MAJOR ARTICLE

Recent Introduction and Rapid Dissemination of Chikungunya Virus and Dengue Virus Serotype 2 Associated With Human and Mosquito Coinfections in Gabon, Central Africa

Mélanie Caron,[1,2] Christophe Paupy,[2,3] Gilda Grard,[1] Pierre Becquart,[1,2] Illich Mombo,[1,2] Branly Bikie Bi Nso,[1] Fabrice Kassa Kassa,[1] Dieudonné Nkoghe,[1,4] and Eric Maurice Leroy[1,2]

[1]Unité des Maladies Virales Emergentes, Centre International de Recherches Médicales de Franceville (CIRMF), Franceville, Gabon; [2]Unité Mixte de Recherche Maladies Infectieuses et Vecteurs: Ecologie, Génétique, Evolution et Contrôle (IRD 224 - CNRS 5290 - UM1-UM2), Institut de Recherche pour le Développement, Montpellier, France; [3]Unité de Recherche de l'Ecologie et de la Santé, CIRMF, Franceville and [4]Ministère de la Santé Publique, Libreville, Gabon

Background. Chikungunya virus (CHIKV) and Dengue virus serotype 2 (DENV-2) were recently introduced in central Africa, along with *Aedes albopictus*. Simultaneous outbreaks of CHIKV and DENV-2 have subsequently occurred, in Cameroon in 2006 and Gabon in 2007.

Methods. To study the spread of the 2 viruses, we conducted active surveillance of acute febrile syndromes throughout Gabon between 2007 and 2010. Diagnostic methods included quantitative real-time reverse-transcription polymerase chain reaction, and molecular characterization was based on partial envelope gene sequences.

Results. Between 2007 and 2010, 4287 acutely febrile patients were investigated for CHIKV and DENV-2 infections, of whom 1567 were CHIKV-positive, 376 DENV-2–positive, and 37 coinfected. We diagnosed 153 CHIKV and 11 DENV-2 cases in 2008, and 5 CHIKV and 9 DENV-2 cases in 2009. In 2010, CHIKV and DENV-2 caused a second large simultaneous outbreak. Among 2826 acutely febrile patients examined during this outbreak, 1112 were CHIKV-positive, 288 DENV-2–positive, and 28 coinfected. Mosquitoes were collected near the homes of coinfected patients, and 1 *Aedes albopictus* specimen was found to be positive for both CHIKV and DENV-2.

Conclusions. These findings show the rapid dissemination of CHIKV and DENV-2 within a nonimmune population in a tropical African country, probably facilitated by the spread of *Aedes albopictus*. This has resulted in major simultaneous outbreaks with numerous coinfections in both human and mosquito.

Widely distributed in tropical and subtropical areas throughout the world, Chikungunya virus (CHIKV) and Dengue virus (DENV) cause acute illnesses mainly characterized by fever and arthralgia. CHIKV has recently caused large outbreaks, and DENV is a global plague, with tens of million of cases each year [1, 2]. CHIKV belongs to the *Togaviridae* family, genus *Alphavirus*, whereas DENV belongs to the *Flaviviridae* family, genus *Flavivirus*, and is composed of 4 closely related serotypes (DENV-1, -2, -3, and -4).

CHIKV and DENV are mosquito-borne and occur through 2 transmission cycles: a zoonotic/sylvatic cycle involving monkeys and simiophilic *Aedes* mosquito species, and an endemic/epidemic cycle involving humans and anthropophilic *Aedes* mosquito species [3]. *Aedes aegypti* is considered to be the main epidemic vector for CHIKV and DENV worldwide, whereas *Aedes albopictus*, a species native to Asia, recently invaded Europe and Africa [4]. *Aedes albopictus* has gradually replaced local species such as *Aedes aegypti* and is a very efficient primary vector, especially in the Indian Ocean islands and central Africa [5, 6].

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Correspondence: Mélanie Caron, Centre International de Recherches Médicales de Franceville, BP 769, Franceville, Gabon (melaniecaron.cirmf@gmail.com).

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Over the last 30 years in Africa, limited outbreaks and sporadic clinical cases of DENV have been reported, affecting countries in the east (Kenya, Mozambique, Sudan, Djibouti, and Somalia) [7–11] and in the west (Nigeria, Senegal, Burkina Faso, Ivory Coast, Cape Verde) [12–17]. First described in Tanzania in 1952 [18], CHIKV resulted in many outbreaks for nearly half a century in Africa. Kenya was hit by a CHIKV outbreak in 2004 that led to the massive Indian Ocean islands outbreak in 2005, with >260 000 clinical cases [1]. CHIKV has caused several major urban outbreaks in rainforest regions of central Africa, including the Democratic Republic of Congo (DRC) in 2000, Cameroon in 2006, and Gabon in 2007 [19–21]. Although CHIKV-affected areas often overlap with DENV-endemic areas [22], simultaneous outbreaks are rare. However, northwest Gabon and Libreville, the capital city, were hit by a simultaneous CHIKV and DENV-2 outbreak in 2007, during which 9 patients were coinfected by the 2 viruses [20]. Although long suspected in India [23], only a few sporadic cases of virologically confirmed coinfection have been reported to date, for example, in Malaysia in 2006, Sri Lanka in 2008, India in 2009, and Singapore in 2009 [24–28].

The rapid geographic expansion of *Aedes albopictus* could potentially lead to an increase in CHIKV and DENV coinfections in humans, with a risk of more severe clinical illness. Through active surveillance of acute febrile syndromes during a 3-year period in Gabon, we found that CHIKV and DENV-2 both continuously caused clinical cases and spread together from northwest to southeast Gabon between 2007 and 2010. The 2 viruses caused a large simultaneous outbreak centered on Franceville in southeast Gabon in 2010, leading to 24 well-documented cases of coinfection. We also report the first documented evidence of CHIKV and DENV-2 coinfection in a wild-caught mosquito.

METHODS

Ethical Considerations

Our team at Centre International de Recherches Médicales de Franceville (CIRMF) partnered with the Gabonese Ministry of Health (MoH) in a surveillance study that formed part of the public health response. The present study was approved by the regional health directors, and given the urgency of diagnosis, only individual oral consent was obtained for blood sampling.

Case Definition of Acute Febrile Syndrome

In the case definition adopted by the Gabonese MoH, an acute febrile syndrome was characterized by acute fever ($\geq$38.5°C) and $\geq$1 of the following symptoms: arthralgia, myalgia, headache, rash, asthenia, nausea, vomiting, diarrhea, jaundice, or bleeding. We excluded patients whose symptoms met these criteria but who had laboratory-confirmed malaria. With the patients' oral consent, the following data were collected: age, sex, residence, time of onset and intensity of symptoms, and location of arthralgia.

Study Area, Patients, and Clinical Samples

Active surveillance of acute febrile syndromes was conducted between September 2007 and August 2010 in the 3 main healthcare centers of Libreville and in the regional hospitals of all major Gabonese towns (Figure 1). One physician in each center acted as the CIRMF correspondent and was in charge of clinical examination, blood sampling, and appropriate symptomatic treatment of all suspected clinical cases seen in the relevant healthcare center during the study period.

From April to July 2010, a large outbreak of acute febrile illness occurred in 2 provinces (Ogooué-Lolo and Haut-Ogooué), starting during the long rainy season and ending at the beginning of the long dry season. The outbreak mainly affected Franceville, capital of Haut-Ogooué province, during a 10-week period between 3 May and 5 July 2010. During this outbreak, 2 physicians investigated acute febrile cases in CIRMF and Franceville healthcare facilities.

Patients who met the case definition were sampled for diagnosis. Blood was collected in 7-mL Vacutainer tubes containing EDTA (VWR International, France). Samples collected during the surveillance period were stored at 4°C until being sent to CIRMF for analysis, and those collected during the 2010 outbreak in Franceville were transported each day to CIRMF. After centrifugation for 10 minutes at 2000*g*, plasma was stored in aliquots at −20°C for molecular and virological investigations.

Molecular and Virological Investigations

Samples were tested for various arboviral RNA genomes as previously described [20], and CHIKV and DENV-2 were detected by using quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) [29, 30]. A partial envelope (E1) gene sequence of CHIKV (692 bp, position 10 138–10 829 nt) and a partial envelope (E) gene sequence of DENV-2 (758 bp, position 1503–2260 nt) were amplified with the SuperScript III One-Step RT-PCR System and Platinum Taq High Fidelity (Invitrogen, Carlsbad, California). Specific One-Step RT-PCR was run in a final volume of 50 µL, with 400 nM of each primer (respectively for CHIKV and DENV-2, CV1R: CGCTTCCGGTATGTCGATG and CV1F: CTATCGCTTGATTACATCACG; Q2R: ARGCVGCYCCRTAGATTG and Q2F: ATGRARTGCTCYCCDAGAAC) and 10 µL of RNA extract. One-Step RT-PCR reactions were incubated in a GeneAmp 9700 Thermal Cycler (PerkinElmer) programmed for 30 minutes at 45°C and 2 minutes at 94°C and then 40 cycles of denaturation (30 seconds at 94°C), primer annealing (30 seconds at 60°C and 52°C for CHIKV and DENV-2, respectively), and primer extension (45 seconds at 68°C), followed by a final extension step for 5 minutes at 68°C.

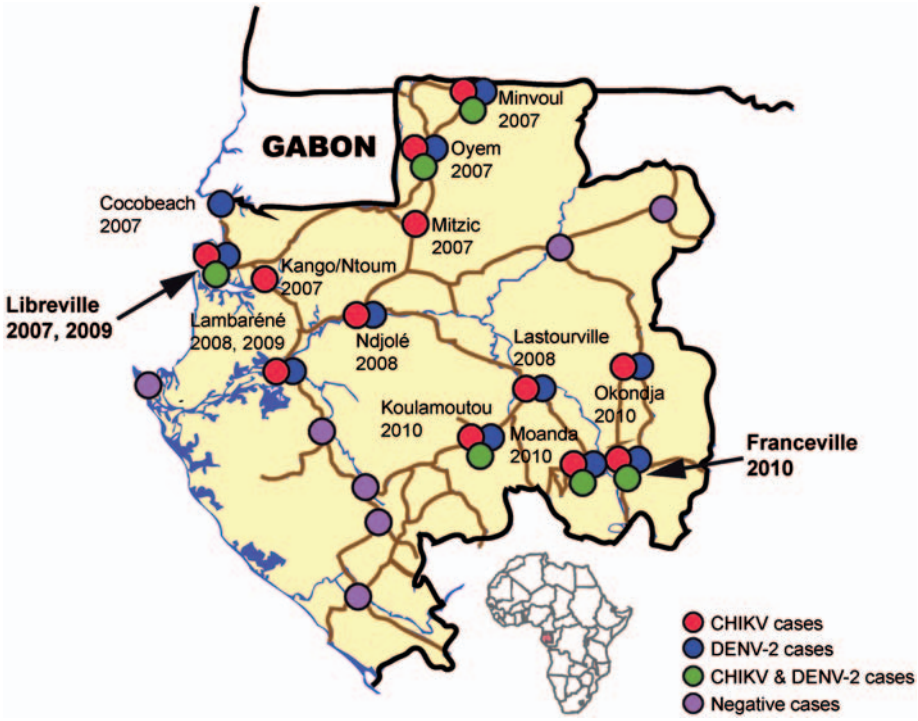

Figure 1. Location of Chikungunya virus and Dengue virus serotype 2 cases between 2007 and 2010 in Gabon, central Africa. Franceville lies in a semiurban area within the transition zone between forest and savanna, near Republic of Congo. Franceville has about 40 000 inhabitants and a low population density. Abbreviations: CHIKV, Chikungunya virus; DENV-2, Dengue virus serotype 2.

Entomological Study

When a human case of coinfection was diagnosed, we captured mosquitoes around the patient's home, outdoors during daytime, using volunteers vaccinated against yellow fever and taking malaria prophylaxis. Signed consent was obtained from each volunteer prior to their inclusion in the study, and institutional clearance was granted by the CIRMF scientific committee and Gabonese MoH. Mosquito collections were carried out by 6 volunteers for 3 days, with 1 session per day between 4:30 PM and 7:00 PM. After species identification, the abdomen of female mosquitoes was removed for monospecific pooling (up to 15 specimens). The carcass remnants (head, thorax, and legs) were transferred individually to microcentrifuge vials and stored at −80°C. The abdomen pools were screened for CHIKV and DENV-2 by qRT-PCR as described above. If an abdomen pool was positive for both CHIKV and DENV-2, the corresponding individual carcass remnants were also screened to detect coinfected mosquitoes.

RESULTS

Epidemiological Findings

Between 2007 and 2010, 4287 acutely febrile patients were tested for CHIKV and DENV-2 infections, of whom 1567 (36.6%) were CHIKV-positive, 376 (8.8%) DENV-2–positive, and 37 (0.9%) both CHIKV-positive and DENV-2–positive (Table 1). There were 1640 men and 2302 women (sex ratio, 0.71), with a mean age of 28.9 years (range, 6 months–89 years). The 1567 CHIKV-positive patients were 627 men and 821 women (sex ratio, 0.76), with a mean age of 27.5 years (range, 6 months–89 years); the 376 DENV-2–positive patients were 159 men and 215 women (sex ratio, 0.74), with a mean age of 32.4 years (range, 6 months–89 years); and the 37 coinfected patients were 14 men and 18 women (sex ratio, 0.78), with a mean age of 36.2 years (range, 6 months–89 years).

Continuous CHIKV and DENV-2 Cocirculation Between 2007 and 2010

In 2007, CHIKV and DENV-2 were responsible for a large simultaneous outbreak centered on Libreville (Figure 1). In total, 1057 patients presented with acute febrile illness, of whom 297 (28.1%) were CHIKV-positive, 68 (6.4%) DENV-2–positive, and 9 (0.9%) both CHIKV-positive and DENV-2–positive (Table 1). In 2008 and 2009, CHIKV and DENV-2 progressed toward southeast Gabon, laboratory-confirmed cases being diagnosed in 2 small towns located in the center of the country (Lambaréné and Ndjolé) and also in Lastourville, a town located in the south, along the main road and the only railroad

Table 1. Distribution of Suspected and Confirmed Cases of Chikungunya Virus and Dengue Virus Serotype 2 Between 2007 and 2010 in Gabon

	Suspected Cases	CHIKV+, No. (%)	DENV-2+, No. (%)	Coinfected, No. (%)
2007 outbreak				
Libreville	968	267	59	7
Cocobeach	19	0	6	0
Kango/Ntoum	10	4	0	0
Mitzic	6	4	0	0
Oyem	48	18	2	1
Minvoul	6	4	1	1
Total 2007	1057	297 (28.1)	68 (6.4)	9 (0.9)
2008 sporadic cases				
Lambaréné	108	80	0	0
Ndjolé	39	10	10	0
Lastourville	104	63	1	0
Total 2008	251	153 (61.0)	11 (4.4)	0 (NC)
2009 sporadic cases				
Libreville	105	2	0	0
Lambaréné	48	3	9	0
Total 2009	153	5 (3.3)	9 (5.9)	0 (NC)
2010 outbreak				
Franceville	2064	882	247	24
Moanda	308	147	8	1
Okondja	29	11	2	0
Koulamoutou	218	64	21	2
Others	207	8	10	1
Total 2010	2826	1112 (39.3)	288 (10.2)	28 (1.0)
Total	**4287**	**1567 (36.6)**	**376 (8.3)**	**37 (0.9)**

Abbreviations: CHIKV, Chikungunya virus; DENV, Dengue virus serotype 2; NC, not calculated.

linking Libreville and Franceville (Figure 1). During these 2 years, respectively, 251 and 153 patients presented with acute febrile illness, of whom 153 (61.0%) were CHIKV-positive and 11 (4.4%) DENV-2–positive in 2008, and 5 (3.3%) were CHIKV-positive and 9 (5.9%) DENV-2–positive in 2009 (Table 1). All the cases occurred during the rainy seasons (April–July and November–December; data not shown).

Finally, CHIKV and DENV-2 caused a large simultaneous outbreak in 2010, centered on Franceville, with cases detected in most towns located in the 2 southeast provinces (Figure 1). A total of 2826 patients with acute febrile illness were screened, of whom 1112 (39.3%) were CHIKV-positive and 288 (10.2%) DENV-2–positive (Table 1). The 2010 outbreak lasted 10 weeks, between 3 May and 11 July (Figure 2). In total, 2064 patients who presented with acute febrile illness were screened during this period in Franceville, of whom 882 were CHIKV-positive and 247 DENV-2–positive. CHIKV and DENV-2 were detected at the same time and uniformly in all parts of Franceville, including the downtown and suburbs (Figure 2*A*). CHIKV-positive cases were 4 times more numerous than DENV-2–positive cases during the first part of the outbreak (Figure 2*B*). This ratio remained stable during the first 3 weeks and increased during the next 2 weeks, peaking at 5:1 in the middle of the outbreak and then falling gradually until the end of the outbreak. This indicated a relative decrease in CHIKV-positive cases compared to DENV-2–positive cases during the second part of the outbreak. Numbers of CHIKV-positive and DENV-2–positive cases were similar at the end of the outbreak.

Frequent CHIKV and DENV-2 Coinfections During the 2010 Outbreak in Franceville

No cases of CHIKV and DENV-2 coinfection were detected by the surveillance program in 2008 and 2009 (Table 1). By contrast, 28 clinical cases of CHIKV and DENV-2 coinfection were detected during the 2010 outbreak, of which 24 occurred in Franceville (Table 1). The number of coinfections followed roughly the same pattern as the number of CHIKV cases, with a peak at week 5 and a decrease toward the end of the outbreak (Figure 3).

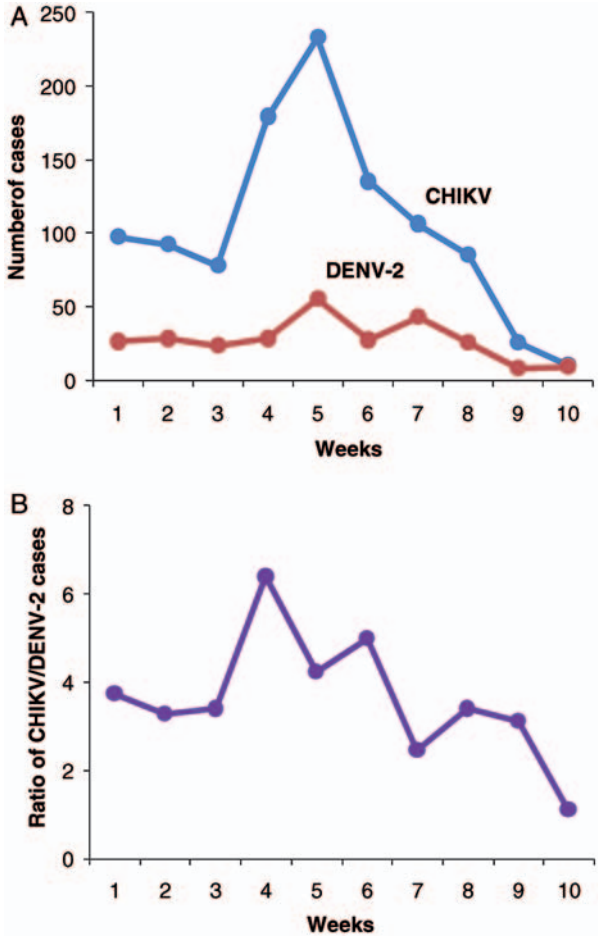

Figure 2. Number (*A*) and ratio (*B*) of Chikungunya virus and Dengue virus serotype 2 cases per week during the 2010 outbreak in Franceville, Gabon. Abbreviations: CHIKV, Chikungunya virus; DENV-2, Dengue virus serotype 2.

Furthermore, 19 of these 24 CHIKV- and DENV-2–coinfected patients in Franceville had a thorough clinical examination (Table 2). None required hospitalization, and none had hemorrhagic or neurological complications. All had fever and arthralgia. Four patients had 2 symptoms, 6 patients had 3 or 4 symptoms, and 3 patients had 5 symptoms. No specific location of arthralgia was noted, and no relation was found between coinfection and sex (sex ratio, 1.1) or age (range, 1–74 years). Coinfection was not associated with particular clinical manifestations.

Complementary DNA (cDNA) viral loads were calculated for 121 CHIKV-positive, 52 DENV-2–positive patients randomly selected during the 2010 outbreak in Franceville, and for the 24 coinfected patients (Figure 4). Mean values were $1.0 \times 10^7 \pm 3.7 \times 10^7$ cDNA copies/mL in CHIKV-monoinfected patients, and $1.6 \times 10^8 \pm 2.2 \times 10^8$ cDNA copies/mL in DENV-2–monoinfected patients. All 24 coinfected patients had high DENV-2 cDNA load (mean $1.0 \times 10^7 \pm 2.1 \times 10^7$ cDNA copies/mL).

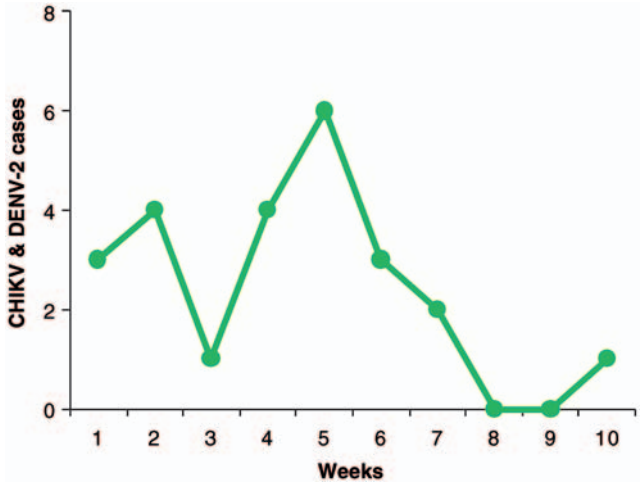

Figure 3. Number of Chikungunya virus– and Dengue virus serotype 2–coinfected patients per week during the 2010 outbreak in Franceville, Gabon. Abbreviations: CHIKV, Chikungunya virus; DENV-2, Dengue virus serotype 2.

Based on CHIKV cDNA load, coinfected patients could be subdivided into 2 groups: a group of 5 patients with both high CHIKV cDNA load (mean $2.2 \times 10^5 \pm 9.6 \times 10^5$ copies/mL) and

Table 2. Clinical and Biological Findings From Patients With Chikungunya Virus and Dengue Virus Serotype 2 Coinfection During the 2010 Outbreak in Franceville, Gabon

Patient	Age (y)	Sex	Symptoms	CHIKV Load (cDNA/mL)	DENV-2 Load (cDNA/mL)
P1	68	F	AM	≤10	1.2×10^7
P2	1	M	FR	≤10	2.2×10^5
P3	29	F	FR	≤10	2.1×10^7
P4	56	M	FH	7.7×10^4	1.4×10^6
P5	22	F	FHR	≤10	1.8×10^5
P6	38	F	FMR	4.8×10^6	2.6×10^5
P7	42	F	FAR	1.0×10^5	2.9×10^4
P8	29	M	FAH	1.7×10^5	1.0×10^4
P9	19	M	FAH	≤10	2.6×10^5
P10	47	M	FAH	≤10	7.1×10^6
P11	74	F	FMHR	≤10	5.5×10^7
P12	23	M	FAHR	≤10	2.8×10^5
P13	40	M	FAMH	≤10	7.0×10^6
P14	41	F	FAMH	1.6×10^1	2.6×10^5
P15	42	F	FAMH	≤10	9.4×10^7
P16	21	F	FAMH	1.4×10^4	9.6×10^4
P17	22	M	FAMHR	≤10	1.8×10^7
P18	22	M	FAMHR	≤10	4.9×10^6
P19	58	M	FAMHR	2.0×10^1	4.4×10^5

Abbreviations: cDNA, complementary DNA; CHIKV, Chikungunya virus; DENV-2, Dengue virus serotype 2; Sex: F, female; M, male; Symptoms: F, fever; A, arthralgia; M, myalgia; H, headache; R, rash.

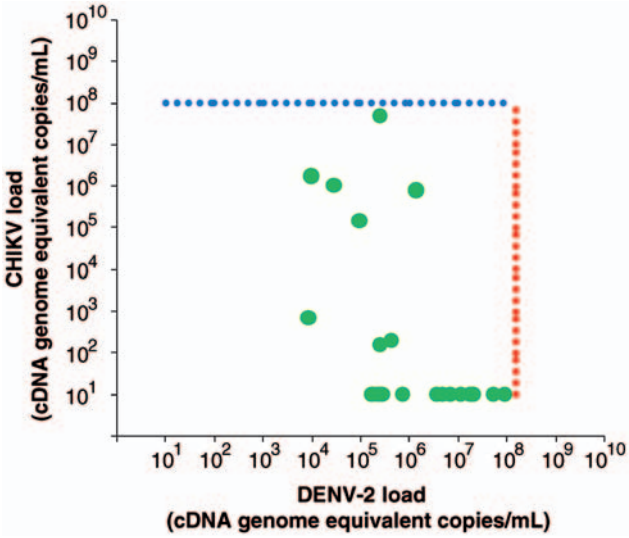

Figure 4. Complementary DNA (cDNA) viral loads from Chikungunya virus (CHIKV)– and Dengue virus serotype 2 (DENV-2)–coinfected patients during the 2010 outbreak in Franceville, Gabon. Mean CHIKV cDNA load in monoinfected patients is shown in red, and mean DENV-2 cDNA load in monoinfected patients is shown in blue. Quantified CHIKV and DENV-2 RNA was used in 10-fold dilutions as a standard for cDNA viral load determination. Exponential regression was then used to determine cDNA viral loads of CHIKV and DENV-2 from the threshold cycle. The minimum of standard linearity was ≤10 cDNA genome-equivalent copies/mL. Abbreviations: cDNA, complementary DNA; CHIKV, Chikungunya virus; DENV-2, Dengue virus serotype 2.

high DENV-2 cDNA load (value indicated above), and a group of 19 patients with low CHIKV cDNA load (positive signal ≤10 cDNA copies/mL) and high DENV-2 cDNA load (value indicated above). Mean values in CHIKV- and DENV-2–monoinfected patients were significantly higher ($P \leq .004$, Student *t* test for continuous variables) than the respective mean values in coinfected patients (data not shown). No association between symptoms and cDNA viral loads was found.

Phylogenetic Characterization

Phylogenetic trees based on partial sequences of the envelope genes showed that CHIKV and DENV-2 isolates recovered from monoinfected and coinfected Gabonese patients belonged to African clusters and grouped together with strains isolated from patients in other parts of Africa (Figure 5). As shown in Figure 5, the most recent common ancestors of the 2010 Gabonese CHIKV (Figure 5*A*) and DENV-2 (Figure 5*B*) strains were the 2007 Gabonese strains, indicating that the 2007 Gabonese strains gave rise directly to the 2010 Gabonese strains.

Entomological Study

In total, 661 mosquitoes were collected around the homes of coinfected patients, comprising 571 *Aedes albopictus*, 38 *Aedes aegypti*, and 52 *Aedes simpsoni* (Table 3). The abdomens were grouped into 52 pools (46 *Aedes albopictus*, 3 *Aedes aegypti*, and 3 *Aedes simpsoni*). All *Aedes simpsoni* abdomen pools tested negative for both viruses, whereas 1 *Aedes aegypti* abdomen pool was CHIKV-positive and 11 and 18 *Aedes albopictus* abdomen pools tested positive for CHIKV and DENV-2, respectively. Three *Aedes albopictus* abdomen pools were positive for both CHIKV and DENV-2. Of these 3 *Aedes albopictus* abdomen pools, 10 of the 32 corresponding individual carcass remnants were positive for CHIKV, 1 was positive for DENV-2, and another was positive both for both CHIKV and DENV-2.

Table 3. Detection of Chikungunya Virus and Dengue Virus Serotype 2 Infection in Wild-Caught Mosquitoes During the 2010 Outbreak in Franceville, Gabon

	Aedes albopictus	*Aedes aegypti*	*Aedes simpsoni*
Mosquito	571	38	52
Pool (abdomen)	46	3	3
CHIKV+	11	1	0
DENV-2+	18	0	0
CHIKV+ & DENV-2+	3[a]	0	0
Individual (carcass)	32	ND	ND
CHIKV+	10	ND	ND
DENV-2+	1	ND	ND
CHIKV+ & DENV-2+	1	ND	ND

Abbreviations: CHIKV, Chikungunya virus; DENV-2, Dengue virus serotype 2; ND, not done.

[a] Abdomen pools positive for both viruses for which carcass remnants were individually tested.

DISCUSSION AND CONCLUSIONS

CHIKV and DENV-2 have rarely been detected in the humid forested countries of central Africa, and only limited CHIKV outbreaks have occurred, in DRC in 2000 and Cameroon in 2006 [19, 31]. A large CHIKV and DENV-2 outbreak suddenly occurred in northeast Gabon between April and July 2007 [20]. To monitor the dissemination of the 2 viruses in Gabon, we conducted active surveillance of acute febrile syndromes throughout Gabon from September 2007 to August 2010.

During this study period, we studied 4287 acutely febrile patients who tested negative for malaria and found that 1567 (36.6%) were CHIKV-positive and 376 (8.8%) were DENV-2–positive. We found that viruses cocirculated continuously since 2007 and caused clinical cases each year in a southward movement from northwest to southeast Gabon. The wave of CHIKV and DENV-2 clinical infection could be divided into 3 periods with distinct epidemiologic characteristics. In the

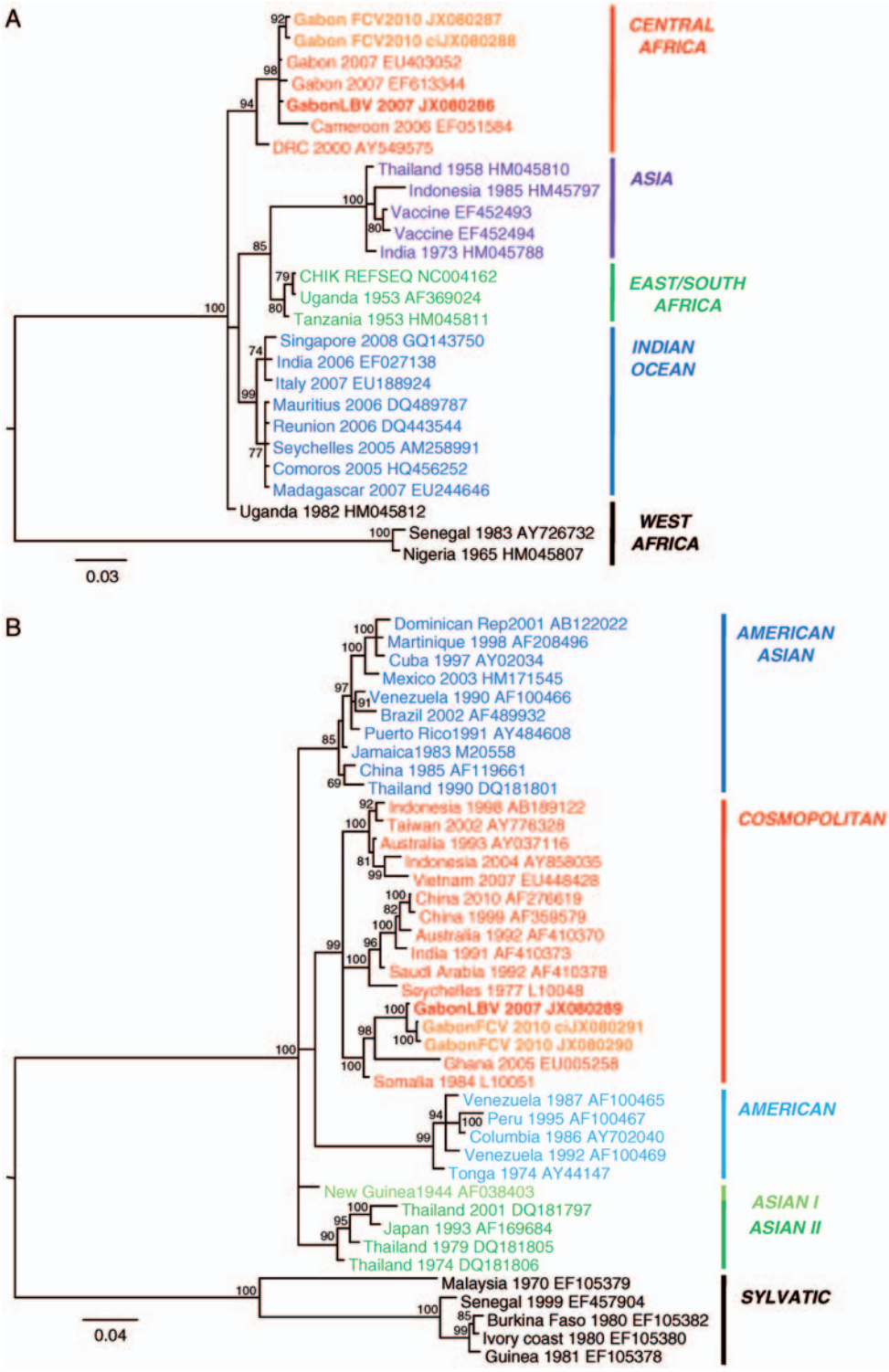

Figure 5. Phylogenic trees of Chikungunya virus (CHIKV) (*A*) and Dengue virus serotype 2 (DENV-2) (*B*) partial E gene sequences. Phylogenic analyses were based on 566-bp CHIKV and 758-bp DENV-2 sequences. Sequences were aligned using the MEGA editor program package [35], with 24 CHIKV and 39 DENV-2 published overlapping sequences available in GenBank. All sequences isolated from clinical cases reported in Africa and representative sequences of each CHIKV and DENV-2 genotype were included. The Gabonese sequences are in bold and are available under GenBank accession numbers JX080286–JX080288 and JX080289–JX080291 for the CHIKV and DENV-2 strains, respectively. The Gabonese sequences characterized from a coinfected patient ends with "ci". Phylogenetic analyses were done with MrBayes version 3.2 software using the default chain for 2 million generations with the GTR + G + I nucleotide substitution model [36]. Trees were sampled every 100 generations, resulting in 200 000 saved trees, the first 5000 saved trees being discarded as "burn-in" samples. Trees were visualized with FigTree 1.3.1 from the BEAST package (http://evolve.zoo.ox.ac.uk/beast/).

first phase, a large CHIKV and DENV-2 outbreak occurred in northwest Gabon and was centered on Libreville during the 2007 long rainy season. During the second phase, numerous sporadic clinical cases of CHIKV and DENV-2 infection occurred in several small towns located in the center of Gabon during the 2008 and 2009 rainy seasons. In the final phase, another large outbreak occurred in southeast Gabon in 2010 and was centered on Franceville. Phylogenetic analyses of partial envelope gene sequences showed an ancestor–descendent relationship connecting the 2007 Gabon strains to the 2010 Gabon strains, and also suggested that the Gabonese CHIKV strains evolved directly from the 2006 Cameroon strain. Our findings clearly suggest that CHIKV spread rapidly across central Africa, from Cameroon to south Gabon, between September 2007 and August 2010, and to Republic of Congo in 2011 [32].

During the 2010 outbreak in Franceville, the number of CHIKV cases far exceeded the number of DENV-2 cases during the first part of the outbreak, and then the ratio fell gradually during the second part of the outbreak. We obtained evidence that *Aedes albopictus* was the only vector of both CHIKV and DENV-2 during the 2010 Franceville outbreak. The higher *Aedes albopictus* infectivity for CHIKV than for DENV-2, associated with the nonimmune status of the population, probably led to a high rate of CHIKV transmission during the first part of the outbreak [33, 34]. DENV-2 would then have spread through the Franceville population as CHIKV transmission declined.

In total, 37 CHIKV- and DENV-2–coinfected patients were identified between April 2007 and August 2010, exclusively during the 2 outbreaks in 2007 and 2010. Our findings indicate that CHIKV and DENV-2 coinfection occurred in densely populated nonimmune areas. Of these, 19 coinfected patients were clinically well-documented. No evidence of particular clinical manifestations was found between the monoinfected and coinfected patients [35, 36]. Rarely observed in the past, the simultaneous appearance and rise of CHIKV and DENV-2 coinfection coincided with the introduction and rapid spread of *Aedes albopictus* in Gabon after 2007.

CHIKV and DENV-2 cDNA loads in coinfected patients were always significantly lower than those in CHIKV- and DENV-2–monoinfected patients, suggesting mutually negative modulation of the 2 viruses. The coinfected patients could be subdivided into 2 groups. One group had high DENV-2 cDNA load but low CHIKV cDNA load, suggesting a blood sampling during the acute phase of DENV-2 infection but at an early or late stage of CHIKV infection. Alternatively, low CHIKV cDNA load could result from inhibition of CHIKV replication by the immune response to DENV-2. In both cases, dual infection likely resulted from 2 mosquito bites separated by a few days. The other group had high cDNA loads of both CHIKV and DENV-2, suggesting a blood sampling during the acute phase of both CHIKV and DENV-2 infection. In this case, dual infection resulted from 2 mosquito bites each infected by 1 virus but close in time, or a single mosquito bite coinfected with the 2 viruses.

Interestingly, we detected 1 CHIKV- and DENV-2–coinfected *Aedes albopictus* specimen, representing the first observation of dual mosquito infection in natura. This demonstrates the possibility that humans could be coinfected with the 2 viruses by the bite of a single mosquito. This is consistent with the experimental evidence that *Aedes albopictus* can be orally coinfected with CHIKV and DENV-2 and harbor infectious particles of both viruses concomitantly in their saliva [6].

Our findings show that the 2 arboviruses spread rapidly through a tropical country of central Africa, probably facilitated by the extraordinary capacity of *Aedes albopictus* to colonize diverse environments and to replace local species. This rapid spread within a nonimmune population, via an invasive mosquito species, led to continuous and growing CHIKV and DENV-2 cocirculation, with cases of coinfection in both human and mosquito. An increase in such coinfections could result in rapid viral genetic evolution, potentially leading to changes in the infectivity or pathogenicity of both viruses.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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References

1. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis **2007**; 7:319–27.
2. World Health Organization. Fact sheet no. 117: dengue and dengue haemorrhagic fever. Available at: http://www.who.int/mediacentre/factsheets/fs117/en/. Accessed 2009.
3. Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral Res **2010**; 85:328–45.
4. Gratz NG. Critical review of the vector status of *Aedes albopictus*. Med Vet Entomol **2004**; 18:215–27.
5. Paupy C, Ollomo B, Kamgang B, et al. Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of dengue and Chikungunya in central Africa. Vector Borne Zoonotic Dis **2010**; 10:259–66.
6. Vazeille M, Mousson L, Martin E, Failloux AB. Orally co-infected *Aedes albopictus* from La Reunion Island, Indian Ocean, can deliver both dengue and chikungunya infectious viral particles in their saliva. PLoS Negl Trop Dis **2010**; 4:e706.

7. Gubler DJ, Sather GE, Kuno G, Cabral JR. Dengue 3 virus transmission in Africa. Am J Trop Med Hyg **1986**; 35:1280–4.
8. Hyams KC, Oldfield EC, Scott RM, et al. Evaluation of febrile patients in Port Sudan, Sudan: isolation of dengue virus. Am J Trop Med Hyg **1986**; 35:860–5.
9. Johnson BK, Musoke S, Ocheng D, Gichogo A, Rees PH. Dengue-2 virus in Kenya. Lancet **1982**; 2:208–9.
10. Kanesa-thasan N, Chang GJ, Smoak BL, Magill A, Burrous MJ, Hoke CH Jr. Molecular and epidemiologic analysis of dengue virus isolates from Somalia. Emerg Infect Dis **1998**; 4:299–303.
11. Rodier GR, Gubler DJ, Cope SE, et al. Epidemic dengue 2 in the city of Djibouti 1991–1992. Trans R Soc Trop Med Hyg **1996**; 90:237–40.
12. Traore-Lamizana M, Zeller H, Monlun E, et al. Dengue 2 outbreak in southeastern Senegal during 1990: virus isolations from mosquitoes (Diptera: *Culicidae*). J Med Entomol **1994**; 31:623–7.
13. Saluzzo JF, Cornet M, Castagnet P, Rey C, Digoutte JP. Isolation of dengue 2 and dengue 4 viruses from patients in Senegal. Trans R Soc Trop Med Hyg **1986**; 80:5.
14. Robert V, Lhuillier M, Meunier D, et al. [Yellow fever virus, dengue 2 and other arboviruses isolated from mosquitos, in Burkina Faso, from 1983 to 1986. Entomological and epidemiological considerations]. Bull Soc Pathol Exot **1993**; 86:90–100.
15. Gonzalez JP, Du Saussay C, Gautun JC, McCormick JB, Mouchet J. [Dengue in Burkina Faso (ex-Upper Volta): seasonal epidemics in the urban area of Ouagadougou]. Bull Soc Pathol Exot Filiales **1985**; 78:7–14.
16. Diallo M, Ba Y, Sall AA, et al. Amplification of the sylvatic cycle of dengue virus type 2, Senegal, 1999–2000: entomologic findings and epidemiologic considerations. Emerg Infect Dis **2003**; 9:362–7.
17. Carey DE, Causey OR, Reddy S, Cooke AR. Dengue viruses from febrile patients in Nigeria, 1964–68. Lancet **1971**; 1:105–6.
18. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond) **1956**; 54:177–91.
19. Peyrefitte CN, Rousset D, Pastorino BA, et al. Chikungunya virus, Cameroon, 2006. Emerg Infect Dis **2007**; 13:768–71.
20. Leroy EM, Nkoghe D, Ollomo B, et al. Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007. Emerg Infect Dis **2009**; 15:591–3.
21. Demanou M, Antonio-Nkondjio C, Ngapana E, et al. Chikungunya outbreak in a rural area of western Cameroon in 2006: a retrospective serological and entomological survey. BMC Res Notes **2010**; 3:128.
22. Mackenzie JS, Chua KB, Daniels PW, et al. Emerging viral diseases of Southeast Asia and the Western Pacific. Emerg Infect Dis **2001**; 7:497–504.
23. Myers RM, Carey DE. Concurrent isolation from patient of two arboviruses, Chikungunya and dengue type 2. Science **1967**; 157:1307–8.
24. Schilling S, Emmerich P, Gunther S, Schmidt-Chanasit J. Dengue and Chikungunya virus co-infection in a German traveller. J Clin Virol **2009**; 45:163–4.
25. Nayar SK, Noridah O, Paranthaman V, et al. Co-infection of dengue virus and Chikungunya virus in two patients with acute febrile illness. Med J Malaysia **2007**; 62:335–6.
26. Hapuarachchi HC, Bandara KB, Sumanadasa SD, et al. Re-emergence of Chikungunya virus in South-east Asia: virological evidence from Sri Lanka and Singapore. J Gen Virol **2010**; 91:1067–76.
27. Chang SF, Su CL, Shu PY, et al. Concurrent isolation of Chikungunya virus and dengue virus from a patient with coinfection resulting from a trip to Singapore. J Clin Microbiol **2010**; 48:4586–9.
28. Chahar HS, Bharaj P, Dar L, Guleria R, Kabra SK, Broor S. Co-infections with Chikungunya virus and dengue virus in Delhi, India. Emerg Infect Dis **2009**; 15:1077–80.
29. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, 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.
30. Leparc-Goffart I, Baragatti M, Temmam S, et al. Development and validation of real-time one-step reverse transcription-PCR for the detection and typing of dengue viruses. J Clin Virol **2009**; 45:61–6.
31. Pastorino B, Muyembe-Tamfum JJ, Bessaud M, 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.
32. Kelvin AA. Outbreak of Chikungunya in the Republic of Congo and the global picture. J Infect Dev Ctries **2011**; 5:441–4.
33. Sanchez-Vargas I, Scott JC, Poole-Smith BK, et al. Dengue virus type 2 infections of *Aedes aegypti* are modulated by the mosquito's RNA interference pathway. PLoS Pathog **2009**; 5:e1000299.
34. Dubrulle M, Mousson L, Moutailler S, Vazeille M, Failloux AB. Chikungunya virus and *Aedes* mosquitoes: saliva is infectious as soon as two days after oral infection. PLoS One **2009**; 4:e5895.
35. Nkoghe D, Kassa Kassa F, Bisvigou U, Caron M, Grard G, Leroy EM. No clinical or biological difference between Chikungunya and dengue fever during the 2010 Gabonese outbreak. Infect Dis Rep **2012**; 4:e5.
36. Nkoghe D, Kassa Kassa F, Caron M, et al. Clinical forms of chikungunya in Gabon, 2010. PLoS Negl Trop Dis **2012**; 6:e1517.
37. Kumar S, Nei M, Dudley J, Tamura K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform **2008**; 9:299–306.
38. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol **2007**; 7:214.