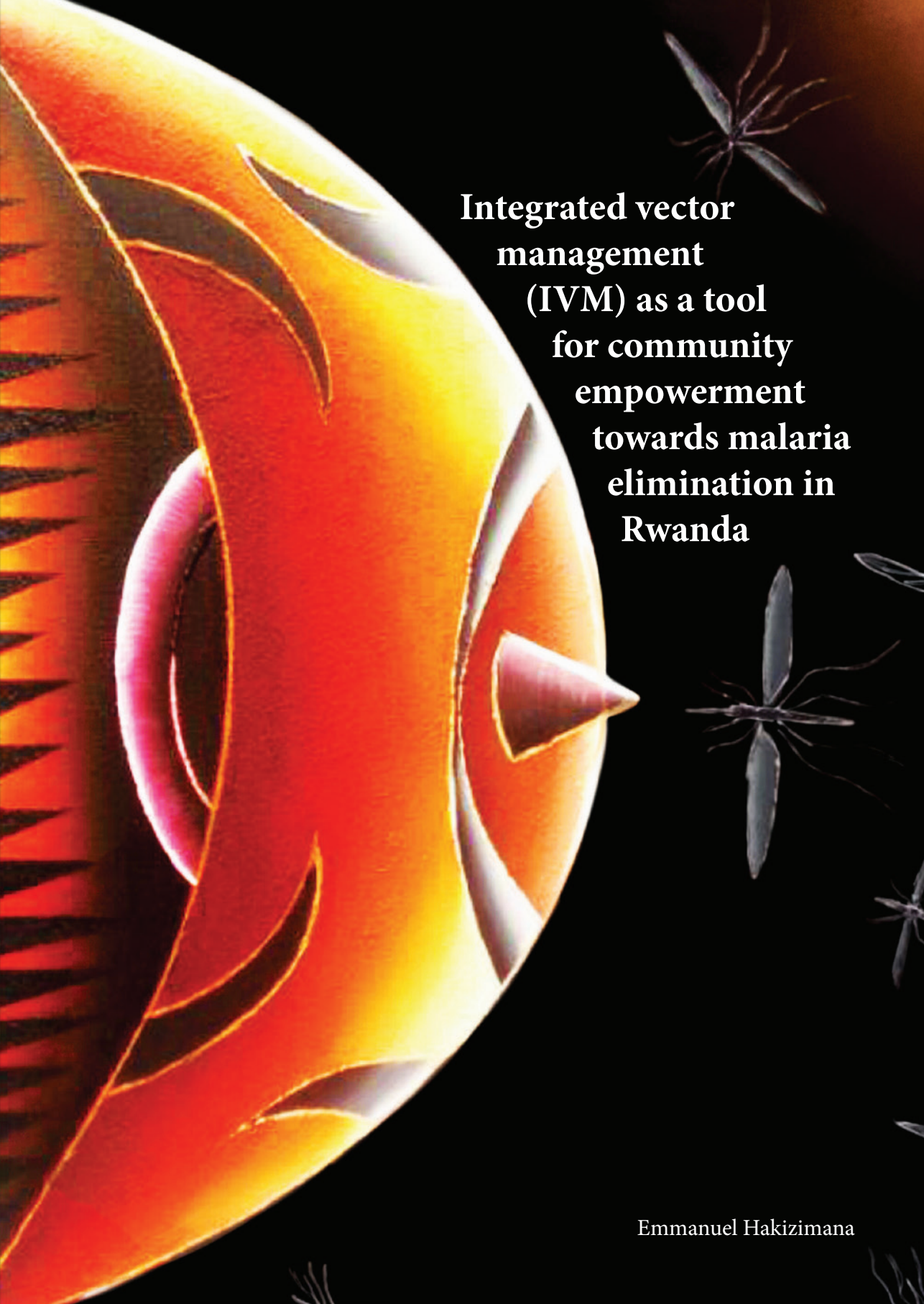

Integrated vector management (IVM) as a tool for community empowerment towards malaria elimination in Rwanda

Emmanuel Hakizimana

Propositions

1. Malaria elimination will not be feasible without timely deployment of effective vector control tools based on location-specific environmental characteristics.
   (this thesis).
2. For successful biological control of malaria mosquito larvae with Bacillus thuringiensis var. israelensis, community education on the mosquito life cycle is essential
   (this thesis).
3. The use of early-warning tools will lead to more accurate predictions of climate-change associated outbreaks of vector-borne disease.
4. The sustainable protection of endangered mountain gorillas needs conservation strategies that are aligned to long-term research of their ecosystem components.
5. Suppression of personal freedom in human society destroys a person's confidence throughout her/his life.
6. Education without practical purpose is a source of learner demotivation.

Propositions belonging to the thesis, entitled
'Integrated vector management (IVM) as a tool for community empowerment towards malaria elimination in Rwanda'

Emmanuel Hakizimana
Wageningen, 19 February 2019

Integrated vector management (IVM) as a tool for community empowerment towards malaria elimination in Rwanda

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General Introduction

Chapter 1

Introduction

Malaria remains the most important vector-borne disease affecting humans in tropical regions (Stratton et al., 2008; WHO, 2016). The disease is caused by a protozoan parasite belonging to the genus of *Plasmodium,* and is transmitted to humans by female *Anopheles* mosquitoes (Diptera: Culicidae) that usually bite at night (Cox, 2010; WHO, 2017a). The burden of malaria is estimated at 216 million new malaria cases and 445,000 deaths in 2016 where respectively 90% and 91% are reported from the African region (WHO, 2017c). From the start of the previous decade, global efforts in fighting malaria were met with substantial success (Bhatt et al., 2015; Mendis et al., 2009; Newby et al., 2016). Since 2000, a decline in the burden of malaria was reported, including in some endemic countries in sub-Saharan Africa (Bhatt et al., 2015; Gatton et al., 2013; Murray et al., 2012; Noor et al., 2014; Takken & Knols, 2009). Since then, 17 countries achieved malaria elimination and many other have reduced malaria cases and are currently moving forward to the ambitious goal of malaria elimination (WHO, 2017a).

Despite tremendous achievements, the gains made in malaria control appear fragile, particularly in Africa (White et al., 2014). Malaria control is currently facing a number of threats, in particular the spread of resistance in mosquitoes to insecticides (Ranson & Lissenden, 2016), and the emergence and further spread of *Plasmodium falciparum* resistance to the drug artemisinin (Fairhurst & Dondorp, 2016). The inadequate performance of health systems and the strong dependence on external donors in low income, malaria endemic countries are other hindrances to sustainable malaria control and elimination (Gosling et al., 2015; Gueye et al., 2016). These challenges have to be addressed with the aim to avert the exposure of vulnerable populations to a resurgence of malaria transmission and epidemic outbreaks (Himeidan & Kweka, 2012). To enable effective and sustainable vector control interventions, proactive strategies and innovative tools are required that are implemented in accordance to the principles of Integrated Vector Management (IVM) (WHO, 2017b). The definition and application of IVM is discussed further below in this section. There is a growing body of evidence that suggests that by application of IVM, malaria can be more effectively reduced and new cases prevented (Chanda et al., 2008; Fillinger et al., 2009; Homan et al., 2016). In this thesis I explore the possibilities for IVM as a strategy for malaria control in Rwanda, and provide information on malaria vector distribution, ecology, insecticide resistance and new tools to be used in the framework of IVM.

Chapter 1

Malaria parasites and transmission

Malaria in humans is caused by five parasite species belonging to the genus *Plasmodium*: *Plasmodium falciparum*, *P. malariae*, *P. ovale, P. vivax* and *P. knowlesi* (Cox, 2010). Their distribution varies by zoo-geographic regions (WHO, 2017a). *Plasmodium falciparum* and *P. vivax* are the most dominant species (Bousema & Drakeley, 2011; Guerra et al., 2010; Killeen, 2014). *Plasmodium falciparum* is responsible for most malaria infections and deaths in Africa, whereas *P. vivax* is widely distributed and causing malaria infection in Asia and Latin America and some limited parts of Africa (White et al., 2014). In the past decade, a fifth parasite species, *P. knowlesi*, was described in South-East Asia, particularly in Malaysia (Cox-Singh et al., 2008). It is associated with cases of zoonotic *Plasmodium* infection, with macaque monkeys as the natural reservoir (William et al., 2013). *Plasmodium knowlesi* currently represents a growing public health concern and may threaten the efforts of malaria elimination undertaken in the region (Barber et al., 2017; William et al., 2014; William et al., 2013).

The development cycle of *Plasmodium* parasites passes through two different hosts: *Anopheles* mosquitoes and humans. The sexual phase or sporogony occurs in the mosquito and comprises a number of stages occurring in different organs of the insect (Aly et al., 2009). In the final stage, the sporozoite forms of the parasite migrate and accumulate in the salivary glands of mosquitoes, from where they are transmitted to humans during the next mosquito bite (Cox, 2010). Inside the human host, the sporozoites undergo the asexual phase (schizogony) which consists of two stages: the exo-erythrocyte cycle within cells of the liver and endo-erythrocyte cycle inside red blood cells (Aly et al., 2009). The duration of each cycle varies according to the parasite species. After invading the hepatocyte (liver) cells, some sporozoites of *P. vivax* and *P.malariae* often become dormant and remain so for a period between three to 18 months, and in rare cases up to five years (Aly et al., 2009). The male and female gametocyte forms of the parasite continue the development cycle in the human host and can be picked up by the mosquito vector upon the next bite (WHO, 2017a).

Malaria vectors

Of approximately 3,500 mosquito species described worldwide (CDC, 2015), an estimated 70 species have been found to transmit malaria, and 41 of these are recognized as dominant

vector species (Hay et al., 2010; Sinka, 2013). Each vector species has developed specific behavioral patterns and occupies a different niche. The host preference and climate conditions are factors explaining the distribution and dynamics of mosquito populations. The typical example is demonstrated by the two major vectors of malaria in Africa where *Anopheles gambiae sensu stricto* dominates in saturated environments, while its sibling species *Anopheles arabiensis* colonizes more arid areas (Lindsay et al., 1998). The life cycle of mosquitoes consists of four distinct stages: egg, larva, pupa and adult (WHO, 2013c). The development time of the various stages depends on the ambient temperature and on nutritional factors. A blood-meal from a vertebrate host is required for female mosquitoes for maturation of the eggs (WHO, 2017a).

Breaking the malaria cycle – interruption of transmission

Human malaria transmission involves complex development cycles of *Plasmodium* parasites and *Anopheles* mosquitoes (Delves et al., 2012). Thus, most malaria control interventions are designed to target the vulnerable stages of each life cycle with the aim to interrupt transmission (Bruce-Chwatt, 1987; Delves et al., 2012). However, the interruption of malaria transmission remains one of the biggest challenges in malaria control particularly in tropical regions (Gonçalves & Hunziker, 2016). The strategies that are used to interrupt the cycle in humans are primarily based on treatment with antimalarial drugs, the development of a vaccine and transmission-blocking interventions (Nilsson et al., 2015). The liver forms, and the sexual stages or gametocytes which thereafter infect mosquitoes are crucial bottlenecks for breaking malaria transmission (Delves et al., 2012).

The life cycle of mosquitoes passes through immature aquatic stages (eggs, larvae and pupae) and the adult stage. The strategies used to break the larval development cycle are based on the suppression of mosquito breeding sites and killing larval stages (Killeen et al., 2013; WHO, 1982). The adult stage of mosquitoes is characterized by the feeding or gonotrophic cycle which is subdivided into host-seeking, feeding, and resting phases (Chitnis et al., 2008). Each phase presents certain risks of mosquito survival. The primary interventions focus on the prevention of man-vector contacts or killing the mosquito vectors. These interventions contribute to shorten the lifespan of the mosquito vector, and to a reduction of mosquito populations and hence a suppression of *Plasmodium* parasite development (Childs et al., 2016). Moreover, a wide range of other technologies are targeting mosquitoes when they enter houses, feed outdoors, attack livestock, feed on sugar or aggregate into mating swarms

(Delves et al., 2012). Conventional and innovative vector control strategies targeting the interruption of malaria transmission at the vulnerable stages of the mosquito life cycle are detailed in the following sections.

Malaria vector control strategies

Learning from the past: dynamic strategies

The Global Malaria Eradication Programme (GMEP) was launched in 1955 by the World Health Organization after the discoveries of dichloro-diphenyl-trichloroethane (DDT) in 1939 as the most effective vector control tool and of the antimalarial drug chloroquine in 1934 (Bruce-Chwatt, 1987; WHO, 1957). This programme was vertically organized, and mainly based on indoor spraying with DDT for vector control and treatment of patients with chloroquine. Therefore, it discouraged traditional vector control measures such as environmental management and house improvement as they were considered as antagonists to the goal of eradication (Najera et al., 2011). DDT was the first long-lasting insecticide with a residual effect that extended from six to 12 months while previously, sprayings with pyrethrum insecticides had to be repeated on a weekly basis (Najera et al., 2011). Hence, it was judged feasible to interrupt malaria transmission and achieve eradication, specifically in endemic areas where malaria was prevailing at low to moderate transmission levels (Mendis et al., 2009). The GMEP was implemented worldwide from 1955, but the tropical African region was not included in this global initiative (Feachem & Sabot, 2008). The GMEP was abandoned in 1969 because of administrative, financial and technical constraints, as well as the withdrawal of professional staff and the resistance of mosquitoes to DDT (Najera et al., 2011) and of *Plasmodium* to chloroquine used for malaria treatment (Payne, 1987). Some countries in temperate regions with well-functioning health programs did achieve malaria elimination, but other endemic countries faced a resurgence of malaria after a prolonged period of interruption (Cohen et al., 2010; Mendis et al., 2009). In much of tropical Africa, malaria remained as endemic as before, with small-scale, localized control efforts.

Based on the lessons from the malaria eradication era, the global community was again mobilized to control malaria with re-orientated strategies focusing on local and progressive malaria elimination in the short term and then eradication in the long term (RBM, 2008; WHO, 2005). This rebirth of global malaria control was triggered by promising results from new malaria control interventions tested in different settings such as Insecticide Treated Nets (ITNs) and later on Long-lasting Insecticide treated Nets (LLINs), new residual insecticides

for Indoor Residual Spraying (IRS), artemisinin-based combination therapy for malaria treatment and rapid diagnostic tests (Bhatt et al., 2015; Newby et al., 2016). This renewed interest for malaria control was also motivated by an increase in external funding channeled through well-structured funding mechanisms (e.g. The Global Fund to Fight AIDS, Tuberculosis and Malaria, the US President's Malaria Initiative and the Bill and Melinda Gates Foundation), the high commitment from regional and local organizations, and the development and endorsement of global guidance frameworks.

These initiatives eventually resulted in an impressive global decrease of malaria morbidity and mortality in many countries including in Africa (Bhatt et al., 2015; Noor et al., 2014; UCSF, 2017; WHO, 2015b). For instance, malaria incidence dropped by 37% and deaths by 60% (WHO & UNICEF, 2015). Moreover, from 2000 until 2015, 17 countries achieved malaria elimination (WHO, 2016), but this was still far from the ultimate goal of the Roll Back Malaria Partnership, which envisioned a world free from malaria with a target of near zero malaria deaths by 2015 (RBM, 2008).

The current frameworks for malaria control set the targets for 2030, and are made of the so-called global technical strategies (GTS) for malaria 2016-2030 (WHO, 2015b), the Action and Investment to Defeat Malaria (AIM) (RBM, 2015), and the "From Aspiration to Action: what will it take to end malaria?" (Gates & Chambers, 2015). The first two strategies clearly outline the required progress for malaria elimination, while the third renewed the global debate on the feasibility of malaria eradication by 2040. More specifically for the global vector control response, the recent document endorsed by the 70th session of the World Health Assembly outlines the strategies and specific actions to tackle the threats of vector borne diseases (WHO, 2017b).

All the recent strategies highlight the need for new tools in the areas of treatment and diagnostics, prevention (vaccines), improved surveillance and response, increased and sustainable funding resources, and partnerships among all relevant sectors (Alonso et al., 2011; Ferguson et al., 2010; Hemingway et al., 2016; WHO, 2005). The implementation of Integrated Vector Management (IVM) has been revealed as one of the most feasible approaches, and there is thus an urgent need to design and develop programmes that incorporate these into malaria control strategies that will be carried out at national level (Birkholtz et al., 2012).

The core interventions for vector control

The World Health Organization categorizes existing vector control interventions into 'core' and 'supplementary' interventions (WHO, 2017a). Bednets (ITNs and LLINs) and indoor residual spraying (IRS) are considered as core interventions and reduce human biting rates and survival of the mosquito, respectively (WHO, 2008a). Both contributed to the drastic decline of malaria prevalence and mortality (Apinjoh et al., 2015; Bhatt et al., 2015; Fegan et al., 2007; Giardina et al., 2014; Lim et al., 2011) and represent more than 60% of the global malaria control investment (WHO, 2012a).

ITNs and LLINs provide both personal and community level protection for the sleepers against infective bites from *Anopheles* mosquitoes (Mutuku, 2011). Although LLINs are still below the universal coverage ratio of one ITN/LLIN per 1.8 persons in the African region (WHO, 2014b), it is by far the largest vector control intervention (Bhatt et al., 2015; Giardina et al., 2014; Le Menach et al., 2007). Optimal protection occurs when used every night by community members and when treated with an effective and recommended insecticide (Gimnig et al., 2003; Smith et al., 2009; WHO, 2017a).

IRS consists of applying a residual insecticide to potential mosquito resting sites within human dwellings such as the interior walls, eaves and ceilings or livestock shelters (WHO, 2015a). IRS repels malaria vectors entering into houses or kills them when resting indoors after taking a blood meal thereby cutting the risk of malaria infection to others in the vicinity (WHO, 2017a). Importantly, the effectiveness of IRS is based on the fact that major malaria vectors usually display indoor biting and resting behaviour when they are searching for blood meals (WHO, 2006). However, in many areas these patterns are changing rapidly as a result of the selective pressures exerted by the insecticides on these behavioural patterns (Killeen, 2014)

Optimal impact of IRS on malaria transmission requires adequate capacity and well established national programmes to ensure its proper planning, implementation and supervision (Phiri et al., 2015; WHO, 2006). This requires a high coverage of sprayable structures reaching above 85% (WHO, 2015a). In combination with other malaria control interventions, the recent scaling up of IRS in Africa has contributed to further declines in malaria morbidity and mortality (Bhatt et al., 2015; Pluess et al., 2010) and in the prevalence

of anemia (Steinhardt et al., 2013). The combination of IRS and ITNs/LLINs provides a higher reduction of malaria transmission than LLINs alone in areas of moderate coverage of LLIN and high resistance of malaria vectors to pyrethroids (Gimnig et al., 2016; Hamel et al., 2011; Lines & Kleinschmidt, 2014; Protopopoff et al., 2015).

Since 2010, however, the proportion of the population protected by IRS is declining due to the need to manage resistance by deploying more expensive insecticides (carbamates and organophosphates) leading to lower household coverage (WHO, 2016). Although the use of carbamates contributes to the management of resistance by reducing the frequency of genetic-based knock-down resistance in *Anopheles* mosquitoes (Abeku et al., 2017), re-establishment of malaria transmission to pre-intervention levels may eventually occur (Cohen et al., 2012). Unfortunately, in high and stable malaria transmission settings from low and middle income countries, there is not any evidence showing that one or a combination of the two interventions could fully interrupt malaria transmission (Ferguson et al., 2010).

The supplemental interventions

There is evidence that residual malaria transmission is maintained in many settings despite the high coverage and usage of both ITNs/LLINs and IRS (Govella et al., 2013; Russell et al., 2011). These interventions alone or in combination not always reduce human exposure to less than one infective bite per year in tropical regions, which is required to successfully implement a malaria elimination program (Shaukat, 2010). Such residual malaria transmission occurs mainly in areas where malaria vectors acquired resistance to existing insecticides or developed behaviours such as outdoor resting, earlier and outdoor biting, preferential biting upon animals and avoiding contact with treated materials with insecticide (Govella et al., 2013; Killeen, 2014). Similarly, also human behaviours contribute to residual transmission, for example living in or paying frequent visits to forest areas, sleeping away from protected houses, specifically during hot seasons, or spending a major part of the night outdoors without any protective measures (WHO, 2014a).

For these reasons, supplemental interventions are required to address the above barriers and sustain interruption of malaria transmission (Hemingway et al., 2016). The development of new vector control tools is based on targeted bionomic and behavioural characteristics of malaria vectors at both the immature and adult stages (Russell et al., 2013), but also

incorporates options to address human behaviours that may stimulate the implementation of these new tools (Ingabire et al., 2014).

Available supplemental vector control interventions

Larval source management (LSM) is one of the oldest malaria control tools extensively deployed to control mosquitoes at the source, targeting the larval stages in water bodies (Fillinger & Lindsay, 2011). The method is categorized into modification or manipulation of habitats through permanent change of land or water, and through recurrent activities such as intermittent irrigation which create unfavorable breeding conditions of mosquito vectors (Keiser et al., 2005). LSM also refers to biological control by introducing the natural predators or enemies of mosquitoes into water bodies, and to larviciding through the application of biological or chemical insecticides into larval habitats. This intervention was broadly used in earlier malaria elimination programs, and currently as a primary vector control intervention to avert the reintroduction of eliminated vector-borne diseases (Killeen et al., 2017; Tusting et al., 2013; WHO, 2013b).

LSM is recommended in mosquito breeding habitats that are "few, fixed and findable" (WHO, 2013b) in high endemic areas. In the context of IVM, LSM presents a wide range of advantages, including the reduction of vector densities (Afrane et al., 2016; Fillinger & Lindsay, 2006; Imbahale et al., 2012), the removal of residual transmission foci in vector elimination programs, the management of insecticide resistance, and the control of other vector-borne diseases such as dengue, filariasis and Japanese encephalitis (Keiser et al., 2005; Killeen et al., 2013; WHO, 2012).

Before the introduction of IRS with DDT in the 1950s, LSM was the back-bone for vector control in many settings in endemic temperate countries (Fillinger & Lindsay, 2011). At present, there is a renewed interest in the LSM strategy, mainly in areas with high levels of insecticide resistance and a predominance of outdoor biting and resting of major malaria vectors (Geissbühler et al., 2009). Recent field trials carried out in Africa and Asia demonstrated that, in appropriate habitats mainly in urban and peri-urban areas, LSM will contribute to the reduction of mosquito densiti, mosquito infection rates, as well as prevalence of malaria infection (Giroux, 2013). However, other field trials revealed that LSM may not work in large and rural larval habitats, for example in extensive flooding ecosystems, such as the flood plains of The Gambia (Majambere et al., 2010). Thus, for LSM to be effective, the

ecology of the vectors needs to be assessed taking the characteristics of the breeding sites into account.

Mosquito proofed housing has promising results, especially in areas with low quality housing. The intervention works through the promotion of new designs or modification of existing houses (von Seidlein, 2017), screening of windows, eaves, and fitting ceilings (Lindsay et al., 2002; Menger et al., 2016). A significant impact of this method was found on the reduction of mosquito entry in Tanzania (Ogoma et al., 2010) and the prevention of anemia in children in The Gambia (Kirby et al., 2009)

Spatial repellents are other products that prevent the entry of mosquitoes inside houses (WHO, 2013a). The main repellent products currently available on market are represented by mosquito coils, vaporizing mats, ambient emanators, liquid vaporizers and aerosols (WHO, 2009). Traditional application of repellent plants inside houses in hanging pots (Seyoum et al., 2002), directly burned or with fresh whole plants or branches, have been tested with significant reduction of mosquito entry (Seyoum et al., 2002). Repellent products through spatial action of emanated vapor or airborne pyrethroid particles induce repellency, deterrence, and mortality in mosquitoes and thus prevent exposure of the house occupants to mosquito bites and malaria infection (Achee, 2012; Ogoma et al., 2012; WHO, 2013a). However, spatial repellents have not yet been promoted as formal vector control methods, and evaluation of their impact at large scale on malaria incidence and mortality remains limited (Ogoma et al., 2012). Meanwhile, a clinical trial conducted in China demonstrated a similar impact of mosquito coils and LLINs implemented alone or in combination on reducing malaria incidence of *P. falciparum* (Hill, 2014). Other repellents delivered for topical applications or vapour-phase emanators are used for personnel protection and prevent outdoor mosquito bites especially when people are active outdoors and cannot be protected by indoor methods (Achee, 2012; Avicor, 2013).

New vector control tools under evaluation

New technologies are emerging and aim to enhance the action of the primary indoor vector control tools (IRS and ITNs) or to provide responses to other mosquito behaviours (Killeen, 2014). The new indoor technologies include novel insecticide-treated materials, such as durable wall lining (Messenger, 2012), new active ingredients formulated alone or in combination for better management of resistance for IRS (Hemingway, 2014), LLINs

combined with a synergist such as PBO (Tungu et al., 2010) and insecticidal wall paints (Mosqueira et al., 2010).

Other vector control tools, not primarily developed for indoor protection, comprise insecticide-treated clothing for personal protection (Banks et al., 2014; Kimani et al., 2006) and attractive toxic sugar baits. Latter have shown to specifically attract infected *Anopheles* and delay behavioral resistance. They are particularly efficient in arid areas (Beier et al., 2012; Gu et al., 2011; Müller et al., 2010; Stone et al., 2016). Other technologies target the development of outdoor barrier sprays, toxic barrier screens (Hemingway et al., 2016), the control of zoophagic mosquitoes by endectocides such as ivermectin (Chaccour et al., 2013) or treating livestock with topical or systemic insecticides (Donnelly et al., 2015; Rowland et al., 2001). The genetic modification of malaria vectors (Deredeca et al., 2011; Hammond et al., 2016; Wilke & Marrelli, 2015) and the control of males in mating swarms (Sawadogo et al., 2017) are other promising vector control tools.

However, none of the above supplemental vector control tools are accompanied by sufficient epidemiological and/or public health evidence to justify their large scale implementation by the national malaria control programs in endemic areas (Killeen, 2014). Further investments and expertise for their continued development and full evaluation are required in the coming years to enhance vector control and, finally to achieve elimination of residual malaria transmission. In order to achieve this, an IVM approach is essential.

The approach of Integrated Vector Management

In past years, the outcomes attained on malaria control encouraged some countries, including Rwanda, to shift to a malaria elimination strategy (WHO, 2017a). To sustain the gains made in malaria control and to rationalize the usage of limited resources, the WHO has advocated and encouraged endemic countries to adopt the concept of IVM (WHO, 2008b). This concept is not new in the history of malaria control (Beier et al., 2008), but its implementation faces a number of challenges which are still prevailing up to date, such as the lack of capacity, not well defined roles for stakeholders, and a lack of collaboration between the health sector and other sectors (Beier et al., 2008).

IVM is officially defined as “a rational decision making process for the optimal use of resources for vector control”(WHO, 2008b). It is based on five principles which have to be

incorporated into national disease control programs: (1) Advocacy, social mobilization and legislation, (2), Collaboration within the health sector and with other sectors, (3) Integrated approach, (4) Evidence-based decision-making, and (5) Capacity-building (WHO, 2004). IVM aims to improve efficacy, cost-effectiveness, ecological soundness and sustainability of vector control interventions with the available tools and resources (WHO, 2008b). The inter-sectorial and multidisciplinary interventions are critical to improve public health and mitigate the potential health and environmental risks (Naranjo et al., 2014). Implementation of IVM was proven to be more cost-effective when it extends beyond the health sector and when it involves multiple stakeholders (Van Den Berg & Knols, 2006; Van den Berg et al., 2007). IVM implies not only the scaling up of combined vector control tools but also the continued monitoring, and evaluation together with a strong commitment and concerted action by governments and international partners (Beier et al., 2008).

Thus, IVM transforms the conventional vertical system of vector control by making it more evidence-based, and enhancing participative synergy at central, decentralized and community level (Van den Berg & Takken, 2010). It emphasizes that key stakeholders should insert IVM within their health systems and establish inter-sectoral partnerships and collaborations to mobilize resources and build capacity for vector control. Each identified partner plays his role throughout his sphere of influence and expertise (WHO, 2012). However, IVM must be actively advocated and communicated in order to become established from national, decentralized and to community level (Mutero et al., 2012). In many settings where IVM was well planned and implemented, it reduced malaria vector densities and the entomological inoculation rate (Killeen et al., 2000), malaria morbidity and mortality (Chanda et al., 2008), and, importantly, produced cross-cutting impact on other vector-borne diseases, such as lymphatic filariasis (Van den Berg et al., 2013).

This thesis

The Government of Rwanda has endorsed the IVM approach as the best approach to achieve malaria pre-elimination. In the meantime, different studies across high endemic countries have shown that malaria control with only the primary vector control interventions are insufficient to achieve the goal of malaria elimination, specifically in high endemic areas. For the planning and implementation of IVM, local evidence is required, as well as the integration of vector control methods and the empowerment of affected communities. Within our project, we hypothesized that this could be achieved by implementing a novel

participatory technique (‘open space’) that involves local communities in developing action plans for malaria control in close collaboration with the National Malaria Control Programme (Ingabire et al., 2014) .

To evaluate the impact of this approach, biomedical, social, economic and entomological questions were addressed. The ultimate aim was to assess the feasibility of malaria elimination in the south of Rwanda. The entomology component highlighted in this thesis therefore focuses on bionomic parameters of malaria vectors and transmission intensity and the options of mosquito sampling (Chapters 2 and 3). Moreover, questions related to the current impact of primary vector control tools are also addressed (Chapters 4 and 5). Besides, it explores community-driven options for malaria vector control (larval source management; Chapter 6). The work focused on the following specific objectives that were defined at the start of the research project:

1) To determine the behavioral characteristics of vector populations over time and space in response to existing and novel vector control tools;
2) To determine the key entomological and environmental parameters which characterize malaria transmission;
3) To strengthen community know-how, implement a larval source management program and evaluate its contribution to malaria elimination in Rwanda;

More specifically, **Chapter 2** determines the bionomics of mosquito populations sampled in study sites across Rwanda and describes the major behavioral parameters of the dominant malaria vectors. The only entomological information existing from Rwanda dated from the malaria eradication era, during the colonial period. This chapter thus provides essential information on mosquito species composition and the biting behaviour including the circadian pattern of malaria vectors. The entomological inoculation rate for estimation of the transmission intensity was also calculated for all study sites.

Chapter 3 assesses the effectiveness of two mosquito sampling traps that can be used for (malaria) vector monitoring and surveillance. Sampling methods routinely used in Rwanda are the human landing catching (HLC), the pyrethrum spraying catch (PSC) for adult mosquitoes, and dipping for the aquatic stages of mosquito larvae. Despite their effectiveness, the two adult sampling methods (HLC and PSC) have limitations associated with the high implementation cost, ethical concerns and variation of attractiveness between

collectors for HLC and early morning disturbance of house occupants for PSC. This chapter therefore describes the effectiveness of the CDC light trap in comparison with a novel adult trap, the Suna trap, for sampling mosquitoes, both indoor and outdoor of local houses. The results will guide the National Malaria Control Program in choosing affordable mosquito sampling options.

Chapter 4 then evaluates the physical durability and attrition of LLINs, the most used vector control intervention in Rwanda as implemented by the National Malaria Control Program. It was hypothesized that the physical shelf-life of bed nets distributed was three to five years according to the information provided by the manufacturers. However, their durability and attrition under realistic field conditions were underexplored at the time of our study. The study shows the attrition and deterioration rates over time, along a period of two years following an interval of surveys conducted every six months.

Chapter 5 describes the resistance status towards the major insecticides used for malaria vector control both for treatment of LLINs and for IRS. The experiments were conducted in different eco-epidemiological foci of malaria transmission throughout Rwanda. It demonstrates the resistance level against each insecticide and the type of resistance mechanisms prevailing per site.

Chapter 6 assesses the effectiveness of a community-based vector control intervention using a biological larvicide, *Bacillus thuringiensis* var. *israelensis* (Bti) as a novel tool in Rwanda to supplement the primary vector control tools. The intervention was applied to the major breeding sites encountered in the study area in Southern Rwanda. This area consists of irrigated rice-fields, land hills and drains in crop lands. The results from two experimental arms are presented and are compared with the control arm (no intervention). The outcomes focus on the abundance of the larval stages and adult mosquitoes.

Finally, **Chapter 7** discusses the outcomes of the different chapters. It highlights the evidence we obtained and which should guide in decision making for vector control in the framework of IVM, with the ultimate aim of malaria elimination in Rwanda.

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Chapter 2

Spatio-temporal distribution of mosquitoes and risk of malaria infection in Rwanda

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Chapter 2

Abstract

To date, the Republic of Rwanda has not systematically reported on distribution, diversity and malaria infectivity rate of mosquito species throughout the country. Therefore, we assessed the spatial and temporal variation of mosquitoes in the domestic environment, as well as to assess nocturnal biting behavior and infection patterns of the main malaria vectors in Rwanda. For this purpose, mosquitoes were collected monthly from 2010 to 2013 by human landing catches (HLC) and pyrethrum spray collections (PSC) in seven sentinel sites. Mosquitoes were identified using morphological characteristics and PCR. *Plasmodium falciparum* sporozoite infection rates were determined using ELISA.

A total of 340,684 mosquitoes was collected by HLC and 73.8% were morphologically identified as culicines and 26.2% as anophelines. Of the latter, 94.3% were *Anopheles gambiae* s.l., 0.4% *Anopheles funestus* and 5.3% other *Anopheles* species. Of *An. gambiae* s.l., *An. arabiensis* and *An. gambiae s.s.* represented 84.4% and 15.6%, respectively. Of all *An. gambiae* s.l. collected indoor and outdoor, the proportion collected indoors was 51.3% in 2010 and 44.9% in 2013. A total of 17,018 mosquitoes was collected by PSC of which 20.5% were *An. gambiae* s.l. and 79.5% were culicines. For the seven sentinel sites, the mean indoor density for *An. gambiae* s.l. varied from 0.0 to 1.0 mosquitoes/house/night. *P. falciparum* infection rates in mosquitoes varied from 0.87-4.06%. The entomological inoculation rate (EIR) ranged from 1.0 to 329.8 with an annual average of 99.5 infective bites/person/year. This longitudinal study shows, for the first time, the abundance, species composition, and entomological inoculation rate of malaria mosquitoes collected throughout Rwanda.

Keywords: Vector distribution; abundance, sporozoite rate; entomological inoculation rate; Rwanda

Chapter 2

Introduction

Understanding heterogeneity in human malaria transmission intensity is important for National Malaria Control Programs in order to identify key differences and similarities that could be exploited to distribute valuable resources in a most optimal way. The most common entomological measure of malaria transmission intensity is the entomological inoculation rate (EIR), which is expressed as the number of infectious bites per person per unit time, usually per year (Tusting et al., 2014). At present, vector control interventions are considered effective tools that are able to interrupt transmission (Shaukat et al., 2010). Unfortunately, both LLINs and IRS are chemical-based and as such, the extensive use of insecticides could have selected for development of resistance and may even lead to changes in feeding and resting behavior (Gatton et al., 2013; Ranson et al., 2011).

In addition, *Anopheles* mosquito breeding sites are created through agricultural and economic activities, such as rice cultivation and other water resource management projects that promote the proliferation of malaria vectors (Keiser et al., 2005; Hunter et al., 1982; Imbahale et al., 2011). These diverse ecological settings result in variation in risk of malaria across African countries, but often this variation has not been well characterized (Keiser et al., 2005; Himeidan et al., 2012). To date, the Republic of Rwanda has not reported on distribution, diversity and malaria infectivity rate of mosquito species. Through the support of the Malaria & Other Parasitic Diseases Division (equivalent to National Malaria Control Program - NMCP) of the Ministry of Health, entomological surveys have been carried out in seven study sites since 2010. For the current study, mosquito data were collected monthly from these sites and analyzed to determine mosquito diversity, distribution (vectors and non-vectors) and entomological inoculation rate. We specifically aimed to characterize nocturnal biting patterns (hourly rates) and spatial biting patterns (endophily versus exophily), because these are under selection pressure with the currently used insecticide-based mosquito control interventions (Russell et al., 2011; Killeen et al., 2014).

Methods

Study sites

In Rwanda, malaria is the most important vector-borne disease and a leading cause of morbidity (Karema et al., 2012). The disease is endemic in 19 out of 30 districts, with the main foci in the Eastern and South-Eastern parts of the country where the altitude is generally

below 1,500m above sea level, the climate is warmer and where the area is characterized by the presence of marshy plains, rice cultivation and brick–making as economic activity.

The study was conducted in seven out of 12 sites established throughout the country by the NMCP and that are used for routine entomological monitoring. The seven sites were set up in 2010, whereas the other five were established in 2012. In this study, data from the seven study sites are presented (Busoro and Karambi in Southern Province, Rukara and Bukora in Eastern Province, Mashesha in Western Province, Bungwe in Northern Province and Kicukiro in Kigali City (Fig. 1).

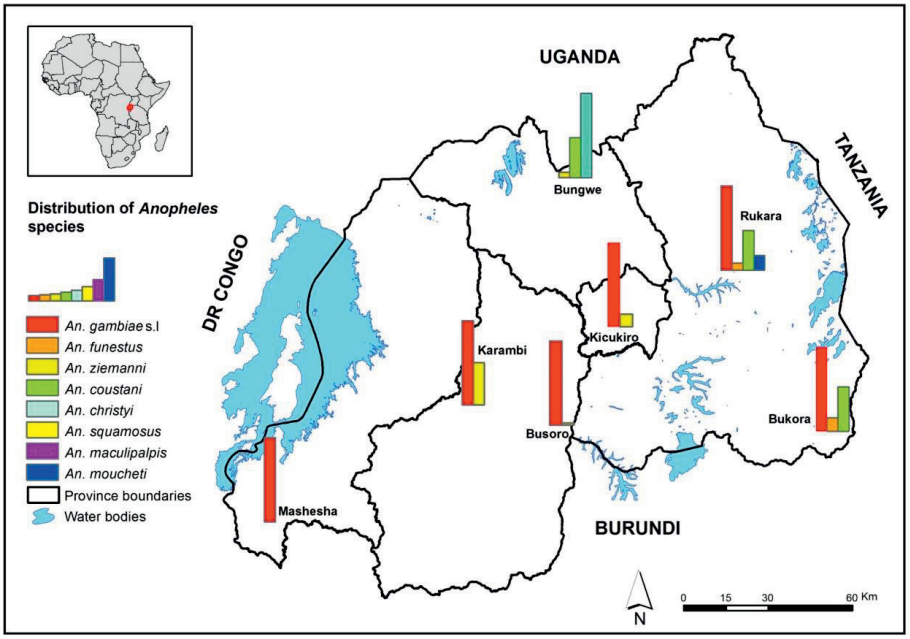

Figure 1: Location of seven study sites and distribution of *Anopheles* spp. collected by human landing catches from 2010 to 2013

Mosquito collection: human landing catches (HLC)

At each of the seven study sites, three villages were selected and from each village, three houses were randomly selected for mosquito collection during two consecutive nights per month. Thus, a total of nine houses were sampled in each study site per month from January 2010 to December 2013. All houses from which mosquitoes were collected were geocoded using a hand-held Global Position System (GPS). Mosquito biting behavior was assessed by collecting mosquitoes inside and outside each house using human landing catches (HLC)

from 6pm to 6am (WHO, 1975; WHO 2013). Two trained volunteers sitting with a torch and an aspirator, one inside the house (at the house entrance) and the other approximately 5-8 m away from the house (AMC, 2009), aspirated mosquitoes that landed on their exposed legs. At each house, the door was usually left open before the sleeping period and closed throughout the sleeping time of house occupants. To correct for differential mosquito attractiveness between the collectors and to avoid fatigue, the collectors took a 10 min. break every hour and switched position at midnight. Due to the risk of receiving an infectious bite before aspirating the mosquitoes, the collectors were screened for malaria every month during the survey, and standard malaria treatment was available if needed. None of the collectors was diagnosed with malaria during the survey period. Mosquitoes collected at each hourly interval were placed in separate paper cups pre-labeled with date, time and location of catch and transported to a field laboratory established at the health center of the study site for morphological identification. A 10% sample of the *An. gambiae* s.l. mosquitoes collected was transported to the central laboratory in Kigali for further processing (see below).

Mosquito collection: pyrethrum spray collection (PSC)

At each study site, three villages were selected from which five houses (other than those used for HLC) in each village were randomly selected for mosquito collection by pyrethrum spray collection (PSC) for two consecutive days per month (Silver, 2008; Service, 1993). A total of 15 houses was thus sampled in each of the seven sentinel sites. Mosquitoes were collected on a monthly basis between 7am and 11am from January 2010 to December 2013. Each house was prepared for spraying by removing food and water and covering the entire floor and furniture with white cotton sheets. One collector sprayed inside the house with a 400 ml can of non-residual Cypermethrin (BOP®, Mac Bride International, Manchester, United Kingdom), while the other sprayed the eaves on the outside to stop mosquitoes from escaping. The house was closed for 10 min. after which the sheets were moved outside to collect the mosquitoes that were knocked down. Mosquitoes were transferred onto a petri dish lined with moist Whatman filter paper n°4 (110 mm diameter). Mosquitoes from each house were sorted and separated into anophelines and culicines, and pooled in 1.5ml vials for transportation to the field laboratory at the health center for further processing.

Mosquito identification

All mosquitoes collected by PSC and HLC were transported to a field laboratory for morphological identification to *An.gambiae* s.l., *An. funestus*, other anophelines and culicines using standard morphological identification keys (Gillies and Coetzee, 1987). Mosquitoes were then pooled per study site and stored in silica gel for transportation to the central laboratory in Kigali for identification of *Plasmodium falciparum* infections and quality control of field mosquito identification. A random sample of 661 *An. gambiae* s.l. from five study sites from 2012 and 2013 were sent to the molecular laboratory of the International Center for Insect Physiology and Ecology (*icipe*), Nairobi, Kenya for molecular characterization using the standard polymerase chain reaction (PCR) assay (Scott et al., 1993).

Sporozoite rates and entomological inoculation rates

The heads and thorax of 10% of *An. gambiae* s.l. mosquitoes collected by HLC at each site were subjected to sporozoite ELISA to determine their infection rates with *P. falciparum*. A sample with an Optical Density (OD) value above the cut-off (cut-off = 2x mean OD of 7 negative samples) was considered positive (MR4, 2011). The sporozoite rate was calculated as the number of mosquitoes infected with *P. falciparum* sporozoites divided by the total number of mosquitoes examined. The entomological inoculation rate was obtained by taking the product of human biting rate and sporozoite rate (WHO, 2013).

Statistical analysis

All data on mosquito collections were entered into Microsoft Excel for the calculation of mean biting densities (number of bites per person per night), the sporozoite index and entomological inoculation rates. Differences in mean biting densities and indoor or outdoor biting behaviour were statistically evaluated using SPSS statistic software, V.20, Chicago, IL, USA. The significance of differences among sentinel sites and years of study (2010-2013) in the proportions of *An. gambiae* s.l. collected indoor or outdoor (endophily) were calculated using Generalized Linear Models with binomial as the distribution and logit as the link function. Hourly biting rates were statistically analyzed by calculating the proportion of mosquitoes biting during the early (6 pm – 10 pm) part of the night and comparing differences between years of study for the six sentinel sites using Generalized Linear Models with binomial as the distribution and logit as the link function.

Results

Mosquito composition by HLC

A total of 340,684 mosquitoes was collected by human landing collection over the 48 months of the study period. Of these, 73.8% were morphologically identified as culicines and 26.2% as anophelines. Of the total anophelines collected (n=89,306), 94.3% was *An. gambiae* s.l. White and 0.4% *An. funestus* Giles. The other six *Anopheles* species included *An. ziemanni* Grunberg (2.3%), *An. coustani* Laveran (2.3%), *An. moucheti* Evans (0.4%), *An. christyi* Newstead & Carter (0.3%), *An. maculipalpis* Giles (0.02%) and *An. squamosus* Theobald (0.004%). Mashesha in the Western Province lowlands with two annual cycles of irrigated rice, recorded the highest number of *An. gambiae* s.l. with 62,018 (73.7%) of the total *An. gambiae* s.l. collected, followed by Busoro with 8,951 (10.6%) (Table1). Only one *An. gambiae* was collected in Bungwe. Four sites reported *An. funestus* (Kicukiro, Bungwe, Rukara and Bukora (Table 1) whereas in the remaining three sites no *An. funestus* was found. *An. christyi* was confined to the highlands of the Northern Province (Bungwe).

A total of 251,378 culicine mosquitoes consisted of 85.3% *Culex* spp., 14% *Mansonia* spp, 0.4% *Coquilletidia* spp and 0.3% *Aedes* spp. As expected, the highest number of culicines were collected from the urban Kicukiro site (Kigali city) with 28.4% (n=71,303) culicines out of which 93.3% were *Culex* spp followed closely by Busoro with 28.3% (n=71,159) culicines out of which 80.3% were *Culex* spp. (Fig. 1 and Table 1).

Table 1: Total number of mosquitoes collected by HLC in sentinel sites from 2010 to 2013, Rwanda

Mosquito species collected	**Sentinel site**							**Total**	**Percentage**
	Bukora	Bungwe	Busoro	Karambi	Kicukiro	Mashesha	Rukara		
Anopheles gambiae s.l.	1,452	1	8,951	952	8,753	62,018	2,062	84,189	94.3
Anopheles funestus	228	4	0	0	1	0	171	404	0.4
Anopheles ziemanni	0	14	223	478	1,307	0	12	2,034	2.3
Anopheles coustani	762	109	32	0	40	139	968	2,050	2.3
Anopheles christyi	0	231	0	0	0	0	0	231	0.3
Anopheles squamosus	0	0	0	4	0	0	0	4	0.0
Anopheles maculipalpis	1	0	0	0	0	18	0	19	0.0
Anopheles moucheti	0	0	0	7	7	0	361	375	0.4
Total *Anopheles* spp.	**2,443**	**359**	**9,206**	**1,441**	**10,108**	**62,175**	**3,574**	**89,306**	**100.0**
Mansonia spp	16,342	5	13,852	89	4,736	29	75	35,128	14.0
Culex spp	22,214	15,046	57,119	14,730	66,495	35,347	3,532	214,483	85.3
Aedes spp	253	1	143	119	41	23	187	767	0.3
Coquilletidia spp	578	265	45	0	31	0	81	1,000	0.4
***Culicinae* spp.**	**39,387**	**15,317**	**71,159**	**14,938**	**71,303**	**35,399**	**3,875**	**251,378**	**100.0**
***Culicidae* spp.**	**41,830**	**15,676**	**80,365**	**16,379**	**81,411**	**97,574**	**7,447**	**340,684**	
% *Anopheles* spp.	**5,8**	**2,3**	**11,5**	**8,8**	**12,4**	**63,7**	**48,0**	**26,2**	

Molecular identification of members of the *An. gambiae* complex

Out of 661 *Anopheles gambiae* s.l. selected for sibling species identification from five study sites, 558 (84.4%) were identified as *An. arabiensis* Patton and 103 (15.6%) as *An. gambiae s.s*. Giles. The former occurs as the dominant species throughout all sites and its relative abundance ranges from 70.6% in Kicukiro, to 76.3% in Mashesha, 84.0% in Bukora, 93.5% in Busoro and 95.5% in Rukara.

Endophily of *An. gambiae* s.l. collected by HLC

Of the 84,189 *An. gambiae* s.l. mosquitoes collected by HLC, 40,570 (48.2%) were caught indoors and the remainder (51.8%) was caught outdoors, i.e. at approximately 5 to 8 m from the house entrance. The GLM (Binomial logit) model indicated a significant interaction between year and sentinel site (Wald Chi-square = 120.81; df = 15, $P < 0.001$), suggesting there was no clear, consistent trend discernable when comparing the effects of years or sites (Fig. 2). For example, analyses on the effect of year within sentinel site indicated that, in comparison with the year 2010, the years 2011-2013 reported similar proportions in endophily in Karambi, while both Mashesha and Rukara reported significantly lower proportions in 2012 and 2013 in comparison with 2010. The other three sites sites reported significantly different proportions in 2011 and 2012 (Bukora), 2011 and 2013 (Kicukiro) or only in 2011 (Busoro) in comparison with 2010 (df = 3, $P < 0.05$).

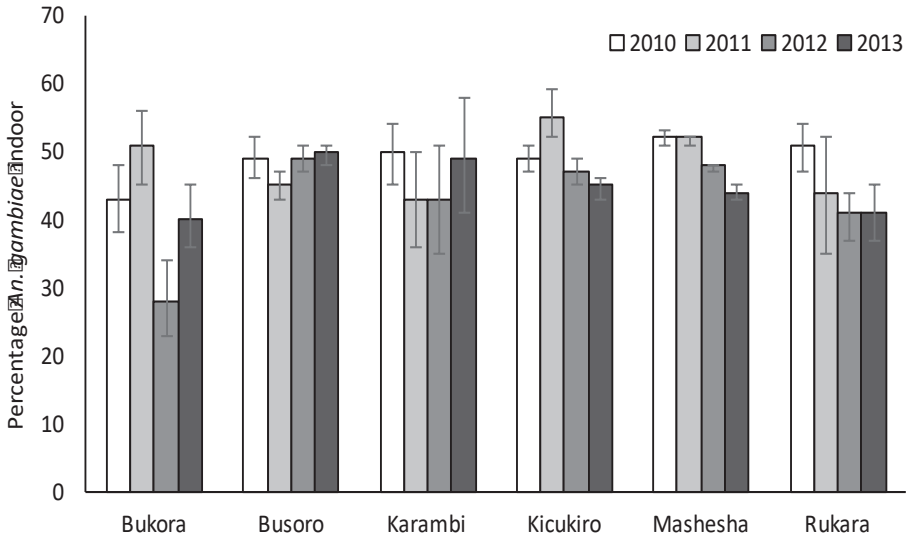

Figure 2: Estimated marginal mean percentages of indoor biting *Anopheles gambiae* s.l. per study site from 2010-2013, collected using HLC. Error bars indicate 95% confidence intervals.

Seasonality of human biting rates of *An. gambiae* s.l

In the four sites that had the highest mean biting intensity (Busoro, Kicukiro, Mashesha and Rukara), two annual peaks were observed. The timing of the first peaks was different for each site and occurred in February, March, April or May and coincided with a South-West to Central-East transect (Mashesha – Busoro – Kicukiro – Rukara). Bukora had a unimodal peak in mosquito density occurring in May, whereas the seasonal pattern for Karambi was less clear (Fig. 3).

Hourly collection of *An. gambiae* s.l. with HLC

Of the 84,189 *An. gambiae* s.l. collected with HLC, the average peak in biting activity was recorded during the second half of the night from 1-2 am for each year of the survey. From 6 pm to midnight, 36.9% of mosquitoes were collected while the remainder (63.1%) was collected during the second half of the night (Fig. 4-A). In Figure 4-B, the percentages of *An. gambiae* s.l. biting between 6 pm and 10 pm ('early biting', i.e. before people sleep under a bednet) are shown for each of six sentinel sites for the four years of study. In comparison with the year 2010, the percentages of early biting mosquitoes were significantly higher for Bukora, Mashesha and Rukara for all three subsequent years (2011-2013; GLM, $P < 0.05$). For Busoro, this was only the case for 2012 and 2013. The remaining two sites were more variable. For Kicukiro, percentages were similar as to 2010, except for 2012. For Karambi, the percentage was either significantly higher (2012) or significantly lower (2011 and 2013) in comparison with the first year of study (2010).

Mosquito resting densities collected by PSC

In addition to HLC, a total of 17,022 mosquitoes were collected by PSC over the four-year period. Of these, 20.5% were *An. gambiae* s.l. and 79.5% were culicines. The average density of all mosquitoes collected was 1.7 per house per night and ranged from 0.3 per house per night in Rukara and Bungwe to 3.8 mosquitoes per house per night in Mashesha. The *An. gambiae* s.l. density averaged 0.3 mosquitoes per house per night, with Mashesha recording the highest density of 1.0 female *An. gambiae* per house per night (Table 2). No *An. funestus* were collected indoors with PSC in all sites.

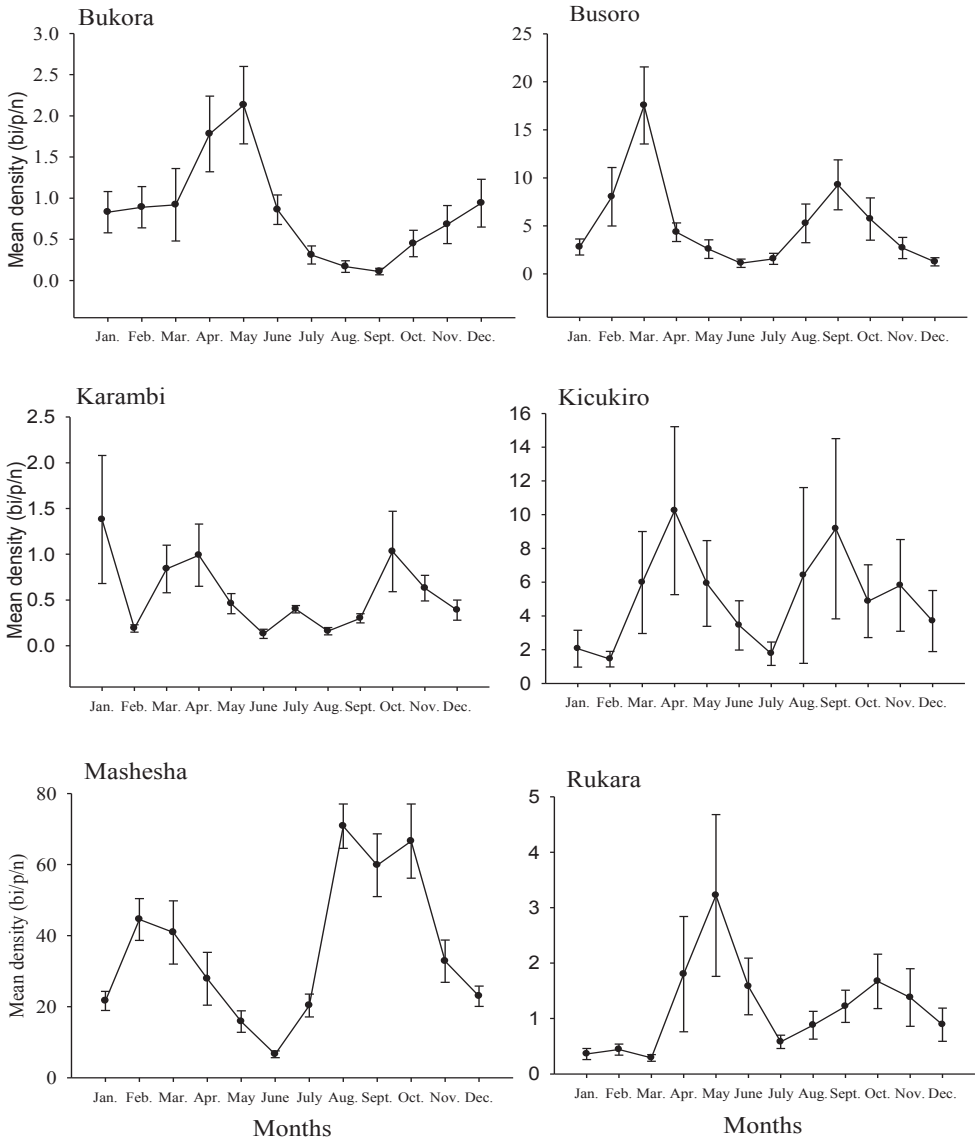

Figure 3: Mean monthly density of *Anopheles gambiae* s.l. collected using human landing collections in six study sites from 2010-2013. Note the different scales of the Y-axes in the Figure.

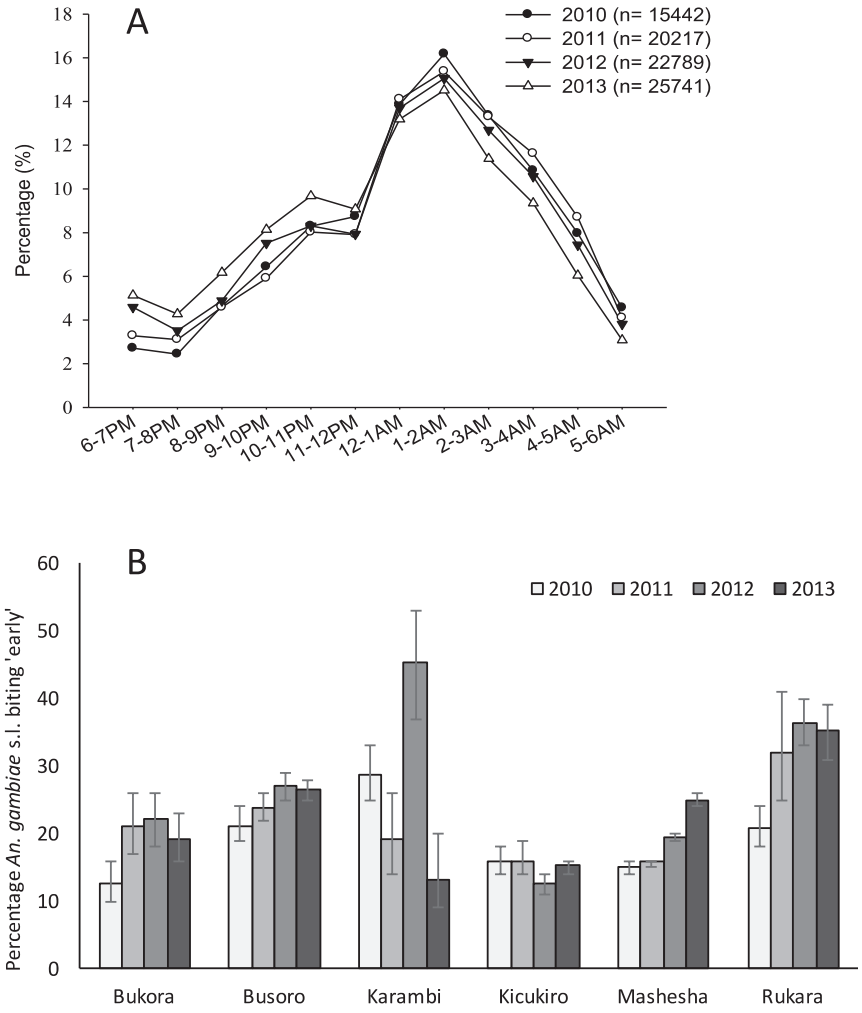

Figure 4: Biting patterns of *An. gambiae* s.l.: (A) Mean percentage of *Anopheles gambiae* s.l. collected per hour (6 pm – 6 am) for the four years of study (2010-2013); (B) estimated marginal mean percentages of mosquitoes biting during the 'early' hours of the biting cycle (6-10 pm) for six sentinel sites over the four years of study. Error bars indicate 95% confidence intervals.

Table 2: Mosquitoes density and composition collected by PSC in sentinel sites from 2010-2013, Rwanda

Mosquito species collected	Bukora	Bungwe	Busoro	Karambi	Kicukiro	Mashesha	Rukara	Total
An. gambiae s.l.	209	0	1,008	206	337	1,504	230	3,494
An. christyi	0	4	0	0	0	0	0	4
Culicinae spp.	652	477	3,103	1,183	3,866	3,987	256	13,524
Culicidae spp.	861	477	4,111	1,389	4,203	5,491	486	17,022
Density of *An. gambiae* s.l. (number/house/night)	0.1	0,0	0.7	0.1	0.2	1,0	0.2	0.3
Density of *Culicidae* (number/house/night)	0.6	0.3	2.9	1,0	2.9	3.8	0.3	1.7

Table 3: Sporozoite infection rate (SIR in %) of *Anopheles gambiae* s.l. per site from 2010 to 2013; N= total number, Pos = # sporozoite positive

Sentinel sites	2010		2011		2012		2013		Total positives/N	SIR (%)
	Pos./N	SIR (%)	Pos./N	SIR (%)	Pos./N	SIR %)	Pos./N	SIR (%)		
Bukora	15/535	2.80	24/381	6.30	7/154	4.55	1/88	1.14	47/1,158	4.06
Busoro	6/673	0.89	75/1,287	5.83	18/583	3.09	1/88	1.14	100/2,631	3.80
Karambi	2/462	0.43	5/188	2.66	0/86	0.00	0/70	0.00	7/806	0.87
Kicukiro	29/925	3.14	66/887	7.44	7/1,399	0.50	1/88	1.14	103/3,299	3.12
Mashesha	20/1,056	1.89	144/3,612	3.99	76/5,722	1.33	1/72	1.39	241/10,462	2.30
Rukara	27/592	4.56	16/194	8.25	7/509	1.38	2/55	3.64	52/1,350	3.85
Total	99/4,243	2.33	330/6,549	5.04	115/8,453	1.36	6/461	1.30	550/19,706	2.79

Sporozoite rates and entomological inoculation rates

The mean sporozoite rate of *An. gambiae* s.l. for the four years period was 2.79% (range 0.87 to 4.06%) with substantial variation over the years and sites (Table 3). The mean annual entomological inoculation rate was 99.5 infective bites per person per year (range 1.0 – 329.8) with Mashesha showing the highest EIR of 329.8, followed by Busoro and Kicukiro with 107.5 and 103.6 respectively (Fig. 5).

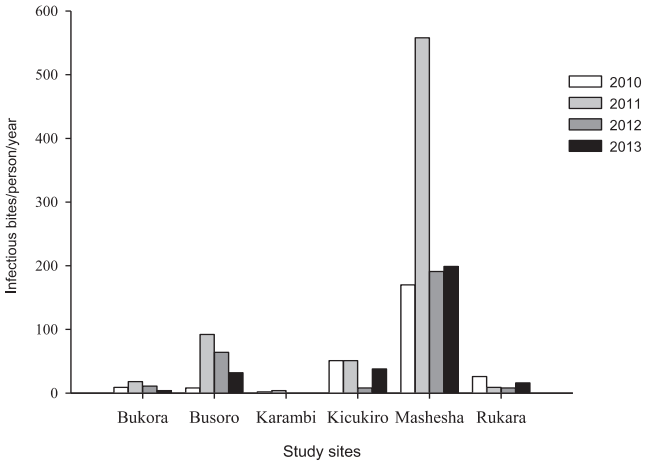

Figure 5: Annual entomological inoculation rate (EIR) of *Anopheles gambiae* s.l. per study site and per year.

Discussion

This is the first comprehensive study on spatial and temporal dynamics in mosquito distribution, seasonality, hourly biting behavior, species composition, *P. falciparum* infection rates, and hence malaria transmission pressure across Rwanda. The results of mosquitoes collected by HLC showed that culicines are the dominant mosquitoes collected in all seven sites except for Mashesha in the lowlands of Western Province where *An. gambiae* s.l was the dominant species. This is a rice production area with high densities of mosquitoes throughout the year, and this site also recorded the highest estimated number of malaria infective bites. The Rwanda health information system reported the highest incidence of malaria in the catchment area of Mashesha ranging from 4.4 to 7.6 malaria cases per 100 inhabitants respectively in 2010 to 2013 (MoH, 2013).

Despite its description as dominant *Anopheles* species in previous studies from Rwanda (Vermylen, 1967; Munyantore, 1989; Loevinsohn, 1994), *An. funestus* was rarely collected in this study, except for two sites (Bukora and Rukara) in Eastern Province. Other anopheline species – *An. ziemanni, An. coustani, An. christyi, An. maculipalpis, An. moucheti* and *An. squamosus* were recorded in low numbers. Although these species have been considered as secondary malaria vectors in Africa (Service, 1993; White, 1974), recent studies indicate that they can act as vectors and should not be neglected in the strategies of malaria control or its elimination. *Anopheles pharoensis* was the main vector throughout Egypt especially in the Nile delta (Wassim, 2014), and transmits *P. falciparum* in Guinea Bissau, Cameroon, Tchad and Ethiopia (Tabue et al., 2014; Sanford et al., 2014; Hinzoumbé, 2012; Kibret, 2012). Similarly, *An. ziemanni* and *An. coustani* contributed to malaria transmission in northern Cameroon and Kenya (Tabue et al., 2014; Mwangangi et al., 2013b). In Cameroon, other *Anopheles* species were also reported as primary or secondary malaria vectors (Tabue et al., 2014).

In Rwanda, *Culex* spp. have not been incriminated as vectors of disease, although lymphatic filariasis is reported to occur in some parts of the country at low prevalence of 0.13% and may be transmitted by anopheline spp. and not by culicines (White, 1974; MoH, 2011; Ruberanziza et al., 2008). Nevertheless, it will be very important to consider *Culex* spp. in the framework of Integrated Vector Management, since it is often the perception of the community that all mosquitoes can be vectors of malaria (Ingabire et al., 2015). Reduction in their numbers would substantially reduce mosquito nuisance, and thus enhance community involvement in and uptake of vector control for malaria prevention.

The molecular identification of *An. gambiae* s.l. showed *An. arabiensis* to be the predominant species in all sites. The species seems to have replaced *An. gambiae s.s*., which was reported by two earlier (non peer-reviewed) technical reports to be the dominant species before the scale-up of vector control interventions in Rwanda (Lansana, 2008; Howell, 2007). *An. arabiensis* are opportunistic indoor or outdoor feeders and can thus better survive indoor-based interventions than *An. gambiae s.s*. (Shililu et al., 2003; Main et al., 2016). The regional shift in species composition in the *An. gambiae* complex has been observed across the East African region in Tanzania, Kenya and Uganda (Russell et al., 2011; Okara et al., 2010; Mwangangi et al., 2013a). Although evaluated with different collection methods and at a different time, a similar trend seems to have taken place in Rwanda.

This change of malaria vector composition is associated with the scaling up of indoor vector control interventions such as LLINs and IRS, and thus the formerly endophilic *Anopheles* were replaced by opportunistic species displaying a tendency of biting outdoor (Russell et al., 2011; Mwangangi et al., 2013a, Kamau et al., 2017). Besides the outdoor feeding of *An. arabiensis*, biting activity before people go to sleep under bed nets (Yohannes and Boellee, 2012) and the degree of anthropophily/zoophily (Mahande et al., 2007) are also under selective pressure. Interestingly, Killeen et al. (2016) recently reported that *An. arabiensis* first attempts to feed indoor, but rapidly exits houses and seeks hosts outdoor. Similarly, *An. funestus* expressed a diurnal biting behaviour in order to evade exposure to LLINs installed inside houses (Sougoufara et al., 2014). Such changes can have a profound effect on any vector control intervention (malERA, 2017, Killeen et al., 2017).

The EIR remains the most direct measurement for assessing the effect of anti-vector actions because it quantifies the parasite-infected mosquito pool and its propensity to transmit infectious parasites to the human population (Shaukat et al., 2010). A 2009 review of annual EIRs illustrated a large gap in EIR data across Africa (Kelly-Hope & McKenzie, 2008). Only 23 of the 54 African countries had reported EIRs with Kenya, the Gambia, Tanzania and Burkina Faso reporting most of these data (56%), with EIR values ranging from 0 to over 500 infective bites per person per year. In our study, the entomological inoculation rate ranged from an estimated 1 to 329, with Mashesha recording the highest EIR followed by Busoro and Kicukiro. Mashesha is a rice production area and has the lowest elevation of all study sites, offering conducive conditions for breeding of mosquitoes and *Plasmodium* development in the mosquito (Paaijmans et al., 2012). The large numbers of *An. gambiae* s.l. collected there are mostly breeding in the inundated rice paddies. Despite the perennial transmission, a low EIR was found in the highland site of Karambi. A similar study conducted in the Kenya highlands (Mumias) also showed a low level of EIRs, but that do contribute to the endemicity of malaria (Shililu et al., 1988; Imbahale et al., 2012). The previous studies showed that it may not be feasible to achieve a dramatic reduction in the prevalence of *P. falciparum* infections in Africa unless control measures sustainably reduce EIRs to below one infective bite per person per year (Shaukat et al., 2010; Beier et al., 1999). Such findings have important implications on the effectiveness of the current vector control interventions that focus on indoor settings. Rwanda has therefore embarked on studies to understand vector bionomics and insecticide resistance patterns (Hakizimana et al., 2016) and currently implements an integrated vector management program. These will guide efficient

and effective control interventions and allow adapting the interventions to the changing vector distribution, ecology and behavior. As part of these efforts, a large field experiment with application of *Bacillus thuringiensis* var *israelensis* (Bti) in different types of mosquito breeding sites was accomplished in Ruhuha wetland areas, in South East of Rwanda in July 2015. This experiment was well perceived by local community and highly increased their knowledge and skills in application of Bti (Ingabire et al., 2017). The results will be used for informed decisions on the expansion of larval source management as supplementary method to the existing vector control tools based on insecticides.

Conclusion

Based on longitudinal indoor HLC and PSC collections across seven sites in Rwanda, the entomological inoculation rate was highest in Mashesha, Busoro and Kicukiro with 329.8, 107.5 and 103.6 infective bites per person per year. Mashesha, a lowland and rice production area, had the highest number of *An. gambiae* s.l. indicating that the highest intensity of malaria transmission in the country strongly relates to land use and altitude. A slightly higher proportion of host-seeking *An. gambiae* s.l. was collected outdoors than indoors in most of the sites in the course of the four-year period suggesting that outdoor transmission can take place. Furthermore, the main malaria vector, *An. gambiae* s.l., was found biting throughout the year with two annual peaks and started to display an earlier biting pattern in the course of the four-year study period in some of the study sites (Bukora, Mashesha and Rukara). The observed trends in mosquito feeding behavior have programmatic implications on effectiveness of indoor interventions with LLINs and IRS that are currently used in Rwanda. Further studies are ongoing in additional study sites to collect mosquito data for the development of a comprehensive database on vector bionomics that will be used to inform decision-making for control of malaria and other vector-borne diseases in Rwanda.

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Chapter 2

Yohannes, M. & Boellee, E. (2012). Early biting rhythm in the afro-tropical vector of malaria, *Anopheles arabiensi*s, and challenges for its control in Ethiopia. *Medical and Veterinary Entomology*, 26**,** 103-105.

Chapter 3

Evaluation of the CDC light trap and odour-baited Suna trap for sampling malaria vectors in Rwanda

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To be submitted

Chapter 3

Abstract

Malaria vector surveillance is one of the central components of malaria control programmes. It requires reliable sampling methods to estimate the entomological indicators of transmission intensity with the ultimate aim of adequate planning and impact evaluation of vector control interventions. The present study aimed to assess the effectiveness of the CDC light trap (CDC-LT) in comparison with the odour-baited Suna trap (with and without light), in Ruhuha, South Eastern Rwanda. Suna traps baited with MB5 alone as attractive lure and CDC-LT were placed indoors and outdoors following a 4x4 Latin Square design in three blocks of four houses and repeated over three consecutive weeks. The study was carried out in August of 2014 and 2015. In 2015, a light bulb was added to the Suna trap baited with MB5 to evaluate the effect of light on trap catches. Traps operated from 06:00 pm to 06:00 am of the next morning. Numbers of *An. gambiae* s.l., other *Anopheles* spp. and *Culex* spp. were compared using generalized linear mixed models, and incidence risk ratios (IRR) were calculated.

In August 2014, CDC-LT caught significantly higher numbers of *Anopheles gambiae* s.l., other *Anopheles* spp. and *Culex* spp. than the Suna trap (IRR = 7.93 (CI: 5.13-12.26), IRR = 6.46 (CI: 1.71-24.51) and IRR = 5.32 (CI: 2.43-11.66), respectively. The results of August 2015 were similar to 2014, with CDC-LT significantly outperforming the odour-baited Suna trap. Addition of a light source to the Suna trap in August 2015 did not increase trap performance. In both 2014 and 2015, significantly more 'other *Anopheles*' were caught outdoors than indoors (IRR = 4.18, (CI: 1.55-11.28) and IRR = 9.24 (CI: 4.06-21.05) for 2014 and 2015, respectively. The relative abundance in sibling species of the *An. gambiae* complex showed that CDC-LT collected significantly more *An. arabiensis* than *An. gambiae s.s.* in comparison with the Suna trap.

CDC-LT outperformed odour-baited Suna traps. Addition of light did not improve the performance of the Suna trap. Addition of carbon dioxide or a carbon dioxide mimic could increase the effectiveness of the latter trap type. Differences in the relative abundance of members of the *An. gambiae* complex between the two types of traps warrant further research.

Key words: CDC light trap, Suna trap, malaria vectors, Rwanda

Introduction

Since the turn of the millennium, there has been a steady decline in malaria in many countries as result of the expansion in malaria control efforts, especially insecticide-based vector control (WHO, 2015b). An increasing number of malaria endemic countries are moving towards elimination or achieving zero indigenous cases of malaria (WHO, 2015a). However, monitoring the success of these interventions requires continued surveillance of malaria vectors (Onyango et al., 2013; Rubio-Palis et al., 2012; Sikaala et al., 2013). As a result of the evolutionary pressure of insecticide-based vector control efforts, different studies report physiological and behavioural changes in vector populations. This includes the development and spread of resistance to insecticides (Ranson et al., 2011; Ranson & Lissenden, 2016; WHO, 2012), as well as changes of vectors towards biting and resting outdoors in order to 'escape' vector control interventions that target indoor mosquito populations (Mwangangi et al., 2013; Russell et al., 2011). This also includes shifts in species composition, where in some malaria transmission settings, secondary vectors have taken over and become principal malaria vectors (Kibret et al. 2012; Mwangangi et al., 2013; Sanford et al., 2014). These changes may not only require alternative methods for vector control, but also new sampling tools for monitoring mosquito populations (Mathenge et al., 2005; Odiere et al., 2007).

The above needs are further justified by the weaknesses of sampling tools such as the Human Landing Collection (HLC), the Pyrethrum Spraying Collection (PSC) and resting collections by means of aspirators (Onyango et al., 2013). Although HLC remains the gold-standard method for the direct measurement of human exposure to malaria mosquito biting rates (Le Goff, Carnevale & Robert, 1993; Wong et al., 2013), the exposure of human volunteers to infected mosquito bites raises ethical concerns. In addition, there is variation among collectors in their relative attractiveness and ability to catch host-seeking mosquitoes which can lead to inaccuracy in estimates (WHO, 2013). Despite the effectiveness of PSC as a tool for estimating densities of indoor resting mosquitoes, it is inconvenient for the occupants of houses, because early in the morning they have to remove furniture and other commodities from their houses (Odiere et al., 2007; Onyango et al., 2013). Finally, both PSC and resting collections are less sensitive in areas where mosquitoes have become exophagic and exophilic due to the evolutionary pressure of indoor vector interventions (Mboera, 2005). Other mosquito sampling tools have been developed and evaluated, with varying success in

different epidemiological systems (Bijllaardt et al., 2009; Laganier et al. 2003; Overgaard et al., 2012; Rubio-Palis et al., 2012). They include methods that target outdoor resting and biting malaria vectors, such as clay pots (Odiere et al., 2007), resting boxes (Govella et al., Killeen, 2011), window exit traps (Mouatcho et al., 2006), Mosquito Magnet-X® (MM-X) traps (Hiwat et al. 2011; Jawara et al. 2009; Krockel et al. 2006) and BG-sentinel traps (Biogents AG, Germany) (Krockel et al., 2006). The latter was initially developed for sampling *Aedes aegypti* as vector of dengue and other viruses (Krockel et al., 2006). Alternative indoor sampling tools include the Prokopack and CDC backpack aspirator (Maia et al., 2011), cattle urine baited traps (Kweka et al., 2009), bednet traps, Mbita traps (Laganier et al., 2003; Mathenge et al., 2005), as well as CDC light traps (CDC-LT) and the latter are considered to be an efficient alternative mosquito trapping method to HLC in some settings (Mboera et al. ,1998; Mbogo et al., 1993). However, the results from some studies comparing CDC-LT with HLC are variable whereby in some settings catches from CDC-LT were higher or lower than those from HLC (Hiwat et al., 2011; Overgaard et al., 2012). It has been shown that the CDC-LT is most effective when positioned indoors near to a human sleeping under a bed net where the human forms the bait for the trap. The CDC-LT is appropriate for sampling *Anopheles arabiensis* in regions where the use of vector control interventions is high and vector densities have declined (Fornadel et al., 2010; Lines et al., 1991; Mboera et al., 1998)

Recently, a new odour-baited trap, the Suna trap, was developed for the mass trapping of malaria vectors as a means of vector control (Hiscox et al., 2014). First evaluations showed that indoor collections in a semi-field setting were equal to those of CDC-LT and MM-X trap for sampling *Anopheles gambiae* s.s. Moreover, the trap proved to be effective in sampling malaria vectors outdoors in the field. The addition of a synthetic blend of attractants replaced the need for human bait. Most importantly, mass deployment of a solar-powered version of this trapping system was effective in lowering *An. funestus* populations by 70% and reducing malaria prevalence by 30% on an island in western Kenya(Homan) . Further evaluation of the Suna trap was recommended in other malaria endemic countries, as the vector populations that are present in these countries will vary (Mathenge et al., 2005).

In the present study, we therefore compared the effectiveness of the Suna trap either with or without light to the CDC-LT with light, used both indoors and outdoors, for the purposes of trapping *Anopheles gambiae* s.l., other *Anopheles* species and culicine mosquitoes. The study was carried out in a village of South Eastern Rwanda which is surrounded by an irrigated rice

field with common water bodies, potentially breeding sites of malaria vectors (Ingabire et al., 2017).

Methodology

Study site

This study was conducted in Gikundamvura village located in the catchment area of Ruhuha health center, in Bugesera district, South Eastern Rwanda. The village is situated at 2°29'17" S and 30°08'49" E, with an altitude ranging from 1438 to 1461 m above sea level. The site is characterized by two rainy seasons (March-May and October - December) which alternate with two dry seasons (January-February and June-September). The village is bordered by two irrigated rice schemes with two annual rice growing cycles. The sentinel houses were mainly made of mud walls and with iron sheet roofs and were organized in grouped resettlements along rural mud streets. At the time of study, the area was inhabited by approximately 24,000 people living in 5,100 houses. People farm subsistence crops and keep cattle (Ingabire et al., 2016). Malaria transmission is low to moderate, with an estimated *P. falciparum* malaria prevalence of 5% (Kateera et al., 2015). *An. arabiensis* occurs as the dominant malaria vector (Hakizimana et al., 2018a). The usage of long lasting insecticide treated nets (LLINs) in the study area was 72% of the household members (Kateera et al., 2015).

Study design

The study was conducted in August 2014 and repeated in a modified form in August 2015. It was done one month post the transplantation of rice plants out of the irrigated fields next to the study area. Earlier work elsewhere in Africa demonstrated that at this stage of rice growth, mosquito densities are at their peak (Klinkenberg et al., 2003; Mutero et al., 2000). Studies were carried out in three blocks of four houses (12 houses in total). Within each block, treatments (= trap configurations) were rotated between the houses according to a 4x4 Latin square design (Tables 1 and 2). The four houses of each block were located on one street and separated from each other by at least 60 meters.

Table 1: Assignment of treatments for comparison of Suna traps and CDC-LT, following a 4x4 Latin square design, August 2014

Nights	House 1	House 2	House 3	House 4	Nights	House 5	House 6	House 7	House 8	Nights	House 9	House 10	House 11	House 12
1	CDC in	Suna out	CDC out	Suna in	1	Suna out	Suna in	CDC out	CDC in	1	CDC out	CDC in	Suna out	Suna in
2	Suna out	Suna in	CDC in	CDC out	2	Suna in	Suna out	CDC in	CDC out	2	Suna in	CDC out	CDC in	Suna out
3	Suna in	CDC out	Suna out	CDC in	3	CDC out	CDC in	Suna in	Suna out	3	Suna out	Suna in	CDC out	CDC out
4	CDC out	CDC in	Suna in	Suna out	4	CDC in	CDC out	Suna out	Souna in	4	CDC in	Suna out	Suna in	CDC out
Nights	House 1	House 2	House 3	House 4	Nights	House 5	House 6	House 7	House 8	Nights	House 9	House 10	House 11	House 12
5	Suna out	CDC in	Suna in	CDC out	5	Suna in	Suna out	CDC in	CDC out	5	Suna out	CDC in	Suna in	CDC out
6	CDC out	Suna out	CDC in	Suna in	6	Suna out	CDC in	CDC out	Suna in	6	CDC in	Suna out	CDC out	Sina in
7	CDC in	Suna in	CDC out	Suna out	7	CDC in	CDC out	Suna in	Suna out	7	Suna in	CDC out	Suna out	CDC in
8	Suna in	CDC out	Suna out	CDC in	8	CDC out	Suna in	Suna out	CDC in	8	CDC out	Suna in	CDC in	Sun out
Nights	House 1	House 2	House 3	House 4	Nights	House 5	House 6	House 7	House 8	Nights	House 9	House 10	House 11	House 12
9	Suna in	CDC in	Suna out	CDC out	9	Suna out	CDC in	CDC out	Suna in	9	CDC in	Suna out	CDC out	Suna in
10	Suna out	CDC out	CDC in	Suna in	10	Suna in	CDC out	CDC in	Suna out	10	CDC out	Suna in	CDC in	Suna out
11	CDC out	Suna out	Suna in	CDC in	11	CDC out	Suna out	Suna in	CDC in	11	Suna in	CDC out	Suna out	CDC in
12	CDC in	Suna in	CDC out	Suna out	12	CDC in	Sun in	Suna out	CDC out	12	Suna out	CDC in	Suna in	CDC out

Table 2: Assignment of treatments for comparison of Suna traps and CDC-LT, following a 4x4 Latin square design, August 2015. Note the grey coloured cells with Suna in/Suna out which indicate the treatments with the Suna traps that were tested without light (n=6).

Nights	House 1	House 2	House 3	House 4	Nights	House 5	House 6	House 7	House 8	Nights	House 9	House 10	House 11	House 12
1	CDC in	Suna out	CDC out	Suna in	1	Suna out	Suna in	CDC out	CDC in	1	CDC out	CDC in	Suna out	Suna in
2	Suna out	Suna in	CDC in	CDC out	2	Suna in	Suna out	CDC in	CDC out	2	Suna in	CDC out	CDC in	Suna out
3	Suna in	CDC out	Suna out	CDC in	3	CDC out	CDC in	Suna in	Suna out	3	Suna out	Suna in	CDC out	CDC in
4	CDC out	CDC in	Suna in	Suna out	4	CDC in	CDC out	Suna out	Souna in	4	CDC in	Suna out	Suna in	CDC out
Nights	House 1	House 2	House 3	House 4	Nights	House 5	House 6	House 7	House 8	Nights	House 9	House 10	House 11	House 12
5	Suna out	CDC in	Suna in	CDC out	5	Suna in	Suna out	CDC in	CDC out	5	Suna out	CDC in	Suna in	CDC out
6	CDC out	Suna out	CDC in	Suna in	6	Suna out	CDC in	CDC out	Suna in	6	CDC in	Suna out	CDC out	Sina in
7	CDC in	Suna in	CDC out	Suna out	7	CDC in	CDC out	Suna in	Suna out	7	Suna in	CDC out	Suna out	CDC in
8	Suna in	CDC out	Suna out	CDC in	8	CDC out	Suna in	Suna out	CDC in	8	CDC out	Suna in	CDC in	Sun out
Nights	House 1	House 2	House 3	House 4	Nights	House 5	House 6	House 7	House 8	Nights	House 9	House 10	House 11	House 12
9	Suna in	CDC in	Suna out	CDC out	9	Suna out	CDC in	CDC out	Suna in	9	CDC in	Suna out	CDC out	Suna in
10	Suna out	CDC out	CDC in	Suna in	10	Suna in	CDC out	CDC in	Suna out	10	CDC out	Suna in	CDC in	Suna out
11	CDC out	Suna out	Suna in	CDC in	11	CDC out	Suna out	Suna in	CDC in	11	Suna in	CDC out	Suna out	CDC in
12	CDC in	Suna in	CDC out	Suna out	12	CDC in	Sun in	Suna out	CDC out	12	Suna out	CDC in	Suna in	CDC out

Two trap types were evaluated. The first was the Centers for Disease Control and Prevention miniature light trap (CDC-LT, model 512, John W. Hock Company, Gainesville, Florida, USA) with an incandescent light bulb and connected to a 6V power supply. CDC-LT were not supplied with any additional synthetic attractant chemical and were thus evaluated in their standard configuration. The second trap type was the Suna trap (Biogents AG, Regensburg, Germany) which was connected to a 12V power supply (Hiscox et al., 2014). In both years of study, only, a nylon strip releasing a volatile synthetic chemical blend of five compounds attractive to mosquitoes (MB5) was added to this trap (Menger et al., 2014).

Each house received one of four trap configurations: a CDC-LT or Suna trap that was placed either indoors or outdoors. After four consecutive trapping nights, each house had received each trap configuration once. This procedure was then repeated two times more (Tables 1 and 2). The sequence of rotation of trap configurations over the sentinel houses was determined beforehand for the entire period of the study covering in total 144 trap nights (12 houses each sampled for 12 nights). In this way, each of the four trapping configurations (CDC-LT indoors, CDC-LT outdoors, Suna indoors and Suna outdoors) was tested on 36 trapping nights.

For the second year of study (2015), we modified the Suna trap by adding an incandescent light bulb of the same specifications as that used in the CDC-LT. We did this to evaluate the performance of the Suna trap with a light source, positioned across the perforated plastic base (Figure 1).

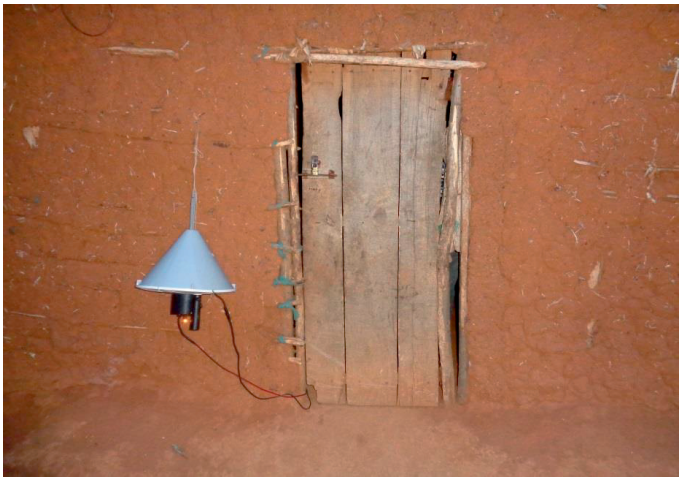

Figure 1: Suna trap modified in 2015 with an addition of an incandescent light bulb, here placed outside a sentinel house.

The same study design was followed as in 2014, except that we also included a number of trapping nights during which we tested the Suna trap without light (Table 2). The total number of trapping nights per trap configuration was six for Suna indoors without light, six for Suna outdoors without light, 30 for both Suna indoors and Suna outdoors with light, and 36 for both the CDC-LT indoors and outdoors with light.

For the indoor collections, the CDC-LT was installed at 1.5 m from the ground at the foot end of a bed net that was occupied by a human sleeper. The Suna trap was installed at 30 cm from

the ground, as this was shown to be the optimal position (Hiscox et al., 2014; Mboera et al., 1998). Outdoors, the two types of traps were hung at the same height above the ground as in their indoor locations and they were suspended near to the window that was closest to the main entrance of the house. The traps ran from 06:00 pm to 06:00 am the next morning and were installed and collected by a team of entomology technicians. The village leader and the head of each selected house were informed about how the traps work and were requested to ensure their security and to report the functioning of the trap during the night. They were also asked to report the number of people sleeping inside the sentinel houses on each experimental night. The entomology team ensured a periodic supervision of functioning and security from thieves of traps throughout the night.

Species identification

The mosquitoes collected per trap per house per night were first sorted by genus as well as sex. All females *Anopheles* mosquitoes were identified to species level using the morphological identification key (Gillies & Coetzee, 1987). A random sample of more than 10% of females *Anopheles gambiae* s.l. was preserved individually in Eppendorf tubes under silica gel, and sibling species were determined by Polymerase Chain Reaction (PCR) at the National Entomology laboratory (Scott et al., 1993). The PCR method used a segment of mosquito leg prior amplified, and the identification of species was carried out using universal primers and specific primers to each sibling species.

Data analysis

The data collected from each house, trap and collection night were analyzed using R version 3.1.2. Comparisons of proportions of *An. gambiae* s.s. versus *An. arabiensis* between the two trap types (CDC-LT and Suna) and between the trap locations (indoors and outdoors) were made using a Generalized Linear Model (GLM) with a negative binomial distribution and a logit-link function. Generalized Linear Mixed Models (using the *glmm ADMB* library in R) were constructed to assess the fixed effects of trap type, location (indoors/outdoors) and the interaction between trap type and position on the numbers of *An. gambiae* s.l., other *Anopheles* and *Culex* mosquitoes, respectively. After evaluation of the fit of the dependent variable to various distributions (normal, lognormal, Poisson and negative binomial), we selected 'negative binomial' (family = "nbinom") for all models. The night of collection and the houses where collections were carried out were included as random variables in the

models. Incidence rate ratios (IRR) were calculated from the parameter estimates resulting from the model.

Ethical considerations

Verbal consent for the study was obtained from the local leaders and the head of the selected houses for the experiment. The Medical Research Center of the Rwanda Biomedical Center approved the research project and the Rwanda National Health Ethic Committee (RNEC) of the Ministry of Health granted ethical clearance (390/RNEC/2012).

Results

Across the 144 trap nights each year, *An. gambiae* s.l. was the major mosquito species identified in the trap collections and this species complex represented 87.7% of the total catches in 2014 (n=3,293) and 66.2 % in 2015 (n=1,644). Other mosquito species caught during the two years of the study were *An. maculipalpis* (7.4%), *An. squamosus* (1.5%), *An. ziemanni* (0.2%), *Culex* spp (9.7%) and *Mansonia* spp (0.6%) (Table 3).

Of a random sample of 288 female *Anopheles gambiae* s.l. identified to species level using PCR, 19.1% were *Anopheles gambiae* s.s. and 80.9% *Anopheles arabiensis*. Quite strikingly, these proportions were significantly different for the two trap types (Figure 2). For the relative abundance of indoor collections, the odds ratio for catching an *An. arabiensis* in a CDC-LT in comparison with Suna was 3.74 (95% CI: 1.44-9.49), whereas this ratio was even larger for outdoor collections: 15.19 (95% CI: 5.77-45.70).

Suna trap versus CDC light trap in 2014

For both types of trap, either indoors or outdoors, the total number of *An. gambiae* s.l. caught per trap per night ranged between 0 and a maximum of 135 females (CDC-LT, indoors; Figure 3-A). CDC-LT collected significantly more *An. gambiae* s.l. than the Suna trap with an estimated IRR of 7.93 (CI: 5.13-12.26). Both the interaction of the effects of location and trap type ($\beta = 0.524 \pm 0.308$, $P = 0.089$) as well as the main effect of location alone ($\beta = -0.020 \pm 0.212$, $P = 0.925$) were not significant.

The numbers of other *Anopheles* ranged between 0 and a maximum of 16 mosquitoes collected by a CDC-LT located indoors (Figure 3-B). There was no significant interaction between location and trap type ($\beta = -0.657 \pm 0.888$, $P = 0.46$). The CDC-LT collected significantly more other *Anopheles* than the Suna trap (IRR = 6.46, CI: 1.71-24.51). In

addition, significantly more other *Anopheles* mosquitoes were collected outdoors than indoors (IRR = 4.18, CI: 1.55-11.28).

The number of *Culex* mosquitoes collected in the traps was within the same order of magnitude as other *Anopheles*. The minimum was 0 and the maximum was 24 *Culex* collected from a CDC-LT indoors (Figure 3-C). Neither the interaction between trap type and location, nor the location of the trap (indoors/outdoors) as a single main effect had an impact on *Culex* catch sizes.

Table 3: Total female mosquitoes caught by CDC-LT and Suna traps in the study area of Ruhuha, south eastern Rwanda, (A) August 2014 and (B) August 2015.

Type of trap	Location	Number of trap nights	*An. gambiae* s.l.	*An. funestus*	*An. maculipalpis*	*An. ziemani*	*An. squamosus*	*Culex* spp.	*Mansonia* spp.	Total
(A) 2014										
CDC-LT	Indoor	36	1352	1	19	0	11	90	0	1473
	Outdoor	36	1063	0	68	1	31	131	2	1296
Suna	Indoor	36	181	0	5	0	0	18	0	204
	Outdoor	36	293	0	9	0	0	18	0	320
	Total 2014	144	2889	1	101	1	42	257	2	3293
	Species composition (%)		87.73	0.03	3.07	0.03	1.28	7.80	0.06	100
(B) 2015										
CDC-LT	Indoor	36	445	0	18	0	2	90	4	559
	Outdoor	36	388	0	205	8	24	88	17	730
Suna+light	Indoor	30	89	0	0	0	0	13	0	102
	Outdoor	30	114	0	40	1	8	23	3	189
Suna (-light)	Indoor	6	9	0	0	0	0	6	0	15
	Outdoor	6	43	0	1	0	0	3	2	49
	Total 2015	144	1088	0	264	9	34	223	26	1644
	Species composition (%)		66.18	0.00	16.06	0.55	2.07	13.56	1.58	100
	Total 2 years	288	3977	1	365	10	76	480	28	4937
	Species composition (%)		80.55	0.02	7.39	0.20	1.54	9.72	0.57	100

(β = - 0.420 ± 0.561, P = 0.45 and β = 0.501 ± 0.328, P = 0.13, respectively). As with *An. gambiae* s.l. and other *Anopheles*, there was a significant effect of trap type on *Culex* catch sizes. CDC-LT indoors and outdoors collected significantly higher numbers of *Culex* females than the Suna trap, with an estimated IRR of 5.32 (CI: 2.43-11.66).

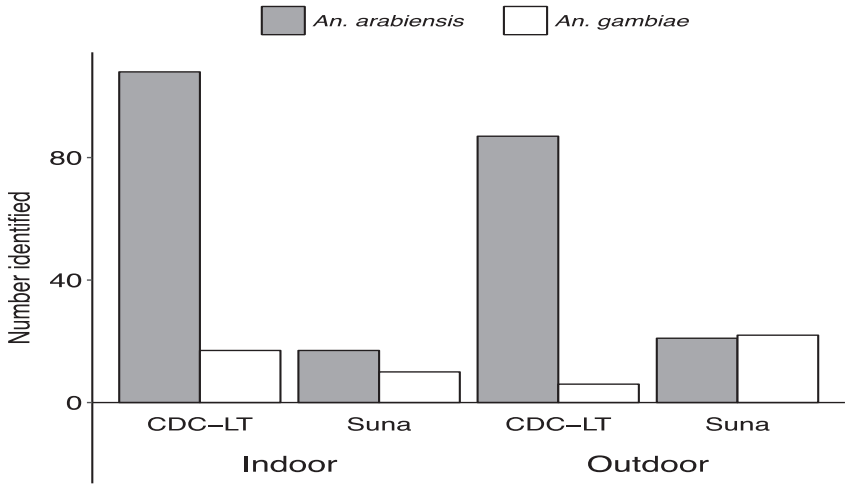

Figure 2: Number of *An. arabiensis* and *An. gambiae s.s.* identified out of 288 randomly selected samples from indoor and outdoor collections with Suna traps (N=70) or CDC light traps (N=218).

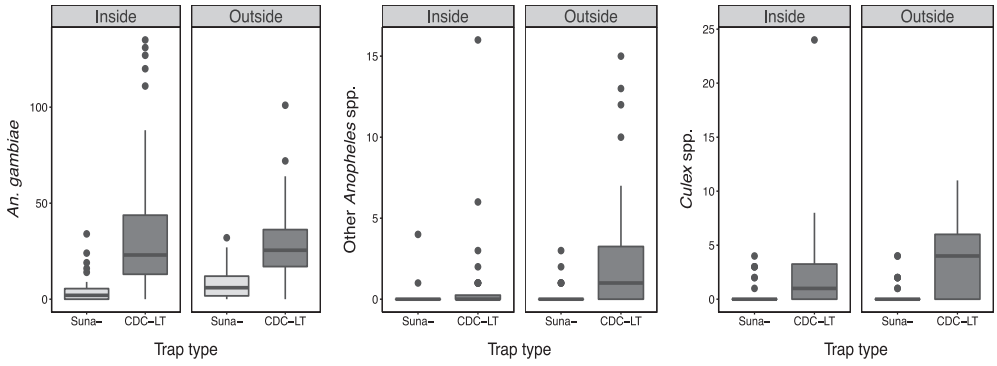

Figure 3: Boxplots showing the number of *An. gambiae* s.l., other *Anopheles* spp. and *Culex* spp. collected in the CDC light trap (36 trapping nights per treatment) or the Suna trap (36 trapping nights per treatment) in South Eastern Rwanda, August 2014.

Suna trap versus CDC light trap in 2015

The number of female *An. gambiae* s.l. collected by the traps ranged between 0 and 97 mosquitoes (Figure 4-A). We first analyzed the data for traps that had a light during the collections. In the model that included the random effects, there was no significant interaction between trap type and location and *An. gambiae* s.l. catch size ($\beta = 0.680 \pm 0.532$, $P = 0.20$). The comparison between CDC-LT and Suna trap (both with light) revealed that the CDC-LT collected significantly more mosquitoes (IRR: 5.09; CI: 2.43-10.70) and there was no significant main effect of trap location ($\beta = -0.479 \pm 0.338$, $P = 0.16$). The separate comparison between the Suna traps with and without light showed that the addition of light did not significantly increase the number of female *Anopheles* caught compared to a Suna trap without light ($\beta = 0.420 \pm 0.933$, $P = 0.65$). In addition, there was no significant interaction effect of trap location ($\beta = 1.030 \pm 1.129$, $P = 0.36$) or the interaction between light and trap location ($\beta = -0.807 \pm 1.301$, $P = 0.54$).

The number of other *Anopheles* ranged between 0 and a maximum of 51 females collected in a CDC-LT outdoor (Figure 4-B). We found that there was no significant interaction between trap type and location with catch sizes of other *Anopheles* species when both traps were using a light ($\beta = 1.366 \pm 1.173$, $P = 0.24$). However, the CDC-LT collected significantly more other *Anopheles* than the Suna with a light when trap type was included in the model as a single main effect (IRR: 20.71, CI: 2.41-177.81). Significantly more other *Anopheles* were collected outdoors compared with indoors with an estimated IRR of 9.24 (CI: 4.06-21.05). No proper statistical comparisons could be made between the Suna trap with and the Suna trap without light. This was a result of the fact that only one individual specimen of 'other *Anopheles*' was collected on 12 trap nights in the Suna trap without light.

Finally, the number of *Culex* females ranged between 0 and 21 (Figure 4-C). Analyzing the results of the two trap types with a light separately, showed that there was no significant interaction between trap type and location and *Culex* catch size ($\beta = 0.586 \pm 0.604$, $P = 0.33$) and no significant main effect of location ($\beta = 0.034 \pm 0.355$, $P = 0.92$). However, CDC-LT collected significantly more than Suna traps with light with an estimated IRR of 6.00 (CI: 2.51-14.35). A comparison between the Suna trap with and without light showed no significant effect of the addition of light ($\beta = -0.652 \pm 0.792$, $P = 0.41$), no significant

difference between the two locations (β = -0.088 ± 1.182, P = 0.94), nor a significant interaction between these two main effects (β = 0.676 ± 1.315, P = 0.61).

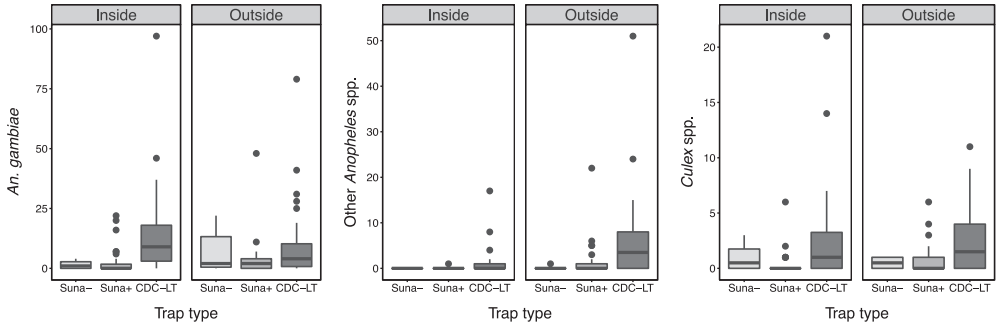

Figure 4: Boxplots showing the number of *An. gambiae* s.l., other *Anopheles* spp. and *Culex* spp. collected in the CDC light trap (36 trapping nights per treatment) or the Suna trap with light "Suna+" (30 trapping nights per treatment) or Suna trap without light "Suna -" (6 trapping nights per treatment) in South Eastern Rwanda, August 2015.

Discussion

The evaluation of CDC light traps and Suna traps in our study area aimed to compare the relative efficacy of each trap type in sampling malaria vectors. The study showed that catch sizes in CDC-LT were significantly higher than those in Suna traps for all groups of mosquito species in the study area in August 2014. This period corresponded to the transplantation of rice plants and is characterized by nuisance from mosquitoes coming from the rice fields. During the same period in 2015, an incandescent light bulb of the same specifications used in the CDC-LT, was added to the Suna trap to supply light with the aim to enhance the performance of the Suna trap. The addition of light did not increase the relative trapping efficacy of the Suna trap compared with the CDC-LT in sampling *Anopheles gambiae* s.l., the main malaria vector found in the area.

The molecular analyses of *An. gambiae* s.l. showed that there were large differences in sibling species composition between the two trap types. Interestingly, this was the case for both the indoor as well as the outdoor population. Earlier research in Kenya with the CDC-LT, Mbita tent traps and HLC showed that no such differences in species composition existed when comparisons were made between different types of trap (Mathenge et al., 2004). It is yet unclear whether our observations are the result of the CDC-LT being relatively more sensitive in collecting *An. arabiensis* or whether the Suna trap collects relatively more *An.*

gambiae s.s.. The Suna trap was baited with the MB5 blend, which was specifically developed for the anthropophilic *An. gambiae s.s.* (Mukabana et al., 2012), thereby supporting the latter hypothesis. On the other hand, it may also be related to the height difference at which the two traps were tested (1.5 m for CDC-LT and 0.3 m for Suna), although it is not clear whether the two sibling species differ in the way in which they approach a house and a host, and thus also a trap.

Our goal was to compare both traps in their most optimal configuration, based on published data (Hiscox et al., 2014; Mboera et al., 1998). Ideally, if traps are to be used for assessing malaria transmission risk, such traps should be evaluated in conjunction with human landing collections (Mburu et al., 2017). Despite the ethical limitations of latter method, different studies have shown that it can reliably collect anthropophagic *Anopheles* (Laganier et al., 2003; Le Goff et al., 1993; Overgaard et al., 2012). It has thus been used as the standard reference method for calibrating other innovative sampling methods (Le Goff et al., 1993; Lines et al., 1991; Mathenge et al., 2005; Overgaard et al., 2012). Interestingly, in comparison with HLC as the standard method, CDC-LT could either collect higher numbers or lower numbers than HLC, depending on specific local factors. For example, it was shown that the performance of CDC-LT was affected by the abundance of mosquitoes (Mathenge et al., 2004) and in some areas by the coverage of vector control (Fornadel et al., 2010). Other studies showed a low sensitivity of CDC-LT in low mosquito abundance of vectors in Bioko island (Overgaard et al., 2012), in an urban area (Govella et al., 2011) and in rural areas of Eastern Coastal (Mbogo et al., 1993) and western Kenya (Mathenge et al., 2005).

The efficiency of CDC-LT has also been compared with other mosquito sampling methods such as BG sentinel-traps and resting boxes. The BG was found more efficient outdoors than indoors for sampling *An. gambiae* s.l. with a relative catching rate (BG/CDC-LT) of 6.4 outdoors and 0.7 indoors, respectively (Pombi et al., 2015). The CDC-LT was also found more effective than clay pots with indoor relative catching rate of 22.64 in collecting female *Anopheles* mosquitoes in a northern arid area of Tanzania (Bijllaardt et al., 2009).

The Suna trap was originally developed to address outdoor biting mosquito populations. The first semi-field trial was carried out in Mbita, western Kenya, and showed that the Suna trap sampled equal numbers of *Anopheles gambiae* s.l. in comparison with the CDC light trap and MM-X trap, all placed inside houses occupied with humans. Used outdoors, the trap with an addition of a synthetic attractive blend demonstrated high performance. The trap was

proposed to be tested in other malaria endemic areas for impact on vector control (Homan) and as future supplement to existing vector control methods (Hiscox et al., 2014). However, in the Lao PDR the odour-baited Suna trap caught fewer mosquitoes than three other mosquito sampling methods: the human double baited net, the odour-baited CDC-LT and the odour-baited BG-sentinel trap (Tangena et al., 2015). The recent study conducted in Kenya showed that the addition of 2-butanone in odour synthetic blends targeting host-seeking malaria vectors proved promising for usage of odour baited traps for mosquito surveillance (Mburu et al., 2017).

Conclusion

The evaluation of the CDC light trap and the Suna trap baited with a synthetic blend of attractants in south eastern of Rwanda demonstrated a higher effectiveness of CDC-LT than Suna traps in collecting the major malaria vectors in the study area. Interestingly, the CDC-LT was promising for sampling outdoor mosquitoes in south eastern Rwanda, and should be further evaluated as alternative method to the human landing catch. The Suna trap, despite its performance under semi-field conditions and impact on mosquito population reduction (Homan et al., 2016) was not highly efficient in sampling mosquito species in comparison with CDC-LT. The addition of light to the Suna trap did not improve its performance in comparison with CDC-LT. Addition of carbon dioxide, for example through yeast-fermentation of sugar or via a carbon dioxide mimic such as 2-butanone (Mburu et al., 2017; Smallegange et al., 2010), could increase the effectiveness of collecting malaria vectors with Suna traps in Rwanda. Difference in sibling species ratio collected by the two traps warrants further research. Accurate assessments of this ratio are needed, because shifts in sibling species ratio have been implicated as drivers in the resurgence of malaria in some parts of Africa (Hakizimana et al., 2018b; Kitau et al., 2012).

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Chapter 4

Monitoring long-lasting insecticidal net (LLIN) durability to validate net serviceable life assumptions, in Rwanda

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Chapter 4

Abstract

To validate assumptions about the length of the distribution–replacement cycle for long-lasting insecticidal nets (LLINs) in Rwanda, the Malaria and other Parasitic Diseases Division, Rwanda Ministry of Health, used World Health Organization methods to independently confirm the three-year LLIN serviceable life span recommendation of WHO. Approximately 3,000 coded LLINs, distributed as part of a national campaign, were monitored in six sites, by means of six–monthly visits to selected houses. Two indicators, survivorship/attrition, a measure of the number of nets remaining, and fabric integrity, the proportion of remaining nets in either 'good', 'serviceable' or 'needs replacement' condition, based on holes in the net material, were tracked. To validate the assumption that the intervention would remain effective for three years, LLIN coverage, calculated using either survivorship, or integrity, by removing nets in the 'needs replacement' category from the survivorship total, was compared with the predicted proportion of nets remaining, derived from a net loss model, that assumes an LLIN serviceable life of three years.

After two years, there was close agreement between estimated LLIN survivorship at all sites, 75% (range 64-84%), and the predicted proportion of nets remaining, 75%. However, when integrity was considered, observed survivorship at all sites, declined to 42% (range 10-54%). Conclusions: More than half, 58%, of the LLINs fell into the 'needs replacement' category after two years. While these nets were counted for survivorship, they were judged to be of little-to-no benefit to a user. Therefore, when integrity was taken into account, survivorship was significantly lower than predicted, suggesting that net serviceable life was actually closer to two, rather than three years, and, by extension, that the impact of the ntervention during year three of the LLIN distribution-replacement cycle could be well below that seen in years one and two.

Keywords: LLIN, Serviceable life, Durability, Survivorship/attrition, Fabric integrity, Loss, Distribution/replacement time

Introduction

Large-scale distribution of LLINs to achieve universal net coverage has been associated with highly successful malaria control outcomes in Rwanda. When insecticidetreated net ownership increased from 15% to 82%, alongside scale-up of other malaria control interventions, there was a 50% decrease in all-cause mortality of children less than five years old Building on this success, Rwanda became one of the first countries in Africa to achieve universal LLIN coverage by distributing over 6.1 million LLINs (2010–2011). In addition to achieving high coverage, the Rwanda LLIN distribution programme is also focused on timely replacement of existing nets. That is maximizing the time between distribution and replacement (for optimum use of resources), while, at the same time, avoiding loss of impact, associated with net failure. Based on LLIN durability monitoring norms (WHO, 2007, 2011; WHO/WHOPES, 2011), the National Malaria Control Program (NMCP), the National Malaria Control Programme (NMCP), in charge of LLIN distribution and replacement, assumes that nets last three years, and that the proportion of nets remaining at any given time is predicted by a three-year NetCALC net loss model (Vector-Works). This report describes monitoring carried out to validate this assumption.

LLIN durability monitoring focuses on three indicators: net survivorship, an estimate of coverage, that is, the percentage of nets still present and in use in the house hold to which they were distributed; fabric integrity, a quantification of the number and size of holes in the LLIN netting and bio-efficacy, a measure of net insecticidal effect. The term 'hole' is used as a general term to describe all damage: tears, burn holes, rodent (chewing)-associated damage, rips in corners and seams. While all three indicators are assessed in Rwanda, two: survivorship and fabric integrity are discussed here. The two should be assessed together, because if a net is present and counted for survivorship, but in such poor physical condition that it offers the user little-to-no protection, then survivorship data alone will, most likely, under estimate net loss. This report presents the results from a prospective, longitudinal assessment of LLIN durability, undertaken by the Malaria and Other Parasitic Diseases Division (MAL & OPDD) to monitor both survivorship and fabric integrity.

Chapter 4

Methods

Site selection

Monitoring was based on a prospective longitudinal assessment, with six-monthly follow up, of six LLIN cohorts. Administrative units, cells (the Rwanda Constitution divides the country into provinces, districts, cities, municipalities, towns, sectors and cells, with borders established by Parliament) containing approximately 500 households, selected by probability sampling, were used as monitoring sites. One LLIN per household at the selected sites was followed. Sites in three settings were included in the assessment: a peri-urban setting with endemic malaria transmission, a rural setting with hypo-endemic transmission, and a rural setting with endemic transmission. Peri-urban sites adjoin urban areas, but are localized outside formal urban boundaries and jurisdictions; such sites are becoming urbanized, and progressively assume many of the characteristics of urban areas. Net distribution was implemented such that one of the two sites in each setting received LLINs, manufactured with polyethylene thread while the other site received LLINs manufactured with polyester thread. Table 1 summarizes relevant information about the LLIN monitoring sites. Maps (Figures 1, 2 and 3) give the geographic location within the country.

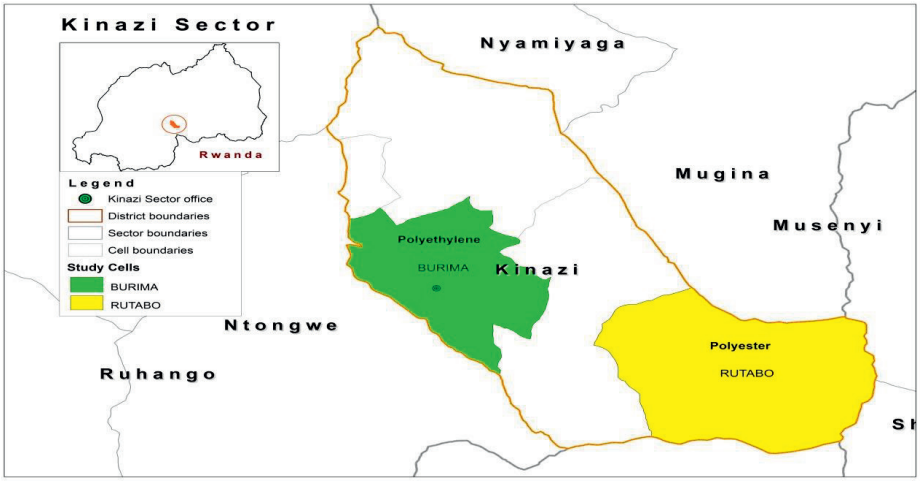

Figure 1: Peri-urban LLIN durability monitoring sites.

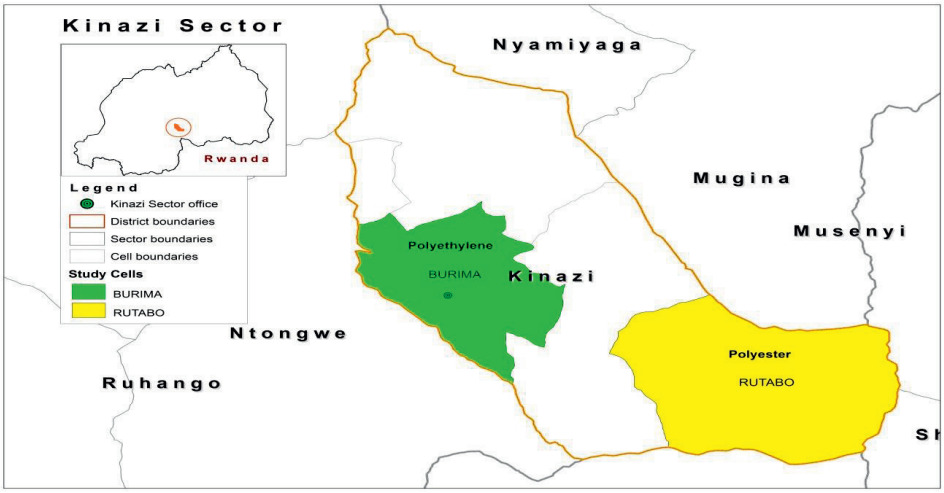

Figure 2: Rural LLIN durability monitoring sites (endemic transmission)

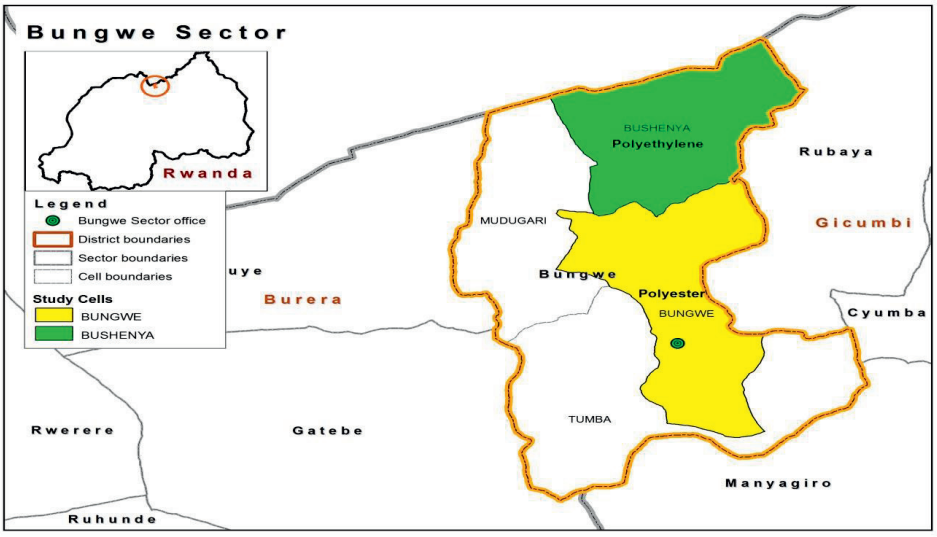

Figure 3: Rural LLIN durability monitoring sites (hypo-endemic transmission)

LLIN distribution and baseline data collection LLINs were distributed to the selected sites as part of a 2010, countrywide, mass net distribution campaign. After one month, LLIN tracking teams visited 500 houses at each site. If the house was open and at least one campaign net was in use, the head of the household was asked to approve participation in the assessment, which included four follow up visits at six (T6), 12 (T12), 18 (T18), and 24 (T24) months post distribution. Upon approval, one of the recently distributed LLINs/household was bar

and color coded (Table 1), and the house, to which the net was distributed, was enrolled in the assessment. There was an initial (T0) inspection of LLIN fabric integrity, and enrolled households were geo-referenced to facilitate follow up visits. Table 2 summarizes the status of the assessment LLINs at T0.

Data measurement and estimation of coverage and integrity

During six-monthly follow up visits, T6, T12, T18, and T24, all households, open for inspection, were re-visited to assess LLIN survivorship, which was estimated using the equation:

$$\frac{\text{LLINs observed x households } (T_0) \text{ x } 100\ (\%)}{\text{households visited}}$$

Fabric integrity was also assessed during each visit. The condition of all nets was derived from an examination of 30 assessment LLINs per site. The nets, randomlyselected from all households inspected, were examined for holes and a pHI, the weighted summary of observed damage (holes), was calculated. In this study a method described at a WHO Vector Control Working Group (VCWG) work stream [7] was used to calculate pHI. The LLINs were temporarily removed from houses, fitted over a frame, and subjected to a side-by-side, plus top, visual examination. Technicians recorded the number and size (length) of each observed hole. Based on the result each hole was assigned to one of four shape/size categories:

(1) A circle with an estimated diameter of 0.5-2 cm (for a hole judged to be 'smaller than one's thumb')

(2) A circle with an estimated diameter of 2–10 cm (for a hole judged to be 'larger than one's thumb, but smaller than one's fist')

(3) A circle with an estimated diameter of 10–25 cm (for a hole judged to be 'larger than one's fist but smaller than one's head')

(4) A circle with an estimated diameter of >25 cm (for a hole judged to be 'larger than one's head')

To calculate the pHI, the number of holes in each category was multiplied by a category weight: 1 for category (1), 23 for category (2), 196 for category (3) and 578 for category (4).

Table 1: LLIN durability monitoring sites: characteristics (sector, setting*), cell, thread (polyethylene or polyester)** and tagging information***

Characteristics		Thread	
Sector (setting*)	**Cell**	**Polyethylene****	**Polyester****
Masaka (peri-urban, endemic*).	***Cell: Rusheshe***	500 nets Ink code: 1 black dot Bar codes A001-A500	
	Cell: Cyimo		500 nets Ink code: 2 black dots Bar codes B001-B500
Kinazi (rural, endemic*)	***Cell: Burima***	500 nets Ink code: 1 red dot Bar codes C001-C500	
	Cell: Rutabo		500 nets Ink code: 2 red dots Bar codes D001-D500
Bungwe (rural, hypo-endemic*)	***Cell: Bushenya***	500 nets Ink code: 1 green dot Bar codes E001-E500	
	Cell: Bungwe		500 nets Ink code: 2 green dots Bar codes F001-F500

* endemic malaria transmission 'constant'; hypo-endemic malaria transmission 'sporadic'
**polyester thread: denier 100; polyethylene thread: denier 150.
***Each LLIN enrolled in the tracking assessment was bar coded. A second site-specific ink code, one or two colored (black, red or green) dot(s), 0.5 cm in diameter, drawn with indelible-ink laundry marker, was also used.

Table 2: LLIN durability: Survivorship (%) at one-month post distribution (T0)

Setting	Cell	Thread	households visited	Dropped*	LLINs (households) Enrolled**	% LLIN Survivorship*** T_0
Urban	Cyimo	polyester	500	93	407	
	Rusheshe	polyethylene	500	32	468	
Low	Bungwe	polyester	500	24	476	100
endemicity	Bushenya	polyethylene	500	17	483	
High	Rutabo	polyester	500	63	437	
endemicity	Burima	polyethylene	500	26	474	

*Households were dropped at T0 because they were either closed, or open, but had not received an. LLIN during the national campaign
**Households re-visited during T6 follow up visit.
***By definition, the percentage (%) of enrolled households with a coded LLIN at T0 is 100%.

Integrity data were entered into the following formula to estimate a pHI for each LLIN:

- pHI = (1) (number of category 1 holes) + (23) (number of category 2 holes) + (196) (number of category 3 holes) + 578 (number of category 4 holes).
- pHI thresholds (Allan et al., 2012), were used to 'translate' observed pHI results into three integrity 'condition' categories:

  (1) A net with a pHI < 64 was classified as being 'in good condition'.

  (2) A net in the 64 ≤ pHI ≤ 768 range, was classified as being 'in serviceable condition' (repairable).

  (3) A net with a pHI > 768, was judged to be 'in need of replacement' and of questionable benefit to user' (Allan et al., 2012)

The estimated median size of a single hole corresponding to each category is:

- 1.6 cm2 for category (1) – good
- 168 cm2 for category (2) – serviceable
- 1,190 cm2 for category (3) - replace

To express the impact of change in fabric integrity on survivorship (net loss), the estimated number of nets in category 3 was removed, and survivorship was recalculated based on the number of nets in categories 1 and 2 only. Estimates of survivorship, based on coverage and integrity, are shown in tabular form, and are plotted against time for comparison with the NetCALC - predicted proportion of nets, based on a three-year serviceable life assumption.

Study clearance

This observational study was planned with, and approved by, the Ministry of Health. Community leaders were consulted before the study began, and all gave verbal consent in advance. Head of households gave their written consent prior to being enrolled.

Results

Survivorship/attrition

On average, LLIN survivorship declined to a mean of 75% (all sites), range 64-84%, after two years (Table 3). There was a small but significant difference between survivorship of polyethylene and polyester LLINs at the peri-urban sites, 84% versus 64% ($p < 0.05$), and at the rural, hypo-endemic sites, 78% versus 70% ($p < 0.05$). While the same pattern was seen at the rural, endemic sites, 79% versus 77%, the difference was not significant ($p > 0.05$). Figure 4 presents the data from Table 3, as well as T6, and T18 survivorship results. NetCALC curves, showing (predicted) proportion of surviving LLINs, based on either a three-year or a five-year net replacement cycle (serviceable life assumption) are shown as dotted lines for comparison. In summary, observed survivorship at most of the assessment sites appear to track the NetCALC curve based on a three-year serviceable life assumption.

Table 3: LLIN durability: Survivorship (%), observed and predicted*, by cell, setting, and LLIN thread type at one (T0), 12 (T12) and 24 (T24) months post distribution

Cell	Characteristics	LLIN type	Observed			Predicted*
			T_0	T_{12}	T_{24}	
Cyimo	Peri-urban endemic	Polyester	100	89	64[1]	75
Rusheshe		Polyethylene	100	93	84[1]	
Bungwe	Rural endemic	Polyester	100	92	77	
Bushenya		Polyethylene	100	94	79	
Rutabo	Rural hypo-endemic	Polyester	100	95	70[2]	
Burima		Polyethylene	100	90	78[2]	

[1,2]p < 0.05 *NetCALC predicted percent of nets remaining after two years, based on a 3-year serviceable life assumption.

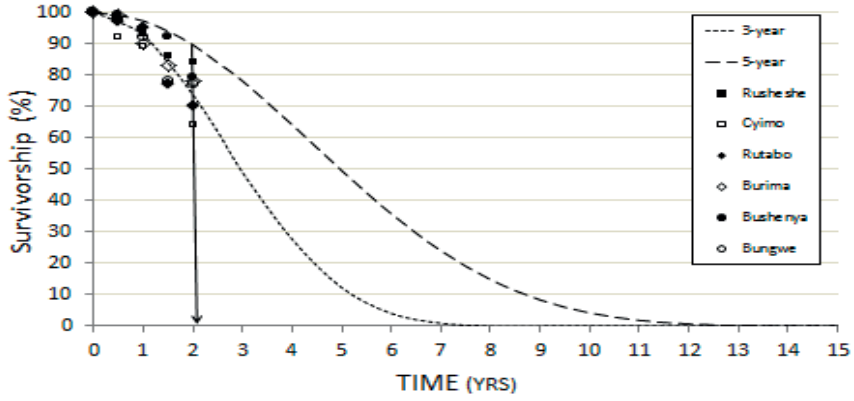

Figure 4: LLIN observed survivorship by site (data from Table 3 with addition of 6-, and 18-month results) versus NetCALC-predicted 'proportion of LLIN's present' assuming either a 3-year (small-dash curve) or a 5-year (larger-dash curve) LLIN serviceable life.

Fabric integrity

Fabric integrity results (Table 4) are presented by site and thread type, based on the three integrity condition categories: 'good', 'serviceable' and 'replace', at baseline and after one (T12) and two (T24) years. After two years, an estimated 77% of the remaining LLINs in the peri periurban sites (Cyimo and Rusheshe) versus 49% of the LLINs in the rural sites (Bungwe, Bushenya, Rutabo, and Burima) fell into the 'replace' category. However, of greater interest than site specific differences in integrity, was the fact that after two years, an estimated 47% to as many as 90% of remaining LLINs fell into the 'replacement' category. When the T24 integrity measurements were converted to survivorship, by discounting LLINs in the 'replace' category, the resulting survivorship estimates (mean values) were between 21 and 65 percentage points below the 75% value predicted by the 3-year serviceable life model (Figure 5).

Table 4: LLIN durability: Fabric Integrity expressed as percentage of LLINs in one of three fabric integrity categories*: good, serviceable or needs replacement (replace) by cell and LLIN thread type at T0, T12, and T24

Cell LLIN thread	Cyimo p-ester	Rusheshe p-ethylene	Bungwe p-ester	Bushenya p-ethylene	Rutabo p-ester	Burima p-ethylene
			T_0 observed			
good	100	100	100	100	100	100
serviceable	0	0	0	0	0	0
replace	0	0	0	0	0	0
			T_{12} observed			
good	47	20	67	37	27	60
serviceable	20	43	13	13	37	27
replace	33	37	20	50	13	13
			T_{24} observed			
good	10	3	3	3	10	6
serviceable	27	7	47	50	37	47
replace	63	90	50	47	53	47

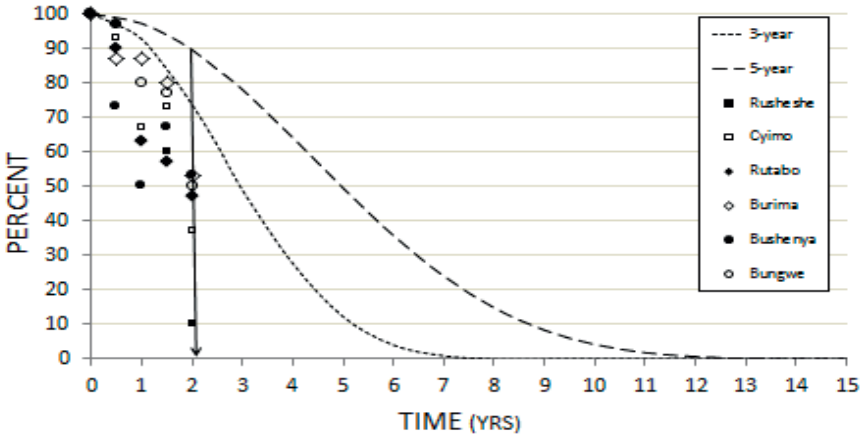

Figure 5: LLIN observed integrity (data from Table 4 expressed as survivorship) versus NetCALC-predicted 'proportion of LLINs present' assuming either a 3-year or a 5-year LLIN replacement cycle. The vertical line bisecting the two years time point (x-axis) facilitates comparison of observed and predicted survivorship.

Discussion

LLIN durability monitoring in Rwanda indicated lower survivorship, viz. greater than anticipated net loss, associated with poor fabric integrity, during year two of a three year LLIN distribution-replacement cycle. The proportion of the remaining nets in need of replacement, after two years, was large enough to suggest that the intervention would lose impact during year three of the distribution replacement cycle. (Karema et al., 2012) suggests low net (ITN) coverage (viz.survivorship), as one explanation for a 2009 resurgence of malaria in Rwanda following a 2006 under-five, countrywide bed net campaign. The present results highlight an additional possibility: that of greater than expected net loss associated with poor fabric integrity. In the present study, coverage, based on survivorship, decreases by 26% to 36% by the end of year two (post-distribution). However, it is the striking loss of fabric integrity, in addition to reduced coverage, that strengthens the argument that LLIN serviceable life and, by extension, the effective life of an LLIN intervention was, perhaps, one year less than the assumed duration of the LLIN distribution – replacement cycle. In this analysis, a 'missing' net reduces survivorship. However, such 'missing' nets could have been

present in another house, not visited by the tracking team, and, therefore, still have contributed to coverage and impact. On the other hand, the poor fabric integrity results after two years, suggest that, regardless of whether or not a net was present and in use somewhere in the community, it was as likely to be torn, in need of replacement, and of little use to the user, as to be in good or serviceable condition. When poor fabric integrity results for surviving nets are considered, survivorship after two years is nearly one half of that predicted by a NetCALC model (41% versus 75%).

The assumption that most of these damaged nets probably provided 'questionable benefit to users' (Allan et al., 2012), raises a red flag with respect to the programmatic assumption that the LLIN intervention would remain effective during year three of the distribution replacement cycle. NetCALC curves, based on a three-to-five years serviceable life, are thought to be realistic estimates for programme planning. However, the results reported here, suggest that many, perhaps most of LLINs, in use in Rwanda, are of questionable benefit to users, due to poor fabric integrity, after two years. If one assumes that such nets, more appropriately, belong in the nets lost (associated with survivorship) category, then LLIN survivorship estimates decrease dramatically. When such results are compared with expected survivorship, derived from NetCALC predictions, the discrepancy is significant. Factors that affect LLIN durability, act to a greater or lesser extent, in different settings. For example, in the present assessment, the rate at which holes appear (loss of fabric integrity) in nets is higher in urban locations. Given the number of factors that affect LLIN durability, and the variation between settings where they are distributed, it is not surprising that reliance on generalizations about how long nets last could be misleading.

LLIN monitoring based on the approach outlined by WHO, is recommended as the best way to address the programmatic question of how long nets last. If faster-than-expected fabric degradation is a significant determinant of LLIN impact, what are the possible ways to ameliorate its effect? Unfortunately, reducing the time to LLIN replacement is problematic, based on current funding models (nonetheless, the Ministry of Health, Rwanda is considering LLIN replacement every 30 months, versus the current 36–40 months). Other alternatives: working with manufacturers to develop and test more durable net products, fostering a stronger net care and repair culture, and 'pushing' more nets to communities via routine channeling and social marketing, as well as national campaigns, are also planned to close the gap between observed and expected net loss rates. Finally, as stated earlier, a comprehensive

programme of LLIN durability monitoring to reduce reliance on assumptions, that may not apply everywhere, is needed to accurately inform programme about 'on-the-ground' net loss rates. When LLINs remain in use after 'failing' due to loss of fabric integrity, communities, where LLIN coverage, as well as compliance with nightly net use, appears to be adequate, may still experience a resurgence of malaria. For this reason, indicators of LLIN coverage, based on questions such as "did you sleep under a bed net last night?" (DHS, 2009), should not, necessarily, be interpreted as a confirmation of protection from malaria transmission. This study did not address the third WHO durability indicator, bio-efficacy. Could, for example, insecticidal effect compensates for poor fabric integrity? Unpublished data from Rwanda suggests that after two years, when net loss associated with fabric integrity has taken its toll, LLIN bio-efficacy also declines, permitting some vector survival following exposure to nets. As vector survival increases, vector entry via holes, man-vector contact and transmission rates also increase (Gnanguenon et al., 2013). The added effect of vector-pyrethroid resistance, the ability of vectors to navigate through holes in nets, and the observation of living vectors resting inside 'older' LLINs, all suggest that bioefficacy, later in the LLIN distribution replacement cycle, no longer compensates for poor fabric integrity.

It is important to note that the thresholds used to evaluate fabric integrity in this study, e.g. pHI-based fabric integrity condition categories, reflect limited observations and should be thought of as more arbitrary than evidence-based. Therefore, their accuracy with respect to the question of net replacement merits additional evaluation. Nonetheless, what pHI threshold-based monitoring does document, regardless of the interpretation, viz. condition category, assigned to each threshold, is the rapid degeneration of fabric integrity during years one and two following distribution in Rwanda. In the present study 0% (T0), 28% (T12), and 58% (T24) of nets were estimated to have crossed the most dangerous pHI threshold, equivalent to approximately one square foot of missing net (pHI equivalent to a single hole of 1,190 cm2 = 1.2 ft2). While the interpretation of pHI thresholds, should be refined, they currently provide a much needed reference for 'real time' evaluation of LLIN interventions, in Rwanda and elsewhere.

Conclusion

Two years after a national LLIN campaign, as many as three in ten LLINs had been removed from the houses where they were hung at distribution. However, more surprising, was the fact that five to as many as nine of every ten remaining LLINs showed loss of fabric integrity to a

degree that called into question their ongoing serviceability, during year three of the planned distribution replacement cycle. If loss of fabric integrity also means loss of protection from man-vector contact, as assumed, then adjustments to LLIN distribution planning are needed, along with more comprehensive monitoring of LLIN durability. As countries continue to scale-up LLIN coverage, it will be important to have specific information on LLIN durability in a variety of settings. This information should be generated in as short a time frame as possible to 'inform' decisions on how best to replace failing LLINs before they compromise the efficacy of the intervention.

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Chapter 5

Susceptibility of *Anopheles gambiae* sensu lato (Diptera: Culicidae) to insecticides used for malaria vector control in Rwanda

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Chapter 5

Abstract

The widespread emergence of resistance to pyrethroids is a major threat to the gains made in malaria control. To monitor the presence and possible emergence of resistance against a variety of insecticides used for malaria control in Rwanda, nationwide insecticide resistance surveys were conducted in 2011 and 2013. Larvae of *Anopheles gambiae* s.l. mosquitoes were collected in 12 sentinel sites throughout Rwanda. These were reared to adults and analyzed for knock-down and mortality using WHO insecticide test papers with standard diagnostic doses of the recommended insecticides. A sub-sample of tested specimens was analyzed for the presence of knockdown resistance (*kdr*) mutations.

A total of 14,311 mosquitoes were tested and from a sample of 1,406 specimens, 1,165 (82.9%) were identified as *An. arabiensis* and 241 (17.1%) as *An. gambiae s.s*. Mortality results indicated a significant increase in resistance to lambda-cyhalothrin from 2011 to 2013 in 83% of the sites, permethrin in 25% of the sites, deltamethrin in 25% of the sites and DDT in 50% of the sites. Mosquitoes from 83% of the sites showed full susceptibility to bendiocarb and 17% of sites were suspected to harbour resistance that requires further confirmation. No resistance was observed to fenitrothion in all study sites during the entire survey. The *kdr* genotype results in *An. gambiae s.s*. showed that 67 (50%) possessed susceptibility (SS) alleles, while 35 (26.1%) and 32 (23.9%) mosquitoes had heterozygous (RS) and homozygous (RR) alleles, respectively. Of the 591 *An. arabiensis* genotyped, 425 (71.9%) possessed homozygous (SS) alleles while 158 (26.7%) and 8 (1.4%) had heterozygous (RS) and homozygous (RR) alleles, respectively. Metabolic resistance involving oxidase enzymes was also detected using the synergist PBO.

This is the first nationwide study of insecticide resistance in malaria vectors in Rwanda. It shows the gradual increase of insecticide resistance to pyrethoids (lambda-cyhalothrin, deltamethrin, permethrin) and organochlorines (DDT) and the large presence of target site insensitivity. The results demonstrate the need for Rwanda to expand monitoring for insecticide resistance including further metabolic resistance testing and implement an insecticide resistance management strategy to sustain the gains made in malaria control.

Keywords: Insecticide resistance, Rwanda, *Anopheles gambiae*, vector control, pyrethroids, bendiocarb, DDT, fenitrothion, *kdr* mutation

Chapter 5

Introduction

Most countries in Africa depend heavily on two vector control interventions in their battle against malaria: long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). These tools use insecticides from four chemical classes: organochlorines, pyrethroids, carbamates and organophosphates. Whereas 14 formulations belonging to these classes are approved by WHO for use in IRS (WHO, 2013a), only pyrethroids are approved for use in LLINs (WHO, 2013c) because of their low mammalian toxicity, excito-repellent properties and rapid knock-down and killing effect (Zaim, Aitio, & Nakashima, 2000). It has been estimated that since 2000 more than 670 million cases of malaria have been averted by combining IRS and LLINs with case management and community education (Bhatt et al., 2015; WHO, 2014). In Rwanda, the national scale-up of vector control interventions has contributed to a steady reduction of malaria cases from 1.6 million in 2005 to 472,000 cases in 2012 (Karema et al., 2012; WHO, 2013a). This reduction has been attributed to the combined effects of universal coverage with LLINs (Karema et al., 2012) and targeted IRS operations in districts with the highest malaria endemicity (5 out of 30 districts) based on epidemiologic and entomologic data. Overall LLIN coverage is high with 83% of the households owning at least one LLIN and 74% reported to have slept under a LLIN the previous night for pregnant women and children under five (NISR, 2013). From 2005 to 2013, the National Malaria Control Programme (NMCP) of the Rwanda Ministry of Health has distributed approximately 11.2 million LLINs to a population of an estimated 11 million people (NISR, 2012).

From 2007 to 2012, nationwide distributions of LLIN have been conducted in conjunction with annual IRS applications of pyrethroids in high malaria transmission districts either in focal sectors or district-wide by blanket spraying, covering an estimated 98% of the targeted structures. In 2013, NMCP shifted from pyrethroids to the use of carbamates (Bendiocarb 80% WP) for IRS as part of an insecticide resistance management strategy. This because of confirmed pyrethroid resistance and following the WHO guidance of using active ingredients with different modes of action in rotation (MoH, 2013a; WHO, 2012). The main mechanisms by which mosquitoes display resistance to insecticides are the expression of elevated levels of detoxifying enzymes (metabolic resistance) and target site insensitivity (knock-down mutations or altered acetycholinesterase) (Ranson et al., 2000; WHO, 2012). Two point mutations in the voltage-gated sodium channel are associated with knock down resistance (*kdr*) to DDT and pyrethroids in the malaria mosquito *Anopheles gambiae s.s.* (Santolamazza et al., 2008) One

mutation involves a leucine (TTA) to phenylalanine (TTT) substitution at residue 1014 of the gene (L1014F). This mutation is mainly found in West Africa and hence named *kdr*-west(Martinez-Torres et al., 1998). The other mutation involves a leucine (TTA) to serine (TCA) substitution at the same residue (L1014S) and is mostly found in East Africa (*kdr*-east) (Kabula et al., 2012), although both mutations co-occur in some parts of Africa (Pinto et al., 2006).

In 2012, WHO reported that insecticide resistance in malaria vectors had already been found in more than 64 malaria endemic countries worldwide, with the majority reporting resistance to pyrethroids (WHO, 2012). This spread is alarming as it poses serious threats to the efficacy of vector control interventions and the gains made in malaria control over the last ten years. Therefore, it is concerning that most national malaria control programmes (NMCPs) continue to use pyrethroid insecticides for vector control. The situation is also compounded by the extensive use of pyrethroids in agriculture, which poses an additional selection pressure on malaria vectors, for example via insecticide-contaminated ground water that permeates to mosquito larval habitats (Kabula et al., 2012; Hilary Ranson et al., 2009; Yadouléton et al., 2014).

The WHO calls for all countries to develop and implement insecticide resistance management strategies in their malaria control programmes in order to curb the spread of resistance as well as preserve the effectiveness of LLINs (WHO, 2012). Many African countries have now implemented entomological monitoring and susceptibility testing. To inform decisions in the control of malaria in Rwanda, the NMCP has been conducting epidemiological and entomological monitoring of malaria vectors in 12 sentinel sites throughout the country since 2010. Mosquito susceptibility to the WHO recommended classes of insecticides are included annually in this routine survey to monitor resistance to organochlorines, organophosphates, pyrethroids and carbamates (WHO, 1998). Here we report the findings of a three-year survey of susceptibility tests conducted to detect knock-down mutations and mortality rates in female *An. gambiae* s.l. mosquitoes. This is the first report presenting nationwide results on the composition of the *Anopheles gambiae* complex and its susceptibility to insecticides used for malaria control.

Chapter 5

Methods

Study area

The study was carried out in 2011 and 2013 in 12 sentinel sites that are distributed over the four provinces of Rwanda (Northern, Eastern, Southern and Western province) and Kigali City, all having different levels of malaria endemicity. The 12 sentinel sites are: Busoro and Karambi in Southern Province; Rukara, Mimuri, Bukora and Mareba in Eastern Province; Mashesha, Kivumu, Mubuga and Nyamasheke in Western Province; Bungwe in Northern Province and Kicukiro in Kigali City (Figure 1).

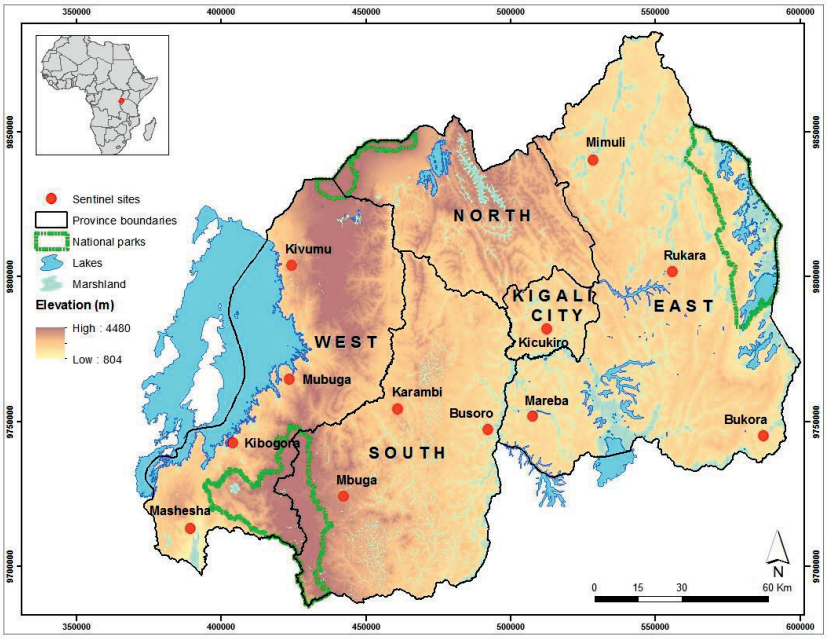

Figure 1: Geographic location of the 12 sentinel sites used for insecticide resistance studies, Rwanda, 2011 and 2013

Mosquito collection

Mosquito larvae were collected in the 12 sentinel sites with standard dippers (350 ml) from stagnant water bodies particularly in rice paddies and other temporal breeding habitats typical for *Anopheles* mosquitoes. All mosquito larvae were collected between 8 and 11am and brought to a malaria entomology laboratory in the field site where they were reared to adults. Emerging adult *Anopheles* mosquitoes were put in holding cages and fed with 10% sugar solution from cotton wool pads. The adult holding room temperature was between 22 and 28°C with a relative

humidity of 70–80%. Approximately 15,000 *An. gambiae* s.l. mosquitoes were reared to the adult stage, of which 13,807 female mosquitoes were tested for insecticide susceptibility according to WHO protocols (WHO, 1998, 2013b). A sample of 10% of these mosquitoes was sent to the International Centre of Insect Physiology and Ecology (*icipe*), Kenya for molecular identification and *kdr* genotyping (Collins et al., 1987; Scott et al., 1993).

Susceptibility tests

Tests were conducted using kits and insecticide-impregnated filter papers supplied from the WHO collaborating center at the University Sains Malaysia (WHO, 1998). A batch of 20-25 non blood-fed female mosquitoes that were between 2-3 days old were exposed for one hour to the standard diagnostic concentrations of deltamethrin (0.05%), permethrin (0.75%), lambda-cyhalothrin (0.05%), DDT (4%), bendiocarb (0.1%) and fenitrothion (1%). Each test was run in four replicates and one control of 25 field-collected *An. gambiae* s.l. per test. The control mosquitoes were exposed to silicone oil impregnated paper for a similar period. The number of knocked down mosquitoes was recorded at 10, 15, 20, 30, 40, 50 and 60 min. (WHO, 1998). After 60 min, tested mosquitoes were transferred into the holding tube and supplied with 10% sugar solution for 24 h after which the final mortality was scored.

In order to explore metabolic resistance, a pre-exposure of mosquitoes for one hour to a synergist (piperonylbutoxide, PBO) was carried out in six sites where resistance to pyrethroids was confirmed. These tests were only carried out in 2013 and conducted alongside the susceptibility testing with the main insecticides used for malaria control, permethrin 0.75% and deltamethrin 0.05%. After one-hour pre-exposure to PBO, the mosquitoes were transferred to the tubes lined with the corresponding insecticide-impregnated paper. The counting of knockdown and mortality of mosquitoes pre-exposed to PBO was conducted as described.

Mosquito identification

All mosquitoes were identified to species based on morphological characteristics (Gillies & Coetzee, 1987) and stored individually over silica gel awaiting molecular identification and detection of *kdr* mutations. From each sentinel site, 10% of female mosquitoes plus all mosquitoes classified as 'resistant' from each site were sent for molecular characterization to the *icipe* laboratory in Kenya where genomic DNA was extracted from the mosquitoes and amplified using specific diagnostic primers for *An. gambiae* (Collins et al., 1987; Scott et al., 1993). Mutations associated with knock-down resistance (*kdr*) genes were assayed (Ranson et

al., 2000) and the DNA products electrophoresed on a 2% agarose gel with ethidium bromide stain and visualized under UV light to identify the presence of susceptible and resistant alleles.

Interpretation of susceptibility test results

In all tests conducted, the observed mortality in the control tubes was less than 5% and therefore Abott's correction was not applied (WHO, 1998, 2013b). Mortality was calculated as the percentage of individuals that died within 24 h of exposure. Mosquito populations were considered 'susceptible' when mortality was between 98-100%. When mortality was below 90%, the mosquitoes were classified as 'resistant'. According to WHO protocol, an intermediate mortality of 90-97% is suggestive of the existence of resistance and indicates that further investigations are needed (WHO, 1998). For the prior exposure of mosquitoes to the synergist, a return to full susceptibility to the insecticide in the WHO tube test compared to the WHO tube test with the insecticide-impregnated paper alone indicates that metabolic resistance plays a role in the insecticide resistance observed.

Statistical analysis

The mortality data were analysed and compared using a Generalized Linear Model with a binomial distribution (IBM SPSS statistics V.20, Chicago, IL, U.S.A.). The output provided estimated marginal means of mortality, 95% confidence intervals, standard errors and p-values based on Chi-square tests.

Results

Morphological and molecular identification

A total of 14,311 mosquitoes tested for insecticide resistance was morphologically identified as *An. gambiae* s.l.. Of these, 1,406 samples collected from 10 sentinel sites were identified by PCR of which 1,165 (82.9%) were *An. arabiensis* Patton and 241 (17.1%) *An. gambiae* Giles *s.s.* (Table 1). Except for Mimuri, *An. arabiensis* was the dominant species in all sentinel sites.

Table 1: Species composition of *Anopheles gambiae* s.l. collected in Rwanda, 2011 and 2013 for insecticide resistance tests

Collection site	Total number tested	An. gambiae s.s.	An. arabiensis
Bukora	200	32 (16%)	168 (84.0%)
Busoro	154	10 (6.5%)	144 (93.5%)
Kicukiro	17	5 (29.4%)	12 (70.6%)
Kivumu	56	3 (5.4%)	53 (94.6%
Mareba	261	16 (6.1)	245 (93.9%)
Mashesha	224	53 (23.7%)	171 (76.3%)
Mimuri	144	89 (61.8%)	55 (38.2%)
Mubuga	183	5 (2.7%)	178 (97.3%)
Nyamasheke	101	25 (24.8%)	76 (75.2%)
Rukara	66	3 (4.5%)	63 (95.5%)
Total	1406	241 (17.1%)	1165 (82.9%)

Mortality rates

In 2011, mortality results showed that out of the 12 sentinel sites, mosquitoes were fully susceptible to lambda-cyhalothrin in all sites except for Mashesha, where resistance was potentially emerging with a mortality rate of 97.6%. Mosquitoes were fully susceptible to permethrin in 33% of the sites, were suggested to be resistant in 33% of sites (i.e. had an intermediate mortality of 90-97%) and were classified as resistant in the remaining 34% of sites. The permethrin resistant populations were from Bukora (84% mortality), Kibogora (89% mortality), Mimuri (86% mortality) and Rukara (84% mortality). For deltamethrin, mosquitoes were fully susceptible in 58% of sites. In 33% of sites, the existence of resistance

was suggested. Resistance to deltamethrin was confirmed for one site: Bukora (85% mortality). For DDT, 28% of sites showed full susceptibility, 36% of sites had a suspicion of resistance and resistance was confirmed in another 36% of sites surveyed: Bukora (77% mortality), Kibogora (75% mortality), Kicukiro (52% mortality) and Mashesha (88% mortality). Mosquitoes were fully susceptible to bendiocarb in 92% of sites. In one site (Kibogora), the mortality of 96% is suggestive of the existence of resistance to bendiocarb. Fenitrothion was the only insecticide to which mosquitoes showed full susceptibility in all the sites tested with 100% mortality after 24 h (Table 2).

Two years later, in 2013, susceptibility to lambda-cyhalothrin was recorded in one site only, while recorded mortalities in 25% of sites were suggestive of the existence of resistance. Resistance was confirmed in 66% of sites. *Anopheles gambiae* s.l. was resistant to permethrin in 25% of sites, showed susceptibility to permethrin in 16% of sites, and mortalities from 58% of the sites were suggestive of the existence of resistance. For deltamethrin, the mosquitoes showed susceptibility in 25%, a suggestion of the existence of resistance in 25%, and full resistance in 50% of sites. The test of resistance to DDT was carried out in 10 sites only, and susceptibility was recorded in 10% of sites, whereas 30% of sites suggested the existence of resistance, and full resistance was observed in 60% of sites. Mosquitoes were fully susceptible to bendiocarb in 83% of sites, and two sites (Bukora and Kivumu) suggested the existence of resistance. Mosquitoes were susceptible to fenitrothion in all sites.

Comparing the mortality rates of 2011 and 2013, there was a significant increase in resistance levels to lambda-cyhalothrin in 83% of the sites, to permethrin in 25% of sites, to deltamethrin in 25% of sites and to DDT in 50% of sites. A significant decrease of susceptibility of *An. gambiae* s.l. to bendiocarb was found in one site (Bukora) and, as in 2011, all mosquitoes tested remained 100% susceptible to fenitrothion in 2013 (Table 2).

Table 2: Mortality rates of female *Anopheles gambiae* s.l tested for insecticide resistance to six insecticides in 2011 and 2013 in 12 sentinel sites in Rwanda

		2011			2013				
Insecticide	Site	N	# Replicates	Mortality (%)	N	# Replicates	Mortality (%)	χ2 value	P-value
Lambdacyhalothrin 0.05%	Bukora	86	4	99±1	100	4	63±5	51.3	<0.001*
	Busoro	82	4	100	200	8	72±3	77.78	<0.001*
	Karambi	84	4	100	99	4	93±3	7.53	0.006*
	Kibogora	81	4	99±1	100	4	85±4	13.29	<0.001*
	Kicukiro	82	4	100	191	8	75±3	64,112	<0.001*
	Kivumu	82	4	100	100	4	100		
	Mareba	88	4	100	100	4	43±5	132.1	<0.001*
	Mashesha	92	4	98±1	94	4	87±3	8.37	0.04*
	Mbuga	81	4	100	97	4	95±2	5.27	0.02*
	Mimuri	85	4	100	100	4	57±5	75.44	<0.001*
	Mubuga	83	4	100	85	4	98±2	2.05	0.15
	Rukara	82	4	100	100	4	78±4	28.02	0.000*
Permethrin 0.75%	Bukora	85	4	84±4	200	8	84±3	0.1	0.92
	Busoro	100	4	100	100	4	95±2	5.26	0.02*
	Karambi	88	4	91±3	99	4	91±3	0	0.98
	Kibogora	84	4	89±3	100	4	92±3	0.367	0.54
	Kicukiro	83	4	99±3	97	4	97±2	0.772	0.38
	Kivumu	80	4	100	100	4	100		
	Mareba	92	4	99	100	4	63±5	53.9	<0.001*
	Mashesha	183	8	96±3	94	4	90±3	3.04	0.08
	Mbuga	87	4	95±3	97	4	100		
	Mimuri	87	4	86±4	100	4	66±5	11.03	<0.001*

		2011			2013				
Insecticide	Site	N	# Replicates	Mortality (%)	N	# Replicates	Mortality (%)	χ2 value	P-value
	Mubuga	90	4	97±2	85	4	94±3	0.72	0.39
	Rukara	86	4	84±5	100	4	92±3	2.95	0.09
Deltamethrin 0.05%	Bukora	168	8	85±3	97	4	74±4	3.85	0.05
	Busoro	84	4	100	200	8	88±2	28.57	<0.001*
	Karambi	82	4	99±1	100	4	97±1	0.72	0.4
	Kibogora	83	4	93±3	97	4	89±3	0.92	0.34
	Kicukiro	86	4	90±3	194	4	82±3	3.1	0.08
	Kivumu	85	4	100	100	4	100		
	Mareba	97	4	99	100	4	67±5	45.1	<0.001*
	Mashesha	99	4	99±2	95	4	97±2	1.22	0.27
	Mbuga	89	4	97±2	86	4	100	2.05	0.15
	Mimuri	86	4	98±2	100	4	81±4	15.42	<0.001*
	Mubuga	90	4	99	90	4	98±2	1.34	0.56
	Rukara	87	4	94.5±2	100	4	90±2	1.37	0.24
DDT 4%	Bukora	86	4	77±5	94	n/a	n/a		
	Busoro	84	4	100	100	4	89±3	12.36	<0.001*
	Karambi	86	4	95±2	95	4	87±3	3.8	0.05*
	Kibogora	84	4	75±2	0	4	n/a		
	Kicukiro	83	4	52±6	93	4	43±5	1.37	0.242
	Kivumu	88	4	95±2	100	4	100	4.2	0.04*
	Mareba	88	4	99	100	4	81±4	19.9	<0.001*
	Mashesha	94	4	88±3	99	4	70±3	14.07	0.001*
	Mbuga	83	4	100	84	4	96±2	3.11	0.08
	Mimuri	85	4	95±2	100	4	76±5	13.22	<0.001*
	Mubuga	90	4	96±2	89	4	96±2	0.000	0.99
	Rukara	n/a	n/a	n/a	100	4	94±2		

		2011			2013				
Insecticide	**Site**	N	# Replicates	Mortality (%)	N	# Replicates	Mortality (%)	$\chi 2$ value	P-value
	Busoro	88	4	100	100	4	100		
	Karambi	84	4	99±1	83	4	98±1	0.202	0.65
	Kibogora	83	4	96±2	97	4	100	3.11	0.08
	Kicukiro	93	4	100	96	4	100		
	Kivumu	82	4	100	100	4	96±2	3.1	0.08
	Mareba	87	4	100	100	4	100		
	Mashesha	93	4	100	100	4	100		
	Mbuga	86	4	99	88	4	100	1.01	0.31
	Mimuri	83	4	100	88	4	100		
	Mubuga	91	4	100	81	4	100		
	Rukara	86	4	99	96	4	100		
Fenitrothion 1%	Bukora	83	4	100	97	4	100		
	Busoro	93	4	100	100	4	100		
	Karambi	82	4	100	94	4	100		
	Kibogora	81	4	n/a	90	4	100		
	Kicukiro	85	4	100	98	4	100		
	Kivumu	84	4	100	100	4	100		
	Mareba	84	4	100	100	4	100		
	Mashesha	183	8	100	100	4	100		
	Mbuga	81	4	100	192	8	100		
	Mimuri	82	4	100	88	4	100		
	Mubuga	82	4	100	88	4	100		
	Rukara	82	4	100	100	4	100		

Kdr genotypes

Seven hundred and twenty-five (134 *An. gambiae s.s.* and 591 *An. arabiensis*) mosquitoes collected in 2011 and 2013 were genotyped for *kdr*-east (L1014S). Of the 134 *An. gambiae s.s*. genotyped, 67 (50%) possessed susceptibility (SS) alleles, while 35 (26.1%) and 32 (23.9%) had heterozygous (RS) and homozygous (RR) alleles, respectively. Of the 591 *An. arabiensis* that were genotyped, 425 (71.9%) possessed homozygous (SS) alleles, while 158 (26.7%) and 8 (1.4%) had heterozygous (RS) and homozygous (RR) alleles, respectively. *Anopheles gambiae s.s.* and *An. arabiensis* from Mimuri (Eastern Province) contributed disproportionately to the total observed frequency of the homozygous resistance genotype (RR), with 36% and 12% for the two species, respectively (Table 3).

Table 3: Variation of *kdr* genotypes (RR, RS and SS) in *An. gambiae s.s.* and *An. arabiensis* collected from seven sentinel sites in 2011 and 2013. RR denotes the homozygous resistant L1014S genotype and SS denotes the homozygous susceptible wild genotype. The *An. gambiae s.s.* and *An. arabiensis* genotypes count for the two years of study were pooled.

Site	Total tested	*An. gambiae s.s.* genotype count (allele frequency)				*An. arabiensis* genotype count (allele frequency)			
		N	SS (%)	RS (%)	RR (%)	N	SS (%)	RS (%)	RR (%)
Busoro	44	4	0 (0.00)	4 (1.00)	0 (0.00)	0	12 (0.30)	28 (0.70)	0 (0.00)
Kicukiro	17	5	0 (0.00)	5 (1.00)	0 (0.00)	12	0 (0.00)	12 (1.00)	0 (0.00)
Kivumu	56	3	3 (1.00)	0 (0.00)	0 (0.00)	53	53 (1.00)	0 (0.00)	0 (0.00)
Mareba	256	17	16 (0.94)	1 (0.06)	0 (0.00)	239	226 (0.96)	13 (0.04)	0 (0.00)
Mashesha	24	12	0 (0.00)	12 (1.00)	0 (0.00)	12	1 (0.08)	11 (0.92)	0 (0.00)
Mimuri	145	88	43 (0.49)	13 (0.15)	32 (0.36)	57	49 (0.86)	1 (0.02)	7 (0.12)
Mubuga	183	5	5 (1.00)	0 (0.00)	0 (0.00)	178	84 (0.47)	93 (0.52)	1 (0.01)
Total	725	134	67 (50.0)	35 (26.1)	32 (23.9)	591	425 (71.9)	158 (26.7)	8 (1.4)

Pre-exposure of *Anopheles gambiae* s.l to piperonyl butoxide (PBO)

The pre-exposure of *Anopheles gambiae s.l.* to the synergist piperonyl butoxide (PBO) was conducted in six sites where resistance to pyrethroids was confirmed. The test was conducted only with permethrin 0.75% and deltamethrin 0.05%. In all sites, susceptibility was restored and ranged from 98% to 100% (Tables 4a and 4b).

Table 4a: Comparison of mortality rates of *Anopheles gambiae* s.l. exposed to permethrin 0.75% alone and permethrin 0.75% + piperonyl butoxide (PBO) per site in 2013.

Sites	Permethrin 0.75%		Permethrin 0.75%+PBO		Chi-Square		
	Total tested	Mortality (%)	Total tested	Mortality (%)	$\chi 2$	df	P
Bukora	200	84±3	100	98±2	9.4	1	0.02
Kibogora	100	92±3	100	99±1	5.9	1	0.015
Kicukiro	97	97±2	100	100	3.1	1	0.08
Mareba	100	63±5	100	100	58.7	1	<0.001
Mimuri	100	66±5	100	98±2	41.9	1	<0.001
Rukara	100	75±5	100	100	32	1	<0.001

Table 4b: Comparison of mortality rates of *Anopheles gambiae* s.l. exposed to deltamethrin 0.05% alone and deltamethrin 0.05% + piperonyl butoxide (PBO) per site in 2013.

Sites	Deltamethrin 0.05%		Deltamethrin 0.05%+PBO		Chi-Square		
	Total tested	Mortality (%)	Total tested	Mortality (%)	$\chi 2$	df	P
Bukora	97	89±3	100	100	12.4	1	<0.001
Kibogora	97	89±3	100	100	12.4	1	<0.001
Kicukiro	194	84±4	98	100	17.8	1	<0.001
Mareba	100	67±5	100	100	49.2	1	<0.001
Mimuri	100	81±4	100	98±2	13.9	1	<0.001
Rukara	100	84±4	100	100	19	1	<0.001

Chapter 5

Discussion

In Rwanda, integrated malaria control interventions (artemisin combination therapy (ACT), LLIN and targeted IRS) have been in use since 2006. This has contributed to a significant reduction in clinical malaria cases in the country (Karema et al., 2012). However, the gains made are fragile due to the decrease of efficacy of interventions, partially as a result of insecticide resistance development that has spread throughout Africa (Ranson & Lissenden, 2016; Ranson et al., 2011; WHO, 2014) . Rwanda achieved universal coverage with LLINs in 2011, but the major challenge is to maintain this coverage and use with effective mosquito nets, especially after we recently reported for Rwanda that LLIN effectiveness lasts less than three years due to the rapid loss of insecticidal activity and physical deterioration in the field (Hakizimana et al., 2014). LLIN deterioration problems were also shown in recent findings in Senegal where damaged nets provided less protection from malaria compared to intact ones (Gnanguenon et al., 2013).

The fact that millions of nets may have lost their effectiveness and thus continue to expose mosquitoes to a sub-lethal dose of pyrethroids is of major concern, because this may contribute to the further development of resistance. Similarly, annual consecutive use of pyrethroids in IRS, combined with extensive use of pyrethroids in agriculture has also implications for emerging insecticide resistance (Thomas et al., 2012). Hence, Rwanda with the support of PMI and the Global Fund has been keen to monitor insecticide resistance to mitigate the emergence of resistance. In 2010, an initial susceptibility survey was conducted in eight sites in which mosquitoes were tested with deltamethrin, permethrin, bendiocarb, malathion and DDT using the CDC bottle assay (Aïzoun et al., 2013; Brogdon & Mc Allister, 1998). These mosquitoes were found to be fully susceptible, except to DDT in two sites (Hakizimana et al., 2018).

The data collected in the current surveys (2011 and 2013) confirm that resistance to DDT has been on the rise since 2011. In addition, these results show resistance to the pyrethroids (deltamethrin, permethrin, and lambda-cyhalothrin) being present in 2011 and further increasing in 2013. Although DDT was banned for usage in Rwanda in 1989, high levels of resistance are of concern because resistance to DDT confers cross-resistance to pyrethroids (WHO, 2012). Mosquitoes were fully susceptible to fenitrothion in all the sites, while the first cases of the emergence of resistance to bendiocarb were found in the present study. The distribution of bendiocarb resistance throughout the country, however, needs to be further

established. The high frequency of *kdr* mutations recorded in some sites can be explained by intense indoor interventions with IRS and LLINs, as was reported in Burundi and Tanzania (Kabula et al., 2014; Protopopoff et al., 2008). Metabolic resistance involving oxidases was proven by using piperonyl butoxide (PBO). Susceptibility was fully restored in the six selected sites where resistance to pyrethroids had been identified. This suggests metabolic resistance also plays a role as mechanism of resistance in malaria vectors of Rwanda.

The resistance level in Mimuri (Nyagatare District) could explain the high number of malaria cases occurring in this district: about 42% of all malaria cases of the 30 districts in 2011 were found here (MoH, 2011) before the introduction of bendiocarb for IRS. This site, as well as Mashesha, Mareba, and Kibogora, is characterised by rice growing in which agricultural pesticides, and pyrethroids in particular, are extensively used.

In response to these findings, Rwanda developed and implemented an insecticide resistance management (IRM) plan in 2013 that recommended transitioning to non-pyrethroid IRS to mitigate pyrethroid resistance (MoH, 2013a). Rwanda transitioned to carbamate (bendiocarb) for IRS and the country will switch to a long-lasting organophosphate (Actellic – pirimiphos-methyl CS) in 2016 due to the reported trends of emerging resistance to bendiocarb. The question remains, however, whether Rwanda should continue with blanket IRS treatment in an entire district when data on resistance is reported from only one sentinel site per district. To answer this question, it is recommended that additional sites should be identified in the targeted districts in order to determine whether or not the spread of resistance is homogeneous throughout the district. Similar to a recent report from Malawi, we conclude that the use of long-lasting IRS formulations, such as pirimiphos-methyl, may be costly initially, but cost-effective in the longer term in managing insecticide resistance (Chanda et al., 2015).

In this study, characterization of *An. gambiae* s.l. from 10 sentinel sites revealed that the predominant sibling species is *An. arabiensis* (83%). This is contrary to a study conducted in one site near Kigali City in 2007 by PMI-Rwanda, in which it was reported that *An. gambiae s.s*. accounted for 93.6% of the total 157 *An. gambiae* s.l. examined by PCR while *An. arabiensis* accounted for only 6.4% (Lansana, 2008). Although the earlier sampling was carried out in one site, the results suggest that *An. gambiae s.s.* was the predominant species before the scale-up of interventions with LLINs and IRS. Such a shift in species composition has been reported in neighbouring countries, for instance in Kenya, Uganda and Tanzania

(Kabbale et al., 2014; Mwangangi et al., 2013; Russell et al., 2011). This phenomenon has important implications for malaria epidemiology and control given that *An. arabiensis* is an opportunistic feeder which has a tendency to rest and feed on humans outdoors (Verhulst & Takken, 2013). Outdoor-biting mosquitoes are less susceptible to indoor interventions and therefore outdoor interventions that supplement LLINs and IRS will need to be instituted in the context of an integrated approach to vector management (Russell et al., 2011; Tchouassi et al., 2012).

With the goal of reaching the pre-elimination phase of malaria by 2018, the Ministry of Health developed an integrated vector management (IVM) strategy which aims at improving the efficiency, effectiveness, ecological soundness and sustainability of vector control interventions in Rwanda (MoH, 2013b). Entomological monitoring, including testing for insecticide resistance, is now a major part of the NMCP in Rwanda and a rotation strategy for management of insecticide resistance has been adopted in line with IVM.

Meanwhile, with spreading insecticide resistance and behavioral change of malaria vectors, there is an interest to integrate other innovative interventions which do not rely on insecticides (Mukabana et al., 2006; WHO, 2004). Larval control interventions have proven cost-effective across a range of different settings and include application of environmental management, insect growth regulators, and biological control (Beales & Gilles, 2002). Recently, successful field experiments have been carried out with microbial larvicides in Tanzania, Kenya, the Gambia and Benin. These showed a substantial impact on malaria disease (Geissbuhler et al., 2009; Majambere, Lindsay, Green, Kandeh, & Fillinger, 2007; Worrall & Fillinger, 2011). It was demonstrated that for a sustainable solution, a horizontally organized community-based programme that takes the needs and wishes of people into account has to be established and technically empowered (Chaki et al., 2014; Chaki et al., 2009). Currently, Rwanda is supporting a research project in Ruhuha (South East Rwanda) that aims to involve communities in the application of the microbial larvicide *Bacillus thuringiensis* var. *israelensis* (Bti) (E.H. Unpublished data). This may form the basis for the integration of alternative vector control interventions for the management of insecticide resistance (Mukabana et al., 2006).

Conclusion

The results of the study show that resistance to pyrethroids and organochlorines (DDT) was documented in 2011 and has been on the rise between 2011 and 2013. An emerging

resistance to carbamates (bendiocarb) was found in few sites and gives reasons for concern of its efficacy in the near future. No resistance was recorded for organophosphates (fenitrothion). Probably due to the use of indoor insecticide-based interventions, the more opportunistic and outdoor feeding *An. arabiensis* was the dominant sibling species in the country. To curb further spread of pyrethroid resistance and to preserve the effectiveness of LLINs, Rwanda implemented an insecticide resistance management strategy in 2013 and switched to carbamates (bendiocarb) for IRS. It plans to implement a rotational strategy of insecticides, including organophosphates, every two to three years. The results reported here are the first results on the status of insecticide resistance in Rwanda and indicate that the NMCP should continue monitoring insecticide resistance for target site insensitivity and metabolic resistance so as to guide future decisions on insecticide use for public health.

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Chapter 6

Community-based control of malaria vectors using *Bacillus thuringiensis* var. israelensis (*Bti*) in Rwanda

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To be submitted

Chapter 6

Abstract

Although current malaria vector control interventions have contributed to a significant decline in malaria in several countries, they have shown limitations to sustain the gains of malaria control. The present study used the biological larvicide *Bacillus thuringiensis* var. israelensis (*Bti*) as a supplement to existing interventions. The trial was implemented by members from Community Malaria Actions Teams (CMATs) and cooperatives of rice farmers. The three study arms consisted of an expert-supervised *Bti* treatment (ES1), a community based *Bti* treatment (CB1 and CB2) and an untreated control. In addition, to rice fields, the trial was extended to water drains of inter-crop land (ES2) and in water reservoirs created by man-made dams (ES2). *Bti* was applied weekly and larval and adult mosquito populations were sampled every two weeks from February to July 2015.

In comparison with the baseline, the application of *Bti* in a rice habitat reduced significantly the density of late instars of anopheline larvae from 2.40 to 0.06 (97.5%) in ES1, from 1.19 to 0.104 (91.2%) in CB1, from 0.585 to 0.105 (82.1%) in CB2 and from 1.19 to 0.69 (48.3%) in control. The proportion of habitat occupancy with pupae declined from 9.4% to 0.7% (92.6%) in ES1, from 59.4% to 4.2% (92.9%) in CB2 and from 48% to 14.4% (70%) in control. The impact was not significant (χ^2=2.219, p=0.136) in CB1, where the habitat occupancy of pupae dropped from 2.8% to 0.9% (67.4%). The decrease in anopheline larval density and pupal occupancy in rice habitats was mainly observed during the first round of monitoring, two weeks following the start of larviciding, while the density of anopheline larvae in the control arm increased.

Besides the rice habitats, in breeding sites made of open water bodies, the reduction of anopheline larval density was 92.8% in ES2 and 100% in ES3. The pupal occupancy was suppressed by 100% in the latter two types of breeding sites following the application of *Bti.* In the adult collections, catches per trap and per night of *Anopheles gambiae* s.l., declined from 19.35 to 3.30 (82.9%) in the expert supervised arm and from 12.85 to 3.09 (75.9%) in the community based area. The adjusted mean density of culicine mosquitoes was not different from the baseline in the expert supervised (mean difference = -0.20, P=0.876) and in the community based arm (mean difference = 5.95, P=0.053), while in the control, the culicines increased significantly with 113% (mean difference = -7.27, p <0.001).

The application of *Bti* led to a reduction of anopheline larval densities of more than 80% and habitat occupancy in rice and open water habitats, both in expert-supervised and community-

based arms. This was accompanied by significant declines in adult anopheline (mainly *Anopheles gambiae* s.l.) densities. The application of *Bti* was most effective in open water bodies and moderate in marshlands with rice cultivation. The effectiveness of *Bti* in rice paddies may be investigated for multiple seasons of rice growing. The rice farmers and leaders of community-based organizations are the most suitable groups for these activities and should be empowered with minor resources in knowledge on bio-ecology and larval source management and then integrate larviciding for mosquito control into their farming activities.

Keywords: malaria, mosquitoes, larval control, *Bacillus thuringiensis* var. israelensis, community engagement, Rwanda.

Introduction

The impact of long-lasting insecticide treated nets (LLIN) and indoor residual spraying (IRS) in the global fight against malaria, encouraged several malaria-endemic countries, including Rwanda, to set up the ambitious goal of malaria elimination (Bhattarai et al., 2007; MoH, 2014; WHO, 2016b). At the same time, the occurrence of residual malaria transmission and local immigration of infected people, represent challenges to the ultimate goal of malaria elimination in highly endemic regions. In other words, elimination may not be feasible without additional, innovative interventions (Gu et al., 2003; Killeen, 2014).

Residual malaria transmission can result from changes in the behaviour of malaria vectors. Several studies demonstrated that mosquitoes have developed the ability to avoid contact with insecticide-treated surfaces (Killeen, 2014; WHO, 2012) . Other examples of behavioural change include the earlier biting when people are not protected by bed nets, and trends of outdoor biting and resting of malaria vectors that were formerly active inside houses (Russell et al., 2011; Sougoufara et al., 2014; Yohannes & Boelle, 2012). Other malaria vector species have developed the habit to bite domestic animals and in this way escape the risk of contact with insecticide (Killeen & Smith, 2007; Mutero et al., 999). Residual transmission can also result from a change in vector species composition, whereby secondary vectors acquire high transmission capacity and replace the primary vectors, mainly due to the ecological and climatic changes (Durnez et al., 2013; Giglioli, 1963; Govella et al., 2013). The scaling up of insecticide-based vector control interventions has contributed to the selection of resistant mosquito strains, which are not killed by the standard dose of the different types of insecticides (Kabula et al., 2013; WHO, 2012). This insecticide resistance has been spreading rapidly and is therefore alarming (Ranson & Lissenden, 2016). The resistance of malaria parasites to anti-malarial drugs is another hindrance to malaria control which continuously requires adaptation and a search for new, cost-effective measures (Butcher, 1997; RBM, 2008).

To tackle the challenge of insecticide resistance, alternative interventions are required that can be implemented in Integrated Vector Management (IVM) programmes as part of malaria control (Gu et al., 2008; Russell et al., 2013; Takken & Knols, 2009). Larval control interventions have proven effective across a range of different settings and include environmental management, application of insect growth regulators, as well as chemical and biological control (Bayoh et al., 2010; Fillinger et al., 2009; Killeen et al., 2002; Tusting et al., 2013). Encouraging field trials using the biological larvicide *Bacillus thuringiensis* var.

israelensis (*Bti*) have been carried out in Tanzania, Kenya, the Gambia and Benin. These showed a substantial impact on key parameters of malaria transmission, such as a reduction of the occupancy rates of aquatic habitats, the density of larvae and pupae inside aquatic habitats, the risk of human biting by mosquitoes, the entomological inoculation rate and the prevalence or incidence of malaria (Fillinger & Lindsay, 2006; Fillinger et al., 2009; Geissbühler et al., 2009; Kinde-Gazard & Baglo, 2012).

A key question that remains is whether such approaches can be implemented by local communities themselves, rather than through a vertically organized malaria control program. The present study was therefore implemented in the framework of the Rwanda IVM strategy. Its specific aim was to evaluate the impact of community-based application of *Bacillus thuringiensis* var. israelensis in a rice irrigation scheme. The current study reports on the entomological impact of the intervention. At the same time, the community acceptance, participation and economic implications were investigated, and these are reported elsewhere (Ingabire et al., 2017).

Materials and methods

Study site

The study was carried out in Ruhuha, one of the fifteen sectors of Bugesera district in south-eastern Rwanda (Figure 1) and located 42 km south from the capital Kigali. The sector is divided into five administrative cells and 35 villages. The elevation ranges from 1,300 m to 1,573 m above sea level. It covers an area of 54 km^2 with an estimated population of 23,893 persons and 5,098 households (Ingabire et al., 2015).

The sector counts one health centre with a network of 140 community health workers (CHWs) and 105 members of community malaria actions teams (CMATs) (Ingabire et al., 2014), who support implementation of health activities in the community. The area is drained by five marshlands and rice is grown in four irrigated rice fields covering an estimated area of 93 ha: Kibaza (27 ha), Gatare (25 ha), Nyaburiba (33 ha) and Kizanye (8 ha). The remaining marshland of Nyagafunzo (8 ha) is used to grow subsistence crops. The major malaria prevention strategies implemented are Long Lasting Insecticide treated Nets (LLINs) and two annual rounds of indoor residual spraying (IRS) using non- pyrethroid insecticides.

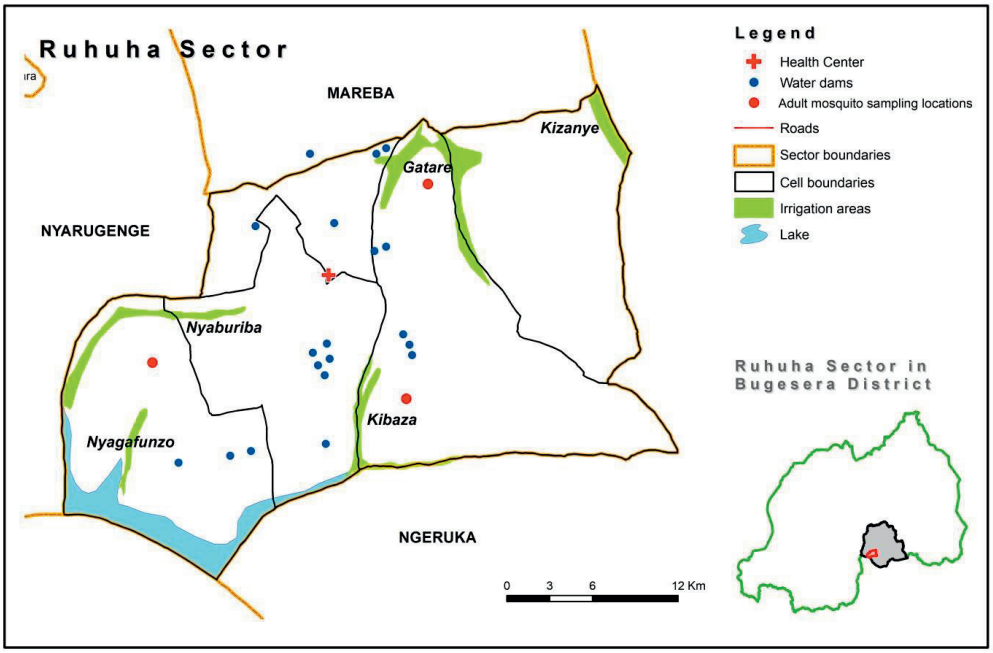

Figure 1: Location of the experimental sites in Ruhuha, South-East Rwanda. Nyaburiba: rice field control, Gatare: rice field-1for community based application of *Bti* (CB1), Kizanye: rice field-2 for community-based application of *Bti* (CB2), Kibaza: rice field-1 for expert supervised application of *Bti* (ES1), Nyagafunzo: crop land-2 for expert supervised application of *Bti* (ES2). Blue dots represent the 19 water hill dams-3 at which expert supervised application of *Bti* (ES3) was also carried out.

Treatment arms for Larval Source Management using *Bti*

According to WHO guidelines, our intervention study is classified as a 'non-randomized trial with control (WHO, 2017) and included one control and two Larval Source Management (LSM) arms. Baseline information on potential mosquito breeding sites was collected in the study area, as well as on stakeholder pre-engagement and socio-economic status (Ingabire et al., 2016). All permanent or semi-permanent water bodies were mapped prior to the intervention (Figure 1). These included the four marshlands used for irrigated rice growing (Nyaburiba, Kibaza, Gatare and Kizanye), one marshland used by the community for growing seasonal subsistence crops (Nyagafunzo), and 19 so-called hill dams for harvesting rain water (each approximately 200 m^2 in size). Nyaburiba (35 ha) was selected as the control arm, without any application of *Bti*. For the 'expert supervised' arm (hereafter referred to as 'ES'), Kibaza (27 ha; entitled 'ES1'), Nyagafunzo (8 ha; 'ES2') and all 19 water hill dams

('ES3') were selected. For the third arm, 'community-based' application of *Bti* (hereafter referred to as 'CB'), Gatare (25 ha, 'CB1') and Kizanye (8 ha; 'CB2') marshlands were selected. The minimum distance between intervention arms was estimated at five km.

One month before the *Bti* application, a baseline survey on the perception, engagement and willingness to pay for LSM was carried out by a team consisting of a sociologist and an economist (Ingabire et al., 2016). At the same time, the entomological sampling points were identified, numbered and mapped. This activity was followed by baseline entomological surveys on larval and adult stages of mosquitoes and led by trained entomology technicians in collaboration with community members.

The selection of implementers for *Bti* application

The 39 community members deployed for application of *Bti* were selected in collaboration with the four local cooperatives of rice farmers and CMATs. Of them, 11 (28%) were CMAT members and 28 (72%) were rice farmers. Initially, the heads of rice farmer cooperatives and representatives of CMATs participated in the process to define the selection criteria for sprayers as well as entomology monitoring surveyors. Two criteria were mutually agreed upon: (1) sprayers of *Bti* should be members of CMATs and/or be a rice farmer, as well as inhabit one of the villages neighboring the areas targeted for treatment with *Bti* and (2) surveyors for monitoring of larval and adult mosquitoes should be selected from the members of CMATs.

For the CB intervention arm, a team of 20 sprayers was selected by the heads of cooperatives of rice farmers and approved by the research team. Another team of 19 sprayers for the ES intervention was recruited by the research team through a semi-structured interview. In addition to the sprayers, two independent teams of community members were deployed for larval and for adult mosquito monitoring of the intervention, respectively. These were separately recruited by the research team among the members of CMATs.

Organizational structures

The major distinction between the two intervention arms (ES and CB) was based on the organization and degree of supervision, the logistical management of *Bti* and the frequency of reporting (Table 1). For ES, the supervision of sprayers and the logistical management was carried out by members of the research team (author EH). The reporting was done daily to the office of the research team established at the Ruhuha health center, located centrally in the

study area. For the CB arm, the application of *Bti* was entirely the responsibility of the cooperative of rice farmers. The head of the cooperatives ensured supervision of the spraying with an overall management of logistics (distribution of spray pumps, the *Bti* product etc.) and reported weekly to the research team. The research team did not interfere in this arm, and deliberately sought to find out potential hurdles (logistical, financial and social) for community-based LSM (Ingabire et al., 2016).

For the CB arm, the *Bti*, spray pumps, personal protective equipment and reporting forms were stored free of charge at the office of the rice cooperative, while all *Bti* spraying commodities and reporting material used by the ES arm, were stored and managed at the Ruhuha health center.

Spraying took place from 7:00 am until 1:00 pm in the ES arm. Working times for coordinators of the CB team ranged from 7:00 am to 2:00 pm. The monitoring of larval and adult mosquitoes was conducted by independent teams selected from CMAT members and supervised by a trained technician for each marshland that was part of the intervention.

Training and calibration of equipment

A five days training of sprayers for application of *Bti* was supported by Valent Biosciences Corporation (Valent Biosciences, Libertyville IL, USA). It covered the techniques of application of *Bti*, including the calibration of sprayer pump nozzles, the spraying speed, flow and application rates, as well as the reporting procedures and schedule. A simulation of spraying using water was conducted in the field after the theoretical training sessions.

The application was calibrated at a spraying speed of 50 m per min, a swath width of eight m, a flow rate of 1.2 liters per min and an application rate of 30 l of *Bti* dilution per hectare (ha). As the application dosage recommended by the manufacturer was 300 g of *Bti* per ha (0.3 kg/ha), this quantity was obtained by mixing three times 100 g of the granules in 10 l of water in the sprayer tank.

Table 1: Main characteristics of the experimental sites, organizational structures of *Bti* application and entomology monitoring of mosquito larval and adult stages in the study site; n.a.: not applicable, CMAT: Community Malaria Action Team, ET: Entomology Technicians

	Control (C) arm	Expert supervised (ES) arm	Community-based (CB) arm
Characteristics			
Name of site (type of crop, size)	Nyaburiba (rice, 33 ha)	ES1 - Kibaza (rice, 27 ha) ES2 - Nyagafunzo (cropland, 8 ha) ES3 - Water dams (n/a, 0.28 ha)	CB1 – Gatare (rice, 25 ha) CB2 - Kizanye (rice, 8 ha)
Total size	33 ha	35.4 ha	33 ha
***Bti* application**			
Organization of Bti sprayer teams	n.a.	4 teams of 3 sprayers 4 team leaders 2 porters 1 coordinator 1 project supervisor (not from community)	4 teams of 3 sprayers 4 team leaders 2 porters 2 coordinators
Logistic management	n.a.	Project research team	2 cooperatives of rice farmers
Reporting of Bti application	n.a.	Daily to research team	Weekly to research team
Monitoring			
Larval stages	3 members of CMATs 1 ET	6 members of CMATs 2 ET	6 members of CMATs 2 ET
Adult mosquitoes	2 members of CMATs 1 ET	2 members of CMATs 1 ET	2 members of CMATs 1 ET

Bacillus thuringiensis var. *israelensis* as biological larvicide

The larvicide applied for LSM was *Bacillus thuringiensis* var. *israelensis*, strain AM 65-52 (*Bti*), commercially traded under the name of VectoBac® Water-Dispersible Granules (WDG), 3000 International Toxic Units (ITU) per mg. The product was supplied by Valent Biosciences Corporation. The active ingredient of the product is based on a mixture of free endotoxin protein crystals produced by *Bti* strain AM65-52 and the spores and cells bearing them (WHO, 2016a).

Entomological surveys

Baseline surveys for larval and adult stages of mosquitoes were carried out two consecutive days/nights one month before the application of *Bti*, as well as every two weeks for six consecutive months, from February to July 2015, covering one cycle of rice cultivation. The individual farming plots of 20 to 20 meters for larval sampling per marshland were marked using a Global Position System (GPS) device at every 100 meters and following three transects respectively along the central irrigation channel and the two edges of rice fields (Figure 2). The geo-referenced plots were then numbered, coded, digitized and mapped using Geographic Information System software. The owner's name of each sampling plot was also recorded for easier identification by the local surveyors. At each intervention site, the representative of rice farmers, who later participated in larval monitoring contributed to the geo-coding of sampling plots and identification of the respective owners.

The aquatic stages were collected with standard dippers (350 ml) with 10 dips per surveyed plot of 20 x 20 m (WHO, 1992). Throughout the surveys, early instars (L1+L2), late instars (L3+L4) and pupae were separately recorded on a sampling form. At each round and one to two days post *Bti* application, the larval survey was conducted for two consecutive days. The density of late instars *Anopheles* larvae and habitat occupancy for *Anopheles* larvae and pupae were calculated.

Adult mosquitoes were sampled every two weeks using miniature CDC light traps (Model 512; John W. Hock Company, Gainesville, FL) suspended one meter above the floor and at the foot end of the bed where an occupant was sleeping (Mboera, Kihonda, Braks, & Knols, 1998). In one village bordering the control and each *Bti* intervention arm, 10 houses were purposively selected for adult collection. The traps were set up at 6:00 pm and retrieved the next morning at 6:00 am by the trained members of CMATs. At the laboratory, mosquitoes were first identified to species level using standard morphological identification keys (Gillies & Coetzee, 1987). The sibling species were identified using a polymerase chain reaction (PCR) assay (Scott, Brogdon, & Collins, 1993) from a sample of the total *An. gambiae* s.l. collected.

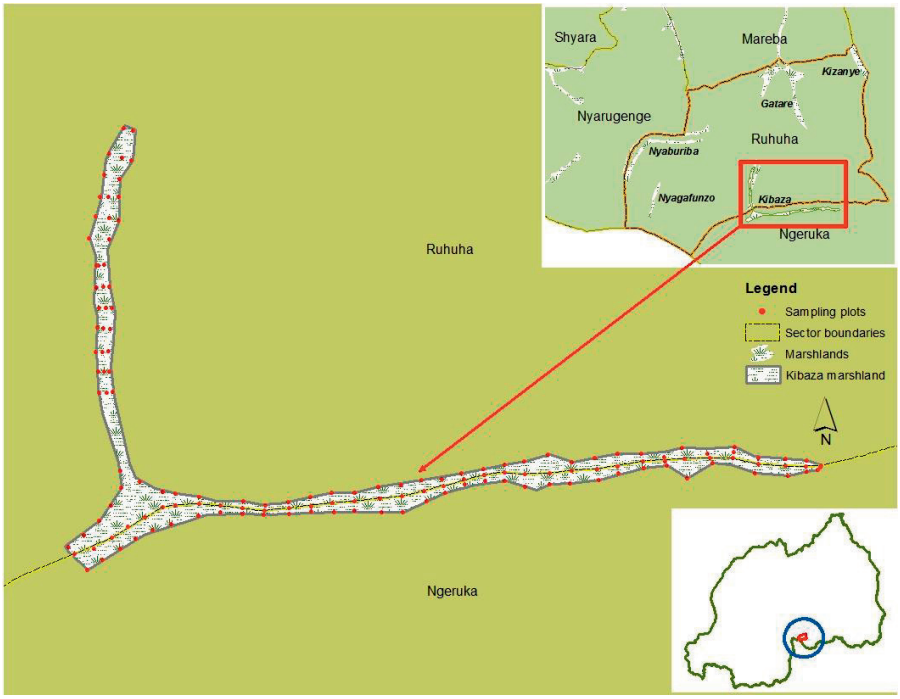

Figure 2: Example of sampling plots for mosquito larval stages in the Kibaza rice field-1 (ES1), Ruhuha, Rwanda**.**

Ethical considerations

The study was presented to the National Health Research Committee (NHRC) and received approval number 390/RNEC/2012 from the Rwanda National Ethic Committee (RNEC). The importation and usage of *Bti* was authorized by the Ministry of Health (20/6375/DGCS/PH/2014). Before application of *Bti* and larval monitoring, verbal consent was obtained from local leaders, the head of the rice farmer cooperative, as well as from the owner of hill dams. Informed consent was also acquired from house owners selected for collection of adult mosquitoes.

Data analysis

To evaluate the impact of the intervention on larval abundance in the rice fields and open water breeding habitats, the mean number of late instar anopheline and culicine larvae collected per dip were compared using non-parametric Mann-Whitney-U tests. The proportion of habitat occupancies by anopheline and culicine larvae as well as pupae was analyzed using the chi-squares tests. The P-values were adjusted using the Bonferoni method. The analysis of the effect of fixed variables, respectively the type of intervention (baseline *versus* treatment), location (villages) and covariates (number of sleepers, number of LLINs) on catch size of mosquitoes fitted a Generalized Linear Model (GLM) with a negative bionomial distribution, log link function. The fixed and covariate variables with significant effect were included into the model for calculation and adjustment of the mean number of mosquito catches per dependent variable. The comparison of the mean number of catch sizes between the baseline *versus* the *Bti* intervention was performed using post-hoc test pairwise comparison of the estimated marginal means for each taxon (anopheline and culicine) of mosquito trapped. The statistical analysis was conducted using SPSS software (IBM SPSS statistics Version 20, Chicago, IL, U.S.A).

Results

Density of larval stages of mosquitoes

The density of mosquito larvae, expressed as the number of *Anopheles* larvae per dip, was high in all three experimental arms during the baseline and the first round following the application of *Bti* in all intervention areas, except for ES2 where the density in water drains of inter-crop lands was low from the start (Figure 3-E). Thereafter, the number of anopheline larvae decreased drastically in ES (Figure 3-D, E and F), and more progressively so in CB (Figure 3-B and C). A rebound in larval numbers occurred in the last two entomology monitoring rounds, particularly in the non-intervened control arm (R8 and R9, Figure 3-A).

In the control arm, both the mean density of anopheline and culicine larvae per dip significantly reduced by 48.3% and 28.2%, respectively, from 1.19 to 0.615 and from 1.03 to 0.74 larvae per dip (Table 2). In the ES1 arm, the mean density of anopheline larvae declined by 97.5%, from 2.41 at baseline to 0.06 larvae per dip during the following nine rounds of larval surveys. For the culicines, the reduction was 97.6% (1.68 at baseline to 0.04 larvae per dip). In the ES2 arm, the reduction was 94.6% for anophelines, and culicine larvae were absent from the baseline to the end of the nine survey rounds. The density of all mosquito

larvae was reduced to zero in the ES3 arm after repeated *Bti* application. In the CB1 rice arm, anopheline and culicine larval density was reduced by 91.2% (from 1.19 to 0.104 larvae per dip) and by 98.3% (from 0.463 to 0.008 larvae per dip). In the CB2 rice arm, the decline was 82.1% and 62.6 % for anopheles and culicines, respectively (Table 2).

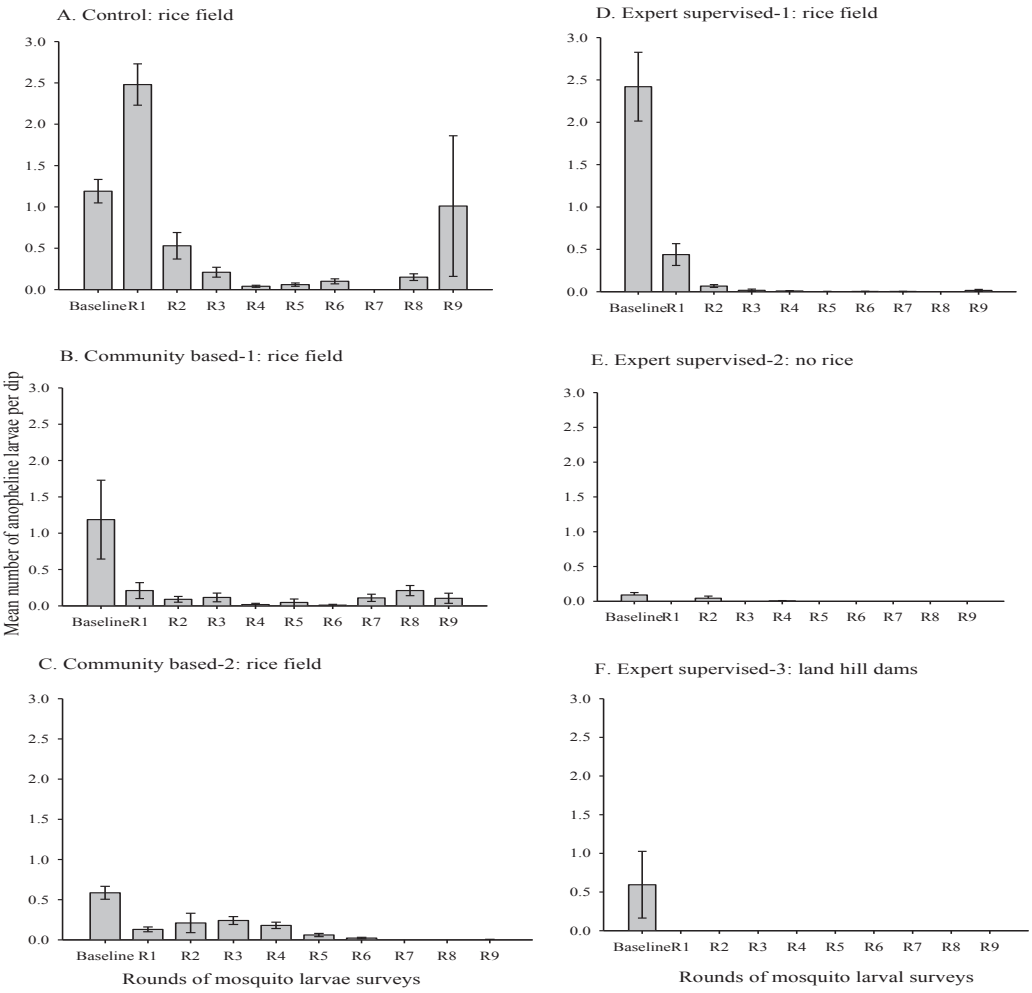

Figure 3: Mean densities of anopheline mosquito larvae from the baseline period and per round during the period of Bti application from February to July 2015 in Ruhuha, Rwanda

Table 2: Mean density ± S.E. of anopheline and culicine late instar larvae per dip collected during the baseline period (one month before) and the *Bti* application from February to July 2015. For "n", the figures indicate the total number of plots surveyed per study arm respectively during the baseline and intervention.

Intervention	Mosquito larvae	Baseline (pre-intervention)	Post-intervention	Reduction (%)	MW-U value	MW-U, Z-value	P-value
Control	anophelines	1.19 ± 0.14	0.62 ± 0.120	48.3	42,203	-4.30	<0.001
(n= 123/857)	culicines	1.03 ± 0.11	0.74 ± 0.009	28.2	36,692	-5.85	<0.001
Expert supervised-1	anophelines	2.40 ± 0.41	0.06 ± 0.015	97.5	44,727	-19.18	<0.001
(n= 148/1332)	culicines	1.68 ± 0.31	0.04 ± 0.013	97.6	31,304	-18.51	<0.001
Expert supervised-2	anophelines	0.09 ± 0.03	0.01 ± 0.003	94.6	504	-7.05	<0.001
(n=15/135)	culicines	0.00	0.00				
Expert supervised-3	anophelines	0.59 ± 0.43	0.00	100.0	1,197	-6.78	<0.001
(n = 19/171)	culicines	0.02 ± 0.01	0.00	100.0	1,453	- 4.35	<0.001
Community based-1	anophelines	1.19 ± 0.54	0.10 ± 0.02	91.2	215,251	-4.65	<0.001
(n= 67/601)	culicines	0.46 ± 0.28	0.01 ± 0.004	98.3	17,360	-6.79	<0.001
Community based-2	anophelines	0.59 ± 0.08	0.11 ± 0.01	82.1	7,421	-7.80	<0.001
(n= 62/483)	culicines	0.42 ± 0.06	0.16 ± 0.02	62.6	9,314	-5.59	<0.001

Habitat occupancy by aquatic mosquito stages

In the control arm, the mean proportion of habitats with presence of mosquito pupae dropped significantly with 70%, from 48% at the baseline survey carried out one month before the *Bti* application to 14.4% in the remaining survey rounds. Furthermore, the habitats surveyed and positive for anopheline and culicine larvae had also significantly reduced with 61.1% (73.2% to 28.5%) and 54.2% (76.4.9% to 35.0%), respectively (Table 3).

Total control (100%) of pupal stages (ES2) and larvae (ES3) was observed in the expert supervised arm. A strong reduction in pupae of 92.6% (from 9.4% to 0.7%) was shown in ES1. A similar reduction of 82.7% (64.3% to 11.1%) and 92.2 % (48.6% to 3.8%) was seen in ES1 in the occupancy of anopheline and culicine larvae, respectively. In ES2 the occupancy of anopheline larvae was reduced by 92.8% (from 93.3% to 6.7%) and culicine larvae controlled from the baseline to end of the *Bti* application.

For the community-based arm, the reduction in pupal occupancy rates was more pronounced in CB2 (92.9%) than in CB1 (67.9%). The decline was again more pronounced for culicine larvae in CB1(92.0%) but less for anopheline larvae in both community arms (58.7% in CB1 and 59.3 % in CB2). The occupancy rate with culicine larvae was only reduced by 54.7% in CB2 (Table 3). It should be noted that the seemingly lower impact of CB than ES on pupal occupancy rates is strongly driven by pupae that were still collected in round 1 and round 2 of CB (Figure 4-B and 4-C). Thereafter, no more pupae were observed in CB, except for round 8 in CB1.

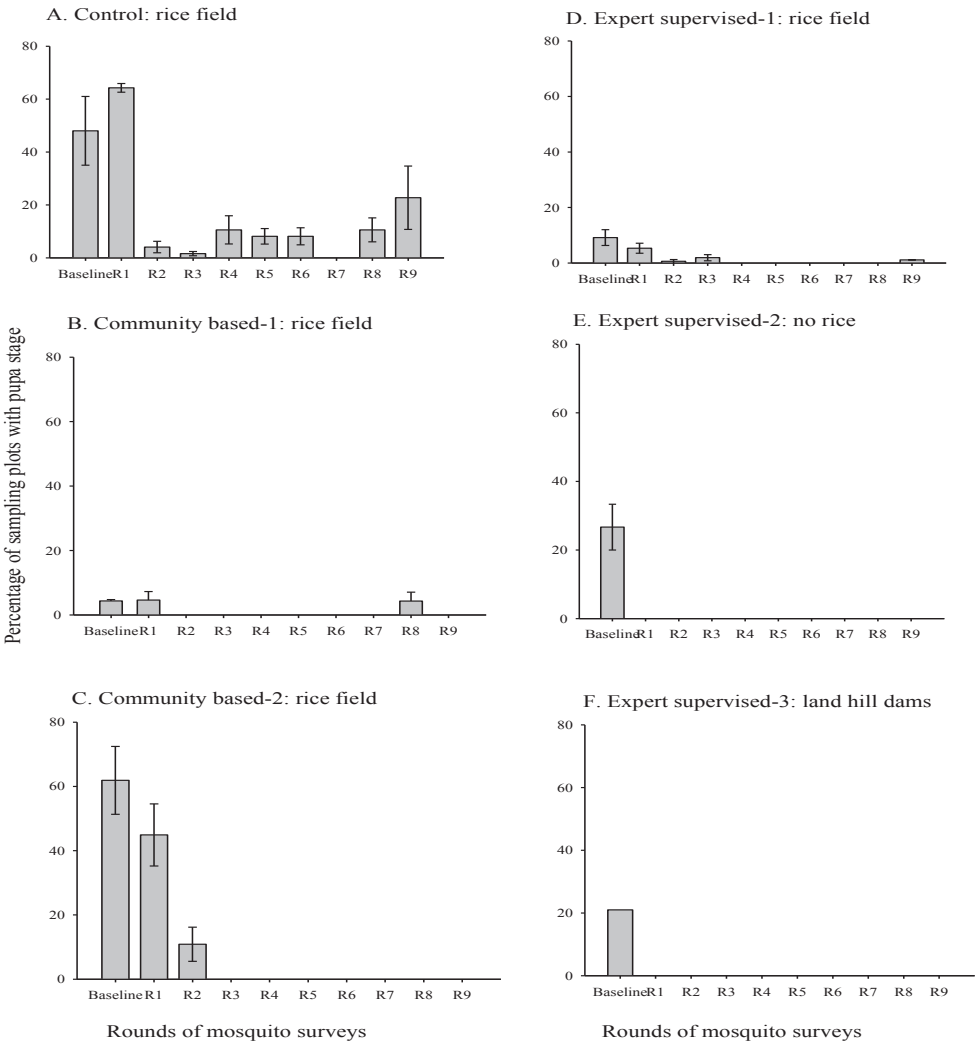

Figure 4: Habitat occupancy by the pupal stage of mosquitoes from the baseline period and per round along the period of *Bti* application from February to July 2015 in Ruhuha, Rwanda.

Table 3: Mean proportion of habitat occupancy of aquatic mosquito life stages (pupae and late instar: L3 and L4 larvae) during the baseline period (one month before) and after the *Bti* application from February to July 2015 in Ruhuha, Rwanda

Intervention	Mosquito life stage	Baseline (pre-intervention)	Post-intervention	Reduction (%)	Chi-square value (df=1)	P-value
Control (n= 123/857)	Pupae	48	14.4	70.0	85.73	<0.001
	anopheline larvae	73.2	28.5	61.1	99.69	<0.001
	culicine larvae	76.4	35.0	54.2	79.92	<0.001
Expert supervised-1 (n=148/1332)	Pupae	9.4	0.7	92.6	79.13	<0.001
	anopheline larvae	64.3	11.1	82.7	332.18	<0.001
	culicine larvae	48.6	3.8	92.2	3.55	<0.001
Expert supervised-2 (n=15/135)	Pupae	26.7	0.0	100.0	36.99	<0.001
	anopheline larvae	93.3	6.7	92.8	78.99	<0.001
	culicine larvae	0.0	0.0			
Expert supervised-3 (n = 19/171)	Pupae	0.0	0.0			
	anopheline larvae	31.6	0.0	100.0	55.76	<0.001
	culicine larvae	5.3	0.0	100.0	9.05	<0.001
Community based-1 (n= 67/601)	Pupae	2.8	0.9	67.9	2.22	0.136
	anopheline larvae	36.6	15.1	58.7	20.98	<0.001
	culicine larvae	14.1	1.1	92.0	46.88	<0.001
Community based-2 (n= 62/483)	Pupae	59.4	4.2	92.9	219.61	<0.001
	anopheline larvae	75.4	30.7	59.3	54.65	<0.001
	culicine larvae	58.0	26.3	54.7	29.99	<0.001

Adult mosquito density in houses neighbouring rice fields

In total, 9,937 adult mosquitoes were collected inside houses with CDC light traps, of which 48.2% were anophelines and 51.8% were culicines. *Anopheles gambiae* s.l. was the main malaria vector caught with 96.05% (n=4,784) of the total number of anophelines. Other anopheline species collected were *An. maculipalpis* (2.12%), *An. ziemanni* (1.59%), *An. funestus* (0.13%) and *An. coustani* (0.08 %). Further molecular analysis showed that *An. gambiae* s.s. and *An. arabiensis* occurred at proportions of 28.8% and 71.2%, respectively (n=445).

Comparing the mosquitoes caught at the baseline and during *Bti* intervention using generalized linear model (GLM), numbers of *Anopheles gambiae* s.l significantly reduced by 66.8% in the control arm, from 38.30 to 11.73 mosquitoes per trap per night (mean difference = 26.62, $p = 0.003$). In contrast, the number of culicine mosquitoes collected increased significantly by 113% (mean difference = -7.27, $p < 0.001$) (Table 4 and Figure 5-D).

The populations of *An. gambiae* s.l. declined more significantly in expert supervised and community based application arms than in the control arm, and the adjusted means dropped from 19.3 to 3.3 and from 12.8 to 3.1 mosquitoes per trap per night, respectively (mean difference = 16.05, $p < 0.001$; mean difference = 9.76, $p = 0.001$). Also, the mean number of culicines collected did not significantly increase in expert supervised or decline in community based interventions (mean difference = -0.20, $p = 0.876$; mean difference = 5.95, $p = 0.053$) (Table 4). The adjusted mean number of *An. gambiae* s.l. caught during the *Bti* application did not differ between expert supervised and community based *Bti* application (mean difference = 0.397, $p = 0.603$).

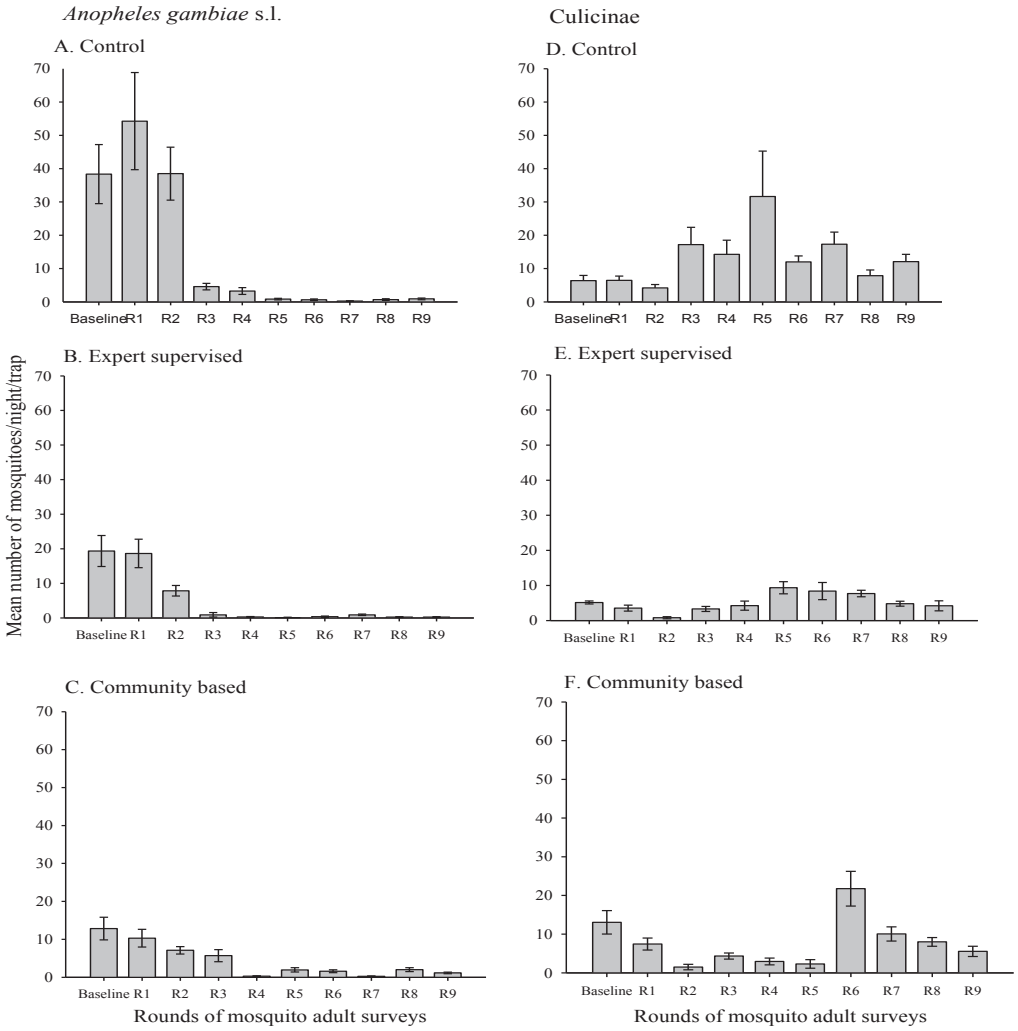

Figure 5: Adult mosquito density during the entomological surveys conducted during the baseline, and per round throughout the period of *Bti* application, from February to July 2015 in Ruhuha, Rwanda.

Table 4: Adjusted mean number ± S.E. of adult mosquitoes collected in three villages during the entomological surveys conducted during the baseline period (one month before) and during the *Bti* application, from February to July 2015, in Ruhuha. The differences were analyzed using the Generalized Linear Model (GLM), Post Hoc analysis with pairwise comparison of the mean differences at 95% of confidence interval. The data fit the negative bionomial distribution with log link.

Study sites	Species composition	Baseline (n= 20 trap nights)		Intervention (n= 180 trap nights)		Reduction in percentage	Mean differences	P. Value
		Adjusted mean number	CI:95%	Adjusted mean number	CI:95%			
Control	*An. gambiae* s.l.	38.30 ± 8.86	24.38-60.31	11.73±0.93	10.04-13.70	66.8	26.62	0.003*
	Tot. culicinae	6.40 ± 1.54	3.99-10.25	13.67±1.05	11.75-15.90	-113.6	-7.27	<0.001*
Expert supervised	*An. gambiae* s.l.	19.35 ± 4.46	12.32-30.40	3.30±0.29	2.78-3.91	82.9	16.05	<0.001*
	Tot. culicinae	4.95 ± 1.21	3.06-8.00	5.15 ± 0.42	4.39-6.04	-4.0	-0.20	0.876
Community based	*An. gambiae* s.l.	12.85±2.98	8.15-20.26	3.09+0.27	2.61-3.67	75.9	9.76	0.001*
	Tot. culicinae	13.05±3.03	8.28-20.56	7.10+0.56	6.07-8.30	45.6	5.95	<0.053

The effects of random factors and covariates on adult mosquito density

The GLM test on the effect of villages and types of intervention as random factors alone or their interaction showed an important effect on the catch size of all collected mosquitoes for the total *An. gambiae* s.l. (χ^2 = 267.504, df=5, p < 0.001) and for culicines (χ^2 = 88.726, df=5, p < 0.001). The number of bed nets found in sampling houses had a small, but significant effect only on *An. gambiae* s.l. (χ^2 = 8.161, df=1, p= 0.004). The number of sleepers did not have an effect on mosquitoes trapped (χ^2 = 0.501, df=1, p= 0.702). The calculation of mean number of each independent variable was based on the interaction between village and intervention variables and then the means number were adjusted to the effect of LLINs only for the catches of *An. gambiae* s.l.

Discussion

Earlier studies have shown that larval control can supplement existing vector control tools, especially because it targets the immature stages of mosquitoes in their breeding sites and it can be readily applied with simple technology (Majambere et al., 2010). The current study explicitly aimed to assess the impact of *Bti* applied through an intervention programme that was led and executed by the (rice farming) community in comparison with an intervention programme that was coordinated and supervised by experts. The intervention trial with *Bti* covered one cycle of rice farming for a period of six months. It was extended to other important mosquito breeding habitats of economic importance, such as water drains in inter-crop lands and hill water dams for rain water management. These were perceived by the local community as inadequate for habitat modification measures usually implemented by the local community (Ingabire et al., 2014).

The impact of *Bti* was similar in declining of anopheline and culicine larval density for more than 90% in both an expert supervised rice field and a community based rice field (CB1). The decline also was more than 80% for anopheline larvae and 62.6% for culicine larvae in another rice field-2 (CB2) treated by community-based teams. In other non-rice habitats treated by the expert supervised teams, larval stages were completely eliminated in dams during the intervention and a reduction of more than 90% was observed in inter-crop land mosquito breeding habitats.

Moreover, the impact on habitat occupancy with aquatic stages (pupae, anopheline and culicine larvae), notably pupae, was also highly significant in rice fields treated by the expert

supervised team and the community based application (CB2). The impact on habitat occupancy with anopheline and culicine larvae was less in all rice fields with community-based application, except for CB1, where the culicine larvae were reduced with 92.%. The density of larval stages was remarkably high during the baseline period, just before the transplantation of rice seedlings and the first round of *Bti* application. Thereafter, a notable decrease of larval stages was mainly observed during the first two weeks following the start of larviciding in intervention areas while the larval densities increased strongly in the control arm. During the following surveys, mosquito larval density incrementally decreased in expert-supervised and community-based interventions with rice farming but also in the control area. A slight recovery was observed in the control arm closer to the harvest period. Densities of adult anophelines, mostly consisting of *An. gambiae* s.l.,, declined significantly in all study arms, including the control. The decline was, however, more significant in treatment arms than in the control arm, demonstrating that the *Bti* treatment had an impact on densities of host-seeking stages of the malaria vectors. This in contrast to the effects on adult stages of culicine mosquitoes, which were not affected or even increased during the study.

Mosquito population dynamics as observed in our study seem typical for populations from irrigated rice fields, e.g. in the inner delta of the Niger River in Mali (Klinkenberg et al., 2003). Here, the development of malaria vectors mostly took place in the first six weeks after transplantation of rice, then decreased as the plant height increased, and malaria vectors were almost absent close to the harvest of the rice. A re-establishment of *An. gambiae* s.l. occurred in small pools due to improper drainage during and after harvesting of the rice. A similar pattern was observed in the Mwea rice scheme in Kenya by Mutero and colleagues, where the highest larval density was recorded in the three weeks post transplantation of rice seedlings and then the larval number dropped dramatically with development of rice until the harvesting period (Mutero et al., 2000).

The impact of *Bti* on the aquatic stages of mosquitoes observed in the present study corresponds with results from recent studies conducted in urban and rural areas in Tanzania and Kenya. Larviciding resulted in a reduction of anopheline larvae of 96% in urban Dar es Salaam, Tanzania, after one year of intervention, although it had a moderate impact on malaria transmission. A higher impact was obtained during the second year of intervention in comparison with the first year. The authors reported that a lower impact was obtained during the two main rainy seasons (Fillinger et al., 2009). During the first year, the density of culicines remained high as some peri-domestic pits or water tanks, and other new stagnant

water bodies generated by rains were not included in the planning and not covered by a larviciding application. The study highlighted that if the impact is limited to *Anopheles* only, this may result in a lack of support from the community because the community perceives a direct, positive impact from a reduction in the intensity of human biting of *Culex* mosquitoes. In urban settings, the effect of larviciding should therefore be enhanced by environmental management measures and implemented by the community itself by controlling domestic mosquito breeding sites (Fillinger et al., 2008; Mukabana et al., 2006).

In flooded riverine habitats in the Gambia, however, the impact of larviciding on larval density was found to be moderate and without a major impact on malaria transmission. It was found that application of the larvicide with simple spraying equipment was not effective in complex habitats that are unstable over time and difficult to access by foot (Majambere et al., 2010).

Besides the spatial extent of mosquito breeding sites being relevant to the success of *Bti,* also timing of the application is important. Larviciding conducted with *Bti* in the rural highlands of western of Kenya contributed to a reduction of > 90% in larval mosquito stages and of > 80% in adult mosquitoes. The impact was less during the rainy season and hence the study suggested that larviciding may be more effective during the dry season and the beginning of the rainy season for controlling malaria (Fillinger & Lindsay, 2006; Fillinger et al., 2009). During this period, mosquito breeding sites are well defined and contained (Imbahale et al., 2012; Tusting et al., 2013).

In our study, several challenges were reported by *Bti* sprayer operators. These were mainly related to the creation of pits for wetting vegetable crops at the bottom of the hills surrounding the rice fields, as well as the new pools and puddles generated by rains. An upstream water dam constructed for storage of rain water and irrigation of the rice field was also not treated, because this site was inaccessible to the operators with simple knapsack sprayers and required a motorized knapsack to ensure a long swath of the spray. The sprayers encountered specific challenges in rice habitats mainly related to the type of soil: in some areas or during a day with rain, the sprayer faced muddy and slippery soils. Walking along the narrow ridge of rice plots was reported as another obstacle as it could limit full coverage of some larger rice plots. The social assessment confirmed a high increase in awareness and acceptance from the local community on larviciding, especially because they were involved in the actual monitoring (larval dipping etc.) and thus observed an impact. In addition, they

perceived a high impact on mosquito nuisance and probably on malaria infection (Ingabire et al., 2017).

Conclusion

This study conducted in complex rural mosquito breeding areas, mainly rice fields, showed a significant reduction in density of anopheline and culicine larval mosquitoes after application of *Bti*, and of adult stages of anophelines, but not culicines where implemented either by expert supervised or community based teams. The impact was more noticeable in open water bodies where the density and the habitat occupancy of mosquito larvae and pupae were reduced to near zero in inter-crop lands and to zero in dams. Although, this study covered only one cycle of rice growing, it demonstrated that the application of *Bti* may lead to the elimination of larval mosquitoes in open water bodies and to gain a moderate impact in complex habitats of rice fields. Importantly, this method of larval control caused a strong reduction in the biting fraction of malaria vectors, and can be expected to impact on malaria transmission.

The effectiveness of *Bti* in rice paddies has to be investigated more for multiple seasons of rice farming and explore the suitable phase of rice growing to be targeted for impact on mosquitoes. Moreover, some inaccessible water bodies with simple spraying equipment have been reported in this study and appropriate equipment per types of breeding sites has to be defined and made available before larviciding is conducted. Based on their technical skills on pest management and handling spraying equipment, the rice farmers and other leaders of community-based organizations seem to be the appropriate community groups to lead and sustain implementation of larval control using a biological larvicide. They should be empowered with affordable resources through additional knowledge and skills on the bio-ecology and control of mosquitoes and then integrate this innovative vector control tool into their usual farming activities.

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Chapter 7

General discussion

Chapter 7

Although malaria continues to be a public health problem, Rwanda has set an ambitious goal to achieve near zero deaths from malaria and pre-elimination with a vision of a country free of malaria in the long term (MoH, 2016). Numerous strategies have been implemented, among them the core vector control interventions, for instance long lasting insecticide treated nets (LLINs) with universal coverage and targeted indoor residual spraying (IRS). These interventions were carried out in the framework of an integrated vector management approach (MoH, 2017a). Despite these efforts, and since 2006, the success in malaria control lasted only for two years post distribution of LLINs and was then followed by a rise in malaria cases (Karema, 2012, MoH, 2016a). From 2013, the annual increase in malaria cases was not interrupted by universal distribution of LLINs or the targeted IRS campaigns using pyrethroid insecticides (MoH, 2016). Nevertheless, a substantial impact on malaria morbidity was accomplished in districts which received the IRS intervention using new generation of non-pyrethroid insecticides in targeted districts with high burden of malaria (MoH, 2016). Up to date, two insecticides, bendiocarb® (a carbamate) and Actelllic® (with pirimiphos methyl, an organophosphate, as active ingredient), have been used in rotation (MoH, 2013).

Several causes have been proposed to explain the observed increase in malaria. The low usage and delay in replacement of LLINs and the potential inadequate efficacy of deployed vector control interventions have been suggested as possible reasons for the increase. Moreover, the entomological information used for decision making was old and predated the eradication period (MoH, 2016). The findings presented in this thesis contribute to elucidate the above dilemma of recurrent malaria increases and to guide the decisions for appropriate intervention strategies with a view to bring malaria under control in the entire country. Chapter 2 provides updated entomological information on species composition, the major behaviours of malaria vectors, and then the distribution of malaria transmission intensity. *Anopheles arabiensis* was found to be the dominant malaria vector and replaced *An. gambiae s.s.* and *An. funestus*, previously considered primary malaria vectors. Different mosquito sampling methods, including the innovative Suna trap and the CDC Light Trap (CDC-LT), were tested and showed that the CDC-LT collected more mosquitoes than the Suna trap, and it was concluded that the CDC-LT could be used for sampling mosquitoes both indoors and outdoors. This method should address the ethical aspects and the high cost related to the sampling methods currently used for mosquito monitoring in sentinel sites, respectively the human landing catch and the pyrethrum spray catch (Chapter 3).

Furthermore, the major issue related to the quality and efficacy of primary vector control tools were addressed in Chapters 4 and 5. Previous studies on LLINs proved that they can reduce man-vector contact by providing the physical barriers between human sleepers and mosquito vectors. This protection is enhanced by their treatment with an insecticide, as the insects are repelled from the impregnated nets and/or get killed by the toxic effect of the nets (Skovmand, 2010). But the critical question was the real operational life of its physical durability as the distribution replacement was based on the assumption that LLINs have a lifespan of 3-5 years under field conditions (Gnanguenon et al., 2013; RBM, 2010).

It was found out in Chapter 4, that the serviceable shelf life of physical durability and the attrition of LLINs under field conditions in Rwanda are less than two years, thus less than the period claimed by the manufacturers. These findings have programmatic implications mainly related to the distribution-replacement period of nets, thus increasing the annual cost of a protected person per distributed net. It was also found that resistance to pyrethroid insecticides emerged in 2011 and spread two years later across most of the high malaria endemic areas of Rwanda (Chapter 5). The major resistance mechanisms prevailing in the study sites involving the knock down (KDR) mutation and metabolic resistance with oxidase enzymes (Chapter 5) are described. This emergence of insecticide resistance would have triggered the ineffectiveness of vector control tools which were in use before the enforcement of insecticide resistance strategy, endorsed in 2013.

In the search for alternative control strategies, the efficacy of the bio-larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) was assessed as a community-based option and to supplement the primary vector control tools (Chapter 6). This trial with *Bti* showed a significant reduction of *Anopheles* larval occupancy and density in rice fields and open water habitats, and the impact was similar in expert-supervised and community-based arms. Larval stages were suppressed in rice fields and water reservoirs in rural areas, and remained low for the duration of the intervention. Moreover, the study demonstrated that rice farmers and leaders of community-based organizations, when empowered with minor resources, should be able to integrate larval control into their usual farming activities and thus complement the effect of primary indoor vector control strategies (Chapter 6, Ingabire et al., 2017).

Linking key findings to the research questions

The effectiveness of vector control interventions depends to a large extent on the patterns in vector behaviour (Pates and Curtis, 2005, WHO, 2014a). The primary vector control interventions, LLINs and IRS, target indoor feeding or resting mosquitoes. The long term application of insecticide-based vector control interventions may lead to behavioural change of malaria vectors. This was reported in several malaria endemic countries (Sougoufara et al., 2017, Reddy et al., 2011, Meyers et al., 2016, Killeen et al., 2016, Wamae et al., 2015)

The first objective of this thesis was **to determine the behavioral characteristics of vector populations over time and space in response to existing and novel vector control tools**. A literature review on mosquito behaviour and background information on vector control interventions implemented specifically in Rwanda were the basis for the selection of sampling methods, the representative study sites and the period required to generate data. At the start of the studies it was assumed that the prevailing malaria vectors are anthropophagic and endophagic.

In line with previous sampling methods used in Rwanda and the aim to collect comparable data, the Human Landing Collections (HLC) and Pyrethrum Spray Collections (PSC) were the methods of choice to address the first research questions. Despite the ethical considerations and the relative high cost and other challenges associated to the above sampling methods, they still represent reliable methods to measure the direct exposure of humans to malaria mosquito bites (Chapter 2).

The mosquito behaviours identified have programmatic implications in terms of vector control interventions. The indoor vector control interventions remain relevant as the important population of malaria vectors feeds and rests inside houses. The highest risk to receive *Anopheles* bites in Rwanda occurs at mid- and late night (Chapter 2). The new generation of LLINs and insecticides should provide adequate protection for the community against indoor transmission of malaria. To return the trend of earlier and outdoor transmission as described in Chapter 2, adequate strategies have to be developed in order to address this new challenge in malaria control. Further research should focus on measuring the intensity and the trend of outdoor transmission. It will be also crucial to evaluate existing or new outdoor mosquito control technologies, such as biological control, space spraying, repellents, mosquito proof housing, larviciding, push-pull systems, endocticides and others (Killeen et al., 2017b). The exophilic and zoophilic behaviours of malaria vectors in Rwanda have not been investigated

and should be the priorities of upcoming studies. The time when most of the human population goes to bed and gets up should also be evaluated in order to estimate the level of residual malaria transmission.

Chapter 3 provides information on alternative mosquito sampling methods and addresses ethical issues, such as exposure of collectors to infective mosquito bites, and sampling bias, such as variation in attractiveness to mosquitoes. Thus, two mosquito sampling traps were evaluated, the CDC light trap (CDC-LT) and the Suna trap equipped with a nylon strip of mosquito attractant blend consisting of five components, the MB5 (van Loon et al., 2015b, Mweresa et al., 2016) and set up with or without a light. CDC light traps proved effective in sampling malaria vectors inside and outside houses. The effectiveness of CDC-LT in areas with *An. arabiensis* as dominant malaria vectors was confirmed by prior studies (Fornadel 2010). Regarding the Suna trap, it showed a lower performance in comparison with CDC-LT in our study site. However, it was more sensitive in sampling the high ratio of *An. gambiae s.s.*. As the experiment was conducted in one study site only, similar experiments are recommended to be carried out in other malaria transmission settings with other dominant malaria vectors. A prior study conducted in western Kenya, proved the high performance of Suna traps in sampling and reducing the populations of *An. gambiae* s.l. (Hiscox et al., 2014, Homan et al., 2016). Our primary aim was to compare the Suna trap to the CDC-LT which is usually not baited with CO_2 for malaria vector sampling. This trap should be evaluated in more detail with the addition of carbon dioxide or one of its mimics, such as 2-butanone (van Loon et al., 2015a, Mburu et al., 2017). Furthermore, the ecology and the role in malaria transmission of other anopheline mosquitoes collected have to be elucidated in future research.

The second objective was **to determine key entomological and environmental parameters which characterize malaria transmission**. For this purpose, data were collected from seven study sites across Rwanda in collaboration with trained community members and local leaders using HLC and PSC as primary mosquito sampling methods (Chapter 2). The findings showed that despite the ambitious goal set by the government to reach malaria elimination, the risk of infective mosquito bites remained high, specifically in study sites located in proximity of rice irrigation schemes. In particular, the 'early biting' intensity of *An. gambiae* s.l. showed an annual increase in some of the selected sites (Figure 4, Chapter 2). This reflects the increasing risk of malaria transmission, and possibly the ineffectiveness of deployed vector control interventions. Our findings also showed that *An. arabiensis* has replaced *An.*

gambiae s.s. and *An. funestus* previously reported as the primary vectors in Rwanda. The molecular forms of *An. gambiae s.s.* have not been identified and should be subject of future studies. These studies should also include the host-seeking preferences and resting behaviour of *An. arabiensis*, which has become the dominant species of malaria transmission in the country.

Other parameters that characterize malaria transmission are the attrition and the physical durability of LLINs and thereby the protection that they give against infective mosquito bites. This subject has received little attention by other researchers. Under field conditions, the attrition of LLINs was found to vary according to the type of net fabric tested (polyester and polyethylene) and the level of malaria endemicity. The serviceable life of LLINs was found to be close to two years, which is lower than the distribution-replacement cycle of three to five years indicated by bed net suppliers. These findings confirmed previous observations on the decreasing impact of LLINs on malaria burden and which is frequently found after two years following the countrywide mass distribution of LLINs. These findings stress the importance of maintaining universal coverage of LLINs in low income malaria endemic countries, but also imply higher costs as LLIN replacement cycles need to be shortened to two years. I recommend that national malaria control programmes set up continuous LLIN durability monitoring programmes and include parameters such as insecticide content, bio-efficacy and bursting strengths of net fibers. Further assessment of the reasons of high attrition and the physical deterioration of LLINs under field conditions will help to identify potential solutions which could be implemented at community level. The latter should include the promoting of the proper use, care and repair of nets (Massue, 2016, Wills, 2013, WHO, 2014e); avoiding storage of food and crops in bed rooms, which are a source of attraction of rodents (Kilian, 2015).

Besides the physical integrity and quality of a bed net, also insecticide resistance in the vector population has an impact on malaria transmission. This was addressed in Chapter 3 where six insecticides currently used for vector control or their alternatives (carbamates and organophosphates) were tested on vector populations throughout the country. For the first time, resistance to pyrethroids was detected in 2011 in few sites, and its onward spread was confirmed for many sites across the country in 2013. Pyrethroids were used for treatment of LLINs as well as for indoor residual spraying. These results help to explain failure of LLINs and the continued increase of malaria in regions where communities are only protected by LLINs (MoH, 2016b, MoH, 2016a). Consequently, the findings were used by the Ministry of

Health of Rwanda to adopt strategies related to insecticide resistance management including the rotation of insecticide products every two years of non-pyrethroid insecticides. This rotation cycle aims to maintain the efficacy of LLINs and IRS. Information on the prevailing resistance mechanisms, i.e. *kdr* and metabolic detoxification associated to esterase enzymes, were made available for decision making through the development of the national malaria contingency plan (MoH, 2016a). Future research should emphasize the extent of each resistance mechanism, the resistance intensity and to determine the presence of other potential resistance mechanisms involving mono-oxygenases and gluthathione S-transferases enzymes. This will further guide decision making on when a given insecticide should be stopped for usage in vector control and to make an appropriate choice of an alternative insecticide.

The third objective was **to strengthen community know-how, implement larval source management and evaluate its contribution to malaria elimination in Rwanda**. The Open Space method for community engagement that we specifically employed in our research (Ingabire et al., 2014) enabled us to establish a preliminary list of community members and obtain their expectations on the planning and implementation of the overall research program. The needs expressed by community members for specific education in environmental management by clearing mosquito breeding sites was of particular interest in the context of the current thesis. The next step consisted of identification of stakeholders and to design the strategies required for their sustained engagement (Ingabire, 2016). The targeted community representatives identified for empowerment in know-how and larval source management consisted of rice farmers, community health workers and local leaders at village level.

Two local community teams were set up and assigned separate responsibilities: the application of *Bti* and the entomological surveillance to monitor the impact of *Bti*, respectively. The first team was mainly made up of rice farmers, familiar with crop protection using sprayer pumps as it was recommended by the rice farmer cooperatives. The members were then trained in the techniques for calibration of spraying equipment, preparing the dosages and applying the bio-larvicide *Bti* in different types of breeding sites. During the application of *Bti*, the members were split into two teams respectively one supervised by the experts of the project and another as community-based independent team. Importantly, the performance of the two teams was similar in terms of impact of *Bti* on reduction of mosquito larvae and adult mosquitoes. These findings demonstrate that communities can themselves take up the responsibility of larviciding and thus contribute to vector control in the framework of integrated approach.

The second team which conducted the entomological monitoring of the intervention was also split into two teams. One consisted of community health workers trained in techniques related to monitoring of larval stages and another consisted of community leaders empowered in adult mosquito sampling using CDC light traps. This community-based entomological monitoring provided data for the calculation of habitat occupancy of larval stages, density of larvae and adult mosquitoes which were the indirect measurements of the impact of *Bti* on malaria transmission. The findings showed that the community can be successfully empowered and participate in monitoring the impact of malaria vector control interventions such as larval source management.

The rice farmers were identified as the most efficient community organization in controlling mosquito larvae in rice fields, because they are familiar with control of crop pests. The application of *Bti* drastically reduced the risk of biting by mosquitoes in the study areas and thus increased the time spent on rice farming (Ingabire, 2017). Moreover, in a parallel study on health economics (A. Rulisa et al., unpublished), communities expressed their willingness to contribute financially to obtain the larvicide and technical support for the application of *Bti* in rice fields. In the context of IVM, the cooperatives of rice farmers represent the key stakeholders for implementation of larval source management. They should be the basis of mobilization of extra funds from their cooperatives, implementation of larval source management as supplement tool to the existing indoor interventions. They could also offer a collaborative channel between the Ministry of Health and other public and private institutions involved in irrigation and rain water harvesting projects.

Implications for vector control policies

The presentation and publication of the results of this thesis have influenced the Ministry of Health to develop new policies or readjust existing strategies governing the vector control interventions, such as the plan for Insecticide Resistance Management, the Extended Malaria Strategic Plan 2013-2020 and the Malaria Contingency Plan 2016-2020. The threat of changes in mosquito feeding behavior, the shift of vector composition (Chapter 2) and spread of insecticide resistance (Chapter 5) are among findings which influenced decision makers in Rwanda. Larvicides and mosquito repellents have now been included into the national list of essential medicine published in 2015. Mosquito repellents are recommended to be available at each level of the health system in order to face outdoor and earlier night malaria transmission.

The aim of the policy is to improve the protection of people before they go to bed, as well as people that work at night or that travel from low to high endemic areas.

The development of national insecticide resistance management and integrated vector management strategic plans, endorsed by the Ministry of Health in 2013, were guided by the results presented in Chapters 4 and 5 of this thesis. The insecticide resistance management strategic plan was proposed to be implemented as part of the integrated vector management plan. These plans aimed to improve the impact of vector control interventions, to ensure the quality control of vector control products, to strengthen local capacity development and then to bring together other stakeholders into the roadmap towards sustainable malaria control and elimination.

The impact of vector control interventions will be guided by the rotation of non-pyrethroid insecticides for IRS on a two years basis in order to reduce the spread of resistance and to protect the efficacy of LLINs at any cost. The IRS will be implemented with blanket coverage instead of focal spraying in high malaria endemic districts. The number of spray rounds that have to be conducted per year will be determined by the residual efficacy of the insecticides in order to ensure that the annual two peaks of malaria vector biting (Chapter 2) and malaria transmission are covered by the intervention. It was also recommended that the monitoring of impact of vector control interventions has to be based on entomological parameters collected from permanent sentinel sites to ensure earlier detection of changes in insecticide susceptibility, species composition as well as changes in vector behaviour. The continuous monitoring of insecticide resistance and LLIN durability, as well as the evaluation of new generations of vector control products were additional recommendations based on the research presented in this thesis and specifically formulated into the IRM strategic plan (MoH, 2013a).

Capacity for entomological monitoring and vector control has been strengthened by advocating and implementation of annual retraining of entomology technicians based at sentinel sites. In terms of infrastructures, the entomology sentinel sites were integrated into their nearby health centers for sustainable monitoring of vector-borne diseases. The functioning experimental huts, insectary rearings of insecticide susceptible *Anopheles gambiae s.s.* Kisumu strains, and of a modern entomology laboratory were set in place in order to respond to the needs of advanced research and the quality control of products and vector control interventions in Rwanda. A budget for entomological and insecticide resistance

surveillance, and capacity building was proposed and approved by the Ministry of Health and implemented through collaboration with different partners mainly the University of Rwanda, the Global Fund and PMI.

The vector control working group has been put in place in order to create synergy with other health and non-health sectors, specifically with the aim to coordinate vector control interventions and to monitor the safe use and management of public health pesticides. In the context of IVM, this group was mandated to provide adequate guidance of implementation and monitoring of cost-effective vector control interventions which have an impact both on malaria and other vector-borne diseases in Rwanda. Actions to be implemented in the framework of cross-border collaboration were also proposed such as regional surveillance and control of vector-borne diseases. The IVM strategic plan was developed with the view to sustain and further build on the existing vector control efforts against malaria and other vector-borne diseases. The document was disseminated at all levels including the stakeholders and the representative of communities under the coordination of the Ministry of Health.

The findings of this thesis also influenced the development of the malaria strategic plan 2013-2018 which aimed to achieve malaria elimination. The plan was extended to 2020 with the revised goal to achieve a reduction of malaria mortality by 30% and then keeping Rwanda free from malaria as a long term vision. The major constraint was the availability of funding to increase the coverage of vector control interventions such as the financial access to the new generation of insecticides and other products which are more cost-effective than the previous products.

The development of malaria contingency plan 2016-2020 was partially influenced by the results presented in this thesis. Its development involved different sectors that should have a role in resurgence or management of malaria including the ministries in charge of agriculture, environment, land use and mining, infrastructure development, gender, local government, education and finance. The final document was approved with a recommended inter-sectorial synergy towards its effective implementation and participative mobilization of financial resources. The proposed interventions addressed all aspects of malaria control including indoor and outdoor vector control interventions implemented in the framework of integrated approach. The role and responsibilities of each stakeholder were clarified with interventions identified per partner. The document supported the role of entomological surveillance and

operational research, the importance of inter-sectorial and cross-border collaboration, as well as a coordination mechanism and framework for monitoring and evaluation of interventions.

The link with Integrated Vector Management for Rwanda

The research objectives of this thesis as well as the field surveys of this study have been aligned with the priorities of the Ministry of Health for malaria control and the implementation of IVM approach. The implementation of surveys and experiments as well as the results all aimed to strengthen the five pillars of IVM (WHO, 2004), within the context of Rwanda.

The need for '**evidence based decision making'** emerged in several chapters. The attrition and waning physical integrity of LLINs within a period of two years was evidence that justified the revision of the LLIN distribution-replacement cycle to two years instead of three to five years. The routine distribution of LLINs through the Expanded Programme of Immunization (EPI) and Antenatal Care (ANC) channels were strengthened in order to respond in time to the high deterioration and attrition of LLINs and to maintain universal coverage.

The confirmation of insecticide resistance (Chapter 5) contributed to the decision to shift and to ensure rotation of insecticides among the non-pyrethroid insecticides for IRS, in order to ensure a toxic effect of the insecticide(s) and to slow down the process of resistance development. It also offered evidence for the country to make a choice towards the new generation of insecticides despite their high cost in order to protect LLINs. This insecticide cost increased four and ten folds, respectively, for carbamates (Bendiocarb®) and Organophosphates (Actellic®). Rwanda was among the first countries to be party to the new generation IRS project. This was stipulated in a Memorandum of Understanding between the Innovative Vector Control Consortium (IVCC) and the Rwanda Biomedical Center in 2016. This MoU allows Rwanda to be eligible to a co-payment mechanism for procurement of new generation insecticides needed for IRS. This co-payment is funded by Unitaid, a global health initiative working in partnership with WHO and collaborating with IVCC to cover the gap from manufacturer's unit cost of new generation insecticides to 15 US$ for eligible malaria endemic countries.

Changes in mosquito host-seeking behaviour and as well as changes in mosquito population composition (Chapter 2) after the scale-up of indoor vector control interventions, demonstrated the prevailing gaps in vector control interventions. Residual malaria transmission was not properly addressed, and other new strategies and capacity building have to be put in place to address the above vector control gaps. Chapter 2 also provides evidence that justifies the design of interventions that cover the perennial malaria transmission which occurs throughout the year with two peak periods of mosquito biting preceding the known peaks of malaria transmission. Additionally, indoor vector control interventions remain relevant, as an important proportion of malaria vectors still bites indoors with peaks displayed at the second half of the night when people are sleeping (Chapter 2).

Other strategies have to be implemented to address the emerging issue of earlier and outdoor biting of mosquitoes. Evidence on the role of secondary vectors that are responsible for residual malaria transmission have to be investigated more and thus guide appropriate strategies for their effective control. The potential role of irrigation and rain water harvesting projects in malaria transmission justifies the importance of a multi-sectorial approach in vector control, as outlined by the Global Vector Control Response (WHO, 2017). It was investigated if communities, when fully engaged, by themselves can carry out larval source management in different mosquito breeding habitats and measure its impact on malaria transmission (Chapter 6).

Results of this thesis contributed in various forms to the **'advocacy, social mobilization and legislation'** pillar of IVM. The launch of the overall project programme "Empowering the community towards malaria elimination" was organized prior to the field surveys and the experiments. It brought together decision makers, researchers, funders, decentralized authorities as well as the community. A new approach for community engagement in the form of Open Space meetings was experimented with. The goals of this approach were to identify the community needs and to establish a preliminary list of community members willing to be engaged in research activities (Ingabire et al., 2014).

At the study site of Ruhuha, this new community engagement approach led to the formation of community malaria action teams (CMATs). These CMATs designed and implemented their action plans mainly based on environmental management of larval sources, as well as on monitoring the usage of LLINs at households. In terms of legislation, the results of this thesis influenced the development of mosquito repellent standards in collaboration with Rwanda

Standards Board, guidelines on integrated malaria control, policies for LLIN distribution and replacement, as well as strategic plans related to vector control.

In terms of "**integrated approach"** as a pillar of IVM, LLINs and IRS are the primary vector control interventions in use. Each of the two interventions solely has proved its effectiveness to reduce malaria disease (Choi et al., 2017, WHO, 2014b). In the framework to accelerate the reduction of malaria transmission, some countries including Rwanda have implemented IRS in combination with universal coverage of LLINs (WHO, 2014c). However, recent studies have shown contradictory findings. Some of them pose that there is an added protection conferred to the combination of the two core interventions (Protopopoff et al., 2018) whilst other studies showed no such effect (Pinder et al., 2015, Bakhiet et al., 2017). As the rollout of the two interventions requires considerable further resources, it is important that such approach needs scientific evidence based on the extent of effectiveness of combining in comparison with either LLINs or IRS alone (WHO, 2014d). This thesis provided information on the potential limitations that affect the effectiveness of the above interventions deployed alone or in combination, such as the gradual decline of coverage and the required period of LLINs replacement, and the spread of insecticide resistance to pyrethroid insecticides (Chapters 4 and 5). In addition, LLINs and IRS seemed not to be the appropriate strategies to tackle the malaria vectors with tendency to bite outdoors, or earlier at night, as it was shown in several sites in Rwanda (Chapter 2). A supplemental intervention based on mosquito larval source management using *Bti* was successfully evaluated in collaboration with beneficiary communities. Based on the results of this thesis, the Rwandan malaria control programme plans to integrate this intervention in its current malaria control strategy, because it represents an appropriate response for involving the community to control malaria transmission at the source. Importantly, because LSM is not focused on the indoor environment, it may tackle the challenge of outdoor transmission (Killeen et al., 2017b, Killeen et al., 2017a).

Collaboration, as the fourth pillar of IVM, was addressed primarily through the collaboration between the academic and research institutions which hosted the overall research programme. At national level, the collaboration involved the Ministry of Health, Ministry of Education, and the Ministry of Labor and Public services to allocate the research permissions and study leaves to the government employees. The Rwanda Biomedical Center collaborated in management of human and financial resources allocated to the project. The University of Rwanda, through the School of Public Health provided the space to establish the entomology

laboratory and insectarium while Caritas Rwanda, as a non-governmental organization, made space available for field work and the construction of experimental huts. At decentralized level, the administrative districts and hospitals, sectors and health centers were involved in delivery of verbal research authorization and introduction of researchers to the community members. The community participated in sensitization meetings from where the purpose of the research was explained and community priorities identified. The collaboration mainly involved the community health workers, the CMATs, the local leaders of villages and members of rice cooperatives. Also, the community participated in planning and implementation of entomology surveys and monitoring of research experiments specifically for the application of *Bti* and impact monitoring of larval source management.

As the fifth and final pillar of IVM**,** the research presented here contributed to '**capacity building'.** For the surveillance at the different sentinel sites throughout Rwanda, infrastructure was developed that includes the entomology posts established at the seven sentinel sites for mosquito distribution surveys. The entomology posts were integrated into their nearby health centers for sampling, morphological identification and preservation of mosquitoes. At central level, a modern entomology laboratory was put in place for molecular analysis, mostly PCR and ELISA. Its goal was to analyze samples collected from the study sites, as well as to check quality of vector control products. An insectarium was established in order to make the biological material for bioassay tests available, while the experimental huts were designed for evaluation of new generation vector control products.

In terms of human capacity, two entomology technicians were recruited and assigned at each study site. The mosquito sampling activities were organized by community members trained in each sampling method used for research. At central level, senior officers also have been recruited and trained in collaboration with regional research institutions for instance NIMR in Tanzania, and *icipe* and KEMRI in Kenya. They are supporting vector control, entomology surveillance, and operational research and mapping using GIS.

The required funds for research have been directly management by the Rwanda Biomedical Center. They were mobilized from different donors including the Netherlands organization for scientific research/WOTRO science for global development, the global fund to fight AIDS, tuberculosis and malaria (GF), the Presidential Malaria Initiative/USAID (PMI/USAID), and the Government of Rwanda. The funds to allow the functioning of the seven study sites were transferred to the health centers hosting the entomology studies on annual basis.

Chapter 7

How to bring in leadership into IVM?

It is often challenging to come up with research outcomes that bring changes and that are also positively perceived by beneficiary communities and owned by decision makers. The hard work is not to obtain the evidence, but rather the "how" and "when" to change decisions and translate it into behaviours that have a positive impact and enabling to achieve a goal. It was also shown that decision making always involves some form of leadership (Dyer et al., 2009). In fact, IVM aims to achieve vector control goals in specific settings with different challenges (WHO, 2008). Considering the definition of leadership as "a process of influencing an organized group towards accomplishing specific goals" (Hughes et al., 1999), the abilities of leadership may play a key role to overcome different challenges and to ensure successful implementation of IVM throughout its different pillars.

The discovery of emerging of insecticide resistance in Rwanda was followed by the shifting from pyrethroids to a new generation of insecticides. On the one hand, this decision contributed to the drastic reduction of the malaria burden in sprayed districts, but on the other hand, the cost of insecticide increased more than 8-fold with introduction of the organophosphate "Actellic". The direct implication was the decline of coverage of structures and populations previously protected by IRS intervention. Follow-up decisions then require strong leadership in order to tackle challenges that emerge after the first changes have been implemented.

Similarly, the serviceable life of LLINs was shorter than recommended in technical specifications of LLINs (2 years and not 3-5 years) and this evidence contributed to the revision of distribution and replacement cycles of LLINs. This decision also increased the annual cost per person protected by LLIN, and created a gap to maintain universal coverage, which required additional funding. Moreover, the experiment of *Bti* also demonstrated the capability of community members for leading to research or operational opportunities for saving costs.

It was found that the implementation of the five IVM pillars and management of changes induced by the decisions require capacity development and reallocation of extra or continuous funds. IVM as an approach was perceived as complex. Sometimes, it is viewed by managers or experts as an intervention rather than an approach. Taking into account the above definition of leadership, this notion of leadership should be integrated into the pillars to guide the

implementation of IVM. Leadership aims to translate a vision into reality, and thus leaders have to set achievable targets, explore the means and ways to deliver the related outcomes (Bennis, 1989). The IVM managers and implementers should thus be empowered in leadership skills and these skills should then be integrated into the IVM training guidelines and national training manuals.

Feasibility of malaria elimination in Rwanda

Regardless of the level of malaria transmission intensity, all malaria endemic countries are requested to move forward to the goal of malaria elimination (WHO, 2017). Based on past experience and best practices, guidance on tools and strategies for malaria elimination has been presented and discussed in different reports and publications (Tatem et al., 2010, Feachem et al., 2010, Mendis et al., 2009, Takken and Knols, 2009, Sokhna et al., 2013).

In Rwanda, vector control interventions successfully reduced the malaria burden at country level and in high endemic districts using new generation insecticides for IRS. The complete interruption of malaria transmission was not possible due to the rise in insecticide resistance, the inadequate quality and household attrition of LLINs and the changes in mosquito behavior and composition as demonstrated in this thesis. Other programmatic challenges have been pointed out such as the scaling up of IRS due to a shift to expensive insecticides and a delay in the universal coverage with LLINs (Otten et al., 2009, MoH, 2016a).

The country presents opportunities and strengths which should be used to mitigate the above threats. Other alternative vector control measures are not well known and not made available for community usage (MoH, 2016b). Previous behaviour change programmes disseminated messages focusing on treatment of malaria symptoms, and the core indoor vector control interventions relying on the distribution of LLINs and IRS campaigns. Unfortunately, knowledge on the ecology of mosquitoes, in particular the larval stage of mosquitoes and associated control measures, remain largely unknown in the community (Ingabire, 2015). Communities have to be empowered in order to implement the strategies that are affordable at community level, such as demonstrated by the implementation of *Bti* in the rice fields in Ruhuha (Chapter 6).

An important key for successful malaria elimination is the strong health system in Rwanda, which is built on the accountability of managers at different levels of the system, and on adequate infrastructures and monitoring systems based on information technology. The health

management information system (HMIS) reports countywide data on a monthly basis, while the integrated disease surveillance system provides immediate case reports and weekly reporting of epidemic diseases including malaria. The major challenge is the active response to the bi-annual malaria outbreaks which in 2017 occurred in many districts despite the large distribution of LLINs (MoH and ICF, 2018). The recognized country leadership on accountability in management of public resources is another opportunity for implementation of vector control interventions according to the IVM principals.

The best approach to be advocated is to implement malaria elimination strategies gradually from low to high endemic districts. In this regard, **capacity building** is a high priority in different aspects of vector control, such as capacity for quality control of products and impact measurement of interventions. The transfer of capacity will generate the required **evidence** to implement the remaining three pillars of IVM (integration of interventions, collaboration, and advocacy, social mobilization and legislation).

Moreover, the dynamics of malaria transmission vary according to the local conditions which are influenced by the spatial variations or landscapes (Lambin et al., 2010). This was confirmed by the results of this thesis respectively related to vector distribution and malaria transmission in Rwanda (Chapter 2) and insecticides resistance status (Chapter 5), which vary according to study sites characterized by specific features of landscape. However, the current vector control interventions are solely designed following the administrative boundaries and not aligned to the combination of geographical and epidemiological variations of malaria transmission. **The landscape approach** referring to the similar eco-epidemiological features has to be considered in planning and implementation of vector control interventions for sustainable outcomes in malaria control and its elimination.

Importantly, the current interventions in Rwanda are mainly supported by external funding and therefore scaling up and sustaining the impact of interventions is a major challenge. It was found that while some stakeholders address the malaria burden, others are involved in the creation of environments favorable for malaria transmission (MoH, 2016a). Therefore, the principle of **"polluter pays"** should be enforced for the mobilization of local funds. Each development project in Rwanda is subject to an environmental impact assessment, because an authorization certificate is mandatory before implementation of any project (REMA, 2005). Any development project that contributes to the spread of malaria or any other vector-borne disease should thus be requested to contribute to malaria control. Potential projects are

particularly those involved in irrigation, rain water harvesting, and production of electricity from hydropower, mining, and road construction. For the coordination, an inter-sectorial group involving the donor agencies, the public and private sectors from health and non-health sectors should be mandated to own and to guide the technical and operational issues. These include monitoring and mobilization of financial resources needed to cover the gap of funds for scaling up, sustain the achievement and thus make malaria elimination a feasible option in the future.

Conclusion

Despite the ambition of the Government of Rwanda to achieve malaria pre-elimination by 2018 and the tremendous achievements in reduction of malaria cases and deaths in the past years (MoH, 2013b), these gains appear fragile. This thesis demonstrates the limitations and weaknesses of the core indoor vector control interventions, such as LLINs and IRS, and provides new (biological) alternative tools and options to involve communities in vector control. In the future, these should be integrated into the existing strategies to sustain malaria control towards its elimination.

The changes in vectors host-seeking behaviour, species composition, the rise of potential secondary vectors and the trends in earlier biting, as demonstrated in this thesis, indicate the urgent need for new strategies to address the challenges that generate residual transmission. Larval source management using *Bti* proved to be effective to supplement existing indoor strategies and should be implemented through communities themselves and integrated into best practices of community-based organizations. The community showed the willingness to participate and invest in larval source management if they are empowered in knowledge and if the products are made available at affordable cost. Malaria elimination is feasible, but requires adaptive strategies based on the principles of IVM. IVM should be enhanced by integrating 'leadership' into its core concept.

Chapter 7

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Summary

Most malaria control interventions are designed to target the life cycle of the malaria vector or the malaria parasite with the purpose to interrupt malaria transmission. However, the interruption of malaria transmission and eventual elimination of the disease remain one of the hardest challenges in malaria control, particularly in countries with low income-economies. Based on lessons from past malaria control strategies, and the promising results from insecticide treated nets (ITNs), long-lasting insecticide treated nets (LLINs) and new insecticides for indoor residual spraying (IRS), the global public health community was mobilized to aim for malaria elimination. Thus, the outcomes obtained on malaria reduction encouraged some countries, including Rwanda, to move forward to a malaria elimination phase. At the same time, malaria endemic countries have been encouraged to adopt the concept of integrated vector management (IVM) and, consequently, to rationalize the usage of limited resources.

This approach intends to transform the existing vertical system of vector control by making it more evidence-based, and by enhancing the participation of decentralized communities. For the current thesis, the concept of IVM, with an emphasis on empowerment of the local community, was implemented for malaria control in Rwanda. The goal was to investigate the feasibility of malaria elimination in Rwanda and evaluate potential hindrances in rolling out IVM. Key questions therefore were: (1) what are the key entomological and environmental determinants which characterize malaria transmission in Rwanda? (2) what are the behavioral characteristics of vector populations over time and space in response to existing vector control interventions? and (3) will community based know-how and larval source management interventions next to the National Malaria Control Programme (NMCP) policy on LLINs, IRS and case management, achieve and sustain malaria control and contribute to elimination?

The study began in the context of a lack of accurate entomological information as previous studies were carried out during the malaria eradication programme (1955-1969). **Chapter 2** therefore provides updated entomological knowledge on malaria vector species composition, their major behaviours (feeding and resting), and the distribution of malaria transmission intensity across Rwanda. Human Landing Collections (HLC) and Pyrethrum Spray Collections (PSC) were the methods used in order to collect comparable data in line with the sampling methods previously used in Rwanda. It was shown that *Anopheles arabiensis* became the dominant malaria vector and replaced *An. gambiae s.s.* and *An. funestus*, previously considered as primary malaria vectors. The peaks of *Anopheles gambiae* s.l. bites

occur at the second half of the night and consequently effective indoor vector control interventions should provide adequate protection to the population against indoor transmission of malaria. However, we also observed trends towards earlier and outdoor transmission, although this was variable across study sites. Vector control strategies have to be adapted to the above challenges. However, the intensity of outdoor transmission has not been measured and future research should focus on this issue as well as on the evaluation of existing and new outdoor mosquito control methods. The patterns of exophilic and zoophilic behaviours of malaria vectors, and the time when the majority of the human population goes to bed and gets up should be prioritized in future research in order to better estimate the risks of residual malaria transmission in Rwanda.

Results from Chapter 2 made clear that alternative mosquito sampling strategies are desired for objective monitoring, especially because HLC and PSC are labour-intensive and not favoured for ethical reasons. **Chapter 3** therefore describes the results from a comparison of mosquito sampling methods, namely Centers for Disease Control (CDC) light traps and Suna traps equipped with a nylon, odour-baited strip, and set up with or without a light. The CDC light traps proved effective in sampling *An. arabiensis*, the dominant malaria vector species in the study site, both inside and outside houses. Interestingly, the Suna trap was more sensitive in sampling the high ratio of *An. gambiae* s.s. The experiment was limited to one study site and similar experiments should therefore be conducted in other malaria transmission settings or with different dominant malaria vector species. Furthermore, for improvement of its catches, the Suna trap should again be evaluated with an addition of carbon dioxide, as an activator of host-seeking behaviour, or with the CO_2 mimic 2-butanone.

Because current malaria control strongly depends on the proper use of LLINs by local communities, there is a need to assess their physical durability under real-life conditions. **Chapter 4** therefore presents the outcomes on the physical durability and attrition rate of LLINs under usage at community level, and addresses the assumption of protection that they provide to the users against infective mosquito bites. It was found that the physical serviceable life of LLINs in Rwanda was closer to two years, which is lower than the distribution-replacement cycle of three to five years indicated by bed net suppliers. The attrition of LLINs at community level varied according to the net fabric and the level of malaria endemicity. The findings confirmed previous observations on the decreasing impact of LLINs on malaria burden, frequently found after two years following the countrywide

mass distribution of LLINs. The results stress the importance of maintaining universal coverage of LLINs through the annual routine LLINs distribution, to shorten the LLIN replacement cycle to two years, and then highlight the importance of continuous LLIN durability monitoring by National Malaria Control programmes. Further assessment of the reasons of high attrition and the physical deterioration of LLINs under field conditions will help to identify potential solutions which could be implemented by community members with technical support from other malaria control stakeholders.

Besides attrition of LLINs hampering malaria control, there are also increased reports of resistance of the mosquito vectors towards the insecticides used on LLINs or used within IRS campaigns. In **Chapter 5,** six insecticides were tested for measuring resistance levels of local vector populations throughout the country. For the first time, resistance to pyrethroids, used both for treatment of LLINs and for IRS application, was detected in 2011 in a few sites, and its onward spread was confirmed for many sites across the country in 2013. These results helped to explain the continued increase of malaria that occurred from 2013 in regions where communities benefit from universal protection with LLINs. Results also provided information for decision making on the prevailing resistance mechanisms, such as genetic-based *kdr* resistance and metabolic detoxification associated with esterase enzymes. Future research should emphasize the extent of each resistance mechanism, the resistance intensity and determine the presence of other potential resistance mechanisms involving mono-oxygenases and gluthathione S-transferase enzymes. This information will further guide decision making on when the use of a given insecticide should be stopped for vector control and to make an appropriate choice of an alternative insecticide. Moreover, the findings presented in this chapter provided basic information for the development and adoption of a national strategy for insecticide resistance management including the rotation of insecticide products every two to three years, and consequently sustain the efficacy of LLINs and IRS interventions.

Because insecticide-based strategies pose more and more challenges (Chapters 4 and 5), there is a need to evaluate and implement alternative malaria control interventions. With the principles of IVM in mind, we therefore evaluated the impact of a new biological control tool for the larval stages of mosquito vectors, the bacterium *Bacillus thuringiensis* var. *israelensis* (*Bti*), and focused on the role that local communities could play themselves in implementing such programmes. **Chapter 6** therefore explores the feasibility of empowerment of local

communities for implementing mosquito larval control. First, by using the Open Space method as community engagement strategy, we established a preliminary list of community members, identified their expectations and co-designed the strategies adapted to their commitment. Mosquito larval source management was most often expressed by community members as an area for empowerment in knowledge. The most engaged community representatives consisted of rice farmers, community health workers and local leaders at village level. Then, two local community teams were set up and assigned separate responsibilities: the application of *Bti* and the entomological surveillance to monitor the impact of *Bti*, respectively. The first team was mainly made up of rice farmers, familiar with crop protection using sprayer pumps. Then, they were trained in the techniques for calibration of spraying equipment, preparing the required dosages and on how to apply *Bti* in different types of breeding sites. During the application of *Bti*, participants were split into two teams, one supervised by the project experts and another as community-based independent team. The findings showed that the community can be successfully empowered and participate in monitoring the impact of malaria vector control interventions such as larval source management. Moreover, the entomological outcomes resulting from the *Bti* application between the two separate teams, were similar in terms of reduction of larval and adult mosquitoes in the study area. This chapter demonstrates that communities can themselves take up the responsibility of larviciding and thus contribute to vector control in the framework of an integrated approach. The cooperatives of rice farmers, the community health workers and the community leaders represent the key stakeholders to supplement the existing vector control interventions using larval source management.

The general discussion**, Chapter 7**, links the key findings to the primary research questions and to their implications for national vector control policies and strategies. The link between the results and the current implementation of the five pillars of IVM in Rwanda is discussed, as well as how to bring in the concept of leadership into IVM and the feasibility of malaria elimination in Rwanda.

It is concluded that despite the ambitious goal to achieve malaria pre-elimination in Rwanda, the achievements previously obtained in malaria control appeared fragile. The major limitations and weaknesses of the core indoor vector control interventions related to the required quality of interventions and to new behaviours of malaria vectors. Furthermore, the changes in vector host-seeking, the shift in species composition, and the trends in earlier and outdoor biting indicate the potential existence of residual transmission. Thus, supplemental

vector control tools are required and preferably should involve local communities and be implemented under the IVM approach. Larval source management using *Bti* proved to be effective and should be implemented through community networks and integrated into best practices of community-based organizations. The community showed the willingness to participate in larval source management if community members are empowered in knowledge and then products made available at an affordable cost. Malaria elimination is still feasible in Rwanda, but requires adaptive strategies based on the principles of IVM and integrating 'leadership' into its primary concept.

Acknowledgments

I frequently suffered from malaria when I was 12 years old until my enrollment in the trials that evaluated the community effectiveness of bed nets in 1993. In other words, am I a survivor of malaria disease? I would not have believed that the largest part of my life would be dedicated to the fight against malaria, and that one day I would be getting a PhD degree in this field. Combining my work duties aligned to the ambitious goal of malaria elimination set by my country, keeping the responsibilities for my family and the demanding research work were not an easy task. But with the friendly advice, the encouragements and the contributions from different people, none is impossible.

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Martijn and Josine family, in the Hindelaan, I appreciate your unconditional good neighborhood. Your frequent advice to improve my personal quality-time and the gift of poems at the end of my visit in 2011, changed my views of the world. The quote that you later shared with me ("Yesterday I was clever so I wanted to change the world, today I am wise, so I am changing myself") remains for ever in my mind. My thanks to the Terpstra and Mahoro family for making our visit to Groningen enjoyable by exploring modern cow farmers and visiting the man-made dyke at the border with the North Sea. Thanks to Dr. Eugene Rurangwa, for the unforgettable fun during a week-end tour to Brussels, the warm welcome to your family and the visits of different tourist attractions of the city. To the Rwandese community of Wageningen University, thanks for the agreeable company for shopping, biking tours around Wageningen city and the delightful barbecues and drinks we used to organize in order to break the long weekly working days.

In Ruhuha, my study site, my sincere thanks to Sisters Liberata and Irène for your warm welcome to the health center and the spontaneous arrangement of space to meet with the community. Thanks to Fred, the Executive Secretary of Ruhuha sector for the facilitation and introduction that you provided for the meetings with the community members. To Eugene, Innocent, Naphtal, Emmanuel and Augustin, the presidents of rice farmer cooperatives, thanks for your leadership in mobilizing the rice farmers. You are the pillars of the success of *Bti* evaluation. To Emmy, Jacky, Ernest, Gad and Peter, entomology technicians, you are here acknowledged for your hard work to support the entomology monitoring along the experiment of *Bt*i.

My two parents and siblings, you passed away without getting sick and please receive my heartfelt gratitude through this work. You always encouraged me to go far in education and now I am almost there when you are physically so far to me. My sincere thanks to my wife, Iribagiza Marie Claire, for her encouragements, moral support and patience along this PhD journey. It was not easy but you did it. You have shown strengths to take care of our family alone during my long and frequent absences as part of the training and research works. I thank you very much.

To my sons, Hakizimana Yvan Igor, Hakizimana Dorcy Agape, and Hakizimana Ntwali Elite Nicone, you often ask me "Daddy, when will you go to school again?" Now the outcome is that you are reading and holding my PhD thesis in your hands. It is the result of a hard work and perseverance. I hope that it will be your inspiration for your educational ambition for the coming years. To my beloved twins, Nshuti Brian Kess and Shimwe Brianna Kessy, you were born during my PhD journey when my availability was limited. Now, I guarantee you that a part of my time will be spent helping you doing homework and having fun.

This thesis would not have been possible without the available funds and my gratitude therefore goes to the NWO-WOTRO Science for Global Health Program. Last but not least, I would like to appreciate the creative work of Birasa Bernard and his beloved son Birasa Bruno to translate my thoughts into an artistic design of this thesis cover. The general design of the cover symbolizes the traditional decoration pattern named locally "*Umugongo* or back". In central, the Rwandan traditional shield represents the IVM which aims for a comprehensive prevention against malaria disease for the entire community, represented by the decoration design of spines placed behind the shield. The dying mosquitoes, pictured here in the darkness of the night, symbolize the future success of integrated vector management

for malaria control. The thesis title appears in the light of sunset and this depicts the future era of malaria elimination in Rwanda.

Curriculum vitae

Emmanuel Hakizimana was born on May 3rd 1967 in Bujumbura, Burundi. His family moved back to Rwanda when he was young. He started his primary school in Rwanda, and completed it in Burundi. After accomplishing his secondary education in 1989 at "College Saint Albert" of Bujumbura, he joined the Faculty of Sciences at the University of Burundi where he obtained a Bachelor degree in Biology in 1993. His research dissertation was based on osteology studies for identification of a new fish species belonging to the subfamily of Bariliines. Later he was employed for one year as a teacher of sciences at the secondary school of Gihanga in Burundi. After moving back to Rwanda, he was employed by the Ministry of Environment and Tourism as the National Inspector of Environment and Tourism from 1994 to 1998. During that period, he had the opportunity to act as the National Focal Point of the Biodiversity convention and to contribute to the negotiation of environmental agreements such as the Desertification Convention and the Biotechnology Protocol. He led the development of the first National Biodiversity Strategy and Action Plan of Rwanda. In 1998, he secured a WHO Scholarship to undertake a Master's training in Medical Entomology at the WHO-Center of Medical and Veterinary Entomology of Bouake, Ivory Coast, hosted by the Bouake University. In 2000, he obtained a Master's degree in Medical and Veterinary Entomology. Back home, he was appointed as the head of Vector Control and Entomology Department at the National Malaria Control Program (NMCP). Four years later he joined the wildlife conservation department, and was assigned the Directorate of Planning, Research and Monitoring at the Former Rwanda Office of Tourism and National Parks from 2003 until 2008. He played a major role in the promotion of tourism and wildlife conservation. He was among the initiators of the annual famous wildlife conservation event called "*Kwita Izina*" or "Naming of baby Mountain Gorilla". He was also coordinating the requests of wildlife researches and delivering research permits.

In 2008, he returned to resume his career at the NMCP. At this point, malaria control was in a critical period for country-wide scaling up of Long Lasting Insecticide-treated Nets for young children and pregnant women and for introducing Indoor Residual Spraying (IRS). In 2011, he started his sandwich PhD at Wageningen University on the topic of Integrated Vector Management (IVM). He was convinced that IVM would be an important approach for

malaria prevention after attending two WHO regional IVM training courses held at the International Center for Insect Physiology and Ecology *(icipe*), Kenya, in 2003 and 2008. During these courses, he enlarged his network and relationships with senior medical entomologists from whom rose up the motivation to undertake the long training towards obtaining a PhD degree. Currently, he is the director of the Vector Control Unit at the Malaria and Other Parasitic Disease Division of the Rwanda Biomedical Center, Rwanda Ministry of Health. Although he started as the only vector control employee in 2008, he built the vector control capacity, and is now managing a large team of 10 entomology officers and technicians based at central level, 24 entomology technicians managing entomology monitoring posts based at peripheral levels. The entomology facilities include a modern (molecular) entomology laboratory, an insectary and animal house, six experimental huts and 12 entomological sentinel posts to support the evidence base for quality and efficacy monitoring of new vector control interventions and strategies.

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Hakizimana E, Karema C, Munyakanage D, Githure J, Mazarati JB, Tongren JE, Takken W, Binagwaho A, Koenraadt CJM. (2018) Spatio-temporal distribution of mosquitoes and risk of malaria infection in Rwanda. Accepted for publication. *Acta Tropica* **182**, 149–157.

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Hakizimana E, Ingabire CM, Rulisa A., Kateera F, Homan T, Van Den Borne B, Muvunyi CM, van Vugt M, Mutesa L, Takken W, Koenraadt CJM. Community-based control of malaria vectors using Bacillus thuringiensis var. israelensis (Bti) in Rwanda. **To be submitted.**

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Biological and environmental control of mosquito larval and adult stages for malaria control

Writing of project proposal (4.5 ECTS)

- Using the Integrated vector management (IVM) approach to empower the community towards malaria elimination in Ruhuha, Rwanda

Post-graduate courses (4.5 ECTS)

- Multivariate analysis; PE&RC (2011)
- Integrated vector management and insecticide resistance monitoring; RBC, WHO & Institut Pierre Richet of Bouake-Ivory Cost (2018)

Laboratory training and working visits (3 ECTS)

- Data collection using mobile technology training course; African Insect Science for Food and Health (ICIPE) (2012)

Deficiency, refresh, brush-up courses (13.5 ECTS)

- Ecological methods; PE&RC (2011)
- Ecological aspects of bio-interactions; PE&RC (2011)
- Basic statistics; PE&RC (2011)

Competence strengthening / skills courses (1.8 ECTS)

- Project management; WGS (2011)
- Scientific Publishing; WGS (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)

- PE&RC Weekend (2011)
- PE&RC Day (2011)

Discussion groups / local seminars / other scientific meetings (11 ECTS)

- MEPR Project discussion group (2011-2015)
- PhD Discussion Group; WUR (2011, 2014, 2015, 2016)
- Vector Control discussion meetings; WUR (2011, 2014, 2015, 2016)
- Malaria Technical Working Group meetings and local seminars; Rwanda (2012-2018)

International symposia, workshops and conferences (11.9 ECTS)

- Malaria Technical Working Group meetings and local seminars; poster presentation; Rwanda (2012)
- The 6th Miltilateral Initiative on Malaria (MIM) conference; poster presentation; Durban, South Africa (2013)
- First Pan African Mosquito Control Association (PAMCA) conference; poster presentation; Nairobi-Kenya (2014)
- American Society of Tropical Medicine and Hygiene (ASTMH) 63st annual meeting; poster presentation; Atlanta-Georgia, USA (2014)
- American Society of Tropical Medicine and Hygiene (ASTMH) 64th annual meeting; poster presentation; Philadelphia, USA (2015)

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