SHORT COMMUNICATION

Dengue 2 Outbreak in Southeastern Senegal During 1990: Virus Isolations from Mosquitoes (Diptera: Culicidae)

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ABSTRACT During 1990, Dengue-2 (DEN-2) virus was isolated for the first time from mosquitoes (*Aedes furcifer*, six isolates; *Ae. taylori*, six isolates; *Ae. luteocephalus*, seven isolates) collected during an epidemic in which DEN-2 virus also was isolated from humans. Numerous isolations have been made previously from mosquitoes in the absence of human infection. In Senegal, DEN-2 virus appears to be maintained in an enzootic cycle and, therefore, plays an expanding role in human disease and increases the need for effective surveillance in mosquito populations.

KEY WORDS Dengue 2, Aedes, Senegal

DENGUE 2 (DEN-2) VIRUS appears to be maintained enzootically in southeastern Senegal, because the virus has been isolated repeatedly from mosquitoes in the absence of widespread human disease. DEN-2 virus was isolated first in the Kedougou area of Senegal from *Aedes (Stegomyia) luteocephalus* (Newstead) in 1974 (Robin et al. 1980). This focus has remained intermittently active and from 1981 to 1982 DEN-2 was again detected when isolations also were made from *Aedes (Diceromyia) furcifer* (Edwards) and *Ae. (D.) taylori* (Edwards) (Cornet et al. 1984). A sylvatic cycle was indicated further in 1981 by the isolation of DEN-2 virus from an *Erythrocebus patas* monkey. Unexpectedly, illness was not reported in humans during this period and a serosurvey in 1988 produced negative findings (Monlun et al. 1993).

During October and November 1989, 36 isolations of DEN-2 virus were made from mosquitoes collected at the Kedougou focus (J.-P.H., unpublished data). Although *Ae. furcifer*, *Ae. taylori*, and *Ae. luteocephalus* were infected most frequently, isolates also were made from *Aedes dalzieli* and *Mansonia africana* (J.-P.H., unpublished data). A serosurvey of children (ages, 1–15 yr) in November 1990 indicated a 4.1% infection rate, and two isolates of DEN-2 virus were made from febrile patients indicating the existence of an epidemic (Monlun et al. 1992, Zeller et al. 1992). The present study describes the abundance of DEN-2 strains and DEN-2 virus infection rates in mosquitoes collected at human bait from the Kedougou study during the summer and fall of 1990 and determines whether additional vectors may have been responsible for this unexpected transmission of virus to humans.

Materials and Methods

The Kedougou area (12° 11′ W–12° 33′ N) is situated in the extreme southeast of Senegal, between Guinea and Mali. The area is hilly and contrasts with the low flat plain that constitutes the rest of Senegal. The hills represent the last spur of the Fouta Djalon. The region belongs to the Sudan-Guinean phytogeography and is crossed by the Gambia River, which is fed from many seasonal tributaries draining the Fouta Djalon. In 1990, the rains started in June and peaked in August (Fig. 1) with a yearly total rainfall of 807.3 mm. Temperature averaged 23.5°C, ranging from 25.2°C in January to 33.0°C in April. The density of human population is low with 2.5 inhabitants per square kilometer living in small, dispersed agricultural villages.

Mosquito Collection and Processing. Study areas and sampling periods for adult mosquitoes were selected on the basis of previous studies (Taufflieb et al. 1973; Cornet et al. 1978). Capture sites were located 10 km north of Kedougou (Fig. 2) and included the edge of the forest gallery at ground level (site 1), the forest gallery with three platforms 4 m high (sites 2, 4, and 6) and one platform 10 m high (site 3), and the savanna area (site 5). Mosquitoes were collected for 10 consecutive days each month on human bait during June, September, October, and No-

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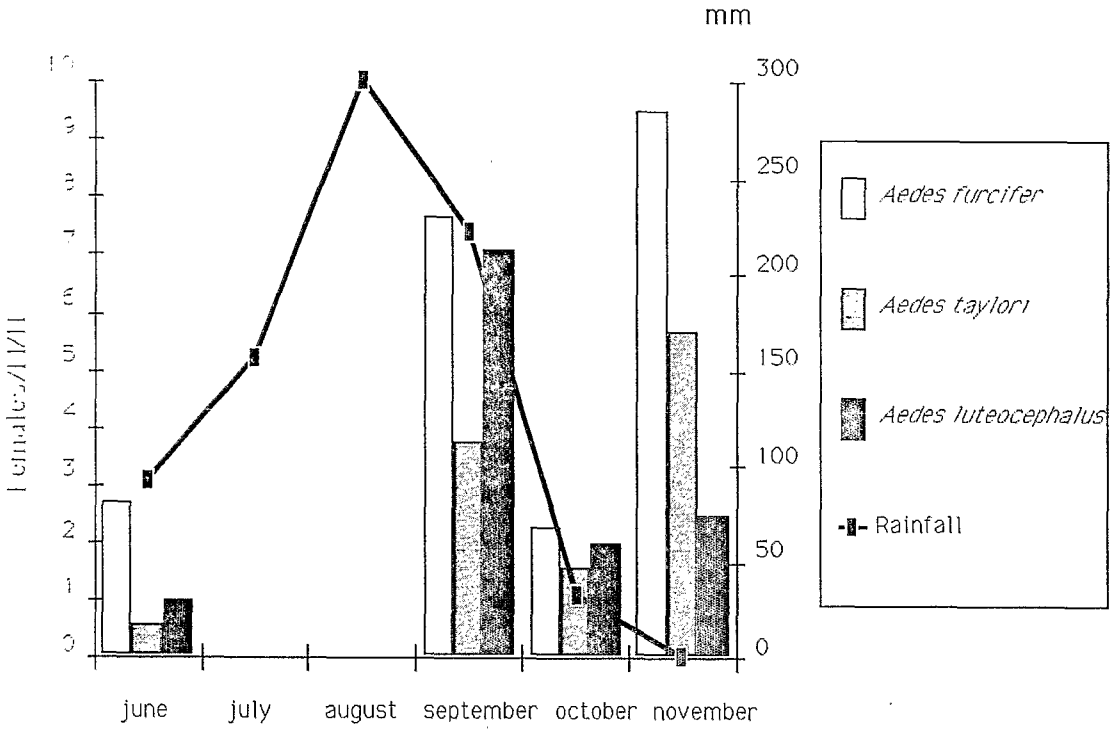

Fig. 1. Dengue-2 vector abundance (females/person hour and rainfall in Kedougou area, 1990.

vember. Eighteen volunteers in six groups collected mosquitoes from 17:30 to 20:30 h.

Mosquitoes were killed by quick freezing at −18°C, and then pooled on a chill table by species, locality, and date (≤100 specimens per pool). Pools were stored in liquid nitrogen and then at −70°C in the laboratory in Dakar before testing. Each pool was ground in 1 ml of Leibovitz 15 medium with 5% fetal calf sera, penicillin-streptomycin and Actinomicin D (Calbiochem. Behring, La Jolla, CA), centrifuged, and the supernatant inoculated into AP 61 *(Ae. pseudoscutellaris)* or Vero cells as described previously (Digoutte et al. 1992). Cells were incubated at 28°C (AP 61) or 37°C (Vero), and cytopathogenic effects were recorded daily. Within 10 d, slides were prepared for immunofluorescent assay and were tested against seven immune ascitic grouping fluids, including most of the African mosquito-borne arboviruses. Monoclonal DEN-2 antibodies were used for type determination (Digoutte et al. 1992). Other viruses were identified by complement fixation and seroneutralization tests in the WHO collaborative Center for Research on Arboviruses.

Results

In total, 18,534 mosquitoes in seven genera and 50 species were collected in 1990 and processed in 375 pools. *Aedes* mosquitoes represented 42% of the total of mosquitoes collected (Table 1). *Stegomyia* and *Diceromyia* were by far the most anthropophilic; 20% of the total mosquitoes were *Diceromyia*, 13% *Aedimorphus*, and 8% *Stegomyia*. Adults from five other genera also were collected and processed for virus with negative results; *Anopheles* (10 species, 2,500 females in 69 pools), *Aedeomyia* (2 species, 15 females in 5 pools), *Culex* (9 species, 1,397 females in 47 pools), *Mimomyia* (6 species, 392 females in 11 pools), *Uranotaenia* (5 species, 266 females in 12 pools) and *Mansonia uniformis* (4,601 females in 52 pools).

In June, at the beginning of rainy season, the *Aedimorphus* mosquitoes constituted 60% of the total females, of which *Ae. vittatus* comprised 26% and *Ae. dalzieli* 10%. *Stegomyia* mosquitoes were 14.6% of the total females and included 53% *Ae. luteocephalus* and 43% *Ae. aegypti* (L.) as well as *Ae. africanus* (Theobald), *Ae. neoafricanus* (Cornet) and *Ae. opok* (Corbet and Van Someren). Overall, *Ae. furcifer* (30.4% of the total mosquitoes) was the dominant species collected at human bait, ranging from 20.3% in June to 38.7% in November. The mean landing rate per person hour indicated that *Ae. furcifer* was least abundant at the beginning of the rainy season (2.5 females/person hour) and most abundant after the rainfall peak (9.5 females/person hour). The maximum number of *Ae. luteocephalus* and *Ae. taylori* were collected in September and November respectively (Fig. 2).

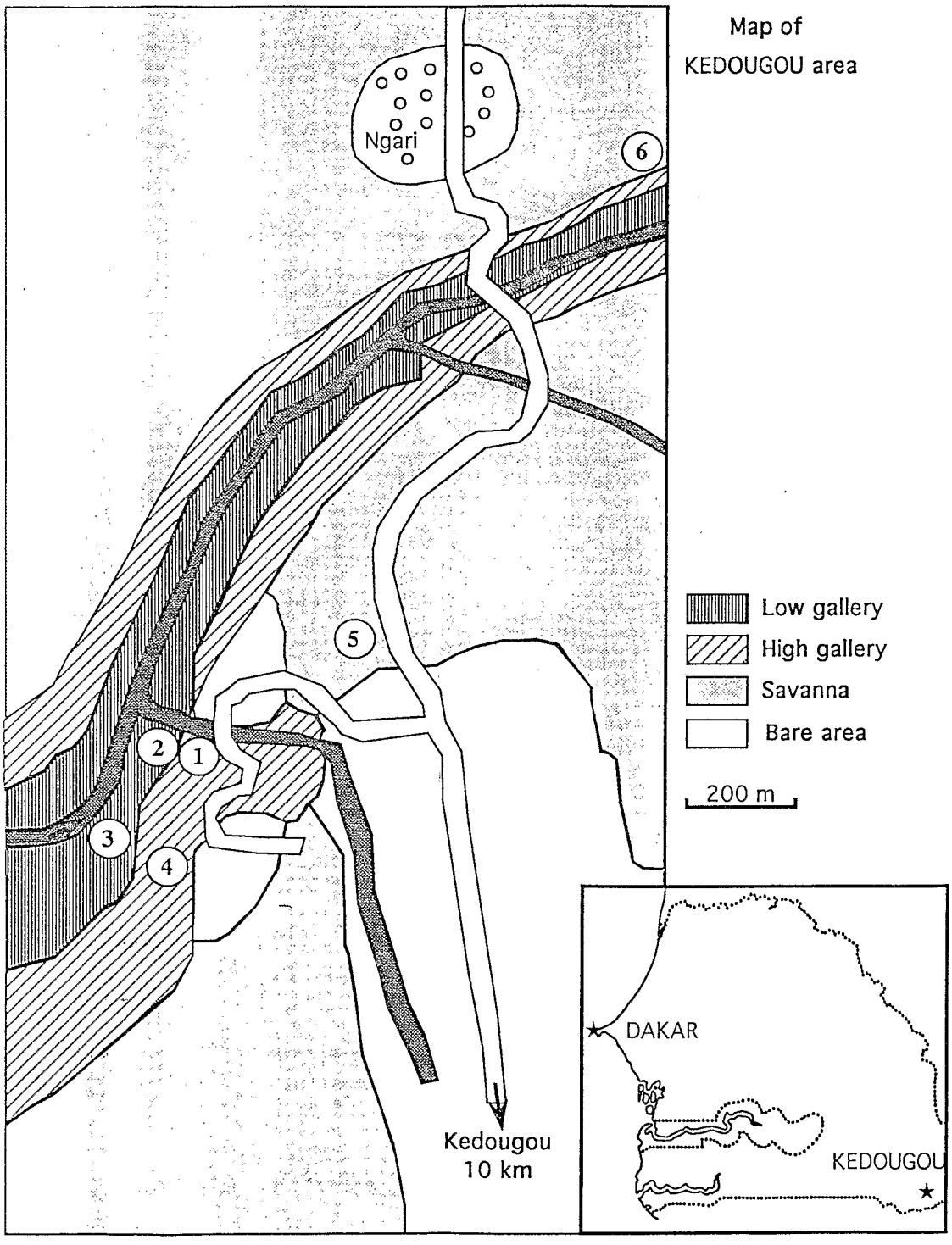

Fig. 2. Map showing capture sites. 1, Forest gallery at ground level. 2, 4, 6, Forest gallery with platforms 4 m high. 3, Forest gallery with platform 10 m high. 6, Savanna area.

Nineteen DEN-2 isolates were obtained during the September–November period (Table 1) from 32% of *Ae. furcifer* pools tested: 23% of *Ae. taylori* and 30% of *Ae. luteocephalus*. These three species comprised 45% of the total pools tested. The minimal field infection rate was 2.5

Table 1. *Aedes* collected in the Kedougou area in 1990, pools tested, and dengue-2 virus isolations

Genus	Species	June			September			October			November			Total		
		No.	Pools		No.	Pools		No.	Pools		No.	Pools		No.	Pools	
			No.	(+)*		No.	(+)		No.	(+)		No.	(+)		No.	(+)
Aedes																
(*Diceromyia*)	*furcifer*	245	3		838	9		251	3		1,030	11	(6)	2,364	26	(6)
	taylori	57	3		411	6		171	2		617	8	(6)	1,256	19	(6)
(*Stegomyia*)	*luteocephalus*	93	4		770	9	(1)	218	3	(2)	272	7	(4)	1,353	23	(7)
	aegypti	77	3		25	3		14	2		45	7		161	15	
	unilineatus	3	1								2	1		5	2	
	cozi	3	1								1	1		4	2	
	neoafricanus										6	1		6	1	
	opok										1	1		1	1	
(*Aedimorphus*)	*vittatus*	524	6		219	4		113	2		69	7		925	19	
	dalzieli	204	3		212	4		82	2		731	10		1,229	19	
	cumminsii	2	1											2	1	
	argenteopunctatus				300	5		11	2		5	1		316	8	
Aedes spp.	*other*				104	9		16	3		27	3		147	15	
Total		1,208	25		2,879	49	(1)	876	19	(2)	2,806	58	(16)	7,769	151	(19)

per 1,000 females tested for *Ae. furcifer*, 4.8 for *Ae. taylori*, and 5.2 for *Ae. luteocephalus*. The first DEN-2 isolate was recovered from *Ae. luteocephalus* at site 2 in September. In October DEN-2 virus was obtained from *Ae. luteocephalus* at sites 2 and 3 (two isolates). In November the virus was isolated at all sites from *Ae. luteocephalus* (sites 1, 3, 4, and 6), from *Ae. furcifer* (sites 2, 3, and 6), and from *Ae. taylori* (sites 4, 5, and 6). Other flaviviruses were isolated in June; five isolates of Kedougou virus from *Ae. dalzieli* (four isolates) and from *Ae. aegypti* (one isolate), one isolate of Bagaza virus from *Culex perfuscus* (Newstead), three isolates of Zika virus from *Ae. furcifer* (one isolate), *Ae. luteocephalus* (one isolate) and *Ae. dalzieli* (one isolate). Single isolates of yellow fever and Chikungunya viruses were obtained from *Ae. furcifer* in November. Another yellow fever strain was isolated from *Ae. luteocephalus*.

Discussion

DEN-2 virus was first isolated at site 2 in a low-gallery forest during September and October, and then spread to other gallery forests and savanna sites in November. Probably the expansion of the outbreak occurred by infected host movements rather than by vectors themselves that are known to remain near their breeding sites (Cornet et al. 1978). The repeated, but intermittent, isolation of DEN-2 virus from *Aedes* mosquitoes over the past 17 yr. without extensive human involvement has indicated the persistence of sylvatic transmission in the Kedougou area. Vertical transmission could explain the maintenance of virus in nature without vertebrate involvement (Rosen 1987); however, the isolation of virus from adults emerging from field-collected immatures is necessary to verify this mechanism. Alternatively, a horizontal sylvatic cycle could exist similar to yellow fever virus involving monkeys and *Aedes* mosquitoes; however, a sylvan cycle has yet to be established for DEN-2 virus and considerable research is necessary to determine the role of monkeys in virus maintenance.

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References Cited

Cornet, M., R. Chateau, M. Valade, P. L. Dieng, M. Raymond & A. Laurand. 1978. Donnees bioecologiques sur les vecteurs potentiels du virus amaril au Senegal Oriental. Role des differentes especes dans la transmission du virus. Cah. ORSTOM., ser. Entomol. Med. Parasitol. 16: 315–341.

Cornet, M., J. F. Saluzzo, J. P. Hervy, J. P. Digoutte, M. Germain, M. F. Chauvancy, M. Eyraud, L. Ferrara, G. Heme & P. Legros. 1984. Dengue 2 au Senegal oriental: une poussee epizootique en milieu selvatique, isolement du virus a partir de moustiques et d'un singe et considerations epidemiologiques. Cah. ORSTOM., ser. Entomol. Med. Parasitol. 22: 313–333.

Digoutte, J. P., M. A. Calvo-Wilson, M. Mondo, M. Traore-Lamizana & F. Adam. 1992. Continuous cell lines and immune ascitic fluid pools in arbovirus detection. Res. Virol. 143: 417–422.

Monlun, E., H. G. Zeller, B. Le Guenno, M. Traore-Lamizana, J. P. Hervy, F. Adam, L. Ferrara, D. Fontenille, R. Sylla, M. Mondo & J. P. Digoutte. 1993. Surveillance de la circulation des arbovirus d'interet medical dans la region du Senegal oriental. Bull. Soc. Pathol. Exot. 86: 21–28.

Monlun, E., H. G. Zeller, M. Traore-Lamizana, J. P. Hervy, F. Adam, M. Mondo & J. P. Digoutte. 1992. Caracteres cliniques et epidemiologiques de la dengue 2 au Senegal. (Clinical and epidemiological features associated with dengue 2 virus infection in Senegal). Med. Malad. Infect. 22: 718–721.

Robin, Y., M. Cornet, G. Heme & G. Le Gonidec. 1980. Isolement du virus de la dengue au Senegal. Ann. Virol. (Paris) 131 E: 149–154.

Rosen, L. 1987. Sur le mecanisme de la transmission verticale du virus de la dengue chez les moustiques. C.R. Acad. Sci. France 13: 347–350.

Taufflieb, R., M. Cornet, G. Le Gonidec & Y. Robin. 1973. Un foyer selvatique de fievre jaune au Senegal oriental. Cah. ORSTOM. ser. Entomol. Med. Parasitol. 11: 211–220.

Zeller, H. G., M. Traore-Lamizana, E. Monlun, J. P. Hervy, M. Mondo, F. Adam & J. P. Digoutte. 1992. Dengue 2 virus isolation from humans during an epizootic in Southeastern Senegal in November 1990. Res. Virol. 143: 101–102.

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