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Short Report: Different Lineages of Chikungunya Virus in Equatorial Guinea in 2002 and 2006

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Abstract. Chikungunya virus is a mosquito-borne virus that causes an acute febrile infection and severe arthralgia and is considered a re-emergent pathogen. During a study investigating arboviruses causing febrile infection in infants in Bata, Equatorial Guinea, the genome of this virus was amplified from blood samples during near two rainy seasons (2002–2003). In 2006, this virus was isolated from a traveler returning to Spain from Equatorial Guinea. These results show that chikungunya virus is present in this country and two lineages are circulating. Thus, this virus should be considered in the differential diagnosis of febrile syndromes in inhabitants and in travelers returning from this country.

Tropical Africa is the likely site of origin of many of the arboviruses of modern medical importance. However, there is little data about the prevalence of arboviral infections in this region.

Equatorial Guinea is located in the equatorial part of Africa between Cameroon and Gabon and is divided into a continental part (area = 26,017 km²) and some islands (area = 2,034 km²). It has a population of approximately 500,000 persons, with a high percentage (45%) less than 15 years of age. This country has an equatorial climate with an annual average temperature of 25°C. The average annual precipitation (> 2,000 mm) is divided into two rainy seasons: from April to May and from October to December.

In west and central Africa, data on the circulation of chikungunya virus (CHIKV) are rare. In Cameroon, serologic prevalence assays have shown that the alphaviruses CHIKV and O'nyong nyong virus are the most common arboviral infections.¹ In Gabon, an outbreak of a dengue-like syndrome caused by CHIKV was described in 2006–2007.² The virus was phylogenetically related to strains isolated in 2006 in Cameroon and seen years earlier in the Democratic Republic of Congo, where 50,000 persons were infected.³

Chikungunya virus belongs to the family *Togaviridae* and the genus *Alphavirus*. It causes an acute infection with an abrupt onset of fever, headache and severe joint pains and is transmitted mainly by *Aedes* mosquitoes.⁴ In recent years, it has been considered a re-emergent virus and has become a public health problem in countries such as the Democratic Republic of Congo.³ More recently, CHIKV has caused large outbreaks that affected different Indian Ocean territories and continental India.^{5,6} This epidemic was the source of an outbreak in Italy caused by an infected traveler from India.⁷

In 2002–2003, a project to study the presence of some viruses in Equatorial Guinea was conducted. Samples from febrile children attending the Reference Center for the Control of Endemic Diseases located in Bata (continental region of Equatorial Guinea) were obtained. From June 2002 to January

2003, 720 blood samples were obtained. RNA was preserved in a guanidinium isothiocyanate buffer. Samples were sent to the National Center for Microbiology in Madrid, Spain, for testing. Samples used were not given personal identifiers to comply with bioethics guides.

After RNA extraction,⁸ samples were assayed. We used generic primers to potentially detect arboviruses in different polymerase chain reaction (PCR) assays. We searched for arenaviruses,⁹ hantaviruses, and orthobunyaviruses (using in-house methods); no positive results were obtained. Arboviruses belonging to the genera *Flavivirus*, *Phlebovirus*, and/or *Alphavirus* were tested by using a generic multiplex real-time nested PCR in which consensus primers for each of the three genera were mixed.¹⁰ Positive amplification with the generic multiplex method was achieved in eight samples obtained in the rainy seasons when vector activity is high. Sequences of amplified fragments corresponding to 195 base-pairs of the non-structural protein 4 gene of alphaviruses identified a homogeneous cluster of CHIKV belonging to the Central-East/South Africa genotype. Chikungunya virus was found in 2002 (three samples in June, one sample in July, and two samples in December) and 2003 (two samples in January). Four of these eight virus-positive samples were also positive for *Plasmodium falciparum*.

To obtain a sequence with more phylogenetic information, primers designed by Powers and others (sense₁₀₂₄₆ 5'-TTAC CCNTTYATGTGGGG-3'₁₀₂₆₂ and antisense₁₀₇₉₃ 5'-CTTA CSGGGTTTGTGTC-3'₁₀₇₇₇),¹¹ were used in combination with primer₁₀₇₁₄ 5'-TRAAGCCAGATGGTGCC-3'₀₆₉₈ to amplify a fragment of a region of the envelope 1 (E1) gene often used for CHIKV phylogenetic analysis. Three of the eight positive samples (probably those with the highest concentration of virus) showed a 469-basepair product whose sequences formed a unique cluster within the Central/East African genotype clade (Figure 1).

In Spain, CHIKV was considered in the differential diagnosis of a febrile syndrome in travelers returning from Equatorial Guinea. In 2006, one of three such travelers returning from this area was diagnosed in Spain as being infected with CHIKV on the basis of a positive PCR result, which showed amplification of part of the E1 gene.¹² This sequence is similar to others described at the same time from Cameroon and Gabon,^{1,2} but is different from sequences obtained in Equatorial Guinea in 2002. There is evidence of endemic circulation of a genetically

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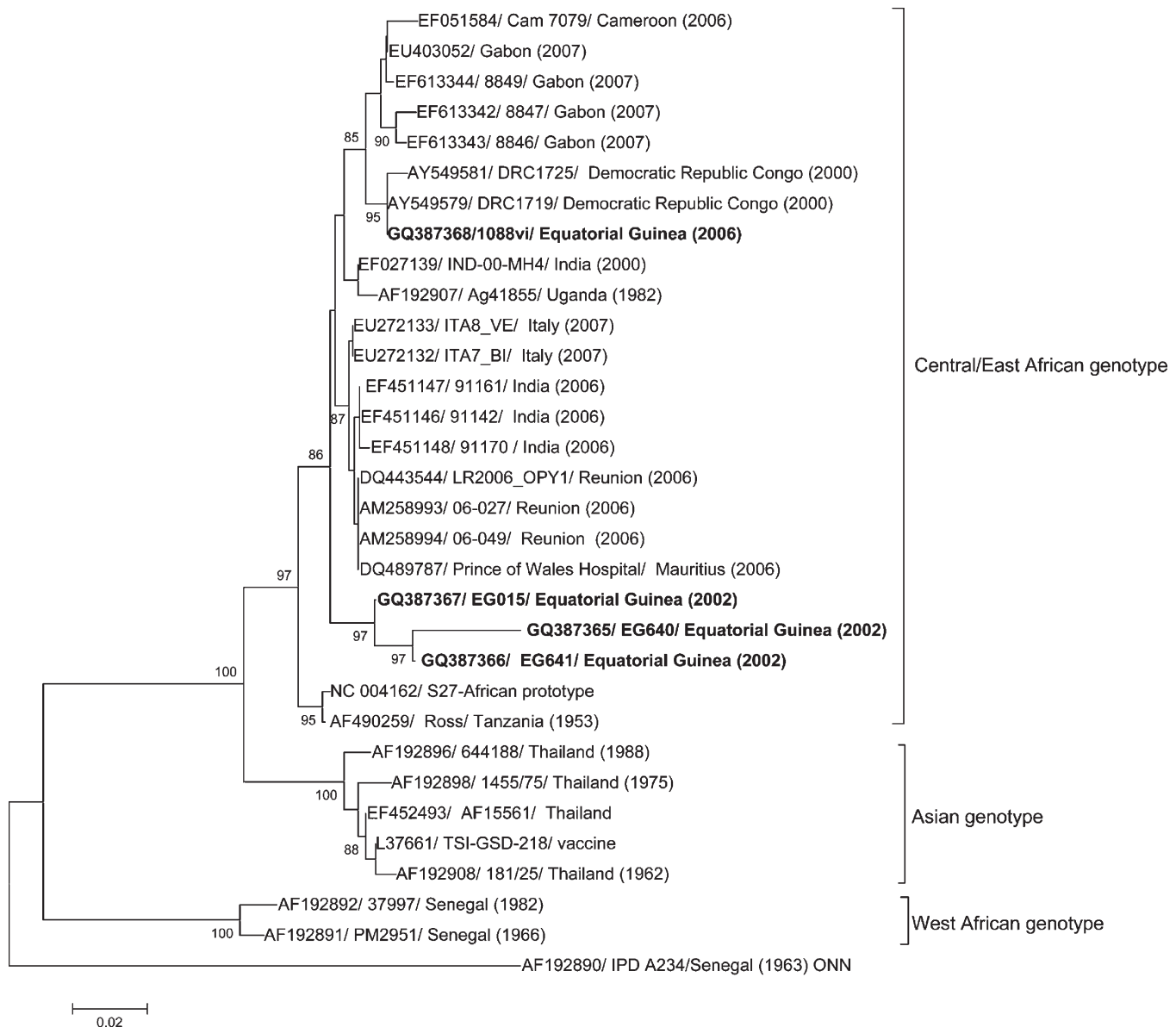


FIGURE 1. Phylogenetic tree showing the relationships of chikungunya virus (CHIK) strains. A 434-basepair fragment from the envelope 1 gene was analyzed by using the neighbor-joining method, the number of differences model, and bootstrap percentage corresponding to 1,000 replicates with the MEGA 4 software. O'nyong nyong virus (ONNV) was used as an outgroup. Accession number, name of the strain, and place and year of isolation are indicated. Strains sequenced in this paper are shown in **bold**. Bootstrap values > 80 are shown.

stable monophyletic CHIKV population during 2000–2007 in west central Africa.²

Our results suggest that a paraphyletic population of CHIKV was circulating in Equatorial Guinea in 2002. Our results indicate that CHIKV is likely endemic in Equatorial Guinea, and suggest that either two (or more) CHIKV populations were co-circulating in Equatorial Guinea or the western Central African lineage has replaced the lineage detected during 2002–2003. Our data clearly identified three genotypes but cannot be used to unequivocally demonstrate the endemicity of CHIKV in Equatorial Guinea. To conduct a robust phylogenetic analysis, complete sequences of viruses belonging to each group are needed. Therefore, studies of CHIKV in patients infected in Equatorial Guinea are ongoing.

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