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Surveillance of dengue fever cases using a novel *Aedes aegypti* population sampling method in Trinidad, West Indies: the cardinal points approach

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Abstract

A novel dengue surveillance method is described and used to evaluate 100 suspected dengue fever (DF) cases in county St. Patrick, Trinidad, West Indies. From the 30 confirmed DF cases fully investigated within 48 h of diagnosis, 63% (19/30 houses) of their homes were found harboring *Aedes aegypti* immature stages. Only houses at the four cardinal points of the index case rather than the entire neighborhood were investigated. The results showed significantly ($P < 0.001$) more *Ae. aegypti* positive houses were observed to the east ($P < 0.04$) and west ($P < 0.01$) than to the north and south ($P > 0.9$). In addition, from the 150 houses inspected a total of 474 artificial containers were inspected and treated, of which 20.8% (99) were infested with *Ae. aegypti* immature stages. More than 49% of the containers inspected were small miscellaneous containers, but they only produced 4.0% of the *Ae. aegypti* immatures, of which only 0.4% were pupae. Water tanks (41.7%), drums (40.4%) and buckets (24.2%) produced over 98% of the pupae. The results of this study imply that dengue vector control programs in Trinidad could increase their efficiency by applying the cardinal points surveillance approach during DF case investigations and concentrating their vector control measures on the most productive containers located at the east and west of the index cases.

Keywords: Dengue; *Aedes aegypti*; Cardinal points method; Surveillance; Container profile and productivity; Trinidad

1. Introduction

In Trinidad, Insect Vector Control Division (IVCD), Ministry of Health embarked on an *Aedes aegypti* eradication program in 1976 which has been maintained with varying levels of efficiency up to 2003 (Rosenbaum et al., 1995; Chadee and Rahaman, 2000; Chadee, 2004). There is no doubt that this eradication program initially suppressed the vector populations and prevented the spread of dengue fever (DF) in Trinidad until 1981, and further delayed the onset of dengue haemorrhagic fever (DHF) until 1995 when only 5 cases were detected, increasing to 114 in 1998 (Teelucksingh et al., 1997; Chadee et al., 2004). During the period 1998–2004, the *Ae. aegypti* program continued to fail with major DF/DHF epidemics occurring during 2002 and 2003 (Chadee et al., 2007). The method used to investigate DF cases involved

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inspection and insecticidal treatment of breeding sites in and around the home after laboratory confirmation, which could range from 7 to 21 days after initial diagnosis (Chadee et al., 2004, 2005). The time lag associated with this approach resulted in a lack of precision with respect to time and space as *Ae. aegypti* populations may have died due to natural attrition or householders application of insecticides may have repelled the adults thus causing dispersal of mosquitoes to other areas within the community thus making the data collected from this protocol unreliable.

The flight range of *Ae. aegypti* has been found to vary depending on the method used and the geographical location where the study was conducted (Sheppard et al., 1969; Reiter et al., 1995). However, it is generally accepted that *Ae. aegypti* dispersal may not exceed 300 m (Christophers, 1960). In addition, during a DF outbreak in Puerto Rico, a cluster of homes with similar human and vector densities only 27.4 m from two foci of dengue remained free of infection for more than 2 months, which strongly suggested a limited flight range for *Ae. aegypti* in the natural environment (Neff et al., 1967). The results also suggest that infected mosquitoes may not travel far distances from breeding sites but rather may rest, blood feed and oviposit within a defined space, the house. The traditional case investigation approaches have been adopted because epidemiological surveillance and vector control remains the best practice in preventing dengue outbreaks (since an effective vaccine is not currently available).

Beckett et al. (2005) conducted epidemiological surveillance within 48 h of the identification of an index case and tested family members and nearest neighbors who lived within a 10-m radius of the index case's home, for the presence of the dengue virus or antigen. Ali et al. (2003) used a geostatistical method (Kriging) to estimate the risk for dengue transmission around houses (within a 50 m buffer zone) and concluded that the clustering of dengue cases was linked to clustering of *Aedes albopictus* and *Ae. aegypti*. This approach is supported by the observations that spatial clusters beyond the household were not evident but that *Ae. aegypti* dispersal of the virus within neighborhoods is significant (Morrison et al., 1998). These studies suggest that households infested with *Ae. aegypti* are randomly distributed within communities (Morrison et al., 2004) and the temporal and spatial clusters of clinically ill dengue patients were possibly from the same house or adjacent houses (Halstead et al., 1969; Chan, 1985; Waterman et al., 1985; Gubler, 1998). However, during these studies the vector population densities were not quantified at the same time. Indeed, the vector density within households when dengue transmission occurred or within a few days after dengue fever was detected would serve as a more reliable prophylactic prerequisite than pupae per person, breteau, house and container indices collected during routine surveillance programs.

This study was conducted to determine the presence or absence of *Ae. aegypti* within houses with dengue positive cases and at houses located at the four cardinal points of the positive premises. This novel "cardinal points" sampling approach is evaluated and discussed with respect to entomological surveillance and dengue cases at the household level and provides a snapshot of the mosquito density at the time or very close to the time dengue transmission occurred in individual houses in county St. Patrick, Trinidad, West Indies.

2. Materials and methods

2.1. General

2.1.1. Study site

This study was conducted in County St. Patrick located on the south western peninsula of South Trinidad (10° north latitude) (Fig. 1). Within the county there are over 43,000 houses with a population of approximately 152,000 people. There are four major industrial areas: Pt. Fortin, a borough and major producer of oil and natural gas; La Brea, the pitch lake and asphalt plant; Penal with an oil refinery and electrical plant, and Palo Seco with another oil production facility. The rest of the county is less developed with major areas covered with primary and secondary forest. However, there are numerous rural towns and villages which are essentially under developed. This county was a major focus of DF/DHF during 1998, with the incidence of DHF being 12.93 per 100,000 of population (Chadee et al., 2004) and with an *Ae. aegypti* house index of 18.1 (Chadee et al., 2007). The climate is tropical with a rainy season from May to November and a dry season from December to May. Details about climate, physical features and vegetation have been previously described (Beard, 1946).

2.2. Case definition

The DF case definition was framed to detect children and adult persons with temperatures of 38 °C or higher for ≤5 days, accompanied by headache, myalgia, and other non-specific signs and symptoms (WHO, 1997). All such cases were closely monitored for signs of haemorrhage, the DHF cases being characterized by high fever, haemorrhagic phenomena and, often, hepatomegaly and circulatory failure. Moderate

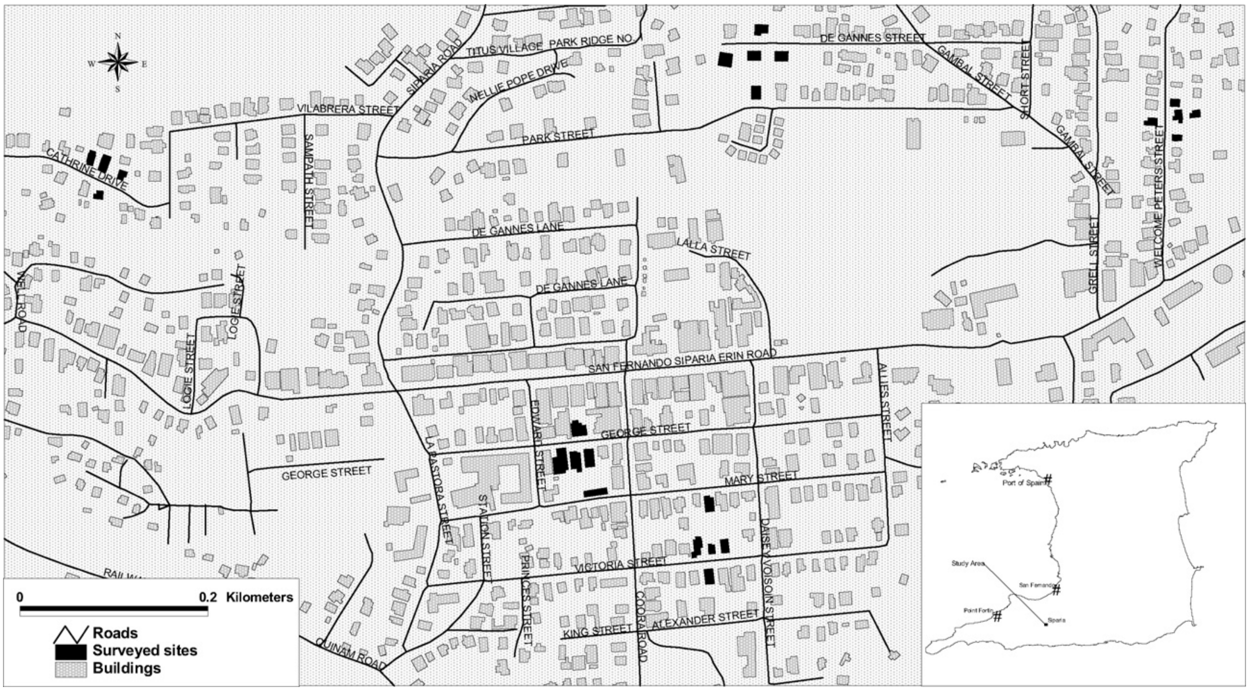

Fig. 1. Map of residential address locations of some suspected dengue cases in County St. Patrick, Trinidad: with examples of the cardinal points surveillance method (2003).

to marked thrombocytopenia with concurrent haemoconcentration was strongly indicative of DHF (WHO, 1997). All suspected cases of DHF were confirmed by virus isolation, detection of specific IgM antibody and/or sero-conversion. Since most DHF cases are admitted to hospital for treatment, it was assumed that over 85% of the cases occurring during the period in 2003 were detected.

2.3. Study design and case data

A population-based dengue case investigation study was conducted in county St. Patrick using house inspections within 48 h after the identification of a suspected/confirmed DF case in the study area. This study was conducted during the period May to December 2003, which is reported to be the dengue season in Trinidad (Chadee et al., 2007). Data on clinically reported dengue fever cases were obtained from sentinel physicians, private practitioners and hospital reports. Laboratory confirmed cases of dengue fever were obtained from consecutive serum samples submitted for dengue fever virology determination at the Trinidad Public Health Laboratory and National Surveillance Unit of the Ministry of Health, Trinidad. During 2003 all patients with symptoms of dengue fever or suspected of having dengue haemorrhagic fever visiting hospitals, health centres and private practitioners were evaluated by public health nurses using a standard questionnaire asking about demographic and clinical data of patients (Chadee et al., 2007).

2.4. Entomological surveillance

The homes of 100 confirmed/suspected cases of DF were investigated and used as the “index house” for this study. One house at each cardinal point of the index case house was inspected (Fig. 1) by staff from IVCD, Ministry of Health using the Pan American Health Organization guidelines (PAHO, 1968). All natural and artificial containers in every house and compound both indoor and outdoor were inspected, and all water holding or potential water holding containers were treated with temephos 1% (one of the few insecticides recommended by the World Health Organization for treatment of potable water at 1 part per million) (PAHO, 1994).

All indoor and outdoor containers, including natural habitats such as tree holes and leaf axils, which might harbor *Ae. aegypti* and other mosquitoes (*Culex quinquefasciatus* Say and *Limatus durhamii*), were inspected to determine whether they were wet or dry and for the presence or absence of *Ae. aegypti* immatures (larvae or pupae). Containers located in dark or shaded areas were inspected using flashlights. Samples from each positive container were collected using ladles and pipettes, placed in phials, labeled, recorded on standard forms and sent to the IVCD laboratory where they were identified or the pupae allowed to emerge and adults identified using appropriate taxonomic keys.

2.4.1. Data analysis

The distribution of *Ae. aegypti* infestations in the index cases and at the cardinal points houses were analyzed by subjecting the data to a X^2 test to determine whether the proportion of *Ae. aegypti* were different in the index cases than that found at the cardinal points. To determine whether there were any differences in the number of *Ae. aegypti* infested houses at all four cardinal points, the data were transformed into contingency tables (4 × 2) and subjected to a *G*-test (Petrie and Sabin, 2000). The distribution of the mosquito indices were calculated as follows: the *House index* as the percentage of houses positive with immatures and the *Breteau index* as the number of positive containers per 100 houses (PAHO, 1994).

3. Results

3.1. Dengue positive houses

From the 100 suspected DF/DHF cases studied, 70 were confirmed as DF/DHF cases. Of the 70 confirmed cases, 30 were fully investigated using the cardinal points approach while 20 cases did not fulfill the cardinal points criteria. That is, houses were not present at all four points of east, west, north and south of the positive dengue case or index case (see Fig. 1). Twenty (20) of the cases were partially investigated because of incorrect street addresses, absence from home and closed houses; therefore these houses were not fully inspected

Table 1
Number of houses located at the four cardinal points including the dengue index case house infested with *Ae. aegypti* immature stages in County St. Patrick, Trinidad, West Indies (2003)

Dengue case premises	*Ae. aegypti* + premises	Houses located at the four cardinal points of positive premises				Total +
		East	West	North	South	
1	+	−	+	−	−	2
2	−	+	+	−	−	2
3	−	−	+	−	+	2
4	+	−	−	−	−	1
5	+	−	+	+	−	3
6	+	+	−	−	−	2
7	−	+	−	−	−	1
8	+	−	+	−	−	2
9	+	+	+	+	−	4
10	+	−	−	−	−	1
11	+	−	−	−	−	1
12	+	+	+	−	−	3
13	−	−	+	−	+	2
14	+	−	+	−	−	2
15	+	−	+	−	+	3
16	−	+	+	−	−	2
17	+	−	+	−	−	2
18	+	+	−	−	+	3
19	−	−	+	−	−	1
20	−	+	−	−	−	1
21	+	−	−	−	−	1
22	−	−	+	−	−	1
23	+	+	−	+	−	3
24	+	−	+	−	+	3
25	+	−	−	−	−	1
26	−	−	+	−	−	1
27	+	−	+	+	+	4
28	+	+	+	+	−	4
29	−	+	−	−	−	1
30	−	+	+	+	−	3
Total	19	12	19	6	6	62/150

within the 48 h time frame and were excluded from this study.

The results of the *Ae. aegypti* inspections conducted at the 30 homes of the DF/DHF confirmed cases, within 48 h of diagnosis, are summarized in Table 1. However, when DF was diagnosed 63% (19 houses) were found positive with containers harboring *Ae. aegypti* immature stages.

3.2. Cardinal points survey

From the 150 houses inspected similar numbers of *Ae. aegypti* positive houses (19) were observed among the "index houses" (30%) and houses located at the four cardinal points ($X^2 = 1.28$; d.f. 4; NS) (Table 1). However, when the number of positive houses at the four cardinal points were compared significant differences ($G = 15.9$, d.f. 1) were observed among houses located to the east ($P > 0.04$) and west ($P < 0.01$) ($G = 63.90$, d.f. 1) and the houses located to the north and south ($P > 0.9$) (Table 1). These results suggest that houses located to the east and west may harbor more *Ae. aegypti* immatures than other houses and may be point sources for infesting other houses and for the transmission of DF.

3.3. Container types

A total of 474 containers were inspected from 150 houses (30 index cases and 120 houses at the cardinal points) in county St. Patrick, of which 20.8% (99) were positive for *Ae. aegypti* immature stages, From the 99 positive containers, 2101 *Ae. aegypti* larvae and pupae were counted (Table 2), of which 1470 (70%) were from drums and buckets. More than 49% of the containers inspected were small miscellaneous containers, but they only produced 4.0% of the *Ae. aegypti* immatures of which only 0.4% were pupae. However, water tanks (41.7%), drums (40.4%) and buckets (24.2%) produced together over 98% of the pupae (Table 2). In terms of ranking containers according to adult productivity levels (Table 2), the inspections showed drums (31.9%), water tanks (31.0%), tubs and basins (25.0%) to be the primary producers, followed by tires (3.6%), brick holes (1.9%), and plants and saucers (1.1%). In addition, the results showed that buckets, drums, tubs and basins, tires and brick holes were the primary producers of *Ae. aegypti* in county St. Patrick.

4. Discussion

The results of the present study clearly demonstrated that 63% of the "index cases" or dengue positive houses were positive for *Ae. aegypti* breeding when inspected within 48 h of clinical diagnosis. Using the traditional entomological indices, to assess the houses of dengue positive cases indicated that the House (% of houses positive for larvae) and Breteau (number of positive containers per 100 houses) indices were extremely high, 19 and 66, respectively. These figures far exceeded the suggested critical threshold for dengue transmission which is suggested to be HI $\geq$ 1% or BI $\geq$ 5 indices, which were proposed for yellow fever transmission and have been applied to dengue transmission. These threshold levels are quite controversial and have been further complicated by the addition of the untested pupae per person index (Focks and Chadee, 1997; Focks et al., 2000).

When the cardinal points surveillance method was conducted, a similar number of index houses and houses at the cardinal points were found infested ($P = 1.28$) with *Ae. aegypti* (41%). Houses located to the west (19)

Table 2
Number and type of containers with *Aedes aegypti* larvae and pupae (in parenthesis), number of pupae and daily female productivity (in parenthesis) from 30 dengue positive premises and 120 houses at their 4 cardinal points in St. Patrick, Trinidad (2003)

Containers	Dengue fever case investigation within 48 h post diagnosis	
	No. of containers inspected and +	No. of pupae and adults produced
Tanks	33 (3)	321 (144)
Drums	61 (40)	329 (148)
Tubs and basins	31 (8)	52 (23)
Buckets	47 (24)	259 (116)
Tires	21 (8)	37 (17)
Brick holes	10 (6)	19 (9)
Small miscellaneous containers, e.g. cans	231 (4)	4 (1.8)
Plant saucers/vases	18 (6)	13 (5)
Bottles	22 (0)	0 (0)
Total	**474** ((**99**)	**1034((463.8)**

Daily production of females is calculated as 45% of emerging pupae.

and east (12) of the "index house" were significantly ($P > 0.04$) more infested with *Ae. aegypti* than at other cardinal points (Table 2). At the moment we are uncertain of the factors which contributed to the aggregation of positive houses to the east and west of the index houses. It is likely that the prevailing winds may be a factor but further studies are recommended to characterize this feature.

The combined results of the index house and the cardinal points investigations suggest that this method may show higher infestation levels than that observed using blocks (Sanchez et al., 2006), neighborhoods (Morrison et al., 1998), buffer zones (Ali et al., 2003) or house clusters (Chan, 1985; Waterman et al., 1985). This method gives similar results to Beckett et al. (2005) who tested nearest neighbors within 10 m for dengue antibodies but did not examine for *Ae. aegypti* breeding sites. The results of the present study suggest that the cardinal points surveillance method may be less time consuming, less labor intensive and less costly because only five houses are inspected and treated as opposed to whole blocks, buffer zones or neighborhoods used for dengue case investigations (Sanchez et al., 2006; Ali et al., 2003; Morrison et al., 1998).

The cardinal points method seems an effective and efficient surveillance tool given the short flight range or flight distance of *Ae. aegypti* mosquitoes (Christophers, 1960). Getis et al. (2003) reviewed the various studies on the flight distances in Kenya (MacDonald, 1977; Trpis and Hausermann, 1986), in Puerto Rico (Edman et al., 1998), and in Mexico (Ordonez et al., 1997) and concluded from these studies and their work done in Peru that "most adult *Ae. aegypti* do not fly far from containers where they developed as larvae and pupae". More importantly, Neff et al. (1967) reported that although a cluster of houses with similar human and vector densities were located within 27.4 m from two foci of dengue, transmission was not initiated for more than 2 months. Similar results were observed in Cuba during the dengue outbreak of 2000 (Sanchez et al., 2006). These results suggest that mosquitoes may not travel far distances from breeding sites or where blood meals were acquired but rather rest, blood feed and oviposit within individual houses. The large number of positive houses observed using the cardinal points method suggests that this approach may be sensitive in detecting this behavior and may prove to be more efficient than the conventional methods such as blocks, and neighborhoods used by others. However, further studies are required to determine the sensitivity and specificity of the different approaches.

The present results suggest that, if a targeted source reduction program was directed at small containers, less than 2% of the mosquito breeding would be removed. In contrast, if large water storage containers such as tanks, drums and buckets (combined totals) could be removed the *Ae. aegypti* immature populations would be reduced by >98% in county St. Patrick. Similar results were observed by Focks and Chadee (1997) and Chadee (2004) who found the best approach may involve profiling containers in each household and targeting the most productive containers (Table 2). In addition, the results suggested that indoor small containers may be inconsequential because of the small number of immatures and adults produced and can be addressed by educating householders on improving hygiene and water management.

It is noteworthy that the results of this study suggest that the presence of a large number of containers does not bear a relationship with level of positives and adult productivity. For example, 231 small miscellaneous containers produced only 4 adults while 61 drums produced over 329 adults per day (Table 2). However, when the daily female productivity of drums, tubs and basins and buckets were calculated over 87.9% of the females were produced during the cardinal point inspections (Table 2). These results suggest that these four containers were possibly the main producers and the point source for the production and dispersal of *Ae. aegypti*, which effected transmission of DF within the cardinal points given their short flight range observed in field (Christophers, 1960; Clements, 1999).

Finally, the results of this study suggest that vector control programs should now consider using the cardinal points approach in case investigations of DF cases because these houses may not only be the point source for *Ae. aegypti* breeding but also for DF transmission. With this information and the cardinal points surveillance method, targeted and sustained vector control measures can be implemented to reduce adult production and thus reduce DF transmission since dengue vaccines are not going to be available for sometime to come.

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