Community-based use of the larvivorous fish *Poecilia reticulata* to control the dengue vector *Aedes aegypti* in domestic water storage containers in rural Cambodia

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ABSTRACT: A community-based study of the distribution of larvivorous fish, *Poecilia reticulata* (common name: guppy), in water storage containers for dengue control was undertaken in 14 villages and approximately 1,000 households in Cambodia. Community volunteers reared guppies and distributed them in water jars and tanks in households for which they were responsible. A nearby control area received no intervention. One year after project commencement, 56.9% of eligible containers contained guppies and there was a 79.0% reduction in *Aedes* infestation in the intervention community compared with the control. Smaller or discarded containers unsuitable for guppy distribution in the intervention area also had 51% less infestation than those in the control area, suggesting a "community-wide" protective effect. In addition, there was less infestation in villages with higher rates of fish uptake, suggesting that the presence of fish was responsible for a reduction in *Aedes* infestation. This applied vector control model was well-accepted, effective, efficient, and shows promise as a sustainable community-based, non-insecticidal intervention for dengue vector control in large domestic water storage containers in rural Cambodia and elsewhere. ***Journal of Vector Ecology*** **33 (1): 139-144. 2008.**

Keyword Index: Dengue, community participation, larvivorous fish, mosquitoes.

INTRODUCTION

Dengue is a disease of increasing public health importance, with over 2.5 billion people at risk globally (Gubler 2002). Over 100 tropical countries are endemic for dengue and report increased epidemics, including more of the severe form of the disease, dengue haemorrhagic fever (DHF). These increases are the results of population growth and urbanization, poor sanitation and hygiene, and an increased range of both virus and vector (Gubler 1998, 2002, Guha-Sapir and Schimmer 2005).

The main mosquito vector, *Aedes aegypti,* breeds in still, clear bodies of water and thrives in human-made receptacles such as discarded pots, tires, and water storage containers. In Cambodia, water from rain, rivers, wells, or other sources is stored for domestic use in large concrete water jars, and previous studies have shown that these jars constitute over 80% of *Ae. aegypti* larval habitats (Socheat et al. 2004). However, due to the sheer number of water jars, the frequency of their use and their unpredictable and seasonally dependent filling/emptying schedules, controlling vector populations in them has proved elusive. They are large and cumbersome and are seldom completely emptied, making regular cleaning of their insides impractical.

A range of interventions have been tested. Since 2001, the larvicide temephos has been added to water jars in stratified dengue high-risk areas and this intervention has reduced the number of dengue cases in these areas by 53% (Suaya et al. 2007). While cost-effective, this or other larviciding interventions are viewed as an interim measure due to high cost, dependency upon donor support, low level of community participation, and the prospect of developing insecticidal resistance. A low-cost community-based intervention that can be consistently practiced has been sought by dengue control programs in various countries for some time and several have been field tested. They include the use of insecticide-treated curtains (Madarieta et al. 1999) and water storage container covers (Socheat et al. 2004; Kroeger et al. 2006), lethal ovitraps (Sithiprasasna et al. 2003), cleaning and removal of breeding sites (Chan et al. 1985), the addition of predators to water jars (Alio et al. 1985, Nam et al. 2002, Jennings et al. 1995), and integrated methods of control (Kittayapong et al. 2006, Cheng et al. 2000).

Guppy fish (*Poecilia reticulata*) have been described as effective biological agents for the control of mosquito larvae (Alio et al. 1985, Nathan 1993, Elias et al. 1995, Rozendaal 1997). They are native to South America, have been introduced into other non-native countries in South America and Asia, and are commonly found in ornamental ponds in Cambodia and elsewhere (Rozendaal 1997). Guppies grow to about 6 cm in length, and females produce 40-50 offspring following a one-month gestation period. In Bangladesh, guppies each ate an average of 41.0 Culex *spp*. larvae per day, with females eating approximately twice as many as males (Elias et al. 1995).

This paper describes an operational-level field trial in 14 villages of Trapeang Kong commune, Kampong Speu province, Cambodia, between April, 2006 and April, 2007, using a community-based model incorporating village meetings, education, and recruitment of unpaid volunteers for guppy breeding and distribution. Entomological assessments of the intervention area and a nearby control area one year after project inception and prospects for further expansion of coverage are discussed.

MATERIALS AND METHODS

Acquisition of guppy stocks

Approximately 100 guppies of mixed sex were purchased from a Phnom Penh pet shop and transferred to the Centre for Parasitology, Entomology and Malaria Control (CNM), Phnom Penh. The fish were reared in water storage jars and fed ground rice husks, where they propagated freely. Within a number of months, there were sufficient stocks for distribution to pilot villages.

Determination of 24 h larval consumption of guppies

A preliminary study was carried out to confirm the observations of others on the rates of consumption of mosquito larvae. Seven plastic containers were each filled with between five and seven liters of tap water and left for five days to dechlorinate in the laboratory. One guppy from the CNM stock was released into each of seven containers (four males and three females) before 200 laboratory-reared *Ae. aegypti* larvae of stages 1-4 were added to each. After 24 h, uneaten larvae were removed and counted. A further 200 larvae were added to each container and the process repeated a total of seven times on seven consecutive days. The 24-h larval consumption of each guppy for each of the seven days was recorded.

Selection of communities

Trapeang Kong commune encompasses approximately 2,300 households, is easily accessible from Phnom Penh, and has entomological and environmental similarity to most dengue-endemic areas of Cambodia. An area of the commune containing 14 villages and approximately 1,000 households on one side of national road number 4 was chosen as the intervention area. An area of the same commune containing 260 households, located at least 500 m away on the opposite side of national road 4, was chosen as the control community. The communities were similar to each other and typical of many Cambodian communities in that they had similar social and economic backgrounds, consisting primarily of paddy farmers; there was an abundance of water storage jars and proliferation of *Ae. aegypti* breeding in most households; and the villages were outside the areas where the large scale national larviciding program was carried out. All villages and houses in the participating areas were assigned unique identification numbers to facilitate field sampling.

Selection and activities of volunteers: guppy rearing and distribution

A total of 28 Village Health Volunteers (VHVs) was selected following discussion with village and commune chiefs. Each VHV was responsible for no more than 50 households. They were trained to breed guppies in water jars at their homes, transfer and distribute them to water storage jars or tanks with a volume of over 200 liters containing water for domestic use, and inspect water storage containers for the presence of guppies and mosquito larvae. They were instructed in the elimination of vector breeding sites and were encouraged to disseminate these messages to villagers.

Each volunteer was provided with two 400 liter water storage jars for guppy breeding, a net and bucket for transporting the fish, and a bag of ground rice husks for feeding. Approximately 100 guppies of mixed sex were also provided. Volunteers fed the fish daily and after approximately three months had sufficient stocks to distribute (500-1,000 fish). Volunteers visited households and added two or three guppies to each water jar or storage tank on properties using the bucket and net to transfer the fish. Volunteers were asked to make such visits to every household two times per month for one year when they inspected containers and replenished lost guppies. Villagers could additionally report to volunteers' homes to obtain replacement guppies at any time.

End-of-year entomological evaluation

One year after project inception, a 25% sample of houses was chosen from each village by randomly choosing a number from one to four for each study village and commencing assessments at the house corresponding to this identification number. Every fourth house was then visited by trained CNM staff to check water jars and tanks for the presence of at least one fish, or one *Aedes* larvae or pupae, and each container was then recorded as positive for fish, *Aedes*, both, or neither. Other in-use containers and discarded containers capable of holding water were counted and the presence or absence of larvae or pupae was recorded. Because jars and tanks are the two container types eligible for fish they were designated "eligible" containers, whereas discarded or other in-use containers were designated "other" for data analysis purposes.

Statistical methods

All statistical analyses were performed with Intercooled Stata 6.0 (Stata Corp., TX, U.S.A.). Statistical means of 24-h larval consumption for both male and female guppies, and the distribution of containers in intervention or control areas were compared by a two-sided Student's t-test. The Z-test of significance was used to compare the proportion of containers infested in the intervention and control areas. The relationship between the percentage of infested containers in each village and level of coverage with fish was assessed by simple linear regression.

Table 1. Number of *Ae. aegypti* larvae eaten by each of four female guppies in containers 1-4 (C1-C4) and three male guppies in containers 5-7 (C5-C7) on each of seven consecutive days. Daily means of each sex and means per day for each fish are shown in the "mean" columns and row, respectively. The "total mean per day" describes all seven fish and the total mean per day for each sex is shown in bold font.

Day	Female					Male				Total mean per day
	C1	C2	C3	C4	Mean	C5	C6	C7	Mean	
1	120	118	125	120	120.8	70	70	75	71.7	102.3
2	121	130	128	123	125.5	75	71	80	75.3	106.7
3	124	115	100	130	117.3	73	72	70	71.7	100.2
4	120	113	120	135	122.0	78	70	75	74.3	104.1
5	126	125	126	132	127.3	73	70	73	72.0	106.5
6	116	123	123	125	121.8	70	70	68	69.3	102.1
7	128	130	125	121	126.0	90	80	82	84.0	110.3
Mean	122.1	122.0	121.0	126.6	**122.9**	75.6	71.9	74.7	**74.0**	102.0

RESULTS

24-h larval consumption of guppies

An average of 102.0 (95% CI 94.9 – 109.2) larvae were eaten each day by each guppy. Males on average ate 74.0 larvae per day (95% CI 71.6 – 76.5), while females ate 122.9 (95% CI 120.3 – 125.6; difference 48.8 (95% CI 45.2 – 52.5); 2-sided t-test p<0.001). There were no significant differences among either individual fish of the same species or consumption on different days (Table 1).

Level of guppy uptake

Of the 1,498 eligible containers in the intervention area (1,452 jars and 46 tanks), 853 (831 jars and 22 tanks; 56.9%) had guppies inside at the time of inspection. This varied between the 14 villages of the commune, ranging between 23.9% (11 of 46 containers, village 4) and 92.8 (64 of 69 containers, village 6; Figure 1).

Entomological indices in containers eligible for fish

Of the 249 intervention households visited, a total of 1,452 water storage jars (5.8 per house) were found, compared to 407 in the 65 control houses (6.3 per house; difference = 0.4; p=0.35). A total of 46 (0.2 per house) and 28 (0.4 per house) water tanks were found in each area, respectively (difference = 0.2; p=0.002). A total of 10.5% of these eligible containers (157/1498) were positive for *Aedes* pupae or larvae in the intervention area compared to 50.1% (218/435) in the control area (difference = 39.6%; Z = -18.4; p<0.001). The proportion of eligible containers positive for *Aedes* larvae or pupae varied between intervention villages from 3.8% (five out of 130 containers positive; village 9) to 22.0% (16 out of 108 containers positive; village 2) (Figure 1).

Entomological indices in "other" containers

A total of 128 "other" (either in-use or discarded) containers were found in the 249 households surveyed in the intervention area (0.51 per house) compared to 106 in 65 (1.63 per house) control area households (difference = 1.1; p<0.001). Seventeen percent (28 of 128) of these containers were positive for *Aedes* pupae or larvae in the intervention area compared with 34.9% (37 of 106) in the control area (difference = 17.7%; Z = -3.1; p=0.002). The container index was 11.0% in the intervention area compared to 47.1% in the control area. The Breteau index in the intervention area was 71.9, in comparison to 392.3 in the control area, and the house index was 45.0 and 93.8, respectively (Table 2).

Table 2. The number of water storage jars and tanks, and the number and percentage of them positive with *Aedes* larvae or pupae discovered on a subset of households in the intervention or control areas of this study. The total percentage is shown in the column "total % positive." The number of houses surveyed in each area is shown in brackets.

Area	Water storage jars			Tanks			Total % positive
	No.	No. positive	% positive	No.	No. positive	% positive	
Intervention (249)	1,452	151	10.4	46	6	13.0	10.5
Control (65)	407	200	49.1	28	18	64.3	50.1

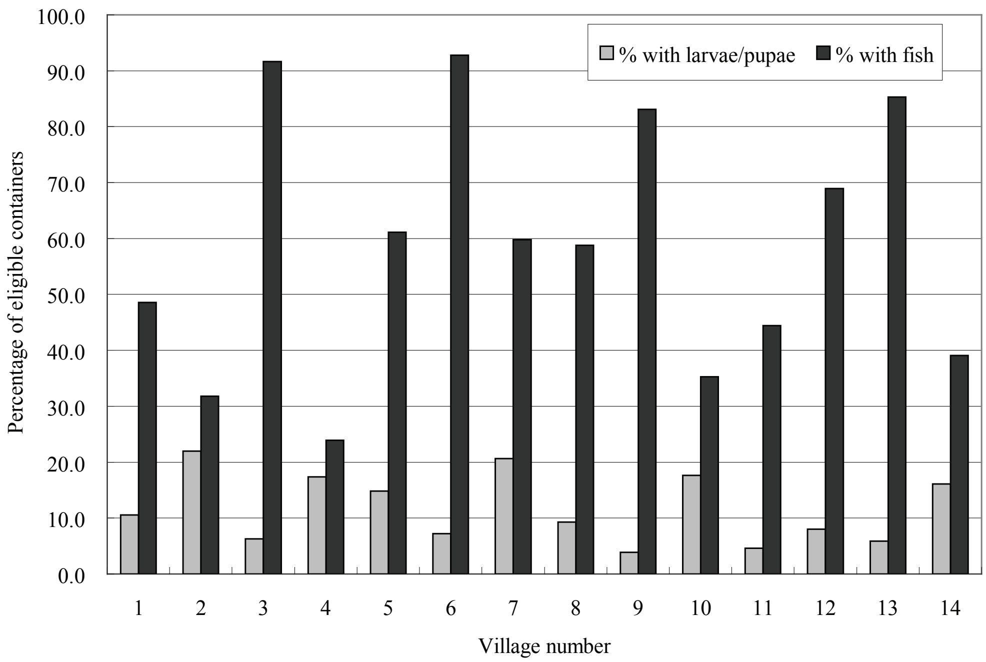

Figure 1. The percentage of eligible containers positive for *Aedes* larvae or pupae (dark grey); or at least one guppy fish (light grey) in each of 14 intervention villages of Trapeang Kong commune one year following project commencement.

Table 3. The number of in-use and discarded containers, and the number and percentage of them positive with *Aedes* larvae or pupae discovered on a subset of households in the intervention or control areas of this study. The combined total percentage is shown in the column "total % positive." The number of houses surveyed is shown in brackets.

Area	In-use			Discarded			Total % positive
	No.	No. positive	% positive	No.	No. positive	% positive	
Intervention (249)	121	21	17.4	7	1	14.3	17.2
Control (65)	63	33	52.4	43	4	9.3	34.9

There was an inverse relationship between the percentage of eligible containers containing guppies and the percentage of such containers being infested with *Aedes* larvae or pupae in each village. For every 1% increase in guppy coverage, there was a corresponding 0.19% decrease in the percentage of containers infested (regression co-efficient -0.19, 95% CI -0.31, -0.07; p=0.006, r^2 = 0.49, Figure 1).

DISCUSSION

After one year of community-managed addition of guppies to water storage containers, the container index of eligible containers in the intervention area was approximately 80% lower than that in the control area, and less than observed in other Cambodian communities where upwards of 50% of water jars are typically infested (Socheat et al. 2004). At our observed coverage rate of 56.9%, only 10.5% of eligible containers (and 11.0% of all containers) were positive with *Aedes* larvae or pupae. The conventional indices of HI, CI, and BI were considerably lower in intervention than control communities and the strong inverse relationship between levels of guppy coverage and levels of infestation in different villages provides evidence that guppies are reducing infestation. Higher levels of guppy coverage will further reduce the proportion of infested containers.

Considering the geographical proximity, social similarity, and similar distribution of water jars, we have no reason to believe that confounding played a role in the considerable differences observed among communities. *Ae. aegypti* migration between areas is unlikely as the distance between the areas exceeds the reported flight range of this vector species (Harrington et al. 2005). The reasons for low coverage in some villages (for example in village 4 only 23.9% of eligible containers contained guppies) require examination. The most important single factor in determining coverage rates is probably the diligence of volunteers. Motivational tools should be

Table 4. The house, container, and Breteau indices of the intervention and control areas of this study one year after project implementation. "n" refers to either number of houses ([a]) or number of containers ([b]). "No. positive" refers to the number of houses ([c]) or containers ([d]).

Area	House Index			Container Index			Breteau Index		
	n[a]	No. positive[c]	Index	n[b]	No. positive[d]	Index	n[a]	No. positive[d]	Index
Intervention	249	112	**45.0**	1626	179	**11.0**	249	179	**71.9**
Control	65	61	**93.8**	541	255	**47.1**	65	255	**392.3**

developed to encourage their continued activity. Villagers should be further encouraged to take responsibility for their own containers and report to volunteers demanding replacement fish, an activity that was rarely reported. If it were possible to create demand for guppies through health education and promotion activities, there is a possibility of villagers raising them independently for sale. Highlighting immediate, tangible benefits of having guppies in containers such as "you won't get bitten by mosquitoes" or "there will be no larvae in your drinking water" may be more effective than strict dengue prevention messages, and further social marketing activities should be explored.

In large water storage containers guppies survive in low densities by feeding on plankton and algae, as well as mosquito larvae, without the need for supplementary feeding. They are easy to breed in the same containers. In Cambodia, breeding guppies were fed on ground rice husks which are a product of local rice production and initial calculations suggest project running costs could be lower than US$0.20 per household per year. In this study, guppies appeared not to jump from jars and consumed all *Aedes* immature larval stages. Volunteers all maintained large stocks of guppies ready for distribution and they reported no serious problems with breeding and maintaining stocks of fish. The rate of loss of guppies from household containers was approximately 10-20% per month so these breeding activities are essential.

We examined levels of infestation in "other" containers into which no guppies were distributed. The intervention area had three times fewer such containers per house than the control area, presumably a product of community education and greater awareness of dengue vector breeding habitats. Approximately half as many of these containers were positive in the intervention area than in the control area. This may be due to a decreased density of ovipositing adult female mosquitoes in villages where guppies were regularly eating larvae and could represent a "community-wide" protective effect.

While larvicide applications elicit a rapid reduction in *Aedes* larval density, the effects are short-lived, and the larvicide must be reapplied in approximately three months or less. This is in contrast to the use of guppies which is a year-round, community-based intervention that enables householders to control vector populations at the household level. We believe that if properly practiced, the reductions in entomological indices arising from guppy distribution represent a long-term, sustainable method of vector control with the potential to reduce the intensity of dengue virus transmission at least in rural areas of Cambodia where it is culturally acceptable, and possibly elsewhere.

A shortfall of this study was the lack of baseline assessments which would have allowed temporal associations to be made, and was due to our initial objective of assessment of guppy coverage using this community-based model. Cambodia's disease surveillance system and the small size of the trial also rendered compilation of dengue case data from the intervention area logistically challenging and inappropriate. There is no entomological indicator threshold correlating to low dengue transmission risk, due to climatic factors and fluctuations in levels of herd immunity that are also determinants of dengue outbreaks (WHO 2003a). However lower Breteau and house indices which have been achieved by this intervention are regarded to correlate with lower dengue risk (WHO 2003b).

The ability of guppies to consume larvae is well documented and we consider their maintenance in containers after one year to be a significant positive finding. Communities were extremely satisfied with the intervention and the lack of mosquito larvae in household water sources, and positive behaviors including additional, unsupported guppy colonization were reported. The methodology employed in Trapeang Kong province seems to require modest inputs from MoH or other local service providers to initiate and monitor project implementation. When local staff are trained, expanding into a wider area should be relatively straightforward, especially given the likelihood of favorable reports from neighboring areas that have adopted the intervention. We plan to scale-up guppy distribution in the near future, incorporating communication for behavioural impact processes that were not feasible for adoption in this small-scale trial. Baseline entomological measures, adult dengue vector population, and rates of dengue disease in treated communities will be assessed.

In addition to disease burden, dengue infections in Cambodia incur indebtedness upon poor households, lead to the sale of productive assets such as land, and can lead to poverty (Van Damme et al. 2004). Few sustainable and affordable methods of dengue vector control exist. Until other operationally feasible interventions are available, particularly for controlling *Ae. aegypti* in large water storage containers, the application of larvicide has necessarily been the mainstay of control activities in Cambodia in recent years. This vertical intervention protects only a limited, high-risk proportion of the population and costly larvicide must currently be applied twice to provide control during the main six-month-long dengue transmission season. An alternative method of vector control is urgently required.

Given the potential for reducing dengue risk, further research into methods of achieving better guppy coverage and adoption in other communities should take place in the immediate future with a view to large-scale implementation. This community-based activity has the potential to decrease the risk to communities of suffering from dengue outbreaks, illness, and death.

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