

# **Control of Aedes breeding sites with a dual-action insecticidal coating is effective in reducing Dengue transmission: Cluster-randomised Controlled Trial Study**

---

## **Study Protocol**

---

Ubication of study: Metropolitan Area of Cucuta, Colombia

Approval of the protocol: 11 March 2019

Update: 12 February 2024

# Study Protocol

---

## Place of study

The base-line study was conducted within 2.5 months, the intervention was applied within 3 months and the follow up study with final evaluation was within 12 months, in 2019–22 in Metropolitan Cucuta in the North-East of Colombia, including the city of Cucuta (629,414), and two adjacent municipalities Villa del Rosario (93,735) and Los Patios (81,411 inhabitants) located in the Norte de Santander state [1]. The climate is warm and dry, with a mean annual temperature of 26.7 °C and annual rainfall of 806 mm. There are two rainy seasons from the end of March to the beginning of June and from the end of September to the beginning of December. DZC are endemic, with occasional epidemic outbreaks [2].

## Study design and sample size to baseline study and evaluation

This is design study for a large cluster randomized trial on the impact of treating water containers with a protective paint. The sample size was calculated for detection of a 50% reduction in the House Index with > 99% power at 5% significance level. Given a baseline HI of 30%, an intra-cluster correlation coefficient (ICC) of 0.01 and a cluster size of 2,000 households it was found that a minimum of 12 clusters per study arm was needed [3]. The same result but with a lower power (68%) was found for a baseline DZC incidence of 3% and a 50% reduction in the intervention arm. A large number of households per cluster was needed as *Ae. aegypti* vectors are day-biters [4,5,6] and it was assumed that many household members stay within their neighbourhoods during the day (mainly housewives, small children and school children when the school is close to their house) where they are at risk to get infected. All estimations were done through The Shiny CRT Calculator which is a web-based app to determine sample size and power for cluster trials [7].

## Sampling procedure for household survey and entomological inspections

On the basis of available maps, the city was stratified into high, middle and low endemic/infested areas for *Ae. aegypti* (strata) using the surveillance information from SIVIGILA of the preceding year as well as information obtained from interviews with the vector control coordinators in Cúcuta. By this way, 24 high and medium endemic areas (clusters) were selected in Cucuta (16 clusters), Los patios (4 clusters) and Villa del Rosario (4 clusters). Figure 1 shows the selected clusters in

Cucuta to be sampled by staff. For the baseline study they were not yet paired and allocated to the intervention and control arm as this was planned to do with the information resulting from the baseline study enabling us to find pairs of clusters with similar characteristics. The following characteristic should be similar in each pair of clusters: (I) High and middle level endemicity for *Aedes*-borne diseases; (II) Similar socio-economic conditions; (III) Similar types of the built environment: houses, schools, industry, health centers, workshops, churches and stores; (IV) Similar characteristics of public spaces. Within each cluster, all blocks of houses were inspected including public spaces. For the entomological/socio-demographic household survey, a proportion of 10% of 2000 houses was sampled in this study, resulting in 200 houses in each cluster. This number was rounded to 250 by convenience. Therefore, the study targeted 6000 households (250 households in each cluster) using a systematic random sampling method. For this we first estimated the sampling interval by dividing the total number of households in each cluster (around 2000) by the number of sample households (250), resulting in a sampling interval of 8. All households located within each cluster were mapped and a randomly defined starting point was selected and from there every 8th household was visited. Clear replacement rules were given to the interviewers and the compliance was checked in sample of families.

Vector control staff and trained interviewers participated in the survey. They were divided into “couples” of one vector inspector and one interviewer. Each couple was assigned one cluster where they had to visit the sample houses. The interviewer did the interview with the head of household and simultaneously the inspector (local vector control staff) did the inspection of water containers both of them filling a form.

### Data collection

The baseline surveys included a household questionnaire and entomological survey (see Additional files). The instruments were discussed in a participatory workshop with experts and pre-tested among the members of vector control staff in the three municipalities leading to some modifications. The first instrument was the questionnaire which was applied by trained interviewers and the other part was the form for the entomological inspections (larvae/pupae survey) which was applied by the vector control staff. As this is part of the national vector control programme only verbal consent of the house owner was obtained to check their water containers. An extensive training was conducted by the research team before starting the field work.

## Household questionnaire

Demographic and health data was collected through face-to-face interviews using a standard questionnaire that included both structured and semi-structured questions. Household questionnaire was adapted from published research in Colombia [8]. The questions referred to socio-demographic parameters (number of people living in the house, age, sex, and educational level), self-reported DZC disease acquired during the last months, population mobility during the day, usage of water in tanks and willingness to accept the intervention trial (see Additional file).

## Entomological survey

The standard entomological survey form was adapted following the guidelines of Standard Operational Procedures (SOPs) by WHO [9]. The questions were adapted to local conditions and survey requirements (see Additional file). The following data were recorded from each household: total number of containers (potential breeding sites), number of mosquito larvae positive and negative containers (any species), pupae count per container, container type, and other container characteristics (if they were covered or uncovered, outdoors or indoors).

## Dengue surveillance and case definition

Data of the national surveillance system were obtained from SIVIGILA (the national health surveillance system), aggregated by year and setting (study areas) over the study period. Notified dengue cases including those classified as dengue fever (DF) and severe dengue, relying on a clinical case definition or lab confirmed or hospitalized patients. This study used annual population data (from the National Institute of Statistics-DANE; [10]) for calculating the incidence rate.

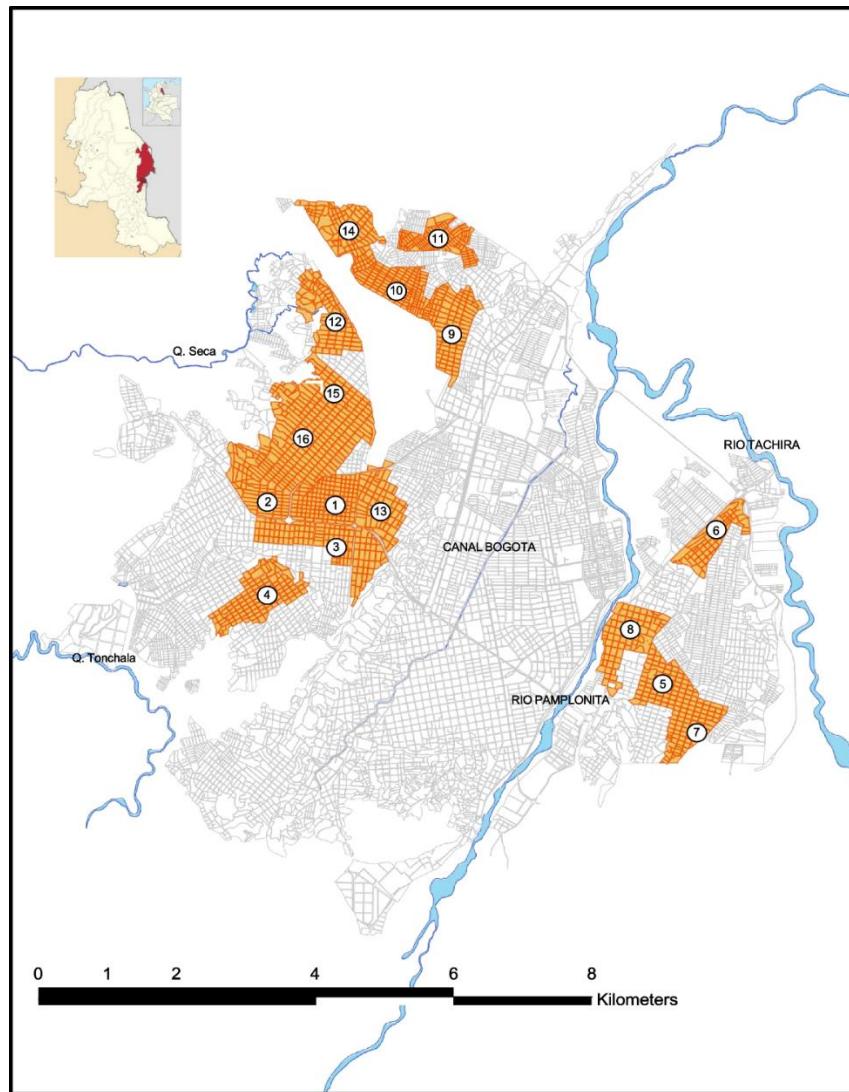
## Ethical considerations

The household questionnaire was only applied to adults who provided information related to the purpose of this study. No child or adolescent below the age of 18 was interviewed in this study. All people participating in the study were informed in local language through the study information sheet in a written and oral way. They were asked to sign the informed consent form. All participants were informed that their participation was voluntary and that their responses remained anonymous, therefore the study used numbers which replaced the names of individuals and codes which replaced the address of house. Before

examining the domestic and peri-domestic water-holding containers, the field team requested permission to enter the house, did the inspection and collected entomological and sociodemographic data.

The study received approval from local health authorities in Cucuta and Norte de Santander and the study protocol was approved by the ethical committee of the Albert-Ludwigs-Universität (application number 141/19) in Freiburg, Germany

## Fig. 1



Selection of clusters in Cucuta

Map shows the 16 clusters selected in Cucuta which were visited by interviewers and vector control staff

**Fig. 2**



A concrete ground tank for washing purpose

Concrete ground tanks for washing (*lavadero*) are made of cement or ceramic tiles with the shape of a rectangle or square and variable sizes

## References

1. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, Lindsay SW. The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Negl Trop Dis.* 2020;14(1):e0007831. <https://doi.org/10.1371/journal.pntd.0007831>.
2. Wolbers M, Kleinschmidt I, Simmons CP, Donnelly CA. Considerations in the design of clinical trials to test novel entomological approaches to dengue control. *PLoS Negl Trop Dis.* 2012;6(11):e1937.

3. DANE. Censo Nacional de Población y vivienda 2018. <https://geoportal.dane.gov.co/geovisores/sociedad/cnpy-2018/>. Accessed 30 Apr 2021.
4. Instituto Departamental de Salud de Norte de Santander (INS Norte de Santander). 2016. Análisis de Situación de Salud con el Modelo de Determinantes Sociales. Coordinación de vigilancia en Salud Pública. Fecha de consulta: 4 de abril de 2018. Available from en: <http://salasituacionalidsnds.weebly.com/uploads/1/0/7/1/10714324/asisdepartamental-2016-nortedesantander.pdf>. Accessed 19 Mar 2018.
5. The Shiny CRT Calculator: Power and Sample size for Cluster Randomised Trials. (2022). <https://clusterrcts.shinyapps.io/rshinyapp/>. Accessed 20 June 2022.
6. Wong J, Astete H, Morrison AC, Scott TW. Sampling considerations for designing Aedes aegypti (Diptera:Culicidae) oviposition studies in Iquitos, Peru: Substrate preference, diurnal periodicity, and gonotrophic cycle length. *J Med Entomol*. 2011;48(1):45–52.
7. Yasuno M, Tonn RJ. A study of biting habits of Aedes aegypti in Bangkok. *Thailand Bull World Health Organ*. 1970;43(2):319–25.
8. Chadee DD. Landing periodicity of the mosquito Aedes aegypti in Trinidad in relation to the timing of insecticidal space-spraying. *Med Vet Entomol*. 1988;2(2):189–92.
9. Hemming K, Kasza J, Hooper R, Forbes A, Taljaard M. A tutorial on sample size calculation for multiple-period cluster randomized parallel, cross-over and stepped-wedge trials using the Shiny CRT Calculator. *Int J Epidemiol*. 2020;49(3):979–95.
10. Higuera-Mendieta DR, Cortés-Corrales S, Quintero J, González-Uribe C. KAP Surveys and Dengue Control in Colombia: Disentangling the Effect of Sociodemographic Factors Using Multiple Correspondence Analysis. *PLoS Negl Trop Dis*.
11. Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization. Operational guide for assessing the productivity of Aedes aegypti breeding sites. <http://www.who.int/tdr/publications/documents/sop-pupal-surveys.pdf>. <http://www.who.int/tdr/publications/documents/sop-pupal-surveys.pdf>. Accessed 20 Jul 2018.

---

12. DANE. Geovisor CNPV 2018: Geoportal DANE GEOPORTAL DANE. <https://geoportal.dane.gov.co/geovisores/territorio/analisis-cnpv-2018/?lt=4.646075&lg=-74.088605&z=20>. Accessed 15 Dec 2021.

13. World Health Organization. Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Haemorrhagic Fever. Revised an. World Health Organization, Regional Office for South-East Asia 2011. Available online at: [http://origin.searo.who.int/entity/vector\\_borne\\_tropical\\_diseases/documents/SEAROTPS60/en/](http://origin.searo.who.int/entity/vector_borne_tropical_diseases/documents/SEAROTPS60/en/).

---

## Household questionnaire

### Form for extraction of socio-demographic data

*We are evaluating a new strategy against dengue. If you allow me, I will ask a few short questions that will not take up much of your time. If you feel uncomfortable with any question, you cannot answer or end the interview whenever you want.*

House code: \_\_\_\_\_

Cluster code: \_\_\_\_\_

**1. ¿ Could you give us the following details about the people who live in your house, including yourself in the following table?**

#### Part A: Household sociodemographic characteristics

Person	Age	Sex 1=F 2=M 3=O	Education Primary school=1 High school= 2 University= 3 No= 4	During this year, did you or anyone in your household have DZC? 1=Dengue 2=Zika 3=Chikunguna 4=No	Which month?	Did you go to the doctor or health unit? 1=Yes 2=No	During the day, where do you spend most of the time (7 am -5 pm)? 1=Inside neighborhood, 2=Outside neighborhood
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

#### Part B: Practices on dengue and water use

Mark the correct answer

**2. Do you apply some type of vector control in your laundry tank to prevent dengue?**

1= Use of fish 2 = Addition of chlorine 3= Use of tank lid 4=Washing and brushing 5= None 6=I don't know

**3. Has the health service applied any type of powder or tablet insecticide in your laundry tank this year?**

1= Yes 2= No 3= I don't know

**4. Which deposits do you use for water consumption?**

1= Ground tank 2=Tap water 3=Other deposits, specify \_\_\_\_\_ 4=None

**5. Do you know where mosquitoes lay their eggs or where they breed?**

1=Clean water 2=Dirty water (e.g. puddle) 3= Grass or pasture 4= I don't know

**6. ¿ which of these media would you like to receive information about dengue? (Multiple choices)**

1=TV 2=Radio 3=Cell phone 4=Internet 5=Newspaper 6=Flyer 6=Visit to your home by a technician 7=Loudspeakers 8=I don't know

#### Part C: Willingness of the community to receive the insecticide coating

**7. Would you like to apply freely a transparent coating as a control method in your laundry tank?**

1 = Yes 2=No 3= I don't know

Thanks for your participation

(Spanish version)

Estamos evaluando una nueva estrategia contra el dengue. Si me permite le realizaré unas preguntas cortas que no le quitarán mucho tiempo. Si usted se siente incómodo con alguna pregunta, puede no contestar o terminar la entrevista cuando lo deseé.

Código casa: \_\_\_\_\_

Código conglomerado: \_\_\_\_\_

**8. ¿Podría darnos los siguientes detalles sobre las personas que viven en la casa, incluyéndose en la siguiente tabla?**

**SECCIÓN A: Características Sociodemográficas del hogar**

Persona	Edad	Sexo 1=F 2=M 3=O	Educación Primaria=1 Bachillerato= 2 Universidad= 3 No= 4	¿En este año ud. o alguien de su hogar tuvo DZC? 1=Dengue 2=Zika 3=Chikunguna 4=No	¿En qué Mes?	¿Asistió al médico o unidad de salud? 1=SI 2=No	¿Durante el día, donde permanece la mayor parte del tiempo (7 am - 5 pm)? 1=En mi hogar o barrio, 2=Fuera del barrio
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

**SECCIÓN B: Prácticas sobre el dengue y uso del agua**

Marque la respuesta correcta

**9. ¿Usted aplica algún tipo de control en su tanque de lavadero para prevenirse del dengue?**

1= Uso peces 2 = Adición de cloro 3= Uso tapa tanque 4=Lavado y cepillado 5= Ninguno 6=No sé

**10. ¿En este año el servicio de salud ha aplicado algún insecticida tipo polvo o pastilla en su tanque de lavadero?**  
1= Si 2= No 3= No sé

**11. ¿Cuáles de los depósitos usa para el consumo de agua?**  
1= Tanque bajo 2=Agua de la llave 3=Otros depósitos, especifique 4=Ninguno

**12. ¿Sabe usted en dónde ponen los mosquitos sus huevos o dónde se crían?**  
1=Aqua limpia 2=Aqua sucia (charco) 3= Monte o Pasto 4= No sé

**13. ¿Con cuál de estos medios le gustaría recibir información sobre el dengue? (opción multiple)**  
1=TV 2=Radio 3=Celular 4=Internet 5=periódico 6=Volante 6=Visita a su casa por técnico 7=Perifoneo 8=No sé

**SECCIÓN C: Disposición de la comunidad para recibir el recubrimiento insecticida**

**14. ¿Le gustaría que pongamos una pintura transparente como método de control de forma gratuita en su tanque de lavadero?**

1 = Si 2=No 3=No sé

Gracias por participar

## Entomological survey

## Form for extraction of entomological data

**House code:** \_\_\_\_\_ **Cluster:** \_\_\_\_\_

**Inspector:** \_\_\_\_\_

Date: \_\_\_\_\_

**Building type:** House:  Car store:  Health unit:  Church:  Grocery store:  School:  Restaurant:  other:  Public space:

Comment:

(Spanish version)

Código casa: \_\_\_\_\_

Conglomerado: \_\_\_\_\_

Inspector: \_\_\_\_\_

Fecha: \_\_\_\_/\_\_\_\_/\_\_\_\_

Tipo de edificación: Casa: \_ Taller: \_ Unidad de salud: \_ iglesia: \_ Tienda: \_ Colegio: \_ Restaurante: \_ otro: \_ Espacio Público: \_

No.	*Tanques de agua		Otros depósitos con agua		*Detalles de cada tanque de agua	Índices entomológicos	Medidas
	Cemento	Plástico	Tanque bajo	Tanque almacenamiento			
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
15							
16							
17							
18							

Observación: \_\_\_\_\_