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Validación de Mesocyclops *(Copépodo) y participación comunitaria como combinación eficaz para el control del Dengue en Vietnam Septentrional*

Jean-Emmanuel Julo-Réminiac, Phong Vu Tran, Yen Thi Nguyen, Hien Tran Nguyen, Diep Bich Vu, Duc Minh Hoang, Le Hoang Nguyen, Thang Trong Vu, Nam Sinh Vu, Luu Duc Do and Paul T. Brey

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« Validation of *Mesocyclops* (Copepoda) and community participation as an effective combination for Dengue control in Northern Vietnam »

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Abstract. The "Programme Meso-Vietnam" was implemented in Vietnam from October 2007 to December 2010 to reduce dengue and dengue hemorrhagic fever incidence and to improve the quality of life in the four project communes. This dengue control project was based on biological control using *Mesocyclops*, a larvivorous micro-crustacean, as well as on the establishment of a strong community involvement to educate the population on dengue transmission and to reduce *Aedes* breeding by removal of containers. During three years, a network of collaborators was responsible for introducing *Mesocyclops* in all the containers defined as key breeding-sites, and regular activities such as community training workshops, school programmes, clean-up campaigns and health promotion through IEC programmess were performed. To make this programme sustainable, local leadership has been strengthened as well.

The use of these larvivorous micro-crustaceans as a biological control strategy against dengue over the medium and long term, when combined with community participation and effective health promotion, demonstrated a significant reduction in dengue vector populations and dengue cases. In Vietnam, this study reconfirms that there is an ecological alternative to systematic use of insecticides to control dengue vector population.

Keywords. Dengue, *Mesocyclops*, *Aedes*, Vector control, Biological agent, Community Participation, Vietnam

1. Background

Dengue is a growing public health problem. In recent years it has become the most important viral disease among arthropod-borne diseases.

About two-fifths of the world's population is now at risk: 50 million dengue infections are estimated worldwide every year, and the disease is now endemic in more than 100 countries (WHO 2009a).

In Southeast Asia, dengue fever and dengue hemorrhagic fever (DF / DHF) has become one of the most important public health issues. In Vietnam, a country of 90 million people, 1.3 million cases and 25,000 deaths were recorded in the last 25 years. After the large epidemic of dengue fever in Vietnam in 1998, which recorded 234,920 cases and 377 deaths, the number of reported cases of DF / DHF in Vietnam has increased from 23,000 in 1999 to more than 100,000 cases in 2010 (Health Statistics Yearbook, 2004 ; Annual Report of Vietnam National Dengue Programme, 2010).

In a few decades, dengue has progressed dramatically worldwide. More frequent and widespread outbreaks show a constant increase in the incidence and range of the disease.

Demographic change, uncontrolled urbanization, new means of transport, natural disasters and poverty are also involved in this increase (Gubler & Trent, 1994).

Despite being a difficult disease to diagnose, dengue is also a difficult disease to treat because there is no specific antiviral treatment at present and a vaccine is not available yet (WHO 2009b).

The fight against the disease is mainly focused on vector control. For now, in the absence of specific drug treatment or a vaccine, the only way to mitigate this disease is to reduce populations of *Aedes* mosquito vectors. To stop the spread of dengue, a policy of integrated control must be conducted, including surveillance of mosquito populations, the reduction of the vector, the introduction of modern information campaigns, as well as effective and efficient community participation (Parks & Lloyd, 2004).

In Vietnam, a national programme for the fight against dengue has been established since 1999. The main strategy of this programme is based on community participation in control activities and prevention of DF / DHF, i.e., through health education, involvement of local authorities and environmental improvement through clean-up campaigns. Vector control remains the main preventive measure.

2. General Information

In Vietnam, ten species of copepods (larvorious micro-crustaceans) exist in natural and artificial environments. The *Mesocyclops* spp. feed on organic matter, but also on the early instars (1 & 2) of mosquito larvae, particularly *Aedes* larvae. Each *Mesocyclops* can kill on the average 16 to 41 larvae of *Aedes aegypti* per day (Nam *et al.,* 2000).

As part of the national fight against dengue, the National Institute of Hygiene and Epidemiology (NIHE) and the French *Groupement d'Intérêt Publique* (GIP) "Alliance pour le Développement" implemented the "Programme Meso-Vietnam", from October 2007 to December 2010. This dengue control project was based on a biological control using *Mesocyclops* and on the establishment of a strong community involvement to educate the population on dengue transmission and reduce *Aedes* breeding sites by removal of containers (rubbish, tires etc).

Figure 1. *Mesocyclops* (left) eating an *Aedes* larva (right) (credit: Phong Vu Tran)

The overall goal of the "Programme Meso-Vietnam" was to reduce dengue and dengue hemorrhagic fever incidence and to improve the quality of life in the four project communes. It aimed to achieve a rapid reduction in vector population and to facilitate long term ecological dengue control through improved prevention practices and interventions on *Aedes* mosquito breeding sites, without requiring chemical insecticides.

The "Programme Meso-Vietnam" focused on the following two objectives:

- to build the local leadership and capacity for surveillance, diagnosis and integrated control of dengue fever / dengue hemorrhagic fever.
- to reduce mosquito populations through low-cost community-based activities.

3. Materials and methods

3.1 Ethical and political permission

Prior to implementation, both the French and the Vietnamese authorities involved approved the "memorandum of understanding" of the project. Furthermore, the project received approval from the "Project Committee" of the Vietnamese Ministry of Health.

3.2 Study site

The geographical location of the municipalities involved in the "Programme Meso-Vietnam" was selected in close coordination with the Vietnamese Ministry of Health, which wanted to strengthen the fight against the disease in the North.

Three communes were selected: Minh Thuan/Vu Ban District (20°26'19"N, 106°02'47"E) and Trung Dong,/Truc Ninh District (20°18'00"N, 106°16'24"E) in the province of Nam Dinh and Dien Kim/Dien Chau District (19° 1'40 "N, 105°37'11 "E) in Nghe An Province.

For comparing the results, a fourth county, Dien Hai/Dien Chau District (19° 3'03"N, 105°37'33 "E) in the province of Nghe An, has been chosen to be the control-commune, in which no intervention other than the entomological surveillance was carried out.

3.3 Intervention description

3.3.1 Strengthening local leadership and establishing a network of collaborators

Upon starting the programme, a Community Management Committee (CMC) was set up in each of the 3 municipalities involved in the project. The CMC was composed of representatives of schools, health services, local authorities (People's Committee) and the community (leaders of associations).

The CMC was responsible for planning and organizing activities within the commune, and played a key role in mobilizing the community.

CMCs were also responsible for selecting collaborators within the commune on criteria such as enthusiasm to participate in the project, spare time, basic knowledge on health issues (Table 1).

Initially, local supplies of *Mesocyclops* were cultivated by collaborators in a dozen of key *Mesocyclops* breeding containers in each commune, following the instructions of NIHE.

Then, each collaborator, provided with a shirt and a cap bearing the logo of the "Programme Meso-Vietnam", played an essential role to educate the members of each of the 100 households he/she was responsible for. Each household was visited on a monthly basis. The collaborators informed the residents on the dengue transmission cycle, the breeding sites of mosquitoes, etc. They urged residents to use simple methods of control such as adding salt water in the vases of flowers, the removal of solid waste, the maintenance of populations of *Mesocyclops* in water containers. If necessary, they reintroduced *Mesocyclops* into these containers collecting the copepods from the key *Mesocyclops* breeding containers previously established, and informed the heads of households about appropriate *Mesocyclops* husbandry techniques (Nam et al, 2005). They also informed the residents on how to detect new cases of dengue, and involved them to fight more effectively against mosquito vectors of dengue.

Each collaborator was equipped with a surveillance kit. This kit consisted of a "dipper" for mosquito larvae and *Mesocyclops*, a flashlight, a transparent plastic cup, a plastic pail and a notebook to record their comments. Collaborators would report their activities to the CMC each month. These monthly meetings between collaborators also allowed them to exchange information and experiences, and to share with the CMC any difficulties encountered.

In addition, to promote exchange of experiences between different municipalities, collaborators and to stimulate their participation, 12 "cross-checks" were held (meetings between employees of 2 communes), 2 competitions (based on quiz tests) involving collaborators from the 3 communes were held in 2008 and 2010, and one important festival involving plays, songs and skits was organized in April 2009.

Table 1. Repartition of population, households, collaborators and CMC members in each project site

Province	Nam Dinh		Nghe An	
District	Vu Ban	Truc Ninh	Dien Chau	
Commune	Minh Thuan	Trung Dong	Dien Kim	Dien Hai (control-commune)
Population	9484	15517	9168	7767
Nb of households	2567	3344	1919	1583
Nb of collaborators	26	33	20	0
Nb of CMC members	7	8	8	0

3.3.2 Community Training

All the collaborators and CMC members (102) received an initial 3-day training tailored to their duties and given by NIHE staff. The training programme was based on dengue etiology, epidemiology, symptoms, vector control methods and use of *Mesocyclops*.

Thirty-five healthcare professionals from municipalities, districts and provinces involved in the "Programme Meso-Vietnam" received an initial 5-day training. During two days, the NIHE team of entomology laboratory taught them generalities on dengue and its vectors, the epidemiology of the disease, different control methods, and aspects of comprehensive surveillance and treatment of patients with suspected and / or suffering from dengue. Participants also learned during practical work how to differentiate *Aedes aegypti* and *Aedes albopictus* towards other mosquitoes, *Aedes* larvae towards other larvae, and how to recognize *Mesocyclops*. This work of differentiation is essential in the field. The next three days were dedicated to field practice. Healthcare professionals from provincial Centers of Preventive Medicine received an additional 2-day training to strengthen their capacity on serological surveillance.

After the training, each participant received a handbook of some fifty pages containing essential information developed during this training. Recycling has occurred each year. Pre-workshop and post-workshop tests demonstrated the improvement of knowledge and ownership of the project.

3.3.3 Schools activities

One hundred and forty teachers belonging to the secondary schools of the three communes were trained four times on dengue and the "Programme Meso-Vietnam" issues, and received a training manual.

They were asked to transfer this knowledge to their

students through weekly lessons on dengue vector mosquitoes and *Mesocyclops*.

Courses and workshops were also established in schools: the creation of drama and songs has also allowed full ownership of the project by the students.

Special courses for civic education in order to prevent dengue were also provided.

3.3.4 Clean-up campaigns

Major clean-up campaigns for collecting solid waste (potential breeding sites) were organized every three months. They involved collaborators, teachers and children, youth associations, women's associations, and anyone who wanted to join. The networking of the various components of the community contributed to create large groups going house by house, thus promoting awareness.

3.3.5 Health promotion

Baseline KAP results were used by NIHE to design appropriate IEC materials and messages following the method of WHO and the National Dengue Control Programme (NDCP) of Vietnam. The appropriate words and photos used were tested by the local people 2 times before being used. With additional ideas from collaborators, CMC and students, communication media have been designed and disseminated to raise awareness, throughout the 3 years of the programme:

- 20,000 time-schedules for pupils, with a design reminiscent of the important information in the fight against dengue and "Programme Meso-Vietnam" such as taught by teachers,
- 35,000 posters for the families, with information on dengue, recognition symptoms, the mosquito life cycle and the importance of the use of *Mesocyclops*,
- 15,000 awareness leaflets were also developed and distributed to the population,
- 3 giant billboards (10m2) were installed in the center of each of the 3 communes,
- various radio messages, to be disseminated through speakers.

Moreover, briefings and discussions on prevention of dengue, intended for the villagers, were also regularly organized by the CMC and local associations.

3.4 Measurements tools

3.4.1 KAP studies

Three KAP studies (before, during and after intervention) were carried out among 100 people in each commune for each study, randomly selected from 34,169 villagers, in order to measure the ultimate impact of the health promotion activities on the populations of the communes involved. At baseline, KAP results were used to design appropriate IEC materials and messages.

3.4.2 *Aedes* and *Mesocyclops* surveys

3.4.2.1 Baseline surveys

Prior to implementation of the programme, baseline surveys for *Aedes* larvae were carried out in each commune among a random sample of 100 households selected from health centre records. These surveys made possible to acknowledge accurately the presence of the different species of *Mesocyclops* in all containers observed, and the distribution of *Aedes* larvae according to the different cottage sites.

Methods for counting the number of larvae in containers included direct counting in small water containers such as flower vases, ant traps, and discards (or pouring into a sample box and counting later); using larvae net to sample larvae in tanks and big jars; and using funnel traps to trap larvae in drums and underground tanks. Investigative methods were standardized: the nets used were the same (diameter: 20cm, deep: 20cm, net mesh: 5 micro meters) and were plunged 5 times from top to bottom in the containers and all containers inspected within the house or yard.

Thence, breeding sites of mosquitoes in local and container types have been determined.

Figure 2. Time-schedule and leaflets (top) and giant billboard in Minh Thuan (bottom)

3.4.2.2 Quarterly surveys

Following the same methodology as for the baseline surveys, *Aedes* larvae and *Mesocylcops* surveillance were held quarterly, allowing information on qualitative and quantitative changes in the mosquito's larvae and *Mesocyclops* populations present in the environment.

To standardize the analysis of the results, 3 indices have been particularly considered in this final analysis:

- The percentage of containers in which Mesocyclops are present
- The Breteau Index: number of containers in which mosquito larvae are present/100 houses.

The Density Larval Index: average number of mosquito larvae per house visited.

3.4.3 Disease surveillance

In the community health centers, (in province and district hospitals if the patients go directly to higher level from the communes), a blood sample was collected and a test was performed in all patients with suspected dengue (following the WHO recommendations: all patients presenting with fever-38,5°C and higher-, headaches and arthralgia and/or myalgia).

Blood samples were sent from the commune health centers to district health centers. These samples were transferred to the Centers for Preventive Medicine of the respective Provinces for serological testing by MAC-ELISA. At this level, the samples tested positive were sent to NIHE laboratory for confirmation and serotyping.

4. Results

4.1 Community involvement

Collaborators activities

During the three years of the "Programme Meso-Vietnam", collaborators have made 277,665 visits, representing 96% of households in three municipalities included in the project. Thirty-five campaigns for the introduction of *Mesocyclops* were held, and 1,614 containers were treated with *Mesocyclops* (more than 85% of key containers). In total, more than 444,000 persons-contacts were informed directly about dengue and the programme.

Clean-up campaigns

With the support and active participation of commune's pupils, as part of Citizenship Day, 35 clean-up campaigns in total were held in the three communes during the study. A total of 294,527 destroyed discards, which are all potential breeding sites, were collected.

Health promotion

All the 20,000 time-schedules, the 35,000 posters and the 15,000 leaflets were distributed to the population.

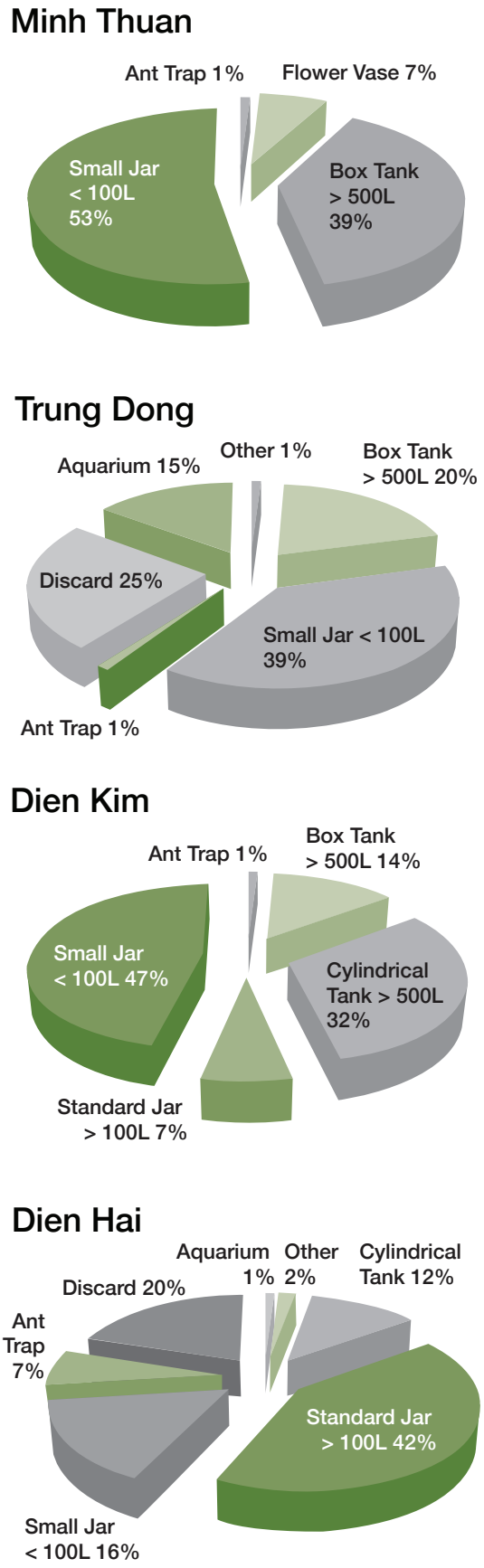

Figure 3. Repartition of *Aedes* larvae found in the different breeding sites in each project site

In total, 102 commune meetings were held, involving more than 27,705 listeners and participants. These meetings have encouraged ownership of the programme by the villagers, and these exchanges have helped to refocus if necessary certain actions or activities of the programme.

Finally, 158 audio communications were also disseminated through speakers in all communities.

School activities

In Trung Dong, 30 speeches from 10 to 15 minutes on the topic of dengue were held for 8,064 pupils. Teachers have also included a course on dengue vector mosquitoes and *Mesocyclops*. These courses were taught in 44 classes (1,807 students). A special course in civic education to prevent dengue was taught in 28 classes.

In Minh Thuan, 37 speeches same as those in Trung Dong were delivered to 16,540 high school students. These courses were taught in 67 classes (4,664 students).

In Dien Kim, 25 teachers were trained (2 training sessions) and specific courses on dengue were taught to 1,260 students (25 classes) in Dien Kim Junior School.

The students of the 3 communes actively participated in 35 clean-up campaigns.

4.2 *Aedes* and *Mesocyclops* surveys

Indices that provide information on larvae density and container productivity were favored over container indices such as the Breteau Index or the Density Larval Index. Survey results permitted to identify the key breeding sites in each project commune, where the target control activities had to be implemented in priority (Figure 3).

4.2.1 *Mesocyclops* baseline surveys

Baseline demonstrated that *Mesocyclops* naturally existed in each project site. Four species of local *Mesocyclops* were detected. The predatory capacity of each species for *Aedes* larvae was tested by the entomology laboratory of NIHE. Results showed that two species of *Mesocyclops* tested in the laboratory had a high predatory capacity for first instars *Aedes* larvae. *Mesocyclops pehpeiensis and Mesocyclops aspericornis* were shown to have the highest predatory capacity followed by *M. affinis, M. wouttersi, M. thermocyclopoides* and *M. ogunnus)*.

4.2.2 Quarterly surveys

In total, throughout the 3 years of implementation of the "Programme Meso-Vietnam", 13 *Aedes* and *Mesocyclops* surveys were conducted. The number of *Mesocyclops* and mosquito larvae was collected and the species of mosquito larvae were analyzed. Based on the collection method, the estimate number of *Mesocyclops* and *Aedes* larvae of each water container was calculated by calibration

Figure 4. Typical box tank from Nam Dinh Province (top) and typical cylindrical tank from Nghe An Province

factor referred by Texa Knox. These data were collected through Excel and analyzed by IPC (software provided by QIMR Australia).

The capacity of *Mesocyclops* to survive in water containers was evaluated through quarterly entomology surveys and from field data collected by collaborators. *Mesocyclops* were shown to be able to survive in toilet tanks and aquariums. However, *Mesocyclops* were found to be unsuitable in containers of small volume such as small jars, discards and flower vases. *Mesocyclops* need time and microscopic algae enough to be able to reproduce. Small containers are more easily dry soon, and do not contain enough microscopic algae to feed the *Mesocyclops*. Therefore, big containers were shown the most adapted for both *Mesocyclops* and *Aedes* larvae.

There was a significant increase in the percentage of containers with the presence of *Mesocyclops* in the 3 communes of the programme, from less than 20% in December 2007 to over 65% in December 2010. However, in Dien Hai, the control-commune, this index never exceeded 17% (Figure 5).

Simultaneously, in the 3 intervention communes (Minh Thuan, Trung Dong and Dien Kim) where the Breteau Index was higher than 45 at an early stage of the programme, it dropped respectively to 0, 0 and 1, while it was at 12 in Dien Hai, the control-commune in December 2010. Similarly, in the same 3 communes where the Density Larval Index was higher than 20 at an early stage of the programme, it respectively dropped to 0, 0 and 1,

while it was at 24 for Dien Hai in December 2010 (Figure 6).

The significant decrease of these two indicators reflects a sharp reduction in the population of mosquito larvae (100% to Minh Thuan and Dien Kim, > 99% at Trung Dong) in the 3 districts, which have benefited from the programme.

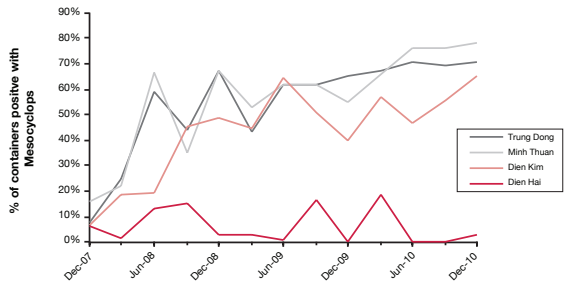

Figure 5. Percentage of containers positive with *Mesocyclops*

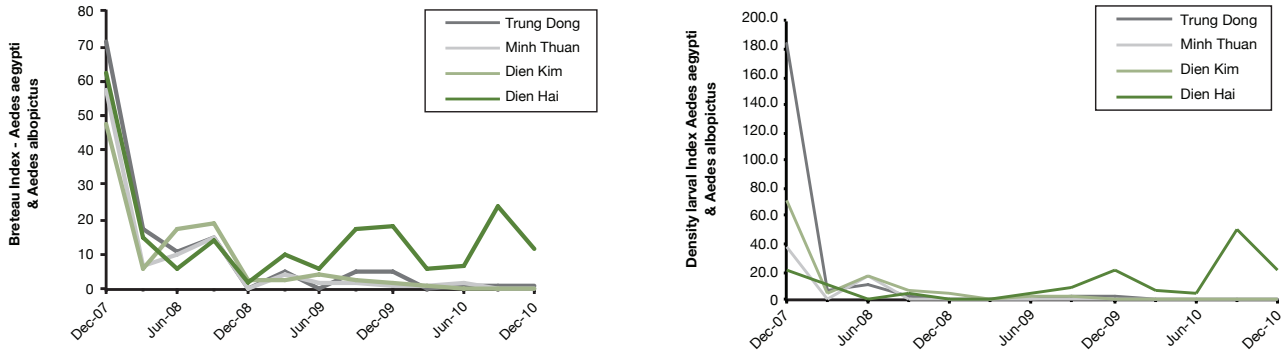

Figure 6. Evolution of Breteau Index (left) and Density Larval Index (right) for both *Aedes aegypti* & *Aedes albopictus*

Table 2. Results of the three KAP survey in the three project communes, 2007-2010

No	Question	% of correct answers			
		2007	2009	2010	Increase from 2007 to 2010
1	Have heard of DF/DHF	84.7	90.9	100.0	15.3
2	Knowing signs and symptoms of DF/DHF	48.2	35.2	78.7	30.5
3	Knowing DF/DHF is dangerous	79.9	88.3	100	20.1
4	Knowing DF/DHF is transmitted by mosquitoes	74.7	85.2	96.3	21.6
5	Knowing dengue mosquito is striped	60.4	59.6	95.3	34.9
6	Knowing dengue mosquito bites at daytime	27.5	36.8	87.0	59.5
7	Knowing dengue vector live indoor	50.8	46.7	93	42.2
8	Larvae habitats of dengue vector				
	Drinking Water container	67.6	72.9	87.3	19.7
	Discards, flower vase, ant trap	69.2	79.5	88	18.8
9	Dengue control methods are mosquitoes and larvae control	54.5	69.2	96	41.5
10	Knowing larvae control methods				
	larvivorous fish	38.1	40.4	72.3	34.2
	cleaning water container regularly	28.2	29.3	72.7	44.5
	cleaning up and eliminating discards	34.5	45.5	85.3	50.8
	Introducing *Mesocylops*	0.3	35.4	92.3	92
11	Knowing about *Mesocyclops*	5	59.7	99.7	94.7
12	Accepting *Mesocyclops* release	90.3	98.3	99.7	9.4
13	Volunteer Involved	97	99.7	99.7	2.7

4.3 KAP studies

Amongst the villagers, knowledge has significantly increased between the beginning and the end of the "Programme Meso-Vietnam".

At the end of the project, 100% of the inhabitants of the municipalities involved had heard of dengue, an increase of more than 15 points compared to 2007. 100% of the target population knows that dengue can be dangerous, and 78.7% is able to recognize the symptoms of a suspected case (respective increase of more than 20 and 30 points). Over 96% of the target population involved is aware that dengue is transmitted by mosquitoes, and more than 95% knows that the vectors are "tiger mosquitoes", (respective increase of over 21 and 34 points for these two items).

96% of the target population knows that the methods for dengue control are essentially methods to control mosquitoes and larvae, against 54.5% in 2007. And 72% of commune's people now knows that larvivorous fish can also be used (increase of 34 points), as well as regular cleaning of water containers (up 44 points), solid waste disposal (up 50 points) and using of *Mesocyclops* (increase of 92 points). The latter figure is to be compared with that in 2010, 99.7% of the target population had ever heard of *Mesocyclops*, against 5% in 2007 (table 2).

4.4 Disease surveillance

While the three communes totaled 2,160 cases of dengue between 1997 and 2007, they have totaled 118 suspected cases over the 3 years of implementation of the project. Of the 45 valid samples studied, 8 were positive (7 in Trung Dong and 1 in Dien Kim). However, epidemiological monitoring shown that these 8 positive cases were imported cases (especially during the outbreak in Hanoi in 2009 and the one in Dien Hai in October 2010).

In the control-commune, Dien Hai, 39 suspected cases and 13 valid samples have revealed 10 positive cases.

5. Discussion

The use of these larvivorous micro-crustaceans as a biological control strategy against dengue over the medium and long term, when combined with community activities and effective health promotion, allows a significant reduction in larval of mosquitoes present in the environment, thus limiting in an ecological way the risk of epidemics.

This study confirms the success already reported in northern and central Vietnam, where respectively 6 and 3 municipalities had not experienced new cases of dengue after similar interventions (Kay *et al.,* 2001; Kay *et al.,* 2002; Nam *et al.,* 2005).

Even if the water storage containers are not designed in the same way all over the country, they represent the major *Aedes* breeding sites that facilitate this model of intervention.

The organization model has proven effective and the local leadership has been strengthened at national, provincial and local levels. This organizational model has generally improved capacity for surveillance, diagnosis and integrated control of dengue and dengue hemorrhagic fever at all levels of the Vietnamese health system.

Furthermore, the general increase in knowledge of dengue, its symptoms, means of transmission and means of prevention in all 3 communes, shows how community activities have been efficient.

These community activities, driven by the CMC, were shown to be the keystone of the "Programme Meso-Vietnam". Nevertheless, it should have been relevant to run KAP studies in the commune control as well, in order to prove the real impact of IEC and health promotion interventions.

The involvement of the school system, including teachers who educate young generations for a change of behavior regarding the environment around them, seems to receive the agreement of all. It could be considered to officially include a training programme on dengue fever in the curriculum of secondary schools in Vietnam at the national level.

In order to compensate for the cessation of external funding at the end of the programme, a micro-credit income generating system has been established in all 3 communes. The profits will support local community vector control activities and pay collaborators. Further studies to evaluate the sustainability of this source of funding are needed. The Vietnamese government, through its National Dengue Control Programme, will continue to play a catalytic role, and foster multidisciplinary interactions.

Copepods have the potential for local source reduction of *Aedes aegypti* and *Aedes albopictus* in many other countries besides Vietnam. With preliminary studies on local *Aedes* behavior and *Mesocylops* utilization capable of integrating local Vietnamese customs, this model could possibly be extended to other countries.

However, unlike Vietnam, professional capacity for copepod management and social institutions for community participation to help with implementation and maintenance are the main factors limiting broader use of copepods for operational mosquito control in other countries (Marten GG, Reid JW, 2007).

In Vietnam, this study confirms that there is an alternative to systematic use of insecticides to control dengue vector population. An extension to the national level could be considered, but would require a strong successful multisectoral involvement to empower the community participation and the behavior changes.

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