Dengue vectors in a rural area of Timor-Leste: Can small-scale mosquito control have a place in an integrated dengue control program?

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Introduction

Dengue virus infection is a major health problem in many countries in Southeast Asia and the Western Pacific region, including Timor-Leste (WHO 2009; Shepard et al 2013). Dengue is endemic in over 100 countries worldwide, with an estimated 390 million cases/year; of these, an estimated 96 million are moderate to severe (Bhatt et al 2013). Dengue incidence is generally underestimated, as dengue fever may be misdiagnosed if the diagnosis is made on clinical grounds only (Capeding et al 2013) and mild cases may be unreported. Serological tests for dengue are expensive, and, in rural areas of Timor-Leste where resources are limited, diagnosis is likely to be made on the basis of symptoms only. In addition, a proportion of the population may not seek medical advice for a variety of reasons, including travel distance to a health facility, absence of a health practitioner and use of traditional medicines (Deen et al 2013) and in these circumstances, cases of dengue would not be reported. Undurraga et al (2013) devised a model to estimate the burden of dengue in 12 countries in Southeast Asia, including Timor-Leste, based on existing health data but using an expansion factor that took into account under-reporting and misdiagnosis of dengue, and estimated that during the period 2001 to 2010, the average number of people infected with dengue virus was over 6,000 per year.

Dengue is transmitted primarily by *Aedes aegypti*, with *Ae albopictus* considered to be a less important vector (Lambrechts et al 2010). *Ae polynesiensis*, *Ae scutellaris* and *Ae hensilli* are also minor vectors of dengue in the Pacific region (Lambdin et al 2009; Moore et al 2007; Ashford et al 2003). *Ae albopictus* was first recorded in Timor-Leste (then Portuguese Timor) in 1974 (Pinhao 1974), although the exact location was not stated, and *Ae aegypti* was first recorded in Timor-Leste in Dili in 1991, together with *Ae albopictus* (Whelan 1999).

Dengue prevention programs focus on controlling *Aedes* mosquito populations, as at present there is no vaccine for this disease (Chang et al 2011; Lambrechts and Failloux 2012). There are several large-scale population control methods in development, for example, laboratory breeding of adult mosquitoes that carry a dominant lethal gene, then release of these *en masse* to compete with non-modified adults. Specific lethal genes include those resulting in flightless female offspring, sterility in males and failure to develop (Lacroix et al 2012). Another method is mass release of male mosquitoes that have been sterilized by radiation (Wise de Valdez et al 2011) or mosquitoes infected with a strain of the bacteria *Wolbachia* that suppresses transmission of dengue virus (Hoffmann et al 2011). However, it is not known when these large-scale methods may be widely applied in Timor-Leste, there are some disadvantages in their use, and efficacy is not guaranteed. For example, experimental releases of sterilised males of the malaria vector *Anopheles albimanus* in a small breeding population in El Salvador successfully eliminated *An albimanus*, but releases of sterilised males in larger scale experiments in the USA failed to control either *Ae aegypti* or *An quadrimaculatus*. Factors that affected efficacy included the low ability of laboratory-bred mosquitoes to compete with wild mosquitoes and the movement of fertilized females into the release zones (Dame et al 2009; Fanchinelli et al 2003).

Spraying adulticide or larvicide over large areas as fog/aerosol mist by plane, helicopter or land vehicle is commonly used to control *Aedes* mosquitoes (WHO 2009). Chemicals used are mainly pyrethroids or organophosphates. *Bacillus thuringiensis israelensis (Bti)* a bacterium that kills mosquito

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larvae may also be sprayed or misted onto larval habitats (Tan et al 2012; Jacups et al 2013). These large-scale methods may not be applicable or cost effective in some rural areas of Timor-Leste because of lack of vehicle access and difficulty of covering all larval habitats. Resistance to pesticides is also a problem (Tan et al 1999), as is harm to non-target organisms.

Larval control methods that can be implemented at the village level in rural areas include reducing available larval habitats by emptying, screening or covering water-containers (source reduction), adding natural predators or parasites of mosquito larvae to breeding sites (Nam et al 2005; Regis et al 2013; Paily et al 2013), adding larvicides (conventional insecticides, insect growth regulators or chitin synthesis inhibitors or *Bti*) to water containers and placing ovitraps (artificial egg traps) that incorporate a kill mechanism in and adjacent to houses. Each of these methods has disadvantages; for example, it is not usually possible to eliminate all possible breeding sites, the use of predators such as fish or copepods is restricted to relatively large containers that do not dry out, resistance has arisen to commonly-used organophosphate larvicides (e.g. temephos) (Lima et al 2011) and pyrethroids (Marcombe et al 2011a), and insect growth regulators (e.g. pyriproxyfen) and chitin synthesis inhibitors (e.g. novaluron) that kill larvae by interfering with their growth are expensive. The biological larvicide *Bti,* is useful in mosquito control because it kills rapidly and there is no cross-resistance with conventional pesticides. However *Bti* must be frequently replaced in natural breeding sites, especially in polluted habitats (Marcombe et al 2011b) or if there is exposure to direct sunlight (Mahilum et al 2005). The biolarvicide spinosad, may have a greater residual activity (Marcombe et al 2011b). Lethal ovitraps using chemical or biological larvicides as the kill mechanism have the disadvantages mentioned above, and ovitraps that have a mechanical kill mechanism, e.g. a wire mesh screen that stops the emergence of adults, or glue that traps ovipositing females), may become breeding sites for mosquitoes if the kill mechanism fails, and if the ovitraps are not monitored.

Lethal ovitraps have been used in *Aedes* control programs in, for example, Australia, Brazil and Thailand using pyrethroid-impregnated oviposition strips (Rapley et al 2009; Ritchie et al 2010b; Perich et al 2003; Sithiprasasna et al 2003), in Pakistan using *Bti* and insect growth regulators (Jahan et al 2011), in the Philippines using a pepper-based larvicide (Philippine information agency 2012), in Australia using sticky oviposition strips that trap adult females (Rapley et al 2009) and in Singapore, Thailand and the United States using a mesh barrier to trap emerging adults (Chan, 1972; Chan et al 1977; Peace Corps 2012; Lok, 1977; Cheng et al 1982). Attractants, usually plant infusions, may be added to lethal ovitraps (lure and kill strategy) (Reiter et al 1991; Mackay et al 2013). Ovitraps are also widely used in surveillance of Aedes mosquitoes, and the addition of a kill mechanism reduces the risk of surveillance ovitraps becoming breeding sites (Regis et al 2013). Female mosquitoes acquire dengue either by biting an infected person, or by infection at the egg stage (as infected females can transmit the virus to their eggs), so the number of mosquitoes capable of transmitting dengue can be reduced by ovitraps, as they remove the eggs of infected females who choose ovitraps as oviposition sites, i.e. the ovitraps act as ‘egg sinks’.

Although there have been some published surveys of the mosquitoes of various areas of Timor-Leste (Pinhao 1974; Whelan, 1999; Whelan and Petitt 2005; Cooper 2010) to date there has been little, if any, published data from Manufahi. In this study, larval surveys were conducted in a small rural community in Same sub-district, Manufahi District over 3 years with the aim of detecting the presence of the dengue vectors *Ae albopictus* and/or *Ae aegypti.* In an area where a dengue vector was detected, two methods of small-scale control were investigated, firstly, the use of copepods native to the area that are natural predators of mosquito larvae, and secondly the use of lethal ovitraps with wire screens to trap emerging adults.

Material and methods

Larval surveys were conducted in a rural area on the outskirts of the town of Same, in the district of Manufahi. The survey area bordered the Welala River, with an elevation ranging from approximately 460m to 600 m, and included forest, land that had been cleared for grazing and agricultural planting, and houses. The surveys were carried out in September 2010, January, July and November 2011, January and July 2012 and January 2013. Larval surveys were also carried out in Maliana in 2010 and 2011.

Natural habitats for container-breeding mosquitoes included tree-holes, rock holes, puddles, tree and bamboo stumps, and broken or gnawed coconut shells. Artificial habitats included containers for washing (mandis), smaller plastic containers for carrying and storing water, splits in bamboo fences, animal drinking troughs, potplant holders, and discarded metal drums, cans, bottles and tyres. Sampling these natural and artificial habitats for container-breeding larval Culicidae was conducted using a mosquito dipper for large containers, and by emptying small portable containers such as coconut shells into a large white plastic dish and removing larvae with a transfer pipette. Small tree holes and rock holes were sampled directly using a transfer pipette to empty the hole. Stage 4 larvae and pupae were killed in 70% ethanol, examined under a digital microscope and identified using the keys of Mattingly (1971) Huang (1979) and Steffan (1968). Some larvae and pupae were retained and raised to adults in breeding vials or large mosquito breeders for identification. Representative larvae, killed and preserved in 70% ethanol were taken to the Medical Entomology Unit, Darwin, Australia for confirmation of identification. Pools and large water containers were sampled for copepods using a plankton net. Copepods, killed and preserved in 70% ethanol, were sent to Dr. Maria Hołyńska, Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw, for identification.

Ovitraps were constructed by cutting the sloping top section off discarded plastic water bottles to make straight-sided containers. For some trials the top was completely removed, for others, only half of the top part of the bottle was cut away, leaving the cap and a sloping portion, that would partially protect the contents from rain and falling debris. The reason for recycling these discarded bottles rather than buying the standard black plastic cups or buckets that are traditionally used as ovitraps was one of cost and availability, as, at present, discarded plastic water bottles are very common in Timor-Leste. The containers were darkened by wrapping them in black plastic (recycled shopping bags obtained locally), as studies have shown that *Ae albopictus* and *Ae aegypti* prefer to oviposit in dark containers (Hoel et al 2011). A 1 cm diameter drainage hole was cut approximately 2 cm from the top of the container. Mosquito screen was cut into circles (diameter about 2mm less than the diameter of the bottles) and a smaller ring shape was cut from black polystyrene 'foam' food trays. As *Aedes* mosquitoes oviposit predominantly on the sides of containers on moist rough surfaces, a 2.5cm wide strip of either red velour paper or white cotton cloth was clipped to the inside of the container with a paperclip, which was also used to secure the black plastic wrapping. Spring water, or solutions containing various attractants were placed in the containers, and the polystyrene ring and mosquito wire were floated on the surface of the liquids. These ovitraps were placed in various locations, both inside and outside houses, with the permission of the landowners/householders. The purpose and mechanism of action of the ovitraps were explained, and householders were asked to choose a place inside their houses where the ovitraps would not inconvenience them and where they would not be easily accessible to young children. The ovitraps were checked daily for mosquito eggs using a hand lens. Constraints on ovitrap placement outdoors included the requirement to protect them from wandering livestock (pigs, poultry, goats, dogs, and cattle), heavy rain, and also from young children. Stones and pebbles were placed against the sides of the outside ovitraps for added stability.

Results

Ae albopictus was the only potential dengue vector found during the larval surveys in a rural area in Same sub-district. Opportunistic human landing catches also confirmed the presence of *Ae albopictus* as a highly prevalent day-biting mosquito. Larval identification was confirmed by Dr Huy Nguyen, Medical Entomology Unit, CDC, Royal Darwin Hospital. *Ae aegypti* was not found in this area, although a larval survey in Maliana in September 2010 found both *Ae albopictus* and *Ae aegypti*. Predatory copepods, identified as *Mesocyclops aspericornis* were found in a blocked drain in Same, Manufahi (the first confirmed record of this species from Timor-Leste). This identification was confirmed by Dr. Maria Hołyńska, Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw. *M aspericornis* has been effectively used elsewhere in Asia to control *Aedes sp.* However, it became apparent that *M aspericornis* would have limited use as a control agent in this area, as the main natural breeding sites of *Ae albopictus* in this particular rural community were too small and transient to be suitable long-term habitats for *M aspericornis*. Except for mandis, the community did not in general use the large water storage containers that were common in communities where *M aspericornis* has been successfully used for mosquito control, instead people generally collected water daily from springs and streams, and used communal springs, and also the riverside, as laundry and washing places. In addition, a study published during 2011 raised concerns about *M aspericornis* as a potential agent of transmission of *Gnathostoma spinigerum*, the cause of gnathostomias (Janwan et al 2011) which suggests that adding cyclopoid copepods to water containers that could be used for drinking or washing now may be contraindicated because of public health concerns.

Overall the ovitraps functioned effectively in that they were attractive oviposition sites for *Ae albopictus*, suggesting they could be used in both screening/monitoring and control programs. Trials in which larvae were allowed to develop to pupae showed that the mesh screen was effective in preventing the emergence of adults. In some cases, the mesh screen became dislodged from the supporting ring, by e.g. in several instances small toads jumping into the ovitraps or large chunks of debris falling in, indicating that regular monitoring would always be necessary to prevent the ovitraps becoming breeding sites. Ovitraps in houses functioned well in preventing emergence except in houses where they were placed in reach of young children. After the purpose and mode of action of the ovitraps was explained, people were encouraging about their use, however in general, their preference for long-term use was for ovitraps to be placed outside, not inside their houses.

Discussion

The presence of *Ae albopictus* in this rural area, and the absence to date of *Ae aegypti* has implications for control options. *Ae albopictus* is considered to be a less effective vector of dengue than *Ae aegypti*, (possibly due to its wider host range, and also because *Ae albopictus* is naturally infected with a strain of *Wolbachia* that reduces viral infection of the mosquito's salivary glands (Mousson et al 2012; Lambrechts et al 2010). However *Ae albopictus* has caused dengue epidemics in areas where *Ae aegypti* is absent, although these are generally less severe than those caused by *Ae aegypti* (Peng et al 2012; Xu et al 2007; Effler et al 2005).

The incidence of dengue in Same sub-district is reported to be low (Same hospital administration and Manufahi Department of Health personal communication). However, as in many other countries where dengue is endemic, it seems likely that mild cases of dengue are not reported. Although a survey of health service usage was not a component of this study it was impossible to avoid noticing a strong reliance on traditional medicine among the householders in the sample area. The two traditional medicine clinics supplied locally grown herbal products to treat fever, some local people regularly harvested plants for medicinal purposes, and people with mild fever tended initially not to seek advice from doctors, and therefore mild fever due to dengue would not be recorded in these cases. *Ae albopictus* is thought to act as a maintenance vector of dengue in rural areas (Gratz et al 2004) and control measures that decreased the population of this mosquito would reduce the risk of a major dengue outbreak in the future.

Ae albopictus is also a competent vector of at least 22 other arboviruses including chikungunya, (Vazeille et al 2010, Gratz 2004, Samuel et al 2009) *Ae albopictus* is of veterinary importance too, as it is a vector of dog heartworm, *Dirofilaria immitis (*Cancrini et al 2003) and a nuisance day-time biter. For these reasons control of *Ae albopictus* in this rural area is desirable.

The WHO recommends a dengue vector management plan incorporating several methods of mosquito control including include source reduction, biological control if applicable and environmental management through community participation (Chang et al 2011). Current WHO research strategies for dengue control includes the use of lethal ovitraps (WHO 2013). Efficacy of ovitraps depends on the attractiveness as oviposition sites compared with other available oviposition sites, placement and density of traps, efficacy of the kill mechanism long-term, and acceptance of ovitraps by the local community. Norzahira et al (2011) and Lim et al (2010) in ovitrap surveys in a suburban area in Malaysia found significantly more *Ae albopictus* larvae in outdoor ovitraps than in indoor ovitraps. This was not the case for *Ae aegypti*. However in a study in Taiwan, significantly more *Ae aegypti* females were collected indoors than outdoors, but more *Ae aegypti* eggs were collected from ovitraps placed outside houses (Wu et al 2013). In the present study, because house windows and doors were unscreened, house doors were frequently left open all day, and traditional house construction left many gaps where mosquitoes could enter, it was hypothesized initially that ovitraps inside would collect as many eggs as ovitraps outside, but this was not so. Placement of ovitraps inside houses had the advantage of protecting the ovitraps from rain, debris and disturbance by animals, but the disadvantages of a lower egg count, and householders' general preference for outside placement of traps.

Conclusion

The dengue vector, *Ae albopictus* is present in the sub-district of Same, Manufahi District. Lethal ovitraps made from recycled plastic bottles with a mesh screen as a kill mechanism are a low-cost, environmentally friendly method of potentially reducing *Ae albopictus* populations in this rural area, if combined with source reduction and other control methods, and if ovitraps are monitored at approximately weekly intervals. Biological control using the copepod *M aspericornis* may have a very limited application.

Ethics statement

Approval from the Timor-Leste Ministry of Health Research Committee, the District Administrator of Manufahi and the Manufahi District Minister of Health was obtained to conduct a field study in Timor-Leste. Verbal permission from the landowners was obtained before placing any ovitraps, or sampling any natural or artificial habitats for larval Culicidae. After explanation of study objectives and procedures, verbal consent was obtained from the adults living in each household for placement of ovitraps in and adjacent to houses.

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