Effects of *Bacillus Thuringiensis* var., *israelensis* and *Bacillus Sphaericus* Mosquito Biolarvicides on incidences of Malaria in selected areas of Lusaka Urban District, Zambia

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ABSTRACT

Effects of spraying the mosquito biolarvicides *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* (Bs) over freshwater bodies in four selected areas of Lusaka urban district, on incidences of malaria in the areas were investigated. Incidences of malaria prior to and after larviciding of the study areas were determined by reviewing and analyzing health centre records of laboratory confirmed positive malaria cases in the study areas prior to and after larviciding. There were relatively higher incidences of malaria cases in all study areas prior to larviciding. Malaria cases dropped drastically by 53-72 % immediately after larviciding in all study areas. However, though numerically very small percent-wise, there were observed marked rises in incidences of positive diagnosed malaria cases in Chelstone study area by the second month. Three study areas; Chainda, Mtendere and Ng'ombe showed continued decline or had slight rises in the incidences of malaria two months after larviciding. Possible reasons for the observed slight rises in incidence in the areas were; importation of malaria by travelers from outside the Lusaka urban district. The deployment of Bti and Bs larvicides in the context of integrated vector management is likely to have long term impacts on incidences of malaria in Zambia.

INTRODUCTION

Malaria remains among the world's greatest killer diseases. Globally, 216 million cases and over 600,000 deaths due to malaria were reported in 2010, with 81 % and 91% of cases and deaths occurring in sub Saharan Africa respectively[1, 2]. Several national malaria control programmes in the continent are implementing vector control as an essential component of the fight against malaria[3]. Presently, insecticide treated bed nets (ITNs) and indoor residual insecticide house spraying (IRS) are the main mosquito vector control interventions[4, 5]. When optimally employed, these interventions are reported to reduce malaria parasite transmission by up-to 90%[5, 6, 7, 8].

In Zambia, IRS deployment has been integrated with the use of ITNs to control malaria mosquito vectors[9, 10, 11, 12, 5, 1]. A more recently introduced intervention in the fight against malaria mosquito vectors in the country is larviciding, using *Bacillus thuringiensis* var., *israelensis* (Bti) and *Bacillus sphaericus* (Bs) larvicides. These are biotoxin-producing bacteria belonging to the *Bacillus* group that has been used in many parts of the world to control aquatic insect larvae. They are reported to be very efficacious against and are said to be preferred control options for aquatic stages of vectors of malaria, dengue and filariasis[14, 15, 16].

In 2011, *Bacillus thuringiensis* var., *israelensis* and *B. sphaericus* larvicides were sprayed over freshwater bodies in selected areas of Lusaka urban district. This pilot malaria mosquito vector control project was a result of a bilateral agreement between the Cuban and Zambian governments on assistance to Zambia in the field of health. This study reports on the effects of larval source management, the spraying of the Bti and Bs larvicides, on the incidences of malaria among communities members in operational areas of Lusaka urban district.

Materials and Methods

Study areas

This study was conducted in four areas of Lusaka urban district in Zambia (Latitude, 15-16° S; Longitude, 28-30° E). These were: Ibex hill/Kalikiliki area Latitude, 15° 24.707' S; Longitude, 28° 22.296' E); Venta/Manzi valley area (Latitude 15° 22.564' S; Longitude, 28° 24.148' E); Chamba valley area (Latitude, 15° 21.587' S; Longitude, 28° 20.01' E) and Chelstone area (Latitude, 15° 21.924' S; Longitude, 28° 23.836' E) (See Appendix 1).

Product application

Large mosquito breeding sites with high submergent, emergent and surrounding vegetation in these areas were aerial sprayed with *Bacillus sphaericus* larvicide on 20, 21, 23 and 24 June 2011, respectively, using a fixed-wing, single-engine aircraft. Smaller and more accessible water bodies were sprayed with

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the larvicide *Bacillus thuringiensis* var. *israelensis* by spray men on foot using Hudson X-pert pressure spray pumps. The recommended dose of 5 ml of *Bacillus thuringiensis* var., *israelensis* larvicide per square meter of surface of active mosquito breeding site was applied in manual Larvicide applications[17]. For aerial sprays, the recommended dosage of 15 liters of larvicide per hectare was used[17]. Both aerial and manual larvicide applications were done by a combined team of Cuban and Zambian technical personnel and trained community volunteers.

Data collection and analysis

Incidences of malaria in study area communities were determined by reviewing and analyzing health centre records of malaria cases prior to and after the larviciding exercise. These were centrally located health facilities that were assumed to be attended by members of communities of the study areas, namely: Chainda, Chelstone, Mtendere and Ng'ombe health facilities, whose catchments areas were Venta/Manzi valley area, Chelstone area, Ibex hill/Kalikiliki area and Chamba valley area, respectively. Only laboratory confirmed malaria cases using either rapid diagnostic tests (RDTs) or microscopy were considered in this study. The health facility data collected included travel history of patients who tested positive for malaria.

Frequencies of malaria positive cases derived from health centre records prior to and after the intervention were used to plot the incidences of malaria for the two periods of time. Data analysis was done in Microsoft excel computer package.

RESULTS

There was great heterogeneity in the incidence of malaria in study areas prior to the larviciding exercise (Figures 1 and 2). The incidences of malaria in Chainda compound during the period January to August, 2010 peaked in April-May (Figure 1). This health facility lacked microscopy services and hence only rapid diagnostic test (RDT) records were used in assessing the effects of the larvicides in this study area. The zeros in the curve show times when there was RDT stock out at the health facility during the period of interest. During the same period, the Chelstone health centre showed an incidence of malaria peak in January and March (Figure 1). In Mtendere compound, the highest peak for malaria was in May- June, 2010 (Figure 2). This health facility did not have the RDTs in stock during the period, March to August, 2010, and hence malaria diagnosis done by microscopy alone was considered for analysis.

In 2011 Mtendere compound had the highest peak of malaria incidences in May-June prior to larviciding (Figure 2), however, this facility did not have RDT services. Instead microscopy diagnosed malaria cases were used. Incidences of malaria in the catchment area of the Ng'ombe health facility peaked in April-May 2010 (Figure 2). Finally, unlike the Mtendere health centre, Ng'ombe clinic only diagnosed malaria by RDT.

Effects of spraying freshwater bodies with Bti and Bs in the study areas showed various results (Figure 2 In Chainda compound, there was a slight rise in the incidence of malaria in the catchment area of the Chainda health facility a month following larviciding which however tended downward in second month (Figure 2). The Chelstone health facility which had both RDT and microscopy malaria diagnoses facilities also showed drops in incidences of malaria immediately following larviciding, for both method of malaria diagnosis (Figure 2). But like the Chainda situation, there was a rise in the incidence of malaria to about 4% using RDT diagnoses, while the incidence of malaria as diagnosed by microscopy services continued to drop two months following the larviciding.

Incidences of malaria following larviciding in the Mtendere health facility catchment area were similar to those of Chelstone area. There was a drop in the incidence of malaria by both diagnostic methods following larviciding (Figure 2). This was followed by a slight rise in the incidence of the disease two months after larviciding by RDT test and a very slight increase by the microscopy test. Incidences of malaria in Ng'ombe continued to reduce drastically into the second month following of spraying the study area with larvicides (Figure 2).

Figure 1: Malaria rates in Chelstone and Chainda compounds, January-August, 2010 and 2011

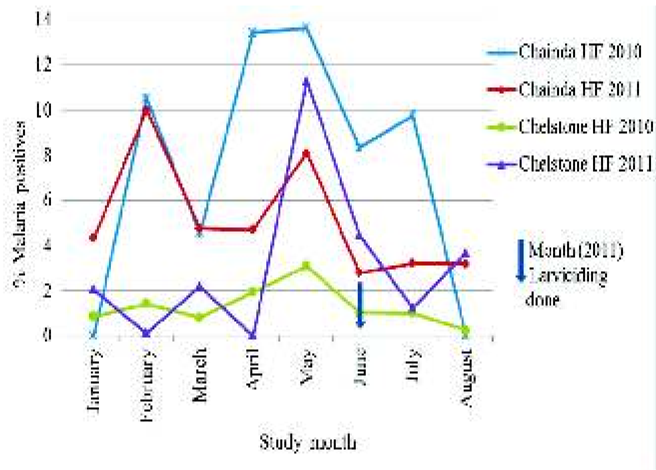
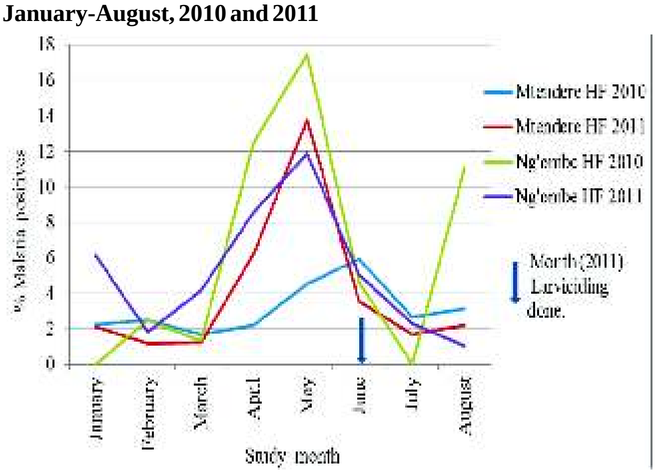


Figure 1: Malaria rates in Mtendere and Ng'ombe compounds, January-August, 2010 and 2011



DISCUSSION AND CONCLUSION

Both larvicides, *Bacillus sphaericus* and *B. thuringiensis* var., *israelensis* have been reported to be very efficacious against mosquito larvae and malaria incidence when sprayed on water bodies by aeroplane and hand, respectively[18, 8]. In this study, trained community volunteers conducted the manual spraying of breeding sites in their residential catchment with the view of enhancing community ownership of the programme. Results from this work corroborate these earlier findings[19, 20].

The observed reduction in the incidence of malaria in all four study areas, following larviciding using Bti and Bs biolarvicides is attributable to the reduction in densities of malaria vector populations. However, there was also a marked disparity in the incidences as detected by the RDTs and microscopy. To illustrate, in Chelstone and Chainda there was a rise in the incidence of malaria as diagnosed by RDTs, while the incidence of malaria as diagnosed by microscopy services continued to drop two months following the intervention. This could be a function of the difference in levels of specificity and sensitivity of the two diagnostic tools[21].

It should be mentioned here that other malaria vector control interventions were being implemented in Lusaka urban district at the time that this study, was undertaken.

The national malaria control programme re-introduced IRS in the district in 2003 with a coverage of 16,000 housing units, in integration with ITNs. The programme was expanded each subsequent year, reaching a coverage of 300,000 houses by the year 2007 and 336,000 houses in 2010[22]. Over 336,000 houses in the district, including the study areas, were already protected against malaria through IRS and ITNs by the time the present study was embarked upon[21]. The prevalence of malaria in the district at the time of introducing the larviciding programme was estimated at 2 percent[1].

However, the closest date of application of insecticides by the IRS programme was September, 2010, which was in the range of nine to eleven months post spraying and far beyond the residual efficacy of the IRS products which are; four to six months for Pyrethroids and two to six months for Organophosphates and Carbamates[5]. Similarly, the closest date of acquisition of ITNs or LLINs and retreatment of ITNs was December, 2010, i.e. Six months prior to the larviciding in the district. This suggests that some ITNs might have been efficacious against human-host seeking mosquitoes entering the houses, supplementing the impacts of larviciding. The malaria positive rates showed reductions of 53- 72% in the study areas in the first month and 1-54 % in the second month in two compounds, and an unexpected 66 percent increase in the Chelstone compound.

Similar observations were made by Majambere et al., (2010)[23] in the Gambia where larviciding did not correlate positively with malaria trends in the control areas. Studies in Tanzania associated larviciding with 78% reductions in parasite infections[24] and 92% reductions in human exposure to malaria conferred by the two biolarvicides[19, 20].

Malaria control efforts through larviciding with mosquitocidal bacterial products of *Bacillus thuringiensis* var. *israelensis* and *B. sphaericus* have historically and recently impacted positively on vector mosquito species and malaria incidences[16, 8, 24, 25, 26]. Fillinger and co-authors (2009)[24] have highlighted that the costs of implementing larval source management with larvicides can be equated to those of Insecticide Treated Nets and Indoor Residual Spraying and could be cheaper if the larviciding could be targeted spatially and temporally, and breeding sites were reduced by environment management.

In one of the four study areas, the observed slight rises in the incidences of malaria a month following the drastic drop in the incidence of the disease could have been as a result of imported malaria cases from outside Lusaka urban district. This view is further supported by the fact that no primary malaria vector mosquito species were collected both as larvae and adults in this study. Two cases of suspected local transmission of malaria i.e. cases of malaria involving people who did not travel outside Lusaka urban district but later tested positive for malaria, were reported in Ng'ombe and Mtendere compounds. These observations were however, within the observed prevalence rate of two percent reported for Lusaka urban district. The malaria incidence rate for the rest of Zambia has been estimated at 2-30%[1].

In conclusion, spraying *Bacillus thuringiensis* var., *israelensis* and *Bacillus sphaericus* mosquito biolarvicides over freshwater bodies in this study, led to drops in malaria incidences in the study areas. Malaria positive cases diagnosed in the study areas following larviciding were probably all imported from outside Lusaka urban district. The study thus recommended the expansion of targeted larval source management using the biolarvicides Bti and Bs as a supplementary malaria control strategy in parts of the country where IRS and ITNs are the primary vector control interventions in integrated fashions or alone in areas without vector control activities but are eligible.

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Appendix 1: Study areas in Lusaka urban district