## B

Vector borne infectious diseases, including dengue, are an important public health problem, linked to poverty, in Latin America and the Caribbean [1]. It is estimated that there are 390 million dengue infections globally each year, 96 million of which produce clinical disease [2]. The main dengue vector is the *Aedes aegypti* mosquito, which generally inhabits urban habitats and breeds in artificial water containers [3], including those used to store water and those that incidentally accumulate water [4, 5]. *Aedes aegypti* is also the vector for other infections of public health importance, including yellow fever, chikungunya, and zika [6–8].

The main strategy for controlling dengue (as well as chikungunya and zika) is to control the vector, and in particular to control its breeding sites in water containers in and around households. Measurements need to be able to reflect the impact of interventions on vector breeding. Pupae indices are the best estimators of dengue transmission risk, because pupa mortality is minimal compared with larvae mortality [9, 10]. Several pupae indices have been described, including: pupae per household; pupae per person; pupae per hectare; and even more specific ones, such as an index of sexual dimorphism focused on the female pupae. Trials of chemical and other interventions for dengue vector control have reported the impact on different pupae indices [11–14].

After the failure of the dengue eradication programme of the 1950s and 1960s the Pan American Health Organization urged countries to focus their efforts on dengue *control* using an integrated approach, giving priority to environmental management (eliminating mosquito breeding opportunities wherever possible and properly covering the remaining containers), with chemical control using larvicides restricted to containers that could not be controlled by any other means and space sprays reserved for emergency situations [15]. Community participation and health promotion were encouraged but in practice the main activity in most countries was a periodic household visit by personnel from the government's vector control authority to apply the organophosphate temephos to water containers complemented by occasional space spraying to control the adult mosquito. Between 2007 and 2009, three systematic reviews, although limited by the quality of available evidence, pointed to the value of community participation in reducing *Aedes aegypti* vector density [16–18].

In a cluster randomised controlled trial conducted in Mexico and Nicaragua, we showed that community participation based on socialising evidence for participatory action reduced rates of recent dengue infection, rates of self-reported dengue illness, and four entomological indices of the *Aedes aegypti* vector [19]. The main trial analysis compared entomological indices between intervention and control clusters in the final impact survey, which took place in the dry season. An earlier survey in both intervention and control clusters took place in the preceding rainy season. This article reports an analysis of pupae measurements and other entomological indices in the rainy and dry seasons and examines the impact of the trial intervention on *Aedes aegypti* pupal production in both rainy and dry seasons in the Mexican arm of the trial. Data on pupal productivity from the Nicaraguan arm of the trial are reported elsewhere [20].

Details of the Camino Verde trial methods are described elsewhere [19]. Briefly, the trial tested the impact of evidence-based community mobilisation for control of *Aedes aegypti* breeding sites as a means to reduce dengue virus infections and clinical disease, in addition to continuing normal prevention efforts, such as application of temephos to household water containers. The trial took place in sites in Managua, Nicaragua, and in 90 representative clusters in the three coastal regions of Guerrero State, Mexico. In Mexico, half the clusters were randomly allocated to receive the intervention, with the remaining clusters acting as controls. The trial impact survey took place in two phases in 2012; the first phase in August–September 2012 was in the rainy season, and the second phase in November–December 2012 was in the dry season. Both phases included an entomological survey of the households in intervention and control clusters.

### En omological s r e

Trained fieldworkers, working in pairs, undertook entomological inspections in the 90 clusters, each of around 130 households, while other fieldworkers undertook household interviews in the same households. The field teams re-visited closed households up to three times. With the consent of the householder and accompanied by a household member, the fieldworkers recorded

The entomologists used a stereoscopic microscope (Olympus ® CX41) and classified and quantified larvae and pupae according to recognized taxonomic codes [21, 22]. They identified and counted as pupae any exuviae and adult mosquitoes found in the samples. We preserved the larvae and pupae samples in a 70% alcohol solution after examination.

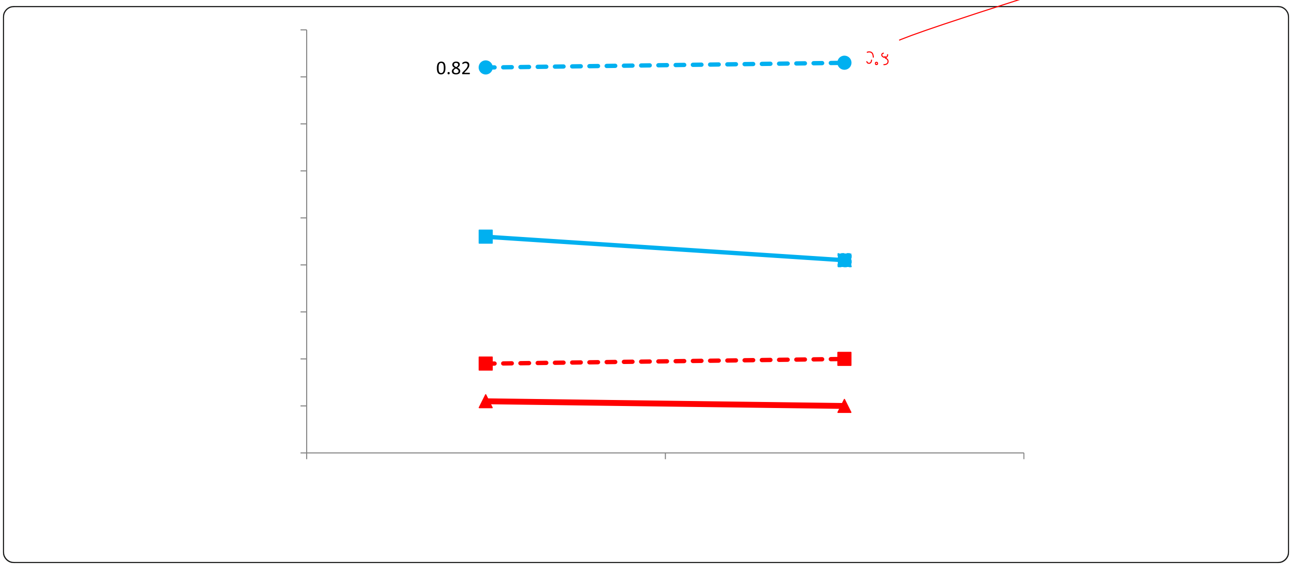
### S a is ical anal sis

Statistical analysis relied on the open-source software CIETmap [23] which provides an interface with the R statistical programming language. We calculated pupae indices for two groups of containers: water storage containers, and containers which incidentally accumulated water (such as discarded items, flower pots, and tyres). We calculated several different indices:

1. Pupal productivity percentage for different container types, calculated as the total number of pupae in the container type, divided by the total number of pupae in all containers, multiplied by 100.

and/or pupae. Most of these containers were found outside the households (73.8%, 29,758/40,323) and just over half were uncovered at the time of the inspection (53.3%, 21,501/40,323). In the dry season later in the year, the CI was lower: 5.8% (2542/43,461) of inspected containers were positive for *Aedes aegypti* larvae and/or pupae. In the dry season, most of the containers were inside the household (75%, 32,626/43,461) and just over half were uncovered (53.6%, 23,287/43,461).

As shown in Tables 1 and 2, almost all the con-




0.018; cluster adjusted 95% CI 0.009 to 0.028). In the dry season (Table 2), the mean number of pupae per container was 0.10 in intervention clusters and 0.20 in control clusters. The proportion of containers with above the overall mean number of pupae (0.15) was again significantly lower in intervention clusters (0.10) compared with control clusters (0.20) (difference 0.012; cluster adjusted 95% CI 0.002 to 0.023).

The PHI and PPI were consistently lower in intervention clusters than in control clusters in rainy and dry

lower in intervention than control clusters, in both seasons. The tests are summarized in Table 5.

## D

The entomological field teams found more containers in and around the households during the dry season than during the rainy season (43,461 vs. 40,323). Despite this, the overall number of pupae found in the households was higher during the rainy season than during the dry season (7070, vs. 6552), and the CI was also higher during the rainy season. These results are similar to those reported by Garelli in Argentina [25] and Maciel de Freitas in Rio de Janeiro (Brazil) [4].

During the rainy season, most inspected containers were found outside the household, whereas most containers were inside the households in the dry season. This reflects the practice of placing containers outside the household during the rainy season in order to collect rainwater, in

Camino Verde trial, made significant contributions to the final manuscript. All authors read and approved the final manuscript.

## Compe ing in eres s

The authors declare that they have no competing interests.

## Consen for p blica ion

Not applicable.

## E hics appro al and consen o par icipa e

The Institutional review board at the CIET Canada research ethics board (16 November 2009) and the ethics committee of the Centro de Investigación de Enfermedades Tropicales at the Universidad Autónoma de Guerrero (27 November 2009) approved the study. All boards performed annual review and approval throughout the study. All participants gave informed consent.


## Abo his s pplemen

This article has been published as part of BMC Public Health Volume 17 Supplement 1, 2017: The Green Way to Aedes aegypti mosquito control: aspects and implications of the Camino Verde trial in Mexico and Nicaragua. The full contents of the supplement are available online at https://bmcpublichealth.biomedcentral.com/articles/supplements/volume-17-supplement-1.


Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.


## A hor de ails

[1]Centro de Investigación de Enfermedades Tropicales (CIET), Universidad Autónoma de Guerrero, Acapulco, Guerrero, Mexico. [2]Unidad Académica de Ciencias Químico Biológicas, Universidad Autónoma de Guerrero, Chilpancingo, Guerrero, Mexico. [3]Departamento de Prevención y Control de Enfermedades Transmisibles por Vector, Servicios Estatales de Salud Guerrero, Av. Rufo Figueroa 6, Colonia Burócratas, Chilpancingo, Guerrero, Mexico. [4]Centro Regional de Investigación en Salud Pública, 19 Poniente Esquina 4ª Norte s/n, C.P30700 Colonia Centro Tapachula, Chiapas, Mexico. [5]CIET International, New York, NY, USA. [6]Department of Family Medicine, McGill University, Montreal, Canada. [7]CIET Trust, Gaborone, Botswana.



Published: 30 May 2017


## References


1. Dujardin JC, Herrera S, Do Rosario V, Arevalo J, Boelaert M, Carrasco HJ, et al. Research priorities for neglected infectious diseases in Latin America and the Caribbean region. PLoS Negl Trop Dis. 2010;4(10):1371–5.
2. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496(7446):504–7.
3. WHO. World Health Organization, Fact sheet 117, Geneva, April 2017, dengue and severe dengue. http://www.who.int/mediacentre/factsheets/fs117/en/. Accessed 28 Apr 2017.
4. Maciel de Freitas R, Marques WA, Peres RC, Cunha SP, de Oliveira RL. Variation in Aedes aegypti (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. Mem Inst Oswaldo Cruz. 2007;102(4):489–96.
5. García Rejón JE, López Uribe MP, Loroño Pino MA, Farfán Ale JA, Nájera Vázquez MR, Lozano Fuentes S, et al. Productive container types for Aedes aegypti immatures in Mérida. México J Med Entomol. 2011;48(3):644–50.
6. Barnett ED. Yellow fever: epidemiology and prevention. Clin Infect Dis. 2007;44:850–6.
7. Weaver SC, Lecuit M. Chikungunya vhra


a(with)parahave3(la8((with)ne9n4ibuta(Con)-8.8(4299.6(R,)-3larvasrD[dengue)-298.6(and)-303303.3(comos:)]Jhe)-385.6-29un)-301.1(ale1Tfin)-las.7(h