Chikungunya Virus, Cameroon, 2006
Christophe Peyrefitte, Dominique Rousset, Boris A.M. Pastorino, Regis Pouillot, Maël Bessaud, Fabienne Tock, Helene Mansaray, Olivier Merle, Aurelie Pascual, Christophe Paupy, et al.

To cite this version:
Christophe Peyrefitte, Dominique Rousset, Boris A.M. Pastorino, Regis Pouillot, Maël Bessaud, et al.. Chikungunya Virus, Cameroon, 2006. Emerging Infectious Diseases, Centers for Disease Control and Prevention, 2007, 13 (5), pp.768-771. 10.3201/eid1305.061500 . hal-03002514

HAL Id: hal-03002514
https://hal.umontpellier.fr/hal-03002514
Submitted on 31 May 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Chikungunya Virus, Cameroon, 2006

Christophe N. Peyrefitte,* Dominique Rousset,† Boris A.M. Pastorino,* Regis Pouillot,† Maël Bessaud,* Fabienne Tock,* Helene Mansaray,‡ Olivier L. Merle,* Aurelie M. Pascual,* Christophe Paupy,§ Aurelia Vessiere,† Patrice Imbert,¶ Patrice Tchendjou,† Jean-Paul Durand,* Hugues J. Tolou,* and Marc Grandadam*

We report the isolation of chikungunya virus from a patient during an outbreak of a dengue-like syndrome in Cameroon in 2006. The virus was phylogenetically grouped in the Democratic Republic of the Congo cluster, indicating a continuous circulation of a genetically similar chikungunya virus population during 6 years in Central Africa.

Chikungunya virus (CHIKV), formerly only an anecdotaly described arbovirus, is now a worldwide public health problem (1). Recently, numerous cases of CHIKV infection have been reported from a major outbreak of febrile illness around the Indian Ocean, which included Comoros, Mauritius, Réunion Island (2,3), and southern India (4).

CHIKV is widely distributed in tropical Africa (5,6) and in Asia (7). In Africa, until 2000, the virus was described as endemic, perpetuated through a sylvatic cycle involving wild primates, humans, and mosquitoes of the genus Aedes (2,8). During the past 6 years, the urban cycle has also tended to play a role in Central Africa (6). Nevertheless, although recent serologic surveys suggest a high prevalence of Togaviridae, Flaviviridae, and Bunyaviridae (9,10), understanding of the circulation and effects of arboviruses in Cameroon remains imprecise. This lack of understanding may reflect confusion between arboviral infections and hyperendemic Plasmodium falciparum infection.

We report the first isolation, to our knowledge, of CHIKV in Cameroon. The virus was identified during an outbreak of a febrile syndrome in French soldiers in Douala and in patients from an urban medical center in Yaoundé. We also found evidence of cocirculation of CHIKV and dengue virus (DENV).

The Study

In Douala, Cameroon, 2 sporadic cases of a dengue-like syndrome were recorded in French soldiers (patients 1 and 2) on April 3 and May 22, 2006, respectively (Table). From the end of May through the end of July 2006, more cases of dengue-like syndrome, which included fever, asthenia, maculopapular rashes, and arthralgia, were observed in Yaoundé. The number of patients who sought treatment at the Yaoundé Medical Center peaked in mid-June 2006. Blood samples were collected from 30 of the 40 patients who visited the medical center. The 30 patients’ ages ranged from 1 to 54 years. The delay between the onset of symptoms and the sampling ranged from 0 to 39 days with a median of 4 days (Table). All but 1 patient lived in Yaoundé, and none of these patients had a history of travel abroad or from Yaoundé. Nine patients were Cameroonian, and all other patients were from other countries; 15 patients were female. A blood sample from a 53-year-old woman who returned to France from Yaoundé was also received. All patients had negative results for P. falciparum according to rapid test (Core Malaria Pf, Core Diagnostics, Birmingham, UK) and thick smear examination.

Serum specimens were tested for immunoglobulin M (IgM) and IgG antibodies specific for DENV, West Nile virus (WNV), Wesselsbron virus, Rift Valley fever virus, Bunyamwera virus, and CHIKV by IgM-antibody capture (MAC-ELISA) and IgG sandwich ELISA, respectively (11). A serum sample was considered positive if the optical density (OD) ratio of viral antigen to uninfected cells was >3. The presence of CHIKV, DENV, and WNV genomes was tested for by specific real-time reverse transcription PCR (RT-PCR) (12). Virus isolation on C6/36 and Vero cells was attempted on samples that were positive by RT-PCR (11).

The serologic follow-up of patient 1 (Table) for a 3-week period detected seroconversion to a virus antigenically related to CHIKV virus (the OD ratios obtained with the second sample were >3) for IgM and IgG. A sample from patient 2 was obtained the day after the onset of symptoms, and no antibodies to all tested arboviruses were detected. However, the specimen was positive by real-time RT-PCR for CHIKV. The patient’s sample yielded CHIKV when cultured, and the envelope gene was partially sequenced (position 10,238–11,367, GenBank accession no. EF051584). The 1.2-kb sequence genetic analysis did not show any codon deletion or insertion when compared with other African CHIKV sequences available in the GenBank database (3,6,8). A high degree of identity was observed when the sequence was compared with the Democratic Republic of the Congo (DRC) strains isolated in 2000 (6). Paired identity ranged from 97% to 98.1% at the nucleotide level and from 98.7% to 99.3% at the amino acid level. The Cameroon isolate displayed a higher nucleotide diver-
gence (paired identity ranging from 95% to 95.5%) when compared with the 2006 Réunion Island strains (2,3,13). However, amino acid sequences were highly conserved (99%–99.5%). The sequence identity among these isolates highlights their common origin and particularly the genetic stability of CHIKV despite the 6 years and the geographic distance from the DRC outbreaks. As shown in the phylogenetic tree (Figure), the CHIKV Cameroon strain clustered with DRC CHIKV strains with a high bootstrap value of 100. This genotype of CHIKV was closely related to strains from the Central African Republic and the 1982 Uganda isolate (6,8). The close genetic relationship suggests a continuous circulation of a homologous CHIKV population in Central Africa with a high degree of genetic stability. The genetic stability of the Central African CHIKV strains during 24 years, whether associated with epidemic or sporadic cases, highlights the peculiar importance of the few mutations detected in the recent Réunion Island isolates (3). This also suggests that the Central African strain CHIKV zone of circulation now includes India (4), the Indian Ocean, and Cameroon.

The phylogenetic tree also illustrates the differences between the Cameroon isolates and the Asian subgroup isolates. Moreover, when compared with Asian CHIKV, including the 2006 isolates, the Cameroon strain showed 91%–91.9% and 96.8%–98.8% identity at the nucleotide and amino acid levels, respectively. Despite the similarity, cross-neutralization experiments must be conducted to confirm the protective effect of the Asian CHIKV-based vaccine against Central African strains (2).

Among patients from Yaoundé, 1 (patient 11) had only IgM antibodies specific to CHIKV, while patients 18 and

| Patient no. | Sex/age, y | City            | Symptom onset | Sampling date | Delay, d | IgM† | IgG† | PCR‡ |
|------------|------------|-----------------|---------------|---------------|---------|------|------|------|
| 1          | M/35       | Douala          | 3 Apr         | 7 Apr         | 4       | Neg  | Neg  | Neg  |
| 2          | M/36       | Douala          | 22 May        | 23 May        | 1       | Neg  | Neg  | Pos CHIKV |
| 3          | F/54       | Yaoundé         | 11 Jun        | 12 Jun        | 1       | Neg  | Neg  | Neg  |
| 4          | F/49       | Yaoundé         | 10 Jun        | 13 Jun        | 3       | Neg  | Neg  | Neg  |
| 5          | F/42       | Yaoundé         | 11 Jun        | 12 Jun        | 1       | Neg  | Neg  | Neg  |
| 6          | F/41       | Yaoundé         | 7 Jun         | 7 Jun         | 0       | Neg  | Pos Flavi | Neg  |
| 7          | M/38       | Yaoundé         | 15 Jun        | 19 Jun        | 4       | Neg  | Neg  | Neg  |
| 8          | M/30       | Yaoundé         | 18 Aug        | 19 Jun        | 1       | Neg  | Neg  | Neg  |
| 9          | M/21       | Yaoundé         | 15 Jun        | 19 Jun        | 4       | Neg  | Neg  | Neg  |
| 10         | F/32       | Yaoundé         | 21 Jun        | 22 Jun        | 1       | Neg  | Neg  | Neg  |
| 11         | F/22       | Yaoundé         | 19 Jun        | 26 Jun        | 7       | Pos CHIKV | Neg  |
| 12         | F/53       | Imported        | 20 Jun        | 26 Jun        | 6       | Pos CHIKV | Pos CHIKV | Neg  |
| 13         | M/42       | Yaoundé         | 24 Jun        | 27 Jun        | 3       | Neg  | Pos Flavi | Neg  |
| 14         | F/42       | Yaoundé         | 18 Jun        | 28 Jun        | 10      | Neg  | Neg  | Neg  |
| 15         | F/27       | Yaoundé         | 18 Jun        | 29 Jun        | 11      | Neg  | Pos Flavi | Neg  |
| 16         | F/43       | Yaoundé         | 26 Jun        | 30 Jun        | 4       | Neg  | Neg  | Neg  |
| 17         | M/31       | Yaoundé         | 16 Jun        | 30 Jun        | 14      | Neg  | Neg  | Neg  |
| 18         | F/37       | Yaoundé         | 22 May        | 30 Jun        | 39      | Pos CHIKV | Pos CHIKV | Neg  |
| 19         | F/45       | Yaoundé         | 10 Jun        | 30 Jun        | 20      | Neg  | Neg  | Neg  |
| 20         | M/45       | Yaoundé         | 22 Jun        | 30 Jun        | 8       | Neg  | Pos Flavi and CHIKV | Neg  |
| 21         | M/1        | Yaoundé         | 23 Jun        | 30 Jun        | 7       | Neg  | Neg  | Neg  |
| 22         | F/9        | Yaoundé         | 10 Jun        | 30 Jun        | 20      | Neg  | Neg  | Neg  |
| 23         | M/4        | Yaoundé         | 26 Jun        | 30 Jun        | 4       | Neg  | Neg  | Neg  |
| 24         | M/54       | Yaoundé         | 4 Jul         | 6 Jul         | 2       | Neg  | Neg  | Neg  |
| 25         | F/45       | Yaoundé         | 14 Jun        | 5 Jul         | 21      | Neg  | Neg  | Neg  |
| 26         | M/48       | Yaoundé         | 24 Jun        | 6 Jul         | 12      | Neg  | Neg  | Neg  |
| 27         | M/20       | Yaoundé         | 30 Jun        | 1 Jul         | 1       | Neg  | Neg  | Neg  |
| 28         | M/37       | Yaoundé         | 4 Jul         | 11 Jul        | 7       | Pos CHIKV | Pos CHIKV | Neg  |
| 29         | M/36       | Yaoundé         | 9 Jul         | 11 Jul        | 2       | Neg  | Neg  | Neg  |
| 30         | M/33       | Yaoundé         | 28 Jun        | 10 Jul        | 12      | Neg  | Neg  | Neg  |
| 31         | M/32       | Yaoundé         | 10 Jul        | 12 Jul        | 2       | Neg  | Neg  | Pos DENV  |
| 32         | F/38       | Yaoundé         | 16 Jul        | 17 Jul        | 1       | Neg  | Neg  | Neg  |
| 33         | M/45       | Yaoundé         | 21 Jul        | 26 Jul        | 5       | Neg  | Neg  | Neg  |

*IgM, immunoglobulin M; Neg, negative; Pos, positive; CHIKV, chikungunya virus; Flavi, flavivirus; DENV, dengue virus.
†DENV, West Nile virus, Wesselsbron virus, Rift Valley fever virus, Bunyamwera virus, and CHIKV antibodies tested.
‡DENV, West Nile virus, CHIKV tested by real-time reverse transcription–PCR.
§CHIKV isolation successful.
28 had both IgM and IgG antibodies specific to CHIKV (Table). One patient from Cameroon (patient 20) had IgG specific to both CHIKV and flavivirus. Three patients (nos. 6, 13, and 15), 2 of whom were Cameroonian, had antibodies specific for flavivirus. All samples were negative for WNV and CHIKV by RT-PCR. One sample (from patient 31) was positive for DENV; however, no virus was detected by cell culture. These results suggested a cocirculation of CHIKV and dengue virus during the same period, which is consistent with the suspected circulation of dengue virus, CHIKV, and yellow fever virus observed in a study from 2000 through 2003 in Cameroon (9).

In Cameroon, as in DRC (6), patients were likely infected in urban or periurban centers (Yaoundé, the capital of Cameroon; Douala, a major city). These infections occurred in a context where *Aedes albopictus* tends to replace indigenous *Ae. aegypti* in rural and urban Cameroonian environments (14). This finding suggests that urban cycles and urban vectors, in addition to the traditional forest-dwelling vectors, may play an important role in the maintenance and amplification of CHIKV in Africa.

**Conclusions**

Since its first isolation in 1953 (8), CHIKV has been isolated in different Central African countries (8,6). Until now, only 2 alphavirus strains antigenically suspected to be CHIKV had been isolated from human patients in Camer-oon (15). Recent serosurvey studies suggested a possible CHIKV circulation in Cameroon (9,10). Our Cameroon CHIKV isolate confirmed its circulation in this country. Our study suggests a 6-year continuous circulation of genetically stable and indigenous strains in Central Africa rather than importation of CHIKV from the recent Indian Ocean or Asian outbreaks. Moreover, the genetic stability of the Central African CHIKV highlights the importance of the unique molecular features that was shown in Réunion Island isolates (7).

**Acknowledgments**

We are indebted to Jon Davis for manuscript revision.

Dr Peyrefitte is involved in the diagnosis and epidemiology of arboviruses. He also researches arbovirus-cell interactions.

**References**

1. Gubler DJ. The global emergence/resurgence of arboviral diseases as public health problems. Arch Med Res. 2002;33:330–42.

2. Bessaud M, Peyrefitte CN, Pastorino BAM, Tock F, Merle O, Col-part JJ, et al. Chikungunya virus strains, Réunion Island outbreak. Emerg Infect Dis. 2006;12:1604–6.

3. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 2006;3:e263.

4. Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, Ganghe SS, et al. Chikungunya outbreaks caused by African genotype, India. Emerg Infect Dis. 2006;12:1580–3.

5. Saluzzo JF, Adam C, Castagnet P, Chapelle P, Cornet M, Heme G, et al. Une poussée épidémique due au virus chikungunya dans l’ouest du Sénégal en 1982. Med Afr Noire. 1982;30:427–30.

6. Pastorino B, Muyembe-Tamfum JJ, Bessaud M, Tock F, Tolou H, Durand JP, et al. Epidemic resurgence of Chikungunya virus in Democratic Republic of the Congo: identification of a new central African strain. J Med Virol. 2004;74:277–82.

7. Lam SK, Chua KB, Hooi PS, Rahmat MA, Kumari S, Tharmaratnam M, et al. Chikungunya infection—an emerging disease in Malaysia. Southeast Asian J Trop Med Public Health. 2001;32:447–51.

8. Powers AM, Braught AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya and O’nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. J Gen Virol. 2000;81:471–9.

9. Kuniholm MH, Wolfe ND, Huang CYH, Mpoundi-Ngole E, Tamoufe U, Burke DS, et al. Seroprevalence and distribution of *Flaviviridae*, *Togaviridae*, and *Bunyaviridae* arboviral infections in rural Cameroonian adults. Am J Trop Med Hyg. 2006;74:1078–83.

10. N'dip LM, Bouyer DH, Travassos Da Rosa APA, Teich RB, Walker DH. Acute spotted fever rickettsiosis among febrile patients, Cameroon. Emerg Infect Dis. 2004;10:432–7.

11. Peyrefitte CN, Pastorino BAM, Bessaud M, Gravier P, Tock F, Couissinier-Paris P, et al. Dengue type 3 virus, Saint Martin, 2003–2004. Emerg Infect Dis. 2005;11:757–61.

12. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou JJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African Chikungunya viruses. J Virol Methods. 2005;124:65–71.

13. Parola P, de Lamballerie X, Jourdan J, Rovery C, Vaillant V, Minder P, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean islands. Emerg Infect Dis. 2006;12:1493–9.
14. Simard F, Nchoutpouen E, Toto JC, Fontenille D. Geographic distribution and breeding site preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) in Cameroon, Central Africa. J Med Entomol. 2005;42:726–31.

15. Digoutte JP. In: Pasteur Institute and IRD. CRORA (Centre collaborateur OMS de reference et de rechereché pour les arbovirus et les virus de fievs hemorrhagiques) database. [cited 2007 Mar 21]. Available from http://www.pasteur.fr/recherche/banques/CRORA

Address for correspondence: Marc Grandadam, Unité de Virologie Tropicale, IMTSSA, BP 46, 13 998 Marseille Armées, France; email: publi.viro@laposte.net

Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.