Chikungunya Virus Infection: First Detection of Imported and Autochthonous Cases in Panama

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Abstract. Chikungunya virus (CHIKV) is a mosquito-borne pathogen that was only endemic in Africa and south Asia until 2005 and 2006, when the virus spread into the Indian Ocean islands, Europe, and Asia. Autochthonous CHIKV transmission in the Caribbean islands was reported in December of 2013. In Panama, two febrile cases were detected in May of 2014: one traveling from Haiti, and the other traveling from the Dominican Republic. After other imported cases were detected, the first autochthonous case was reported in August of the same year. We detected CHIKV viral RNA and isolated the virus from serum samples. The phylogenetic analysis of the two imported isolates and one autochthonous CHIKV isolate indicated that the viruses belong to the Asian lineage in the Caribbean clade and are related to viruses recently identified in Saint Martin island, British Virgin Islands, China, and the Philippines. Although the circulating CHIKV lineages in the Americas have not yet been described, our results suggest that the Asian lineage is circulating in most American countries reporting autochthonous infection.

Chikungunya virus (CHIKV; *Alphavirus, Togaviridae*) is a mosquito-borne pathogen that is endemic in Africa and some countries in Asia. In 2004, a CHIKV epidemic in coastal Kenya was reported, and by 2005 and 2006, CHIKV had spread to the Indian Ocean island of La Reunion as well as Asia, where it caused major epidemics. Several imported cases were reported in Europe and the Americas.¹ Three major lineages of CHIKV have been described: the east, central and south African (ECSA) lineage, the west African lineage, and the Asian lineage.² A single mutation in the ECSA strain allowed the emergence of the Indian Ocean outbreak lineage (IOL) because of the increase of viral infectivity, dissemination, and transmission of CHIKV in *Aedes albopictus*.^{3,4} The IOL has been related to the explosive CHIKV epidemics in the Indian Ocean and Asia and autochthonous infections in Italy and the south of France as well as several imported cases into the Americas.^{2,5} Therefore, it was believed that the IOL of CHIKV would reach the Americas,⁶ where the two vectors *Ae. aegypti* and *Ae. albopictus* have an overlapping distribution⁷ and adapt to cause autochthonous infection. Autochthonous CHIKV infections caused by the Asian lineage were reported in December of 2013 on the French island of Saint Martin and spread to several other Caribbean islands and Latin American countries in 2014.^{8,9} Here, we report the detection of imported cases of CHIKV in Panama and the establishment of autochthonous infections as well as the results of the genetic characterization of the CHIKV viral strains.

On May 13 and 14, 2014, two suspected cases of Chikungunya fever were detected in two public medical facilities in Panama City, Panama. The first patient (256114) was a 23-year-old male with the following travel history: Brazil to Haiti to Panama to Brazil. The day before his travel to Haiti from Rio

de Janeiro (May 6), he presented fever, myalgia, and general malaise; he later progressively improved. After 5 days in Haiti, he presented with the symptoms described in Table 1. The patient attended Santo Tomás Hospital on May 13 during a 24-hour layover in Panama City. The second patient (256137) was a 58-year-old female who was traveling from the Dominican Republic to Panama. She presented general malaise days before her arrival to Panama (Table 1). On May 12, this patient was seen by a physician at the Panama Airport Clinic; 2 days later, she was also seen by a physician at Parque Lefevre Health Center in Panama City.

After detection of the two first imported cases, the Panamanian Ministry of Health implemented active surveillance of patients with febrile disease that arrived from the countries with confirmed CHIKV circulation. Despite the effort to detect imported cases and contain the establishment of an autochthonous infection, the first autochthonous case was reported in Panama on August 18, 2014. This case was a 26-year-old male (256619) without history of travel who presented the symptoms described in Table 1.

Sera samples from patients were sent to Gorgas Memorial Institute of Health Studies for Dengue and Chikungunya fever diagnostics. Samples were tested first using real-time reverse transcription polymerase chain reaction (RT-PCR) specific for Dengue virus (DENV)¹⁰ and then, for CHIKV non structural proteins nsp 2 and 4 gene.^{11,12} Immunoglobulin M (IgM) antibodies were detected with enzyme-linked immunosorbent assay (ELISA) for both viruses.¹³

The samples from these three cases were RT-PCR DENV-negative and CHIKV-positive. The patient from Brazil and Haiti (256114) also had positive IgM for Dengue. Autochthonous CHIKV infection was not reported in Brazil at that time, and a Chikungunya epidemic was occurring in Haiti.^{14,15} The diagnostic results suggest that this patient had most likely first contracted Dengue in Brazil and then, had a second infection with CHIKV in Haiti. However, we cannot confirm this hypothesis; Dengue IgM response can last up to 3 months. The acute sera should have been tested by viral isolation or RT-PCR, but it was not available.

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TABLE 1
General and clinical information about the two first imported cases and the first autochthonous case of Chikungunya fever

Patient	Age (years)	Sex	Travel history	Symptoms	Laboratory hemogram findings	Laboratory chemistry findings
256114	23	Male	Brazil and Haiti	Fever, myalgia, headache, nausea, vomiting, skin rash*	WBCs at 4,900/ μ L, neutrophils at 79%, lymphopenia of 600/ μ L, platelets at 162,000/ μ L, hemoglobin in 14.7 g/dL	Creatinine in 1 mg/dL; normal: electrolytes and hepatic function
256137	58	Female	The Dominican Republic	Fever, headache, joint pain, eupnoea	Hemoglobin at 11.2, hematocrit at 34.5%, WBCs at 4,410/ μ L, neutrophils at 73%, platelets at 250,000	Not done
256619	26	Male	Autochthonous	Fever, chills, severe headache, retro-orbital pain, severe myalgia, severe arthralgia, nausea, vomiting	Hemoglobin at 14.6 g/dL, hematocrit at 44.5%, lymphocytes at 13.1%	Not done

WBC = white blood cell.

*For patient 256114, only the symptoms suspected to be related to his CHIKV infection and not the symptoms from DENV infection in Brazil are presented.

Virus was isolated from sera using Vero cells (ATCC, Bethesda, MD) maintained with media medium essential minimum (MEM) 1% fetal bovine serum (FBS)/1% penicilline/streptomycin (P/S) and observed for 5 days for cytopathic effects (CPEs). Samples were inoculated two times for CPE confirmation. When the CPEs were evident, RNA was extracted using the QIAamp RNA Viral Extraction Kit (Qiagen, Hilden, Germany) and tested using alphavirus genus-specific RT-PCRs.¹⁶ Amplicons generated were purified directly using the Qiaquick PCR Purification Kit (Qiagen, Germany) and sequenced in both directions with the RT-PCR primers using an Applied Biosystems (Foster City, CA) 410 Genetic Analyzer following the manufacturer's protocols. After its identification using Basic Local Alignment Search Tool (BLAST) Software, CHIKV RT-PCR-specific reactions for the entire E1 gene and primer-walking sequencing were performed (primer sequences for RT-PCR and sequencing were provided by Scott Weaver and Rubing Cheng). Consensus sequences covering the structural E1 gene of the CHIKV isolates were aligned with 36 representative homologous sequences from the GenBank library and The European Virus Archive using the MUSCLE program amino acids and then returned to nucleotides to conserve codon homology.¹⁷ An optimal maximum likelihood (ML) tree was then generated using a heuristic search, and the evolutionary relationships were undertaken with the general time reversible + gamma distribution (GTR+G) model. One hundred bootstrap replicates were calculated. Similar tree topologies and evolutionary relationships were observed with the optimal ML tree for E1 (Figure 1) or the neighbor-joining tree for nsp4 (data not shown). Phylogenetic analyses were conducted using MEGA*, version 6.¹⁸

During December of 2013, the French government detected autochthonous transmission of CHIKV on the French island of Saint Martin. It was shown that the isolated strains from Saint Martin were from the Asian lineage and not from the IOL,⁸ which was previously expected. The Chikungunya outbreak in the Americas began on the Saint Martin island and spread until November of 2014 to several French Caribbean islands and the Caribbean islands, including the Caymans, Curacao, the Dominican Republic, Guadeloupe, Puerto Rico, St. Vincent and the Grenadines, and the US Virgin Islands, as well as Mexico, Belize, El Salvador, Guatemala, Honduras, Nicaragua, Brazil, Colombia, and Venezuela.¹⁹

Here, we report the first two imported cases and the first autochthonous case of Chikungunya fever in Panama. One of

the imported cases also had a probable sequential dengue infection. The clinical findings of CHIKV imported and autochthonous cases detected in Panama are consistent with previous reports.^{20,21}

The Panamanian isolates 256114 and 256137 from the imported cases and 256619 from the autochthonous case occupy the Asian lineage in the Caribbean clade (Figure 1) and are closely related to strains identified in the British Virgin Islands (BRITISH VIRGIN ISLANDS/99659/2014) and Saint Martin (St. Martin/H20235/2013). Panamanian strains are nearly identical to these strains, and a single synonymous mutation in the E1 gene of the strain 256119 was found; no mutation of adaptation of others vectors was found. These strains are related to isolates from Asia that circulated in 2012 and 2013 in the state of Yap of Micronesia (MICRONESIA/3807/2013 and MICRONESIA/3462/2013), China (CHINA/JC2012/2012 and CHINA/chik-sy/2012), and the Philippines (PHILIPPINES/13-112A/2013).

The circulating lineages in Haiti, the Dominican Republic, and the rest of the Americas have not been yet described.⁹ Our results suggest that the Asian lineage is circulating in most countries in the Americas that have confirmed autochthonous circulation of CHIKV. Both principal vectors of CHIKV are present in Panama, with a prevalence of *Ae. aegypti* in urban areas and with a prevalence of *Ae. albopictus* in rural areas (Valderrama-Cumbrera A, personal communication). Panamanian mosquito strains from both species are able to transmit with high efficacy the CHIKV IOL and the Asian lineages.⁷

At the time of publication, 29 imported and 21 autochthonous cases were reported in Panama (Ministry of Health). Interestingly, the expected explosive profile of CHIKV epidemics observed in other regions has not yet been seen in Panama. More studies are being conducted to understand differences in the epidemics. The results may underline the importance of an effective vector control program, early case detection, and differential diagnosis with other fever illness. Taken together, our results support the hypothesis that a single CHIKV strain introduction of Asian genotype on the Caribbean islands later spread to other Latin American countries.²² However, with continuous human travel, migration, and commercial trade, the introduction of other Asian CHIKV strains or genotypes could threaten areas where both vectors are present.

FIGURE 1. CHIKV phylogenetic tree. ML tree of 39 CHIKV strains based in the E1 protein fragment of 1,782 nucleotides. The three major viral lineages and the Indian Ocean and Caribbean clades are shown, and Panamanian strains are bold. Virus labels include country of isolation, strain designation, and year of isolation. The evolutionary relationships were undertaken with the GTR+G model. Bootstrap values are shown in major branches. Evolutionary analyses were conducted in MEGA 6.¹³

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