MICROBIAL CONTROL OF MOSQUITOES IN URBAN AREAS

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INTRODUCTION

In terms of morbidity and mortality dengue is the most serious arbovirus disease confronting mankind. Approximately 2 billion people in the tropics, especially Asia, the Western Pacific region, the Caribbean, as well as Central and South America, live under the risk of dengue infections (Halstead, 1980 and 1982). The economic losses caused by this disease are immense. Due to its excellent adaptation to human habitation, *Aedes aegypti* became the principle vector of dengue and dengue haemorrhagic fever (DHF). The breeding sites of this mosquito vary widely, ranging from water containers, which are the most common breeding site, to tyres, rock pools, leaf axils, and tree-holes.

Due to uncontrolled urbanization in many tropical areas, the provision of drinking water has become a critical problem in less developed areas of the fast growing cities. In the absence of tap water, water for daily use has to be obtained from wells and then stored in containers. Such containers provide excellent developmental conditions for *Aedes aegypti*.

The rapid growth of many large cities raises also the problem of disposal of sewage. The immature stages of *Cx. quinquefasciatus*, as the main vector of lymphatic filariasis, predominantly inhabit sewage drains, cess pits and pools filled with eutrophic waters. Lymphatic filariasis persists as a major cause of clinical morbidity and a significant impediment to socioeconomic development in much of Asia, Africa, the West Pacific as well as in certain regions of the Americans. At least 120 million people are infected with the nematodes *Wuchereria bancrofti, Brugia malayi* and *Brugia timori*. More than 90% of all infections are caused by Wuchereria bancrofti which is mainly transmitted by *Cx. quinquefasciatus*.

Due to high water prices and limited natural water resources in many moderate climatic zones people catch rainwater in artificial containers e.g. to irrigate their garden areas. These containers are the most abundant breeding sites of the so-called house mosquito *Culex pipiens*. In rural areas cess pools provide ideal breeding conditions for this mosquito species due to the high content of organic substances in the water.

INTEGRATED CONTROL OF MOSQUITOES IN URBAN AREAS

Mosquito control can be accomplished with chemical, physical and biological control tools. For the most part control operations are predominantly aimed at the adult stage through application of residual chemical insecticides (Lacey and Lacey, 1990). However, the often quick onset of resistance, the increasing costs for chemical insecticides and the concern for environmental damage have directed increasing interest towards environmentally safe and effective biological control tools as part of an integrated control strategy in order to reduce dependence on chemical insecticides. Integrated mosquito control should be an ecologically based approach that may involve several complementary interventions including e.g. microbial and biological control measures, environmental management and reduction of human mosquito contact based on community participation. The discovery and development of toxin-producing mosquitocidal bacilli such as *Bacillus thuringiensis israelensis* (B.t.i.) and *Bacillus sphaericus* (*B. sphaericus*) inaugurated a new chapter in biological control of mosquitoes and black flies (Becker and Margalit, 1993; Margalit *et al.*, 1995). The rapid exploitation of microbial control agents was aided by their specific properties such as high efficacy against the target organisms, the relative ease with which they can

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be mass produced on an industrial scale, the enormous environmental safety, ease of handling, relative low risk for the development of resistance, cost-effectiveness and their simple integration into control programmes involving community participation. The insecticidal activity is based on the occurrence of proteinaceous parasporal crystalline inclusions (the so-called protein crystals) in the bacterial cells. The parasporal body of B.t.i. contains 5 major proteins (MW: 135, 128, 78, 72 and 28 kDa) and *B. sphaericus* at least two major proteins (MW: 41,9 and 51,4 kDa). The selectivity of the bacilli derives from a variety of factors: (1) The protein crystals (inactive protoxin) must be ingested by the target insect, and this depends on their feeding habits. (2) Midgut proteases cleave the protoxin into biologically active toxins in the high pH of the midgut. (3) The toxins (several toxins act synergistically together) must then bind to cell surface receptors (glycoproteins) of the midgut epithelial cells of the target insect. This disturbs the osmoregulatory mechanisms of the cell membrane, thereby swelling and bursting the midgut cells. The larva consequently becomes paralyzed and dies in a short time, depending upon the amount of toxin ingested. It is assumed that the synergistic effects reduce the likelihood of resistance.

The nontarget organisms that do not activate the protoxin into the toxin or without the specific receptors on their intestinal cells remain undamaged. The toxicity of B.t.i. is restricted to mosquitoes and black flies and at higher dosages to a few other nematocerous families. *B. sphaericus* is toxic to a much narrower range of target organisms. Certain mosquito species such as *Culex quinquefasciatus* and *Cx. pipiens* or *Anopheles gambiae* are highly susceptible whereas *Aedes aegypti* is more than 100-fold less susceptible. Black fly larvae as well as other insects (exception: psychodids), mammals, and other nontarget organisms are not susceptible to *B. sphaericus*. The high potential of *B. sphaericus* as a bacterial control agent lies in its high efficacy against *Culex quinquefasciatus* as the main vector of *Wuchereria bancrofti* and its ability to recycle or to persist in nature even in very polluted water (Becker *et al.*, 1995). For several years both bacilli have been successfully used against mosquitoes especially in the urban environment where usually the breeding sites are defined and easily accessible.

The control of mosquitoes in containers

The main methods of mosquito control in containers are:

- Source reduction by means of environmental sanitation. The elimination of all non-essential water containers functioning as breeding water for mosquitoes is the most effective method in terms of long-term reduction of the mosquito population and of the costs associated with it.
- Protection of water containers, for example by means of lids, to prevent larval breeding.
- Release of larvivorous fish or copepods as predators of larvae.
- Observance of a "weekly dry day", meaning that the containers are to be emptied at least once a week.
- Cleaning the containers before and after the rainy season can also help to reduce mosquito populations.
- Larviciding.

Sustained "community participation" is one of the strongest asset of a successful campaign. An integrated community-based approach, with volunteers, is the most promising method of motivating a community to participate in mosquito control programmes.

MICROBIAL CONTROL OF *AEDES AEGYPTI*

In spite of all efforts, it has not been possible to overcome the dengue problem. All that has been achieved in most epidemic areas is the prevention of an even more drastic increase in the number of cases.

Up until now in most DHF epidemic countries, the organophosphate insecticide Abate (TEMEPHOS) has been the most commonly used chemical for mosquito control in water containers, which usually contain 200 liters of water. Abate is also sold to private people; for example, as a 1%-sand granulate. One package contains 10 grams and is sufficient for 100 liters of water. Application at two-month intervals is recommended.

However, many people do not like the rotten egg-like odour of this chemical in water used for consumption and the household. Furthermore, resistance against Abate could be a problem.

For these reasons, there was the need to develop new approaches for controlling the vector of dengue haemorrhagic fever. One of the most promising microbial agent for mosquito control is *Bacillus thuringiensis israelensis* (B.t.i.), which has been used in Germany for many years with excellent results (Becker and Rettich, 1994).

B.T.I.-TABLETS (CULINEX) – A NEW FORMULATION AGAINST MOSQUITOES BREEDING IN CONTAINERS

In order to provide the public in Germany with an effective, inexpensive and environmental safe tool for the control of mosquitoes breeding in containers, such as *Culex pipiens*, a fizzy tablet based on B.t.i. has been developed and registered as CULINEX®-Tablets. Since 1992 several millions of Culinex-B.t.i.-tablets have been successfully used against *Culex pipiens* which breeds predominantly in containers and other artificial water bodies in Germany.

In comparison to conventional insecticides the Culinex-B.t.i.-tablets show several advantages:

1. They are safe for humans and the environment. This is a considerable advantage over many traditional insecticides as neither the operator nor the house occupier is exposed to danger. For this reason, B.t.i.-tablets are particular suitable for programmes based on *Community Participation* and the use by volunteers, such as house occupiers (Becker 1992).
2. Application is simple. No precautionary measures are necessary. The calculation of the effective dosage is easy. One to two tablet are usually enough to treat a container with 100–200 litres of water.
3. B.t.i. tablets are particular suitable components for integrated control programmes. Because of their selective effect they do not kill mosquito predators, such as fish or copepods which can be introduced in the containers inhabited by larvae of *Aedes aegypti.*
4. B.t.i. tablets can be easily distributed. They can be sold on the market, since this formulation is accepted by the users (costs for one tablet approximately 0.15 cents).

The tablet formulation has to fulfill the following prerequisites in order to be able to fight *Aedes aegypti* larvae:

1. It has to dissolve equally in the water body. Because of the rapid knock-down effect and the high level of efficiency of the B.t.i.-tablets, the success of the treatment can be monitored within a few hours of the application.
2. The toxin crystals have to persist at the bottom and on the container walls in order to serve the larvae as food and to provide a sustained effect for several weeks. Taking and refilling of the water should not significantly affect the long-term effect.
3. The water should not become turbid and should not smell in order to avoid the impression of contamination.
4. Contamination of the water body should be avoided. The B.t.i. material used for the production of the tablets should be hygienically pure and free of bacteria or spores. In this context, sterilized B.t.i. material is recommended for the production of the tablets.
5. Long-term storage can be guaranteed when appropriately stored. The tablets can be kept for more than a year without any significant decrease in their activity.

THE USE OF CULINEX-B.T.I. TABLETS IN TROPICAL COUNTRIES

1. Tests of B.t.i.-tablets in Jakarta, Indonesia

Within the scope of a cooperation programme between the Health Department of Jakarta, the University of Indonesia in Jakarta, the University of Heidelberg and the KABS a new tablet formulation was first tested against Ae. aegypti larvae in Jakarta (Becker *et al.* 1991). The influence of food attractants, stirring of the water as well as the efficacy of the tablets in plastic containers and earthen-ware jars was determined. A long-term effect of about 30 days with a mortality rate of 86.5–97.6 percent could be achieved.

2. Dengue control Cùcuta, Colombia

Within a mutual cooperation between the PHC-Project, NORSSALUD, Cúcuta, the Liverpool School of Tropical Medicine, the University of Heidelberg and the KABS, a study on dengue control was undertaken in a poor urban area in Ccuta, Colombia (Kroeger *et al.*, 1995). We had three objectives: (1) to describe people's knowledge, attitudes and practices regarding dengue fever, the transmission of the disease and possible protective measures; (2) to analyse the infestation of the community with *Aedes* larvae; and (3) to test the efficacy of Culinex- B.t.i. tablets with respect to the level and duration of reduction of *Aedes aegypti* larvae in water tanks.

In the city of Cùcuta (600,000 inhabitants) in the Northeast of Colombia, next to the border with Venezuela, two areas, Comuneros and Dona Nidia, were selected for the field trial. During the baseline study in each of the two areas more than 1000 households (including 5,195 people) were interviewed, and the mapping of the breeding sites was carried out in 967 houses (634 in Comuneros and 333 in Dona Nidia). In spite of all promotional efforts by the local vector control programme the proportion of people who didnot know anything about the disease transmission was high (30% in Comuneros and 47% in Dona Nidia). Therefore, a large number of breeding sites was infested with *Aedes* larvae. The house index was 61 and the Breteaux index 96. Most people (91%) were aware of the insecticide spraying carried out by the control programme. Approximately 33% provided negative comments about spraying. 30% complained about the smell of malathion or because they had got cough or other irritations in the respiratory tract.

The large water tanks (500 litres) for water storage were the most important breeding sites (75.5%).

A total of 180 water tanks infested with Aedes larvae were treated either with one tablet or 2 tablets per 50 litres (each fizzy tablet contains 30% of B.t.i. powder – potency: 8.000 ITU/mg). 69 tanks were checked on a weekly basis. A tank was classified as being reinfested when one or more third or fourth instar larvae had been detected. 22 tanks served as control. Tanks treated with 2 tablets were protected against re-infestation at least for one month. Even after 48 days the infestion in untreated tanks was significantly higher than in the treated ones. The higher the dosage the longer the long-term effect. One tablet per 50 litres provided control for about half a month.

At the end of the observation period, 58% of the 261 interviewees stated that they would prefer fizzy B.t.i.-tablets over Abate. The main reasons given were: water quality decreases with Abate (50%), B.t.i. has a better effect (33%).

LARGE-SCALE FIELD TRIALS OF EFFICACY OF *BACILLUS SPHAERICUS* AGAINST *CULEX QUINQUEFASCIATUS*

Whereas, B.t.i. was found to exhibit a high level of insecticidal activity towards larvae of practically all mosquito species as well as black flies, *B. sphaericus* shows significant residual activity against *Cx. quinquefasciatus* and *Cx. pipiens molestus* even in highly polluted breeding habitats (Ragoonanansingh *et al.*, 1992, Yuan Fang-Yu *et al.*, 1994). Due to these properties, B. sphaericus is already successfully used by tons in many mosquito control programmes mainly in zones with a moderate climate.

In 1990 an informal consultation was organized by TDR in order to develop a strategy for large-scale field trials (WHO, 1994). The goals of these trials were to study the impact of a suitable formulation of *B. sphaericus* on *Cx. quinquefasciatus* populations and, if feasible, its effect on the transmission of filariasis in various parts of the world (Brazil, Cameroun, India, Sri Lanka, and Tanzania).

All projects had to follow a protocol for large-scale field trials with *B. sphaericus*, developed by TDR. The study areas were divided in a operational and check area, and the projects were conducted in four phases.

Phases of the projects

During *phase I* a baseline collection of data was conducted including mapping of the breeding sites in the operational area and parasitological studies to assess e.g. the microfilaria rate and density.

During *phase II*, the preparation phase, basic entomological data such as larval densities, adult biting rates as well as adult infection and invectivity rates were determined.

During *phase III*, the implementation phase, the application procedure was standardized. The actual potency of the formulation in use, the effective dosage based on the susceptibility of local field-collected mosquito larvae as well as the minimum and optimum effective dosage was assessed.

Design of the control strategy

Based on the results achieved during the preparation phases, the strategy for the regular larviciding has been designed and the spraying of *B. sphaericus* has been conducted for about 18 months. All entomological and parasitological indices have been continued during *phase III* and during *phase IV*, the follow-up phase, for additional 6 months, when larviciding has been stopped. In the check area the adult mosquito density and the parasitological data were evaluated during all phases. Only slight modifications and adaptations were allowed due to the specific needs and situations in the trial areas in order to obtain data which could be compared to allow a precise assessment of the impact of *B. sphaericus* treatments on the vector population in various parts of the world under different conditions. The following parameters have been important for the design of the strategy:

1. Sufficient information on the climatic conditions such as occurrence of rainy and dry seasons which influence the mosquito densities and the efficacy of the treatment (e.g. the control agent could be flashed during heavy rainfalls);
2. Precise knowledge on the fluctuation of the mosquito population. Based on this knowledge the timing for the treatment has to be determined in order to reduce the mosquito population at that time, when the population is most sensitive in its development (e.g. treatment before the maximum of adult population is achieved to avoid the maximum or at the beginning of a low level of development to reduce the population to a very low level). Thus the prerequisites for the development of the following mosquito populations are drastically reduced.
3. The efficacy and long-term effect of the product in various breeding types. The long-term effect can vary from breeding type to breeding type. Therefore the sequence of re-treatments had to be adapted to the local situation e.g. shaded, stagnant water bodies had to be treated bi-monthly or less but drains with flowing water weekly or bi-weekly.

   The phases of transmission of the parasite have also to be considered in order to reduce the mosquito population especially when transmission occurs and to such a level that transmission is avoided.

   The output of these projects led to a better understanding of a proper design of mosaic-like control strategies for the use of *B. sphaericus* under different ecological conditions.

In the field tests a remarkable impact of *B. sphaericus* use has been observed in reducing vector biting density by 80% through bi-monthly treatment of mosquito larval habitats; in addition a significant decline in the proportion of Culex carrying filarial infective larvae has been achieved. The cost for vector control programmes using B. sphaericus has been estimated as less than US $0,5 per person per year in areas where breeding habitats of mosquito vectors are very common.

CONCLUSION

From these results the conclusion can be drawn that fizzy Culinex-B.t.i.-tablets are a promising formulation in the fight against *Aedes aegypti* as the vector of Dengue. Culinex tablets are effective and well accepted by the users. The interest in using B.t.i.-tablets in urban areas in Asia is already growing. The fizzy B.t.i.-tablets can be a sensible supplement or even replacement for chemical insecticides such as Abate. It can also be assumed that, by applying B.t.i. in integrated programmes, the onset of resistance can be prevented at least for the time being.

It is already obvious that with the discovery of potent B. sphaericus strains a new weapon in the fight of lymphatic filariasis is found which can be a suitable tool in an intergrated appoach in the control of the disease. It is to be hoped that, with the support of international and national agencies wide-scale programmes for the integrated control of *Cx. quinquefasciatus*, as the main vector of lymphatic filariasis, will be implemented. The onchocerciasis programme in West Africa could serve as a model.

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