VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 0, Number 00, 2009

DOI: 10.1089/vbz.2009.0005

ORIGINAL ARTICLE

Comparative Role of *Aedes albopictus* and *Aedes aegypti* in the Emergence of Dengue and Chikungunya in Central Africa

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Abstract

Since its discovery in Nigeria in 1991, *Aedes albopictus* has invaded much of Central Africa, a region where *Ae. aegypti* also occurs. To assess the relationship between the invasion by *Ae. albopictus* and the recent emergence of dengue virus (DENV) and chikungunya virus (CHIKV), we undertook vector competence experiments on populations collected from Cameroon and conducted field investigations during concurrent epidemics of DENV and CHIKV in Gabon. Overall, infection and dissemination rates were not significantly different between *Ae. albopictus* and *Ae. aegypti* when exposed to titers of $10^{8.1}$ mosquito infectious dose 50/mL and $10^{7.5}$ plaque forming units/mL of DENV type 2 and CHIKV, respectively. Field investigations showed that *Ae. albopictus* readily bit man, was abundant, and outnumbered *Ae. aegypti* to a large extent in Gabon, particularly in suburban environments. Nevertheless, *Ae. aegypti* was predominant in the more urbanized central parts of Libreville. In this city, CHIKV and DENV were detected only in *Ae. albopictus*. These data strongly suggest that *Ae. albopictus* acted as the major vector of both viruses in Libreville in 2007, impacting on the epidemiology of DENV and CHIKV in this area.

Key Words: *Aedes aegypti*—*Aedes albopictus*—Central Africa—Chikungunya—Dengue—Vector competence.

Introduction

Within the last 30 years, the mosquito *Aedes albopictus*, a species native to Southeast Asia, has invaded American, European, and African countries (Gratz 2004). In Africa, *Ae. albopictus* was first reported in Nigeria in 1991 (Savage et al. 1992), where it had presumably been introduced through the used tire trade. It was subsequently recorded in Cameroon and Equatorial Guinea (Fontenille and Toto 2001, Toto et al. 2003), where it colonized the same larval habitats as the indigenous *Aedes aegypti* (Simard et al. 2005). Recent reports of *Ae. albopictus* in Gabon (Coffinet et al. 2007) strongly suggested that this species now occurs in most countries of the Congo Basin in Central Africa.

The invasion of this area is especially worrying because *Ae. albopictus* readily transmits major arthropod-borne viruses, such as dengue virus (DENV) (Reiter et al. 2006) and chikungunya virus (CHIKV) (Delatte et al. 2008), and could therefore affect the epidemiology of these emerging infections. In West and Central Africa, human-to-human DENV transmission in urban environments is very limited, despite viral isolation of DENV types 1, 2, and 4 (Diallo et al. 2008). The situation remains poorly understood, and the absence of major epidemics could be the result of low vector competence in local populations of *Ae. aegypti*, the main vector of DENVs throughout the world (Failloux et al. 2002). However, the number of reported cases of DENV acquired in Africa has been on the rise since 2000, especially in Central Africa (Krippner and von Laer 2002, Peyrefitte et al. 2007, Leroy et al. 2009), indicating a change in DENV epidemiology in this region. CHIKV, which recently emerged in several countries in the Indian Ocean (Pialoux et al. 2007) and Europe (Bonilauri et al. 2008), is endemic in rural areas of Africa. Similar to DENV, an increase in CHIKV fever epidemics has been reported in

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Central Africa during the last decade, suggesting changes in CHIKV epidemiology. Massive urban outbreaks were recorded in the Democratic Republic of Congo in 1999–2000 (Pastorino et al. 2004), Equatorial Guinea and Cameroon in 2006 (Peyrefitte et al. 2008), and in Gabon in 2007 (de Lamballerie et al. 2008, Leroy et al. 2009). The invasion of *Ae. albopictus* was concurrent with this apparent increase in DENV/CHIKV circulation in Central Africa.

Here we report the results of entomological and virus detection studies conducted during the recent DENV/CHIKV epidemic that occurred in Libreville, Gabon, in 2007. In addition, we present results from a comparative study of the vector competence for DENV and CHIKV involving the indigenous *Ae. aegypti* and invasive populations of *Ae. albopictus* from Cameroon. The latter study builds on recent experiments on mosquito populations from Libreville (Vazeille et al. 2008), with the aim of assessing their potential role in recent arbovirus emergence and to further explore the risk of increased and sustained virus transmission in the region.

Materials and Methods

Vector competence experiments

Mosquitoes. The *Ae. aegypti* Paea strain, originating from Tahiti, French Polynesia (Vazeille-Falcoz et al. 1999), was used as a control throughout the experiments. Mosquitoes were sampled from different urban locations in Cameroon in 2007 (Fig. 1). Larvae and/or pupae were collected from domestic containers (e.g., jars and tanks) and discarded containers (e.g., tires). In each locality, immature stages were collected from 2 to 5 larval habitats and stored in cylindrical plastic boxes (depth, 8 cm; height, 12 cm). Larvae and pupae were transferred into pans containing dechlorinated tap water and reared to adults in the insectaries, under ambient conditions and a photoperiod of 12 h. Emerging adults were readily identified as *Ae. aegypti* or *Ae. albopictus*, and mosquitoes from the same species and the same locality were pooled together in separate cages. This was done to help prevent inbreeding and to increase samples sizes to a minimum of 50 females. Females were allowed to feed blood on a restrained rabbit, according to the standard operating procedures in use at "Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale" and conformed with animal ethics guidelines. Eggs were collected on seed germination paper. The F_1 generation was used for experimental infection.

Virus strains. Oral susceptibility to DENV was assayed using D2S32, a strain of serotype 2 isolated in 1974 from a human serum sample collected in Bangkok, Thailand (Vazeille-Falcoz et al. 1999). Experimental infections with

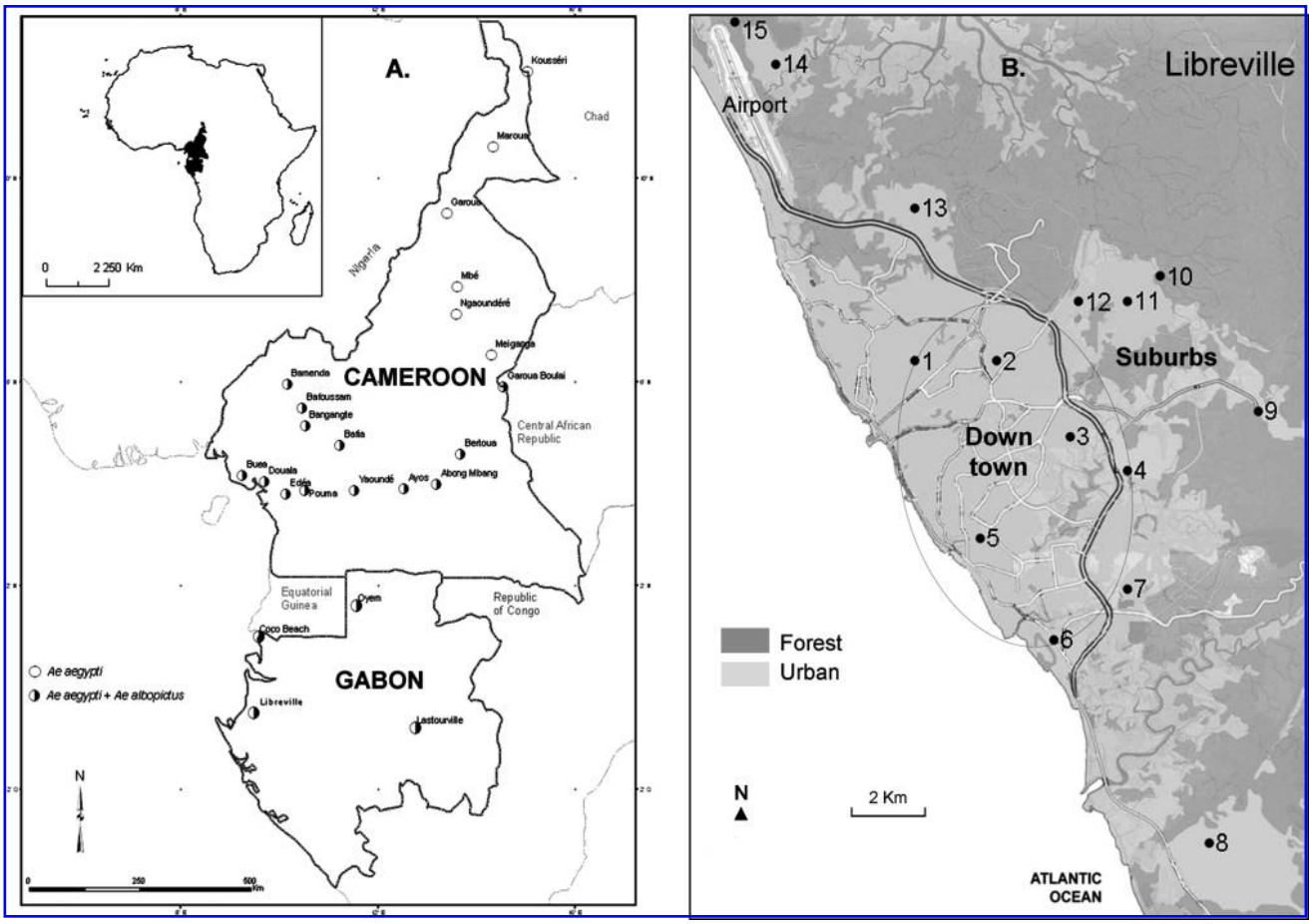

FIG. 1. *Aedes aegypti* and *Aedes albopictus* collection sites in (**A**) Cameroon and Gabon, and (**B**) Libreville, the capital of Gabon.

CHIKV were conducted with the 06.21 strain provided by the French National Reference Center for Arboviruses in Lyon, which was isolated from *Ae. albopictus* (C6/36) cells in serum collected from La Reunion island in 2006 (Schuffenecker et al. 2006, Vazeille et al. 2007). The 06.21 strain was the major genotype isolated from patients during the 2005–2006 outbreak and presented an A226V mutation in the gene E1. Both the D2S32 and CHIKV 06.21 strains have been extensively used in the GMB unit (Pasteur Institute, Paris, France) to generate an extensive data set on the oral susceptibility of *Ae. aegypti* and *Ae. albopictus* populations from around the world. The use of standardized protocols allowed us to compare our results with those from previous studies.

Assessment of oral susceptibility to virus infection. Experimental infection assays were performed by blood-feeding 4-day-old females for 20 min. Females were allowed to feed through a chicken skin membrane covering the base of a glass feeder containing infectious blood maintained at 37°C. The blood–virus mixture was composed of two parts of washed rabbit erythrocytes isolated from arterial blood collected 24 h before blood feeding, and one part of diluted viral suspension (Vazeille-Falcoz et al. 1999). The final titers of the D2S32 and CHIK 06.21 strains were, respectively, $10^{8.1}$ MID_{50} (50% of mosquito infectious dose for *Ae. aegypti*)/mL and $10^{7.5}$ plaque-forming units (pfu)/mL determined using C6/36 cells. For CHIKV, 10^7 pfu/mL corresponds to 10^8 MID_{50}/mL (Vazeille et al. 2008). Adenosine triphosphate (ATP) at a final concentration of 5 mM was added to the blood–virus mixture as a phagostimulant. Fully fed females were transferred to small cardboard containers and maintained with 10% sucrose at 28°C for 14 days (relative humidity $80 \pm 10\%$ and photoperiod 12 h).

Virus assay. The disseminated infection rate (DIR) was measured by checking for DENV and CHIKV antigens by immunofluorescent assay in head squashes from females surviving for 14 days postingestion of the infectious blood meal.

Entomological surveys and virus detection

Sample locations. Mosquito collections were undertaken in Libreville between June and July 2007, in 15 sites where suspected DENV cases were reported (Fig. 1). Based on the geographical position and the degree of urbanization, each sampling site was categorized as belonging to one of two classes: suburbs, where the majority of suspected cases were reported from, and downtown. Additional sampling was performed in epidemic foci located in three other Gabonese cities: Oyem and Cocobeach (North-Western Gabon) in July 2007 and Lastourville (South-Eastern Gabon) in September 2007 (Fig. 1). Environmental conditions in these secondary cities were similar to those of suburban areas in Libreville, and were characterized by low levels of urbanization and persistent vegetation.

Mosquito collection. Mosquitoes were collected outdoors after landing on a volunteer 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. In previous studies in neighboring Cameroon, the peak in biting for both *Ae. albopictus* and *Ae. aegypti* was between 4:00 pm and 6:30 pm (Paupy, unpublished data). As we were also interested in nocturnal mosquitoes (i.e., *Culex, Anopheles*), collection time was extended and performed from 4:30 pm to 8:30 pm by 8–20 volunteers (Table 3). Collected mosquitoes were transported to the laboratory where *Ae. albopictus* and *Ae. aegypti* were divided into pools of 25 individuals and stored at −80°C in the Virology Unit of the University of Medicine in Libreville. The vials were then sent to the Centre International de Recherches Médicales de Franceville (CIRMF, Gabon), where they were stored again at −80°C until testing. Mosquito densities were estimated using the human biting rate (HBR), which corresponds to the number of biting mosquitoes per person and per hour.

Virus detection. Mosquito pools were homogenized using 24-well vial (2 mL) sets provided by OPS Diagnostics (Bridgewater, NJ).The pools were transferred into vials containing 500 μL of nucleic acid lysis solution (Applied Biosystems, Foster City, CA) and ground for 2 min at 1500 stroke/min using a GenoGrinder 2000 (OPS Diagnostics). Extraction of viral RNA was performed using ABI Prism 6100 Nucleic Acid PrepStation kit (Applied Biosystems) according to manufacturers' recommended procedures for 400 μL of homogenate. Reverse transcription was performed on 50 μL of RNA template using high-capacity cDNA synthesis reverse transcription kits (Applied Biosystems) according to the manufacturers' instructions. Q-polymerase chain reaction (Q-PCR) was performed in 50 μL reactions containing 10 μL of cDNA template, 200 nM of probe, and 900 nM of each primer (Drosten et al. 2002, de Lamballerie et al. 2008). CHIKV and DENV primers, respectively, targeted E1 and noncoding regions of DENV genome. For DENV, mosquito pools were first screened using universal primers (3′UTR), allowing detection of the four serotypes. The serotype-specific characterization of DENV was further based on amplification of fragment straddled the 5′UTR and the capside gene. The amplification program in the 7500 real-time PCR system (Applied Biosystems) was 95°C (15 s) and 60°C (1 min) for 45 cycles according to manufacturers' recommended procedures. Maximum likelihood estimation (MLE) of mosquito infection rates were estimated for both viruses at Libreville using the MLE-IR program (Gu et al. 2003).

Results

Oral susceptibility to DENV type 2

Nine experimental infections were conducted with the D2S32 strain of DENV (Table 1). DIRs in the control strain (*Ae. aegypti* Paea strain) ranged from 85.7% to 98% across the nine experimental infection runs and were homogeneous (Fisher exact test; $p > 0.05$). In the 19 *Ae. aegypti* field samples from Cameroon, the DIR ranged from 17.2% in Maroua to 59.7% in Douala and were heterogeneous among sites (Fisher exact test; $p < 10^{-5}$). The DIR ranged from 13.3% (in Bangante) to 47.5% (in Edea) for the 12 *Ae. albopictus* sampling sites, and were homogenous among sites (Fisher exact test; $p > 0.05$, Table 1). Out of 12 possible comparisons between sympatric samples, only in Douala were the DIRs of *Ae. aegypti* (59.4%) and *Ae. albopictus* (30.4%) significantly different (Fisher exact test; $p < 0.05$).

Table 1. Disseminated Infection Rates in *Aedes aegypti* and *Aedes albopictus* 14 Days After Being Exposed to $10^{8.1}$ Mosquito Infectious Dose 50/mL of the D2S32 Strain of Dengue Virus Type 2

	Aedes aegypti		Aedes albopictus	
Location	*Assay*	*Control*	*Assay*	*Control*
West and North West				
Bangante	35.7 (14)	93.3 (30)	13.3 (15)	89.7 (29)
Bafoussam	40.0 (15)	91.1 (45)	–	–
Bamenda	42.9 (28)	93.3 (30)	30.6 (49)	85.7 (42)
Littoral				
Buea	47.4 (38)	90.2 (51)	29 (22)	85.7 (42)
Douala	59.7 (62)	89.7 (87)	30.4 (23)	97.3 (75)
Edea	36.7 (60)	89.7 (87)	47.5 (61)	97.3 (75)
Pouma	17.7 (62)	90.2 (51)	32.4 (37)	85.7 (42)
Centre				
Bafia	28.6 (49)	91.1 (45)	40.7 (27)	89.7 (29)
Yaoundé	42.7 (82)	89.7 (87)	27.9 (61)	97.3 (75)
Ayos	35.8 (67)	93.3 (30)	34.8 (46)	89.7 (29)
East				
Abong Mbang	40.7 (27)	98.0 (51)	38.5 (26)	98.0 (51)
Bertoua	30.8 (39)	98.0 (51)	47.1 (17)	98.0 (51)
Garoua Boulaï	27.4 (62)	91.1 (45)	42.9 (35)	85.7 (42)
Adamaoua				
Meiganga	29.2 (89)	93.3 (30)	–	–
Ngaoundéré	46.9 (49)	90.2 (51)	–	–
North and far North				
Mbé	37.9 (58)	90.2 (51)	–	–
Garoua	27.0 (63)	82.5 (80)	–	–
Maroua	17.2 (99)	82.5 (80)	–	–
Kousseri	44.4 (54)	82.5 (80)	–	–

Locations indicate main cities (grouped according province) in Cameroon where mosquitoes were collected. In parentheses are sample sizes.

Oral susceptibility to CHIKV

Results of three experimental infections with CHIKV 06.21 are shown in Table 2. DIRs in controls (*Ae. aegypti* Paea strain) ranged from 95.2% to 98.8% across the three infections and were homogeneous (Fisher exact test; $p > 0.05$). DIRs from the five *Ae. aegypti* samples ranged from 80.3% in Abong-Mbang to 95.0% in Douala and were homogeneous (Fisher exact test; $p > 0.05$). In sympatric populations of *Ae. albopictus* DIR ranged from 74.2% in Bertoua to 100% in Douala and were significantly heterogeneous (Fisher exact test; $p < 0.05$), revealing variability across locations. All comparisons between sympatric *Ae. aegypti* and *Ae. albopictus* samples showed that both species exhibited similar DIRs (Fisher exact test; $p > 0.05$).

Virological and entomological surveys

Both *Ae. albopictus* and *Ae. aegypti* mosquitoes were collected in all sites in Libreville, except in site 7, where only *Ae. albopictus* was recorded (Table 3). *Ae. aegypti* was generally more prevalent than *Ae. albopictus* in urbanized areas (sites 1–6). Mean hourly HBR ranged between 0.2 and 1.2 bites per person per hour (BPH) for *Ae. albopictus*, and between 0.2 and 4.8 BPH for *Ae. aegypti*. In the suburban areas (sites 7–15), *Ae. albopictus* largely outnumbered *Ae. aegypti* with a ratio ranging from 1.5 (site 8) to 28.7 (site 7), with mean HBR in the range between 0.7 and 15.7 BPH for *Ae. albopictus* and between 0 and 3.9 BPH for *Ae. aegypti*. Values of HBR obtained in other Gabonese cities confirmed that *Ae. albopictus* readily bites humans (Table 3), without, however, excluding it could also feed on other nonhuman hosts. The ratio of *Ae. albopictus* to *Ae. aegypti* (10.7, 35.4, and 45.3 at Cocobeach, Oyem, and Lastourville, respectively) clearly demonstrated that *Ae. albopictus* also largely outnumbered *Ae. aegypti* in these locations (Table 3).

A total of 104 pools of *Ae. albopictus* and 42 pools of *Ae. aegypti* collected in Libreville were analyzed using Q-PCR. The presence of CHIKV RNA was detected in seven pools of

Table 2. Disseminated Infection Rates in *Aedes aegypti* and *Aedes albopictus* 14 Days After Being Exposed to $10^{7.5}$ Plaque-Forming Units/mL of the Chikungunya Virus 06.21 Strain of the Chikungunya Virus

	Aedes aegypti		Aedes albopictus	
Location	*Assay*	*Control*	*Assay*	*Control*
Littoral				
Douala	95.0 (107)	95.2 (83)	100.0 (19)	94.3 (105)
Edea	86.0 (43)	95.2 (83)	76.5 (68)	94.3 (105)
Centre				
Yaoundé	90.3 (113)	95.2 (83)	91.5 (82)	94.3 (105)
East				
Abong Mbang	80.3 (61)	98.8 (82)	94.4 (18)	98.8 (82)
Bertoua	82.7 (52)	98.8 (82)	74.2 (31)	98.8 (82)

Locations indicate main cities (grouped according to province) in Cameroon where mosquitoes were collected. In parentheses are sample sizes.

Table 3. Mosquito Collection and Virus Detection During the Outbreak of Dengue and Chikungunya in Libreville, Gabon, June–July 2007

						Aedes albopictus					Aedes aegypti					
	Site number	*Location name*	*Geographic coordinates*	*Duration of capture* (H)	*Number of volunteers*	*Number of females collected*	*HBR*	*Number of mosquito pools*	*Number of CHIKV+ pools*	*Number of DENV+ pools*	*Number of females collected*	*HBR*	*Number of mosquito pools*	*Number of CHIKV+ pools*	*Number of DENV+ pools*	*Ratio* Aedes albopictus/ Aedes aegypti
Libreville																
Downtown	1	Vallée-Ste Marie	0°24′51″N; 9°26′25″E	4	8	28	0.9	2	–	–	152	4.8	7	–	–	0.2
	2	Nkembo	0°24′45″N; 9°27′25″E	8	20	45	0.3	2	–	–	88	0.6	4	–	–	0.5
	3	Kinguélé	0°23′57″N; 9°28′35″E	8	12	20	0.2	2	–	–	52	0.5	2	–	–	0.4
	4	Plein-ciel	0°23′36″N; 9°28′55″E	8	12	22	0.2	1	–	–	18	0.2	2	–	–	1.2
	5	London	0°23′05″N; 9°27′18″E	4	8	38	1.2	1	–	–	141	4.4	3	–	–	0.3
	6	Lalala	0°22′07″N; 9°27′53″E	4	8	36	1.1	2	–	–	150	4.7	6	–	–	0.2
		All				189	0.7	10	–	–	601	2.5	24	–	–	0.3
Suburbs	7	IAI Golf	0°22′11″N; 9°28′52″E	8	12	148	1.5	6	–	–	0	0.0	0	–	–	–
	8	Alenkiri	0°18′39″N; 9°30′30″E	4	8	503	15.7	20	2	3	34	1.1	2	–	–	14.8
	9	PK9	0°24′22″N; 9°31′07″E	8	12	179	1.9	6	1	–	8	0.1	1	–	–	22.4
	10	Nzeng Ayong	0°26′25″N; 9°29′54″E	12	18	141	0.7	6	1	–	38	0.2	2	–	–	3.7
	11	Nzeng Ayong	0°25′58″N; 9°29′15″E	8	12	252	2.6	10	–	–	41	0.4	2	–	–	6.1
	12	Nzeng Ayong	0°25′41″N; 9°28′25″E	4	6	213	8.9	9	–	–	93	3.9	4	–	–	2.3
	13	Bel-Air	0°26′13″N; 9°26′ 38″E	8	12	290	3.0	11	1	–	38	0.4	2	–	–	7.6
	14	Avore Mbam	0°28′01″N; 9°24′53″E	8	11	546	6.2	22	2	–	19	2.2	2	–	–	28.7
	15	Okala village	0°29′22″N; 9°24′12″E	8	12	78	0.8	4	–	–	51	0.5	3	–	–	1.5
		All				2,350	4.6	94	7	3	322	1.0	18	–	–	7.3
All Libreville						2,539	3.0	104	7	3	923	1.6	42	–	–	2.8
CocoBeach	16	–	0°59′31″N; 9°35′22″E	15	12	1409	7.8	NT	NT	NT	132	0.7	NT	NT	NT	10.7
Oyem	17	–	01°36′14″N; 11°34′49″E	13	8	743	7.14	NT	NT	NT	21	0.2	NT	NT	NT	35.4
Lastourville	18	–	0°49′54″N; 12°42′57″E	6	6	272	7.55	NT	NT	NT	36	1	NT	NT	NT	45.3

CHIKV, chikungunya virus; DENV, dengue virus; HBR, human biting rate (number of biting mosquitoes/person/hour); NT, nontested.

Ae. albopictus collected from five sites, and the MLE of infection was 3.31% (CI 95%: 1.41–6.51) (Table 3). DENV type 2 was detected from 3 *Ae. albopictus* pools collected in a single site, and the MLE of infection was 1.51% (CI 95%: 0.31–3.81). All *Ae. aegypti* pools were negative for CHIKV and DENV. In addition to *Ae. aegypti* and *Ae. albopictus* specimens, 52 (10 pools) *Ae. simpsoni*, 72 (8 pools) *Anopheles gambiae* sensus lato, 645 (28 pools) *Culex quinquefasciatus*, and 185 (10 pools) *Mansonia unifromis/africana* collected in Libreville were also analyzed for the presence of CHIKV and DENV RNA. All these pools were negative for both viruses.

Discussion

Our data show that all *Ae. aegypti* and *Ae. albopictus* populations from Cameroon are susceptible to DENV and CHIKV. The susceptibility of *Ae. albopictus* is commensurate with field investigations we conducted in neighboring Gabon at Libreville in 2007, during a simultaneous outbreak of CHIKV and DENV. We report the detection of CHIKV from natural *Ae. albopictus* populations and the first report of DENV from this species in continental Africa. Despite *Ae. albopictus* having only recently invaded Libreville, it was already abundant and readily biting humans throughout the study area. This was especially true in suburban environments, where it frequently outnumbered the endemic *Ae. aegypti* populations from which no virus was detected. Moreover, the invasive mosquito also predominated over the indigenous one at CocoBeach, Oyem, and Lastourville. Taken together, these findings suggest that *Ae. albopictus* acted as the primary vector during the 2007 Gabonese epidemic and showed that this mosquito is vector of CHIKV and DENV.

The widespread distribution of *Ae. albopictus* (Toto et al. 2003, Simard et al. 2005, Coffinet et al. 2007) together with its high local densities found during the outbreak period in Libreville and three other Gabonese localities further testifies to the extraordinary invasive behavior of the species and the rapidity with which it has been able to establish in Central Africa (Benedict et al. 2007). As formerly observed in Asia (Tewari et al. 2004) and South America (Braks et al. 2003), the highest densities of this species in Gabon were found in the outskirts of the city, where patches of vegetation are readily available (Chan et al. 1971, Cox et al. 2007). In the more urbanized downtown, *Ae. aegypti* was more prevalent than *Ae. albopictus*, although the HBR due to this species was relatively low when compared to sites where *Ae. albopictus* predominated. Given *Ae. albopictus* only recently invaded Central Africa, the current situation is likely to evolve further and sustained monitoring of these natural populations should be implemented to assess the spatial and temporal dynamics of the spread of *Ae. albopictus* and its interactions with resident *Ae. aegypti* populations. Further, *Ae. albopictus* was repeatedly found infected with CHIKV and/or DENV in Libreville, whereas no virus was detected in *Ae. aegypti*. Similarly, a recent study based on mosquitoes collected in a single site in Libreville, lead to the detection of CHIKV only in *Ae. albopictus* (Pages et al. 2009). Thus, although *Ae. aegypti* has been shown to be a major vector of DENV and CHIKV in several parts of the world, including Africa (Failloux et al. 2002), our results from the field suggest that this species represented at best a secondary vector in the Libreville epidemics of 2007, whereas *Ae. albopictus* played a prominent role.

West and Central African populations of *Ae. aegypti* are known to be much less susceptible to DENV than those originating from other parts of the world (Failloux et al. 2002). This character, which is under genetic control (Black et al. 2002), is thought to explain the lack of DENV outbreaks in the region. Our experiments, based on 19 *Ae. aegypti* populations from Cameroon, confirmed lower levels of vector competence for DENV type 2 (17.2% to 59.7% in our study) compared to those found in Asia (93.2% to 100%, Vazeille et al. 2003) and South America (21.57% to 99.02%, Lourenço-de-Oliveira et al. 2004) when assessed under standard experimental conditions. We found similar oral susceptibility levels to DENV type 2 in both *Ae. aegypti* and *Ae. albopictus* populations from throughout Cameroon. However, DIRs observed in 12 *Ae. albopictus* samples from Cameroon (13.0% to 47.5%) indicated that the level of susceptibility to DENV type 2 virus was greater than recently reported from Gabon (13% and 21%, Vazeille et al. 2008). Levels of DIRs as reported here for *Ae. albopictus* were also consistent with those reported from Southeast Asia (7% to 45.8%), where *Ae. albopictus* is not considered as the primary vector of DENV (Vazeille et al. 2003).

Similar to what was found with DENV, our experimental infections with CHIKV performed on *Ae. albopictus* and sympatric *Ae. aegypti* populations from Cameroon showed that vector competence was high and comparable between the two species (74.2% to 100% and 86.0% to 95% for *Ae. albopitus* and *Ae. aegypti*, respectively). Similar levels of vector competence for the CHIKV 06.21 strain were recently found for three *Ae. albopictus* samples from Libreville (66.7% to 86.0%) (Vazeille et al. 2008). As in our experiment, this study failed to detect any clear statistical difference between DIRs from *Ae. albopictus* and *Ae. aegypti* (Vazeille et al. 2008).

Taking into account that both species exhibited a similar level of vector competence for both DENV and CHIKV, we assumed that additional factors (i.e., bionomical) were probably influential in determining the vector capacity of *Ae. albopictus* in Central Africa. Low vector competence can easily be countered by specific increases in epidemiologically relevant biological and/or behavioral traits, such as high local vector density, increased longevity or strong anthropophilic preferences, which can result in producing a very efficient vector. Our data provide evidence that *Ae. albopictus* is currently abundant and readily bites humans in outbreak areas of Gabon, just as it is in most urban environments in southern Cameroon where it is the most prevalent *Aedes* (*Stegomiya*) spp. (Paupy, unpublished data). In La Reunion Island, where *Ae. albopictus* was found to have similar levels of vector competence to DENV than those we found in Cameroon (18% to 59%; Paupy et al. 2001), the species was shown to be the main vector during a large DENV type 2 outbreak occurring in 1977–1978. Additionally, *Ae. albopictus* could favor the selection, emergence, and spread of mutant viral strains responsible for large outbreaks, as exemplified for CHIKV during three independent events in Gabon, La Reunion, and Italy (de Lamballerie et al. 2008). In these areas, the emergence of a strain with a single adaptive mutation, E1-A226V (i.e., a Valine residue at position 226 of the E1 gene), presumably resulted from independent viral exposures to *Ae. albopictus* (de Lamballerie et al. 2008). It was demonstrated that such a mutation (E1-A226V) provided a selective advantage for the replication and transmission of CHIKV by this mosquito

(Tsetsarkin et al. 2007, Vazeille et al. 2007). Our estimates of vector competence in Central Africa reveal the general pattern of oral susceptibility of *Ae. aegypti* and *Ae. albopictus* to DENV and CHIKV. A more definitive picture will be possible if future studies estimate vector competence using viral strains endemic to Central Africa.

Overall, our results provide evidence that *Ae. albopictus* populations from Central Africa are involved in CHIKV and DENV virus transmission, and probably played a pivotal role during the Libreville epidemics in 2007. The species was at least as susceptible as the resident *Ae. aegypti* populations to both exogenous viral strains we tested. Updates on geographic distribution throughout Central Africa and comparative data on the biology and vector–virus interactions (i.e., vertical transmission and dynamics of infection) between both species would be helpful to comprehensively assess the role of *Ae. albopictus* in current viral emergence in Central Africa. These data would also be useful to assess the risk of DENV, CHIKV, and the transmission of other arboviruses in this area.

Acknowledgments

We thank Elysée Nchoutpouen for significant help during field work and mosquito rearing, Wilson Toussile for his help with statistical analyses, and Philip Agnew for his critical review of the article. We are grateful to the editor and two anonymous reviewers for comments that greatly improved previous versions of this article.

This research was supported by the French government through the "Agence Nationale pour la Recherche" that funded the Epidengue project (ANR 05 SEST 010-01) and the Institut de Recherche pour le Développement with a "credits incitatifs" grant to C.P. CIRMF is supported by the Government of Gabon, Total-Fina-Elf Gabon, and the Ministère de la Coopération Française.

Disclosure Statement

No competing financial interests exist.

References

Benedict, M, Levine, RS, Hawley, WA, Lounibos, LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonot Dis 2007; 7:76–85.

Black, W, IV, Bennett, KE, Gorrochótegui-Escalante, N, Barillas-Mury, CV, et al. Flavivirus susceptibility in *Aedes aegypti*. Arch Med Res 2002; 33:379–388.

Bonilauri, P, Bellini, R, Calzolari, M, Angelini, R, et al. Chikungunya virus in *Aedes albopictus*, Italy. Emerg Infect Dis 2008; 14:852–854.

Braks, MAH, Honorio, NA, Lourenco-De-Oliveira, R, Juliano, SA, et al. Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. J Med Entomol 2003; 40:785–794.

Chan, KLB, Ho, C, Chan, YC. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore city 2. Larval habitats. Bull World Health Organ 1971; 44:629–633.

Coffinet, T, Mourou, JR, Pradines, B, Toto, JC, et al. First record of *Aedes albopictus* in Gabon. J Am Mosq Control Assoc 2007; 23:471–472.

Cox, J, Grillet, ME, Ramos, OM, Ammador, M, et al. Habitat segregation of dengue vectors along an urban environmental gradient. Am J Trop Med Hyg 2007; 76:820–826.

de Lamballerie, X, Leroy, E, Charrel, RN, Ttsetsarkin, K, et al. Chikungunya virus adapts to tiger mosquito via evolutionary convergence: a sign of things to come? Virol J 2008; 5:33–36.

Delatte, H, Dehecq, JS, Thiria, J, Domerg, C, et al. Geographic distribution and developmental sites of *Aedes albopictus* (Diptera: Culicidae) during a chikungunya epidemic event. Vector Borne Zoonot Dis 2008; 8:25–34.

Diallo, M, Ba, Y, Faye, O, Soumare, ML, et al. Vector competence of *Aedes aegypti* populations from Senegal for sylvatic and epidemic dengue 2 virus isolated in West Africa. Trans R Soc Trop Med Hyg 2008; 102:493–498.

Drosten, C, Göttig, S, Schilling, S, Asper, M, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-congo hemorrhagic fever virus, Rift valley fever virus, Dengue virus, and Yellow fever virus by real-time reverse transcription-PCR. J Virol 2002; 2323–2330.

Failloux, AB, Vazeille, M, Rodhain, F. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti.* J Mol Evol 2002; 5:653–663.

Fontenille, D, Toto, JC. *Aedes* (*Stegomyia*) *albopictus* (Skuse), a potential new dengue vector in southern Cameroon. Emerg Infect Dis 2001; 7:1066–1067.

Gratz, NG. Critical review of the vector status of *Aedes albopictus*. Med Vet Entomol 2004; 18:215–227.

Gu, W, Lampmann, R, Novack, RJ. Problems in estimating mosquito infection rates using minimum infection rate. J Med Entomol 2003; 40:595–596.

Krippner, R, von Laer, G. First confirmed dengue-1 fever cases reported from Cameroon. J Travel Med 2002; 9:273–274.

Leroy, E, Nkoghe, D, Ollomo, B, Nze-Nkogue, C, et al. Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon. Emerg Infect Dis 2009; 15: 591–593.

Lourenço-de-Oliveira, R, Vazeille, M, de Filippis, AMB, Failloux, AB. *Aedes aegypti* in Brazil: genetically differentiated populations with high susceptibility to dengue and yellow fever viruses. Trans R Soc Trop Med Hyg 2004; 98:43–54.

Pages, F, Peyrefitte, CN, Toung Mve, M, Jarjaval, F, et al. *Aedes albopictus* mosquito: the main vector of the 2007 outbreak in Gabon. PLoS ONE 2009; 4:e4691.

Pastorino, B, Muyembe-Tamfum, JJ, Bessaud, M, Tock, F, et al. Epidemic resurgence of chikungunya virus in Democratic Republic of the Congo: identification of a new central African strain. J Med Virol 2004; 74:277–282.

Paupy, C, Girod, R, Salvan, JM, Rodhain, F, et al. Population structure of *Aedes albopictus* from La Réunion island (Indian Ocean) in relation with susceptibility to a dengue virus. Heredity 2001; 87:273–283.

Peyrefitte, CN, Bessaud, M, Pastorino, BA, Gravier, P, et al. Circulation of chikungunya virus in Gabon, 2006–2007. J Med Virol 2008; 80:430–433.

Peyrefitte, C, Rousset, D, Pastorino, B, Pouillot, R, et al. Chikungunya virus, Cameroon. Emerg Infect Dis 2007; 13:768–771.

Pialoux, G, Gaüzère, BA, Jauréguiberry, S, Strobel, M. Chikungunya, an epidemic arbovirosis. Lancet Infect Dis 2007; 7:319–327.

Reiter, P, Fontenille, D, Paupy, C. *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? Lancet Infect Dis 2006; 6:463–464.

Savage, HM, Ezike, VI, Nwankwo, ACN, Spiegel, R, et al. First record of breeding populations of *Aedes albopictus* in continental Africa: implication for arbovirus transmission. J Am Mosq Control Assoc 1992; 8:101–103.

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

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; 42:726–731.

Tewari, SC, Thenmozhi, V, Kathoi, CR, Manavalan, R, et al. Dengue vector prevalence and virus infection in a rural area in south India. Trop Med Int Health 2004; 9:499–507.

Toto, JC, Abaga, S, Carnevale, P, Simard, F. First report of the oriental mosquito *Aedes albopictus* on the west African island of Bioko, Equatorial Guinea. Med Vet Entomol 2003; 17:343–346.

Tsetsarkin, KA, Vanlandingham, DL, McGee, CE, Higgs, S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog 2007; 3:e201.

Vazeille, M, Moutailler, S, Coudrier, D, Rousseaux, C, et al. Two chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus*. PLoS ONE 2007; 2:e1168.

Vazeille, M, Moutailler, S, Pages, F, Jarjaval, F, et al. Introduction of *Aedes albopictus* in Gabon: what consequences for dengue and chikungunya transmission. Trop Med Int Health 2008; 13:1–4.

Vazeille, M, Rosen, L, Mousson, L, Failloux, AB. Low oral receptivity for dengue type 2 viruses of *Aedes albopictus* from Southeast Asia compared with that of *Aedes aegypti*. Am J Trop Med Hyg 2003; 68:203–208.

Vazeille-Falcoz, M, Mousson, L, Rodhain, F, Chungue, E, et al. Variation in oral susceptibility to dengue type 2 virus of populations of *Aedes aegypti* from the islands of Tahiti and Moorea, French Polynesia. Am J Trop Med Hyg 1999; 60:292–299.

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