Nam, Vu Sinh, Yen, Nguyen Thi, Phong, Tran Vu, Ninh, Truong Uyen, Mai, Le Quyen, Lo, Le Viet, Nghia, Le Trung, Bektas, Ahmet, Briscombe, Alistair, Aaskov, John G., Ryan, Peter, & Kay, Brian (2005) ***Elimination of dengue by community programs using Mesocyclops(Copepoda) against Aedes aegypti in central Vietnam.*** American Journal of Tropical Medicine and Hygiene, 72(1), pp. 67-73.

Am. J. Trop. Med. Hyg., 72(1), 2005, pp. 67–73

ELIMINATION OF DENGUE BY COMMUNITY PROGRAMS USING *MESOCYCLOPS* (COPEPODA) AGAINST *AEDES AEGYPTI* IN CENTRAL VIETNAM

VU SINH NAM, NGUYEN THI YEN, TRAN VU PHONG, TRUONG UYEN NINH, LE QUYEN MAI, LE VIET LO, LE TRUNG NGHIA, AHMET BEKTAS, ALISTAIR BRISCOMBE, JOHN G. AASKOV, PETER A. RYAN, AND BRIAN H. KAY

National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; Pasteur Institute of Nha Trang, Vietnam; Australian Foundation of Peoples of Asia and the Pacific, Hanoi, Vietnam; Queensland University of Technology, Brisbane, Queensland, Australia; Australian Centre for International and Tropical Health and Nutrition at the Queensland Institute of Medical Research, Brisbane, Queensland, Australia

Abstract. From September 2000 to June 2003, a community-based program for dengue control using local predacious copepods of the genus *Mesocyclops* was conducted in three rural communes in the central Vietnam provinces of Quang Nam, Quang Ngai, and Khanh Hoa. Post-project, three subsequent entomologic surveys were conducted until March 2004. The number of households and residents in the communes were 5,913 and 27,167, respectively, and dengue notification rates for these communes from 1996 were as high as 2,418.5 per 100,000 persons. Following knowledge, attitude, and practice evaluations, surveys of water storage containers indicated that *Mesocyclops* spp. already occurred in 3–17% and that large tanks up to 2,000 liters, 130–300-liter jars, wells, and some 220-liter metal drums were the most productive habitats for *Aedes aegypti.* With technical support, the programs were driven by communal management committees, health collaborators, schoolteachers, and pupils. From quantitative estimates of the standing crop of third and fourth instars from 100 households, *Ae. aegypti* were reduced by approximately 90% by year 1, 92.3–98.6% by year 2, and *Ae. aegypti* immature forms had been eliminated from two of three communes by June 2003. Similarly, from resting adult collections from 100 households, densities were reduced to 0–1 per commune. By March 2004, two communes with no larvae had small numbers but the third was negative; one adult was collected in each of two communes while one became negative. Absolute estimates of third and fourth instars at the three intervention communes and one left untreated had significant correlations ($P = 0.009–< 0.001$) with numbers of adults aspirated from inside houses on each of 15 survey periods. By year 1, the incidence of dengue disease in the treated communes was reduced by 76.7% compared with non-intervention communes within the same districts, and no dengue was evident in 2002 and 2003, compared with 112.8 and 14.4 cases per 100,000 at district level. Since we had similar success in northern Vietnam from 1998 to 2000, this study demonstrates that this control model is broadly acceptable and achievable at community level but vigilance is required post-project to prevent reinfestation.

INTRODUCTION

Since 1995, with the assistance of the World Health Organization and various consultants, the national strategy for dengue surveillance and control for Vietnam was redrafted to involve a paradigm shift from emergency responsiveness to a preventative approach using community-based delivery of locally collected predacious copepods of the genus *Mesocyclops*. Ten species of copepods have been recovered from ponds, lakes, and even in water storage containers in Vietnam.[1] The water storage containers were inoculated unknowingly by the process of water transfer or by flooding.[2] The utility of predacious copepods, particularly of the genera *Mesocyclops* and *Macrocyclops*, has been widely reported as being efficient predators of early instars of *Aedes*, *Ochlerotatus*, and *Anopheles.*[3,4] The predatory behavior of *Mesocyclops* is facultative, since these cyclopoid genera also feed on a range of microorganisms, including algae, protozoans, and rotifers.[5]

In 1998, we reported the first eradication of *Aedes aegypti* (L.) without using insecticides at Phan Boi village (400 households)[6] and by 2000, detailed expansion to six communes (11,675 households) in the three northern provinces of Hung Yen, Haiphong, and Nam Dinh.[7]

From the national notifiable disease records of Vietnam, the mean incidence of dengue reported from 1996 to 1999 for the central provinces of Quang Nam, Quang Ngai, and Khanh Hoa was 69–419 cases per 100,000, but the incidence in the three communes in these provinces that were selected for intervention was far higher (467–2,418 per 100,000) than for the provincial data. From September 2000 to June 2003, we implemented phase 2 of this community-based control program with the same previous goals: 1) to reduce the incidence of dengue and dengue hemorrhagic fever (DHF) by controlling or eliminating *Ae. aegypti*, thus raising the quality of life; and 2) to strengthen the capacity of health staff at all levels to implement a community-based program for dengue vector control using the biologic agent *Mesocyclops*. This study details further success using our model of prioritized control based on container productivity and community-driven biologic control supplemented by clean up of discarded articles.

MATERIALS AND METHODS

The key project strategies were based on creating community self sufficiency, recognition of key container types[8] as primary targets of control, recognition of *Mesocyclops* as a local resource mainly for treatment of large water storage containers with a capacity > 100 liters coupled with collection of smaller discarded containers, and aided where possible by intersectoral promotion of recycling for economic gain. Program management combines "top down" (professional advice from the health sector) and "bottom up" (local management, community collaborators, teachers, school children) approaches.[9]

Prior to implementation, the procedures to be used were fully reviewed and approved by the Vietnam Ministry of Health and the Peoples Aid Coordination Committee to ensure that they complied with the guidelines for health projects as specified in a memorandum of understanding between the governments of Australia and Vietnam. At each commune

specified in this study, informed consent was initially obtained from the commune and hamlet chairmen, Youth Union leaders, Peoples Committee leaders, and Women's Union leaders prior to canvassing individual support from all community members.

The following activities were done in setting up the program.

Site selection and community survey. The three communes were selected on the basis of frequent dengue, a committed health staff, a concerned community, and a container-type configuration predisposed to large water storage and therefore suitability for *Mesocyclops* inoculation. Knowledge, Attitude, and Practice (KAP) surveys were carried out later to confirm community attitude that dengue (DHF) was dangerous or a serious problem, and to establish levels of understanding about its etiology, possible control options, and the acceptability of various modes of delivering health messages.

Cam Thanh, Binh Chanh, and Ninh Xuan communes (Table 1) in the central provinces of Quang Nam, Quang Ngai, and Khanh Hoa, respectively, were chosen for interventions, whereas Ninh Binh commune next to Ninh Xuan was selected as an untreated entomologic control.

Establishing community management committees and collaborator networks. Community management committees (CMCs) were established in each project commune (Table 2). The CMCs were composed of representatives of the school, the health service, the local authority, and the community. They were responsible for planning and managing the program activities within the commune and played a key role in mobilizing the community.

Following the formation of CMCs, project collaborators were selected. Key criteria upon which collaborator selection was based included enthusiasm and having spare time and a basic knowledge of health issues. Collaborators were responsible for approximately 100 households each and played a key role in mobilizing the community and bringing vector control activities to the household level by monthly inspection. The numbers of CMC members and collaborators in Cam Thanh, Bin Chanh, and Ninh Xuan communes were 31, 36, and 29 persons, respectively.

Community training. The first training workshop was conducted for 15 health staff from provincial level to commune level in Nha Trang (September 2000). The 12-day workshop combined both theoretical and practical components. Training topics included dengue etiology, biology of the dengue vector, and vector control methods (especially community based methods using the biologic agent *Mesocyclops)*. Pretraining and post-training evaluation showed that participants had developed a good understanding of these core concepts.

Training courses were conducted for CMCs and collaborators from each project site. Courses were conducted by the health staff who attended the workshop in Nha Trang. These courses provided theoretical and practical training in methods for surveillance and detection of mosquito breeding sites; identification of different larvae and mosquitoes; identification of *Mesocyclops*; techniques for breeding and using *Mesocyclops;* and community activities based on community participation. All collaborators participated in the training course and practical field-work activities. Commune level collaborators were generally able to distinguish *Aedes* from *Culex* and *Anopheles* larvae and *Mesocyclops* (or more correctly large cyclopoids) from other organisms. Annual refresher courses were conducted to update knowledge on dengue control methods and to strengthen practical skills on dengue vector surveillance and larval detection.

TABLE 1
Key statistics for project communes in central Vietnam

Province	District	Commune	Status	Houses	Population
Quang Nam	Hoi An	Cam Thanh	Treated	1,511	6,556
Quang Ngai	Binh Son	Binh Chanh	Treated	2,530	11,372
Khanh Hoa	Ninh Hoa	Ninh Xuan	Treated	1,872	9,239
Khanh Hoa	Ninh Hoa	Ninh Binh	Untreated	2,165	10,419

TABLE 2
Numbers of community management committees (CMCs), collaborators, teachers, and school children receiving training in the three communes

Community group	Quang Nam	Quang Ngai	Khanh Hoa	Total
CMC	10	9	9	28
Collaborators	21	27	20	68
Teachers	35	37	48	120
School children	1,151	2,495	1,622	5,268

Each collaborator was equipped with a manual on community-based mosquito control, a uniform and a field kit for surveillance including a net, torch, glass cup, plastic bucket, and notebook for recording data. Collaborators recorded their activities to report to the CMC at the monthly collaborator's meeting. This meeting also served as a forum for collaborators to share information and experiences with each other, raise any difficulties with the CMC, and devise solutions to these problems. The CMC regularly monitored collaborators' activities by accompanying them on household visits. This enabled CMC members to keep abreast of difficulties and develop appropriate strategies.

To improve skills of collaborators and the quality of their activities, project communes regularly conducted collaborator crosschecks in which collaborators were paired to check each others houses. A further quality improvement activity was the "collaborator exchange festival" held in Nha Trang in June 2002. During this activity, collaborators from different provinces were brought together to share experiences. The exchange activity resulted in a number of valuable outputs, including the identification by each group of specific targets for their commune. Collaborators devised and agreed to implement the "three don'ts": don't leave a household unchecked, don't leave a water container unchecked, and don't leave a breeding site untreated.

Schools program. Eleven training courses were conducted for 120 school teachers. All teachers were provided with a manual on community-based dengue control using *Mesocyclops*. These teachers supported the implementation of school-based dengue education activities for students at schools within the project communes: Cam Thanh (1,151), Binh Chanh (2,495), and Ninh Xuan (1,622).

Baseline *Aedes* and *Mesocyclops* surveys. Samples of 100 households were drawn randomly from health center records. Methods for counting the number of larvae in containers included direct counting in small water containers such as flower vases, ant traps, and discards; using a 20 × 20 × 10 cm piece of 100-μm netting to sample larvae in tanks and big jars;

and using funnel traps to trap larvae in drums and underground tanks. Productivity was assessed on the basis of counts of third and fourth instars and for large containers, calibration factors were applied as before (500–4,250-liter outdoor concrete tanks × 17; 220-liter metal drums × 5; and wells/underground tanks × 3.5).[7] Survey results were used to identify the key breeding sites in each project commune and then used to target control activities. Two collectors also collected any resting adult *Ae. aegypti* from inside houses for 15 minutes each by placing tubes over specimens and corking the tube with cotton wool. For each survey, the number of adults was divided by the number of houses surveyed to give an adult density index based on 30 minutes of collecting.

At the same time, the presence or absence of *Mesocyclops* was recorded for all containers surveyed. The predatory species most commonly found in each site were bred and mass produced in the entomology laboratories at the National Institute of Hygiene and Epidemiology and the Institute Pasteur in Nha Trang. *Mesocyclops* were then packed in rubber foam blocks (15 × 15 × 5 mm) and distributed to the project sites as base stocks for inoculation into selected key containers within the communes. In laboratory conditions (temperature = 28–31°C, relative humidity = 85%) 62.2% of the *Mesocyclops* were shown to survive in these foam blocks after 20 days, 40.5% after 30 days, and 6.7% after 40 days. Thus, this method was seen as an effective means for transporting *Mesocyclops* to the provinces from Hanoi. Once *Mesocyclops* populations were established in these containers, collaborators and school children collected aliquots using nets and funnel traps for release into other containers in the community.

Intervention and efficacy. Control methods used included introducing *Mesocyclops* into water containers with capacities > 100 liters, putting salt into ant traps, and/or cleaning-up all discards in and around houses. Twelve entomologic surveys were conducted on a quarterly basis to monitor changes in the estimated numbers of *Ae. aegypti* larvae, and also to redefine key container type as the most important were progressively eliminated. Control efficacy was scored on the basis of numbers remaining in subsequent surveys and expressed as a percentage of the numbers in the pre-intervention survey.

The capacity of *Mesocyclops* to survive in water containers was evaluated through quarterly surveys by the project team and from monthly field data collected by collaborators. The same nets were used to capture both mosquito immature forms and copepods.

Disease surveillance. Patients presenting at the Health Center in study communes with symptoms of dengue fever (DF) but no vomiting or diarrhea and without cough or evidence of upper respiratory tract infection provided a blood sample to be tested for the presence of anti-dengue virus IgM in an IgM-capture enzyme-linked immunosorbent assay (ELISA)[7] and or for virus isolation. At the district and provincial level, patients continued to be identified by whatever criteria were in use prior to this study and, in accordance with the guidelines of the National (Vietnam) Dengue Control Plan, sera from at least 5% of these patients were tested for the presence of IgM antibody to dengue virus. Each provincial laboratory performing IgM-capture ELISAs for this study participated in two serology Quality Assurance Programs each year and, where necessary, results from the provincial laboratories were confirmed at the National Institute of Hygiene and Epidemiology in Hanoi.

RESULTS

Baseline *Mesocyclops* surveys. *Mesocyclops* naturally existed in each project site. Five, four, and six species of local *Mesocyclops* were detected in Cam Thanh (Quang Nam province), Binh Chanh (Quang Ngai), and from Ninh Xuan (Khanh Hoa), respectively, in 322 habitats sampled. *Mesocyclop*s were found to pre-exist in 3–17% of the water containers at these communes with *M. aspericornis* Daday and *M. ogunnus* Onabimiro present in all three, *M. thermocyclopoides* Harada at Cam Thanh and Ninh Xuan, *M. pehpeiensis* Hu and *M. affinis* Van der Velde in Binh Chanh and Ninh Xuan, *M. ferjemurami* Holynska at Ninh Xuan, and *M. woutersi* Van der Velde and *M. shenzhensis* Van der Velde at Cam Thanh.

Knowledge, attitude and practice surveys. Three KAP surveys were conducted at 930 randomly selected households during October 2000–2002 (Table 3). Initial survey results showed that people's pre-project knowledge of dengue (85.6%) was relatively high, but their knowledge about the "striped mosquito" that transmitted dengue viruses (42.4%) and its breeding sites (59.1–62.4%) was low. Only 7.7% of the residents knew of *Mesocyclops,* but after an explanation that the program only involved expanded release of a locally occurring organism, almost all (95.7%) agreed to accept the program.

During the three years of project implementation, regular community-based health education programs were implemented. Results of the final KAP surveys showed a significant increase in the proportion of people who knew of the dengue vector mosquito and its breeding sites (increased to 97.2%). In addition, the household level activities were shown to have had a noteworthy impact on community attitude and behavior toward dengue control, with 95.1–98.3% of the people having factual knowledge to apply at their own house.

Community programs. *Collaborator activities.* From January 2000 to June 2003, 159,206 collaborator visits were made to households on a monthly basis (Table 4). This was considered to be the most important activity of the community programs. With the motto of "No Larvae, No Dengue", collaborators visited households to provide education on dengue, how it was transmitted, and where dengue mosquitoes bred. Collaborators guided householders to practice simple control methods such as putting salt into flower vases, removing discards, and maintaining *Mesocyclops* in water containers (average of 71% overall). They also informed householders how to detect new dengue cases and mobilize family members to take action against dengue vectors.

School activities. School activities on dengue control varied between the different project sites. Ninh Xuan and Cam Thanh had only primary schools within the commune, while in Binh Chanh both primary and secondary schools were involved in dengue control activities. There were a total of 6 training courses for 120 schoolteachers, 830 lessons on DF/DHF, and 5 competitions on DF for 5,268 school children. In addition, school children were involved in implementing key community campaigns such as the commune-wide clean-ups and the *Mesocyclops* introduction campaigns. Students in Cam Thanh and Binh Chanh also conducted "dengue plays" for community audiences.

Health education and clean-up campaigns. Between 9 and 14 community education campaigns were implemented to in-

TABLE 3
Results of KAP survey of householders in the Cam Thanh, Binh Chanh, and Ninh Xuan communes, 2000–2003*

Question	% Correct answer			
	Oct 2000	Oct 2001	Oct 2002	Improvement
Number interviewed	310	311	309	
Have heard of DF/DHF	85.6	98.5	99.2	13.6
Know symptoms of DF/DHF	25.8	68.8	91.9	66.1
Know DF/DHF is dangerous	97.5	97.7	99.7	2.2
Know DF/DHF is transmitted by mosquitoes	78.1	96.8	98.2	20.1
Know dengue mosquito is the striped mosquito	42.4	91.8	97.2	54.8
Know dengue mosquito bites by day	50.9	66.6	80.3	29.4
Know dengue vector lives indoors	65.2	80.4	93.6	28.4
Understood larval habitats	60.8	70.1	97.2	36.4
Drinking water containers	59.1	67.3	97.2	38.1
Discards, flower vases, ant traps	62.4	72.8	97.2	34.8
Know that dengue control methods are aimed at mosquitoes and larval control	76.7	89.5	95.9	19.2
Knowledge of larval control methods	28.5	56.1	98.3	69.8
Larvivorous fish	19.8	26.2		
Cleaning water containers regularly	53.5	70.2		
Cleaning up and eliminating discards	39.3	60.0		
Introducing *Mesocyclops*	1.2	57.5	95.1	93.9
Previous knowledge of *Mesocyclops*	7.7	83.9	100.0	92.3
Accept *Mesocyclops* release	95.7	97.9	100.0	4.3
Trust and compliance of				
Health workers	94.2	83.5		5.6
Local staff	28.8	54.5		57.7
Mass meetings	19.6	37.5		17.9
Television	24.5	32.6		8.1
Local mass media	11.7	17.6		5.9

* KAP = knowledge, attitude, and practice; DF = dengue fever; DHF = dengue hemorrhagic fever.

crease householder's knowledge of DF/DHF and methods for vector control. Community education activities included commune meetings (total = 690, average audience = 63); loudspeaker announcements (total = 695); 14 locally arranged video shows (average audience = 126); and dengue performances including plays and live performances of folk songs; posters, leaflets, and billboards (total = 13,000). Local CMCs, collaborators, school leaders, and mass organizations were all actively involved in the implementation of these activities. Regular commune-wide clean-up campaigns served to remove discarded waste (101 campaigns resulted in removal of 64,903 containers) resulting in an average 95% reduction of mainly small containers that could act as breeding sites for mosquitoes. This was reinforced by some radio and television coverage of the project.

Vector surveys. Prior to the intervention, the frequency of different container types was established, including their relative contribution to population size estimates of third and fourth instar *Ae. aegypti* (Figure 1). Tanks > 500 liters, tanks < 500 liters, 130–300-liter jars, wells and some 220-liter metal drums were judged suitable for treatment with *Mesocyclops*. At Cam Thanh, Binh Chanh, and Ninh Xuan, respectively, such containers comprised 54.5%, 37.2%, and 44.3% of all containers surveyed and contributed 81.4%, 73.3%, and 62.2% of total immature *Ae. aegypti* estimates.

Twelve surveys for immature and resting adult *Ae. aegypti*

TABLE 4
Statistics of community program activities, September 2000–June 2003

Activity/statistic	Cam Thanh	Binh Chanh	Ninh Xuan	Total
No. of houses visited by collaborators (% of total houses)	41,245 (100)	70,152 (98)	47,809 (96)	159,206 (98)
No. of containers treated by collaborators	12,605	4,476	2,747	19,828
No. of *Mesocyclops* campaigns	5	8	4	17
% containers with *Mesocyclops* (June 2003)	82	67	65	71
No. of education talks with villagers	45,646	84,109	64,647	194,402
No. of education campaigns	10	14	9	33
No. of loudspeaker messages	89	196	410	695
No. of community meetings	215	182	293	690
Total participants at meetings	15,039	15,250	14,057	44,346
No. of video shows	10	4	0	10
Total video audience	1,123	636	0	1,759
No. of posters for families	3,600	5,500	3,900	13,000
No. of cleanup campaigns	26	32	43	101
No. of discards removed	13,819	37,088	13,996	64,903
Total people involved in cleanups	5,239	13,908	3,044	22,191
No of discards; Oct 2000 and June 2003	1,284/89	7,899/250	1,014/174	10,197/513

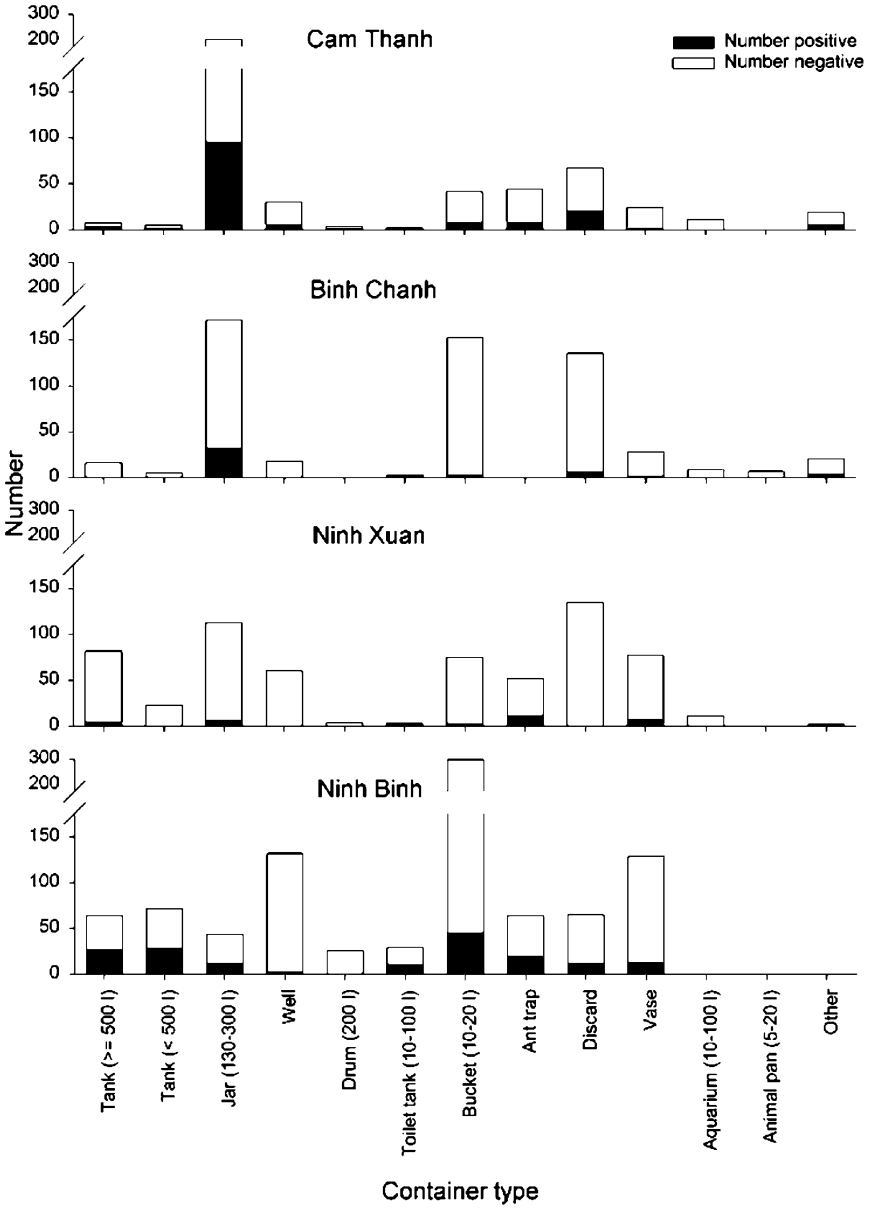

FIGURE 1. Frequency of different container type categories and their positivity (**shaded**) for *Aedes aegypti* larvae at the intervention (Cam Thanh, Binh Chanh, and Ninh Xuan) communes compared with the untreated control commune (Ninh Binh) in central Vietnam.

were conducted from September 2000 (baseline) until June 2003, when the project officially ended (Table 5), using a sample size of 100 houses. Data for intervention communes post-project were available for September and December 2003 and March 2004. Based on our quantitative estimates, *Ae. aegypti* larval populations in the three communes were reduced by approximately 90% after one year, by 92.3–98.6% after two years of intervention, and *Ae. aegypti* populations had been eliminated from Cam Thanh and Binh Chanh with 11 larvae being detected at Ninh Xuan by June 2003. After three years, the September 2003 survey indicated a similar pattern, but the December 2003 and March 2004 surveys indicated residual larval numbers at Cam Thanh and Binh Chanh with Ninh Xuan becoming negative.

Adult indices showed similar reductions from 0.12–1.16 in September 2000 to 0–0.01 in June 2003. Subsequent surveys until March 2004 indicated adult densities of 0–0.03.

Using logistic regression, we demonstrated a positive correlation between estimates of log (third and fourth instar population size + 1) and the log (mean adults per house + 1) *Ae. aegypti* for each of 15 survey periods ($R^2 = 0.505$, n = 57; $P < 0.001$). This was the case at all four localities: Cam Thanh ($R^2 = 0.641$, n = 15; $P < 0.001$), Binh Chanh ($R^2 = 0.621$, n = 15; $P < 0.001$), Ninh Xuan ($R^2 = 0.516$, n = 15; $P = 0.003$), and at untreated Ninh Binh commune ($R^2 = 0.515$, n = 15; $P = 0.009$). When examined by the Pearson product moment correlation, the *P* values were 0.000000397, 0.00137, 0.000611, and 0.00223, respectively, for these communes.

Disease surveillance. The data from 1996 to 1999 prior to implementation of our project demonstrate a high incidence of dengue, especially in the selected communes (Table 6). Since the field intervention did not commence until September 2000, the average incidence in the communes chosen for interventions was similar to district figures, but by 2001 there

TABLE 5

Estimated numbers of third and fourth instars and adult *Aedes aegypti* density index at treated Cam Thanh (Quang Nam province), Binh Chanh (Quang Ngai), and Ninh Xuan (Khanh Hoa) communes compared with untreated the Ninh Binh commune (Khanh Hoa) from surveys of 100 households, September 2000 to March 2004*

	Cam Thanh		Binh Chanh		Ninh Xuan		Ninh Binh	
Survey date	No. of larvae	Adult index	No. of larvae	Adult index	No. of larvae	Adult index	No. of larvae	Adult index
Sep-2000	10,759	1.16	2,900	0.12	2,466	0.13	2,568	0.65
Dec-2000	6,568	0.43	3,270	0.22	1,610	0.25	2,580	0.70
Mar-2001	2,138	0.34	1,034	0.16	424	0.04	540	0.33
Jun-2001	1,690	0.42	1,010	0.17	374	0.07	973	0.10
Sep-2001	1,100	0.35	300	0.17	232	0.04	1,130	0.23
Dec-2001	692	0.21	582	0.08	576	0.10	2,573	0.23
Mar-2002	253	0.07	224	0.01	286	0.03	424	0.12
Jun-2002	129	0.06	100	0	147	0.08	1,558	0.22
Sep-2002	153	0.11	100	0	190	0.04	2,284	0.61
Dec-2002	71	0.04	12	0.01	125	0.05	4,698	0.84
Mar-2003	45	0.02	15	0	40	0	1,955	0.35
Jun-2003	0	0.01	0	0	11	0.01	960	0.40
Sep-2003	0	0.02	0	0	25	0.01	ND	ND
Dec-2003	49	0.03	13	0.01	18	0.01	ND	ND
Mar-2004	13	0.01	15	0	0	0.01	ND	ND

* ND = not done because the project officially ended after the June 2003 survey. Entomologic data from intervention communes was provided with community assistance but at untreated Ninh Binh, surveys were carried out only by the project team.

was some evidence to suggest the control strategy was becoming effective with a reduced incidence of dengue of 76.7% (11 versus 47.2 cases/100,000 residents). No dengue cases were identified in any of the three intervention communes in 2002 and in 2003 until June when the project officially ended, despite an incidence in the surrounding districts of up to 112.8 (2002) and 14.4 (2003) cases/100,000 residents.

DISCUSSION

Clinical dengue infections were eliminated in three rural communes in central Vietnam that prior to the implementation of this vector control program had a high incidence of this disease (Table 6). This follows similar success in northern Vietnam where five rural communes and one urban commune were subjected to similar interventions.[7]

In northern Vietnam, jars < 50 liters in capacity were filled daily from large tanks and wells and the water was used for household requirements. Therefore, they posed little problem in terms of breeding sites for *Ae. aegypti.* In central Vietnam, a major water storage vessel is the 150-liter jar, and during the dry season in some villages within these communes water is purchased. At Ninh Xuan, 2,000-liter tanks were common. Thus, in this latter context, there is no possibility of simply emptying these containers to eliminate larvae because of their size, but mainly because the water was too precious.

TABLE 6

Incidence (per 100,000 population) of reported cases of dengue at Cam Thanh, Binh Chanh, and Ninh Xuan communes compared with district and provincial data, 1996–2003

Year	Three project provinces	Three project districts	Three project communes
1996	170.6	357.2	1,641.7
1997	166.2	360.4	2,418.5
1998	419.1	1,543.9	1,597.5
1999	69.0	159.6	467.5
2000	23.1	58.1	55.2
2001	28.9	44.2	11.0
2002	51.2	112.8	0.0
2003	6.6	14.4	0.0

During the project, *Mesocyclops* populations were shown to develop well in water containers commonly found in the central provinces such as cement tanks, drums, and big jars. In the early stages of the project, the number of containers positive for *Mesocyclops* fluctuated. One reason for this is that during the dry season, the water in many of the containers was exhausted and on refilling particularly in the early stages of the project, collaborators did not have sufficient experience in breeding and maintaining *Mesocyclops* populations for public use. Householders did not know how to reintroduce *Mesocyclops* each time they refilled a water container. By the end of the project, *Mesocyclops* populations were being successfully maintained in these containers. After three years of project implementation, the percentage of large water containers positive for *Mesocyclops* had increased to an average of 71%. We have previously commented on the egg-sink effect,[5] but we now have unpublished laboratory data from Vietnam that confirms findings from Mexico[10] that *Ae. aegypti* selectively oviposits in waters with *Mesocyclops* or in waters where they have been. These micropredators were accepted by 100% of the householders by the end of the project and although seven local species were involved; mainly *M. woutersi* and *M. aspericornis* were mass released.

Our community-based dengue control strategy using *Mesocyclops* was highly effective in controlling the dengue vector mosquito at the project sites. Specifically, *Ae. aegypti* were eliminated (or reduced to extremely low levels) as indicated by both larval and adult surveys.

Key factors influencing the effectiveness of the community-based vector control program included the attitude and willingness of collaborators and CMCs to be involved in program activities. The communities willingly participated through the involvement of school children, local authorities, health staff, and collaborators to target activities at the household level. In contrast to our community approach in the northern provinces, collaborators were given an expanded list of duties that included differentiation of *Aedes* larvae from mosquito larvae

generally, and cyclopoids from other copepods. This proved to be no problem and their records were comparable with those from the quarterly surveys done by the project team. It also seems clear that post-project surveillance is necessary because if left unchecked, the colonization process by *Ae. aegypti* may be rapid. It is not known whether the low numbers that appeared after June 2003 are due to subsequent hatches of desiccation-resistant eggs in existing containers or due to importation of new ones.

Currently, there is considerable interest in using pupal counts to estimate adult abundance and risk of dengue transmission.[11] Because the focus of control efforts are directed against larvae, and because sampling precision is better for third and fourth instars compared with pupae (at least for 220-liter drums),[12] we have continued to use late instars as our monitoring tool. The regression analyses and correlations on crude numbers of larval versus adult estimates per 100 houses for Cam Thanh, Binh Chanh, Ninh Xuan, and Ninh Binh were all highly significant, suggesting that there is some merit in pursuing this methodology. However, we believe that this would be better followed-up at the individual house level, and does not imply that we believe it is realistic to ascribe such numbers to generalized regional risk tables for transmission to occur.

Fortunately, our control efficacy is such that it is clear that all three intervention communes had zero or insufficient numbers of *Ae. aegypti* (or *Ae. albopictus*, which also occurred in low numbers at all three communes) to transmit dengue from 2002 onwards. As we learned in our phase 1 project, the selection of a neighboring village or even commune as an untreated control was problematic to measure any reductions in morbidity associated with our interventions. This was because of the randomness with which dengue appears. We suspect that similar problems will occur when attempting to define *Ae. aegypti* threshold numbers in relation to transmission at the village or commune level. To measure control efficacy in relation to dengue transmission, we elected for a broad comparison between intervention communes against the average district rates, and also included the rates for the project provinces. This was also confirmed by the numbers of serologically confirmed cases in the participating communes each year of the study. Our data from these communes contrasted with the increase in the number of cases reported from the districts and provinces between 2001 and 2003.

The community-based dengue control model using *Mesocyclops* has been replicated and expanded in other areas in Vietnam both under the National Dengue Control Program and with some support from external donors. In northern Vietnam in Hung Yen, Haiphong, and Nam Dinh, post-project activities by local provincial, district and community leaders has resulted in unparalleled success (Kay BH, Nam VS, unpublished data), whereas in Kien Giang in the south, the Netherlands-Vietnam Medical Committee project (Nam VS, Marchand R, unpublished data) has also used this model successfully. The key issue now is not whether the model works, but rather the creation of sufficient capable leadership with adequate resources to affect broad-scale national and regional expansion. However, it is also seems clear that continuous community inputs are required post-project to prevent reinfestation.

Received May 26, 2004. Accepted for publication July 8, 2004.

Acknowledgments: We thank AusAID for their support and encouragement, and the provincial, district, and communal health personnel who provided local technical support. We especially thank Dr. Bui Trong Chien (Director, Institut Pasteur Nha Trang) and the community management committees and communities of Cam Thanh, Binh Chanh and Ninh Xuan for their hard work and hospitality throughout the project. Without them, none of this would have been possible.

Financial support: This intervention was supported by a grant from AusAID to the Australian Foundation of Peoples of Asia and the Pacific.

Authors' addresses: Vu Sinh Nam, Nguyen Thi Yen, Tran Vu Phong, Truong Uyen Ninh, and Le Quyen Mai, National Institute of Hygiene and Epidemiology, 1 Yersin Street, Hanoi, Vietnam. Le Viet Lo and Le Trung Nghia, Institut Pasteur, Nha Trang, Vietnam. Ahmet Bektas and Alistair Briscombe, Australian Foundation of Peoples of Asia and the Pacific, PO Box 12, Crows Nest, New South Wales, Australia 1585. John G. Aaskov, School of Life Sciences, Queensland University of Technology, Garden Point Campus, Brisbane, Queensland, Australia 4000. Peter A. Ryan and Brian H. Kay. Australian Centre for International and Tropical Health and Nutrition, Queensland Institute of Medical Research, Post Office Royal Brisbane Hospital, Brisbane, Queensland, Australia 4029.

Reprint requests: Brian H. Kay, Queensland Institute of Medical Research Post Office, Royal Brisbane Hospital Brisbane, Queensland, Australia 4029, Telephone: 61-7-3362-0350, E-mail: brianK@qimr.edu.au.

REFERENCES

1. Nam VS, Yen NT, Holynska M, Reid JW, Kay BH, 2000. National progress in dengue vector control in Vietnam; survey for *Mesocyclops* (Copepoda), *Micronecta* (Corixidae) and fish as biological control agents. *Am J Trop Med Hyg 62:* 5–10.
2. Kay BH, Nam VS, Yen NT, Tien TV, Holynska M, 2001. Successful dengue vector control in Vietnam: a model for regional consideration. *Arbovirus Res Aust 8:* 187–193.
3. Brown MD, Kay BH, Greenwood JG, 1991. The predation efficiency of north-eastern Australian *Mesocyclops* (Copepoda: Cyclopoida) on mosquito larvae. *Bull Jpn Plankton Soc Special Volume:* 329–338.
4. Marten GG, Bordes ES, Nguyen M, 1994. Use of cyclopoid copepods for mosquito control. *Hydrobiologia 292/293:* 491–496.
5. Williamson CE, 1991. Copepoda. Thorp JH, Covich AP, eds. *Ecology and Classification of North American Freshwater Invertebrates*. New York: Academic Press, 787–822.
6. Nam VS, Yen NT, Kay BH, Marten GG, Reid JW, 1998. Eradication of *Aedes aegypti* from a village in Vietnam using copepods and community participation. *Am J Trop Med Hyg 59:* 657–660.
7. Kay BH, Nam VS, Tien TV, Yen NT, Phong TV, Diep VTB, Ninh TU, Bektas A, Aaskov JG, 2002. Control of *Aedes* vectors of dengue in three provinces of Vietnam by use of *Mesocyclops* (Copepoda) and community-based methods validated by entomologic, clinical, and serologic surveillance. *Am J Trop Med Hyg 66:* 40–48.
8. Gubler DJ, 1989. *Aedes aegypti* and A*edes aegypti*- borne disease control in the 1990s: Top-down or bottom-up. *Am J Trop Med Hyg 40:* 571–578.
9. Tun-Lin W, Kay BH, Barnes A, 1995. Understanding productivity, a key to *Aedes aegypti* surveillance. *Am J Trop Med Hyg 53:* 595–601.
10. Torres-Estrada JL, Rodriguez MH, Cruz-Lopez L, Arredondo-Jimenez JL, 2001. Selective oviposition by *Aedes aegypti* (Diptera: Culicidae) in response to *Mesocyclops longisetus* (Copepoda: Cyclopoidea) under laboratory and field conditions. *J Med Entomol 38:* 188–192.
11. Focks DA, 2003. A review of entomological sampling methods and indicators for dengue vectors. WHO/TDR/IDE/Den/03.1, 1-36. http://www.who.int/tdr/publications/pdf/dengue_vectors.pdf.
12. Tun-Lin W, Kay BH, Burkot TR, 1994. Quantitative sampling of immature *Aedes aegypti* in metal drums using sweep net and dipping methods. *J Am Mosq Control Assoc 10:* 90–396.