JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Outbreak of Chikungunya virus in the north Caribbean area of Colombia: clinical presentation and phylogenetic analysis

Salim Mattar[1], Jorge Miranda[1], Hernando Pinzon[2], Vanesa Tique[1], Amada Bolaños[1], Jose Aponte[1], German Arrieta[1,3], Marco Gonzalez[1], Katerine Barrios[2], Hector Contreras[1], Jaime Alvarez[1], Ader Aleman[1]

[1] *Universidad de Córdoba, Instituto de Investigaciones Biológicas del Trópico, Montería, Colombia*
[2] *Clínica del Niño, Cartagena, Universidad de Cartagena, Colombia*
[3] *Corporación Universitaria del Caribe (Cecar), Grupo de Salud Pública, Sincelejo, Colombia*

Abstract

Introduction: The Caribbean area of Colombia has been severely affected by a Chikungunya virus (CHIKV) outbreak since 2014.

Methodology: The study was carried out on 100 patients during a fever outbreak from August to September 2014 in two small rural villages in the northern Caribbean area of Colombia. The molecular assays performed by reverse transcription polymerase chain reaction (RT-PCR) on acute patient sera were collected within one to five days of the appearance of symptoms. Sequence analyses were carried out based on phylogenetic analyses of genes NS1 and E2. For serological assays, 49 (49%) patients at ≥ 6 days of disease onset were tested with NovaLisa Chikungunya IgG/IgM μ-capture enzyme-linked immunosorbent assay (ELISA).

Results: The main signs or symptoms associated with Chikungunya infection were arthralgia of the lower limbs (96%), fever (91%), arthralgia of the upper limbs (85%), rash (64%), and headache (57%). Ninety-four percent (46/49) of patients were positive for either IgM or IgG; the remaining three (6%) patients were seronegative. Viral loads were detected in 25 patients. Based on phylogenetic analysis of NS1 and E2, the characterization of the Colombian CHIKV indicated that it was a strain closely related to the British Virgin Islands strain and to the Asian genotype.

Conclusions: This study shows the phylogenetic and clinical description of CHIKV in Colombia. The main symptoms shown were: arthralgia, fever, and rash. CHIKV sequences detected in Colombian patients were within the Asian genotype and closely related to the British Virgin Islands strain.

Key words: Chikungunya; vector-borne diseases; *Culicidae*; epidemiology.

J Infect Dev Ctries 2015; 9(10):1126-1132. doi:10.3855/jidc.6670

(Received 31 January 2015 – Accepted 15 June 2015)

Introduction

Chikungunya infection is a neglected tropical disease caused by the Chikungunya virus (CHIKV), an arthropod-borne virus belonging to the alphavirus group [1,2]. The clinical manifestations of CHIK are well defined [1]. The infection can be diagnosed by serology to detect IgG and/or IgM. In its classical form, the illness appears as a dengue-like disease, sometimes confused with dengue fever, specifically in regions where the two viruses co-circulate [3]. In Latin America, it is believed that the most common manifestation occurs after an incubation period of one to five days, with sudden onset of high fever, followed by severe and often incapacitating and painful long-term polyarthralgia, sometimes for months. In Latin America, common symptoms also involve a severe exanthema, myalgia, and headaches. On Reunion Island, severe forms were reported, mainly in patients with underlying medical conditions [4]. These forms included neurological and cardiovascular disorders, hepatitis, skin diseases, and respiratory and renal failure [2,3,5]. Miscarriages and neonatal infections have been also described, and some deaths have been directly attributed to CHIKV [4].

In December 2013, autochthonous CHIKV cases on Saint-Martin island in the Caribbean were confirmed [6,7]. From there, the epidemic spread very rapidly in the Caribbean and reached Colombia in August 2014. Since then, it is believed that 340,629 cases have been detected in Colombia [8]. CHIKV is a new arbovirus in South America; however, its appearance was not unexpected because of the tropical climate and the presence of *Aedes aegypti*, the mosquito vector for dengue virus (DENV), another common arbovirus in the region [9-14].

The aim of this study was to report the clinical, epidemiological and genetic phylogeny of CHIKV in the Caribbean area of Colombia.

Methodology

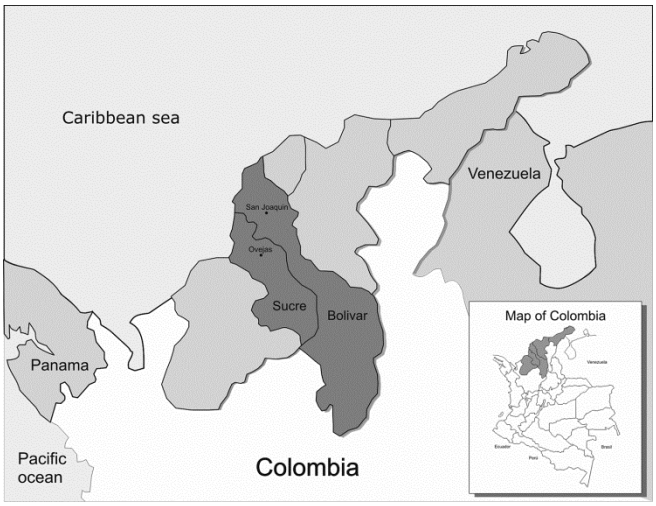
Outbreak area, patients and clinical data

The study was carried out during a fever outbreak between August and September 2014 in northern Colombia in two small rural villages, San Joaquin in Bolivar province (10°14'00.15"N, 75°11'21"W), and Ovejas in Sucre province (9°30'00.21"N, 75°09'59"W); both provinces were affected seriously by the disease (Figure 1). The data, sample collection, and clinical examination of 100 consecutive patients with an acute febrile illness and signs or symptoms compatible with Chikungunya fever (fever, joint pain, or rash) were included in the study. A blood sample was taken from each patient, kept at 4°C, and transported to the laboratory within 12 hours. The samples were either tested on the same day or stored at -80°C until testing. Physicians in healthcare facilities in the two villages performed the clinical study. Patients were suspected of having Chikungunya fever if they presented with at least one of the following symptoms or signs: fever, arthralgia, myalgia, headaches, rash, or fatigue. The data, which included age, sex, residence, time of onset and severity of symptoms, and site of arthralgia were recorded in a questionnaire. Analgesics and non-steroidal anti-inflammatory drugs were prescribed to patients, none of whom were hospitalized.

Molecular detection, viral load and sequence

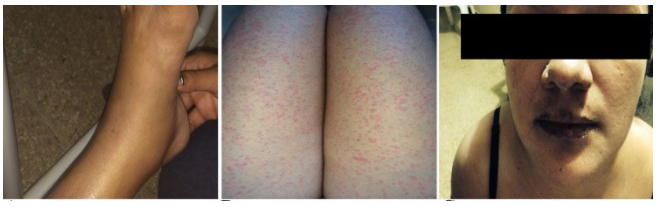
Molecular analysis was performed by reverse transcription polymerase chain reaction (RT-PCR) on 25 acute patient sera that were collected within one to five days of the appearance of symptoms. RNA was extracted from 140 uL of serum by using the QIAamp Viral RNA Mini Kit according to the manufacturer's protocol (Qiagen, Courtaboeuf, France). cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Indianapolis, USA) according to the manufacturer's instructions. Finally, 5 uL of synthesized cDNA was used as template in the real-time RT-PCR assay. For detection and quantification of CHIKV cDNA, a LightMix Chikungunya Virus Kit (TIB MOLBIOL, Berlin, Germany) containing primers and probes and LightCycler FastStart DNA Master HybProbe (Roche Diagnostics, Indianapolis, USA) were used according to the manufacturers' instructions. Real-time RT-PCR assays were performed in a LightCycler instrument.

Figure 1. Geographical location of the study area during the fever outbreak from August to September 2014. The rural villages, San Joaquin in Bolivar province and Ovejas in Sucre province are showed.



The assay used a hybridization probe labeled to detect a 181 bp fragment of the CHIKV E1 gene. The PCR reaction was monitored by an internal control that does not interfere with CHIKV. The viral load was determined by comparing it to a standard curve, following the manufacturers' instructions. Exponential regression was used to determine the CHIKV loads from the threshold cycle in unknown samples. For sequence analysis, two conventional PCRs were carried out. First, a 434 bp fragment of the nonstructural protein NS1 gene was amplified by standard PCR using the primers M2W - YAGAGCDTTTTCGCAYSTRGCHW and cM3w - ACATRAANKGNGTNGTRTCRAANCCDAYCC for forward and reverse, respectively [15]. Second, a 427 bp fragment of the E2 gene was amplified with the primers Chik-1–TAATGCTGAACTCGGGGACC and cChik-4 ACCTGCCACACCCACCATCGAC for forward and reverse, respectively [16]. For sequencing, only clinical samples with the highest viral load were selected; both strands of each gene

Figure 2. Clinical manifestations of Colombian patients with chikungunya infection.



A: arthritis with edema in ankle; **B:** severe exanthema on legs; **C:** Hemorrhagic manifestations in lips.

fragment were directly sequenced and each sample was sequenced twice in an automatic ABI 3730XL1 sequencer. Partial DNA sequences obtained from the amplified PCR products (NS1 and E2) were aligned by Basic Local Alignment Sequence Tool (BLAST) with the corresponding sequences of other CHIKV available in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic distances between homologous sequences were estimated using Kimura's two-parameter model. For each analyzed gene, a phylogram was constructed by the neighbor-joining method using Molecular Evolutionary Genetics Analysis (MEGA) version 6 [17].

Serology

For serological assays, only 49 (49%) patients whose disease onset had begun six or more days ago were tested. NovaLisa Chikungunya IgG/IgM μ-capture enzyme-linked immunosorbent assay (ELISA) (NovaTec Immunodiagnostica GMBH, Dietzenbach, Germany) was used; the kit is intended for the qualitative determination of IgG- and IgM-class antibodies against CHIKV in human serum.

Ethics

Institutional standard guidelines of the Minister of Health of Colombia and University of Cordoba ethics committee were followed for the collection of patients' blood samples after their written informed consent for involvement in the study was obtained.

Results

Epidemiological, clinical, and serological characteristics of study population

The outbreak started in August 2014 in San Joaquin village, moved throughout the Caribbean north region, and reached Ovejas in Sucre province. The peak incidences were reached at epidemiological weeks 33 and 38 in each province, respectively. According to the National Health Institute in December 2014, 1,136 cases were reported in San Joaquin and 985 in Ovejas province. The present study enrolled 64 females and 36 males (age range, 6–81 years; average, 39 years). The principal occupations of people studied were housewives (n = 35), students (n = 23), salesmen (n = 22), farmers (n = 14), and others (n = 6). The mean duration of symptoms in the acute phase was six days (standard deviation [SD] 3.2). With respect to the time to onset of symptoms, 51 patients saw symptoms after zero to five days (average of 2.9 days) and 49 patients saw symptoms after six or more days (average of 13 days). Table 1 shows the signs and symptoms of 100 patients; Figure 2 shows other clinical manifestations. No complications or deaths were reported. With respect to serology, 46 patients were positive for either IgM or IgG; the remaining three patients were seronegative.

Viral load, sequence, and phylogenetic analyses

Twenty-five patients were positive by RT-PCR; signs and symptoms of these patients are shown in Table 2. Only clinical samples that gave a high viral load in the primary detection RT-PCR were selected to ensure that sufficient DNA would be available for sequencing reactions.

Table 1. Main symptoms and sings in patients with Chikungunya virus in Caribbean area, Colombia.

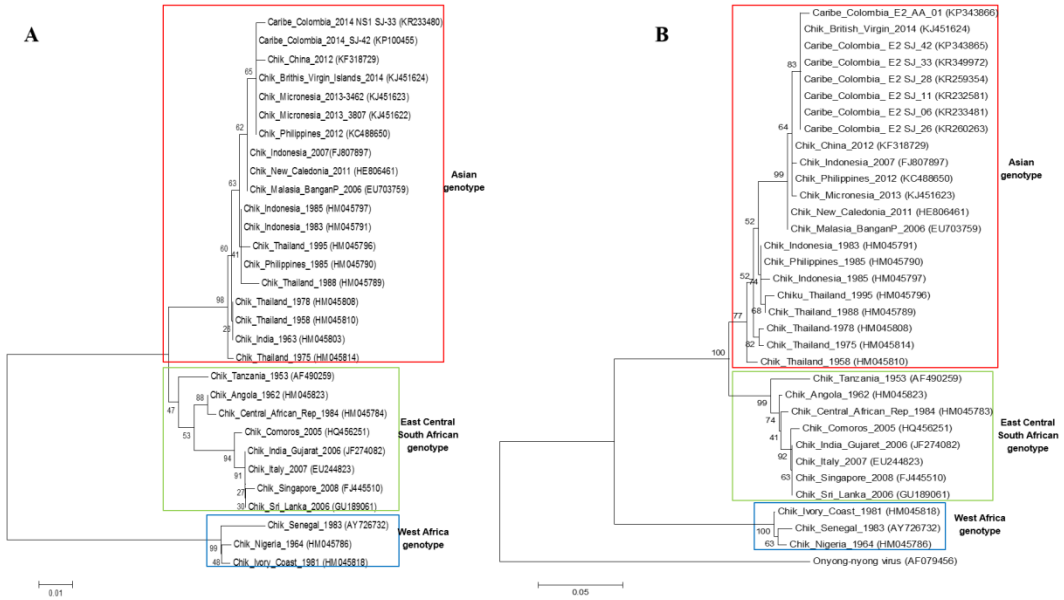
Symptoms	n (%)
Arthralgia lower limbs	96 (96%)
Fever	91 (91%)
Arthralgia Upper limbs	85 (85%)
Rash	64 (64%)
Headaches	57 (57%)
Myalgia	24 (24%)
Pruritus	17 (17%)
Adenophatia	17 (17%)
Vomiting	14 (14%)
Nausea	12 (12%)
Diarrhea	10 (10%)
Asthenia	10 (10%)
Dizziness	10 (10%)
Conjunctivitis	8 (8%)
Haemorrhagic signs	2 (2%)

Table 2. Description of symptoms, onset of disease, viral load and unusual symptoms of 25 patients with positive RT-PCR.

Patients	Time of onset	Age	Gender	Viral load	Frequently symptoms*	Unusual symptoms**
1	1	27	F	$6,2\times10^5$	Fever, arthralgia lower limbs, rash	None
2		6	M	$1,3\times10^5$	Fever, arthralgia	None
3		71	F	$2,5\times10^6$	Fever, polyarthralgia, rash, pruritus	None
4	2	38	F	$4,5\times10^5$	Fever, arthralgia, rash	None
5		12	F	$1,71\times10^5$	Fever, arthralgia, headache	Vomiting
6		76	F	$4,19\times10^4$	Polyarthralgia	None
7		52	M	$5,6\times10^3$	Fever, polyarthralgia, rash	Pruritus
8		13	F	$8,2\times10^4$	Polyarthralgia, rash	None
9	3	63	F	$1,44\times10^6$	Fever, arthralgia	Edema
10		28	F	$9,1\times10^5$	Fever, arthralgia, rash, headache	None
11		19	F	$6,2\times10^3$	Polyarthralgia	Vomiting
12		45	F	$2,9\times10^3$	Polyarthralgia	None
13		15	M	$1,9\times10^3$	Polyarthralgia, rash	Dizziness
14		59	F	$1,51\times10^3$	Polyarthralgia	Dizziness
15		19	F	$1,04\times10^3$	Fever, polyarthralgia, rash, headache	None
16		32	F	$7,4\times10^2$	Fever, arthralgia	Sore throat
17		35	F	$4,2\times10^1$	Fever, rash, arthalgia	Vomiting, pruritus
18		19	F	$3,5\times10^1$	Rash, fever	None
19	4	62	M	$1,8\times10^3$	Polyarthralgia	None
20		39	M	$3,9\times10^1$	Polyarthralgia	None
21		70	F	$9,7\times10^0$	Polyarthralgia	None
22	5	46	F	$1,07\times10^6$	Polyarthralgia	None
23		32	F	$4,5\times10^1$	Polyarthralgia, rash, headache	Vomiting
24		15	F	$1,9\times10^1$	Polyarthralgia, rash	None
25		31	F	$1,06\times10^1$	Arthralgia lower limbs, rash	Adenophaty

* Frequently symptoms: Fever: 25 (100%), Polyarthralgia: 22 (88%), Rash: 14 (56%), headache: 16 (64%), Arthralgia lower limbs: 3 (12%); ** Unusual symptoms: Myalgia: 7 (28%), Vomiting: 6 (24%), Dizziness: 5 (20%), Pruritus: 4 (16%), Adenopathy: 2 (8%), Diarrhea 1 (4%), Sore throat: 1 (4%), Cough: 1 (4%).

Figure 3. Molecular Phylogenetic analysis of Colombian Chikungunya virus detected.



The evolutionary history was inferred using the Neighbor-Joining protocol for both genes: **A.** NS1 and B E2. Evolutionary analyses of NS1 and E2 were conducted in MEGA 6. The bootstrap consensus of both trees (NS1 and E2) inferred from 1000 replicates were taken to show the evolutionary history of the taxa analyzed. **A**. NS1 gen, was used a fragment of 380 pb of the NS1 gen of Chikungunya virus, the analysis involved 30 nucleotide sequences. **B.** E2 gen, was used a fragment of 380 pb of the E2 gen of Chikungunya virus, The analysis involved 34 nucleotide sequences. Both analyses of genes E2 and NS1, demonstrates that the CHIKV strains from Colombia and Virgin islands, they are closely related to Yap, BVI, China, and the Philippines and they constitute a strongly supported clade (bootstrap of 1,000) within the Asian genotype.

Four fragments of the NS1 gene (samples SJ_6, SJ_33, SJ_25, and SJ_42) and seven fragments of the E2 gene (samples AA_1, SJ_11, SJ_6, SJ_26, SJ_33, SJ_28, and SJ_42) of the same number of patients were sequenced. Based on phylogenetic analyses of NS1 and E2 sequences, the characterization of the Colombian CHIKV indicated that it is a strain closely related to British Virgin Islands-99659 (KJ451624), and also closely related to the Asian genotype isolated in China (KF318729) and the Philippines (KC488650). Partial sequences of the NS1 gene from strain Caribe_colombia_SJ-42 and strain Caribe_colombia_2014_NS1 SJ-33 were deposited in GenBank under the accession numbers KP100455 and KR233480, respectively. The other two fragments of NS1 (SJ_6 and SJ_25) were inconclusive. Partial sequence of the E2 gene were deposited in GenBank under the accession numbers KP343865 for the strain Caribe_colombia_E2_SJ-42, KP343866 for the strain Caribe_colombia_E2_AA_01, KR349972 for the strain Caribe_colombia_E2_SJ-33, KR259354 for the strain Caribe_colombia_E2_SJ-28, KR232581 for the strain Caribe_colombia_E2_SJ-11, KR233481 for the strain Caribe_colombia_E2_SJ-06, and KR260263 for the strain Caribe_colombia_E2_SJ-26 (Figure 3).

Discussion

From September to October 2014, 100 suspected cases of CHIKV in the Caribbean area of Colombia were analyzed. According to the Instituto Nacional de Salud (INS) of Colombia, the first cases in Colombia appeared in the first week of August 2014; the INS reported a total of 106,592 cases in 2014 [8]. Several countries in South America have also reported cases of CHIKV; in Venezuela, more than 200 suspected cases were described, and the Ministry of Health of Brazil has reported autochthonous transmission of CHIKV in ten cities of the country [18,19].

On the other hand, the clinical presentation of patients in the present study showed some slightly different features from those seen in African and Asian countries [1-3,5,9]. We found the same percentage of fever and arthralgia; however, we observed a lower incidence of myalgia and headache [1,4]. Nevertheless, skin lesions (macular or maculopapular exanthema or rash) have been reported with more frequency in contrast to recent outbreaks [1,4]. Hemorrhagic signs were rare in our study (Table 1). In our work, persistent arthralgia was observed in many patients for more than four months (data not shown). Severe forms (requiring hospitalization) and deaths were not seen in our study population, in contrast with the report of Economopoulou *et al*. of the outbreak on Reunion Island during 2005 and 2006, where the overall mortality rate was 10% (65/610 cases) [20]. The economic impact of the disease due to loss of workdays and wages, money spent on medications, and consultation costs has been calculated in some states in India, which is one of the countries that was most seriously affected by CHIKV pandemic of 2006–2007. Vijayakumar *et al*. reported economic losses estimated at USD 13 million in 2007 in the state of Kerala, which accounted for 56% of cases in all of India [21]. It is therefore necessary as soon as possible to calculate the economic impact of Chikungunya fever in Colombia.

With respect to phylogenetics, analysis of the partial nucleotide sequences of NS1 (380 bp) and E2 (384 bp) demonstrated that the Colombian CHIKV strain was closely related to the CHIKV strain found in the British Virgin Islands in 2014 and in Dominica [6,22]. The phylogenetic trees generated showed that the Colombian CHIKV strain sits in the clade of Asian genotype with strains isolated in China, the Philippines, Indonesia, Thailand, and Malaysia (Figure 3). In the present study, we examined two genes: one non-structural (NS1) and one structural gene (E2). The genotypes of the Chikungunya virus can be defined by the phylogenetic clustering of several genes like NS1 and E2. In our study, we compared only the relationship and similarity of the Colombian strains with other strains from Asia, Africa, and other isolates around the world. Although E1 is more often used in phylogenetic studies than is E2, both encode structural proteins. However, E2 has more major nucleotide variation; therefore, we suggest that our choice of E2 is valid for phylogenetic analysis [12].

Different genotypes have been found in the Americas; the British Virgin Islands and other Caribbean Islands have confirmed the presence of Asian genotype [6], whereas the ECSA genotype was found recently in Brazil [23]. It is important to identify the genotype involved in the Colombian outbreak because the presence of particular genotypes has been associated with more severe clinical manifestations, high pathogenicity or virulence, mortality, and even enhancement of mosquito infectivity [24].

The predominance of *Ae. aegypti* as a competent vector for DENV in the Caribbean area of Colombia is well established, and we therefore believe that it was responsible for the rapid dissemination of CHIKV [9-11]. Although minor populations of *Ae. albopictus* are present, their role in the outbreak appears unlikely, at least at the beginning of the outbreak.

Hence, the presence of DENV and other arboviruses in Colombia may present a difficulty for physicians in diagnosing these arboviruses, because dengue infections have a similar clinical presentation [2,7]. In addition, the antibody responses in patients with a concomitant infection are completely unknown.

Finally, we believe that the real epidemiologic impact of Chikungunya in Colombia is underestimated by the Minister of Health. One reason is that many patients with CHIKV symptoms do not seek medical attention and instead opt for self-medication. Thus, the actual number of cases in this epidemic remains underestimated. If the current outbreak of CHIKV infection in South America follows the same trend seen in previous dengue and encephalitis outbreaks, increased circulation of the virus can be expected in the short term. In addition, the co-circulation of other arboviruses will be a public health concern in the Americas.

Conclusions

This study shows a phylogenetic and clinical description of CHIKV in Colombia. Phylogenetic analysis revealed that the strains involved in the outbreak were closely related to CHIKV strain found in the British Virgin Islands in 2014. This Asian genotype that is currently moving throughout the Caribbean region is believed to come from China and the Philippines.

Acknowledgements

The authors acknowledge Professor Ben Adler, Monasch University of Australia for critical reading of the manuscript, to Dr. Albeiro Perez for the partial analysis of the phylogenetic tree, to Leydi Mendoza, Marcela Durango, Luis Causil, Nelson Mendez for the recollection of clinical information, and the University of Cordoba for supporting the study. To people of San Joaquin and Ovejas. To Dr. Manolo Olivares and Miladys Lambrano, Secretary of Health (Sucre).

References

1. Nkoghe D, Kassa RF, Caron M, Grard G, Mombo I, Bikie B, Paupy C, Becquart P, Bisvigou U, Leroy EM (2012) Clinical forms of chikungunya in Gabon, 2010. PLoS Negl Trop Dis 6: e1517. doi:10.1371/journal.pntd.0001517
2. Lebrun G, Chadda K, Reboux AH, Martinet O, Gauzere BA (2009) Guillain-Barre syndrome after chikungunya infection. Emerg Infect Dis 15: 495-496. doi:10.3201/eid1503.071482
3. Dupuis-Maguiraga L, Noret M, Brun S, Le Grand R, Gras G, Roques P (2012) Chikungunya disease: infection-associated markers from the acute to the chronic phase of arbovirus-induced arthralgia. PLoS Negl Trop Dis 6: e1446. doi:10.1371/journal.pntd.0001446
4. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L, Lassalle C, Thiria J, Rachou E, de Valk H, Ilef D, Ledrans M, Quatresous I, Quenel P, Pierre V (2007) A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. Am J Trop Med Hyg 77: 727-731.
5. Kosasih H, de Mast Q, Widjaja S, Sudjana P, Antonjaya U, Ma'roef C, Riswari SF, Porter KR, Burgess TH, Alisjahbana B, van der Ven A, Williams M (2013) Evidence for endemic chikungunya virus infections in Bandung, Indonesia. PLoS Negl Trop Dis 7: e2483. doi:10.1371/journal.pntd.0002483
6. Lanciotti RS, Valadere AM (2014) Transcontinental movement of Asian genotype chikungunya virus. Emerg Infect Dis 20: 1400-1402. doi:10.3201/eid2008.140268
7. Omarjee R, Prat C, Flusin O, Boucau S, Tenebray B, Merle O, Huc-Anais P, Cassadou S, Leparc-Goffart I (2014) Importance of case definition to monitor ongoing outbreak of chikungunya virus on a background of actively circulating dengue virus, St Martin, December 2013 to January 2014. Euro Surveill 19. pii: 20753. doi:10.2807/1560-7917.es2014.19.13.20753
8. INSTITUTO NACIONAL DE SALUD. (National Health Institute of Colombia). http://www.ins.gov.co/Noticias/Chikungunya/Resumen%20Chikungu%C3%B1a%20SEMANA%2039%202015.pdf Accessed October 12th, 2015.
9. Powers AM, Logue CH (2007) Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. J Gen Virol 88: 2363-2377. doi:10.1099/vir.0.82858-0
10. de Lamballerie X, Leroy E, Charrel RN, Ttsetsarkin K, Higgs S, Gould EA (2008) Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? Virol J 5: 33. doi:10.1186/1743-422X-5-33
11. Vega-Rua A, Zouache K, Girod R, Failloux AB,and Lourenco-de-Oliveira R (2014) High level of vector competence of Aedes aegypti and Aedes albopictus from ten American countries as a crucial factor in the spread of Chikungunya virus. J Virol 88: 6294-6306. doi:10.1128/JVI.00370-14
12. Solignat M, Gay B, Higgs S, Briant L, Devaux C (2009) Replication cycle of chikungunya: a re-emerging arbovirus. Virology 393: 183-197. doi:10.1016/j.virol.2009.07.024
13. Leparc-Goffart I, Nougairede A, Cassadou S, Prat C, de Lamballerie X (2014) Chikungunya in the Americas. Lancet 383: 514. doi:10.1016/S0140-6736(14)60185-9
14. Yap G, Pok KY, Lai YL, Hapuarachchi HC, Chow A, Leo YS, Tan LK, Ng LC (2010) Evaluation of Chikungunya diagnostic assays: differences in sensitivity of serology assays in two independent outbreaks. PLoS Negl Trop Dis 4: e753. doi:10.1371/journal.pntd.0000753

15. Pfeffer M, Linssen B, Parke MD, Kinney RM (2002) Specific detection of chikungunya virus using a RT-PCR/nested PCR combination. J Vet Med B Infect Dis Vet Public Health 49: 49-54. doi:10.1046/j.1439-0450.2002.00535.x
16. de Morais Bronzoni RV, Baleotti FG, Ribeiro Nogueira RM, Nunes M, Moraes Figueiredo LT (2005) Duplex reverse transcription-PCR followed by nested PCR assays for detection and identification of Brazilian alphaviruses and flaviviruses. J Clin Microbiol 43: 696-702. doi:10.1128/JCM.43.2.696-702.2005
17. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725-2729. doi:10.1093/molbev/mst197
18. Figueiredo ML, Figueiredo LT (2014) Emerging alphaviruses in the Americas: Chikungunya and Mayaro. Rev Soc Bras Med Trop 47: 677-683. doi:10.1590/0037-8682-0246-2014
19. Donalisio MR, Freitas AR (2015) Chikungunya in Brazil: an emerging challenge. Rev Bras Epidemiol 18: 283-285. doi:10.1590/1980-5497201500010022
20. Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, Quenel P, Germonneau P, Quatresous I (2009) Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005-2006 outbreak on Reunion. Epidemiol Infect 137: 534-541. doi:10.1017/S0950268808001167
21. Vijayakumar K, George B, Anish TS, Rajasi RS, Teena MJ, Sujina CM (2013) Economic impact of chikungunya epidemic: out-of-pocket health expenditures during the 2007 outbreak in Kerala, India. Southeast Asian J Trop Med Public Health 44: 54-61.
22. Ahmed S, Francis L, Ricketts RP, Christian T, Polson-Edwards K, Olowokure B (2015) Chikungunya virus outbreak, Dominica, 2014. Emerg Infect Dis 21: 909-911. doi:10.3201/eid2105.141813
23. Teixeira MG, Andrade AM, Costa Mda C, Castro JN, Oliveira FL, Goes CS, Maia M, Santana EB, Nunes BT, Vasconcelos PF (2015) East/Central/South african genotype chikungunya virus, Brazil, 2014. Emerg Infect Dis 21: 906-907. doi:10.3201/eid2105.141727
24. Kumar A, Mamidi P, Das I, Nayak TK, Kumar S, Chhatai J, Chattopadhyay S, Suryawanshi AR, Chattopadhyay S (2014) A novel 2006 Indian outbreak strain of Chikungunya virus exhibits different pattern of infection as compared to prototype strain. PLoS One 9: e85714. doi:10.1371/journal.pone.0085714

Corresponding author

Salim Mattar,
Universidad de Córdoba, Instituto de Investigaciones Biológicas del Trópico, Campus Universitario, Montería, Colombia.
Phone: + 57-4-756920.
http://www.unicordoba.edu.co/index.php/investigacion/inst-investigacion/instituto-de-investigaciones-biologicas-del-tropico
Email: mattarsalim@hotmail.com;
smattar@correo.unicordoba.edu.co

Conflict of interests: No conflict of interests is declared.