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Comparison of *Aedes aegypti* arbovirus transmission thresholds in two communities with differing water supply infrastructure

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Abstract

Background To assess whether the 'economic boom' in the tropical seaport city of Barranquilla improved tapped water supplies to socio-economically poor neighbourhoods resulting in: (1) their reduced use for domestic water-storage in large (> 1,000-litre) custom-made cement tanks which are their principal *Aedes aegypti* breeding sites and (2) their pupae/person index (PPI) values to below their established 0.5–1.5 PPI arbovirus transmission-threshold value, compared to matched neighbourhoods in the: (a) pre-economic boom (2004) period in Barranquilla and (b) economically-neglected seaport city of Buenaventura.

Methods The simple, accurate and robust water surface sweep-net/calibration factor or total count methods were used to determine the total *Ae. aegypti* pupae numbers in greater or less than 20-litre water-holding container types located 'inside' or 'outside' these neighbourhood premises. The women residents also participated in questionnaire-based responses about their domestic water supplies, water-storage and maintenance and mosquito life stages and disease transmission knowledge, to subsequently plan appropriate resident education programmes. Microsoft Excel 8.0 with OpenEpi was used to determine the samples sizes and the statistical values.

Results Tapped water supplies to the three poor Barranquilla neighbourhoods were dramatically increased from 2004 to 2023 resulting in their residents significantly reducing their: (a) large cement water-storage tanks from 1 per 6.9 (2004) to 1 per 31.2 (2020) premises ($z = 10.5$: $p = 0$) and (b) PPI values to 0.16, 0.19 and 0.53 (mean: 0.29: 95% CI ± 0.4) in each study neighbourhood. In contrast, tapped water supplies remained inadequate in the Buenaventura neighborhoods, thereby resulting in their continued use of many large (> 1,000-litre) water-storage containers (Barranquilla: 1 per 31.2 and Buenaventura: 1 per 1.5 premises: $z = -9.26$: $p = 0$), with unacceptably high 0.81, 0.88 and 0.99 PPI values in each study neighbourhood (mean 0.89: 95% CI ± 0.12).

Conclusions Improved tapped water supplies resulted in reduced numbers of large custom-made stoneware water-containers, as are employed by poor residents throughout the world, as well as their *Ae. aegypti* PPI transmission threshold values which, together with appropriate residents' education programmes, are also urgently to reduce to prevent/reduce *Ae. aegypti* transmitted human diseases globally.

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Keywords *Aedes aegypti*, Community-practices, Water-storage, Arboviruses, Disease, Control, Dengue

Introduction

The mosquito species *Aedes aegypti* is the main vector of the dengue viruses (DENVs), but it is also an efficient vector for other arthropod-borne viruses such as chikungunya, Zika and urban yellow fever [1]. Since *Ae. aegypti* has a highly domestic and anthropophilic nature and is present throughout most tropical and subtropical areas [1, 2], it represents a major global public health problem through its estimated 100 million annual human arbovirus-transmitted infections [1, 3]. This vector species nearly always breeds in clean water but while their populations are increased during wet seasons, its principal breeding sites throughout the year are large domestic water-storage containers in Barranquilla [4–8]. Importantly, large water containers were known for decades to be their principal breeding sites in Africa and Asia and other regions of the world for decades [9, 10] and was performed prior to the effective yellow fever vaccine programme through the increased provision of water supplies, storage tank and domestic water container covers and their maintenance through cleaning [11]. More recently, domestic tanks were also demonstrated as principal *Ae. aegypti* breeding sites in the international WHO-TDR funded Pupae Demographic Survey [12]. As such, these large custom-made containers could produce up to 92% of the *Ae. aegypti* pupae populations with a pupae/person index (PPI) value of 11, which were dramatically above their established 0.5–1.5 PPI arbovirus transmission-threshold value [5, 13]. These high productive domestic water-storage tanks and drums, used due to irregular tapped water supplies are therefore the main targets for the control of the *Ae.aegypti* aquatic stages by the local Health Authorities, usually using temephos treatment [14] (see below).

We previously performed a complete *Ae.aegypti* larvae and pupae survey in all water holding containers in three poor (socio-economic strata 1 and 2) dengue virus 'hotspot' neighbourhoods in a principal Caribbean seaport city of Barranquilla during 2004 [6]. As expected, roof-covered large custom-made domestic water-storage cement tanks and drums were the principal *Ae.aegypti* breeding sites during the dry season [6]. Barranquilla subsequently underwent dramatic economic growth due to international investment resulting in a reduction in poverty and unemployment [15, 16] and increased the tapped water supplies to even the poorest residents and resulting in supplying 99.4% of the premises throughout the city [17].

We therefore opted to perform and compared an updated *Aedes aegypti* pupae survey in (a) three very poor (socio-economic stratum 1 and 2) neighbourhoods of Barranquilla located immediately adjacent to those previously surveyed during the dry season in 2020, as well as (b) three matched neighbourhoods in the economically-neglected seaport city of Buenaventura which is located on the Pacific Coast of Colombia and were approximately only 55% of the population have access to the tapped water supply which is only accessible for 6 h/day in 2014 [18], but which was reduced to 17.4% of the premises not having a tapped water supply in 2020, but which was only available for 4 h/day every second day in a Colombian Government report [19]. In addition, the women residents in these neighbourhoods were questioned about their domestic water practices and beliefs.

Materials and methods

Ethical considerations

This study was approved by the ethics committee of the Universidad del Norte, through act number 180. These studies were performed during the COVID-19 pandemic at a time when travel in public spaces was permitted under strict biosafety conditions. The team performed the information-gathering activities following the protocols and measures established by the Colombian government. Importantly, informed consent was obtained, before performing the entomological study and the anonymity of the residents who were interviewed and who responded to the questionnaire from each premise visited was maintained using a neighbourhood, block and premise and resident numbering system and the publication of the findings was agreed by the Health Authorities of both Barranquilla and Buenaventura.

Study sites

Premises in three neighborhoods of Barranquilla, Atlantico (Caribbean coastal seaport city: population: 2,775,756) and Buenaventura, Valle de Cauca (Pacific coastal seaport city: population: 451,000) were surveyed during 2020 (Fig. 1). These neighborhoods were chosen due to being matched neighborhoods which were classified as belonged to the lowest socio-economic 1 and 2 stratum levels using the Colombian Estratificacion Socio-Económica (ESE) and SISBEN systems [20] and maps for the study neighbourhoods in each city are shown (Fig. 1). Importantly, the socio-economic stata classifications in Colombia are determined by multiply different parameters and therefore the increased tapped water access did not alter their socio-economic stratum 1 and 2 premise or neighbourhood classifications in Barranquilla. Since a subsequent water-storage tank cleaning and education programme was performed in those surveyed from 2007 [21], three matched neighborhoods, located immediately

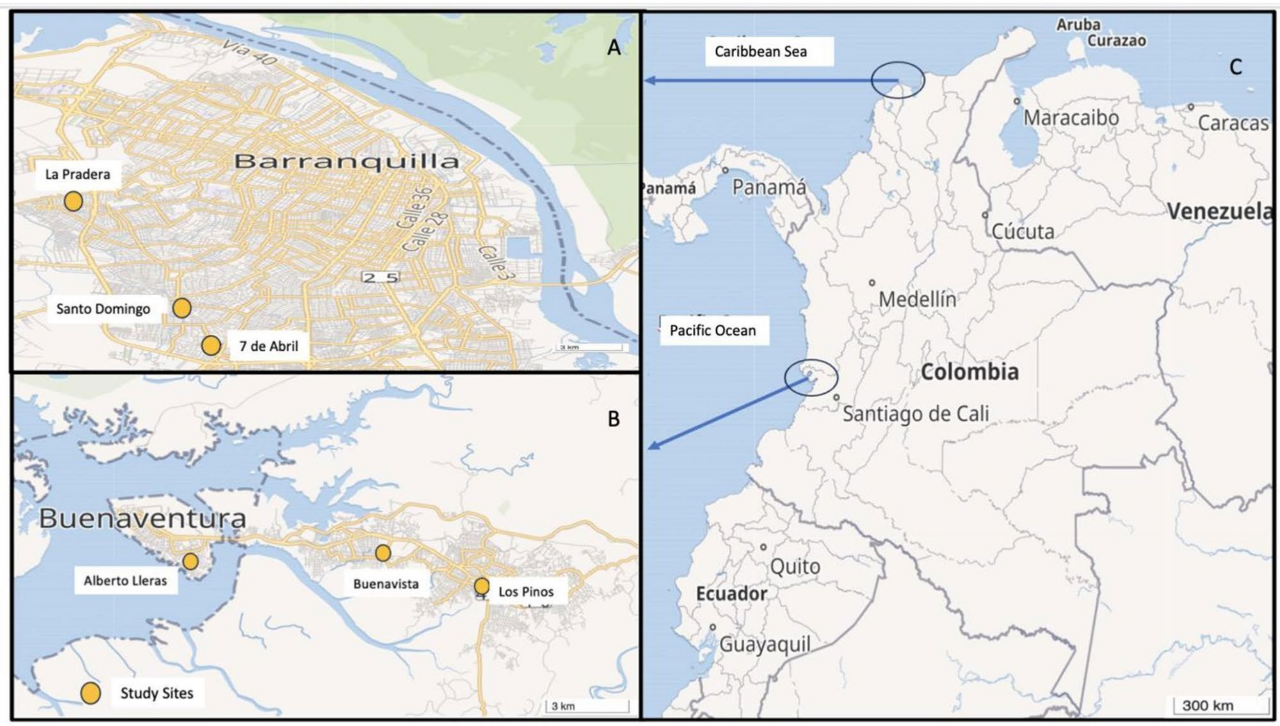

Fig. 1 Location of the study sites. **A**. Barranquilla and **B**. Buenaventura, showing the three neigborhoods in **C**. Colombia

adjacent to those surveyed in 2004 [6], were instead surveyed in this 2020 study.

During these surveys, the annual average temperatures and rainfall for Barranquilla and Buenaventura were 21-30ºC and 25-32ºC and 126–820 mm and 6821–7673 mm, respectively.

Premise sample size

The Epidemiological Calculator OpenEpi [22] was used to calculate the number of premises required based on the total number of premises and their previous premise index (PI: percentage of premises with *Ae. aegypti* larvae). Since the PPI value for each neighbourhood in Barranquilla and Buenaventura was previously above 10%, the premise numbers required to survey with a 99% confidence level were calculated as the total number of premises/calculated sample size in the three Barranquilla study neighbourhoods were 3,221/223 (6.92%) for La Pradera, 4,609/228 (4.95%) for Santo Domingo and 5,113/229 (4.48%) for 7 de April and in the three Buenaventura study neighbourhoods were 1,516/215 (14.18%) for Carlos Lleras, 1,307/203 (15.53%) for Bellavista and 992/193 (19.46%) for Los Pinos/NC. After the sample size was determined, the number of premises required to be visited were divided by the number of blocks, as well as the resident numbers required to provide their answers to the questionnaires, in each study neighborhood in Barranquilla and Buenaventura.

Entomological study

Premise inspections

In each premise, a thorough search was performed for water containers and *Ae. aegypti* larvae and pupae in them in both: (a) the roof-covered areas designated as 'inside' with or without walls and (b) the uncovered areas designated as 'outside', such as uncovered patios and gardens. Adjacent parks, rainwater sewers (sinkholes), open fields, public roads, and streams were also inspected.

Aedes aegypti breeding site productivities

In this study, the type and number of containers with water and the number of containers with *Ae. aegypti* pupae were reported for each inspected premise and they were transported to the laboratory to identify their *Aedes* species using morphological keys [23].

For water containers of less than 20 L, the total number of *Ae. aegypti* pupae were collected and counted. For those with greater than 20-litre capacities, the total numbers of *Aedes aegypti* pupae were accurately estimated by the water-surface sweep net collection coupled with the calibration factor method, whereby those pupae collected were multiplied by either a 2.6, 3.0 or 3.6 calibration factor, depending upon the containers' 1/3, 2/3 or 3/3 water levels as described [4–8]. These studies were performed during the dry season between November and December 2020 by four groups, each containing three people, and each study premise was inspected on one occasion and no rainfall was reported this period. The premises in each of the three study neighborhoods in Barranquilla and Buenaventura were assigned a code number for their neighbourhood, block and premise, with the appropriate biosecurity measures required during the COVID-19 pandemic.

Residents' questionaires

At the same time as the entomological surveys were performed, structured interviews, based on questionaries, were provided to and by the women residents in each premise visited for the entomological survey using the same premise code for their anonymity, since they played a dominant role in the domestic activities. These questions included three main topics:

1). Water: (i) 'What was the source of water they used in their homes?', (ii) 'What did they consider to be the advantages and disadvantages of the cost and quality of that water?', (iii) 'Did they use containers to store domestic water supplies?', (iv) 'What did they consider to be the advantages and disadvantages of storing water?' and if they stated that they stored water in containers: **2. Washing their water-storage containers**: (i) 'How often did they wash those containers with water?', (ii) 'Did they discard the water used to wash those containers?', and (iii) 'What made them decide to clean those water-storage container?', and **3. Mosquitos and their larvae and pupae and disease**: (i) 'What do they call these animals (after samples of *Ae. aegypti* larvae and pupae were shown to them)?, (ii) 'Where do they believe they come from?, (iii) What health problems do they believe that they cause?, and (iv) What do they do to get rid of these animals from their water?'.

Statistical analysis

The descriptive statistical analyses were performed using Microsoft Excel 18.0. The sample size for the entomological study, the z-test, and ANOVA values were obtained using OpenEpi [22].

Results

Entomological study

In Barranquilla, a total of 749 premises in the three study neigbourhoods were inspected (Fig. 1), in which there were 3939 residents while in Buenaventura a total of 627 premises were inspected in the three study neighborhoods (Table 1). In Barranquilla, 13.8% (103/749) of the premises contained pupae-positive containers, and Santo Domingo had the highest number of them with 27.0% (62/231) of their inspected premises having pupae-positive premises and 19.2% (55/231) of them were *Ae. aegypti* pupae-positive. A higher percentage of positive premises (17.2%: 108:627) was recorded in Buenaventura, with the Alberto Lleras neighbourhood containing the highest percentage (23.0%) of positive premises, while the highest percentage (7.5%: 77/212) of *Ae. aegypti* pupae-positive containers was reported in the Los Pinos NC neighbourhood, but which was therefore lower than either the Santo Domingo (19.2%: 55/231) or 7 de April (7.6%: 34/287) neighborhoods in Barranquilla.

The total number of water tanks in the three study neighborhoods of Barranquilla ($n = 941$) was considerably lower than in the study neighbourhoods of Buenaventura ($n = 3{,}044$), while the total percentage of pupae-positive containers in Barranquilla (10.7%: 101/729) was double that of Buenaventura (5.1%: 156/627). The total number of *Ae. aegypti* pupae in Buenaventura ($n = 1881$) was however 1.6 times higher than in Barranquilla ($n = 1184$). Importantly, the average pupae/person index (PPI) value in Barranquilla was 0.29 (95% CI±0.4), with their neighbourhoods obtaining 0.16, 0.19 and 0.53 PPI values, while the average PPI value in Buenaventura was 0.89 (95% CI±0.12), with their neighbourhoods obtaining 0.81, 0.88 and 0.99 PPI values. Each neigbourhood in Buenaventura, therefore, had pupae/resident values dramatically above the minimum 0.5 (0.5–1.5) PPI arbovirus transmission-threshold value, thereby placing their residents at higher risk of dengue virus (and other *Ae. aegypti*-transmitted arbovirus) infections **(see Discussion)**.

Table 1 Premises, residents, water containers and *Aedes aegypti* pupae numbers in each study neighbourhood

Neighbourhood Information[a]	Barranquilla Neighbourhood			Total	Buenaventura Neighbourhood			Total
	Santo Domingo	7 de Abril	La Pradera		Alberto Lleras	Bellavista	Los Pinos/NC	
Inspected Premises	231	287	231	749	223	192	212	627
Inspected Premise Resident Numbers	1385	1454	1100	3939	934	307	815	2056
Pupae Positive Premises (%)	62 (27.0)	31 (11.0)	9 (4.0)	103 (13.8)	52 (23.0)	26 (14.0)	30 (14.0)	108 (17.2)
Water Container Numbers	286	447	208	941	1285	733	1026	3044
Pupae Positive Containers (%)	55 (19.2)	34 (7.6)	12 (5.8)	101 (10.7)	53 (4.1)	26 (3.5)	77 (7.5)	156 (5.1)
Pupae Numbers (%)	730 (61.7)	271 (23.4)	177 (14.9)	1184	825 (43.9)	250 (13.3)	806 (42.8)	1881
Pupae number/Resident (Mean)	0.53	0.19	0.16	(0.29)	0.88	0.81	0.99	(0.89)

a. Details of resident numbers residing in the inspected premises, *Aedes aegypti* pupae numbers, numbers and percentages of positive containers, and pupae numbers/resident in three different study neighbourhoods of Barranquilla and Buenaventura

Types of containers with water and pupae productivity in Barranquilla

Ten different categories of water containers were identified as potential or actual *Aedes aegypti* breeding sites in the study neighbourhoods of both Barranquilla and Buenaventura (Table 2), as had been reported previously in Barranquilla [5–7]. These containers were also designated as being located 'inside' (roof-covered areas even with no walls) and 'outside' (uncovered in patios and gardens) the premises.

Of the *Ae. aegypti* pupae-positive containers/ total number of containers, 54.5% (55/101) of were located inside the premises and which produced 58.5% (693/1184) of the pupae. The majority of the containers inspected in these three neighbourhoods in Barranquilla were plastic tanks (48.7%: 458/941) and 'other used' containers (28.7%: 270/941), which were mainly located inside (67.7%: 310/458) the premises. The ground-based plastic tanks, 'others discarded' and 'other used' containers contributed 40.5% (296/730), 23.2% (164/730) and 22.5% (169/730) of the total *Ae.aegypti* pupae in these neighbourhoods respectively and 53.1% (629/1184) of the pupae in all three neighbourhoods. These three container types therefore contributed the majority (80.1%: 948/1184) of *Aedes aegypti* pupae in the inspected premises of these three Barranquilla neighbourhoods.

Types of containers with water and pupae productivity in Buenaventura

By contrast to Barranquilla, the highest percentage of the total containers (75.4%: 2389/3169), pupae-positive containers (76.9% 120/156) and total pupae numbers (74%: 1393/1881) were reported inside the inspected premises in the three study neighborhoods of Buenaventura (Table 3). The great majority of these *Aedes aegypti* pupae were produced in large (>1000-litre) custom-made cement ground tanks (38.0%: 715/1881), plastic tanks (25.6%: 482/1881), medium-sized cement tanks (22.32%: 420/1881) and 'others used' containers (7.4%: 139/1881) (Table 3). As such, these four container types contributed 93.4% (1756/1881) of the pupae in the inspected premises in these study neighbourhoods of Buenaventura.

Unlike that found in the premises inspected in the three Barranquilla neighbourhoods, the greatest number of *Ae. aegypti* pupae were produced in large (>1,000-litre) custom-made cement ground tanks (Buenaventura: 715 pupae versus Barranquilla: 28 pupae) and these water-storage containers were much more commonly used in each study neighborhood in Buenaventura (Alberto Lleras: $n=101$, Bellavista: $n=147$, and Los Pinos: $n=169$) versus Barranquilla (Santo Domingo: $n=12$, 7 de Abril: $n=15$, and La Pradera: $n=1$).

In Barranquilla these large custom-made domestic water-storage containers were reduced from 1 per 6.9

Table 2 Pupae numbers (bold) in inside and outside pupae-positive Container types in the Barranquilla study neighbourhoods

Container Type[a]	Pupae Numbers (bold): Number of Pupae-Positive Containers/Number of Water Containers Located Inside and Outside the Residents' Dwellings[b]											
	Barranquilla Neighbourhood											
	Santo Domingo			7th de Abril			La Pradera			Total		
	Inside	Outside	Total	Inside	Outside	Total	Inside	Outside	Total	Inside	Outside	Total
Plastic Tanks	**80**:7/85	**216**:8/28	**296**:15/113	**120**:12/146	**26**:8/69	**146**:20/215	**9**:1/79	**9**:3/51	**18**:4/130	**209**:20/310	**251**:19/148	**460**:39/458
M Cement Tanks	0:0/4	**14**:3/11	**15**:3/15	**47**:5/7	0:0/0	**47**:5/7	0:0/1	0:0/1	0:0/2	**47**:5/12	**14**:3/12	**61**:8/24
L Cement Tanks	**18**:1/6	0:0/6	**18**:1/12	0:0/4	**6**:1/11	**6**:1/15	0:0/0	**4**:1/1	**4**:1/1	**18**:1/10	**10**:2/14	**28**:3/24
Metal Drums	**49**:1/1	0:0/0	**49**:1/1	0:0/1	0:0/2	0:0/3	0:0/0	**3**:1/1	**3**:1/1	**49**:1/2	**3**.1/3	**52**:2/5
Raised Tanks	0:0/2	**7**:1/1	**7**:1/3	0:0/0	0:0/6	0:0/6	0:0/1	0:0/0	0:0/1	0:0/3	**7**:1/7	**7**:1/10
Others Used	**124**:8/41	**40**:3/9	**164**:11/50	**25**:1/74	0:0/90	**25**:1/164	**102**:1/5	0:0/51	**102**:1/56	**247**:10/120	**40**:3/150	**287**:13/270
Others Discarded	**83**:10/15	**86**:7/16	**169**:17/31	**1**:1/3	**5**:1/18	**6**:2/21	0:0/1	**26**:2/2	**26**:2/3	**84**:11/19	**117**:10/36	**201**:21/55
Flower Vases	**4**:2/15	**1**:1/11	**5**:3/26	**18**:3/15	**23**:2/4	**41**:5/19	**13**:2/7	**11**:1/5	**24**:3/12	**35**:7/37	**35**:4/20	**70**:11/57
Bottles	0:0/5	0:0/18	0:0/18	0:0/0	0:0/0	0:0/0	0:0/0	0:0/2	0:0/2	0:0/5	0:0/20	0:0/25
Tyres	0:0/2	**8**:3/10	**8**:3/12	0:0/1	0:0/0	0:/0/1	0:0/0	0:0/0	0:0/0	0:0/3	**8**:3/10	**8**:3/13
Total	**358**:29/176	**372**:26/110	**730**:55/286	**211**:22/251	**66**:12/196	**271**:34/447	**124**:4/94	**53**:8/114	**177**:12/208	**693**:55/521	**491**:45/420	**1184**:101/941

a. Container type as plastic, medium (M: < 1,000 L capacity) or large (L: >1,000 L capacity) cement ground tanks, metal (220 L capacity) drums, raised (elevated) plastic tanks, other used containers (usually for watering animals), other discarded (unused) containers, flower vases, glass bottles and tyres

b. Pupae numbers accurately estimated using total counts or the sweeping method coupled to calibration factors from pupae-positive/total number of different water containers located inside (indoors: including containers located under a roof without walls) or outside (outdoors) in three different neighbourhoods of Barranquilla

Table 3 Pupae numbers (bold) in inside and outside pupae-positive container types in the Buenaventura study neighbourhoods

Container Type[a]	Pupae Numbers (bold): Numbers of Pupae-Positive Containers/Numbers of Water Container Types Located Inside and Outside the Residents' Premises[b]											
	Buenaventura Neighbourhood											
	Alberto Lleras			Bellavista			Los Pinos/NC			Total		
	Inside	Outside	Total	Inside	Outside	Total	Inside	Outside	Total	Inside	Outside	Total
Plastic Tanks	**63**:6/357	**158**:12/182	**221**:18/539	**59**:10/205	**4**:1/44	**63**:11/249	**143**:9/234	**55**:6/38	**198**:15/272	**265**:25/796	**217**:18/264	**482**:43/1060
M Cement Tanks	**0**:0/0	**0**:0/1	**0**:0/1	**0**:0/2	**0**:0/0	**0**:0/2	**225**:1/1	**195**:1/1	**420**:2/2	**225**:1/3	**195**:1/2	**420**:2/5
L Cement Tanks	**479**:10/83	**35**:2/18	**514**:12/101	**70**:8/137	**5**:1/10	**75**:9/147	**126**:49/153	**0**:0/16	**126**:49/169	**675**:67/373	**40**:3/44	**715**:70/417
Metal Drums	**28**:5/35	**17**:2/20	**45**:7/55	**0**:0/14	**0**:0/1	**0**:0/15	**28**:2/10	**0**:0/2	**28**:2/12	**56**:7/59	**17**:2/23	**73**:9/82
Raised Tanks	**16**:2/9	**0**:0/4	**16**:2/13	**3**:1/19	**0**:0/3	**3**:1/22	**6**:2/19	**0**:0/1	**6**:2/20	**25**:5/47	**0**:0/8	**25**:5/55
Others Used	**18**:6/222	**7**:5/87	**25**:11/309	**89**:1/80	**1**:1/29	**90**:2/109	**15**:2/170	**9**:3/120	**24**:5/290	**122**:9/472	**17**:9/236	**139**:18/708
Others Discarded	**0**:0/6	**1**:1/3	**1**:1/9	**10**:2/6	**0**:0/1	**10**:2/7	**2**:1/6	**0**:0/8	**2**:1/14	**12**:3/18	**1**:1/12	**13**:4/30
Flower Vases	**2**:1/10	**1**:1/3	**3**:2/13	**9**:1/13	**0**:0/1	**9**:1/14	**2**:1/9	:**0**:0/22	**2**:1/31	**13**:3/32	**1**:1/26	**14**:4/58
Bottles	0:0/186	**0**:0/58	**0**:0/244	**0**:0/120	**0**:0/47	**0**:0/167	**0**:0/282	**0**:0/58	**0**:0/340	**0**:0/588	**0**:0/163	**0**:0/751
Tires	**0**:0/0	**0**:0/1	**0**:0/1	**0**:0/0	**0**:0/1	**0**:0/1	**0**:0/1	**0**:0/0	**0**:0/1	**0**:0/1	**0**:0/20	**0**:0/3
Total	**606**:30/908	**219**:23/377	**825**:53/1285	**240**:23/596	**10**:3/137	**250**:26/733	**547**:67/885	**259**:10/266	**806**:77/1151	**1393**:120/2389	**488**:36/780	**1881**:156/3169

a. Container type as plastic, medium (M: < 1,000 L capacity) or large (L: >1,000 L capacity) cement ground tanks, metal (220 L capacity) drums, raised (elevated) plastic tanks, other used containers (usually for watering animals), other discarded (unused) containers, flower vases, glass bottles and tyres

b. Pupae numbers accurately estimated using total counts or the sweeping method coupled to calibration factors from pupae-positive/total number of different water containers located inside (indoors: including containers located under a roof without walls) or outside (outdoors) in three different neighbourhoods of Barranquilla

residents in the 2004 survey [6] to 1 per 32.2 residents in 2020 (z=10.5; p=0) and which was also significantly lower than the 1 per 1.5 resident value in Buenaventura (z = -9.26; p=0).

The total *Ae. aegypti* pupae (number/mean/standard deviation) in Barranquilla versus Buenventura were therefore 28/1.1/0.16 and 715/25.4/8.4 for the large (>1,000 L) cement tanks and 61/9.8/0.87 and 420/5.4/0.8 for medium-sized (<1,000 L) cement tanks, which therefore provided significantly lower (ANOVA: $p \leq 0.00$) total pupae numbers in Barranquilla. These results, therefore, strongly implicated these custom-made cement tanks being major producers of *Ae. aegypti* pupae in these Buenaventura study neighbourhoods.

To reduce the effects of: (a) low numbers of highly productive *Ae. aegypti* pupae containers in these container-type classifications and (b) neighborhood differences in each city affecting the analyses, the mean numbers of *Ae. aegypti* pupae in each container type in all three neighbourhoods in Barranquiila and Buenaventura were calculated (Table 4). In this study, only 2.3% (18/693) of the average total numbers of *Ae. aegypti* pupae were identified in the large (>1,000-litre) custom-made cement tanks located inside the premises in Barranquilla, compared to 53.1% (675/1393) in Buenaventura (z = -10.08; p=0). In addition, medium-sized (<1,000-litre) custom-made tanks contributed only an average of 5.9% and 2.6% compared to 17.7% and 40.0% of the total average number of pupae inside and outside the premises in Barranquilla and Buenaventura respectively.

However, high average pupae numbers were produced in both cities in plastic tanks located both inside (Barranquilla: 26.3%; Buenaventura 20.8%) and outside their premises (Barranquilla: 46.8%; Buenaventura 44.5%) their premises.

In contrast to the large ground tanks, the numbers of plastic tanks per premise were only slightly reduced between the 2004 and 2020 Barranquilla surveys from 1 per 1.7 premises in 2004 to 1 per 1.6 premises. These plastic tanks produced an average of 26.4% and 46.8% of the total *Ae. aegypti* pupae inside and outside the Barranquilla premises in 2020 respectively, compared to 20.8% and 44.5% in the Buenaventura, which also had 1.7 plastic tanks per premise (Table 4). There were also high average *Ae.aegypti* pupae numbers in the 'others used' containers located inside the premises (Barranquilla: 31.5%; Buenaventura: 9.6%), and 'others discarded' located both inside and outside their premises in Barranquilla (23.4% inside: 21.8% outside). Thus, while the very large custom-made cement tank numbers had been significantly reduced in the 2020 Barranquilla survey, increased average *Ae. aegypti* pupae numbers were observed in plastic tanks and 'others used' and 'others discarded' containers located both inside and outside their premises.

Table 4 Mean numbers of *Aedes aegypti* pupae in each Container type located inside or outside the premises in the Barranquilla and Buenaventura study neighbourhoods

Container Type	Mean Number (Standard Deviation) of *Aedes aegypti* Pupae			
	Barranquilla		Buenaventura	
	Inside	Outside	Inside	Outside
Plastic Tanks	69.7 (45.9)	83.7 (93.8)	88.3 (38.7)	72.3 (64.1)
M (< 1,000 L) Cement Tanks	15.7 (22.2)	4.7 (5.6)	75.0 (106.1)	65.0 (91.9)
L (> 1,000 L) Cement Tanks	6.0 (8.5)	0.0 (0.0)	225.0 (181.1)	13.3 (15.5)
Metal Drums	16.6 (23.1)	1.0 (1.4)	18.7 (13.2)	5.7 (8.0)
Raised Tanks	0.0 (0.0)	2.7 (3.3)	8.3 (5.6)	0.0 (0.0)
Others Used	83.7 (42.4)	13.3 (18.9)	40.7 (34.2)	5.7 (3.4)
Others Discarded	62.0 (43.8)	39.0 (34.3)	4.0 (4.3)	0.3 (0.5)
Flower Vases	11.7 (5.8)	11.7 (9.0)	3.7 (3.9)	0.3 (0.5)
Bottles	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Tyres	0.0 (0.0)	2.7 (3.8)	0.0 (0.0)	0.0 (0.0)
Mean Total	265.4	178.8	423.7	162.6

Residents' questionaires

Questionaires were provided to residents of the inspected promises and collected from 749 women residents in Barranquilla and 627 women residents in Buenaventura (Table 5). In this study, all of the 749 women residents from Barranquilla reported that tapped water was their only source for their domestic water and the majority stated that its main disadvantage was the high cost. The majority in these residents in the Barranquilla neighbourhoods stated that they used plastic tanks for domestic water-storage and that the main advantage was to act as a reserve when the tapped water supply was halted (74.2–81.8%) and some residents (18.2-25.8%) stated that it reduced the water bill cost (Table 5), while very few (0.9–2.2%) stated that they used large custom-made cement ground tanks, as was confirmed in the pupae survey (Table 2).

In contrast, many of the interviewed residents of Buenaventura (11.5–32.5%) stated that they collected rainwater as an alternative source of domestic water, while the majority of them (67.5–88.5%) used tapped water supplies, but the option of many of the residents (16.4–30.2%) to continue to employ large custom-made cement ground tanks (Table 4) accounted for their *Ae. aegypti* PPI values being maintained above the established arbovirus transmission threshold (Table 1).

Interestingly, while the interviewed residents in both study sites stated that the main advantage of domestic water container storage was to act as a reserve, but some of them (Barranquilla: 4.8–17.3%, Buenaventura: 2.7–18.2%) stated that the stored water became dirty, led to mosquito production (Barranquilla: 48.1–81.4%, Buenaventura: 75.3–84.0%) and caused disease (Barranquilla only: 6.1–23.8%), none of them in either city claimed to discard any water or to clean/wash their water-storage containers to reduce or prevent those stated concerns.

Discussion

Despite knowing for decades that large water-holding tanks are the principal *Ae. aegypti* breeding sites in Africa, Asia [9] and the Americas [11], very large custom-made stoneware ground tanks continue to be used in other regions of Colombia [4, 5, 7], Latin America and the Caribbean [24, 25], as well as in most countries in Asian as reported, for example, in Vietnam [26], Laos [27], Cambodia [28], Thailand [29], India [30, 31], Bangladesh [32], Pakistan [33] and Saudi Arabia [34] and also in East [35] and West [36] Africa. As shown previously, these very large custom-made concrete ground tanks were the principal *Ae. aegypti* breeding sites, in many isolated Colombian towns [4, 5, 7], as well as in two of the three neighborhoods of Barranquilla during the 2004 survey, followed by plastic, cement and metal tanks during the dry season survey [6]. There was a significant reduction in the numbers of large custom-made cement water-storage containers identified between the 2004 and 2020 surveys in Barranquilla when the tapped water supply was dramatically increased throughout Barranquilla, due to investment and incentives for poor customers by the commercial water company [17]. Despite the improved tapped water supplies, these Barranquilla residents continued to store domestic water supplies in plastic tanks, which were located both inside and outside their premises and which contained high numbers of *Ae. aegypti* pupae, together with their 'others used' and 'others discarded' containers mainly located inside their premises (Table 4). While the 'others used' (0.0 to 9.1%) and 'others discarded' (0.0 to 19.0%) containers only contributed a low percentage to total *Ae. aegypti* pupae in in the 2004 Barranquilla dry season survey [6], they contributed much higher percentages in 2020 survey (Tables 2 and 4). While these residents claimed that they neither discarded water nor washed any of their water containers, despite them being high infested with *Ae. aegypti*

Table 5 Residents' answers to questionnaires regarding water and water storage

Topic Question[b]	Barranquilla Neighbourhood[a]			
	Santo Domingo	**7 de Abril**	**La Pradera**	**Total**
	***n*=231**	***n*=287**	***n*=231**	***n*=749**
	% (number) of Residents' Responses[c]			
Water Source				
Tapped water from the aqueduct	100 (231)	100 (287)	100 (231)	100 (749)
Advantages of this Water Source				
Good Quality	57.1 (132)	60.3 (173)	63.6 (147)	60.3 (452)
None	42.9 (99)	39.7 (114)	36.4 (84)	39.7 (297)
Disadvantage of this Water Source				
Expensive	64.9 (150)	99.3 (285)	71.4 (165)	80.3 (600)
None	35.1 (81)	0.7 (2)	28.6 (66)	39.8 (149)
Water Containers Used				
Plastic Drums	53.3 (123)	68.9 (198)	53.7 (124)	59.5 (445)
Large Cement Ground Tanks	2.2 (5)	2.1 (6)	0.9 (2)	1.7 (13)
None	40.3 (93)	26.8 (77)	44.6 (103)	36.5 (273)
Advantage of Water Container Use				
Reserve When Water Supply is Halted	81.8 (189)	74.2 (213)	74.5 (172)	76.6 (574)
Reduced Water Bill Charge	18.2 (42)	25.8 (74)	25.5 (59)	23.4 (175)
Disadvantage of Water Container Use				
Mosquito Production	81.4 (188)	56.5 (162)	48.1 (111)	61.6 (461)
Disease Production	6.1 (14)	16.7 (48)	23.8 (55)	15.6 (117)
Water Becomes Dirty	4.8 (11)	15.7 (45)	17.3 (40)	12.8 (96)
Topic Question[b]	**Buenaventura Neighbourhood[a]**			
	Alberto Lleras	**Bellavista**	**Los Pinos NC**	**Total**
	***n*=223**	***n*=192**	***n*=212**	***n*=627**
	% (number) of Resident's Responses[c]			
Water Source				
Tapped water from the aqueduct	88.8 (188)	88.5 (170)	67.5 (143)	79.9 (501)
Rain Water	15.7 (35)	11.5 (22)	32.5 (69)	20.7 (126)
Advantage of Tapped Water Source				
Good Quality	37.6 (84)	11.5 (22)	85.3 (181)	51.9 (320)
None	55.6 (124)	0.0 (0)	0.0 (0)	19.8 (124)
Disadvantage of the Tapped Water Source				
Expensive	44.4 (109)	83.3 (160)	76.8 (163)	67.3 (432)
None	16.1 (36)	14.5 (28)	14.2 (30)	15.0 (94)
Water Containers Used				
Plastic Drums	62.3 (139)	33.3 (64)	36.3 (77)	44.7 (280)
Large Cement Ground Tanks	16.4 (36)	30.2 (58)	22.1 (47)	22.5 (141)
Advantage of Water Container Use				
Reserve When Water Supply is Halted	33.1 (74)	45.3 (87)	44.3 (94)	40.7 (255)
Disadvantage of Water Container Use				
Mosquito Production	75.3 (168)	76.6 (178)	84.0 (178)	78.6 (493)
Water Becomes Dirty	2.7 (6)	18.2 (35)	11.8 (25)	11.0 (66)

a. Number (n) of the residents who participated in the questionaires from each neighbourhood in Barranquilla or Buenaventura

b. Topic and particular question provided to each resident in the questionnaire

c. Percentage and (number) of the questioned residents who provided that answer to that particular question

pupae, appropriate residents' education programmes are urgently required for them to further reduce their PPI values as low as possible.

Due to the high ambient temperatures in Barranquilla and Buenaventura, the large custom-made ground tanks identified and surveyed in the 2004 [6] needed to be washed frequently (less than 7 days) to remain 'mosquito-free' [21] due to the known *Ae. aegypti* egg to adult life-cycle duration of 7 days at 28°C [37]. Washing these containers would however require the residents to discard some of the stored water, but which the women residents stated they did not performed in the questionnaire survey, probably due to 80.5% (64.9–99.3%) of them stating their tapped water supply was expensive (Table 5). Tank-washing is therefore unlikely to be acceptably performed by the residents in the Barranquilla and Buenaventura. As an alternative method, simple and inexpensive framed net-covers were designed, constructed and tested in a Colombian town where the principal *Ae. aegypti* breeding sites were very large custom-made cement ground tanks [4]. While these framed net-covers significantly reduced the *Ae. aegypti* larvae-positive container percentages, they were required to be custom-constructed for these tanks with different dimensions. As such, smaller and more uniform sized water containers with user-friendly covers are, instead, more suitable (see below).

While Stegomia indexes: house index (HI), Container index (CI) and Breteau index (BI) have been critisised due to being based on *Ae. aegypti* larvae and which has been replaced by the pupae index (PI) as a better correlate for dengue virus (DENV) transmission in a study performed in India [38], the *Ae. aegypti* pupae/person index (PPI) arbovirus transmission-threshold value of 0.5 to 1.5 was proposed to be a more suitable parameter to assess DENV transmission risk [13]. As such, we also believe that this PPI value should be the standard to gauge the efficacy of *Ae. aegypti* control teams throughout the world. Elsewhere in Colombia, 0.22 to 2.04 PPI values were reported, which were therefore above the 0.5 PPI value in 18/24 of the neighbourhoods surveyed [39]. Since many of these residents were, therefore, at high risk of *Ae. aegypti* transmitted arbovirus (DENV, CHIKV and ZIKV) infections, their PPI values urgently need to be reduced.

We therefore suggest that improved/effective *Ae. aegypti* control throughout the world should be focused on:

a) improved tapped-water services with an 'incentive-scheme' to make it easier for poor residents to pay for that service [17], b) reductions in the numbers and use of the very large (> 1,000 L) custom-made earthenware domestic water-storage tanks and jars (see references above), c) their replacement by inexpensive smaller uniform-sized (e.g. 220-litre) (preferably white-coloured to be less attractive for gravid adult female *Ae. aegypti*) plastic drums which can be maintained 'mosquito-free' either through: (i) washing every 5 days, (ii) the use of 'mosquito-proof' netted covers or (iii) the addition of chemicals (e.g. temephos or insect growth regulators) or biological (e.g. Bti) agents as reviewed [24, 25], d) the extensive use of the accurate, rapid and robust sweep-net/calibration factor method for local residents use to accurately determine and gauge the effective reduction of their *Ae. aegypti* PPI values to below the 0.5 PPI target value and e) the design and delivery of highly effective education campaigns based at the community level for *Ae. aegypti* control, as were reviewed from meta-analyses [24, 25].

Conclusions and limitations

Improved tapped water supplies throughout Barranquilla significantly reduced their: (a) use of very large (> 1000-litre) custom-made earthenware ground tanks, which were previously shown to be the principal *Ae. aegypti* breeding sites and which are employed by poor residents where the tapped water supplies are inadequate elsewhere in Colombia and in other DENV-endemic regions throughout the world and (b) PPI values to below the established *Ae. aegypti* arbovirus transmission threshold. Residents' education programmes, as identified through the residents' responses to the questionnaires provided to them in this study are, however, required to further reduce *Ae. aegypti* breeding in and around their premises. This study was however limited to surveys performed in only three matched socio-economic strata neighbourhoods in each city due to the logistics required to design and adequately conduct much larger studies and was performed during the dry season to more effectively reduce assess *Ae. aegypti* pupae productivity in their principal breeding sites which maintain their populations throughout the year.

While we attempted to select and compare matched neighborhoods in these two study sites based on their very similar socio-economic strata as defined by the Colombian Government, but which had clear differences in their tapped-water supplies, this study did not include an intervention that would account for and control for any confounding variables or potential biases. As such, well-designed intervention studies should also be performed in subsequent studies.'

Abbreviations

DENV dengue virus

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Author contributions

CMRV, PJAP and AKF designed and co-ordinated the study, CMRV, DP and CERG collected the data and CMRV, DP, CERG and AKF performed the data analysis and wrote and revised the article. All authors read and approved the final manuscript.

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Data availability
All data supporting the findings of this study are available within the paper.

Declarations

Ethics approval and consent to participate
This study was approved by the ethics committee of the Universidad del Norte, through act number 180. These studies were performed during the COVID-19 pandemic at a time when travel in public spaces was permitted under strict biosafety conditions. The team performed the information-gathering activities following the protocols and measures established by the Colombian government. Importantly, an informed consent was obtained, before performing the entomological study and questionnaire for each premise visited and the publication of the findings was agreed by the Health Authorities of both Barranquilla and Buenaventura.

Consent for publication
The Health Authorities of both study sites (Barranquilla and Buenaventura) where the data were obtained, as well as all of the authors have agreed to the submission of this article for publication.

Previous publication
This article is not presently been submitted elsewhere for publication and none of the data contained within this article have been published previously.

Competing interests
The authors declare no competing interests.

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