

Dengue Serotype Differences in Urban and Semi-Rural Communities in Ecuador

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Abstract

Dengue is a major vector-borne infection causing large outbreaks in urban communities in tropical regions. During the 2010-2014 period; 434 serum samples from febrile patients were collected from a semi-rural community hospital located in the northwestern region of Ecuador. Dengue virus (DENV) was investigated by reverse transcriptase PCR; a total of 48 samples were positive for dengue. During our study we detected DENV-2 and DENV-3 from 2010 to 2013 and the four DENV serotypes during the period 2013-2014. Surprisingly, our results contrasted with surveys carried out in urban centers throughout the Ecuadorian Coast in which DENV-1, DENV-2 and DENV-4 were prevalent during years 2010-2013 and only 2 serotypes (DENV-1 and DENV-2) in 2014. These results suggest that dengue viruses in semi-rural communities didn't originate in the Ecuadorian cities.

Keywords: Dengue, Borbon, semi-rural community, urban community, Esmeraldas, Dengue serotypes.

Diferencias en los Serotipos de Dengue en Comunidades Urbanas y Semi-rurales en Ecuador

Resumen

El dengue es una importante infección transmitida por vectores que causa grandes brotes en las comunidades urbanas de las regiones tropicales. Durante el período 2010-2014; Se colectaron 434 muestras de suero de pacientes febriles de un hospital rural ubicado en la región noroccidente del Ecuador. Se investigó el virus Dengue (DENV) mediante PCR transcriptasa reversa; un total de 48 muestras fueron positivas para dengue. Durante nuestro estudio se detectaron los serotipos DENV-2 y DENV-3 de 2010 al 2013 y los cuatro serotipos de DENV durante el período 2013-2014. Sorpresivamente, nuestros resultados contrastaron con las encuestas realizadas en los centros urbanos de la costa ecuatoriana en las que DENV-1, DENV-2 y DENV-4 fueron prevalentes durante los años 2010-2013 y sólo 2 serotipos (DENV-1 y DENV-2) en el 2014. Estos resultados sugieren que los virus del dengue en comunidades rurales no se originaron en las ciudades ecuatorianas.

Palabras clave: Dengue, Borbon, comunidad rural, comunidad urbana, Esmeraldas, serotipos de dengue.

INTRODUCTION

Dengue is one of the most important vector borne diseases in many tropical and subtropical regions of the world [1]. The etiological agent is a flavivirus comprising 4 serotypes (DENV1-4), which originated in Old World non-human primates 1000 years ago [2]. This virus is transmitted by 2 species of mosquito vectors: *Aedes aegypti* and *Aedes albopictus* which become infected when they feed on the blood of infected hosts [3]. Dengue virus has disseminated worldwide; in 2013, 3.6 billion people were estimated to live in areas where dengue virus could be transmitted; 50 to 200 million dengue infections occur annually and 500,000 cases correspond to severe dengue which causes 20,000 deaths. Severe dengue is characterized by 2 to 3 days of fever, haemorrhagic manifestations in mucosal and skin, vomiting, plasma leakage and hepatomegaly [4]. Dengue has an endemo-epidemic pattern with outbreaks every 5 years. Between 2008 and 2012, more than 1.2 million people were infected, from these, 28,333 were severe cases [5]; in the Americas, 19% of the cases occurred in the Andean countries [6]. Since dengue fever was introduced in Ecuador in 1988 the number of cases has increased; in 2014 there were 15,446 cases reported, 67 were severe dengue, resulting in 11 fatalities [7]. In 2015 there were 42,483 cases, 51 were severe dengue, no fatalities were reported [8].

The *Aedes* mosquitoes travel short distances (500 m), therefore transmission is facilitated by human mobility in urban centers where infected individuals live in close proximity to susceptible individuals [9]. This is especially critical in developing countries where cities suffer from uncontrolled urban growth, poverty and lack of basic infrastructure such as water distribution and garbage management. Water storage in open containers and accumulation of garbage favors the proliferation of mosquitoes [10]. Dengue in rural communities is thought to be the result of viral spillover from urban centers [11].

Control of dengue fever requires understanding the factors involved in the transmission of the virus. This report describes a discrepancy of serotype distribution between urban and semi-rural communities in Ecuador which may be important in future public health measures to control the disease.

MATERIALS AND METHODS

Sample collection

The study was conducted at the Civil Hospital in Borbon (HCB), in the Northwestern Coast of Ecuador. All the subjects accepted an oral informed consent which was approved by the USFQ Bioethics Committee and the University of Michigan Institutional Review Board.

A total of 434 serum samples from febrile patients were collected during the period of 2010-2014.

Viral RNA extraction

For viral RNA extraction, QIAamp Viral RNA Mini Kit (Qiagen, USA) was used with 140 µl of serum sample, according to manufacturer's instructions. The serum sample was added to 560 µl of AVL lysis buffer, then 560 µl of ethanol 96% was added. 640 µl were



taken and put on a spin column. Centrifuge for 1 min at 4,651 X g. Residue of ethanol was discarded. Then 500 µl of AW1 was added to the spin column. This was centrifuged for 1 min at 4,651 X g. Again the residue was discarded. The collection tube was changed and 500 µl of AW2 was added and centrifuged for 3 min at 12,281 X g, and one more centrifuged was performed for 1 min at 12,281 X g to obtain the column dried. After that, 41 µl of elution buffer was added and centrifuged for 1 min at 4,651 X g. This step was repeated one more time. 82 µl of viral ARN was obtained and this was stored at -80°C until used.

For testing the quality of RNA extractions, β-actin gene amplification was used with the following primers: (primer forward, 5' CGG AAC CGC TCA TTG CC 3' and, reverse: 5' ACC CAC ACT GTG CCC ATC TA) [12].

Reverse transcriptase (RT-PCR) and sequencing

Multiplex RT-PCR was performed with the SuperScript III One-Step RT-PCR and Taq DNA Polymerase System with 5 µl of viral RNA sample, according to the manufacturer's instructions (ThermoFisher, USA). The primers, previously reported by Harris *et al*, 1998, amplify different amplicon sizes for dengue serotypes as follows: D1: 5'-TCA ATA TGC TGA AAC GCG CGA GAA ACC G, TS1: 5'-CGT CTC AGT GAT CCG GGG G (482 bp, D1-TS1), TS2: 5'-CGC CAC AAG GGC CAT GAA CAG (119 bp, D2-TS2), TS3: 5'-TAA CAT CAT CAT GAG ACA GAG C (290 bp, D3-TS3), TS4: 5'-TGT TGT CTT AAA CAA GAG AGG TC (389 BP, D4-TS4) [13]. An electrophoresis was performed in a 1.5% of agarose gel with ethidium bromide. Furthermore, crude PCR products were sequenced by a third party (Functional Biosciences, Inc., WI).

Positive isolated dengue virus controls for each serotype were kindly donated by Instituto Nacional de Investigación en Salud Pública (INSPI), Guayaquil-Ecuador.

RESULTS

From 2010 to 2014, 11% of 434 samples (95% CI: 8.3-14.4) were positive for dengue in semi-rural communities. From 2010 to 2013, DENV-3 was detected in semi-rural communities but not in urban ones according to INSPI, while DENV-4 was detected in urban but not in semi-rural communities (Table 1). From 2013 to 2014 all dengue serotypes were detected in semirural communities but only DENV-1 and DENV-2 in urban communities (Table 2).

DISCUSSION

We found that semi-rural communities in the northern coast of Ecuador (in close proximity to the Colombian border) have different dengue virus serotypes than urban coastal communities including the capital of the province, the port of Esmeraldas (Table 2) according to INSPI. In Colombia all the dengue serotypes have been reported since 2006 [14].

This phenomenon may indicate that people in these remote communities move more frequently to rural regions of the Colombian coast than to cities in Ecuador. There is no road connecting Ecuador and Colombia in this region, however there is an active commerce between semi-rural communities of these two countries which includes regular goods and illegal traffic of goods subsidized by the Ecuadorian government



(such as gasoline and propane) and illegal drugs [15].

The other possibility is sylvatic dengue transmission, although no report of sylvatic transmission of dengue virus has been confirmed in the Americas. The Chocó humid forest has suffered massive deforestation, however there are some areas where howler monkeys (*Alouatta palliata*) and the machin monkey (*Cebus albifrons aequatorialis*) could still be found [16]. This means that there is the possibility that a sylvatic cycle is helping for the maintenance and transmission of the virus in a minor scale.

This report highlights the need for more research in this region. The potential entry of dengue virus (and other arboviruses) from Colombia to Ecuador by this route may require additional measures to control these diseases in Ecuador.

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AUTHOR'S CONTRIBUTIONS

Joseph N. Eisenberg, Josefina Coloma, conceived and design the analysis.
Julio Carrera, J., Emilia Espín, Sara Cifuentes, collected the samples from semi-rural communities and performed the analysis from 2010 to 2014.
Sully Márquez Gabriel Trueba, collected the data of each period of time, and wrote the paper.



Table 1. Number of samples identified within different Dengue serotypes in a semi-rural community located in northern coast of Ecuador from 2010 to 2014.

YEAR	SEROTYPES			
	DENV-1	DENV-2	DENV-3	DENV-4
2010-2011	0	5	1	0
2011-2013	0	3	2	0
2013-2014	20	7	8	2

Table 2. Comparison between the presence of different dengue serotypes in Ecuador in semi-rural and urban communities with serotypes in Colombia (Santander department).

Communities	Years		
	2010-2011	2011-2013	2013-2014
Serotypes in semi-rural communities	2, 3	2, 3	1, 2, 3, 4
Serotypes in Urban communities (Esmeraldas, Manabí, Guayas, El Oro, Zamora Chinchipe, Morona Santiago, Napo provinces) ¹	1, 2, 4 ¹	1, 2, 4 ¹	1, 2 ¹
Serotypes detected in (Santander-Colombia)	1,2,3,4 ¹⁷	1,2,3,4 ¹⁷	1,2,3,4 ¹⁷

¹INSPI, 2012

¹⁷Villabona-Arenas, 2016