A Chikungunya Outbreak Associated with the Vector *Aedes albopictus* in Remote Villages of Gabon

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Abstract

Chikungunya virus (CHIKV) recently caused major urban outbreaks in central African countries such as the Democratic Republic of Congo (DRC), Cameroon, and Gabon. In Gabon, the tiger mosquito *Aedes albopictus* was shown to be the main CHIKV vector during the 2007 outbreak. This invasive Asian species was first identified in Gabon in early 2007, and was thought to be restricted mainly to coastal provinces where urban epidemic CHIKV foci were recorded. Here we report a CHIKV outbreak in a small cluster of villages isolated in the deep forest of southern Gabon, in which *A. albopictus* was the main vector. This observation indicates concomitant geographic and ecological spread of CHIKV activity and *A. albopictus* in remote environments in central Africa, as well as an enhanced risk of propagation of epidemic arboviruses.

Key Words: *Aedes albopictus*—Chikungunya—Deep rain forest—Gabon.

Introduction

IN THE PAST 20 YEARS, CHIKUNGUNYA VIRUS (CHIKV) HAS CAUSED MAJOR AND GEOGRAPHICALLY WIDE-RANGING EPIDEMICS, mainly affecting urban areas (Charrel et al. 2007; Pialoux et al. 2007; Rezza et al. 2007). Recently, CHIKV has emerged in new regions, including the tropical rain forest of central African countries such as the Democratic Republic of Congo (DRC), Cameroon, and Gabon, where it has caused outbreaks centered on major cities (Pastorino et al. 2004; Peyrefitte et al. 2007; Leroy et al. 2009). CHIKV emerged in Gabon between April and July 2007, causing a large outbreak with about 20,000 cases (Leroy et al. 2009). Although this outbreak mainly affected the capital, Libreville, epidemic foci subsequently developed in several Gabonese towns along the route north towards Cameroon. The *Aedes albopictus* mosquito was first identified in Libreville in early 2007 (Coffinet et al. 2007) and was shown to be the primary CHIKV vector during the 2007 outbreak (De Lamballerie et al. 2008; Leroy et al. 2009; Paupy et al. 2010). Here we report an unexpected CHIKV outbreak in a small cluster of isolated villages located in the deep forest of southern Gabon, in which *A. albopictus* was the main vector.

On April 12th, 2010 the local health authorities in Lastourville, the second largest town of Ogooué-Lolo province, southern Gabon, informed the Centre International de Re-

cherches Médicales de Franceville (CIRMF) of numerous cases of a febrile arthralgic syndrome compatible with CHIKV infection in Ndangui, a cluster of small gold-washing villages. The Ndangui villages, located in the Mouloundou department of Ogooué Lolo province (L 13.0969-1 0.50131), are situated in deep rainforest 80 km north of Lastourville (Fig. 1), and are home to 640 people. Access to Lastourville is difficult, owing mainly to poor road conditions during the rainy season. In order to investigate this CHIKV-like outbreak, we conducted on-site medical and entomological surveys from April 21–29, 2010.

The medical survey included a first active phase to identify patients with febrile arthralgic syndromes in order to identify the causative virus. Persons who presented with compatible symptoms (fever, arthralgia, and/or rash) during the investigation were examined by our team's doctors, and were sampled with their oral informed consent, as recorded by Ministry of Health (MoH) and CIRMF teams. The patients then received symptomatic treatment and care at home. One blood sample per suspected case was collected by arm venipuncture in EDTA tubes and kept at 4°C until their return to the CIRMF laboratory. At CIRMF, plasma samples were tested for several arbovirus RNA genomes, including CHIKV, Dengue, Yellow, West Nile, Zika Rift Valley Fever, and O'nyong nyong virus, using specific TaqMan qRT-PCR assays (Leroy et al. 2009). A total of 24 blood samples from febrile and

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FIG. 1. Map of Gabon, showing the location of Ndangui village.

arthralgic patients were collected during a 4-day period. Specific CHIKV TaqMan qRT-PCR (De Lamballerie et al. 2008) was positive in 12 samples, while all assays for other viruses were negative.

In order to assess the extent and duration of this CHIKV outbreak in Ndangui, we conducted epidemiological investigations during the same period, based on household interviews. A probable case of CHIKV was defined as an individual who declared having suffered fever and multiple arthralgias for at least 4 days within the previous month. Interviews were conducted in 90 of 137 households, representing 453 of the 640 Ndangui inhabitants. Seventy percent of the interviewees ($n=325$) declared having experienced a syndrome compatible with CHIKV infection, indicating a massive CHIKV outbreak in Ndangui. Moreover, such a percentage indicated that our intervention probably took

TABLE 1. CHIKV DETECTION IN FEMALE MOSQUITOES COLLECTED DURING THE NDANGUI OUTBREAK, GABON, 2010

Mosquito species	n Sampled	n Pools	CHIKV-positive pools		
			N	%	MLE (95% CI)
<i>A. albopictus</i>	627	45	32	71	10.3 (7.1,14.5)
<i>A. aegypti</i>	12	6	1	17	8.3 (0.5,31.9)
<i>A. simpsoni</i>	1	1			

n Sampled, number of female mosquitoes sampled; *n* Pools, number of mosquito pools per species; *n* and %, number and percentage of CHIKV-positive pools per species; MLE, maximum likelihood estimate of mosquito infection rates, using the MLE-IR program (Gu et al. 2003).

place at or near the end of the epidemic, and also suggested that the major period of transmission occurred over 4–5 weeks. The high attack rate and explosive nature of the outbreak suggested that the population of Ndangui was largely naïve to CHIKV.

In order to identify a potential vector species involved in this outbreak, mosquitoes were collected outdoors during daytime after landing on volunteers vaccinated against yellow fever and taking malaria prophylaxis. Informed consent was obtained from every volunteer prior to their inclusion in the study, and institutional clearance was granted by the Health Ministry of Gabon. Mosquito collections were carried out by 5 volunteers during 2 days at the rate of two sessions per day (8:00–10:15 AM and 4:00–6:15 PM). The mosquitoes were then identified and stored as single-species pools of up to 15 specimens in liquid nitrogen. The mosquito pools were then assayed for CHIKV using the same specific qRT-PCR TaqMan assay (De Lamballerie et al. 2008). A total of 640 female mosquitoes were collected, and were identified as *A. albopictus* ($n=627$), *A. aegypti* ($n=12$), and *A. simpsoni* ($n=1$; Table 1). The only *A. simpsoni* specimen was negative for CHIKV, whereas 32 (71%) of 45 *A. albopictus* pools, and 1 (17%) of 6 pools of *A. aegypti* were positive. The maximum likelihood estimates (MLE) of mosquito infection rates, 10.3 and 8.3, respectively, for *A. albopictus* and *A. aegypti*, were similar for the two CHIKV-infected mosquitoes species. As *A. albopictus* was the main human-biting species, it was probably the primary CHIKV vector during the Ndangui outbreak. Given its small population size, and assuming the ratio between both species remained stable during the transmission period, *A. aegypti* probably played a secondary role.

Overall, these findings suggest that CHIKV massively infected the population living in the isolated villages of Ndangui. They also highlight the spread of *A. albopictus* in Gabon, even in isolated villages located in the deep rainforest. Most Ndangui villagers are gold miners who travel frequently between Ndangui and Libreville, always via Lastourville, a town located on the only rail line and the main road through Gabon. Thus, CHIKV and its vector were probably introduced, directly or indirectly, between 2007 and 2010, following the 2007 Libreville outbreak. The main potential human consequence of the growing geographic range of *A. albopictus* and its ability to colonize new ecosystems in Gabon, is the spread of epidemic pathogens, as exemplified here by Chikungunya virus. As *A. albopictus* also feeds on other animals (Gubler, 2003), there is a clear risk of human emergence of zoonotic viruses circulating in forest wildlife, and monitoring programs in central Africa need to be encouraged.

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Author Disclosure Statement

No competing financial interests exist.

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