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## A large-scale field trial to evaluate the efficacy of bacillus larvicides for controlling malaria in western Kenya: Study design and methods

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**Abstract:** Since malaria vaccine development is slow and parasite resistance to anti-malarial drugs is developing rapidly, vector control is still the most practical method for reducing malaria transmission in developing countries. House spraying and treated bed nets have been popular control measures targeting indoor resting mosquitoes; however, chemical insecticides should be treated and managed with great care. In this proposed study, we will evaluate the efficacy of bacterial larvicides combined with environmental management strategies for controlling malaria vectors and transmission in western Kenya. These control methods are less harmful to the environment. The article describes the study design and methods.

### BACKGROUND

Despite the long history of malaria control efforts, malaria remains a major threat to human health. Malaria is responsible for over 300 to 500 million clinical cases and more than a million deaths each year globally (the World Health Organization world malaria report 2005, <http://rbm.who.int/wmr>). Around 90% of these deaths occur in Sub-Saharan Africa, mostly in children under five years of age. Malaria is still the leading cause of death, and controlling malaria is the key to improvement of health and economic conditions in Africa [1, 2, 3, 4, 5].

Since malaria vaccine development is slow and parasite resistance to anti-malarial drugs is developing rapidly, vector control is still the most practical method for reducing malaria transmission in developing countries [6, 7, 8]. As a result of the discovery of Dichlorodiphenyl-trichloroethane (DDT), the primary vector control method became indoor residual insecticide spraying targeting adult mosquitoes. Although the international community discouraged the use of DDT in developing countries because of its toxic effects, house spray using other chemical insecticides remained a popular method for mosquito control. In September 2006, the World Health Organization (WHO) recommended the wider use of indoor spraying with DDT, and so indoor residual insecticide spraying will become an ever more popular method for malaria vector control in African countries. Another effective control intervention is the use of insecti-

cide treated bed nets targeting adult mosquitoes, a method that has become increasingly popular in recent years [9, 10, 11, 12].

However, chemical insecticides should be treated and managed with great care. Excessive use and mismanagement of insecticides easily pollute the environment. In particular, contamination of agricultural lands and water with DDT is a great concern [13, 14, 15, 16, 17]. House spraying and insecticide treated bed nets are also vulnerable to the development of resistance, vector avoidance behavior and reduction of predators [18, 19, 20].

Before the introduction of house spraying, modifying and reducing mosquito breeding sites (referred as environmental management) was the most successful method for malaria control in many countries [3, 21]. One advantage of targeting immature stages is that they are confined within relatively small aquatic habitats and can not readily escape control measures whereas adults are highly mobile flying insects that can readily detect and avoid intervention measures [6]. This approach may be particularly effective in regions where the primary malaria vector is exophilic, making indoor residual spraying and treated bed nets less effective [22]. However, physical reduction of aquatic habitats may be restricted when local inhabitants use the habitats in their everyday life [7, 23]. Moreover, a large scale source reduction may affect local ecosystems.

Vector control can also be achieved through treating breeding sites directly with chemical or biological agents

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that kill the larvae. Larviciding may be more effective in an area where breeding sites are relatively few in number, or during the dry season [23, 24]. Because most chemical insecticides are toxic to non-target organisms and quickly disperse in water, they are not recommended for application to natural bodies of water. On the other hand, biological control agents such as predacious fish are safe to humans and most non-target organisms. Although the larvivorous fish, *Tilapia zilli*, and crayfish have been considered for the control of *Anopheles gambiae* s.l. in Kenya [25, 26], this method will not be effective in small temporary habitats in which the African malaria vectors usually breed. The fish have to be frequently reintroduced into each of numerous small habitats, which is a time consuming and unrealistic.

In recent years, there is increasing interest in two bacterial species, *Bacillus thuringiensis* var. *israelensis* (Bti) and *B. sphaericus* (Bs), for the control of African malaria vectors. Bti and Bs are highly effective larvicides against mosquitoes and other nematoceran dipterans including blackflies [27] and exhibit little or no toxicity against other organisms [28]. The bacteria produce a few types of protein in crystal form during sporulation. The crystal proteins become toxic when ingested and solubilized in the larval midgut. Their selectivity is determined by the structure of the proteins and the presence of proteolytic enzymes and receptors in the midgut of mosquitoes [29, 30]. The feeding habits (filter feeding) of anophelines larvae also facilitate ingestion of the toxin. The safety of Bti and Bs for non-target organisms is a clear advantage over chemical insecticides. Other advantages of the microbial larvicides include the fact that the development of resistance by insects is apparently slow due to a complex mechanism of toxin, and that the toxins do not typically persist or accumulate in the environment or in body tissue.

In 1982, a commercial formulation of Bti was first used on a large scale to control blackflies, which are vectors of onchocerciasis in West Africa [31]. The campaign considerably reduced blackflies and consequently cases of onchocerciasis as well. Formulations of Bti and Bs have also been routinely used for control of nuisance culicine mosquitoes in Europe and North America [32, 33]. Despite these successes, the microbial larvicides have not been used to control anopheline mosquitoes and malaria transmission in Africa. Anopheline larvae demonstrate lower susceptibility than culicines larvae to most formulations of these bacteria, because of their surface feeding behavior and the rapid settling of the toxins [27, 34]. Recently, small scale field trials revealed that a new water-dispersible granule (WDG) formulation is highly effective against the African malaria vectors [35,36].

## OBJECTIVES

The general objective of this study is to evaluate the efficacy of Bti and Bs combined with environmental management strategies for controlling malaria vectors and transmission in a large-scale field setting. The specific objectives are 1) to determine whether this control measure significantly reduces vector population, 2) to determine whether the reduction of vectors is sufficient to suppress parasite transmission, 3) to determine whether this control method significantly reduces malaria morbidity and mortality, and 4) to evaluate the feasibility of the method.

## MATERIALS AND METHODS

### Study site

The study sites were established within the area monitored by the demographic surveillance system (DSS) operated by the Institute of Tropical Medicine, Nagasaki University, in Suba District, western Kenya (see the article by Kaneko et al. in this issue; Figure 1). The sites include four islands in Lake Victoria (Ngodhe Island, Kibuogi Island, Rushinga Island, Takawiri Island) and four villages (Kaugege, Kisui, Mbita, Nyamanga) on the adjacent mainland. Rushinga Island, the largest, was divided into 8 sections.

Most houses in the area feature mud walls and iron-sheet roofing. The main economic activities are fishing and farming. The rainfall pattern is bimodal with a long rainy season from March to June and a shorter one in October

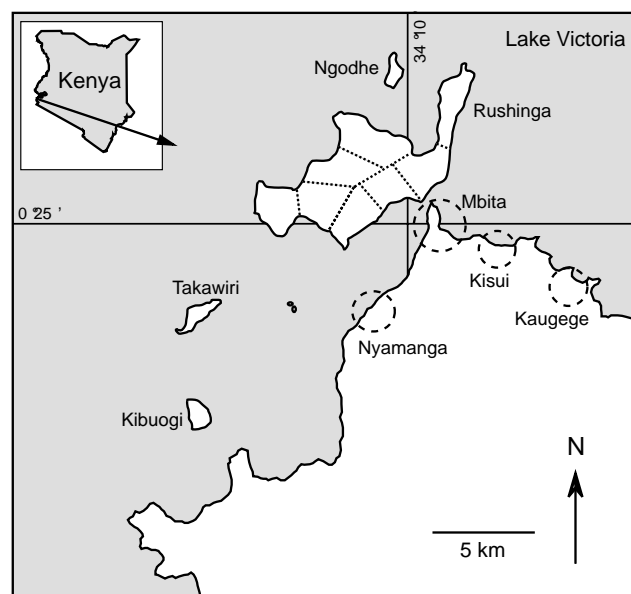


Figure 1. Study sites in western Kenya. Rushinga Island will be divided into 8 sections. The mainland villages are shown as dotted circles.

and November. Malaria is endemic and poses a major health problem in this area. *Plasmodium falciparum* parasite prevalence may reach as high as 70-80% among children less than 5 years old [37, 38]. The primary malaria vectors are *A. gambiae* and *A. arabiensis*, while *A. funestus* occurs less commonly along the lakeshore [23].

#### Indoor resting mosquito population

For the preliminary survey, we have already started sampling indoor resting mosquitoes biweekly using the pyrethroid spray catch method [39]. Mosquitoes have been sampled from 10 houses in each section of Rushinga Island, 30 houses on Takawiri Island, 20 houses on the other islands, 40 houses in Mbita, and 10 houses in the other mainland villages. The total of 220 houses were randomly selected using the DSS data. The sampled mosquitoes were stored in a freezer, and female anophelines were identified to species using the PCR method in the Nagasaki University field station in Nairobi [40]. Malaria vectors have been also tested for the presence of malaria sporozoites in the head and thorax using standard enzyme-linked immunosorbent assay procedures.

#### Mosquito breeding sites

As part of the preliminary survey, we have also started monitoring all standing bodies of water in the study sites for the presence of anophelines larvae (Figure 2). The bodies of water are monitored biweekly. The presence of anophelines larvae is examined using a standard dipper (350ml) at each body of water, and the coordinates are recorded using a handheld GPS. The conditions of each body of water are also described.

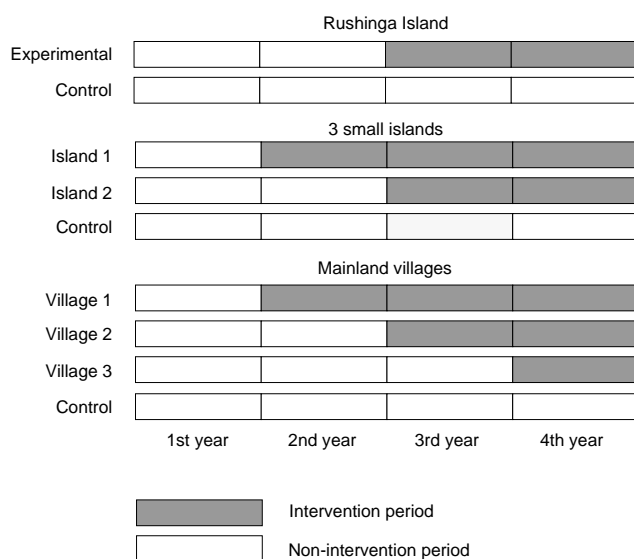


Figure 2. Project time table. We will use “Staircase” designs for the small islands and the mainland villages.

#### Malaria-related morbidity and mortality

Malaria-related infection rates and morbidity among children under 5 years old will be estimated at the end of the two rainy seasons each year. Caregivers will be interviewed to determine the child’s history of illness during the previous 2 weeks. Axillary temperature will be measured using a digital thermometer. The presence of *Plasmodium falciparum* in the child’s body will be tested using rapid diagnostic tests (WHO: [www.wpro.who.int/rdt](http://www.wpro.who.int/rdt)). Clinical malaria will be defined as an axillary temperature of 37.5C or higher and any parasitemia. The children will be randomly selected within each study site.

Mortality rates among children under five years old will be estimated from household censuses of the study population in each study site. Census data will be obtained from the DSS. Because malaria infections may affect mortality both directly and indirectly, all-cause mortality will be used rather than malaria-specific mortality [11].

#### Intervention

We will use the microbial larvicides and environmental management methods to control mosquitoes. Prior to the application of larvicides, we will reduce potential breeding sites by modifying or filling them completely. The microbial agents will be VectoLex<sup>®</sup> and VectoBac<sup>®</sup> (Valent BioSciences Corporation, IL, USA) containing the active ingredient *Bs* and *Bti*, respectively. We will use two formulations, water-dispersible granules for liquid application with sprayers, and corn granules for hand application, depending on habitat conditions [36]. The optimum dosages will be determined in accordance with the data provided by Fillinger et al. [7].

“Before/After and Control/Impact-Paired” designs will be used to detect the impact of intervention within an experimental site and between experimental and control sites [41]. On Rushinga Island, we will start treating all standing bodies of water in four randomly selected sections in the third year (Figure 2). The other four sections will serve as controls. For the small islands, we will use a “Staircase” design where the intervention will start in two successive years, because of few replications [42]. We will intervene on one randomly selected island in the second year and another randomly selected island in the third year, and the third island will serve as a control. We will also use a staircase design for the mainland villages; the intervention will start in three villages in three successive years, and the fourth village will serve as a control. The intervention will continue in the experimental sites until the end of the study.

#### Ethical clearance

We will obtain a research permit for the use of insecti-

cides from the Pest Control Products Board, Nairobi, and ethical clearance from the Kenya Medical Research Institute, Nairobi. Community and individual consent will be obtained for any activities involving human subjects and access to houses. We will provide opportunities to explain potential risks and benefits to local residents and to answer their questions.

#### Data analysis

We started the preliminary survey in 2006. The data from the survey and the DSS will be used to calculate adequate sample sizes of houses and children. Time-series analysis will be used to determine whether intervention is associated with significant changes in the number of indoor resting mosquitoes, number of breeding sites, infection rates, morbidity, and mortality. We will analyze data separately for Rushinga Island, the small islands, and the mainland villages. For better generalization, we will also analyze data from all sites together.

#### ANTICIPATED RESULTS AND BENEFITS

We anticipate that the bacillus larvicides will reduce vector populations and human-mosquito contact, and that we will be able to demonstrate a decrease in malaria incidence in the intervention sites as compared to the control sites. If our goal is achieved, we will be able to urge organizations involved in malaria control to incorporate this method into their programs. The conventional methods targeting adult mosquitoes will become more efficient and effective if they are combined with this method.

This study will benefit the children involved by closely monitoring their health condition and immediate malaria treatment free of charge. Through our activities, the local community will also gain an awareness of malaria-related problems and values in vector control. Our activities will recruit and train several field assistants in the local community, people who, we hope, will go on to play a leading role in controlling malaria in the future.

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