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Improving Sterile Insect Technique (SIT) for tsetse flies through research on their symbionts and pathogens

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Abstract

Tsetse flies (Diptera: Glossinidae) are the cyclical vectors of the trypanosomes, which cause human African trypanosomiasis (HAT) or sleeping sickness in humans and African animal trypanosomiasis (AAT) or nagana in animals. Due to the lack of effective vaccines and inexpensive drugs for HAT, and the development of resistance of the trypanosomes against the available trypanocidal drugs, vector control remains the most efficient strategy for sustainable management of these diseases. Among the control methods used for tsetse flies, Sterile Insect Technique (SIT), in the frame of area-wide integrated pest management (AW-IPM), represents an effective tactic to suppress and/or eradicate tsetse flies. One constraint in implementing SIT is the mass production of target species. Tsetse flies harbor obligate bacterial symbionts and salivary gland hypertrophy virus which modulate the fecundity of the infected flies. In support of the future expansion of the SIT for tsetse fly control, the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture implemented a six year Coordinated Research Project (CRP) entitled “Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens”. The consortium focused on the prevalence and the interaction between the bacterial symbionts and the virus, the development of strategies to manage virus infections in tsetse colonies, the use of entomopathogenic fungi to control tsetse flies in combination with SIT, and the development of symbiont-based strategies to control tsetse flies and trypanosomiasis. The results of the CRP and

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Disclosures

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the solutions envisaged to alleviate the constraints of the mass rearing of tsetse flies for SIT are presented in this special issue.

Keywords

Tsetse; Glossinidae; Salivary gland hypertrophy virus; SIT; Symbionts; Pathogens

1. Introduction

Tsetse flies(Diptera:Glossinidae) are the cyclical vectors of the trypanosomes, which cause human African trypanosomosis (HAT) or sleeping sickness in humans and African animal trypanosomosis (AAT) or nagana in animals (Simarro et al., 2003) (Fig. 1). Human sleeping sickness is a zoonosis caused by the protozoan *Trypanosoma brucei rhodesiense* in East Africa and *Trypanosoma brucei gambiense* in West and Central Africa. The nagana-causing trypanosomatids, *Trypanosoma vivax* and *Trypanosoma congolense*, are major pathogens of cattle and other ruminants, whereas *Trypanosoma simiae* causes high mortality in domestic pigs and *Trypanosoma brucei* infects all livestock. Vaccines are not available and are unlikely to be developed due to the antigenic variation exhibited by the trypanosome in the mammalian host. The drug treatment of both HAT and ATT is in a perilous state and relies on old, often dangerous drugs and resistance is becoming an increasing problem (for more information see Holmes, 2013).

It is conservatively estimated by the World Health Organization (WHO) that there are currently 10,000–45,000 cases of human African trypanosomosis (HAT) with 60 million people at risk in 36 countries covering ~40% of Africa (almost 10 million km²). After a devastating epidemic in the early 20th century when a million people died of HAT the disease almost disappeared from Africa by the 1960s. In the 1990s we have witnessed yet another epidemic that killed tens of thousands of people. Through an ambitious campaign led by WHO and many Non-Governmental Organizations (NGOs) and thanks to a public-private partnership with Sanofi-Aventis and Bayer to donate the necessary drugs to WHO for distribution to affected countries, the latest epidemic has come under control although several foci in Congo, Uganda and Angola remain of major concern. Sustainable management of such diseases poses a formidable challenge to endemic countries and health ministries that are faced with limited infrastructure and financing. If the decline in the reported HAT cases trigger African governments to abandon their local control efforts and for funding agencies to relax their disease research priorities, it is certain that epidemics will continue to flare up in the near future as has happened in the recent past (Aksoy, 2011).

The impacts of African trypanosomosis are multidimensional and affect human health, livestock production, agricultural production (lack of draught animals and manure), rural socio-economic development, national economies (import and export of animal products), and the environment (insecticide applications). About fifty million cattle and tens of millions of small ruminants are at risk from AAT. Tsetse and trypanosomosis (T&T) are the major factors preventing the establishment of sustainable agricultural systems in many parts of sub-Saharan Africa. The Programme Against African Trypanosomosis (PAAT) estimates that AAT causes approximately 3 million cattle deaths per year. Farmers are required to

administer approximately 35 million doses of costly trypanocidal drugs. Unfortunately, many of these chemicals fail because of development of resistance by the parasites. Direct losses in meat production and milk yield and the costs of programs that attempt to control trypanosomosis are estimated to amount to between US\$600 million and \$1.2 billion each year. In the absence of the AAT problem, a family that is currently dependent on manual labour alone could use draught animals and thus increase its income from agricultural work by 45% per unit of land and by 143% per unit of labor. If one includes this loss in livestock and crop production, trypanosomosis is estimated to cost sub-Saharan Africa US\$4 billion or more each year (Budd, 2000), equivalent to the value of one-quarter of the area's total livestock production.

The lack of effective vaccines and high costs of disease treatment, disease control, via control of the tsetse vector, has been found to be the most effective strategy. Present vector control efforts, which depend on trapping or killing the tsetse flies with insecticides, have been difficult to sustain at the local community level for human disease control. However these methods are widely used for control of animal diseases and this CRP has focused on one complement to current vector control methods, the Sterile Insect Technique (SIT). The eradication of the tsetse fly *Glossina austeni* from Unguja Island of Zanzibar by means of an area-wide integrated pest management program concluding with the release of sterile flies stimulated interest to expand this strategy to large areas on mainland Africa (Vreysen et al., 2000). In July 2000, at a summit in Lomé, Togo, the African Heads of State and Government passed a resolution (AHG/Dec.156 XXXVI), giving birth to the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC). Several UN Organizations, including WHO, the Food and Agricultural Organization (FAO) and the International Atomic Energy Agency (IAEA) subsequently passed resolutions supporting the PAT-TEC initiative. In May 2002, PATTEC and PAAT identified priority areas for T&T intervention and for related sustainable agriculture and rural development. Specific national/sub-regional strategies were developed so that the necessary national and international funding could be generated, including grants and soft loans from the African Development Bank.

2. FAO/IAEA sponsored Coordinated Research Project

The Joint Division of Nuclear Techniques in Food in Agriculture of the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) sponsors Coordinated Research Projects (CRPs), research networks that focus participating scientists from both developing and developed countries on applying nuclear techniques to specific problems relevant to agriculture. A CRP entitled "Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens" was initiated in 2006. This CRP involved four Research Coordination Meetings (RCMs) held in Vienna, Austria (October, 2007), Bobo-Dioulasso, Burkina Faso, (February 2009), Nairobi, Kenya (July, 2010) and Vienna, Austria (March, 2012) to review results and plan future research. Twenty-three research teams from eighteen countries (Austria, Belgium, Burkina Faso, Cameroon, Canada, China, France, Germany, Ghana, Greece, Italy, Kenya, Slovakia, South Africa, Tanzania, The Netherlands, Uganda and the United States) participated and conducted research on different aspects of tsetse symbionts and pathogens.

The long-term goal of the CRP is to improve the quality of decision-making related to field implementation of SIT projects. Achieving the specific objectives outlined in the CRP (see below) is directed at improving the mass production of healthy colonies for production of sterile males, developing parasite-resistant SIT lines that can be applied in disease endemic areas, and incorporating natural incompatibilities for production of robust sterile males. The beneficiaries include: (i) people and livestock in the endemic areas through reduced incidence of trypanosomiasis; (ii) livestock producers through increased profitability; (iii) the environment through reduced insecticide use; and (iv) tsetse-free countries by reducing, or possibly eliminating, the risk of introduction of these devastating diseases.

3. Objectives of the CRP

The overall objective of the CRP was to exploit interactions between tsetse flies and their microbes to enhance the efficacy of tsetse SIT programs. The specific objectives and the expected output of this CRP are listed in Table 1.

4. Current status

4.1. Tsetse symbionts

Tsetse flies feed exclusively on vertebrate blood and exhibit an unusual adenotrophic viviparity mode of reproduction during which all embryonic and larval stages develop within the female's uterus (modified common oviduct). The mother develops a single oocyte at a time, then carries and nourishes the resulting embryo and larva in an intrauterine environment for the duration of its immature development. To supplement their single diet, which is restricted in many vitamins and coenzymes, tsetse have entered into symbiotic association with bacterial microbes. Throughout larvagenesis maternal milk gland secretions provide developing offspring with nourishment as well as 3 distinct endosymbiotic bacteria (Aksoy, 2000; Attardo et al., 2008). To date, members from the genera *Wigglesworthia*, *Sodalis*, and *Wolbachia* have been identified in both natural field populations as well as in colonies of different tsetse fly species. The paper presented by Balmand et al. (2013) in this volume describes the spatial distribution of tsetse's three symbionts in different host tissues by applying fluorescence in situ hybridization (FISH) with symbiont specific 16S rDNA probes. These studies showed that *Sodalis* is absent from the germ cells (oocyte and embryo) while *Wolbachia* are present in these cells. Examination of the milk glands showed that *Sodalis* and *Wigglesworthia* were localized in the duct while *Sodalis* cells were also infecting the milk gland cells. In addition to the extracellular cells in the lumen of the milk gland, *Wigglesworthia* are also localized within bacteriocytes in the midgut bacteriome organ. Thus, it appears that while *Wolbachia* are transmitted transovarially, *Sodalis* and *Wigglesworthia* are acquired by the developing intrauterine larva from the mother's milk glands.

4.1.1. Genus *Wigglesworthia*—The association between *Wigglesworthia* and its tsetse host is apparently ancient (50–80 million years old) and phylogenetic studies show that *Wigglesworthia* species display concordance with their insect host species, a phenomenon indicative of strict vertical transmission of the symbiont from generation to generation (Chen et al., 1999). The elimination of *Wigglesworthia* via antibiotic supplementation of

tsetse's diet results in loss of host fecundity (Nogge, 1976; Pais et al., 2008). The genome sequences of two *Wigglesworthia* species (from tsetse hosts *Glossina brevipalpis* and *Glossina morsitans*) indicates a drastic reduction to about 700 kb in size and a high A + T bias of over 80% (Akman et al., 2002; Rio et al., 2012). The related free living enterics typically have a genome size of 4000–10,000 kb (Wernegreen, 2002). Despite this reduction, and hence functional capacity, the small genome has retained many vitamin biosynthetic pathways, confirming the nutritional role of *Wigglesworthia* symbiosis in tsetse. The small *Wigglesworthia* genome has retained the ability to synthesize a functional flagellum. Gene expression studies indicate that the flagellum is expressed in the milk gland tissue possibly suggesting a role for flagella functions in the transmission process (Rio et al., 2012). Knowledge of *Wigglesworthia* products that support host fecundity and transmission biology to progeny is central for successful maintenance of tsetse colonies given its indispensable nature.

4.1.2. Genus *Sodalis*—The second organism, the commensal *Sodalis*, is harboured in all tsetse flies in the insectary colonies but is heterogeneous in natural populations. Symbionts closely related to *Sodalis* have been identified in insects from different taxa such as hippoboscids (Diptera), weevils (Coleoptera) and chewing lice (Phthiraptera) (Kaiwa et al., 2010; Novakova and Hypsa, 2007; Snyder et al., 2011). The taxonomic origin of *Sodalis* needs to be further clarified. The genome sequence of *Sodalis* is also available, further mediating functional studies into *Sodalis*' biology in the tsetse host. Unlike *Wigglesworthia* the *Sodalis* genome, which is about 4.5 Mb in size, has not undergone a size reduction and is more in line with free-living enteric microbes. It does however contain many pseudogenes, especially in pathways that are unlikely to be active in the restricted nutritional ecology of its host biology. It represents an organism that is apparently in transition from a free-living state to a symbiotic microbe (McCutcheon and Moran, 2012).

Sodalis's functional role in tsetse is unknown. An interesting hypothesis has focused on the influential role of *Sodalis* for trypanosome transmission. The presence of *Sodalis* has been implicated in enhancing the vectorial capacity of tsetse (Baker et al., 1990; Maudlin et al., 1990). Flies that harbour greater *Sodalis* densities have been suggested to be more susceptible to trypanosomes. In addition to density effects, it is also possible that different *Sodalis* genotypes may confer greater transmission ability to the host. These inferences can be evaluated further in populations both for density effects and for potentially different genotypes that may exist in natural populations that confer enhanced parasite transmission ability to host insects. In a previous work, a large tsetse fly sampling campaign had been conducted in Bipindi and Campo where that are two historical sleeping sickness foci located in the south of Cameroon. Each fly species was identified along with the presence/absence of *Sodalis glossinidius*, and the trypanosome species infections they may carry. Given the wide differences observed between the two fly populations, the genetic diversity in symbiont populations was investigated. Preliminary results using about 200 flies have confirmed the existence of *Sodalis* diversity in field flies and allowed for the identification of about twenty different genotypes (Tchoumene-Labou et al., personal communication).

It has been possible to establish *in vitro* culture conditions for *Sodalis*, to introduce and express foreign genes and to reintroduce the modified symbionts into tsetse (Beard et al.,

1993a). Other desirable traits can now be introduced and expressed in *Sodalis*. One application of the paratransgenic process for SIT is the ability to engineer release lines that are unable to transmit trypanosomes in the field since both male and female tsetse blood feed and transmit trypanosomes. The establishment of such lines will further enable the ongoing sterile male release programs and those planned in human disease endemic areas (Aksoy et al., 2008a, 2008b; Rio et al., 2004).

To enable paratransgenic applications two candidate trypanosome-interfering proteins were selected for expression in *Sodalis*: the human trypanolytic serum protein, Apolipoprotein L-1, and a trypanosome targeting Nanobody[®] Nb33. For trypanocidal impact, it is essential that the expressed proteins are released from the symbiont into the midgut lumen (Caljon et al., 2013). This requires improved knowledge of the functional secretion pathways to the periplasmic and/or extracellular environment in *Sodalis*. Expression of a marker gene (Green Fluorescent Protein, GFP) fused to the twin-arginine signal peptide of TMAO reductase (TorA), which is known to target proteins across the cytoplasmic membrane via the twin-arginine translocation (Tat) system was successful. Thus, it appears that heterologous proteins can be exported to the periplasm of *S. glossinidius* in an active form by the Tat pathway. This periplasmic expression is an important first step in the development of a *Sodalis* expression system that will allow the release of the trypanosome-targeting nanobodies into the extracellular environment where they can tackle the trypanosome parasite.

4.1.3. Genus *Wolbachia*—The third symbiont *Wolbachia* is an obligatory intracellular and maternally transmitted alphaproteobacterium that infects many arthropod and filarial nematode species (Saridaki and Bourtzis, 2010; Werren et al., 2008). *Wolbachia* is responsible for the induction of a number of reproductive alterations and cytoplasmic incompatibility (CI) (Saridaki and Bourtzis, 2010; Serbus et al., 2008; Werren et al., 2008). In its simplest form, CI is expressed as embryonic lethality when an infected male is crossed with an uninfected female (Werren et al., 2008; Saridaki and Bourtzis, 2010). *Wolbachia*-induced CI has been proposed as a tool for the control of vector populations and diseases (Aksoy et al., 2001; Beard et al., 1993b, 1998; Bourtzis, 2008; Bourtzis and O'Neill, 1998; O'Neill et al., 1993). A prerequisite for the development of symbiont-based control strategies is the detection and characterization of the symbiotic communities of both laboratory and natural population of the target species. Although the presence of *Wolbachia* has been reported in a limited number of tsetse flies samples (Cheng et al., 2000; O'Neill et al., 1993), a thorough investigation for the presence of this symbiont, particularly in natural populations, as well the genotyping of these infections was lacking. One of the specific objectives of this CRP was to study the prevalence, genotyping and phylogenetic analysis of *Wolbachia* infections in both laboratory and natural populations of *Glossina* species including the investigation of the potential reproductive effects this infection may have in tsetse flies (see Doudoumis et al. 2013; Wang et al. 2013, Schneider et al. 2013; (Alam et al., 2012; Doudoumis et al., 2012).

4.1.4. Intercommunity dynamics—It has been possible to develop tsetse lines that are either devoid of *Wigglesworthia* or that are symbiont free by supplementation of the host

diet with ampicillin and tetracycline along with yeast extract, respectively (Alam et al., 2012; Pais et al., 2008). These lines have allowed for investigations into the functional roles of tsetse's different symbionts. In the absence of *Wigglesworthia* (while still harbouring *Sodalis* and *Wolbachia*) adult flies were found to be unusually susceptible to infections with trypanosomes. Molecular investigations into tsetse's immune responses under these conditions have shown that larvae developed in the absence of *Wigglesworthia* give rise to adults that have compromised cellular immune responses (Weiss et al., 2011, 2012). Many studies are now indicating that interactions between the beneficial microbes harboured by insect hosts and pathogens represent highly dynamic processes. The paper presented by Wang et al., describes the intercommunity influences on symbiont density regulation by following symbiotic densities in these lines through multiple generations. These studies show evidence for cooperation between *Wigglesworthia* and *Sodalis* such that fly lines that lack *Wigglesworthia* while retaining *Sodalis* and *Wolbachia* eventually lose *Sodalis* infections by the third generation, while *Wolbachia* and GpSGHV densities initially decline but eventually reach normal levels. Interestingly, in the absence of tsetse's symbionts, GpSGHV densities display relaxed density levels but reach homeostatic levels by the third generation. Thus, tsetse host responses as well as community interdependencies are apparently influential in shaping symbiotic densities.

4.1.5. Symbiotic influences on female mating behavior—Data have been obtained on spatial/temporal variation in the reproductive behavior of *G. fuscipes fuscipes*. In particular, the occurrence of female mating and remating has been assessed in two natural populations in Uganda (Bonomi et al., 2011). Different mating and remating estimates provide evidence that remating is a frequent behavior in the wild and what is more, females store sperm from multiple males, which are potentially available for insemination. In these two populations, *Wolbachia* infection frequency is high. Work is in progress to assess if the presence of *Wolbachia* infections may result in a selective advantage for polyandry and on the consequent sperm use.

4.2. Tsetse fly pathogens

The majority of research on the SGHV has been conducted under objective 2 *to understand and manage the tsetse SGHV interactions in lab populations*. The **first** of the five verifiable indicators for this objective was the analysis of the GpSGHV genome isolated from the Uganda tsetse strain maintained since 1979 at Seibersdorf. The collaborative research effort of the IAEA laboratories at Seibersdorf, and others have produced and published a full-length sequence of the GpSGHV (Abd-Alla et al., 2008). In addition, the complete genome sequence of the SGHV isolate from Ethiopian *G. pallidipes* colony has been sequenced. The two viruses share 98.7% nucleotide sequence identity, their overall organization is almost identical, and 80% of their ORFs have the same best blastp value, strongly supporting the conclusion that they are two isolates of the same virus (unpublished data). The putative 160 ORFs of both genome isolates correspond to expressed sequences, their transcriptome maps showed that over 90% of the putative ORFs are transcribed (unpublished data).

Phylogenetic analysis conducted on several genes demonstrated that the GpSGHV differs significantly from all known large dsDNA viruses of invertebrates (baculoviruses,

nudiviruses, iridoviruses, ascoviruses, entomopoxviruses and whispoviruses) (Fig. 2). Based on these data and the structure of the virus particle, a new virus family, named *Hytrosaviridae*, has been proposed to and accepted by the International Committee on Taxonomy of Viruses (ICTV) by the newly established Hytrosaviridae Study Group (Abd-Alla et al., 2009b). The name *Hytrosaviridae* is derived from *Hytrosa*, a sigla from the Greek ‘*Hypertrophia*’ for ‘hypertrophy’, ‘*sialoadenitis*’ for ‘salivary gland inflammation and consist of two genera: (1) *Glossinavirus* with the type species *Glossina hytrosavirus*, and (2) *Muscavirus* with the type species *Musca hytrosavirus*. GpHV belongs to the species *Glossina hytrosavirus*. The genus *Muscavirus* includes the SGHV from *Musca domestica*. Its genome, although smaller (124,241 bp) than that of the *GpSGHV*, produces similar SGH symptoms (Fig. 2), and contains a number of putative ORFs which are homologues to those of *GpSGHV* and which are distinct from other insect viruses (Garcia-Maruniak et al., 2008, 2009). Phylogenetic analyses have been performed to elucidate the relationship of SGHVs to the baculoviruses and nudiviruses, as these viruses share a number of core genes involved in DNA replication, RNA transcription and virion host cell interaction (Wang et al., 2011; Wang and Jehle, 2009). There is strong evidence that hytrosaviruses, nudiviruses and baculoviruses comprise a supergroup of invertebrate-specific nuclear-replicating circular dsDNA viruses, warranting the establishment of a new virus Order (Jehle et al., 2013).

The availability of sequence information from multiple closely related and disparate SGHVs has provided a framework to identify and design molecular probes that target common domains. These data have been used to develop universal primer pairs designed to detect SGHVs (Abd-Alla et al., 2011b). Experiments have demonstrated that these primers generated a virus-specific PCR product from both MdHV and GpHV whereas no non-specific PCR product was found when using genomic DNA of healthy flies as templates. Therefore, these primers sets could be used to detect novel hytrosaviruses in other insects. A similar approach can be taken in designing dsRNA to target homologous regions to reduce viral replication. Significantly, this approach will allow validation of the suppressive activities of RNAi in the easily manipulated house fly host.

LC-MS/MS analysis revealed that *GpSGHV* contains 61 virus-encoded proteins (Kariithi et al., 2010). The most abundant proteins (GP10 and GP96) are products of the ORF10 and ORF96 genes. The proteome contained homologues to four *per os* infectivity factors (PIFs), that are essential for oral infectivity in baculoviruses (Slack and Arif, 2007) and a giant protein (511 kDa) encoded by ORF62. Antiserum generated against virions strongly reacted with GP10 and GP96 and a few other virion proteins, such as the PIF proteins. Among the *GpSGHV* structural proteins, 32 had homolog genes in the MdHV genome, although only 12 of these were detected in the MdHV proteome. This supports the recent assembly of *GpSGHV* and MdHV into two separate genera within the accepted family *Hytrosaviridae*. Information on the GpHV proteome is pivotal for both neutralization of virus infection and interruption of virus transmission in tsetse fly colonies. Boucias et al. (2013) described the major envelope protein of MdHV and showed that the neutralization of the virus particle of MdHV with the specific envelope protein antibodies reduced levels of the virus infection. The analysis of the saliva of hypertrophied flies indicated, in addition of the virion proteins,

the presence of a suite of host specific proteins providing new leads for virus control studies (Kariithi et al., 2011).

The **second** verifiable indicator was deciphering the pathology of the SGHVs. Utilizing quantitative PCR-based diagnostic methods (Abd-Alla et al., 2009a, 2010b), non-infected and infected flies have been identified and were used to demonstrate that this virus can be both vertically and horizontally transmitted in colonized tsetse flies. In addition to salivary glands that display overt pathologies, a wide range of tissues was found to support replication (Abd-Alla et al., 2009b). A parallel study, conducted by Lietze et al. (2011c), demonstrated that the MdHVDNA and resulting transcripts can be detected at high levels in a wide range of tissues without inducing hypertrophy. EM studies suggested that the tracheole system extending throughout all tissues is an alternate site of replication for MdHV. In heterologous fly species, evidence is accumulating that the SGHVs can replicate without causing detectable hypertrophy symptoms. For example, challenge of the stable fly (*Stomoxys calcitrans*) and the black dump fly (*Hydrotaea aenescens*) with MdHV sterilized females without inducing hypertrophy (Geden et al., 2011a).

Replication of GpHV (Seibersdorf) and of MdHV (Florida) was attempted in 21 cell lines derived from *Lepidoptera*, *Hymenoptera*, *Hemiptera* and *Coleoptera*. Inoculated cells were observed daily for evidence of virus-induced cytopathic effects and alteration in cell growth. To date, no evidence of replication of either virus was observed in the tested cell lines, which underscores the need to generate novel cell lines from the tsetse fly and house fly (see Arif, 2013).

The **third** verifiable indicator was the establishment and maintenance of asymptomatic colonies. To date, not all colonized flies have been shown to harbour GpHV. In Tanzania, molecular analysis of laboratory *Glossina pallidipes* did not detect SGHV infection; however a prevalence of 5% was recorded from *G. pallidipes* and *G. morsitans* lab flies in Kenya. The relative incidence of this virus in wild populations varies from one location to another. For example, SGHV prevalence studies in different wild tsetse populations from two sites in Tanzania (coastal and inland) did not reveal infection in 200 dissected flies and 30 flies analyzed by PCR (Malele et al., 2013). In Kenya, six out of eight feral populations of *G. pallidipes*, *G. brevipalpis* and *G. austeni*, had infection rates ranging from 0% to 77%. Further screening will be performed to verify the status of colonized laboratory flies, and screening of feral populations from the three countries will continue in order to identify if there are any virus-free populations. Genetic analysis of GpHV from different regions of East Africa suggested a high degree of conservation enhancing the feasibility of a generic strategy for virus control (Kariithi et al., 2013).

The **fourth** verifiable indicator was the development of management strategies to reduce virus loads in tsetse fly mass rearing (Abd-Alla et al., 2011a). Polyclonal antibodies were generated against eight GpHV virion proteins, including those encoded by ORF10, ORF96 and the four PIF genes to study their potential to neutralize GpHV infection and to block virus transmission in tsetse flies. As the ORF10 protein has been localized on the surface of the GpHV virion, the anti-ORF10 has potential to block transmission of GpHV and is, therefore, a good candidate to block transmission of the virus in tsetse fly rearings and

production systems (Kariithi et al., 2010). The impact of several antiviral drugs on the viral infection in *G. pallidipes* is presently being examined as a potential blood supplement. Recently, an effective control of GpSGHV in laboratory condition using antiviral drug valacyclovir has been achieved (Abd-Alla et al., 2012).

Several experiments were carried out to better understand the virus transmission and biology (Abd-Alla et al., 2010b). After demonstrating the role of horizontal transmission through the membrane feeding system used in the tsetse fly laboratory colony, it was recommended to initiate a “clean feeding” system. One clean-feeding colony was established by feeding the fly cages first on the fresh membrane and blood and keeping these cages and their progeny fed in the same way. The remaining blood was used by the other “normal-feeding” colony. The “clean feeding” colony was maintained separately from the other colony and samples were taken regularly to assess the virus load and the prevalence of SGH. The qPCR results indicated significant decreases in the virus load in the “clean feeding” colony in comparison with the “normal feeding” colony. Fly dissections indicated that after three months the clean-feeding colony became a SGH-free while the normal-feeding colony continued to show about 10% SGH (unpublished data).

A **fifth** verifiable indicator was to demonstrate horizontal transmission of entomopathogenic fungal inoculum. Experiments conducted in a large field cage (Calkins and Webb, 1983) to simulate field conditions have confirmed the horizontal transmission of conidia between *Metarhizium anisopliae*-infected *G. pallidipes* and fungus-free flies when the flies were allowed to mate. All the fungus-treated “donor” male or female flies succumbed to fungal infection within 6–10 d with mycosis. First or second line “recipient” flies that succeeded in mating with the “donor” flies, also died from fungal infection with mycosis within 7–15 days after mating. Fungal infection also had an effect on the reproduction potential of flies. Female flies in the control treatment produced more puparia than the fungus treatments, except in the second line recipient where there was no difference between the treatments (Maniania and Ekesi, 2013).

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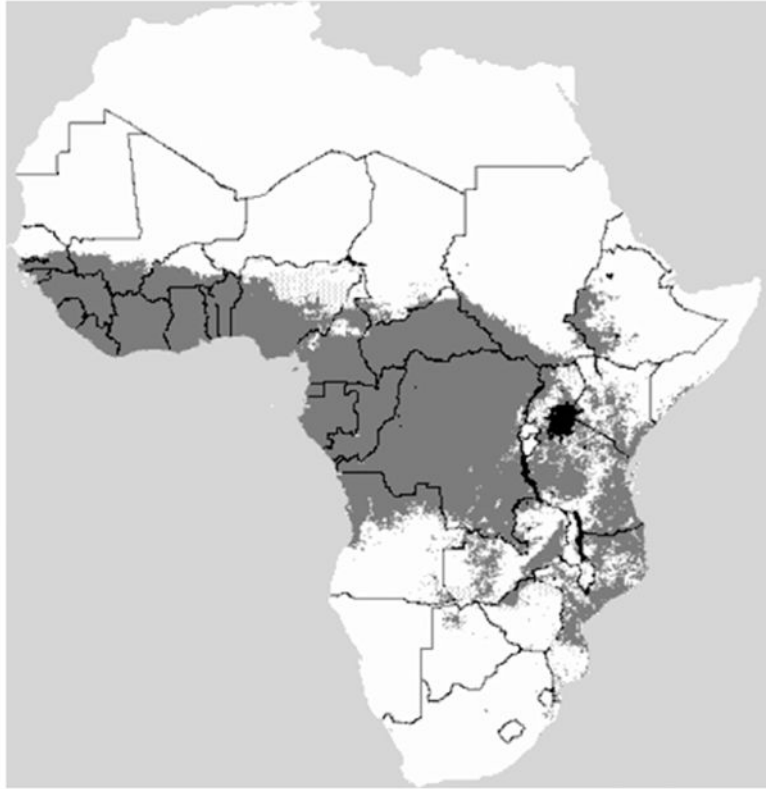


Fig. 1. Tsetse fly distribution in African continent (modified from PAAT information system <http://www.fao.org/ag/againfo/programmes/en/paat/infosys.html>).

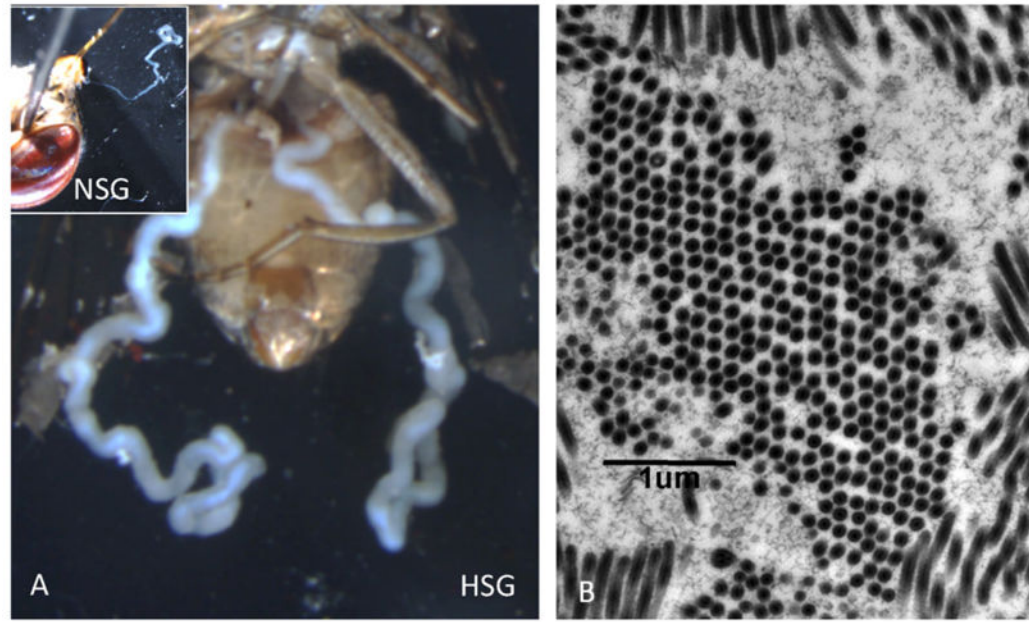


Fig. 2.
(A) Normal (NSG) and hypertrophied salivary gland (HSG) of *Glossina pallidipes* fly. (B) Thin section of a heavily infected salivary gland cell showing bundles of GpHV virions in both longitudinal and cross sections.

Table 1

Specific objectives and the expected output of the CRP.

Objectives of the CRP	Expected output of the CRP	Papers published during the CRP
(i) Decipher the interactions between the tsetse, symbiont and other microbes	<ol style="list-style-type: none"> i. Improvement of the mass rearing procedures ii. Study the population dynamics of tsetse microbial flora 	Alam et al. (2012), Bonomi et al. (2011), Bourtzis (2008), Muturi et al. (2011), Pais et al. (2008) Abd-Alla et al. (2010b), Adams et al. (2008, 2010), Doudoumis et al. (2012), Enyaru et al. (2010), Farikou et al. (2010a, 2010b, 2011a, 2011b), Geden et al. (2008, 2011b), Geiger et al. (2007, 2009, 2010, 2011), Hamilton et al. (2008), Lietze et al. (2011b), Malele et al. (2007, 2011a, 2011b) and Prompiboon et al. (2010)
(ii) Understand and manage tsetse - virus interactions in laboratory populations,	<ol style="list-style-type: none"> i. Characterize salivary gland hyperplasia virus (SGHV) group to develop virus mitigation protocols in colony rearing ii. Manage the impact of the virus to optimize mass production process 	Abd-Alla et al. (2007, 2008, 2009a, 2009b, 2010a, 2011b), Garcia-Maruniak et al. (2008), Garcia-Maruniak et al. (2009), Geden et al. (2011a), Karithi et al. (2010, 2011), Lietze et al. (2010, 2011c, 2011a), Wang et al. (2007) and Wang and Jehle (2009) Abd-Alla et al. (2010a, 2011b) and Sasanya et al. (2010)
(iii) Manipulate microbial flora to express parasite refractoriness traits	<ol style="list-style-type: none"> i. Develop parasite refractory lines ii. Identify trypanosome inhibitory products iii. Incorporate parasite resistance traits into SIT lines 	De Vooght et al. (2011, 2012) Akoda et al. (2009a, 2009b and 2009c) Scolari et al. (2011)
(iv) Harness symbiont-mediated natural mating incompatibilities to improve tsetse suppression technology	<ol style="list-style-type: none"> i. CI-mediated gene drive system ii. Harness CI for SIT application 	Alam et al. (2012), Doudoumis et al. (2012), Ioannidis et al. (2007), Saridaki and Bourtzis (2009) and Saridaki and Bourtzis (2010) Ioannidis et al. (2007), Saridaki and Bourtzis (2009, 2010)
(v) Disseminate knowledge among disease endemic country researchers to improve field application of SIT	<ol style="list-style-type: none"> i. Disseminate discoveries to endemic countries and interested parties and relevant databases 	http://entnemdept.ufl.edu/boucias/hytrasaviridae/Salivary_Gland_Hypertrophy_Viruses/Home6.html , http://www.sanger.ac.uk/cgi-bin/blast/submitblast/g_morsitans , www.cost-fa0701.com

Objectives of the CRP	Expected output of the CRP	Papers published during the CRP
through better decision making and capacity building	ii. Publish results in scientific journals	