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REVIEW OF PROTOCOL FOR THE USE OF BACILLUS THURINGIENSIS VAR. ISRAELENSIS FOR MALARIA CONTROL IN THE HONDURAS HEALTH SECTOR II PROJECT

December 12 - 16, 1988

by

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and

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I. INTRODUCTION

At the request of USAID/Tegucigalpa, Drs. Arata and Lacey of the Vector Biology and Control Project (VBC) of USAID/S&T/H undertook this consultancy. The Scope of Work (SOW) developed by USAID Tegucigalpa for VBC was to review the protocol entitled "Evaluacion de la capacidad y eficacia del Bti en el control de la malaria en areas de alta transmission en Honduras." The implementation of this protocol is planned as a major part of the malaria control program of the newly (1988) established Health Sector II Project of USAID/Honduras.

The basic document contains a good overview of the evolution of the malaria situation in the country. However, the protocol contained within the document lacks sufficient information on laboratory and field trials, is sketchy on details, includes too broad a range of Bti concentrations to be tested, and fails to detail the formulations and methods of application (field trials) to be tested. An alternative plan of action and procedures are provided in this report in the following sections.

The authors spent two days in the field (December 13-14) to evaluate proposed field sites. The sites visited were not an exhaustive representation of all areas suitable for initial application of Bti, but provided sufficient variety to differentiate between potential test sites and sites that would not be suitable for field trials.

Finally, recommendations are made in this report regarding the amount of Bti that would be required for the trials proposed, as well as the equipment and technical assistance (TA) needed during the preliminary phases of laboratory and field testing.

The title and focus of the protocol, "Evaluation of the value and efficacy of Bti in the control of malaria in areas of high transmission in Honduras," should be changed. We suggest that Bti be tested to determine where it will be effective and feasible, and not only in areas with high transmission rates. Some areas described in the text where there is high transmission, such as El Progreso, may be unsuitable for use of Bti without concurrent use of other major control measures.

II. FIELD TRIP

December 13-14, 1988

For two days, Drs. Lacey, Arata and Margalit, accompanied by Ing. José Gomez, Chief of Operations (DCV/MSP), visited four localities in the northwest of the country. The purpose was to visit localities in malarious areas to be considered as potential sites for field evaluation of Bti. Those visited and described below do not represent all of the potential sites in the country. Other areas described in the text of the protocol (e.g., Guapinol, Catacamas, Quebrada de Arena, pp. 9-10) should be investigated in the early stages of the project.

Localities Visited

A. El Progreso - This is an urban setting of high malaria prevalence. Breeding sites were examined in extensive areas of rice cultivation adjacent to the city, where larval populations are reported to be high during the summer. Other breeding sites in the city are caused by clogged drainage canals and rain-filled ponds. There has been no intradomiciliary spraying for several years due to poor community cooperation and lack of resources.

Although rice fields in the area could be treated with Bti, the all-important urban breeding sites cannot. The rice fields are not planted in a synchronized manner -- the fields remain totally inundated for about 5 months a year, but there is some standing water at all times.

Malaria control operations recommended for such areas are:

1) Intensification of case finding and chemotherapy.

2) Identification and elimination of urban breeding sites.

3) Intradomiciliary spraying following a malaria education campaign (also for source reduction).

4) Possible application of Bti in adjacent rice fields if transmission continues in these areas and they are shown to be major production sites of anopheline vectors.

Summary: Because of the impracticality of using Bti in such an urban setting, El Progreso is not recommended as a site for initial field trials with Bti.

B. **Inundated ponds near Potrerrillos** - This area reportedly has low to moderate malaria prevalence. Until two years ago, the Division de Control de Vectores (DCV) monitored larval populations. The area is flooded by rain and river overflow and supports high anopheline larval populations. The surface area varies considerably during the year.

The area is easily accessible and would serve as a good test site for Bti formulations, dosages and application techniques. Geographical reconnaissance during wet and dry seasons will be required, along with estimates of the human population and malaria prevalence during the past years. These figures should be available at the local health post.

Summary: The area is recommended as a possible site for field trials with Bti.

C. El Cajón - This recently completed hydroelectric dam has produced an extensive dendritic reservoir. The human populations are not yet stable and the malaria control personnel are continuing their geographical reconnaissance. Entomological data are poor, but An. pseudopunctipennis, a secondary vector, is reported to be the most common mosquito species. The area is at, or above, the normal altitudinal range of An. albimanus, the major malaria vector in the country. Breeding occurs along the lake margins and in small ponds caused by the fluctuating water level.

Malaria reporting is poor: the area most heavily infected is the municipality of La Victoria (c. 10,000 inhabitants). In 1987, 1,490 blood samples (242 -- 16 percent positive) were taken in the first semester and in 1988, 3,637 (242 -- 3 percent positive) were taken. The resulting API for 1987 was 4.18, which is below the API national average of 4.4 that year.

The DCV explained that a large portion of the population is migratory, practicing slash and burn agriculture, so that sampling and interventions are difficult. The local DCV office has very limited manpower resources. The nature of the shoreline and the area's inaccessibility to vehicles would make larviciding very difficult to carry out and evaluate.

Summary: The El Cajón area is not recommended as a site for field trials with Bti.

D. **Rural Cooperatives near Comayagua** The last area visited was the cooperative, Ivan Betancour de Comayagua. There are reported to be about 20 such cooperatives in the area with populations of 350-400 each. The populations are stable, engaged in small crop production (vegetables, corn, etc.) for local consumption and sale.

In general, one small pond (ca.2 ha) produced by damming serves as the water supply for two adjacent cooperatives. Although no systematic larval surveys have been conducted, these ponds are said to be, and appear to be, heavy anopheline breeding sites. Each cooperative has a volunteer collaborator (VC). In the one visited, 80 slides had been taken in 1987 (20 positive) and 88 taken thus far in 1988 (27 positive). This limited sample would suggest an API of 5.0, slightly above the national API average of 4.4 for 1987. The breeding sites are readily accessible and of a suitable, moderate size for treating the margins of the ponds with backpack sprayers.

Summary: The rural cooperatives in the Comayagua area would be suitable for field trials with *Bti*.

III. LABORATORY BIOASSAY

The standardized assay used by industry and researchers is recommended for determining the activity in International Toxicity Units (ITU) of primary powders and flowable concentrate formulations (FC). Granular formulations should be assayed at an LC_{95} of the active ingredients (A.I.), i.e., the amount of primary powder in the formulation (usually 5 to 15 percent) to determine if the formulation process hinders performance or if the active ingredients are satisfactorily potent. An essential component for conducting rapid laboratory bioassay will be the establishment of three colonies of An. albimanus: one from field-collected larvae, one from adult populations that are susceptible to chemical pesticides and another from an (adult?) population that is resistant. An Aedes aegypti colony is also necessary for standard potency bioassays. It is highly recommended that these colonies be established as soon as possible.

The laboratory procedure described below is adapted from a number of published reports and is provided in detail because the original articles may not be available to the DCV entomologists.

The recommended procedure follows:

Using early fourth instar larvae from colonized field collections, count out 20 larvae/100 ml cup for a total of 18 to 20 cups for each material (primary powders, FC, standardized powder) to be tested. Based on preliminary assays, use five concentrations of powder or FC that range between an approximate LC_{50} and LC_{95}, with two concentrations above and two below the estimated LC_{50}. The test water should be distilled or deionized. If none is available, aged tap water can be used (let the water stand at least 48 hours). Serial dilutions of a stock suspension of the test materials can be prepared by 8:2 dilutions or greater (i.e., 7:3, 6:4, etc.) to obtain the desired endpoint concentration in the test cups.

The minimum amount of fluid to be transferred when making serial dilutions should always be greater than or equal to 10 ml. Magnetic stirrers can be used to suspend and mix test materials. Stock suspensions should be thoroughly shaken and/or mixed for 30 minutes. Subsequent dilutions should be mixed for at least ten minutes. It is important to avoid wetting agents and heat. Magnetic mixers can create a concentrated vortex of material; restir the liquid with a pipette before making transfers.

When the material is added to the test cups, the total amount of water in the cup should equal 100 ml. (i.e., if the treatment suspension that is being added is 10 ml, it should be added to 90 ml of water already in the cup; if the amount of treatment suspension is 15 ml, it should be added to 85 ml, etc.).

Larvae should then be held at 27°C without food. Mortality is assessed in each container 24 hours following treatment. This process should be repeated on two more occasions for a total of three replicated tests over time.

Determination of LC_{50} and LC_{95} will be calculated by Probit analysis after correction for check mortality (untreated larvae) with Abbott's formula.

A replicated series of tests should also be run with Aedes aegypti larvae for each primary powder base of each granular formulation, FCs, and with a standard powder preparation in order to assign or confirm potency in ITU's. Standard powders can be obtained from the Pasteur Institute or the U.S. Department of Agriculture.

Summary of Protocol for Laboratory Bioassay of *Bacillus thuringiensis* var. *israelensis*

Species: Anopheles albimanus and Aedes aegypti

Larval Age: Early 4th instar

No. larvae: 20/cup; 3 cups/conc. and check

Temperature: 27°C

Duration of exposure: 24 hours

Food: None

Volume of water: 100 ml.

Assay container: wax coated paper cups or equivalent (capable of holding over 100 ml)

Number of replicate tests: three (on separate dates)

Procedure: Count larvae; 20/cup; 18 cups/test material. Three cups per conc. (5 conc.), three cups for untreated controls.

Mix and serially dilute stock mixture (30 min. for stock, at least 10 min. for each serial dilution). Apply diluted suspension to cups. Total fluid/cup should equal 100 ml.

Hold larvae at 27°C for 24 hours with no food. After 24 hours determine mortality. Correct for untreated control mortality.

IV. Proposed Time Schedule (1989) for Lab, Preliminary Field Trials and Required TA

1989

	Activity	TA/Time
A.	Begin laboratory assays in April -- finished by June (Colonies of An. albimanus and An aegypti should be started as soon as possible in 1989.)	Approximately two months required for bioassays; TA required for one month to supervise labwork and for final selection of field trial sites.
B.	Late June - early July Preliminary field trials I near Potrerrillos and other locations to test dosages, formulation and frequency of application (malaria data monitored by local V.C.).	One month of TA required. Studies will continue until November to evaluate methodologies.
C.	August - November Preliminary field trials II in Comayagua cooperatives and possibly in experimental rice field growing areas near Choluteca, starting as soon as effective application rates and dosages are determined in field trial I.	One month of TA required. Studies will continue until November.
D.	Evaluation by DC and two consultants (VBC). Objective: Prepare plan for 1990.	Two weeks.

VI. ESTIMATED BUDGET 1989

1)	TA 3 months (contractor's rates)		=	$37,761
2)	Evaluation 2 persons x 2 weeks		=	14,590
				52,351
3)	Equipment			
	Mist blowers			
	Vehicle			
	Other			40,000
				$92,351
4)	Bti			
	granules 400 kg	=	$ 880	
	(200 each, 2 companies)			
	flowable concentrate 100 l.		$1,000	
	(50 l. each, 2 companies)		1,880 x 2	3,760
		Estimated total	US	$96,111

Operational costs not included,
i.e, gas, national salaries and per diem

At least 200 kgs of granular formulation will be required for small plot trials from each of two suppliers (for example, Sandoz, Inc. and Abbott Labs). Total = 400 kgs.

At least 50 liters of flowable concentrate formulations will be required from each of the two suppliers. Total = 100 liters.

A minimum of five backpack granule spreaders and five hand held ULV application sprayers will be required for preliminary trials in 1989.

VII. SPECIAL POINTS

1) **Local Production of Bti** - It is far too early in this program to consider national production of Bti. It is more important at this time to determine whether Bti has an important role to play in suppression of malaria under the tropical conditions of transmission in Honduras. If this is demonstrated, the quantities and formulations required annually should be determined so that a cost-evaluation can be made to determine whether local production is justifiable.

2) **Aerial applications** - Such application has been used successfully to treat large areas of agricultural crops (e.g., rice) with Bti in the U.S. and other countries. Should Bti prove effective in Honduras under such conditions, aerial application (using the same equipment commercially used for insecticide spraying and seeding) should be considered.

   Helicopters made available by the military, which will not be available for normal malaria control operations, should not be used for Bti application in trials. To properly test efficacy and cost of application, only equipment available for routine control measures should be used in trials.

3) **Community Participation** - Although community participation is desirable and even essential in malaria control operations, it is not specific to application of Bti. Bti will be applied as a larvicide under the control of the Division of Vector Control (DCV), not by community action groups.

   Community participation is an integral part of the malaria control program and should be a general activity associated with integrated control. It does not have a specific place in this research program or protocol.

4) **Vector Control with Bti in the Integrated Pest Management (IPM) Context** - Bti will be used as a component in an integrated control program, not as a single measure for malaria control. Therefore, following demonstration of efficacy under local conditions and determination of the proper dosages, formulations and frequency of application, Bti use will have to be integrated with physical larval control measures, residual house spraying in some areas, and distribution of anti-malarial drugs in the overall malaria control program designed for various regions of the country. Therefore, it is important to schedule TA from expert consultants who have worked in IPM programs of mosquito control rather than employing only employ specialists familiar with Bti.

5) **Epidemiological Considerations** - Malaria has decreased in Honduras over the past 10 years, dropping from 41.9 thousand during 1978-82 to 29.4 thousand during 1983-87. The last three years have shown progressive declines in reported cases, from 34,000 (1985) to 29 thousand (1986) to 19,000 (1987). Although these are reported cases (i.e., microscopically positive blood films based on Passive Case Detection (PCD) using the volunteer collaborator system), the number of actual cases may be considerably higher. However, a good indication of the lessening severity is the reduction in the proportion of falciparum malaria cases, which has dropped from 12 percent (1978-82) to 5 percent (1983-87). The Annual Parasite Index (API) has been reduced from an average of 12.5 (1978-82) to an average of 5.0 (1983-87). In 1987, the API was 4.4. Local variations (rise, fall) will be observed, but the general picture, by any malariometric measure, displays a downward trend.

As a result, malaria remains highest in areas where control is difficult for a variety of reasons (e.g., El Progreso) -- limited resources, difficult access, reduced community compliance, insecticide resistance, lack of general sanitation and multiple breeding sites. Such areas will also be difficult to control with Bti because a multiple approach is necessary. This considerably reduces the areas where Bti can be properly tested, i.e., in rural areas of low to moderate prevalence. Therefore, the potential role of Bti in an integrated malaria control program will have to be evaluated in such areas based on cost, manpower requirements and the size of the populations at risk in the areas suitable for Bti application.

The title of the protocol "Evaluacion de la capacidad y eficacia del Bti en el control de la malaria en areas de alta transmission en Honduras" should be changed in order to correctly indicate that Bti will be used in an integrated control program in areas where its use is shown to be effective, not just in areas of high transmission.

6) **Manpower Resources** - The DCV has critical limitations of human resources for routine malaria control operations such as intradomiciliary spraying, as well as shortages of equipment and supplies. Addition of Physical Larval Control (PLC) and biological control activities will make these personnel and equipment shortages more severe and will possibly have an adverse affect on the overall program of malaria control. To our knowledge, no specific provisions for additional national workers were detailed in the Project Paper.

None of the routine control operations should be delayed or stopped during the testing of new methods of intervention. For both PLC and biological control, there is a need for more field staff to conduct assessments of larval populations. Training or retraining of operational staff will also be required.

7) **TA for the Overall Malaria Control Program** - Proper integration of the proposed interventions into the national malaria control program will require consultants familiar with the Central American malaria situation. An apparent danger in the proposal under review is that there is an excessive emphasis on exclusive use of Bti at this stage to the detriment of the other elements of the control program. If extensive TA (greater than 1-2 months) are scheduled, they should be for integrated control, not just one component. In our opinion, the extensive TA requested by Dr. Margalit and his colleagues is excessive. In addition, there is no detailed work plan for the TA they wish to carry out. TA should be aimed at working with the Honduran staff to make the program sustainable. The present approach is more "We will come and carry out the work." This is unacceptable because it may do more harm than good.

Training in Israel seems to be inappropriate and costly. Participant training should be limited. Techniques of Bti application in the U.S. can be demonstrated as planned by USAID/Tegucigalpa, but in-country training will be the most effective.