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Short Communication

Re-emergence of chikungunya in the Republic of the Congo in 2019 associated with a possible vector-host switch

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ABSTRACT

In January 2019, an outbreak of chikungunya virus fever was reported in a rural region near Pointe-Noire, Republic of the Congo. Molecular and phylogenetic analysis of this new CHIKV strain demonstrated the presence of the A226V substitution and a surprisingly close relation with *Aedes aegypti*-associated Central Africa chikungunya strains. These results, combined with the preponderance of *Aedes albopictus* in the outbreak area, suggest a recent vector-host switch facilitated by the emergence and spread of the A226V mutation from a related CHIKV strain previously circulating in *Aedes aegypti*. The proximity of this outbreak to the large city of Pointe-Noire alerts us to a possibly devastating future outbreak in the absence of measures limiting the proliferation of *Ae. albopictus* mosquitoes.

Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) which in the last 20 years has acquired worldwide distribution and is associated with regular regional outbreaks. A large outbreak that occurred on Reunion Island between 2004 and 2005 is notable because of its association with two events that have changed the epidemiology of CHIKV considerably (Chastel, 2005). First, the virus became primarily transmitted by *Aedes albopictus*, a highly invasive mosquito species that can develop high-density populations that quickly outnumber indigenous *Aedes aegyptyi* populations in suburban environments. Second, this vector switch was associated with the emergence and spread of a single adaptive mutation, E1-A226V, that provides a selective advantage for the replication and transmission of CHIKV in *Ae. albopictus* (Schuffenecker et al., 2006; Tsetsarkin et al., 2007). This vector-host switch facilitated the spread of CHIKV throughout the world, including Africa where Cameroon, Gabon, and RC experienced outbreaks between 2006 and 2011 (Leroy et al., 2009; Mombouli et al., 2013; Peyrefitte et al., 2007).

On January 7, 2019, authorities in the Hinda-Loango and Mvouti-Kakamoeka health zones, Kouilou Department in RC, were informed of numerous cases of febrile arthralgia in the villages of Diosso and Matombi (Figure 1). By January 13, 2019, 1,649 suspected cases were reported, including 1,141 in Diosso. Serum and plasma samples from 51 symptomatic patients from Diosso were collected and sent to the French Institute for Research and Development in Montpellier (IRD; UMR 224) for analysis. Viral RNA was extracted from plasma using the Qiamp viral RNA kit (Qiagen). The presence of CHIKV, Dengue virus (1–4) and Zika virus RNA in samples was assessed using two-step quantitative RT-PCR (de Lamballerie et al., 2008; Leroy et al., 2009). Presence of CHIKV RNA was detected in 36 patients (70.6%). Viral RNA from Dengue and Zika was not detected in any of the 51 patients tested. These results demonstrate that CHIKV is responsible for the current outbreak, indicating a re-emergence of CHIKV in Central Africa after the last reported outbreak that occurred in Brazzaville, the capital of RC, in 2011.

We then performed full genome sequencing of virus isolated from E6 Vero cells infected from the diluted serum of a CHIKV-positive patient. Viral RNA was extracted from the cell culture

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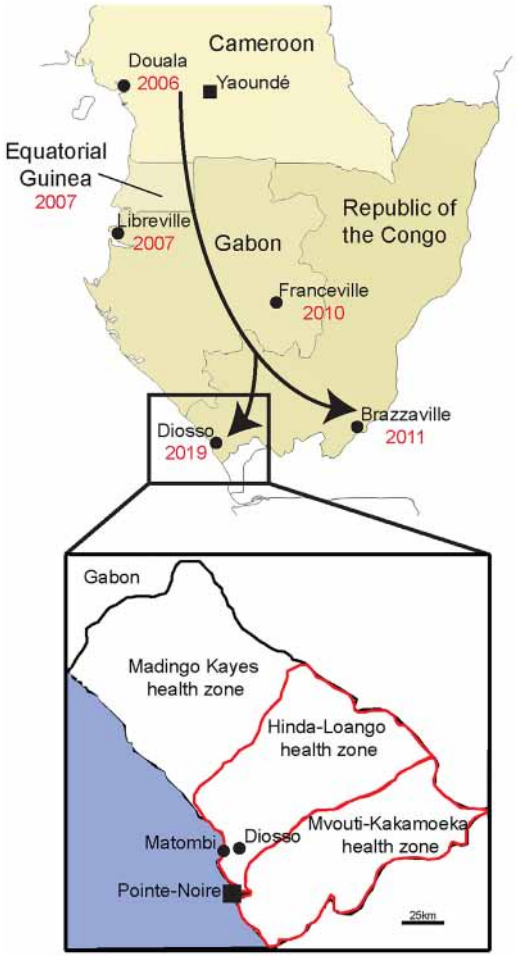

Figure 1. Distribution and progression of chikungunya outbreaks in Central Africa since 2006. The locale (black circle) and date (in red) of each outbreak in Central Africa since 2006 are indicated on the map. The north to south spread of *Ae. albopictus* is represented by black arrows. The Kouilou department is shown in the inset, with the Hinda-Loango and Mvouti-Kakamoeka health zone, where the suspected cases of chikungunya were reported, circled in red.

supernatant using Qiamp Viral RNA, followed by RT-PCR as previously described (Schuffenecker et al., 2006). PCR products were sequenced using the Sanger method and aligned to obtain a complete genome (11,532 nt) (GenBank accession number MK690206).

Phylogenetic analysis revealed that the virus (named RC_Diosso_2019) belongs to the East/Central/South African (ECSA) lineage and falls within the ECSA2 clade, which contains strains isolated from Central African Republic, Cameroon, Gabon, and RC (Figure 2). This study thus provides further evidence that the sustained and active circulation of CHIKV in Central Africa since 1962 is restricted to ECSA2 strains. Our phylogenetic analysis further indicates two distinct groups (95% bootstrap) within the ECSA2 clade. The first group (Group A) contains primarily strains isolated in Angola and CAR prior to 1984 that carry an Alanine at position 226 of the E1 envelope gene. The second group (Group B) contains strains isolated since 2006 in Cameroon, RC, and Gabon and all possess the recently derived E1-A226V mutation facilitating the vector-host switch from *Ae. aegypti* to *Ae. albopictus*. The RC_Diosso_2019 strain falls within Group A and is most closely related to a strain recently isolated in Japan from an Angolan patient (Angola_2016; Takaya et al., 2017). However, unlike the rest of the Group A viruses, RC_Diosso_2019 carries the E1-A226V mutation. The presence of this mutation was confirmed in 30 other samples by E1 envelope characterisation (data not shown). To the extent that this mutation is a marker of vector-host affinity, our analysis suggests that RC_Diosso_2019 likely evolved from an *Ae. aegypti*-vectored African strain circulating in the region and that *Ae. albopictus* is probably the primary vector in this outbreak, which is consistent with other previous outbreaks (Paupy et al., 2010). Additionally, we have investigated the E2-I211T mutation. As expected for a Group A virus, RC_Diosso_2019 does not display the *Ae. albopictus*-adaptive mutation. This observation further may support the idea of a very recent vector host-switch.

Our study suggests that *Ae. albopictus* could have spread continuously in a southward movement from 2006 in Cameroon to Gabon and then into Brazzaville, RC, in 2011. Upon reaching the Kouilou Department where *Ae. aegypti*-associated CHIKV strains were circulating (Figure 1), a vector-host switch occurred, facilitated by the emergence and spread of the A226V mutation following repeated interactions with *Ae. albopictus,* and precipitating the current outbreak.

A recent survey found high densities of *Ae. albopitus in* the suburban environment of Pointe-Noire, compared to downtown where *Ae. aegypti* still prevails (Kamgang et al., 2018). The high density of *Ae. albopictus* in nearby Pointe-Noire, located less than 30 km from the current outbreak, makes us wary of a potentially devastating outbreak in this city of nearly one million inhabitants. Prevention measures aimed at limiting the proliferation of *Ae. albicans* in Pointe-Noire should, therefore, be urgently implemented.

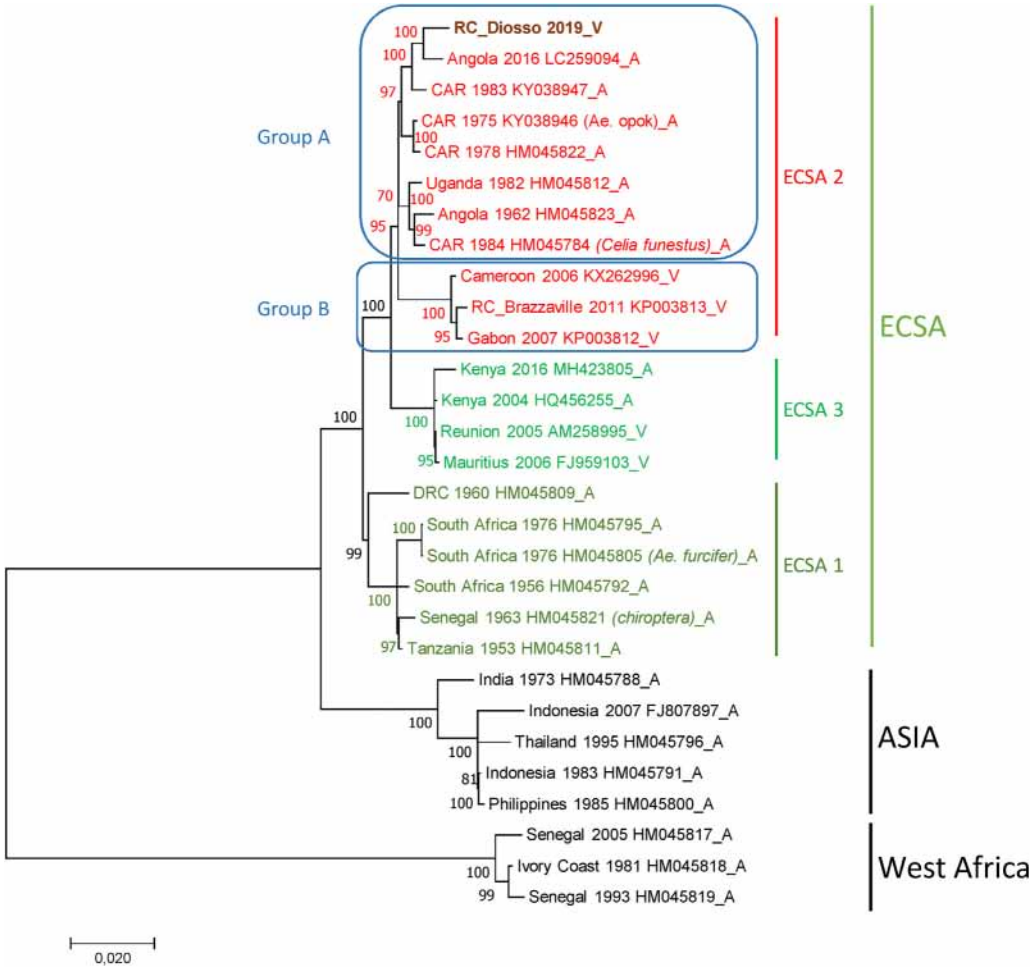

Figure 2. A phylogenetic tree using CHIKV nucleotide complete partial sequences. The RC_Diosso_2019 sequence from this study is marked in dark red bold and falls in Group A of the East/Central/South African lineage 2 (ECSA 2) in red. Sequences belonging to the ECSA1 and ECSA3 are respectively in grass green and light green. For each virus strain, the taxon labels include isolation locale/date/accession number and symbol for the amino acid at the position E1-226. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers along branches indicate bootstrap values. Multiple sequence alignment was performed using MUSCLE. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model. Evolutionary analyses were conducted in MEGA X.

Ethical approval

Not required.

Conflict of interest statement

None of the authors have any conflict of interest (financial or personal) in this study.

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