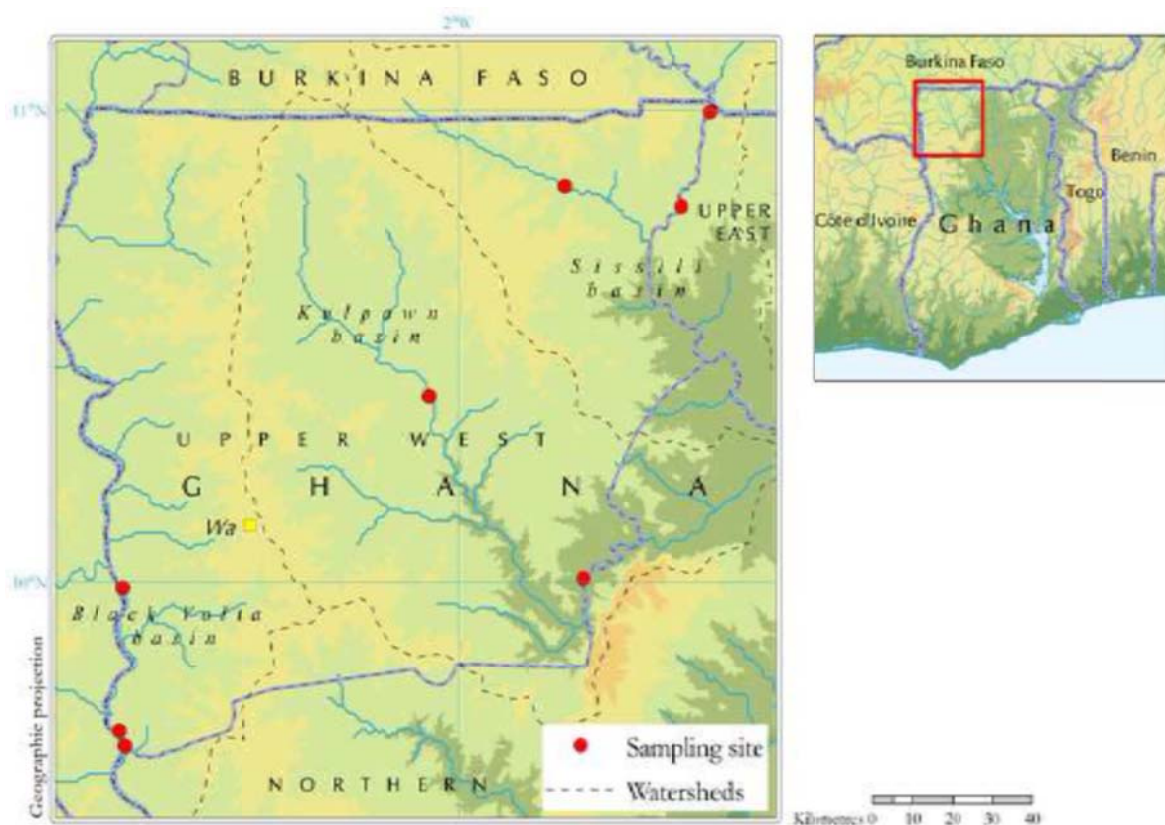


Fig. 1. Study area and location of the genotyped flies.

[Figure options](#)

3.2.2. Entomological sampling

From 19/01/2010 to 12/02/2010, 20 georeferenced biconical traps were deployed in each site. On average 5 flies were sampled per trap and a total of 1,054 flies were trapped, i.e. 884 *G. tachinoides*, 166 *G. palpalis gambiensis* and 4 *G. morsitans submorsitans*.

3.2.3. Genotyping

A total of 266 individuals (130 in Black Volta, 44 in Kulpawn and 92 in Sissili) were used for the genetic analysis at microsatellite loci ([Table 1](#) & [Fig. 1](#)). The objective was to genotype at least 30 flies per site.

Table 1. Number of *Glossina tachinoides* genotyped in each site.

River	Populations	M	F	Total
Black Volta	1	27	19	46
Black Volta	2	39	29	68
Black Volta	3	6	10	16
Kulpawn	4	7	22	29
Kulpawn	5	6	9	15
Sissili	6	9	24	33
Sissili	7	13	18	31
Sissili	8	10	18	28
Total	8	117	149	266

[Table options](#)

A diagnostic PCR was used to confirm the species (*G. tachinoides*) based on length differences of internal transcribed spacer 1 sequences was performed before genotyping ([Dyer et al., 2008](#)).

Nine microsatellite loci were used (preceded by ‘‘X’’ for X-linked loci): XpGp13, pGp24, pGp17, XpGp20, pGp28 and pGp29 ([Luna et al., 2001](#)), XB104, C102 (A. Robinson, FAO/IAEA, pers. com.), GpCAG133 ([Baker and Krafur, 2001](#)). In each 1.5 ml tube containing three legs of the tsetse flies, 200 µl of 5% Chelex chelating resin was added ([Solano et al., 2000](#) and [Walsh et al., 1991](#)). After incubation at 56°C for one hour, DNA was denatured at 95°C for 30 min. The tubes were then centrifuged at 12,000 g for two minutes and frozen for later analysis.

The PCR reactions were carried out in a thermocycler (MJ Research, Cambridge, UK) in 20 µl final volume, using 10 µl of the supernatant from the extraction step. The composition of mix solution for each sample was: 2µl of 10X Buffer, 0.4 µl of 10mM dNTP, 0.2 µl of 10pmol/µl reverse primer, 0.16 µl of 10pmol/µl forward primer with M13, 0.2 µl of 10pmol/µl infrared dye (700nm or 800nm) 0.1 µl of 5u/µl Taq polymerase and 6.94µl of sterile distilled water.

After PCR amplification, allele bands were resolved on a 4300 DNA Analysis System (LICOR, Lincoln, NE) after migration on 96-lane reloadable 6.5% denaturing polyacrylamide gels. This method allows multiplexing of loci by the use of two infrared dyes (IRDye), separated by 100 nm (700 and 800 nm), and read by a two channel detection system that uses two separate lasers and detectors to eliminate errors due to fluorescence overlap. To determine the different allele sizes, a large panel of about 30 size markers was used. These size markers had been previously generated by cloning alleles from individual tsetse flies into pGEM-T Easy Vector (Promega Corporation, Madison, WI, USA). Three clones of each allele were sequenced using the T7 primer and the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA). Sequences were

analyzed on a PE Applied Biosystems 310 automatic DNA sequencer (PE Applied Biosystems) and the exact size of each cloned allele was determined ([Solano et al., 2009](#)).

PCR products from these cloned alleles were run in the same acrylamide gel as the samples, allowing the allele size of the samples to be determined accurately. Allele sizes were determined using SAGA^{GT} Generation 2 Software. Loci located on the X chromosome give an absence of heterozygotes on a subsample of males.

3.2.4. Statistical analyses

During preliminary statistical analyses we encountered some problems that led us to study the comparative behavior of each of our nine microsatellite loci. For this, we considered flies contained in each trap as subpopulation units and studied Wright's F_{IS} and F_{ST} ([Wright, 1965](#)). F_{IS} is individual inbreeding relative to subpopulation (trap) inbreeding and reflects deviation from local random mating of individuals. It varies from -1 to +1. Negative values reflect heterozygote excesses while positive values reflect heterozygote deficits. Under the panmictic model $F_{IS}=0$ or is expected slightly negative in dioecious species ([Balloux, 2004](#)). F_{ST} is subpopulation inbreeding relative to total inbreeding and reflects subdivision. It is expected to be close to 0 when dispersal is free across subpopulations and positively increases with increasing subdivision (up to one). These parameters were estimated with Weir and Cockerham's unbiased estimators ([Weir and Cockerham, 1984](#)), F for F_{IS} and θ for F_{ST} . Unlike its parametric counterpart, θ can display negative values. While slightly negative values are expected by chance under the null hypothesis of no subdivision, strongly negative values might reflect homogenizing selection. The 95% confidence intervals around the value for each locus was estimated through jackknife over subsamples (traps) and across the mean over all loci by bootstrap over loci as described elsewhere ([De Meeûs et al., 2007a](#)). These

computations were undertaken with Fstat 2.9.4 ([Goudet, 2003](#)) (updated from ([Goudet, 1995](#))). Outliers loci were subsequently rejected from further analyses (see Results).

In order to determine the smallest relevant population units we undertook a hierarchical analysis with HierFstat ([Goudet, 2005](#)) which estimates Yang's parameters ([Yang, 1998](#)) with Weir and Cockerham's method ([Weir and Cockerham, 1984](#)) and test their significance with a *G*-based randomization test as described in ([Goudet et al., 1996](#)). The sampling can be subdivided into 63 traps. We considered a group (Trap) as the collection of traps within 0.1 km² of suitable habitat (0.1km of gallery forest ~100m width) as defined elsewhere ([Koné et al., 2011](#)), and traps closer than 300 m were considered to belong to the same sub-group. There were 17 such sub-groups. Sub-groups were included in 8 sites containing traps no more distant than 2-3 km that belonged to a homogeneous river section. In the following level (i.e. 'location') traps were separated by at least 20 km (7 locations), and then the most inclusive level was represented by the three river basins (Black Volta, Kulpawn and Sissili). Each of these levels thus corresponded to a fixation index:

F_{Tr-SG} the inbreeding measured in traps relative to inbreeding in the subgroup it belongs to, which reflects subdividing effect of traps, F_{SG-S} the inbreeding measured in subgroups relative to inbreeding in the sites they belong to, which reflects subdividing effect of subgroups, F_{S-L} the inbreeding of sites relative to inbreeding in the location that reflects the effect of sites on inbreeding, F_{L-RB} the inbreeding of groups relative to inbreeding in river basins and reflecting the subdividing effect of groups and finally F_{RB-T} the inbreeding in river basins relative to the total inbreeding, which reflects the subdividing effect of river basins. The significant departure from 0 of these F was tested by randomizing units contained within the level to be tested (just below in the hierarchy) between these units and within the unit containing the unit to be tested (just above in the hierarchy). For instance, to test F_{Tr-SG} , individuals are

randomized between traps of the same subgroup. Number of randomization was set to 1,000 in each case. When one level was found to not significantly influence genetic distribution, such level was removed from the hierarchy and the whole procedure repeated. A user-friendly tutorial of how to use HierFstat is presented in ([De Meeûs and Goudet, 2007](#)).

Statistical independence between loci was tested with the *G*-based randomization test implemented in Fstat 2.9.4 (([Goudet, 2003](#)) updated from ([Goudet, 1995](#))). It allows a global test over all subsamples for each pair of loci and is more powerful than other combining procedures ([De Meeûs et al., 2009](#)). Because there are as many tests as loci pairs ($k=L(L-1)/2$ with L loci) and because these tests are not independent, we had to adjust *P*-values with the Bonferroni correction. We also tested if no more than 5% significant tests expected under the null hypothesis of random association between loci were observed in the k test series, using a unilateral exact binomial test undertaken under R ([R-Development-core-team, 2010](#)).

Conformity to local panmixia was assessed through estimation of Wright's F_{IS} ([Wright, 1965](#)) with Weir and Cockerham unbiased estimator ([Weir and Cockerham, 1984](#)). The significant departure from 0 of F_{IS} was assessed through 10,000 randomizations of alleles between individuals in each subsample. Estimate and testing was undertaken with Fstat 2.9.4.

We checked for the possible presence of null alleles and stuttering with MicroChecker ([Van Oosterhout et al., 2004](#)). We used Brookfield's ([Brookfield, 1996](#)) second method to estimate the expected proportion of homozygous nulls under the panmictic hypothesis. We then compared this proportion to the actual observed blanks (missing genotypes) in the data with a unilateral exact binomial test (alternative hypothesis: there are fewer blanks observed than expected). In the case of X linked loci, we also used null allele frequency estimate from females to compute expected missing genotypes in males, because it should exactly reflects

allelic frequency, and compared expected and observed values with the same procedure as for autosomal loci.

Sex specific genetic structure was assessed with the “Biased dispersal” procedure implemented in Fstat 2.9.4. For this, as explained elsewhere ([Goudet et al., 2002](#), [Prugnolle and De Meeûs, 2002](#) and [Prugnolle et al., 2003](#)), we only used the corrected mean assignment index mAI_c ([Favre et al., 1997](#)), which measures how well each individual genetically fits into the subsample it belongs to, the variance of this index vAI_c and Wright’s F_{ST} ([Wright, 1965](#)), which is a genetic signature of subdivision. The most philopatric sex should display higher mAI_c and F_{ST} and smaller vAI_c . This is tested by randomizing the sex of individuals within subsamples. F_{ST} and vAI_c are the most powerful ([Goudet et al., 2002](#)) statistics but mAI_c can prove useful when difference between genders is more complex than a simple dispersal difference ([De Meeûs et al., 2002](#) and [Kempf et al., 2010](#)). We tested sex bias dispersal between each unit that appeared significant during the HierFstat analysis. Sex biased dispersal between traps was also tested independently within each subgroup. We then computed the mean over all subgroups of each statistic and combined the tests with the generalized binomial procedure ([Teriokhin et al., 2007](#)) with the software MultiTest V1.2 ([De Meeûs et al., 2009](#)). We also tested sex bias dispersal between subgroups keeping only one male and one female per trap for the sake of effect of trap control (if any) as we know this can have a spectacular effect ([Kempf et al., 2010](#)). Since we expect females to disperse less ([Koné et al., 2011](#)), tests were all unilateral.

Isolation by distance was studied with Rousset’s method ([Rousset, 1997](#)). In a two dimension framework we expect the parameter $F_{ST}/(1-F_{ST})$ to be linearly connected to the neighbourhood size of the population $N_b=4\pi D_e\sigma^2$ in the wide sense ([Watts et al., 2007](#)) with an equation of the form $F_{ST}/(1-F_{ST})=a + b \times \text{Ln}(G_D)$, where the slope $b=1/N_b$, and G_D is the

geographic distance between two subsamples, D_e is the effective density of reproducing adults and σ is the mean distance between reproducing adults and their parents (dispersal). We used subsample units that appeared significant with HierFstat and tested the significance of regressions with a Mantel test as recommended ([Mantel, 1967](#)) with 1,000,000 randomizations. This was undertaken with Genepop 4 ([Rousset, 2008](#)). The software uses subsample units (e.g. trap) coordinates in meters (UTM WGS 84). The software also computes 95% confidence of the slope with ABC bootstrap procedure. The minimum distance for the regression was set at 0.3 km. We also analyzed isolation by distance between individuals with the statistic a and e (equivalent to $F_{ST}/(1-F_{ST})$ and with similar properties) because neighborhood size N_b (86 and 51 respectively) appeared close to the limit of choice between the two statistics ([Watts et al., 2007](#)).

Effective population sizes (N_e) were assessed through the linkage disequilibrium based method of Waples ([Waples, 2006](#)). It was undertaken with LDNe ([Waples and Do, 2008](#)). We also used the within and between locus correlation method ([Vitalis and Couvet, 2001b](#)) with the software Estim 1.2 ([Vitalis and Couvet, 2001a](#)) and the mean value of F_{IS} subsamples, the negative values of which can be connected to the effective population size of dioecious populations as $N_e = [-1/(2F_{IS})] - [F_{IS}/(1-F_{IS})]$ ([Balloux, 2004](#)). Effective population density was then estimated as $D_e = N_e/S$. Slope of regression (b) of Rousset's model in two dimension isolation by distance ([Rousset, 1997](#)) allows estimating mean effective number of immigrants per generation in a subpopulation as $N_e m = 1/(2\pi b)$, the neighbourhood size in the strict sense as $N_b' = 1/(4\pi b)$ ([Rousset, 2008](#)) (note that $N_b' = D_e \sigma^2$) and the mean dispersal distance between reproducing adults and their parents as $\sigma = \sqrt{(\pi b D_e / 4)}$.

There are three X-linked loci that are thus haploid in males. Consequently, males were coded as missing data for these loci for all F_{IS} based analyses and were coded homozygous otherwise.

When using traps as subpopulation units, only traps containing at least five flies were kept.

Most procedures used are more detailed in ([De Meeûs et al., 2007a](#)).

3.3. Results

3.3.1. Locus selection

Traps closer than 300 m (sub-groups, as defined for the HierFstat analysis in the Material and Methods section) were used as population units in order to optimize both subsample sizes and subsample numbers. The locus by locus analysis (Supplementary material 1-2) indicated that locus pGp17 displayed an F_{IS} of almost 1 that was significantly different from the mean. Locus pGp29 displayed an unusually small F_{IS} and F_{ST} that appeared significantly different from the mean over all loci and could be reflecting some kind of selection signature. Both loci were therefore removed from the data for further analyses that was carried out with the remaining seven loci (XB104, XpGp13, XpGp20, C102, GpCAG133, pGp24 and pGp28).

3.3.2. Hierarchical population structure

The significance of the different hierarchical levels was tested with HierFstat. This analysis indicated a non-significant effect of sub-groups within sites ($F_{SG-S} < 0$, P -value=1). When this level was ignored, we obtained a non-significant effect of locations within river basins ($F_{L-RB} < 0$, P -value=0.166). The non significant effect of a hierarchical effect simply means no supplementary information is brought by this level. It does not mean that no genetic differentiation occurs between individuals belonging to different units of this hierarchy (see supplementary material of ([Rougeron et al., 2009](#)) for a detailed explanation). The remaining

levels appeared to significantly affect genetic diversity distribution: traps within subgroups ($F_{T-SG}=0.038$, P -value=0.043), sites within river basins ($F_{S-RB}=0.047$, P -value=0.001) and river basins within the total ($F_{RB-T}=0.056$, P -value=0.037). The level “Trap” displayed the weakest effect, though significant, which is not typical for *G. tachinoides* that usually differentiates genetically at higher scales ([Koné et al., 2011](#) and [Koné et al., 2010](#)). In further analysis we tested and measured local parameters (linkage disequilibrium and F_{IS}) within traps and within sites.

3.3.3. Local population structure

Within traps, one pair of loci significantly displayed a linkage disequilibrium (P -value=0.007) and within subgroups and within sites, one pair of loci also showed significant linkage (P -value<0.03). This is not significantly above the 5% expected under the null hypothesis (unilateral exact binomial test, P -value=0.6594 for 21 tests with 7 loci) and became not significant after Bonferroni correction. We can therefore assume no or very weak statistical association between the seven microsatellite loci.

Across loci there was a significant and highly variable heterozygote deficit within traps as illustrated in [Fig. 2](#). Only two loci displayed heterozygote excess ($F_{IS}=-0.066$ for XpGp13 and $F_{IS}=-0.08$ for GpCAG133) as expected in a dioecious species. Null alleles were probably responsible for the observed pattern. MicroChecker analyses confirmed that all heterozygote deficits can largely be explained by null alleles (smallest P -value>0.13, [Table 2](#)).

Fig. 2. *Glossina tachinoides* from Ghana inbreeding of individuals relative to inbreeding within traps (F_{IS}) for the seven retained loci. The 95% confidence interval around each locus was obtained by jackknife over population (traps). Over all loci (All) 95% confidence interval was obtained by bootstrap over loci. Here, males were coded as missing data for X linked loci.

[Figure options](#)

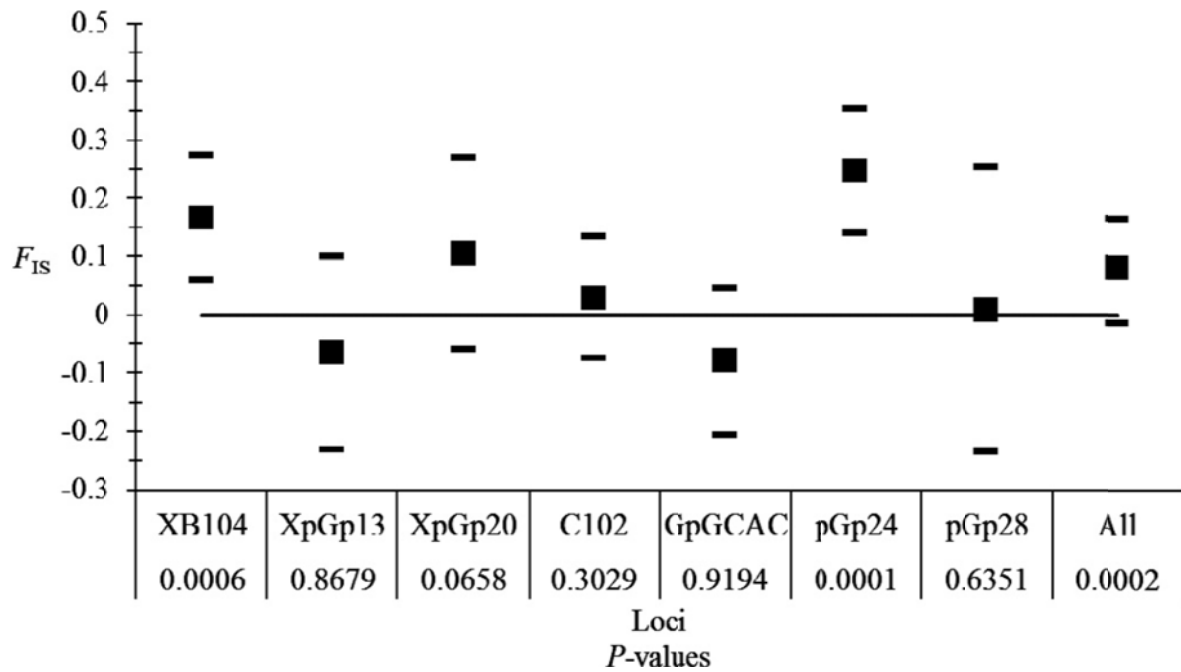


Table 2.

Exact binomial tests (P -value) that there are enough blank genotypes (putative null homozygotes) observed in each trap (Nb_obs) as compared to the expected number under panmictic conditions and using null allele frequency estimate from Brookfield's second method as computed by MicroChecker (Nb_exp). The tests are undertaken over all traps with at least 5 individuals (total numbers of individuals summed over all traps). N is the total number of individuals. For X linked loci, allele frequency of nulls, computed with female data, is also compared to the proportion of missing genotypes in males. For heterosomal loci, less data could be analyzed with Microchecker resulting in globally less individuals for heterosomal data than for autosomal data.

Gender	Locus	N	Nb_exp	Nb_obs	P -value
All	C102	183	1	1	0.819
	GpGCAC133	183	14	19	0.948
	pGp24	183	9	7	0.267
	pGp28	183	1	1	0.738
Females	XB104	57	2	3	0.807
	XpGp13	57	4	6	0.842
	XpGp20	57	2	3	0.780
Males	XB104	17	2	0	0.133
	XpGp13	17	4	6	0.944
	XpGp20	17	2	3	0.804

[Table options](#)

A similar analysis within sites and within river basins showed similar patterns for F_{IS} but with significantly increasing values (Fig. 3). This confirms that pooling traps and then sites produced significant Wahlund effects.

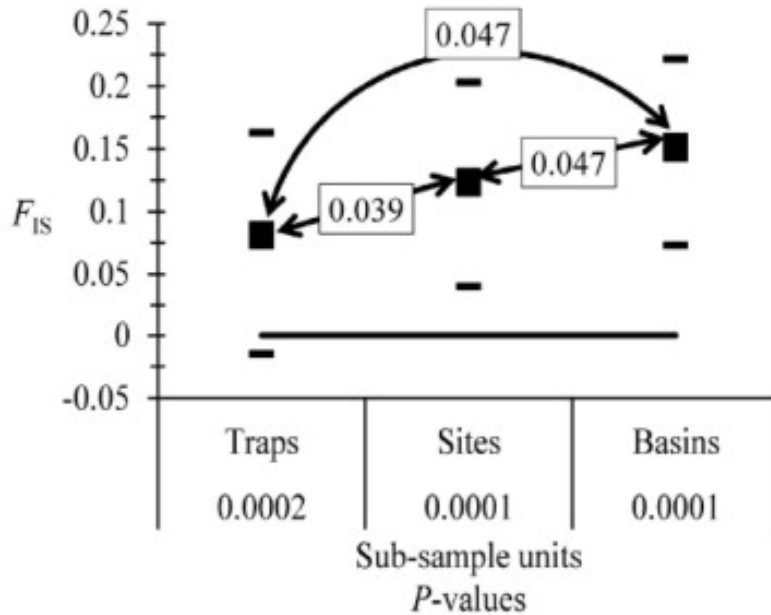


Fig. 3. Testing for Wahlund effect by comparing inbreeding of individuals relative to inbreeding within the subsample unit (F_{IS}) for different subsampling units that significantly affect genetic polymorphism: flies caught in the same trap (Traps), pooling flies from traps no more distant than 3 km from each other (sites) and pooling flies from the same river basin. Double arrows indicate the Bonferroni corrected P -values of comparisons with Wilcoxon signed rank unilateral test for paired data.

[Figure options](#)

3.3.4. Sex biased dispersal

An analysis of sex biased dispersal showed no significant sex biased dispersal between traps either considering traps alone or when tests were undertaken within each site separately and combined with the generalized binomial procedure (Table 3). In fact, the signal tended to be more in agreement with a female biased dispersal. Nevertheless, between sites or between river basins, all parameters (except F_{ST} when corrected for trap effect) indicated a male biased dispersal and were significant for vAI_c in two instances (Table 3). It can be noted that

controlling for the effect of traps was only possible for the test between sites where the drop in sample sizes probably led to the non-significance observed.

Table 3. Results of sex biased dispersal analyses using different components as subpopulation units as defined in the text. Parameters indicating the most philopatric sex (that disperse less) are in bold. Significant differences are in italics.

Subpopulation units	Parameters	Females	Males	<i>P</i> -value
Traps	mAI _c	-0.156	0.205	0.9174
	vAI _c	3.445	3.036	0.4475
	<i>F</i> _{ST}	0.110	0.117	0.9884
Traps in separated MSGs	mAI _c	-0.1563	0.205	0.7
	vAI _c	3.445	3.036	0.2372
	<i>F</i> _{ST}	0.110	0.117	0.7
MSGs	mAI _c	0.233	-0.292	0.0817
	vAI _c	6.570	<i>10.921</i>	<i>0.0187</i>
	<i>F</i> _{ST}	0.095	0.080	0.1518
MSGs corrected for traps	mAI _c	0.138	-0.147	0.273
	vAI _c	5.199	5.750	0.534
	<i>F</i> _{ST}	0.074	0.112	0.9075
River basins	mAI _c	0.192	-0.241	0.1592
	vAI _c	8.862	<i>15.274</i>	<i>0.004</i>
	<i>F</i> _{ST}	0.077	0.069	0.3546

[Table options](#)

3.3.5. Isolation by distance and demographic inferences

All regressions but the one using sites provided a significant isolation by distance. The best adjustment was obtained with traps containing at least five individuals ([Fig. 4](#)), with a regression slope of $b=0.01462$ and a 95% confidence interval (CI) of [0.0076, 0.0229] resulting in a neighborhood size of $N_b=70$ and CI=[44, 131] individuals in the wide sense, of $N_b'=6$ and CI=[3, 10] individuals in the strict sense and with $Nm=11$ and CI=[7, 21] immigrants per generation in each subpopulation.

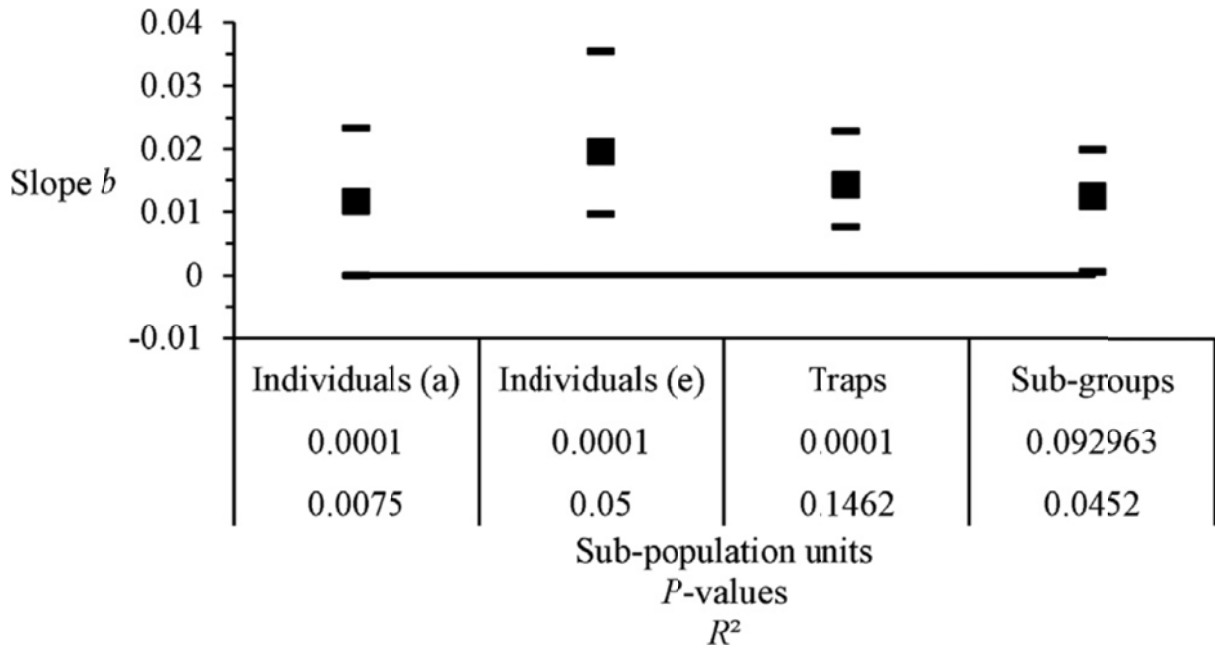


Fig. 4. Comparison of slopes obtained during isolation by distance analyses using either individual flies (with statistics a and e), traps or sub-groups as units. Significance of the regression is given by the P -values obtained during the Mantel test and the quality of adjustment measured by the determination coefficient (R^2) of the regression. The 95% confidence intervals were obtained by the ABC bootstrap method implemented in Genepop 4.

[Figure options](#)

Estimating effective population size in each trap was possible in seven traps with LDNe, six traps with Estim and for only two loci XpGp13 and GpCAG133 with the F_{IS} method ([Fig. 5](#)).

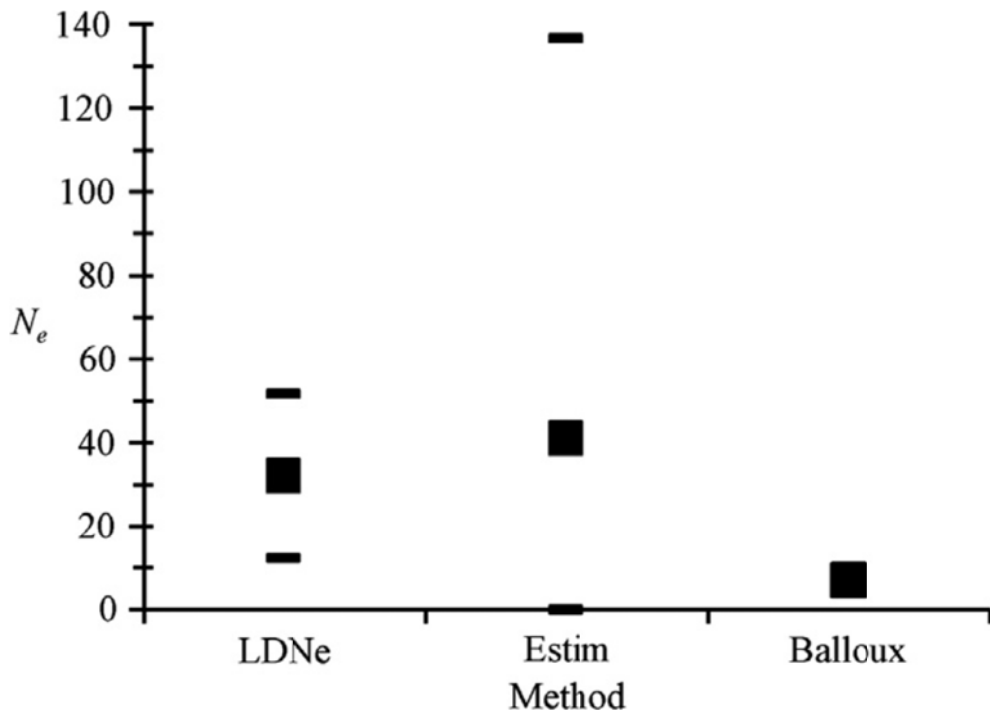


Fig. 5. Results obtained to estimate effective population sizes (N_e) of *Glossina tachinoides* in traps with at least 5 individuals. For LDNe and Estim values, the 95% confidence intervals were computed with the formula: $N_e \pm s^2(N_e) \times t_{0.05, \gamma}$ where t is the student parameter for $\alpha=0.05$ and γ degrees of freedom (number of traps-1). Lower limits were set to 0 when negative. For Balloux's method, only two loci, XpGp13 and GpGCAC, gave a negative (hence usable) F_{IS} and thus only two values (very close: 6 and 8 respectively) were available.

[Figure options](#)

The order of magnitude of N_e was similar for LDNe and Estim that relied on more data and were more in line with N_{em} . We thus assumed N_e to be approximately around 32 and 41 individuals in the zone surrounding each trap. The mean distance between traps in the same site was around 26 m, which corresponds to a surface $S = 0.000673 \text{ km}^2$ approximately occupied by a subpopulation that corresponds to one trap. This allows computing an approximate local density (N_e/S) of $D_e \sim 48000\text{-}61000$ flies/ km^2 with CI $\sim [18000, 77000]$ (or 0.048-0.061 flies/ m^2). This leads to an approximate dispersal distance of $\sigma \sim 11$ m per generation with CI $\sim [9, 17]$.

3.4. Discussion

G. tachinoides populations in the UWR (Ghana) appeared much more subdivided than their northern counterparts in Burkina Faso ([Koné et al., 2011](#)). The HierFstat approach clearly showed that traps and river basins did not have any effect on population structuring in Burkina Faso, whereas in Ghana both had a significant effect with higher F values at all levels as compared to those obtained in Burkina Faso. However, even if neighborhood size and the number of immigrants per generation appeared similar in both areas studied, the geographic size of these units were much smaller in Ghana where apparent densities of the fly populations were 20 orders of magnitude higher and dispersal seven orders of magnitude lower than in Burkina Faso ([Koné et al., 2011](#)). These differences might be due to a higher habitat fragmentation in Ghana than in Burkina Faso, as increased fragmentation results in

higher aggregation of the flies in residual patches which will decrease actual spatial dispersal. The more favorable conditions in these patches will increase population densities and likewise, reduce dispersal i.e. the probability that migrants can establish in these sites at constant habitat saturation. If this density is reduced by a control effort however, the probability that migrants can establish increases. This has important consequences for control since dense habitats are also those where the probability of flies surviving aerial spraying of insecticide droplets (SAT) is more likely ([Adam et al., 2013](#)). In fact, while the impact of the first spraying cycle was relatively important (reduction of 92% in fly density), there was no further reduction of the density of the *G. tachinoides* population after subsequent cycles. Moreover, the impact of targets and live baits, also used during this campaign, is also reduced by a lower dispersal of the flies (Bouyer et al., 2010b).

Sex biased dispersal is more difficult to detect when dispersal is smaller and fragmentation of the populations increases ([Goudet et al., 2002](#) and [Prugnolle and De Meeûs, 2002](#)). This is probably why sex biased dispersal was more difficult to show in this study than in Burkina Faso ([Koné et al., 2011](#)). However, also in Ghana, our results seem to show that it is difficult for female *G. tachinoides* to establish themselves in remote subpopulations, which might be attributed to higher mortality of immigrating females as compared to males, or to difficulties of the migrating female flies to find suitable new breeding sites. It would be very useful to carry out a mark-release-recapture study to compare the relative survival and dispersal of the two sexes in these fragmented landscapes, as most of the existing data date back to the 1980's when experiments were conducted in less fragmented gallery forests ([Cuisance et al., 1985](#)).

Even if the dispersal of *G. tachinoides* is lower in the UWR as compared to that observed in Burkina Faso, it was deemed sufficient for flies to migrate between the river basins and between SAT-treated areas, and for these to be reinvaded from adjacent populations, as

demonstrated by the low value of F_{RB-T} . Since fly densities were not uniform across the study area, with steep variations from less than 1 to more than 100 flies per trap per day ([Adam et al., 2012](#)), it is probable that the apparent pattern of “continuity of isolation by distance” might result from dispersal that adjusts to local conditions (increased dispersal distance when densities drop). These data corroborate results from previous population genetics studies indicating that in West Africa *G. tachinoides* populations from different river basins cannot be considered isolated from one another ([Koné et al., 2011](#) and [Koné et al., 2010](#)). In Uganda and with another riverine species, *Glossina fuscipes fuscipes*, it was found that populations belonging to various river networks were also not isolated, except those separated by Lake Kyoga ([Abila et al., 2008](#)). In the present case, the issue is all the more important as the target area borders Burkina Faso, where the existence of other tributaries and river basins poses further threats of reinvasion. If Governments which have subscribed to the PATTEC initiative are planning a sequential eradication strategy using the rolling carpet approach at the regional level, buffer areas to prevent reinvasion will have to be established between SAT-treated blocks and between untreated adjacent river basins ([Hendrichs et al., 2005](#)). Deltamethrin-impregnated biconical traps deployed at 100m intervals along 7-10km river section successfully prevented migration of *G. p. gambiensis* and *G. tachinoides* in Burkina Faso ([Cuisance and Politzar, 1983](#)). Although no field data exists on the potential dispersal of native riverine tsetse between river basins, insecticide-impregnated cloth targets deployed at $\approx 250m$ intervals established a barrier with a width between 2 and 25 km and so far has successfully prevented reinvasion of *G. m. centralis* in the Okavango delta in Botswana ([Kgori et al., 2006](#)). However, these barriers require proper maintenance, and generally have proven not to be sustainable ([de La Rocque et al., 2001](#)).

Finally, mean population densities of *G. tachinoides* seem to be 10 times higher in Ghana than in Burkina Faso, which might reflect much bigger effective meta-population sizes in

Ghana. It would be interesting to compare the relative impacts of targets in these two countries where control campaigns are being conducted simultaneously.

In the above described tsetse control effort in the UWR of Ghana, the SAT component of the campaign failed to eradicate both riverine species (i.e. *G. tachinoides* and *G. palpalis gambiensis*), probably because the dense tree canopy prevented adequate penetration of the insecticide droplets, therefore not successfully killing all adult female flies. At least 22% of the adult female flies of both *G. tachinoides* and *G. p. gambiensis* dissected after each SAT cycle were survivors or immigrants ([Adam et al., 2013](#)). Furthermore, even if SAT had succeeded in eradicating the tsetse populations in the sprayed blocks, the results of our population genetics study suggest that there would have been a high probability of reinvasion from the unsprayed tributaries of the neighboring eastern populations, and from Burkina Faso. The very high efficient densities suggested by our genetic data also suggest that small zones as narrow as 673 m² can be missed during eradication campaigns and represent thereafter important reinvasion reservoirs.

For future programs aimed at eradication, the establishment of a buffer zone of adequate size using insecticide impregnated targets before the implementation of SAT operations would be recommended ([Kgori et al., 2006](#)). Also, more attention should be given to controlling the tsetse populations in the tributaries, if an area-wide approach is to be pursued within the target area.

After the SAT operations, the Government of Ghana opted for a long-term suppression approach that will be based on the integration of insecticide-impregnated-targets and pour on treatment of cattle to maintain the level of population suppression obtained with the SAT-based integrated campaign. Furthermore, it is expected that farmers will become

progressively more involved, through the promotion of control tactics such as the footbaths, i.e. the restricted application of insecticide to cattle legs ([Bouyer et al., 2011](#)).

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Chapter4: Integrated Tsetse Control

The Sequential Aerosol Technique: A Major Component in an Integrated Strategy of Intervention against Riverine Tsetse in Ghana

Adapted from:

Adam Y., Cecchi G., Kgori PM., Marcotty T., Mahama CI., Abavana M., Anderson B., Massimo P., Mattioli R., Bouyer J. (2013) The Sequential Aerosol Technique: A Major Component in an Integrated Strategy of Intervention against Riverine Tsetse in Ghana. PLoS Negl Trop Dis 7(3): e2135. doi:10.1371/journal.pntd.0002135

Abstract

An integrated strategy of intervention against tsetse flies was implemented in the Upper West Region of Ghana (9.62°–11.00° N, 1.40°–2.76° W), covering an area of ≈18,000 km² within the framework of the Pan-African Tsetse and Trypanosomosis Eradication Campaign. Two species were targeted: *Glossina tachinoides* and *Glossina palpalis gambiensis*.

The objectives were to test the potentiality of the sequential aerosol technique (SAT) to eliminate riverine tsetse species in a challenging subsection (dense tree canopy and high tsetse densities) of the total sprayed area (6,745 km²) and the subsequent efficacy of an integrated strategy including ground spraying (≈100 km²), insecticide treated targets (20,000) and insecticide treated cattle (45,000) in sustaining the results of tsetse suppression in the whole intervention area.

The aerial application of low-dosage deltamethrin aerosols (0.33–0.35 g a.i/ha) was conducted along the three main rivers using five custom designed fixed-wings Turbo thrush aircraft. The impact of SAT on tsetse densities was monitored using 30 biconical traps deployed from two weeks before until two weeks after the operations.

Results of the SAT monitoring indicated an overall reduction rate of 98% (from a pre-intervention mean apparent density per trap per day (ADT) of 16.7 to 0.3 at the end of the fourth and last cycle). One year after the SAT operations, a second survey using 200 biconical traps set in 20 sites during 3 weeks was conducted throughout the intervention area to measure the impact of the integrated control strategy. Both target species were still detected, albeit at very low densities (ADT of 0.27 inside sprayed blocks and 0.10 outside sprayed blocks).

The SAT operations failed to achieve elimination in the monitored section, but the subsequent integrated strategy maintained high levels of suppression throughout the intervention area, which will contribute to improving animal health, increasing animal production and fostering food security.

4.1. Introduction

In sub-Saharan Africa the tsetse fly (Genus: *Glossina*) is the cyclical vector of trypanosomosis, a disease of livestock and humans, caused by unicellular parasites of the genus *Trypanosoma*. Whilst the human form of the disease no longer appears to be a major public health issue in Ghana [1–3], animal trypanosomosis is still widely reported and causes considerable losses in the livestock sector resulting in major impacts on agricultural production, livelihoods and food security [4,5].

In Ghana, *Glossina* species have raised concern from the beginning of the colonial period [6]. Since then, different techniques have been used in a number of control efforts [7–9]. In Ghana, these techniques included, inter alia, the removal of tsetse habitat by bush clearing, the destruction of wildlife, as well as the use of insecticide treated targets (ITT) and insecticide treated cattle (ITC) [5,10–12]. These techniques, albeit successful in suppressing tsetse populations, failed to eliminate the tsetse fly from Ghana [5]. In this paper, the term ‘elimination’ is used to designate a local eradication.

In the control area, located in the Upper West Region, *G. tachinoides* and *G. palpalis gambiensis* are the sole species present [5, 13]. *G. morsitans submorsitans* is absent, but found at 30 km only north-east to the target area, in the Sissili protected forest. Both *G. tachinoides* and *G. palpalis gambiensis* are riverine species, for which riparian vegetation represents the typical habitat [14–16]. In the study area, this type of habitat is mainly found along the rivers Black Volta, Kulpaw and Sissili. All of these rivers flow southwards into Lake Volta, but parts of their catchment basins are located to the North, in neighbouring Burkina Faso. Along the tributaries of the three main rivers, the habitat appears unsuitable for tsetse flies in the dry season (December–May), during which bush fires and the absence of rain seriously limit the availability of appropriate vegetation [13]. At this time of year, tsetse

flies retreat from tributaries to the main rivers, where permanent water and dense vegetation mitigate the harsh ambient macroclimatic conditions (e.g. temperatures often rising above 40°C).

Large-scale aerial spraying of insecticide was adopted as the main intervention tool against tsetse flies in the Upper West Region of Ghana within the frame work of the African Union's Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), whose ultimate goal is to eradicate African trypanosomosis through the progressive and sustainable creation of tsetse and trypanosomosis-free areas [17]. In Ghana, an integrated strategy of intervention was adopted, whereby the sequential aerosol technique (SAT) was complemented by ground spraying, insecticide treated targets (ITT) and insecticide treated cattle (ITC). To promote sustainability and limit the risk of postoperation reinvasion, the integrated intervention strategy was based on regional collaboration. Contrary to previous control campaigns conducted at a local scale, SAT operations were conducted jointly in Ghana and Burkina Faso. The impact of the integrated interventions on non-target aquatic, terrestrial and insectivorous fauna was monitored and will be presented elsewhere.

The present study aimed at evaluating the potentiality of SAT to achieve elimination of the riverine tsetse species in a particularly challenging subsection (dense tree canopy and high tsetse densities as revealed by the baseline survey) and the subsequent efficacy of the integrated strategy including ground spraying, ITT and ITC to sustain the results of tsetse suppression in the Upper West Region of Ghana.

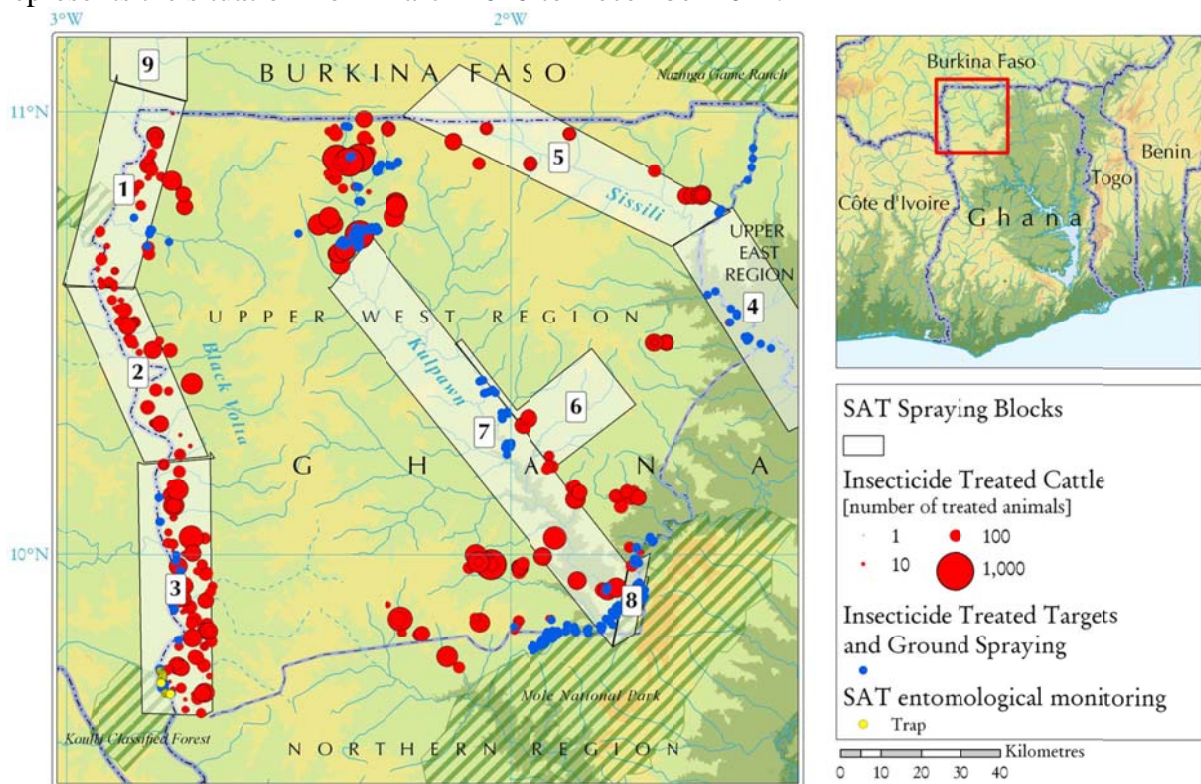
4.2. Materials and Methods

4.2.1. Study area and sequential aerosol technique

The study area is located in north-western Ghana (from lat. 9.62 to 11.00 N and from long. 1.40 to 2.76 W), and it covers a surface of $\approx 18,000$ km² (Figure 1).

SAT, consisting of repeated spraying of non-residual insecticide aerosols from the air [18], was applied to the three main rivers (Black Volta, Sissili and Kulpawm). Eight spraying blocks of different size were defined (Figure 4.1), covering a total area of 6,745 km. A ninth block of 1,940 km² was located across the border in Burkina Faso. A 15 km blocks' width assured the coverage of the tsetse-infested riparian vegetation, whilst the block lengths were selected to allow parallel flying lines.

Figure 4.1 Tsetse control operations conducted in the Upper West Region of Ghana. The map represents the situation from March 2010 to December 2011.



The aerial application of low-dosage insecticide aerosols was conducted following the principles described by Kgori et al. (2006). The insecticide application rates were adjusted to suit local climatic conditions, and 4 cycles were used (as opposed to the 5 conducted in Botswana in the operations described by Kgori et al. (2006)). Air-spraying was conducted at night, along swaths of 275 m width. Occasionally, daytime spraying was carried out under stable and cool overcast weather conditions when suitable temperature inversion was

recorded. Such modification of the standard SAT procedure was necessary to maintain continuity of the spraying programme following, for instance, unavoidable interruptions due to inappropriate weather conditions. The operation was carried out by Orsmond Aviation (Bethlehem, South Africa), using five custom designed fixed-wing Turbo thrush aircraft – fitted with navigation and spray management equipment.

The applied insecticide was Deltamethrin (0.35% (w/v), ultralow volume (ULV) (Deltanex formulation, Avima, Johannesburg, South Africa). The insecticide dispersal units, fitted to each aircraft were made of two boom-mounted, wind-driven Micron-air AU 4000 rotary atomisers (Micron Sprayers Ltd., Bromyard, UK) operated with cage speed of 11,000rpm and average flow rate of 9.7 l/km². The micron-airers were fitted with shut-off valves to prevent spillage in the event of damage to the atomizers. Each aircraft was also fitted with the upgraded GPS-based guidance system - SATLOC M3 (CSI Wireless, Calgary, Canada), to provide precision controlled spray application aided by a moving map display that guided the pilot using an external light-bar. Flight and spraying statistics were captured automatically using the system's integrated data logger [19].

The spectrum of aerosol droplets was assessed using magnesium oxide coated glass slides. While rotating at 330 rpm, the Numerical and Volume Median Diameter values were 17.70 and 35.50 μ m respectively. As the insecticide had been stored locally for about one year before usage, samples of the formulation were sent to a laboratory (Pesticide Analytical Technology, Pretoria, South Africa) for potency testing. Despite storage at high temperatures, both the physical and chemical properties of the product were confirmed to be within the expected ranges.

SAT operations were conducted between 3 April and 5 May 2010 (Figure 4.2). For the first spraying cycle, the application rate was at 0.33 g active ingredient (a.i)/ha in all the sprayed

blocks. Subsequent treatments of blocks 3 and 7 used 0.35 g a.i/ha. The choice of the spraying period, i.e. late dry season, as well as the timing, i.e. from dusk to dawn, aimed at optimising the advantage of temperature inversion conditions with gentle winds that allowed the smaller aerosol droplets to descend into tsetse habitat. Temperature inversion was investigated using probes located at a height of approximately 1.5 and 8 m. The inversion layer was shallow in the early afternoon, improving through the night and breaking down around 0700h in the morning (Figure 4.3). Flying height was about 10 m above tree canopy. Localised areas of thick vegetation in blocks 3 and 7 presented serious challenges to insecticide penetration. Therefore, in those areas three supplementary swaths were added along the edges of the river to increase the application rate during the three last spraying cycles.

In SAT, the sequence of insecticide application must be timed so that female tsetse flies emerging after one treatment have no sufficient time to deposit larvae before the next treatment. This period is called the first larval period (FLP). In planning the intercycle period, estimates of FLP are to be taken into account.

Moreover, the sequence of application has to continue until new adults cease appearing from underground, i.e. until the pupal period (PP) is completed. These two parameters are temperature dependent and can be calculated from the Jackson's formulae [20], where t is the temperature in degrees Celsius (°C):

$$\mathbf{FLP = 1/(0.0661+0.0035(t-24))}$$

$$\mathbf{PP = 1/(0.0323+ 0.0028(t-28))}$$

Figure 4.2 Timeline of the preparation and implementation of the integrated intervention strategy against riverine tsetse. For field operations the bars indicate the start and end date of deployment. More details on the spatial configuration and the frequency of deployment are provided in the text.

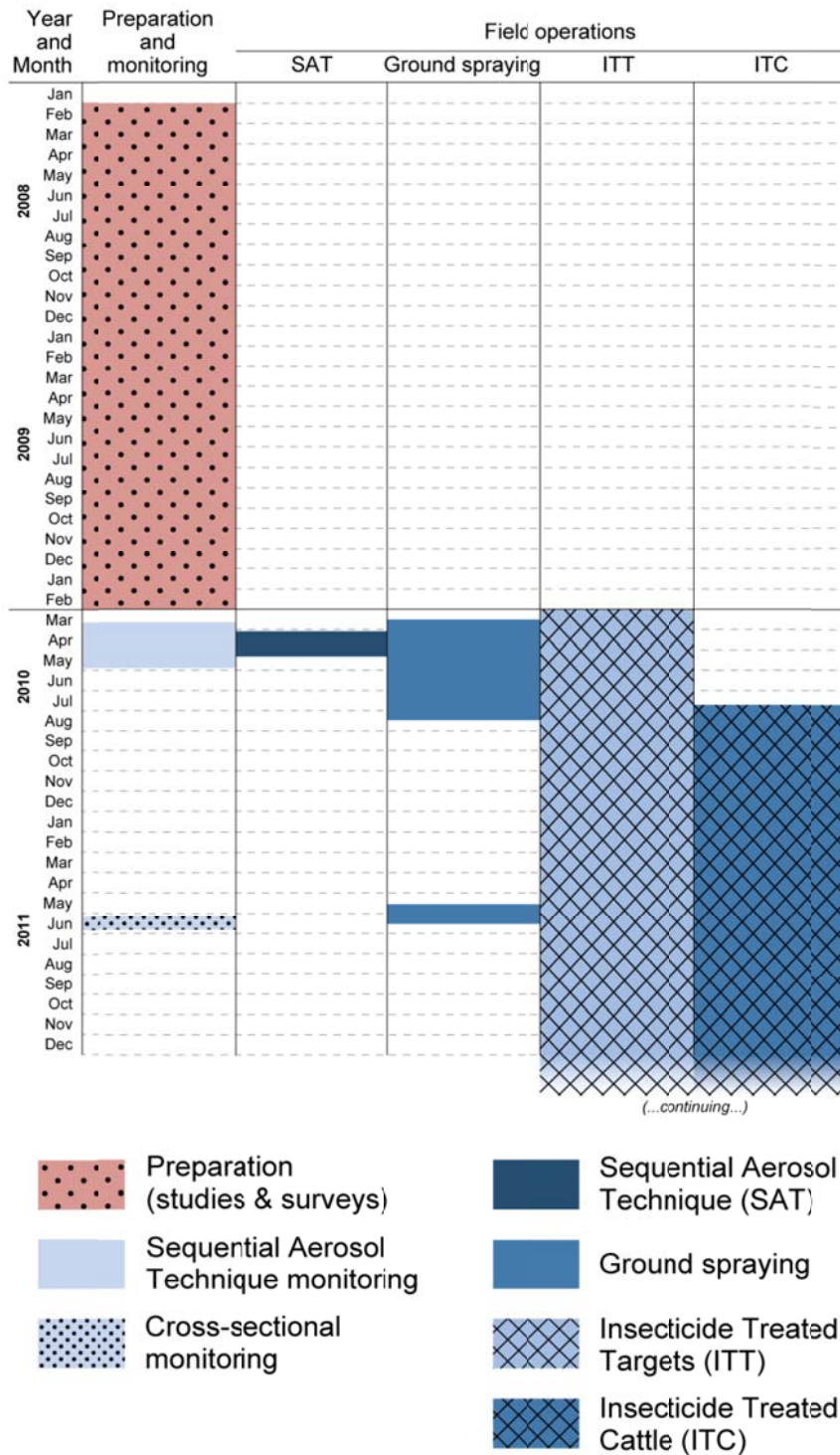
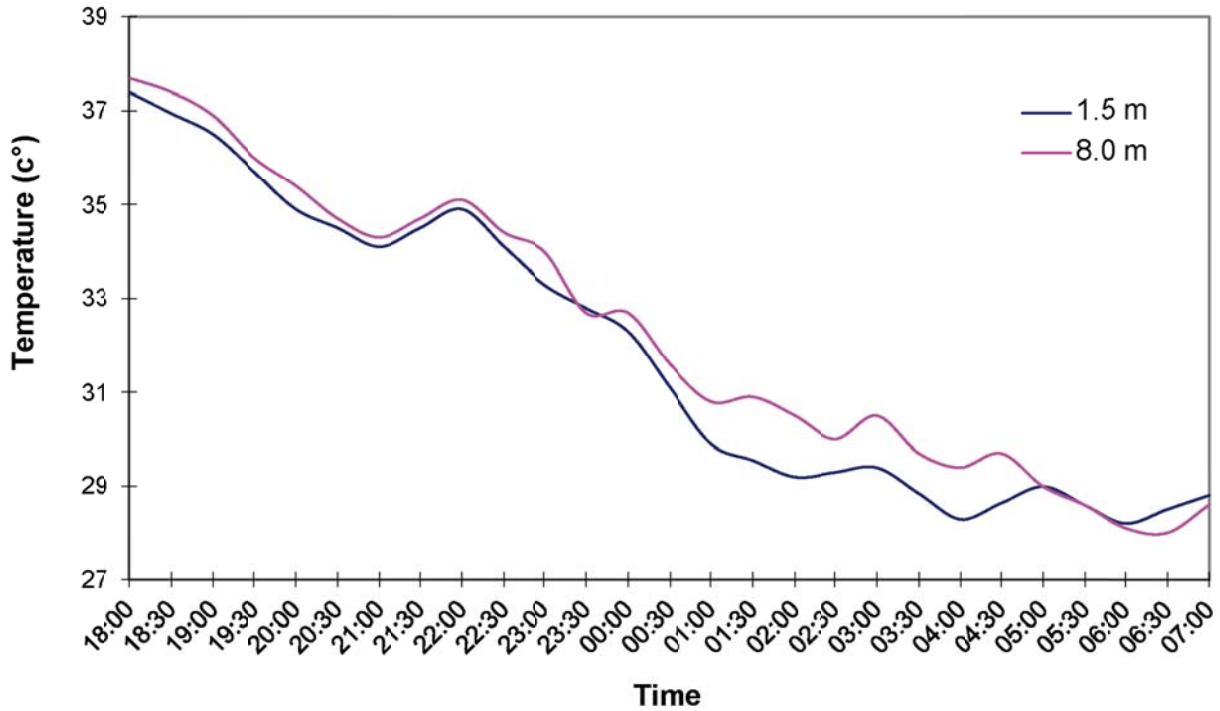


Figure 4.3 Temperatures inversion layer measured from Wa airport on 2 April 2010.

In the Upper West Region, the initial spraying schedule was based on a six-year average of daily temperatures at Wa, the regional capital. The estimated FLP and PP were 11 and 20 days respectively. Spraying began on 3 April 2010. Subsequent cycles started on 12 April, 22 April and 3 May. During operations, meteorological data were collected to control that planning was appropriate.

4.2.2. Other control techniques

Figure 2 provides a timeline of the preparation and implementation of the integrated intervention strategy. Ground spraying of insecticide was one of the complementary control measures deployed after SAT operations in order to sustain the results of tsetse suppression in the intervention area. Ground spraying was conducted in the sites where the tree canopy was too dense to allow a sufficient penetration of the insecticide sprayed by air and where targets were placed to act as barriers against reinvasion from non-treated tsetse infested areas (Figure

1). Motorised hand sprayers were employed using Deltamethrin sc 20% (200 g/litre of water). A dose of 100 ml insecticidal concentrate per 18 litres of water (one knapsack sprayer) was used to obtain a concentrated spray liquid (0.1%), allowing chemical persistence at active levels for 7 months (FAO 1992). Apart from a core staff of 12 organized in 3 teams of 4 persons working simultaneously in different locations, each team recruited and trained ≈ 10 staff from the communities as casual labour for the ground spraying operations. Ground spraying was applied to tree trunks between and around targets, under overhanging rocks, branches and twigs that provide resting and breeding sites for tsetse. Ground spraying operations concerned $\approx 100 \text{ km}^2$ (i.e. $\approx 5 \text{ km}^2$ in the Black Volta river basin, $\approx 54 \text{ km}^2$ in the Kulpawn River basin and $\approx 40 \text{ km}^2$ in the Sissili River basin) and took place from March to August 2010 and from May to June 2011. To compensate for the inability to repeat the spraying more frequently, the Deltamethrin formulation used had an added UV inhibitor to extend the effective life of the insecticide.

Deltamethrin (1% w/v) pour-on formulation (Shandong Luxi Animal Medicine Company-P.R. China) was applied along the backline of cattle at a dose of 1 ml/10 kg of body weight. In selecting animals for pour-on treatment, priority was given to those located in the vicinity of rivers (Figure 1). All the animals of at least 50% and in most cases 100% of all kraals located within 10 km from one of the three main rivers were treated. Over 45,000 cattle were treated using approximately 454 litres of deltamethrin (1%) pour-on formulation. The project conducted the first pour on treatment (start date 22 July 2010) and distributed additional product to the farmers thanks to locally trained staff, so that they could conduct monthly treatments by themselves.

ITT were deployed from March 2010 in the intervention area at locations strategically selected to serve a dual purpose (Figure 4.1). First, targets were placed along sections of the

perimeter of the intervention area, especially at river crossings and at the border with game reserves, in an effort to stem reinvasion from nontreated areas. Second, ITT were deployed both within and outside SAT-treated blocks to act as a complementary suppression tool. The density of ITT was greater at the limit of the Mole National Park, where tsetse densities and reinvasion pressure are at their highest. The targets corresponded to PermaNet 2.0ND, comprising 100% polyester panels, sized W1506H100 cm (Vestergard Frandsen Group) and with a central blue panel flanked by black side panels of 50 cm wide each.

Targets were placed in four rows spaced by 50 m, in order to form 150 m-deep barriers along protected areas, main rivers and their tributaries. Horizontal target-spacing was 25 m for the first row, closest to the potential source of reinvasion, and 50 m for the other rows. Overall, about 20,000 deltamethrin treated targets were deployed on a total linear distance of about 89 km.

4.2.3. Monitoring the impact of the control campaign

The impact of the different SAT cycles on tsetse population density was monitored in one site only, at the southernmost part of the study area in Block3. The site was considered to be particularly challenging for SAT because of its dense vegetation and the high density of tsetse [13]. Monitoring in the other spraying blocks could not be conducted because of budgetary and logistical constraints. Thirty biconical traps [21] were deployed with an average inter-trap distance of 200 meters. Traps were deployed from two weeks before and until two weeks after the four spraying cycles. Fly catches were collected and sorted on a daily basis. Fly species, sex and age were recorded. Age was only estimated in females through ovarian dissection and observation [22]. Catches were analysed using a robust negative binomial regression in Stata 11. Individual traps were considered as primary sampling units whereas

post SAT periods were used as categorical explanatory variables. Suppression rates were estimated for each SAT spraying cycle and for the four cycles as a whole.

From 6 to 24 June 2011, approximately one year after SAT operations, a cross-sectional entomological survey was conducted to determine the cumulative impact of the integrated control measures across the project area. Monitoring sites were selected taking into account (i) the spatial configuration of the integrated tsetse suppression operations (Figure 1), with particular attention to the SAT sprayed blocks, (ii) pre-operation apparent densities of tsetse flies and prevalence of bovine trypanosomosis [13], and (iii) the risk of fly reinvasion.

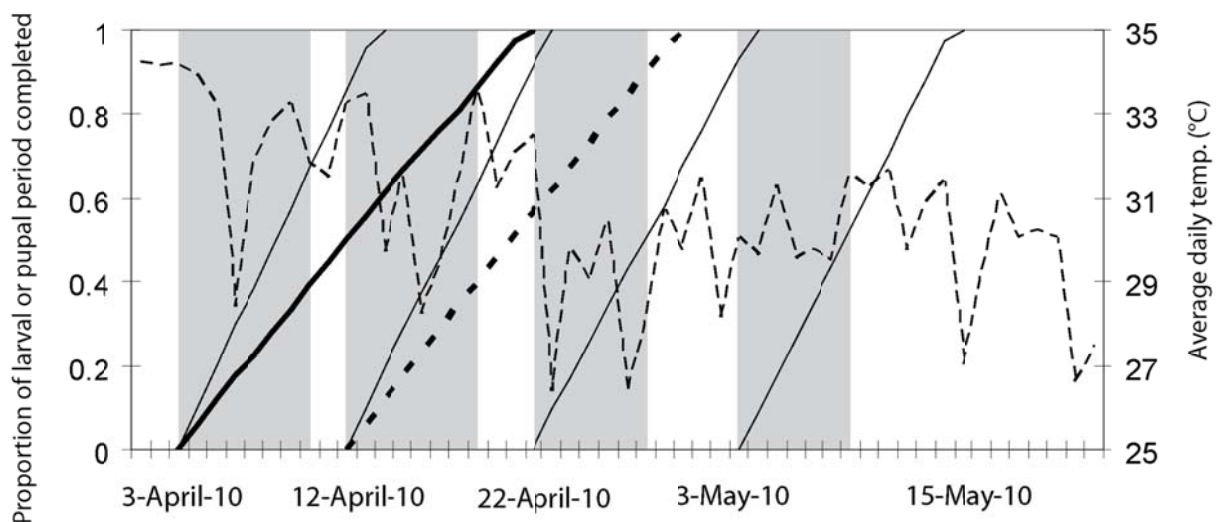
Guided by the above principles, and also using Landsat 7 Satellite images to identify potential tsetse habitat, 20 monitoring sites were selected. At each site, 10 biconical traps spaced 100 m were deployed. Traps were inspected twice a day for fly catches and removed after 48 hours of trapping. Flies caught were sorted by species, physiological status and sex. Data were analysed in a robust negative binomial regressions using the sites as primary sampling units. The total abundance was first estimated as a function of the location (within or outside the SAT areas). Species abundance was estimated in a second model using species as categorical explanatory variable.

4.3. Results

4.3.1. SAT schedule

According to local meteorological data measured during field operations, the SAT schedule fitted exactly the predicted FLP (Figure 4.4). More precisely, each cycle was conducted one to two days before the predicted days of larviposition of primipares. Moreover, the whole treatment period lasted much more than the maximum PP.

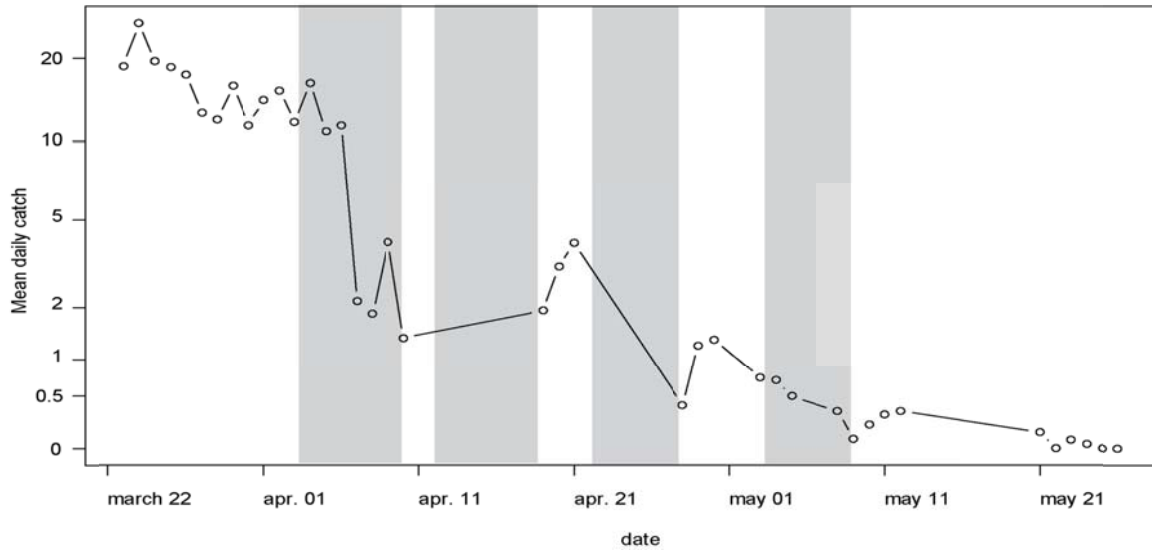
Figure 4.4 Course of larval and pupal developments durations during the SAT operations in Ghana. The thin solid lines present the larval development and the thick solid line the pupal development, estimated using Jackson's formulae (see (Hargrove, 2003)). Mean daily temperature is presented as a dashed line. A second pupal period (thick dashed line), starting at the beginning of the second cycle, shows the potential development of pupae produced by females surviving to the first treatment. Vertical bars indicate the periods of the four SAT applications.



4.3.2. Efficacy of the SAT cycles

In the densely vegetated site chosen for SAT monitoring, the mean apparent density per trap per day (ADT) dropped from 16.7 (95% CI 10.4–26.8) before spraying to 1.4 (0.7–1.7), 3.6 (1.8–6.8), 1.3 (0.6–3.0) and 0.3 (0.1–0.5) during the two days immediately after spraying cycles 1 to 4 respectively. This corresponded to reduction rates of 92% (85–95%, $p < 0.001$), 0% (a significant increase was even observed, $p = 0.001$), 64% (46–76%, $p < 0.001$) and 78% (76–86%, $p < 0.001$) respectively (Figure 4.5). The resulting overall reduction rate was 98% (97–99%, $p < 0.001$). ADT further dropped to 0.04 (0.01–0.11) on the last observation week (12 to 19 days after the last spraying).

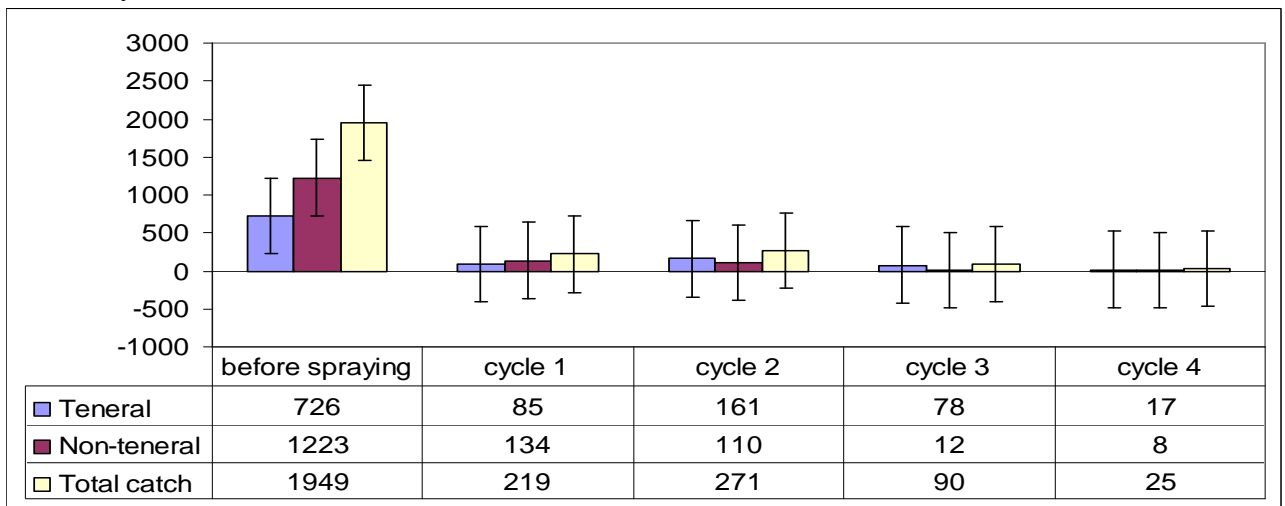
Figure 4.5 Mean daily catch of tsetse before and during the SAT operation in Ghana. Vertical bars indicate the periods of SAT applications.



Tsetse dissection indicated a continuous reduction of nonteneral female flies from 1,223 flies before spraying to 8 flies after cycle 4 (Table 4.1a). The percentage of non teneral flies declined from 63%(s.d.5%) before SAT operations to 61%(s.d.6%), 41% (s.d.5%), 14%(s.d.6%) and 33%(s.d.14%) after cycles 1 to 4 respectively ($X^2 = 34, df= 4, p < 10^{-3}$). However, the amounts of non teneral flies proved that at least 22% of the adult females dissected after each cycle were survivors or immigrants. The emerging juveniles also declined from 726 to 17 after the last cycle.

Table 4.1 Tsetse catches and Disected females during SAT operations

a) Fly catches



b) Dissected females

Period	Total catch	Dissected females		
		Teneral	Non-teneral	Total
Before spraying	1949	35	59	94
Cycle 1	219	30	47	77
Cycle 2	271	57	39	96
Cycle 3	90	32	5	37
Cycle 4	25	8	4	12

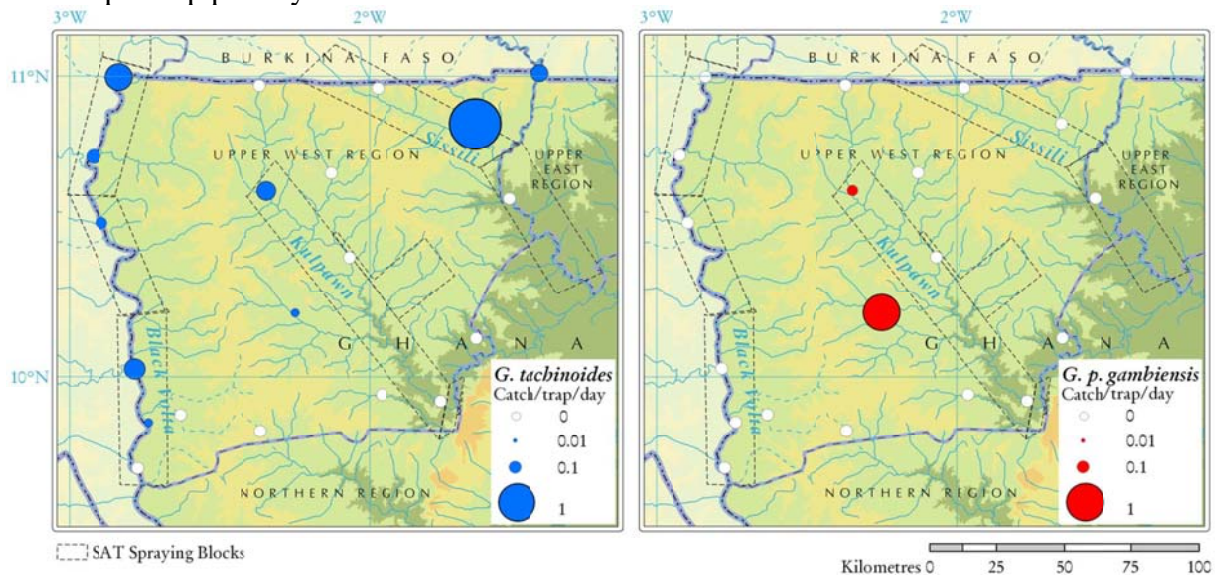
The data (Tables **a** and **b**) correspond to the total tsetse catches; teneral, non-teneral and total dissected female flies before (2 weeks) and after each SAT cycle (2–3 days for cycles 1 to 3 and 2 weeks after cycle 4).

4.3.3. Efficacy of the integrated tsetse control campaign

One year after SAT operations, the apparent density of tsetse flies was still very low throughout the project area (Figure 6), with average ADT of 0.27 (95% CI: 0.1–0.7) inside the sprayed blocks, and 0.10 (95% CI: 0.02–0.5) outside the sprayed blocks. The difference was not significant ($p = 0.35$).

Rigorous comparisons with pre-intervention densities are difficult to make because of differences in the design of the baseline and post-intervention cross-sectional surveys. In terms of species relative abundance, post-intervention entomological data were in line with pre-operation surveys, in that *G. tachinoides* was more abundant and much more widespread than *G. p. gambiensis* ($p = 0.18$), with ADT of 0.17 (95% CI: 0.06–0.47) and 0.03 (95% CI: 0.005–0.2) respectively. Even after a year of the beginning of the integrated tsetse control, *G. tachinoides* was captured in all three river basins in the project area, and all along the Black Volta. The highest density was detected in one site along the river Sissili, where the complementary pressure from ITC, ITC and ground spraying was comparatively lower. *G. palpalis gambiensis* was captured in two sites within the Kulpawn river basin (including one site located along a right-hand tributary that lies outside the SAT sprayed blocks).

Figure 4.6 Results of the entomological survey conducted in Ghana one year after SAT operations. The survey was conducted from 6th to 24th June 2011 to monitor the impact of the integrated tsetse control campaign. Tsetse apparent density is expressed as the number of catches per trap per day.



4.4. Discussion

In southern Africa, SAT was successfully used to eliminate *G. morsitans centralis* from the Okavango Delta of Botswana, as confirmed by sustained post-intervention monitoring [18, 23]. In the Okavango Delta, climatic and biophysical conditions such as the presence of open savannah, together with the ecology of the target tsetse species, enhanced the impact of SAT. Two main lessons can be drawn from this study: first, in the Upper West Region of Ghana, SAT achieved very high levels of suppression in one of the most challenging sites, but failed to achieve elimination of the tsetse population. It must be acknowledged that the density of the vegetation was highest in this section, and that elimination might have been achieved in other sections of the target area, where the gallery is more open. The possibility of extrapolating the present results is therefore limited. However, in an elimination perspective, a partial failure hampers the results in the whole area, since tsetse can then re-invade the cleared areas. Second, the integrated strategy of intervention succeeded in sustaining the results of suppression one year after the SAT operations.

As the predicted FLP and PP used to schedule SAT matched the FLP and PP derived from the real temperature data measured during operations, it is unlikely that inability to eliminate tsetse be ascribable to inappropriate synchronisation of pupae sequestration in the soil and SAT application. Yet, FLP and PP were estimated using average climatic data and, as such, may not account for substantial microclimatic heterogeneity found in riparian habitats [24], which are often characterized by various levels of disturbance and fragmentation of the natural vegetation [14,25]. Moreover, we have no field data for the target species and Hargrove has pointed out a strong variability of FLP (up to 3 days) between individuals [20].

Arguably, the single most important factor affecting the efficacy of SAT operations in Ghana was the presence of patches of thick vegetation, which pose serious challenges to insecticide penetration into tsetse habitat. To corroborate this assumption attempts were made to measure droplet penetration both under the canopy and in the open, but unfortunately the samples were damaged by excessive overnight dew and no detectable chemical was apparent in the results. The loss of data for droplet penetration therefore left us with no unquestionable proof for this assumption. Earlier experiences of aerial spraying against riverine tsetse in West Africa successfully addressed the problem of penetrating riverine forest by using helicopters, based on the assumption that the downdraft from the helicopters would help the insecticide penetrate beneath the canopy [26–28]. However, residual insecticides were used, with significant impacts on non-target organisms. Tsetse female survivors were found in large proportions of the dissected flies in the densely vegetated hotspot monitored during SAT. Given the observed proportion, it is not likely that these survivors were immigrant flies, all the more for the last 3 cycles, when supplementary spraying swaths would have necessitated a dispersal of more than 7.5 km within 2–3 days, which is highly unlikely given the dispersal rate of these species in this environment [29]. Actually, the probability to cross this distance would be almost null given the mean square displacements from 303 m/day to 780 m/day for

G. palpalis gambiensis, and from 316 m/day to 775 m/day for *G. tachinoides*, as measured by markrelease-recapture experiments in similar landscapes in Burkina Faso and Mali [29–31]. Moreover, the tsetse observed densities along these tributaries were already low during the baseline survey [13]. Finally, target barriers deployed by the PATTEC Burkina Faso project along the tributaries coming from Burkina Faso into the target area (not shown in Figure 1) also reduced the probability that the observed non-teneral survivors were immigrant flies.

Trap efficiency is always very low and absence of capture does not mean absence of flies [32]. Considering a trap efficiency of 0.01 for biconical traps against these species, based on raw data collected in the area of Sideradougou, Burkina Faso [33,34], and a mean width of the gallery forest of 100 m, the initial densities in the monitored section can be estimated at 2,783 flies/km² of gallery forest [95%CI 1,733–4,467]. Given the simulation models [23], and for a 4-cycle treatment, the kill rate should be over 95% to warrant elimination, a suppression rate that was never attained here.

The spatial coverage of operations is another important factor to be considered when analysing the overall impact of interventions. Neither SAT nor the ancillary tools were applied across the whole project area. Operations, including aerial spraying, focused on the three main rivers, where suitable habitat for tsetse abounds. Whilst it is arguably a cost-effective approach to target interventions where they will have the largest impact on the tsetse populations, small pockets of flies located away from the main rivers may go untreated. The existence of these small pockets was not ruled out by the baseline data collection, which was limited to the main river systems [13]. The capture of both *G. tachinoides* and *G. palpalis gambiensis* in a right-hand tributary of the Kulpawn during the post-intervention survey supports the notion that small pockets of flies may be sustained through the dry season also in

areas far from the main rivers. A similar situation was observed for *G. palpalis gambiensis* in dryer zones of its distribution area 2 for example in Senegal [35].

Although SAT failed to eliminate tsetse flies, the operations did reduce significantly tsetse abundance in a very short period of time. Considering the temperature in the study area (mean day temperature around 30°C), 4 cycles were required to cover the estimated PP. It was decided to limit SAT operations to a 4-cycle schedule because SAT-monitoring data (i.e. reduction rates and survival of mature females) showed that not even a fifth cycle would have achieved elimination, and it would have only increased the cost of suppression.

Importantly, suppression was achieved with few environmental side-effects. The impact of SAT operations on non-target aquatic, terrestrial and insectivorous fauna was studied and it will be presented in detail elsewhere. In essence, this environmental monitoring showed that the majority of non-target species were broadly unaffected [36].

If we consider the integrated control strategy as a whole, a number of factors must be called upon to explain the results of the post-intervention entomological survey. First, despite the deployment of barriers, possible re-invasion of flies from neighbouring areas may have occurred. In the absence of reinvasion barrier, *G. palpalis gambiensis* was estimated to reinvade at a speed of about 7.5 km/year along the Mouhoun River [30]. Moreover, both riverine tsetse species, and particularly *G. tachinoides*, are probably able to move between river basins [29, 37]. This is all the more of a preoccupation for *G. tachinoides*, which disperse ≈ 3 times faster than *G. palpalis gambiensis* in these fragmented landscapes. In the project area, not all barriers were in place before SAT operations started, and many were deployed during the spraying period. Models have shown that efficiency substantially increases if barriers are set up before the spraying cycles [23].

As far as ITC is concerned, the technique is efficient in reducing riverine tsetse populations [7,38,39] but arguably less so to achieve elimination, because a large proportion of riverine tsetse flies feed on alternative hosts [40,41]. Theoretical models have shown that a daily mortality of 3% would eliminate a tsetse population [20], a result that would be obtained if only $\approx 10\%$ of tsetse fed on ITC. However, Bauer et al. (1999) demonstrated in similar settings and using blood meal analyses, that small pockets of *G. tachinoides* were able to survive by feeding mainly on reptiles [38]. In fact, learning behaviour tends to protect flies that first fed on reptiles because they tend to feed repeatedly on the same host thereafter [40]. ITT, which can partially overcome this problem, could not be uniformly deployed within the project area, thus possibly contributing to the survival of pockets of tsetse. Moreover, these two techniques are very efficient at high tsetse densities, but less so when the abundance is low [8]. In the Loos Islands in Guinea, it was recently observed that, using the same techniques, decrease in tsetse density was sharp during the first months of the control campaign, but it required several years to obtain further sizable reductions in tsetse densities [9]; elimination has still not been achieved to date [42]. Looking at the overall integrated strategy implemented in Ghana by the ‘‘Multinational Project for the Creation of Sustainable Tsetse and Trypanosomosis Free Areas in East and West Africa’’, we can conclude that it achieved a high level of suppression of tsetse populations using SAT as a key component. Baseline surveys had shown an average ADT of 6.51 (s.d. 26.59) and 0.14 (s.d. 0.69) for *G. tachinoides* and *G. p. gambiensis* respectively inside the spray blocks [13]. If we compare this with the data collected inside the spray blocks one year after SAT operations, we obtain relative reductions of $\approx 96\%$ for both species. This result is interesting given the different ecologies of the flies in the target area, *G. tachinoides* favouring open riverine tickets whereas *G. p. gambiensis* preferring closed gallery forests, which are scarce in the study area [14].

Moreover, *G. tachinoides* feed more readily on cattle than *G. palpalis gambiensis* do [41], and might have been more impacted by ITC.

Further epidemiological and socio-economic studies are needed to assess the extent to which suppression of the tsetse populations resulted in a lower AAT risk and in a more conducive environment for livestock production.

Regarding the field costs for the operations, excluding salaries and depreciation of vehicles and equipment, they were \$3,633,979, corresponding to \$202/km², including \$3,000,000 for SAT operations (i.e. \$445/km² for suppressing tsetse in the 6,745 km² sprayed), \$200,000 for ground spraying, \$133,890 for pour on treatments and \$300,089 for impregnated targets (i.e. 35\$/km² for the integrated strategy used to maintain suppression during two years in the whole 18,000 km² area). These costs are broadly in line with available estimates on the costs of tsetse and trypanosomosis control [43].

Tsetse suppression is being sustained in the Upper West Region, and schemes will be implemented to recover part of the cost from farmers who will be encouraged to use cost-effective techniques to protect their animals [44]. At the same time, suppression is planned to be extended to neighbouring Upper East and Northern regions. The regional strategy also foresees that on-going interventions in Western Burkina Faso will progressively expand their scope, targeting the areas at the border with Ghana, and thus reducing the cost of barriers.

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Chapter5: Selection of monitoring sites

Considerations for the Selection of Monitoring Sites in a post SAT Tsetse Control Programme

Adam Y., Cecchi G., Bouyer, J. Considerations for the selection of Monitoring Sites in a post SAT tsetse control programme.

Tsetse flies live in habitats that provide shade for developing puparia and resting sites for adults. Their development, like that of many invertebrates, is temperature limited (Robinson, *et al.* 1997a). Species of the palpalis group need high humidity and deep shade. The best conditions are about 25⁰C and 80-85% R.H. *Glossina palpalis gambiensis* occurs in a wide range of vegetation associated with rivers and streams. In west Africa, *G. p. gambiensis* often cohabitates with *G. tachinoides* but does not extend so far into the dry north (Pollock 1982). In savannah areas, pupae of *G. p. gambiensis* are found especially in denser, forest type vegetation in low shade provided by shrubs, creepers, low branches, fallen logs, overhanging riverbanks, etc. While the underside (0.3-2m) of gallery forest provide a resting place during the daytime, the top upper surfaces of leaves provide tsetse a resting place at night (Pollock 1982). Typical habitats for *G. tachinoides* other than the general conditions of *G. palpalis* mentioned above are open savannah vegetation types. The density of vegetation and the canopy or cover formed by the leafy branches of the vegetation, are important in the ecology of *G. tachinoides*. They provide conditions required for their survival and their breeding. *Glossina* spp in the Upper west region are of the riverine type, inhabiting forest galleries along the three main rivers (Black Volta, Kulpawn and Sissili) that run through the region in southward direction

In the UWR of Ghana, a baseline survey was conducted to determine prevalence and tsetse densities prior to the implementation of an integrated strategy to eliminate tsetse, using the Sequential Aerosol Technique (SAT) as the main suppression tool between March and May 2010. SAT was followed by additional control tools, namely Insecticide Treated Targets (ITT), cattle pour-on and ground spraying (Adam Y., *et al.* 2013). Twelve months after the end of SAT operations (May-April 2011), a first round of monitoring was conducted to determine the presence or absence of tsetse flies. The objective of this study was to compare

the usefulness of the pre and post SAT tsetse monitoring procedures for the selection of tsetse monitoring sites and to recommend an appropriate study design for the selection of tsetse monitoring sites.

Potential tsetse habitat is hypothesized on the basis of land cover/ vegetation (Cecchi G., et al., 2008), water bodies and the availability of hosts (livestock and wildlife), which are known to have a direct relation to tsetse densities and the risk of cyclical trypanosomosis. In the pre-intervention tsetse density determination, a systematic sampling procedure was implemented along the three main rivers and within the SAT spray blocks (Adam Y., et al., 2012 and 2013). The pre-intervention monitoring procedure was based on the hypothesis that tsetse flies retract from tributaries to the riparian forest along the main rivers in the UWR during the dry season after bush fires have cleared the vegetation cover and with prevailing high temperatures (mean day temperatures above 40°C). Sampling was therefore limited to the riparian forest along the three main rivers of the Upper West Region, hence tsetse was not found outside the riparian forest along the main rivers (Figure1) (Adam Y., et al. 2012).

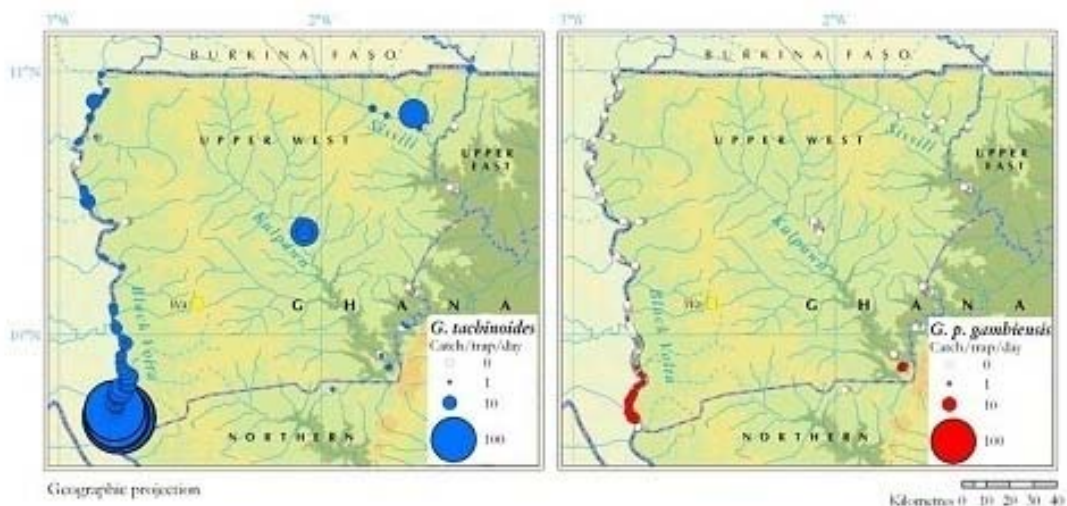


Figure 1 Results of the baseline entomological survey in the Upper West Region (Ghana). Tsetse apparent density is expressed as the number of catches per trap per day [CTD].

On the other hand the post SAT tsetse monitoring procedure was set up to verify the hypothesis of the pre-intervention monitoring procedure and to provide evidence that tsetse flies could still be present outside the SAT treated spray blocks and in some of the river tributaries. Landsat7 Satellite Images, as well as other ancillary GIS data, were used to guide the selection of 20 monitoring sites for the post-SAT tsetse monitoring (Cecchi G., 2011 Mission report). The primary guide in selecting sampling sites for monitoring riverine tsetse was vegetation cover along rivers, tributaries and game reserve borderlines with river crossings. By visual appraisal, 12 sampling sites were identified within the spray blocks and 8 sites outside the treated blocks (Figure2). The number of 20 sampling sites was established taking financial and logistic constraints into consideration (Cecchi G., 2011 Mission report).

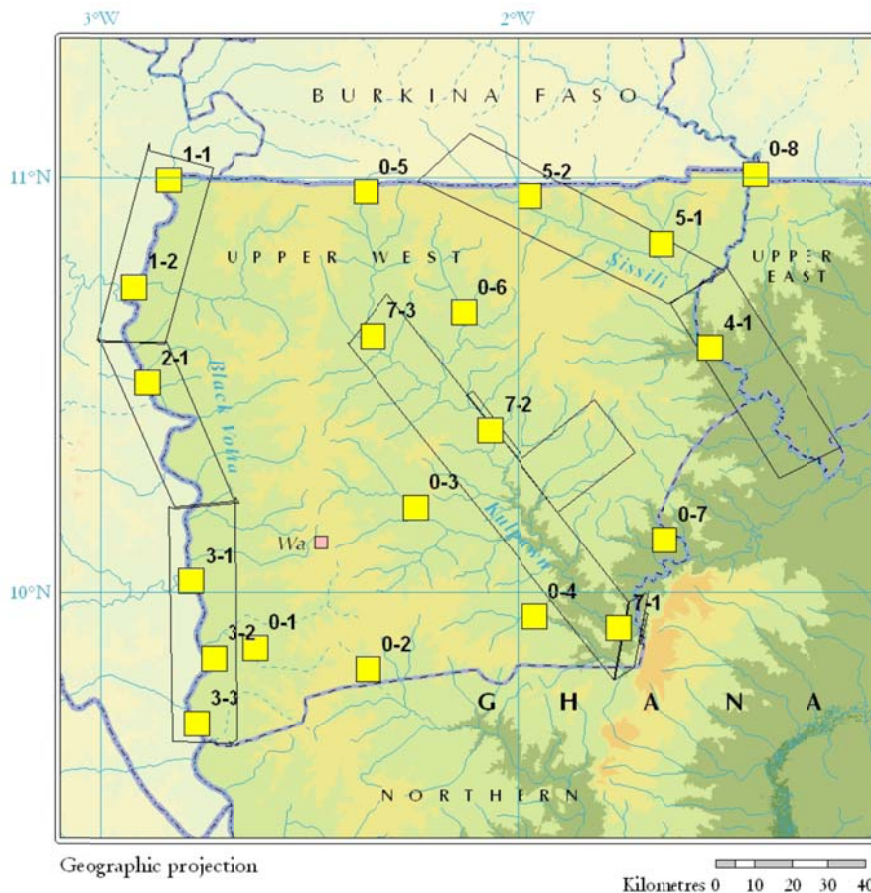


Figure 5.2 Twenty monitoring sites selected to assess the effects of the integrated control strategy on tsetse populations. Numbering of sites is composed by block number and a progressive site number. 0 identifies sites outside spray blocks.

The post-SAT tsetse monitoring survey found tsetse flies outside the riparian forest along the main rivers, contrary to the pre-SAT monitoring hypothesis (Figure 2). Both *G. tachinoides* and *G. p. gambiensis* were captured in two sites outside the SAT sprayed blocks on the tributaries of the main rivers (Adam Y., et al., 2013) (Figure 3).

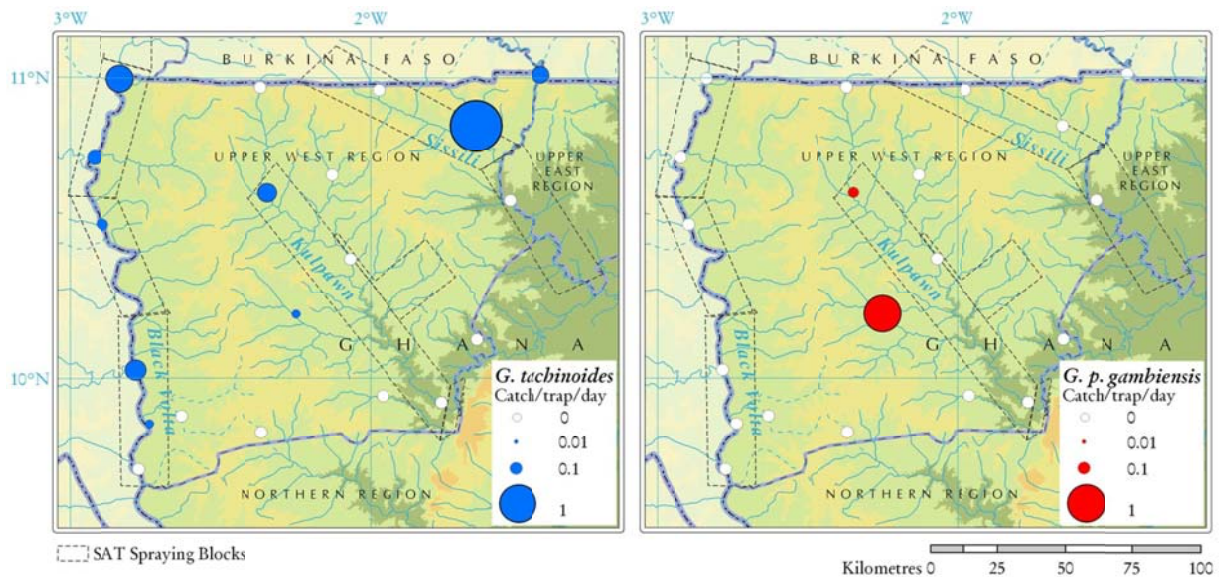


Figure 5.3 Results of the entomological survey conducted in Ghana one year after SAT operations. The survey was conducted from 6th to 24th June 2011 to monitor the impact of the integrated tsetse control campaign. Tsetse apparent density is expressed as the number of catches per trap per day (Adopted from - Adam Y., et al., 2013).

From the results in figure 3, we can conclude that the design of the post-intervention monitoring survey proved more efficient than the pre-intervention tsetse monitoring survey as tsetse were detected also outside the pre-intervention monitoring sites. Absence of capture does not mean absence of flies (Barclay et al., 2005). The efficacy of tsetse monitoring survey to determine presence or absence, largely depends on the methods employed for the selection and considerations for the selection of monitoring sites as presented in this survey.

The spatial distribution of tsetse captured in the post SAT monitoring survey, greatly exposes the inefficiency of the monitoring survey method employed in the pre-SAT and during the SAT operations (Adam Y., *et al.*, 2012 ; 2013). The spatial coverage of operations is an important factor to be considered when analysing the overall impact of interventions. . The capture of both *G. tachinoides* and *G. p. gambiensis* in a right-hand tributary of the Kulpawm during the post-intervention survey supports the notion that small pockets of flies may be sustained through the dry season. A similar situation was observed for *G. palpalis gambiensis* in dryer zones of its distribution area, for example in Senegal (Bouyer J., *et al.*, 2010). The detection of tsetse densities outside the main river basins with riverine vegetation in this study presents the post-intervention cross-sectional survey design more appropriate than the design of the pre-intervention monitoring technique. In conclusion, we recommend the design of post-intervention tsetse monitoring (Cecchi G. 2011, end of mission report) for use in subsequent tsetse intervention programmes.

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Chapter6: Environmental impact assessment

Assessment of the impacts of Sequential Aerosols Technique of Deltamethrin application for the suppression of Tsetse fly populations on aquatic and terrestrial invertebrates in the Upper West Region of Ghana

Adam Y., Bouyer J., Marcotty T., Mahama C.I., Grant I.F. *Assessment of the impact of Sequential Aerosols Technique of Deltamethrin application for the suppression of Tsetse populations on aquatic and terrestrial invertebrates in the Upper West Region of Ghana. Abstract from Pr. I.F. Grant's Report: May 2010*

Trypanosomosis is a vector born disease, mainly transmitted by tsetse flies in sub-Saharan Africa and affects both human beings and livestock. African animal trypanosomosis in Ghana causes a major constraint to economically viable livestock productivity and the optimal utilization of land for low input agricultural production (*Mahama et al.* 2004). In 2010, a large scale integrated tsetse eradication campaign, using the sequential aerosol technique of deltamethrin insecticid control agent in the Upper West Region of Ghana was initiated. Along side the intervention project an environmental impact assessment was conducted with the objective to determining the direct impact of deltamethrin on non-targeted aquatic and terrestrial arthropods in the control area. This aspect of the project also served as training exercise to build local capacity in environmental monitoring¹.

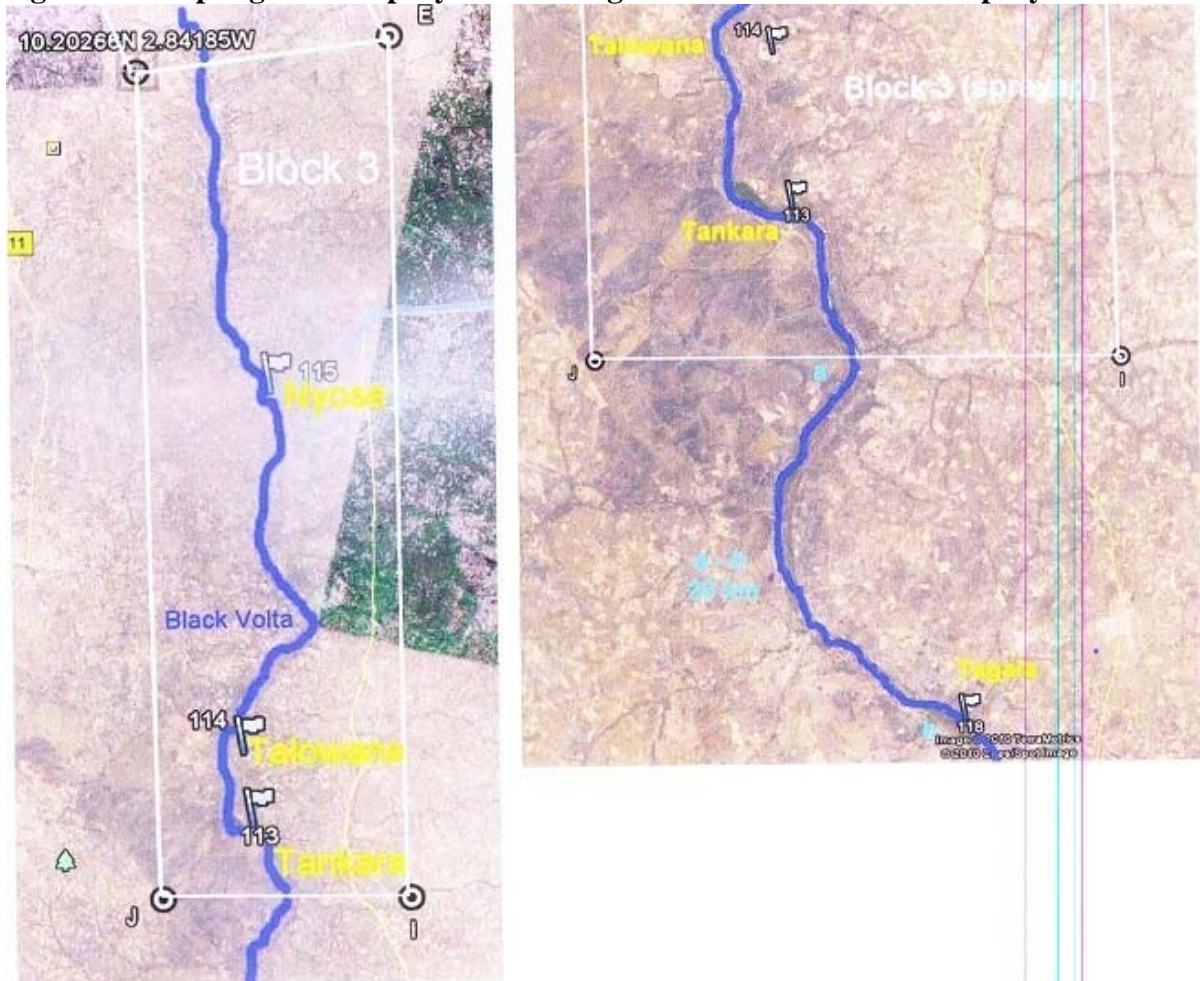
Deltamethrin is recommended as safe to mammalian species (including humans), reptiles, amphibians and fishes at the levels applied for SAT (*Elliot 1976; Grant 2010*). Deltamethrin products are among some of the most popular and widely used insecticides in the world for pest control. It belongs to one of the safest classes of pesticides: synthetic pyrethroids (*Metcalf, 2002*). While mammalian exposure to Deltamethrin is classified as safe, tsetse flies are particularly susceptible to Deltamethrin, even at the low dosage of 0.33a.i/ha, used in the SAT operation. Deltamethrin, like other synthetic pyrethroids, break down quickly in the environment (in a matter of days to weeks) and do not bio-accumulate but get metabolised by plants and animals (*Elliot, Janes & Potter, 1978; Grant and Crick 1987; Perry et al., 1998*).

Deltamethrin aerosols were applied in late dry season (April) from dusk to dawn in gentle winds, using 5 Turbo Thrush aircrafts (Orsmond Aviation, Bethlehem, South Africa). On the whole, 4 cycles of Deltamethrin treatments and 3 clean-ups were applied at an average dose rate of 0.34 g active ingredient (a.i)/ha.

¹ Two environmental consultants from UK

The environmental monitoring operations were conducted along a sprayed section of the Black Volta River, a riverine corridor where suitable conditions for monitoring the effects of SAT on both aquatic and terrestrial life were present. The impact of the SAT operations on aquatic arthropods was investigated using two sprayed and one unsprayed (control) sites along the Black Volta River (at Nyose, Tankara and Tagala respectively), while sampling sites for monitoring the impact on terrestrial arthropods were located in the riparian forest at Talawana, Nyose and Tagala (unsprayed). Temperature at monitoring sites ranged between 26°C and 35°C in the morning and often exceeded 40°C by midday in March and early April. Water temperature at sampling sites recorded between 29°C and 31°C in flowing water. Until the early rains began, suspended and dissolved solids were low. Sampling techniques employed for terrestrial arthropods included the use of Malaise traps for flying insects, pitfall traps for epigeal arthropods activity and funnel traps for knockdown of canopy dwellers. Aquatic arthropods were sampled using heel sampling technique for benthic invertebrates and drift nets for knock-down aquatic invertebrates (aquatic drift).

Figure1 : Sampling sites in spray block3 **Figure2 : Control site below spray block**



(Adopted from Grant's report 2010)

Results of post-spray searches at dawn for affected aquatic organism generally showed no evidence of vertebrate or invertebrate mortality. Juvenile fish, aquatic bugs and decapods (Belastomatidae, Nepidae, prawns and crabs) that usually display symptoms of stress to deltamethrin, such as hyperactivity or erratic swimming, behaved normally. Inspections of the undersides of rock showed the presence of mayfly nymphs and dragonfly larvae. The observed downstream drift of aquatic invertebrates before and after SAT cycles 1, 2, 3 and 4, at all sites, was negligible – perhaps one or two nymphs in 24h. Thus, no significant changes in invertebrate drift were evident before and after the SAT despite the presence of groups normally sensitive to deltamethrine such as the mayflies, dytiscids, dragon fly and naucorid

bugs (SEMG (ed.) 1987; 1993). The situation was not different with benthic invertebrates (Heel sampling) Species diversity and abundance of benthic invertebrates were similar at monitoring sites. The density of benthic invertebrates caught was low – but not unusual for large rivers like the Black Volta. Samples of benthic invertebrates included a number of families with known sensitivity to deltamethrin such as nymphs and larvae of Plecoptera, Ephemeroptera, Anisoptera, Dytiscidae and Nepidae.

Terrestrial arthropods activity was kept low during this period (late dry season) due to the associated harsh environmental conditions - the combination of heat, low humidity and no precipitation – kept arthropods presence and activity suppressed. The only common flying insects observed at all monitoring sites along the Black Volta were muscids, grasshoppers, plant-hoppers, vespids and day-flying Lepidoptera, while ants and cockroaches dominated the epigeal fauna. Fall-out rate of canopy dwelling arthropods was negligible.

Two periods were defined for comparative analysis of the dry matter emanating from trappings at the 3 sites: before and after the spraying of Deltamethrin insecticidal aerosols. A regression analysis was carried out using the periods, the sample sites and the interaction between them as explanatory variables. The regression analysis was run using the STATA 10 statistical tool.

The average biomass before spraying ranged between 0.3g dry wt. and 0.4g dry wt. per trap and the average reduction after the chemical spray was 0.2g dry wt. (95% CI: -0.2 – 0.6). Interactions between the explanatory variables were not significantly different from 0, indicating that the difference between the dry matter before and after spraying was similar in the 3 sites.

Malaise trap catches were fairly evenly matched at Nyose and Talawana. Sample variation at Tagala was greater. Before spraying, the abundance and groups of insects trapped at all sites were broadly similar. Over the spray period, the qualitative picture of non-targeted arthropods across all sites was good enough to observe any large population changes induced by Deltamethrin. There was no evidence that either moths or butterflies were adversely affected by Deltamethrin. Moths were present at both sprayed sites throughout the SAT. Bees were scarce throughout the SAT operation but wasps were regularly trapped at all sites.

The susceptibility of both terrestrial and aquatic arthropods to deltamethrin is largely dependent on a range of factors including dose, exposure and environmental conditions (SEMG (ed.) 1987; 1993). In Ghana's SAT operations, unlike others with bad examples of deltamethrin application in West Africa (Nigeria and Burkina) (*Takken et al. 1978*), the choice of late dry season for the SAT operations under harsh, dry season conditions – no moisture, low humidity and higher temperatures above annual averages served as benign factors and could be responsible for the attenuated neurotoxic effect of deltamethrin that was observed in this study.

We conclude that deltamethrin at low dosages as used in the SAT operations did not lead to significant changes in the non-target terrestrial arthropod populations. At the same dose rate we can also conclude that the effect of deltamethrin on vertebrate or invertebrate aquatic arthropod populations is insignificant.

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I am most grateful to Professor Ian F. Grant (Cybister Environmental Protection UK) for giving me training and guidance through this study.

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Table1 Environmental Measurements: Aquatic & Terrestrial (Grant's report 2010)

SITE	DATE	20.3.10	23.3.10	24.3.10	25.3.10	29.3.10	5.4.10	7.4.10	11.4.10	16.4.10	20.4.10	25.4.10	29.4.10	6.05.10
Tagala 271masl 215	Air Temps			43.4	40.5	35.8	34.8	31.3	30.2	34.1	32.1		31.4	
	% Relative Humidity			7.7	32.2	48.2	49	60.2	66	57.6	60.7		66.8	
	Wind speed			0.9	2.1	0.4	0.4	0.4	0.2	0	0.5		0	
	Light (open/shaded)				open									
	Pressure Mb				987	988	986	986	987.2	986.6	988.6		988.8	
	Water Temps				30.5	31.8	30.8		30.4		31.3			
	pH water				9.92		9.2	8.9	8.96	8.67	8.65	8.81	8.08	
	Dissolved Oxygen				10.2		9.3	10.7	11.7	13.4	15.9	4.7	18.8	
	Conductivity				0.15		0.17	0.15	0.17	0.13	0.17	0.16	0.18	
	Current (m/sec)						0.56	0.52	2.1	1.7	1	1.3	1.2	
	Total Solids				0.07		0.08	0.07	0.08	0.06	0.08	0.08	0.09	
	Colour							hazy	hazy					
	Substratum													
Nyose 236masl	Air Temps			38		29.4	29.5	26.9	30.1	31.8	31.9	30.1	30.1	28
	% Relative Humidity	46	13.5			34.4	64	76	62.5	56	55.4	66.4	72.6	73.2
	Wind speed (x)	1.2	2			1.4		0	0.9	0.2	2.2	0	0	0.2
240 terr	Light (open/shaded)													
	Pressure Mb			985		986	983	982	984	984.6	985.5	986	984.8	983.7
	Water Temps	36	28.2			33								
	pH water		8.6											
	Dissolved Oxygen		6.3											
	Conductivity		0.18											
	Current	0.423	0.57						0.54					
	Total Solids		0.09											
	Colour		hazy											
Talawana/ Tankara 225masl	Air Temps	40.8				36	34.5	33.4	33.6	31.5	32.4	32.2	32	33.2
	% Relative Humidity	22.6				42.6	47	58	55.4	59.9	52	63.5	67.6	61.4
	Wind speed	1.4-4.2				3.9	5.8		1.5	0	0	0	2.4	0.2
	Light (open/shaded)													
	Pressure Mb	989				986			985.6	985.5	986.5	987.5	987.1	987.6
	Water Temps													30.7
	pH water	9.3												8.8
	Dissolved Oxygen	7.9												
	Conductivity	0.1												0.16

Chapter7: General Discussion & Conclusion

Risk Factors of Animal Trypanosomosis in the UWR and monitoring

Impact of Vector Eradication

Intervention in Ghana

7.1. Introduction

Over the years, affected African countries have been battling with the problem of tsetse and trypanosomosis on individual country basis. Understanding the trans-boundary nature and habitat preference of the tsetse fly therefore is fundamental to the development of a sustainable control strategy against AAT in the continent. Commitment at the continental level to address the trans-boundary problem of tsetse and trypanosomosis was demonstrated by African Heads of State and Governments in Lome, in the year 2000, when they passed a resolution that essentially called for a concerted effort between countries in the fight against tsetse flies.

The application of tsetse control methods one at a time though achieving some level of successes, failed to achieve elimination in Ghana (Mahama *et al.*2004). The current thesis was premised on the hypothesis that cross-border collaboration with a combination of multiple control methods could bring about tsetse elimination over a large area.

The objectives of this thesis were ; 1) to confirm the hypothesis that biological vectors of trypanosomosis were still substantially present in spite of the many control efforts that have taken place since the beginning of colonial rule in Ghana ; 2) to confirm that the use of SAT and other complementary control efforts in an integrated approach could sustainably suppress tsetse population to a level that can bring about vector and disease free status in the Upper West Region of Ghana; and 3) to demonstrate that monitoring the impact of the integrated

control effort on non-target species was crucial to ensure the environmental acceptability of the methods used.

7.2. Vector and Trypanosomosis (Disease) surveys

Tsetse and trypanosomosis control surveys come in two forms (pre-and post-intervention surveys). The objective of the pre-intervention survey was to establish baseline tsetse and trypanosomosis prevalence in the targeted control area. The post-intervention survey is equally essential for the analysis of the impact of the intervention, relative to the baseline situation. To embark on a technologically advanced tsetse control programme such as the application of SAT, it was pertinent to conduct baseline survey to establish the intensity of the problem and to guide the planning of control programmes. Results of baseline survey formed the basis for the planning of the control and monitoring strategies as observed in chapter 2 of this thesis. The baseline survey revealed two riverine *Glossina* species (*G. p. gambiensis* and *G. tachinoides*) inhabiting narrow bands of riverine forest galleries of the three main rivers in the study area (Black Volta, Kulpawn and Sissili rivers). It is important to note that these three rivers, traversing the study area, form the major risk factors of bovine trypanosomosis disease in the Upper West Region. Even though mean tsetse apparent density (ADT) of 16.7 and 0.2 was recorded for the pre and post intervention periods respectively, the highest ADT for both periods were recorded in the southern most of the study area with thick forest galleries. This confirms natural forest galleries and their border as risk factor for tsetse and trypanosomiasis (Bouyer et al. 2006, Guerrini and Bouyer 2007). The mean ADT of 0.2 in the post intervention period survey is a confirmation of eradication target failure. Possible reason for the eradication target failure can be attributable to the failure of Deltamethrin aerosols to penetrate the thick forest galleries that form tsetse habitat for the riverine tsetse species in the study area. However no unquestionable proof for this assumption was delivered

and other reasons may have been (partly) responsible for the eradication target failure. The success of a similar tsetse control programme in Botswana (Kgori *et al.*, 2006) was largely because of the open savannah tsetse habitat, the tsetse species and the early placement of insecticide barriers before the intervention. Other methods like the SIT, efficient at very low densities of the target population, might be considered to mop up remnant flies (Vreysen *et al.* 2013b). This notwithstanding, the isolation of the treatment area is as essential for SIT as it is for SAT. The difficulty here, however, is the sustainability of maintaining a tsetse-free zone in the middle of tsetse-infested countries.

While parasitological survey in the study area revealed prevalence of two species of pathogenic trypanosomes (*T.vivax*, and *T.brucei*), a serological survey revealed all the three species including *T. congolense* (Adam *et al.*, 2012). The occurrence of bovine trypanosomosis in the study area as revealed in the baseline survey was probably related to the presence of the two cyclical vectors of trypanosomosis detected parasitologically.

The findings of the baseline survey enabled an integrated tsetse elimination strategy to be developed, which combined aerial spraying (Sequential Aerosol Technique–SAT), ground spraying, insecticide treated targets and cattle pour-on.

7.3. Study of *G. tachinoides* population structuring in the Upper West Region of Ghana.

From the baseline survey (Chapter2) it was found that *G. tachinoides*, the main vector of AAT in the UWR, was widespread along the three main rivers (Black Volta, Kulpawn and Sissili). For a successful implementation of vector control, it was deemed important exploring the dispersal capabilities and isolation status of the target population. Population genetics was therefore employed as a tool to determine structuring of the *G. tachinoides* population so as to inform decision making in the control strategy.

Population genetic studies have been conducted in previous tsetse control campaigns and have proven their usefulness in exploring the dispersal capabilities and isolation status of the target population (Solano et al. 2009, Solano et al. 2010b, Solano et al. 2010a). The application of population genetic methods to disease vectors offers insights into facets of population structuring, dispersal patterns, population dynamics and genetic diversity that can have direct relevance for guiding control programmes. In recent timescales, population genetics has been employed to delineate isolated vector populations that may be appropriate targets for local eradication and can identify dispersal routes that must be disrupted to prevent re-infestation of previously cleared zones (Bouyer et al. 2010, Koné et al. 2011, Vreysen et al. 2013a).

Results of the survey showed that dispersal of *G. tachinoides* in the UWR was estimated to be lower than in Burkina Faso, situated north of the study area. However, the observed dispersal is deemed sufficient for a tsetse-cleared area to be reinvaded from neighbouring populations in adjacent river basins. These data corroborate results from other population genetics studies indicating that in West Africa *G. tachinoides* populations from different river basins cannot be considered isolated from one another (Kone, *et al.*, 2010; Bouyer *et al.*, 2010).

Barriers to prevent reinvasion would have to be established between SAT-treated blocks and between river basins and maintained in a continuous suppression effort to prevent a possible re-invasion of treated blocks. Deltamethrin-treated biconical traps deployed at 100m intervals in riparian forest along a 7km river section successfully prevented migration of *G. p. gambiensis* and *G. tachinoides* in Burkina Faso (Cuisance and Politzar 1983).

7.4. Integrated application of SAT and other complementary control methods

Integrated tsetse control efforts as reported in this thesis re-emphasises the importance of integrating a number of control methods with SAT to ensure a sustainable tsetse and trypanosomosis suppression/elimination over large areas within a relatively short period. Whereas tsetse control techniques such as the use of traps/targets, insecticide treated cattle (ITC) and ground spraying (GS) are considered as supplementary control efforts, SAT is considered a major control effort due to the relatively larger aerial coverage within a relatively shorter period.

The SAT technique is based on spraying an area with an insecticide from aircraft repeatedly at intervals depending on the development time of the first larvae in females and the total duration of pupation. The first spray is designed to kill all adult tsetse flies in the area. . Four subsequent sprays kill tsetse as they emerge from puparia buried in the ground. This technique works well on a large scale and is the most rapid of the techniques, achieving elimination in as little as two months, costing US\$ 590 per sq km (Shaw, *et, al.*, 2013). In Botswana, 16,000 km² of the Okavango Delta were aerial sprayed five times with Deltamethrin, applied at 0.26–0.3 g/ha, to control *Glossina morsitans centralis* Machado over a period of 8 weeks approximately (Kgori, *et, al.*, 2006). And in Ghana a total area of 6,745km² of forest galleries along three main rivers of the Upper West Region was sprayed four times with non-residual insecticide aerosols at a dose rate of 0.33-0.35g a.i/ha, from the air within a period of about one month and achieved 98% tsetse suppression. This level of suppression, covering approximately 18,000km² in one month of SAT operations and one year of continuous suppression effort, even though commendable, failed to achieve the main objective of tsetse elimination, probably because the ultimate integrated tool (SIT) was not applied to mop up remnant flies in the tick gallery forest. Other reasons that may have been

(partly) responsible for the eradication failure, in the absence of unquestionable prove of droplet penetration failure into the thick forest galleries (Cooper JF; Dobson HM 1993) are:

1) Differences in susceptibility to deltamethrin between the different tsetse fly species, which may necessitate additional studies on the appropriate deltamethrin dose to be used in SAT against tsetse species other than *G.m.morsitans*. *G.p.gambiensis* seems to be particularly resistant to the insecticidal action of deltamethrin (De Deken *et, al.* 1998).

2) Differences in fly size: *G. tachinoides* flies have a smaller size than *G.m.morsitans* or *G.p.gambiensis* and thus will capture less insecticidal droplets on their body. This makes *G. tachinoides* less vulnerable to the aerosol application (Johnstone, D.R., *et, al.*, 1989).

3) As reported by Harris, E.G., *et al.* (1990) and Childs, S.J. (2011), there is a negative correlation between the insecticidal efficacy of deltamethrin and temperature. This negative correlation may have been the reason why in this study, notwithstanding a higher dose of deltamethrin was used than in Zimbabwe, the environmental impact of the aerial spraying on non-target organisms was lower than the one observed during the RTTCP campaign in Zimbabwe (Report 1987 – 1990 ; Nagel, P., 1993).

4) The insufficient and late isolation of the SAT-treated areas to prevent invasion of flies from untreated areas within the study area and from outside the study area.

One may wonder if, in the conditions of high environmental temperatures encountered during this study, it wouldn't have been more opportune to use the organophosphorous insecticide endosulfan, which is more efficacious at higher temperatures and had according to the observations of the SEMG (at the then in Zimbabwe prevailing climatic conditions) also had no irreversible impact on non-target organisms (Nagel, p., 1993 and Magadza, C.H.D., 1978).

The occasional daytime spraying carried out as a modification of the standard SAT procedure is to be avoided as anabatic uphill winds may be expected under the weather conditions of Ghana when SAT is carried out over a valley. Even if weather conditions were inappropriate, the aerial spraying could be postponed since The “Jackson” formulae on first larval period

and pupal duration have meanwhile been improved by John Hargrove (2004). According to the formula of Hargrove the FLP at 31.09°C wouldn't have been 11 days but 13.3 days. So it was possible to extend the periods between the spraying cycles up to 2 days.

7.5. Impacts of Deltamethrin Aerosol application for tsetse suppression on aquatic and terrestrial invertebrates in the Upper West Region of Ghana.

Deltamethrin synthetic pyrethroid was the main insecticidal agent used in the tsetse control programme in the UWR. Being a large scale, insecticide-based method, it had the potential to cause adverse side effects on the riverine environment. Provision was therefore made for an environmental monitoring component of the intervention to determine the level of impact on the ecology. Monitoring was focused on the direct impacts of Deltamethrin on aquatic and terrestrial arthropods.

Deltamethrin is recommended as safe to mammalian species (including humans), reptiles, amphibians and fish at the levels applied for SAT (Elliot 1976; Ian-Grant 2010). Deltamethrin products are among some of the most popular and widely used insecticides in the world and have become very popular with pest control. It belongs to one of the safest classes of pesticides: synthetic pyrethroids (Metcalf, 2002). While mammalian exposure to Deltamethrin is classified as safe, tsetse flies are particularly susceptible to Deltamethrin, even at the low dosage of 0.33a.i/ha, used in the SAT operation. However, the use of Deltamethrin synthetic pyrethroids is less problematic to the environment as they break down quickly in the environment (in a matter of days to weeks) and do not bio-accumulate but get metabolised by plants and animals (Elliot, Janes& Potter, 1978; Grant and Crick 1987; Perry *et al.* 1998;).

Post spray searches at dawn for affected organisms showed no evidence of vertebrate or invertebrate mortality. Juvenile fish, aquatic bugs and decapods (Belastamethidae, Nepidae,

prawns and crabs) that usually display symptoms of stress to Deltamethrin, such as hyperactivity or erratic swimming, behaved normally. Inspections of the undersides of rocks showed the presence of mayfly nymphs and caddis fly larvae.

7.6. The integrated Control Strategy

The integrated tsetse control strategy adopted in Ghana allowed the suppression of tsetse and the subsequent control of trypanosomoses. A continuous suppression effort in the intervention areas is however very expensive and its costs should be progressively transferred to the farmers based on local-IPM strategies (Bouyer, *et al.*, 2013). The elimination would be possible only if the “rolling carpet” across common borders could be applied, which is not the case presently.

7.7. Conclusion

The challenge of controlling/eliminating tsetse and trypanosomosis in sub-Saharan Africa is daunting and continues to attract more research work that delve into the realm of possible control strategies with the ultimate objective to ensuring a tsetse free Africa. Contributing to the achievement of this goal, we conclude this thesis with highlights on the major findings and recommendations of the operational research work: 1) the three rivers (Black Volta, Kulpaw and Sissili), traversing the study area, form the major risk factors of bovine trypanosomosis in the Upper West Region; 2) the existence of portions of uninterrupted forest galleries along these rivers provides not only tsetse habitat but also habitat for wildlife that serve as blood meal for tsetse and partial protection against SAT; 3) Despite the limited dispersal capability of tsetse populations estimated in the UWR, the observed dispersal was still enough to trigger tsetse reinvasion in tsetse cleared areas; 4) Failure of the control programme to achieve complete tsetse elimination in the UWR was probably due to the

absence of SIT in the integrated strategy, necessary to mop up remnant flies in the forested southern limits where the impact of SAT was deficient.

Thus, to begin any comprehensive tsetse control programme as envisaged by the technical committee of PATTEC we recommend that: 1) a robust feasibility study be made to select the best strategy (elimination versus suppression) before any control campaign; 2) in sites where elimination is possible, apply an Integrated approach involving SAT, Ground spraying, Insecticide treated cattle/targets and SIT based on the “tsetse without border” concept: eradicating tsetse flies from one “pocket/island” before dealing with the next one by coordinating efficiently all the efforts between countries. The methods should be carefully selected according to the target tsetse species and the type of vegetation; 3) - in sites where elimination is not possible: include local-IPM of trypanosomosis in the general strategy of PATTEC.

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Summary of Thesis

“Theme: Evaluation of Risk Factors of Animal Trypanosomosis in Ghana, and the Monitoring of the Impact of Disease and Vector Eradication Intervention in the Upper West Region of Ghana”

African animal trypanosomosis (AAT) is a major constraint to viable and sustainable livestock production systems in Ghana. The disease limits the utilization of abundant natural pastures across the country with the attendant poverty and malnutrition amongst rural populations. Over the years, the Veterinary Services Directorate of the Ministry of Food and Agriculture in Ghana has been committed to controlling AAT. However, despite past control efforts, the problem of AAT persists. In 2001, the African Union launched the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), advocating eradication of tsetse populations across common borders as the most sustainable way of controlling trypanosomosis in sub-Saharan Africa. Under the umbrella of the PATTEC, Ghana is collaborating with Burkina Faso in a sub-regional initiative aiming at creating tsetse-free areas across their common borders. The objective of this thesis was to conduct research to guide project implementation by providing rational intervention strategies for tsetse and trypanosomosis eradication. Specifically, the research seeks to i) determine the pre-intervention vector and disease situation of the PATTEC intervention area in Ghana, ii) determine tsetse population structuring and the consequences on sustainable tsetse control efforts, iii) evaluate SAT for the control of riverine tsetse species in Ghana, iv) evaluate the environmental risk of the intervention programmes

Chapter one of this thesis deals with a general review of bovine trypanosomosis; epidemiology and risk factors of the disease, vector control methods and the continental approach to tsetse control/eradication. The occurrence of pathogenic trypanosomes (*Trypanosoma vivax*, *T. Congolense* and *T. brucei*) and the wide variation in the prevalence of

trypanosomosis is a major feature of the epidemiology of the disease in Africa and is consistent with the correlation between tsetse apparent density and the risk of trypanosomosis described by (Rogers 1985, Mahama, *et al.*, 2003). The African pathogenic trypanosomes affecting human or livestock belong to the Salivaria group, 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 infected animal. On the other hand two subspecies of *Trypanosoma brucei rhodesiense* and *T. b. Gambiense* cause disease in humans (FAO, 1998). Geographically *T. b. rhodesiense* is found in East Africa but *T. b. gambiense* is found in Central and West Africa. Tsetse flies are the cyclical vectors of trypanosomosis. Risk factors of trypanosomosis are largely attributed to tsetse habitat suitability (Vegetation cover and water sources) and the presence of animals as sources of blood meal for the tsetse fly. The major epidemiological risk factor in African trypanosomosis is contact between humans and tsetse flies. This interaction is influenced by an increasing tsetse fly density, changing feeding habits and by expanding human development into tsetse fly-infested areas. African animal trypanosomosis is a Trans-boundary Animal Disease (TADs) with the potential for spread along transhumance livestock rearing systems in sub-Saharan Africa. Tsetse and trypanosomosis control employs two complementary strategies aimed at reducing or eliminating the disease. The two strategies are chemotherapeutic, targeted at reducing the incidence of trypanosomosis in livestock and entomological, aimed at disrupting the cycle of transmission of the disease by reducing or eliminating the vector of trypanosomosis (Bouyer *et al.* 2010). Vector (tsetse) control techniques have gone through evolutions, with earlier crude methods recently being replaced by methods that are economically cheaper, more directed, and ecologically sustainable. These techniques include: Sequential aerosol spraying (SAT), pesticide campaigns, traps/targets, animal baits and the use of Sterile Insect Technique (SIT).

In the second chapter of the thesis we report on the conduct of a baseline survey in the Upper West Region of Ghana to assess the distribution and densities of tsetse species, as well as the prevalence of bovine trypanosomosis. The entomological survey was designed to cover the suitable tsetse habitats along the three main rivers in the study area (i.e. Black Volta, Kulpaw and Sissili). Biconical traps (*Challier et al.*, 1977) were deployed in the suitable riparian vegetation and at cattle watering points. An average of 20 traps was deployed at each site at 200 m spacing. The location of each trap was recorded with a Global Positioning System (GPS). A total of 564 traps were deployed along the three rivers. Traps deployed at 8 am were recovered the following day at the same time, having been inspected twice within that period. Thus, tsetse flies were collected twice daily and the number, species and sex of flies caught determined and recorded. An index of apparent abundance (IAA) of tsetse was calculated as the number of flies (males and females) caught per trap per trapping day. Results indicated the presence of *Glossina tachinoides* in all three river basins, whilst *Glossina p. gambiensis* was only found close to the southern limit of the study area. The highest values of apparent abundance (IAA > 100) were found in the South-western tip of the Upper West Region, along the Black Volta River where the riverine forest gallery was thickest. For the survey of disease situation, a random sampling of 1800 cattle of the West African Short Horn, Sanga and Zebu breeds of cattle from 36 randomly selected grid cells covering the study area was conducted. Results showed substantial differences between parasitological and serological prevalence. The average parasitological prevalence, using the Buffy Coat measure, was estimated at 2.5% (95% CI: 1.06–5.77) with the majority of the infections due to *Trypanosoma vivax*. Most of the infected cattle were found close to the major river systems (A major risk factor). The serological prevalence measured, using Enzyme Linked Immunosorbent Assay (ELISA) test, was 19% (95% CI: 14.03–25.35). Cattle with anti-trypanosomal antibodies were also found throughout the study area.

We concluded in this study that *G. tachinoides* and *G. p gambiensis* are the main vectors of trypanosomosis in the Upper West Region of Ghana. Entomological surveys conducted along the three main river systems suggest a possible southward retreat of the tsetse fly in line with the existing riverine vegetation cover. However, trypanosomosis remains widespread in the Upper West Region, with the predominance of infections by *T. vivax* and, to a lesser extent, *T. b. brucei*. The findings of the present study enabled an integrated tsetse elimination strategy to be developed, which combines aerial spraying (Sequential Aerosol Technique–SAT), ground spraying, insecticide treated targets and cattle pour-on. Implementation started in the period March–May 2010 with SAT operations, conducted along the three main rivers.

In chapter three we investigated the structuring of *G. tachinoides* populations within and between the three main river-basins of the target area in the UWR and a neighboring population at the north-east of the target area. The background situation that necessitated this study was that: Genetic structuring of tsetse populations in the UWR of Ghana had not been studied before. The riparian vegetation along the three main rivers is heavily fragmented, and their tributaries become largely unsuitable for riverine tsetse during the dry season (December-May). Bush fires and the absence of rain during this season seriously limit the availability of favorable vegetation ([Adam et al., 2012](#)), so that tsetse flies retract from the tributaries to residual spots along the main rivers, where permanent water and vegetation mitigate the harsh ambient macroclimatic conditions (e.g. temperatures often rise above 40°C). Whilst the Black Volta River is a permanent water course, the Kulpawn and Sissili are seasonal. It was therefore hypothesized that it might be more challenging for tsetse to disperse between the main rivers in the UWR of Ghana than between the Mouhoun and adjacent river basins in Burkina Faso. The objective of this study was therefore to determine whether tsetse populations in the UWR of Ghana were able to disperse between river basins, which were

used as the basic operational units for the SAT operations, and hence, whether the tsetse populations of the target area ([Adam et al., 2013](#)) could be considered as isolated from adjacent north-eastern populations. The study focused on *G. tachinoides*. Results of this study was used by managers of the eradication campaign to inform decisions on the potential need to establish adequate barriers to prevent re-invasion of areas where tsetse populations are reduced to very low levels. Eight sites located along the three rivers were investigated. The selected sites had been previously studied in terms of tsetse species composition and abundance ([Adam et al., 2012](#)). Out of a total sample of 884 flies, a sub-sample of 266 was genotyped at nine microsatellite loci. The significance of the different hierarchical levels was tested using Yang's parameters estimated with Weir and Cockerham's method. A significant effect of traps within groups (pooling traps no more than 3 km distant from each other), of groups within river basins and of river basins within the whole target area was observed. Isolation by distance between traps was highly significant. A local density of 0.048-0.061 flies/m² was estimated and a dispersal distance that approximated 11 m per generation [CI 9, 17]. No significant sex-biased dispersal was detected. Dispersal distances of *G. tachinoides* in the UWR were relatively low, possibly as a result of the fragmentation of the habitat and the seasonality of the Kulpawn and Sissili rivers. Moreover, very high fly population densities were observed in the sample sites, which potentially reduce dispersal at constant habitat saturation, because the probability that migrants can establish is reduced (density dependent dispersal). However, the observed spatial dispersal was deemed sufficient for a *G. tachinoides*-cleared area to be reinvaded from neighboring populations in adjacent river basins. These data corroborate results from other population genetics studies in West Africa, which indicate that *G. tachinoides* populations from different river basins cannot be considered isolated.

In the fourth chapter we report on the implementation and evaluation of the efficacy of an integrated strategy of intervention against tsetse flies. The study was conducted in the Upper West Region of Ghana (9.62° – 11.00° N, 1.40° – 2.76° W), covering an area of $\approx 18,000$ km² within the framework of the Pan-African Tsetse and Trypanosomosis Eradication Campaign. Two species were targeted: *Glossina tachinoides* and *Glossina p. gambiensis*. The objectives were to test the potentiality of the sequential aerosol technique (SAT) to eliminate riverine tsetse species in a challenging subsection (dense tree canopy and high tsetse densities) of the total sprayed area (6,745 km²) and the subsequent efficacy of an integrated strategy including ground spraying (≈ 100 km²), insecticide treated targets (20,000) and insecticide treated cattle (45,000) in sustaining the results of tsetse suppression in the whole intervention area. The aerial application of low-dosage deltamethrin aerosols (0.33–0.35 g a.i./ha) was conducted along the three main rivers using five custom designed fixed-wings Turbo thrush aircraft. The impact of SAT on tsetse densities was monitored using 30 biconical traps deployed from two weeks before until two weeks after the operations. Results of the SAT monitoring indicated an overall reduction rate of 98% (from a pre-intervention mean apparent density per trap per day (ADT) of 16.7 to 0.3 at the end of the fourth and last cycle). One year after the SAT operations, a second survey using 200 biconical traps set in 20 sites during 3 weeks was conducted throughout the intervention area to measure the impact of the integrated control strategy. Both target species were still detected, albeit at very low densities (ADT of 0.27 inside sprayed blocks and 0.10 outside sprayed blocks). The SAT operations failed to achieve elimination in the monitored section, but the subsequent integrated strategy maintained high levels of suppression throughout the intervention area, which will contribute to improving animal health, increasing animal production and fostering food security. Failure to achieve elimination was largely due to the difficulty of the SAT aerosols to penetrate the thick gallery forests in the southern limits of the intervention area. Other causes of failure could be as follows, among

others:

- 1) the differences in sensitivity to deltamethrin between different species of tsetse flies. *Glossina palpalis gambiensis* particularly is highly resistant to the action of deltamethrin insecticide.
- 2) The size of the fly: *G. tachinoides* has a size less than *G. m. morsitans* and *G. p. gambiensis* and thus capture fewer droplets containing the insecticide on their body. This makes *G. tachinoides* less vulnerable to aerial application.
- 3) Since there is a negative correlation between the effectiveness of deltamethrin and the ambient temperature, the insecticide was less toxic in the climatic conditions of Ghana.
- 4) Inadequate and delayed isolation of areas treated by aerial application probably does not prevent the invasion of flies from untreated areas within or outside the study area.

The fifth chapter presents an abstract on two comparative study designs for the selection of entomological monitoring sites employed in the pre- and post-integrated Tsetse Control Programmes of the Upper West Region of Ghana, established in collaboration with Giuliano Cecchi, GIS expert at FAO_PAAT. The objective of this study was to recommend an appropriate study design for the selection of tsetse monitoring sites in future control programmes. Potential tsetse habitat is hypothesized on the basis of land cover / vegetation (Cecchi G., *et al.*, 2008), water bodies and the availability of hosts (livestock and wildlife), which are known to have a direct relation to tsetse densities and the risk of cyclical trypanosomosis. In the pre-intervention tsetse density determination, a systematic sampling procedure was implemented along the three main rivers and within the SAT spray blocks (Adam Y., *et al.*, 2012 and 2013). The pre-intervention monitoring procedure was based on the hypothesis that tsetse flies retract from tributaries to the riparian forest along the main rivers in the UWR during the dry season after bush fires have cleared the vegetation cover and with prevailing high temperatures (mean day temperatures above 40°C). Sampling was therefore limited to the riparian forest along the three main rivers of the Upper West Region, hence tsetse was not found outside the riparian forest along the main rivers (Adam Y., *et al.*,

2012). On the other hand the post SAT tsetse monitoring procedure was set up to verify the hypothesis of the pre-intervention monitoring procedure and to provide evidence that tsetse flies could still be present outside the SAT treated spray blocks and in some of the river tributaries, to validate the results of the population genetics study. Landsat7 Satellite Images, as well as other ancillary GIS data, were used to guide the selection of 20 monitoring sites for the post-SAT tsetse monitoring (Cecchi G., 2011 Mission report). The primary guide in selecting sampling sites for monitoring riverine tsetse was vegetation cover along rivers, tributaries and game reserve border lines with river crossings. By visual appraisal, 12 sampling sites were identified within the spray blocks and 8 sites outside the treated blocks (Figure2). The number of 20 sampling sites was established taking financial and logistic constraints into consideration (Cecchi G., 2011 Mission report). The post-SAT tsetse monitoring survey found tsetse flies outside the riparian forest along the main rivers, contrary to the pre-SAT monitoring hypothesis. Both *G. tachinoides* and *G. p. gambiensis* were captured in two sites outside the SAT sprayed blocks on the tributaries of the main rivers (Adam Y., *et al.*, 2013). The spatial distribution of tsetse captured in the post SAT monitoring survey, revealed a lack of efficiency of the monitoring survey method employed in the pre-SAT and during the SAT operations of Adam Y., *et al.*, (2012 and 2013). The spatial coverage of operations is an important factor to be considered when analysing the overall impact of interventions. The capture of both *G. tachinoides* and *G. p.gambiensis* in a right-hand tributary of the Kulpawn during the post-intervention survey supports the notion that small pockets of flies may be sustained through the dry season. A similar situation was observed for *G. palpalis gambiensis* in dryer zones of its distribution area, for example in Senegal (Bouyer J., *et al.*, 2010). In conclusion, we recommend the design of the post-intervention tsetse monitoring effort (Cecchi G. 2011, end of FAO mission report) for use in subsequent tsetse intervention programmes.

In chapter six, we present an abstract of an environmental impact assessment study of Deltamethrin application in an integrated tsetse control programme that was conducted in collaboration with Ian F. Grant, expert in environmental monitoring at Cybister Environmental Protection, UK. The environmental impact assessment was conducted alongside the PATTEC intervention programme of SAT to control two riverine tsetse fly species along the three main rivers of the Upper West Region of Ghana (Black Volta, Kulpaw and Sissili Rivers). The objective was to determine the level of Deltamethrin impact on the ecosystem. Monitoring was focused on the direct impacts of Deltamethrin on non-targeted aquatic and terrestrial arthropods. Of the 8 Spray Blocks targeted for the SAT operations, the environmental monitoring operations were sited in Block3 at the lower stretch of the Black Volta River where suitable conditions for monitoring the effects of SAT on both aquatic and terrestrial life were present. Deltamethrin aerosols were applied in late dry season (April) from dusk to dawn in gentle winds, using 5 Turbo Thrush aircrafts (Orsmond Aviation, Bethlehem, South Africa). On the whole, 4 cycles of Deltamethrin treatments were applied at an average dose rate of 0.34 g active ingredient (a.i)/ha. Two monitoring and one control sites were chosen along the Black Volta River at Nyose, Tankara and Tagala respectively for monitoring the impact of the SAT operations on aquatic arthropods. Sampling sites for terrestrial monitoring and control were located at Talawana, Nyose and Tagala respectively, along the forest gallery of same segment of the Black Volta River. Sampling techniques for terrestrial arthropods included the use of Malaise traps for flying insects, Pitfall traps for epigeal arthropods activity and Funnel traps for knockdown of canopy dwellers. Drift and Heel sampling techniques, on the other hand, were employed for aquatic arthropods. Results of post spray searches at dawn for affected aquatic organism generally showed no evidence of vertebrate or invertebrate mortality. Juvenile fish, aquatic bugs and decapods (Belastamethidae, Nepidae, prawns and crabs) that usually display symptoms of

stress to Deltamethrin, such as hyperactivity or erratic swimming, behaved normally. Inspections of the undersides of rock showed the presence of mayfly nymphs and caddis fly larvae. The average biomass before spraying ranged between 0.3 and 0.4 and the average reduction after the chemical spray was 0.2g (95% CI: -0.2 – 0.6). Interactions between the explanatory variables were not significantly different from 0, indicating that the difference between the dry matter before and after spraying was similar in the three monitoring sites. Malaise trap catches were fairly evenly matched at Nyose and Talawana. Sample variation at Tagala was greater. Before spraying, the abundance and groups of insects trapped at all sites were broadly similar. Over the spray period, the qualitative picture of non-targeted arthropods across all sites was good enough to observe any large population changes induced by Deltamethrin. There was no evidence that either moths or butterflies were adversely affected by Deltamethrin. We conclude that deltamethrin at low dosages as used in the SAT operations did not lead to significant changes in the non-target terrestrial arthropod populations. At the same dose rate we can also conclude that the effect of deltamethrine on vertebrate or invertebrate aquatic arthropod populations is insignificant.

The final chapter (Chapter7) of the thesis presents a general discussion of the major findings of the research work with recommendations for decision support to the Tsetse and Trypanosomosis Control Programme in Ghana. The challenge of controlling/eliminating tsetse and trypanosomosis in sub-Saharan Africa is daunting and continues to attract more research work that delve into the realm of possible control strategies with the ultimate objective to ensuring a tsetse free Africa. Contributing to the achievement of this goal, we conclude this thesis with highlights on the major findings and recommendations of the operational research work: 1) the three rivers (Black Volta, Kulpawn and Sissili), traversing the study area, form the major risk factors of bovine trypanosomosis in the Upper West Region; 2) the existence of portions of uninterrupted forest galleries along these rivers

provides not only tsetse habitat but also habitat for wildlife that serve as blood meal for tsetse and partial protection against SAT; 3) Despite the limited dispersal capability of tsetse populations estimated in the UWR, the observed dispersal was still enough to trigger tsetse reinvasion in tsetse cleared areas; 4) Failure of the control programme to achieve complete tsetse elimination in the UWR was probably due to the absence of SIT in the integrated strategy, necessary to mop up remnant flies in the forested southern limits where the impact of SAT was deficient. Thus, to begin any comprehensive tsetse control programme as envisaged by the technical committee of PATTEC we recommend that: 1) a robust feasibility study be made to select the best strategy (elimination versus suppression) before any control campaign; 2) in sites where elimination is possible, apply an Integrated approach involving SAT, Ground spraying, Insecticide treated cattle/targets and SIT based on the “tsetse without border” concept: eradicating tsetse flies from one “pocket/island” before dealing with the next one by coordinating efficiently all the efforts between countries. The methods should be carefully selected according to the target tsetse species and the type of vegetation; 3) -in sites where elimination is not possible: include local-IPM of trypanosomosis in the general strategy of PATTEC.

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Résumé de Thèse

“Thème: Evaluation du risque de Trypanosomose Animale au Ghana, et suivi de l'impact de l'intervention d'éradication de la maladie et du vecteur dans la region Ouest du Ghana”

La Trypanosomose animale africaine (TAA) est une contrainte majeure pour le développement et la durabilité des systèmes de production animale au Ghana. La maladie limite l'utilisation des pâturages naturels abondants dans tout le pays entraînant une pauvreté et une malnutrition fréquente notamment en milieu rural. Au fil des années, la Direction des Services vétérinaires du Ministère de l'alimentation et de l'agriculture du Ghana s'est engagée dans le contrôle de la TAA. Cependant, malgré les efforts de contrôle consentis dans le passé, le problème de la maladie persiste. En 2001, l'Union Africaine a lancé la Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), prônant l'éradication des populations de glossines à travers les frontières de manière durable pour contrôler la trypanosomose en Afrique Sub-saharienne. Sous l'égide de la PATTEC, le Ghana a collaboré avec le Burkina Faso dans une initiative sous-régionale visant à créer une zone libérée des tsé-tsé à travers leurs frontières communes. L'objectif de cette thèse était de mener des recherches pour guider à la mise en œuvre du projet en fournissant des stratégies d'intervention rationnelles pour l'éradication des tsé-tsé et des trypanosomoses. Plus précisément, la recherche visait à i) faire une situation initiale de la densité vectorielle et la prévalence de la maladie dans la zone d'intervention de la PATTEC Ghana, ii) déterminer la structuration de la population de tsé-tsé et ses conséquences sur les efforts d'une lutte durable contre les glossines, iii) évaluer la SAT (pulvérisation séquentielle d'aérosol) pour le contrôle des glossines d'espèces riveraines au Ghana, et iv) d'évaluer le risque environnemental des programmes d'intervention.

Le premier Chapitre de cette thèse porte sur une synthèse générale de la trypanosomose bovine, l'épidémiologie et les facteurs de risque de la maladie, les méthodes de lutte anti-vectorielle et l'approche continentale du contrôle/éradication de la mouche tsé-tsé. La présence de trypanosomes pathogènes (*Trypanosoma vivax*, *T. congolense* et *T. brucei*) et la grande variabilité de la prévalence de la trypanosomose est une caractéristique majeure de l'épidémiologie de cette maladie en Afrique, en relation avec la densité apparente des glossines et le risque de la trypanosomose décrit par Rogers (1985) et Mahama et al. (2003). Les trypanosomes pathogènes africains affectant l'homme ou l'animal appartiennent au groupe Salivaria et leur transmission se fait via la salive de l'insecte vecteur lors du repas sanguin. Chez les trypanosomes animaux, *T. congolense* savannah est le plus pathogène et est responsable de l'infection aiguë et de la mort de l'animal. Par ailleurs, deux sous-espèces de trypanosomes, *Trypanosoma brucei rhodesiense* et *T. b. gambiense* provoquent la maladie chez l'homme (FAO, 1998). Géographiquement, *T. b. rhodesiense* se trouve en Afrique de l'Est, alors que *T. b. gambiense* se rencontre en Afrique du Centre et de l'Ouest. Les mouches tsé-tsé sont les vecteurs cycliques de la trypanosomose. Les facteurs de risque de la trypanosomose sont en grande partie attribuables aux conditions climatiques et à l'habitat des glossines (couverture végétale et sources d'eau) et la présence d'animaux comme sources de repas de sang pour la mouche tsé-tsé. Le principal facteur de risque épidémiologique dans la trypanosomose africaine est le contact hôte-mouche. Cette interaction est influencée par l'augmentation de la densité de mouches tsé-tsé. La modification des habitudes alimentaires des glossines liée au développement des activités humaines favorise aussi ce contact. La trypanosomose animale africaine est une maladie animale transfrontière (MATs) avec des échanges dans toute l'Afrique sub-saharienne, notamment à partir des systèmes d'élevage du bétail transhumant. Le contrôle de la mouche tsé-tsé et de la trypanosomose passe par deux stratégies complémentaires visant à réduire ou éliminer la maladie. Les deux stratégies sont la

chimiothérapie, qui vise à réduire l'incidence de la trypanosomose chez le bétail, et entomologique, visant à interrompre le cycle de transmission de la maladie en réduisant ou en éliminant le vecteur de la trypanosomose (Bouyer et al. 2010). Les techniques de contrôle du vecteur ont subi des évolutions, où des méthodes antérieures rudimentaires sont récemment remplacées par des méthodes économiquement moins chères, plus spécifiques, et écologiquement durables. Ces techniques comprennent: la pulvérisation séquentielle d'aérosol (SAT), les pièges/écrans imprégnés d'insecticides, les appâts vivants et la technique de l'insecte stérile (TIS) (Bouyer et al. 2013; Vreysen et al. 2013).

Dans le deuxième chapitre de la thèse, nous présentons la conduite d'une enquête de référence dans la deuxième région occidentale du Ghana pour évaluer la répartition et la densité des espèces de glossines, ainsi que la prévalence de la trypanosomose bovine. L'enquête entomologique a été conçue de telle sorte à couvrir les habitats favorables aux glossines le long des trois principaux cours d'eau de la zone d'étude (Volta Noire, Kulpawn et Sissili). Des pièges biconiques (Challier et al., 1977) ont été déployés dans la végétation riveraine et adaptés aux points d'abreuvement du bétail. Une moyenne de 20 pièges ont été déployés dans chaque site espacés de 200 m l'un de l'autre. La position de chaque piège a été enregistrée à l'aide d'un GPS (Global Positioning System). Un total de 564 pièges ont été déployés le long des trois rivières. Les pièges sont déployés à 8 heures et récoltés le lendemain à la même heure. Les pièges ont été prospectés deux fois par jour et les mouches capturées ont été identifiées par espèce et par sexe. Les données ont été exprimées en Densité Apparente (DAP) qui correspond au nombre de mouches capturées par piège et par jour. Les résultats indiquent la présence de *Glossina tachinoides* dans les trois bassins tandis que *G. p. gambiensis* a été retrouvé seulement près de la limite sud de la zone d'étude. Les densités les plus élevées (DAP > 100) ont été obtenues dans la pointe sud-ouest de la région Nord-Ouest, le long du fleuve Volta Noire où la galerie forestière est la plus dense. Pour l'enquête

parasitologique, un échantillon aléatoire de 1800 bovins composés de races ouest africaine à cornes courtes, Sanga et Zébu a été réalisé. Les animaux ont été choisis au hasard dans 36 mailles couvrant la zone d'étude. Les résultats ont montré des différences importantes entre les prévalences parasitologique et sérologique. La prévalence parasitologique moyenne, par utilisation de la méthode du Buffy Coat, a été estimée à 2,5% (IC 95%: 1,06-5,77) avec une prédominance des infections dues à *Trypanosoma vivax*. La plupart des bovins infectés ont été trouvés à proximité des grands systèmes fluviaux. La prévalence sérologique mesurée à l'aide du test ELISA (Enzyme Linked Immunosorbent Assay), était de 19% (IC 95%: 14,03-25,35). Nous avons conclu de cette étude que *G. tachinoides* et *G. p gambiensis* sont les principaux vecteurs de la trypanosomose dans la deuxième région occidentale du Ghana. Les enquêtes entomologiques menées le long des trois principaux cours d'eau suggèrent une possible retraite vers le sud des mouches tsé-tsé suivant la couverture de la végétation riveraine existante. Cependant, la trypanosomose reste très répandue dans la région Nord-Ouest, avec la prédominance des infections à *T. vivax* et, dans une moindre mesure, *T. brucei*. Les conclusions de cette étude ont permis de développer une stratégie intégrée d'élimination des glossines, qui combine la SAT, la pulvérisation au sol, le déploiement de tissus imprégnés d'insecticides et le pour-on. La mise en œuvre a commencé dans la période mars-mai 2010 avec les opérations SAT, menées le long des trois principaux cours d'eau.

Dans le troisième chapitre, nous avons étudié la structuration de la population de *G. tachinoides* à l'intérieur et entre les trois principaux bassins fluviaux de la zone cible dans la UWR (Upper West Region) du Ghana et une population voisine au nord-est de la zone cible. L'absence de données antérieures sur la structuration génétique des populations de glossines dans la UWR a nécessité cette étude. La végétation riveraine le long des trois principaux cours d'eau est très fragmentée, et leurs affluents sont devenus largement inappropriés pour la survie des glossines riveraines pendant la saison sèche (décembre à mai). En effet, les feux de

brousse et l'absence de pluies pendant cette saison limitent sérieusement la disponibilité d'une végétation favorable (Adam et al., 2012), de sorte que les glossines se rétractent des affluents pour se retrouver le long des principaux cours d'eau, où l'eau et la végétation permanente atténuent les dures conditions et créent des conditions microclimatiques favorables (ainsi, les températures montent souvent au-dessus de 40°C à l'extérieur de la galerie). Alors que la Volta Noire est un cours d'eau permanent, le Kulpawn et Sissili sont saisonniers. D'où l'hypothèse qu'il pourrait être plus difficile pour les tsé-tsé de se disperser entre les principales rivières de la UWR du Ghana contrairement à ce qui est observé au Burkina Faso entre le fleuve Mouhoun et ses bassins adjacents. L'objectif de cette étude était donc de déterminer si les populations de glossines dans la UWR du Ghana réussissent à disperser entre bassins fluviaux, qui ont été utilisés comme des unités opérationnelles de base pour les opérations SAT, et donc, si les populations de glossines de la zone cible (Adam et al., 2013) peuvent être considérées comme isolées des populations des bassins adjacents du nord-est. L'étude a porté sur *G. tachinoides*. Les résultats de cette étude peuvent être utilisés par les responsables de la campagne d'éradication pour éclairer les décisions sur la nécessité éventuelle d'établir des barrières appropriées pour empêcher la ré-invasion des zones où les populations de glossines sont éliminées. Huit sites situés le long des trois rivières ont été étudiés. Les sites sélectionnés avaient déjà été étudiés en termes de diversité et d'abondance des tsé-tsé (Adam et al., 2012). Sur un échantillon total de 884 mouches, un sous-échantillon de 266 a été génotypé à neuf loci microsatellites. La signification des différents niveaux hiérarchiques a été testée en utilisant les paramètres estimés de Yang avec la méthode de Weir et Cockerham. Un effet significatif pièges au sein des groupes (pièges à moins de 3 km de distance les uns des autres), groupes au sein des bassins hydrographiques et bassins hydrographiques dans l'ensemble de la zone cible a été observé. L'isolement par la distance entre pièges était hautement significatif. Une densité locale de 0.048-0.061 glossines/m² a été estimée avec une

distance de dispersion approximative de 11 m par génération [CI 9-17]. Aucune influence du sexe sur la dispersion n'a été détectée. Les distances de dispersion de *G. tachinoides* dans la UWR du Ghana étaient relativement faibles, en relation avec la fragmentation de l'habitat et la saisonnalité des rivières Kulpawn et Sissili. En outre, une très forte densité de la population de glossines a été observée dans les sites d'étude, ce qui réduit potentiellement la dispersion dans l'habitat, à saturation constante, parce que la probabilité que les migrants puissent s'établir est réduite (dispersion dépendante de la densité). Cependant, la dispersion spatiale observée a été jugée suffisante pour qu'une zone où *G. tachinoides* serait éliminée soit ré-ennahie à partir des populations des bassins fluviaux adjacents. Ces données corroborent les résultats d'autres études de génétique des populations en Afrique de l'Ouest, qui indiquent que les populations de *G. tachinoides* de différents bassins fluviaux ne peuvent pas être considérées comme isolées.

Dans le quatrième chapitre, nous présentons la mise en œuvre et l'évaluation de l'efficacité d'une stratégie intégrée d'intervention contre les mouches tsé-tsé. L'étude a été menée dans la région UWR du Ghana (9.62°-11.00°N, 1.40°-2.76°W), couvrant une superficie $\approx 18000 \text{ km}^2$, dans le cadre de la PATTEC. Deux espèces ont été ciblées: *G. tachinoides* et *G. p. gambiensis*. Les objectifs étaient de tester le potentiel de la SAT à éliminer les espèces de glossines ripicoles dans une zone difficile (couverture dense des arbres et densités de glossines élevées) sur une superficie totale pulvérisée de 6745 km^2 et l'efficacité ultérieure d'une stratégie intégrée pour le maintien des résultats de la suppression des glossines dans toute la zone d'intervention. Cette stratégie comprenait la pulvérisation au sol ($\approx 100 \text{ km}^2$), le déploiement d'écrans et de pièges imprégnés d'insecticide (20,000) et l'application de produits insecticides sur bovins (45,000). L'épandage aérien de deltaméthrine à faible dosage (0,33-0,35 g a.i./ha) a été fait le long des trois principaux cours d'eau à l'aide de cinq avions Thrush (Orsmond aviation, Bethlehem, Afrique du Sud). L'impact de la SAT sur les densités de

glossines a été contrôlé à l'aide de 30 pièges biconiques déployés deux semaines avant et deux semaines après les opérations. Les résultats du suivi ont indiqué un taux de réduction global de 98% (de 16,7 glossines comme DAP initiale à 0,3 à la fin de la quatrième et dernière période). Un an après les opérations SAT, une deuxième enquête a été menée tout au long de la zone d'intervention avec 200 pièges biconiques posés dans 20 sites pendant 3 semaines afin de mesurer l'impact de la stratégie de lutte intégrée. Les deux espèces cibles étaient encore détectées, mais à de très faibles densités (DAP de 0,27 à l'intérieur du bloc pulvérisé et 0,10 à l'extérieur). Les opérations SAT n'ont pas réussi à atteindre l'élimination dans la section suivie, mais la stratégie intégrée mise en place après la SAT a maintenu un niveau élevé de suppression tout au long de la zone d'intervention, ce qui contribuera à améliorer la santé animale, à l'augmentation de la production animale et à la sécurité alimentaire. L'échec à parvenir à l'élimination a été largement attribuable à la difficulté des aérosols SAT pour pénétrer les épaisses forêts-galeries dans les limites sud de la zone d'intervention. Les autres causes d'échec pourraient être les suivantes, entre autres :

- 1) Les différences de sensibilité à la deltaméthrine entre les différentes espèces de mouches tsé-tsé. Particulièrement *G.p.gambiensis* est très résistante à l'action insecticide de la deltaméthrine.
- 2) La taille de la mouche: *G.tachinoides* a une taille inférieure à *G.m.morsitans* ou *G.p.gambiensis* et donc capture moins de gouttelettes contenant l'insecticide sur son corps. Cela rend donc *G.tachinoides* moins vulnérable à l'épandage aérien.
- 3) Vu qu'il existe une corrélation négative entre l'efficacité de la deltaméthrine et la température ambiante, cet insecticide était moins toxique dans les conditions climatiques du Ghana.
- 4) L'isolement insuffisant et tardif des zones traitées par l'épandage aérien n'a probablement

pas pu empêcher l'invasion de mouches en provenance des aires non traitées au sein de la zone d'étude ou en dehors de la zone d'étude.

Le cinquième chapitre présente un résumé des deux modèles d'étude utilisés pour la sélection des sites de suivi entomologique en phases de pré et post-intervention des programmes de lutte dans la région UWR du Ghana, établis en collaboration avec Giuliano Cecchi, expert GIS à FAO_PAAT. L'objectif de cette étude était de recommander un modèle d'étude pour la sélection des sites de suivi des tsé-tsé dans les futurs programmes de contrôle. L'habitat potentiel des glossines a été identifié sur la base de la couverture du sol / végétation (Cecchi G et al., 2008), des plans d'eau et la disponibilité des hôtes (bétail et la faune), qui sont connus pour avoir une relation directe avec les densités de glossines et le risque de la trypanosomose cyclique. Lors des enquêtes préliminaires, une procédure d'échantillonnage systématique a été mise en place le long des trois principaux cours d'eau et dans les blocs de pulvérisation SAT (Adam et al., 2012; 2013). Les procédures de contrôle avant l'intervention étaient basées sur l'hypothèse que les mouches tsé-tsé se rétractent des affluents à la forêt riveraine le long des principales rivières de la UWR pendant la saison sèche après la destruction de la végétation par les feux de brousse et en raison des températures très élevées (moyenne des températures diurnes au-dessus 40°C). L'échantillonnage a donc été limité à la forêt riveraine le long des trois principaux cours d'eau de la région, où les mouches tsé-tsé sont abondantes (Adam et al., 2012). D'autre part la procédure de suivi des glossines après les opérations de SAT a été mis en place pour vérifier l'hypothèse de la procédure de suivi pré-intervention et de fournir la preuve que les mouches tsé-tsé pourraient encore être présentes en dehors des blocs traités par pulvérisation SAT et au niveau de certains affluents de la rivière. Elle visait également à valider les résultats de l'étude de la génétique des populations. Des images satellites Landsat7, ainsi que d'autres données auxiliaires SIG, ont été utilisées pour guider la sélection des 20 sites de suivi des tsé-tsé après la SAT (G. Cecchi, 2011

Rapport de mission). Le guide principal dans le choix des sites d'échantillonnage pour le suivi des glossines riveraines était le couvert végétal le long des cours d'eau, les affluents et les bordures de la réserve forestière avec les traversées de rivières. Par appréciation visuelle, 12 sites d'échantillonnage ont été identifiés dans les blocs de pulvérisation et 8 sites en dehors des blocs traités (Figure 2). Le nombre de 20 sites d'échantillonnage a été établi en prenant en considération les contraintes financières et logistiques (Cecchi G., 2011; Rapport de mission). L'enquête de suivi post-SAT a montré l'existence de mouches tsé-tsé en dehors de la forêt riveraine le long des principaux cours d'eau, contrairement à l'hypothèse de suivi pré-SAT. Les deux espèces *G. tachinoides* et *G. p. gambiensis* ont été capturées dans deux sites situés en dehors des blocs pulvérisés sur les affluents des rivières principales (Adam et al., 2013). La distribution spatiale des glossines capturées aux enquêtes de suivi post-SAT, a révélé un manque d'efficacité de la méthode d'enquête employée dans l'étude pré-SAT et pendant les opérations SAT de Adam et al. (2012 et 2013). La couverture spatiale des opérations est un facteur important à considérer lors de l'analyse de l'impact global des interventions. La capture des deux espèces riveraines, *G. tachinoides* et *G. p. gambiensis* dans un affluent droite de la Kulpawn lors de l'enquête post-intervention soutient l'idée que de petites poches de mouches peuvent être maintenues pendant la saison sèche. Une situation similaire a été observée avec *G. p. gambiensis* dans les zones sèches de son aire de répartition, par exemple au Sénégal (Bouyer et al., 2010). En conclusion, nous recommandons la conception de ce type d'échantillonnage pour le contrôle pré- et post-intervention (Cecchi G. 2011, rapport de fin de mission de la FAO) pour les programmes de lutte ultérieurs.

Dans le chapitre six, nous présentons un résumé d'une étude d'évaluation de l'impact environnemental d'un programme de lutte intégrée contre les glossines qui a été conduite en collaboration avec Ian F. Grant, expert en suivi environnemental à Cybister Environmental Protection, Royaume-Uni. L'évaluation de l'impact environnemental a été menée

parallèlement au programme d'intervention SAT de la PATTEC pour le contrôle des deux espèces de glossines le long des trois principaux cours d'eau de la région Ouest du Ghana (Volta Noire, Kulpawn et Sissili). L'objectif était de déterminer le niveau d'impact de la deltaméthrine sur l'écosystème. Le suivi a porté sur les impacts directs de la deltaméthrine sur les arthropodes terrestres et aquatiques non ciblés. Sur les 8 blocs de pulvérisation ciblés pour les opérations SAT, les opérations de suivi environnemental ont été implantées dans le Block 3 du tronçon inférieur de la Volta Noire où les conditions sont appropriées (présence de vie aquatique et terrestre) pour le suivi des effets de la SAT sur les insectes non cibles. La pulvérisation de deltaméthrine a été appliquée à la fin de la saison sèche (Avril), du crépuscule à l'aube à vents doux, avec 5 avions Turbo Thrush (Orsmond aviation, Bethlehem, Afrique du Sud). Dans l'ensemble, 4 cycles de pulvérisation à la deltaméthrine ont été appliqués à la dose moyenne de 0,34 g de matière active (a.i)/ha. Deux sites de lutte et un de contrôle négatif ont été choisis le long de la Volta Noire à Nyose, Tankara et Tagala respectivement pour le suivi de l'impact des opérations SAT sur les arthropodes aquatiques et terrestres. Les techniques d'échantillonnage des arthropodes terrestres comprennent l'utilisation de pièges Malaise pour les insectes volants, pièges à fosse pour les arthropodes à activité épigée et les pièges parapluie pour capturer les insectes vivant dans la canopée. Les techniques d'échantillonnage de Drift et Heel, d'autre part, ont été utilisées pour les arthropodes aquatiques. Les résultats de recherches menées sur les organismes aquatiques après les pulvérisations de l'aube n'ont généralement montré aucune preuve de mortalité des vertébrés ou invertébrés. Les poissons juvéniles, les insectes aquatiques et les décapodes (Belastamethidae, Nepidae, crevettes et crabes) qui affichent généralement des symptômes de stress à la deltaméthrine, comme l'hyperactivité ou la nage erratique, se comportaient normalement. Les inspections de la face inférieure de la roche ont montré la présence de nymphes d'éphémères et des larves de mouches trichoptères. La biomasse moyenne avant le

traitement variait entre 0,3 et 0,4 g et la réduction moyenne après la pulvérisation chimique était de 0,2 g (non significatif, 95% CI: -0,2-0,6). Les interactions entre les variables explicatives n'étaient pas significativement différentes de 0, ce qui indique que la différence entre la matière sèche avant et après le traitement était insignifiante dans les trois sites de suivi. Les captures des pièges Malaise ont été similaires à Nyose et Talawana. La variation de l'échantillon à Tagala était plus grande. Avant la pulvérisation, l'abondance des groupes d'insectes piégés dans tous les sites était globalement similaire. Au cours de la période de pulvérisation, l'image qualitative des arthropodes non ciblés sur tous les sites était suffisamment précise pour observer d'éventuels grands changements démographiques induits par la deltaméthrine. Il n'y a eu aucune preuve que les papillons aient été affectés par la deltaméthrine. Nous concluons que la deltaméthrine à faibles doses comme utilisés dans les opérations SAT n'a pas conduit à des changements importants dans les populations d'arthropodes terrestres non cibles. A la même dose, nous pouvons également conclure que l'effet de la deltaméthrine sur les populations d'arthropodes aquatiques vertébrés ou invertébrés est insignifiant.

Le dernier chapitre (chapitre 7) de la thèse présente une discussion générale sur les principales conclusions des travaux de recherche avec des recommandations pour aider à la décision pour le programme de lutte contre la mouche tsé-tsé et de la trypanosomose au Ghana. Le défi de contrôle/élimination des tsé-tsé et de la trypanosomose en Afrique subsaharienne est de taille et continue d'attirer plus de travaux de recherche orientés dans le domaine des stratégies de luttés possibles avec l'objectif ultime d'assurer une Afrique libre de la mouche tsé-tsé. Pour contribuer à l'atteinte de cet objectif, nous concluons cette thèse en faisant ressortir les principales conclusions et recommandations des travaux de recherche opérationnelle: 1) les trois rivières (Volta Noire, Kulpaw et Sissili), traversant la zone d'étude, constituent les principaux facteurs de risque de la trypanosomose bovine dans la

région Nord-Ouest du Ghana; 2) l'existence de portions de galeries forestières contigües le long de ces cours d'eau fournissent non seulement l'habitat pour les glossines mais aussi un habitat pour la faune qui servent d'hôtes nourisseurs pour les glossines et de protection partielle contre la SAT; 3) Malgré la capacité de dispersion limitée des populations de glossines estimés dans le UWR, la dispersion observée était suffisante pour assurer une ré-invasion des tsé-tsé dans les zones assainies; 4) L'échec du programme de contrôle de parvenir à l'élimination complète des mouches tsé-tsé dans le UWR était probablement dû à l'absence de la technique de l'insecte stérile (TIS) dans la stratégie intégrée, nécessaire pour achever les populations résiduelles dans les limites sud boisées où l'impact de la SAT était déficient. Ainsi, pour mener à bien un programme de lutte contre les glossines comme prévu par le comité technique de la PATTEC, nous recommandons que: 1) une étude de faisabilité robuste soit menée afin de choisir la meilleure stratégie (l'élimination versus suppression) avant toute campagne de lutte; 2) dans les sites où l'élimination est possible, d'appliquer une approche intégrée impliquant la SAT, la pulvérisation au sol, bétail/écrans et/ou pièges imprégnés d'insecticide et la TIS sur la base du concept "mouche tsé-tsé sans frontière": éradiquer les mouches d'une "poche/île" avant d'entammer la suivante en coordonnant efficacement tous les efforts entre les pays. Les méthodes doivent être soigneusement choisies en fonction de l'espèce cible et le type de végétation; 3) dans les sites où l'élimination n'est pas possible: inclure localement une lutte intégrée de la trypanosomose dans la stratégie générale de la PATTEC.

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