

VECTORS OF CHIKUNGUNYA VIRUS IN SENEGAL: CURRENT DATA AND TRANSMISSION CYCLES

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Abstract. Chikungunya fever is a viral disease transmitted to human beings by *Aedes* genus mosquitoes. From 1972 to 1986 in Kédougou, Senegal, 178 Chikungunya virus strains were isolated from gallery forest mosquitoes, with most of them isolated from *Ae. furcifer-taylori* (129 strains), *Ae. luteocephalus* (27 strains), and *Ae. dalzieli* (12 strains). The characteristics of the sylvatic transmission cycle are a circulation periodicity with silent intervals that last approximately three years. Few epidemics of this disease have been reported in Senegal. The most recent one occurred in 1996 in Kaffrine where two Chikungunya virus strains were isolated from *Ae. aegypti*. The retrospective analysis of viral isolates from mosquitoes, wild vertebrates, and humans allowed us to characterize Chikungunya virus transmission cycles in Senegal and to compare them with those of yellow fever virus.

As part of monitoring programs for yellow fever, dengue, and Rift Valley fever in Senegal, arboviruses not specifically studied are often isolated. These include Ngari, Chikungunya, Wesselsbron, Zika, and West Nile viruses. These wild vertebrate arboviruses, in equilibrium in their natural environment, can spread in human populations. For that reason, they represent a potential or real threat to public health as shown by fatal cases of infection with West Nile virus in Romania and by isolation of Ngari virus from humans in Senegal.^{1,2} Chikungunya virus is an Alphavirus of the family Togaviridae. It was first isolated by Ross in 1953 during a dengue epidemic that occurred in the Newala district of Tanzania.³ Following 2-4 days of incubation, clinical symptoms include fever, headache, nausea, vomiting, photophobia, and arthralgia. Its association with fatal hemorrhagic forms was reported in India.⁴ It is transmitted by mosquitoes, mainly of the genus *Aedes*. Its geographic distribution covers tropical regions of sub-Saharan Africa, Asia, and South America.

In Senegal, three epidemics of Chikungunya fever were reported in 1966, 1982, and 1996 (Thonnon J, unpublished data) in the western part of the country.⁵⁻⁷ Chikungunya virus was also isolated from different mosquito species. This study describes the occurrence of Chikungunya virus in Senegal after 25 years of entomologic monitoring and its transmission cycles.

MATERIALS AND METHODS

Entomologic surveys. Entomologic surveys were carried out every year during the rainy season in two different bioclimatic zones of Senegal: Kédougou (12°11'W, 12°33'N) located in the Sudano-Guinean region and Barkédji (15°17'N, 14°17'W) located in the Sahelian region (Figure 1). These programs were launched in Kédougou in 1970 following the yellow fever epidemic that occurred in Diourbel in 1965, and in Barkédji in 1990 following the epidemic of the Rift Valley fever that occurred in southern Mauritania in 1987. Results from Kédougou cover the period 1972-1996 and those from Barkédji cover the period 1990-1996.

From 1972 to 1990, entomologic surveys were carried out monthly in Kédougou from June to December. The study protocol was carefully explained to the assembled village population. Informed consent was obtained individually

from all participants or their parents (if children). The study was approved by the Conseil de Perfectionnement of the Pasteur Institute of Dakar and the Senegalese Ministry of Health. The sampling methods used were human bait under forest canopy, catches with mowing nets in vegetation, and oviposition traps. Most of the mosquitoes caught were *Aedes*, which are yellow fever vectors. From 1990 onwards, surveys were carried out in July, October, and November in Kédougou and monthly from July to January in Barkédji using four sampling methods: crepuscular human bait catches, catches with CO₂ light traps set nearby temporary ground pools, light traps without CO₂ set in sheepfolds, and mosquito net traps with animal bait (sheep or chicken). Mosquitoes caught were sorted, classified into monospecific pools, numbered, and frozen in liquid nitrogen for virus isolation. Feeding mosquitoes were tested for blood meal identification by an ELISA according to the method of Beier and others.⁸

Surveys on vertebrates. The virus was studied in wild vertebrates during surveys carried out in the 1970s and 1980s. Any systematic survey was carried out on humans except during epidemics.

Virus isolation. Isolations were performed at the World Health Organization Collaborating Centre for Reference and Research on Arbovirus of the Pasteur Institute in Dakar on Vero and AP 61 cell lines (*Ae. pseudoscutellaris*) as previously described by Digoutte and others⁹ or by inoculation into suckling mice.

Chikungunya virus identification was done using indirect immunofluorescence with a specific immune ascitic fluid and confirmed by complement fixation or seroneutralization tests. The minimum infection rates (MIRs), which is the ratio of the number of isolated strains to the number of mosquitoes inoculated, were calculated.

RESULTS

Kédougou. The mosquito species associated with Chikungunya virus in nature, virus isolations, and the MIR for each species are shown in Table 1. Since 1972, 178 Chikungunya virus strains have been isolated from mosquitoes caught in Kédougou, including associations with Zika virus (one strain), yellow fever virus (seven strains), dengue 2



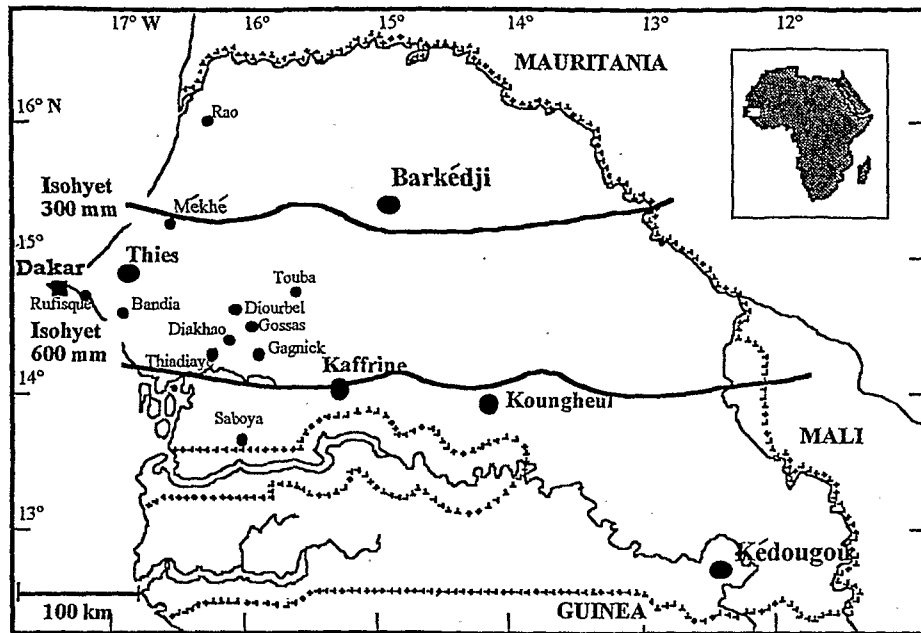


FIGURE 1. Location of the study area.

virus (one strain), and Zika and dengue 2 viruses (three strains).

Of 102 species of mosquitoes caught in Kédougou, 11 were found associated with Chikungunya virus. A total of 72.5% of the isolates were obtained from the *Aedes* subgenus *Diceromyia*, the *furcifer-taylori* group, 16.8% from the *Aedes* subgenus *Stegomyia* mainly from *Ae. luteocephalus* (15.2%), 8.9% from the *Aedes* subgenus *Aedimorphus*, including 6.7% from *Ae. dalzieli*. *Culex* spp. and *Anopheles* spp. accounted for less than 2% of the isolates. Following the separation of species in the *Ae. furcifer-taylori* group,¹⁰ *Ae. furcifer* accounted for 35.4% of the Chikungunya virus isolates whereas *Ae. taylori* accounted for 3.4%. The highest MIRs were observed in *Cx. ethiopicus* (2.16%), *Ae. neoafrikanus* (0.5%), and *Ae. furcifer* (0.44%). However, only 462

of *Cx. ethiopicus* females were tested, from which two virus strains were isolated.

The MIRs varied considerably with time depending on the vector (Figures 2 and 3). When virus was isolated, infection rates in *Ae. furcifer-taylori* were generally high: 4.34%, in 1975, 4.16% in 1979, 2.84% in 1983, and 2.53% in 1992. The highest infection rate in *Ae. luteocephalus* (3.73%) was obtained in 1992.

Observations made in 1991 and 1992 showed that in addition to simio-anthropophilic mosquitoes (*Ae. furcifer-taylori* and *Ae. luteocephalus*), zoophilic mosquitoes such as *Ae. dalzieli*, *Ae. argenteopunctatus*, *Cx. ethiopicus*, and *An. rufipes* carried also Chikungunya virus. Isolates were obtained from these species caught with light traps placed in sheepfolds, traps with animal bait (sheep or chicken), and

TABLE 1
Infection rates and abundance of potential Chikungunya (CHIK) virus vectors captured in Kédougou, Senegal from 1972 to 1996

Species	Mosquitoes captured			Strains of CHIK virus isolated		MIR* (%)
	No.	(%)	No. of pools	No.	(%)	
<i>Aedes furcifer</i>	143,460	(18.38)	4,180	63	(35.4)	0.44
<i>Aedes taylori</i>	38,421	(4.92)	1,206	6	(3.4)	0.15
<i>Aedes furcifer-taylori</i>	68,267	(8.74)	2,858	60	(33.7)	0.88
Subtotal <i>Aedes</i> (<i>Diceromyia</i>)	250,148	(32)	8,244	129	(72.5)	0.51
<i>Aedes luteocephalus</i>	100,469	(12.87)	3,347	27	(15.2)	0.27
<i>Aedes africanus</i>	4,088	(0.52)	211	1	(0.6)	0.24
<i>Aedes neoafrikanus</i>	3,963	(0.51)	272	2	(1.1)	0.50
Subtotal <i>Aedes</i> (<i>Stegomyia</i>)	108,520	(13.90)	3,830	30	(16.8)	0.28
<i>Aedes dalzieli</i>	93,330	(11.95)	2,069	12	(6.7)	0.13
<i>Aedes argenteopunctatus</i>	26,356	(3.38)	894	1	(0.6)	0.04
<i>Aedes vittatus</i>	67,682	(8.67)	3,234	3	(1.7)	0.04
Subtotal <i>Aedes</i> (<i>Aedimorphus</i>)	187,368	(24)	6,197	16	(8.9)	0.08
<i>Anopheles coustani</i>	14,589	(1.87)	630	1	(0.6)	0.07
<i>Anopheles rufipes</i>	38,495	(4.93)	861	1	(0.6)	0.02
<i>Culex ethiopicus</i>	462	(0.06)	77	1	(0.6)	2.16

* MIR = minimum infection rate = number of strains isolated/number of mosquitoes captured.

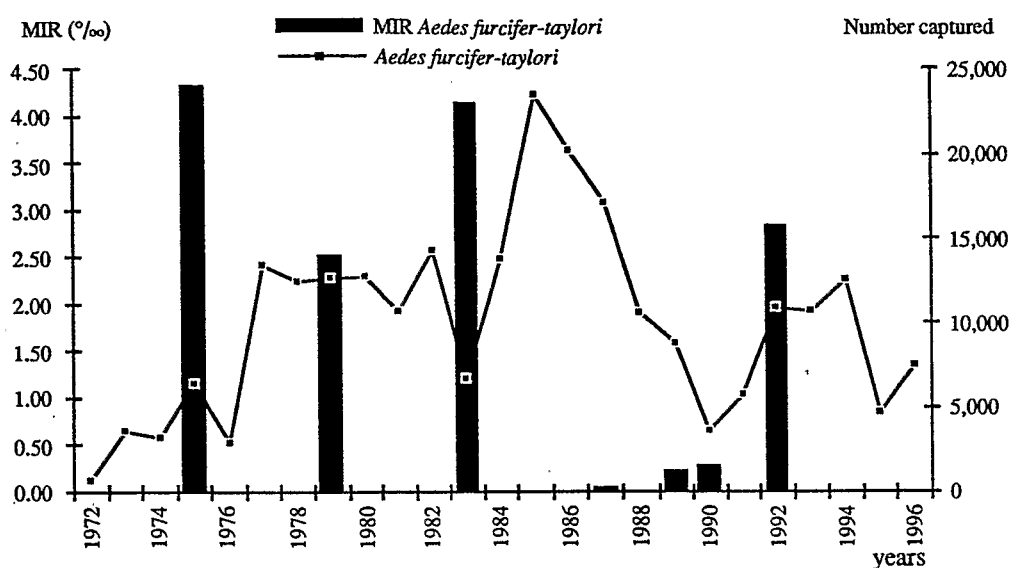


FIGURE 2. Minimum infection rates (MIRs) and number of specimens *Aedes fuscifer-taylori* captured per year in Kédougou, Senegal.

CO₂ light traps (Table 2). Blood meal analysis showed a zoophilic tendency of *Ae. (Aedimorphus) spp.* and *Anopheles spp.*, as well as the high ornithophily of *Cx. ethiopicus* (Table 3).

Four major sylvatic occurrences of Chikungunya virus were observed in 1975, 1979, 1983, and 1992. Except for the 1987–1992 period, periods of three or four years when no viruses were isolated were observed (Figure 4). Three isolates were obtained from humans in Kédougou during intense circulation periods: one strain in 1975 and two strains in 1983 (Figure 4). Isolations were also obtained from monkeys during intense circulation periods: one strain from *Cercocebus aethiops* in 1972, one strain from *Papio papio* in 1975, and one strain from *Erythrocebus patas* in 1983.

Barkédji. In Barkédji, 257,386 mosquitoes of 52 species were tested but no viral strains were isolated.

Other regions. Besides the sylvatic occurrence in the Kédougou area, where yellow fever virus is also endemic,¹¹ isolates were obtained from other locations in western Senegal. In 1966 and 1967, five strains were isolated from *Ae. aegypti* caught at Diakhao (14°27'N, 16°17'W), from *Ae. luteocephalus*, *Ae. irritans*, and *An. gambiae s.l.* captured in a mangrove swamp in Saboya (13°36'N, 16°05'W) and from *An. gambiae s.l.* in Bandia (14°35'N, 17°01'W) during an epidemic in western Senegal. One strain was also isolated from *Ornithodoros erraticus sonrai* (currently known as *Alectorobius sonrai*) ticks collected in a rodent burrow in Bandia.⁶

More recently, during a yellow fever epidemic in the Kafrine area (14°05'N, 15°33'W) in November 1996, two Chikungunya virus strains were isolated from *Ae. aegypti* caught on humans in villages. Serologic tests performed during this

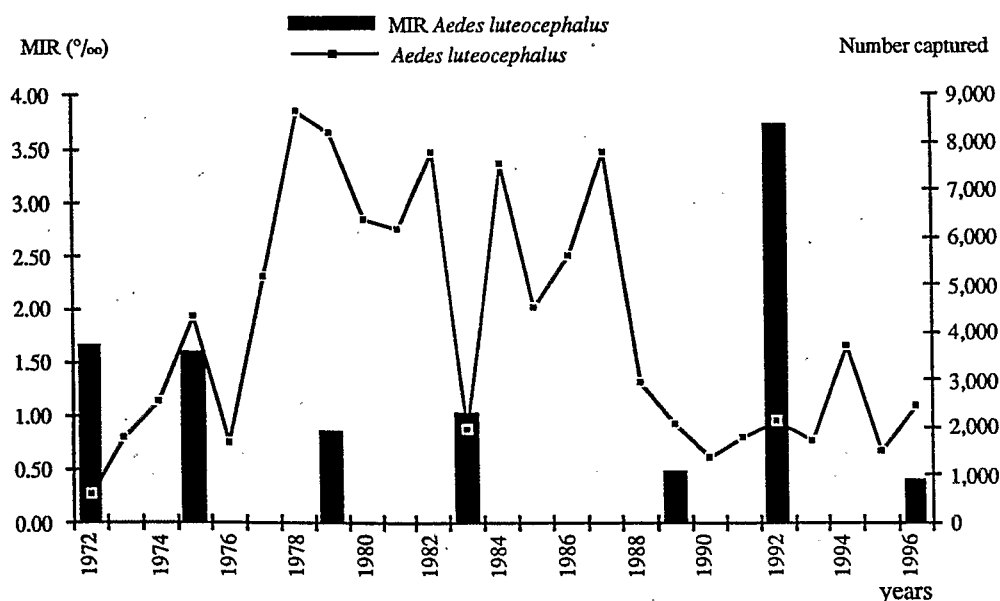


FIGURE 3. Minimum infection rates (MIRs) and number of specimens *Aedes luteocephalus* captured per year in Kédougou, Senegal.

TABLE 2

Number of specimens captured in Kédougou, Senegal per species according to the sampling method from 1991 to 1992, and isolation of Chikungunya virus according to the sampling method*

Species	Number of mosquitoes captured according to collection method					Number of strains isolated /type of trap				
	HB	SBT	CBT	Sh	CO ₂ LT	HB	SBT	CBT	Sh	CO ₂ LT
<i>Aedes furcifer</i>	10,988 (13.1)	5 (0.13)	—	5 (0.05)	7 (0.05)	31	—	—	—	—
<i>Aedes taylori</i>	3,659 (4.4)	—	—	—	1 (0.01)	5	—	—	—	—
<i>Aedes luteocephalus</i>	3,462 (4.1)	1 (0.03)	—	—	6 (0.04)	8	—	—	—	—
<i>Aedes dalzieli</i>	966 (1.15)	3,780 (96.92)	317 (9.32)	1,283 (12.34)	6,104 (42.10)	2	2	1	—	3
<i>Aedes argenteopunctatus</i>	104 (0.12)	860 (22.05)	9 (0.26)	459 (4.41)	1,458 (10.06)	—	—	—	1	—
<i>Aedes vittatus</i>	2,053 (2.44)	405 (10.38)	95 (2.79)	40 (0.38)	448 (3.09)	1	—	—	—	—
<i>Anopheles rufipes</i>	26 (0.03)	1,116 (28.62)	507 (14.91)	2,295 (22.07)	1,256 (8.66)	—	—	—	1	—
<i>Culex ethiopicus</i>	2 (0.0023)	—	—	3 (0.03)	143 (0.99)	—	—	—	—	1

* HB = human bait; SBT = sheep bait trap; CBT = chicken bait trap; Sh = sheepfold; LT = light trap. Values in parentheses are the number of specimens captured/human/night or the number of specimens captured/trap/night. Dashes indicate that no mosquitoes were captured with the collection method and that no Chikungunya virus strain was isolated.

outbreak to assess the prevalence of IgM antibodies against Chikungunya virus confirmed recent circulation of this virus. Among 445 human sera tested, 156 (35.3%) were IgM positive (Thonnon J, unpublished data). The virus was also isolated from humans: four strains in Rufisque in 1966,⁵ eight strains in Touba (14°52'N, 15°55'W) in 1981, and one strain in Touba and four strains in Thiadiaye (14°24'N, 16°25'W) in 1982.⁷

Among wild vertebrates, the virus was isolated from a *Cercopithecus aethiops* monkey, a *Galago senegalensis* galago in Saboya, a *Xerus erythropus* palm squirrel in Bandia, and *Scotophilus sp.* bats in Gagnik (14°19'N, 16° W), Gossas (14°29'N 16°04'W), and Rao (16°N, 16°23'W).⁶

DISCUSSION

Chikungunya virus has been reported in nearly all of Africa, as shown by the prevalence rates of IgM antibodies against this virus and isolation of the virus from mosquitoes in Cote d'Ivoire, the Central African Republic, and Senegal (Centre de Reference OMS de Recherche sur les Arbovirus, Dakar, 1995, unpublished data).¹² In Senegal, 185 strains of Chikungunya virus were isolated from 13 mosquito species. Although the infection rates are high in some mosquito species, epidemics seem to be rare. Repeated isolation of the virus from mosquitoes made us consider these mosquito species as vectors, although their vector competence has never been proved experimentally. All potential vectors are sylvatic except *Ae. aegypti*. The main vectors in the Kédougou area, in order of importance, are *Ae. furcifer*, *Ae. luteocephalus*, and *Ae. taylori*. The scarcity of *Ae. africanus* and *Ae. neafricanus* in catches reduces their role as vectors in this area. The role of *Aedes* from the subgenus *Diceromyia*

has already been proven in South Africa, where the vectors are *Ae. furcifer* and *Ae. cordellieri*. *Aedes aegypti* has never been involved in an epidemic in that region.¹³

In Asia, epidemics have occurred in urban areas where *Ae. aegypti* and *Ae. albopictus* are vectors.¹⁴⁻¹⁶ Seroprevalence studies in *Macaca sinica* in Sri Lanka support the lack of susceptibility of these simian populations to this virus.¹⁷ In Senegal, the major sylvatic vectors of Chikungunya virus (*Ae. furcifer*, *Ae. taylori*, and *Ae. luteocephalus*) are the same as those of yellow fever virus.¹¹ The only domestic vector of yellow fever identified (*Ae. aegypti aegypti*) is also the only one mosquito species found associated with Chikungunya virus in human habitats.¹⁸ The sylvatic form (*Ae. aegypti formosus*) does not appear to be involved on transmission because although it was regularly caught in Kédougou, no strain of Chikungunya virus has ever been isolated from it. As for yellow fever, the major vertebrate hosts of Chikungunya virus are monkeys and humans.

These considerations allowed us to compare the patterns of occurrence of Chikungunya and yellow fever viruses as described by Cornet and others¹⁹ and Cordellier.²⁰ However, Chikungunya virus transmission cycles cannot be superimposed on the yellow fever cycles and this for at least two reasons: 1) the existence of other vectors for Chikungunya virus and 2) the existence of vertebrate hosts other than monkeys and humans for Chikungunya virus.

The transmission cycle of Chikungunya virus is characterized by a periodicity of occurrence with silence intervals of 3-4 years. These cycles, which characterize the movement of this virus in monkeys, are probably related in part to the immune status of the monkeys and to the percentage of the simian population susceptible to the infection. Following the circulation of the virus, nearly all monkeys might be

TABLE 3
Feeding patterns of potential Chikungunya virus vectors caught in Kédougou, Senegal*

Species	Total tested	Human No. (%)	Cattle No. (%)	Sheep No. (%)	Chicken No. (%)	Donkey No. (%)	Other vertebrate No. (%)
<i>Aedes dalzieli</i>	194	2 (1)	53 (27.3)	103 (53)	30 (15.4)	1 (0.5)	5 (2.6)
<i>Aedes argenteopunctatus</i>	176	—	42 (23.8)	118 (67)	8 (4.5)	—	8 (5.1)
<i>Aedes vittatus</i>	36	1 (2.7)	4 (11.1)	26 (72.2)	5 (13.8)	—	—
<i>Anopheles rufipes</i>	126	1 (0.7)	58 (46)	63 (50)	1 (0.7)	1 (0.7)	2 (1.5)
<i>Anopheles coustani</i>	66	1 (1.5)	9 (13.6)	54 (81.8)	2 (3)	—	—
<i>Culex ethiopicus</i>	9	—	—	—	9 (100)	—	—

* — = no blood meal was taken.

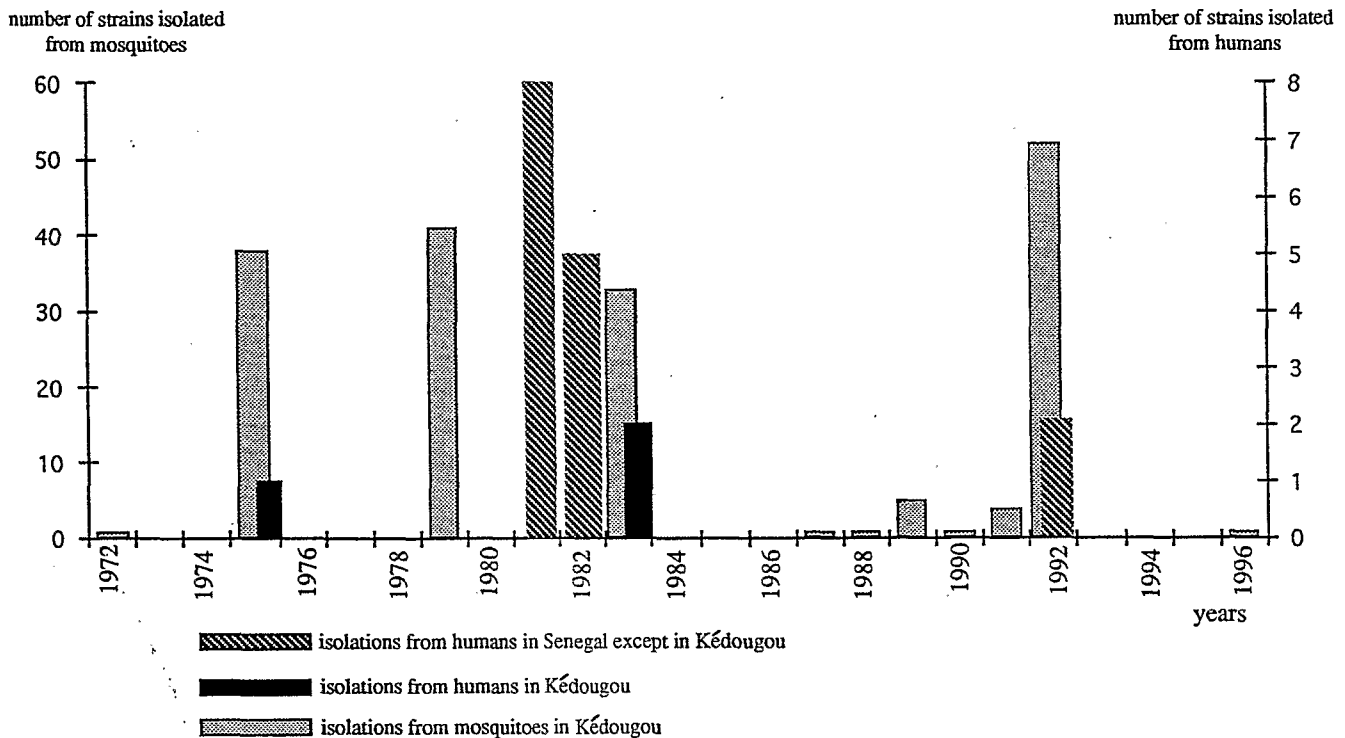


FIGURE 4. Epizootics and epidemics of Chikungunya fever in Senegal from 1972 to 1996.

exposed to it and therefore become immunologically protected. For instance, in 1992 in Kédougou, 53 strains of Chikungunya virus were isolated from simioanthropophilic mosquitoes caught on humans. In this area, the transmission season lasts from July to November. Mosquitoes were captured in the gallery forest by 18 humans volunteers every evening for seven days in July, October, and November, i.e., 378 human-days of catching. Assuming that monkeys were bitten as much as humans, the entomologic inoculation rate was approximately one bite per mosquito carrying the virus every seven days, i.e., approximately 21 bites of infected mosquitoes (not necessarily infective) per monkey for the transmission season.

The natural renewal of monkey populations by birth and migrations leads to an increase in the proportion of nonimmunized monkeys. The beginning of an epizootic disease will then depend on the populations density of susceptible monkeys. These cyclical appearances are well known with other viruses such as measles, rubella, poliomyelitis, and mumps, which, however, are not transmitted by vectors.^{21,22} Among potential vectors, species of the subgenus *Aedimorphus*, which prefer to feed on cattle, may also facilitate circulation of Chikungunya virus. This is the case with *Ae. dalzieli*, which is often found infected in nature, as well as *Ae. vittatus* and *Ae. argenteopunctatus*. The observed infections in *Culex* spp. and *Anopheles* spp. seem fortuitous since isolation of the virus is rare, but *An. gambiae s.l.* and *Cx. quinquefasciatus* are not able to transmit the virus experimentally.^{23,24} This argument also applies to ticks, in which the lack of a vector role has been shown experimentally with *Ornithodoros savignyi*²⁴ and *Alectorobius sonrai*, which is a rodent ectoparasite.²⁵ These isolations are probably related

to the presence of the virus in an undigested blood meal from a viremic vertebrate.

Isolates of Chikungunya virus from zoophilic mosquitoes suggest that the virus circulates in rodents and cattle, although in Central African Republic, the role of ruminants appears to be negligible.²⁶ Isolates obtained from a squirrel, chiroptera, and ticks (*Alectorobius sonrai*), as well as the presence of antibodies specific for Chikungunya virus in rodents and birds, support the assumption that secondary wild cycles exist (Cornet M and others, unpublished data). The existence of such cycles could contribute to maintaining the virus in an endemic region while the simian populations are immunologically protected. Vertical transmission, which was never observed in nature or demonstrated in the laboratory,^{27,28} could not be taken into account in a maintenance cycle. In the Sahelian region, the absence of Chikungunya virus in Barkédji despite the presence of *Ae. aegypti*, could be explained by the scarcity of sylvatic vectors, the absence of monkeys, and by the low density of human populations and exchanges (movement of the populations during weekly market visits).

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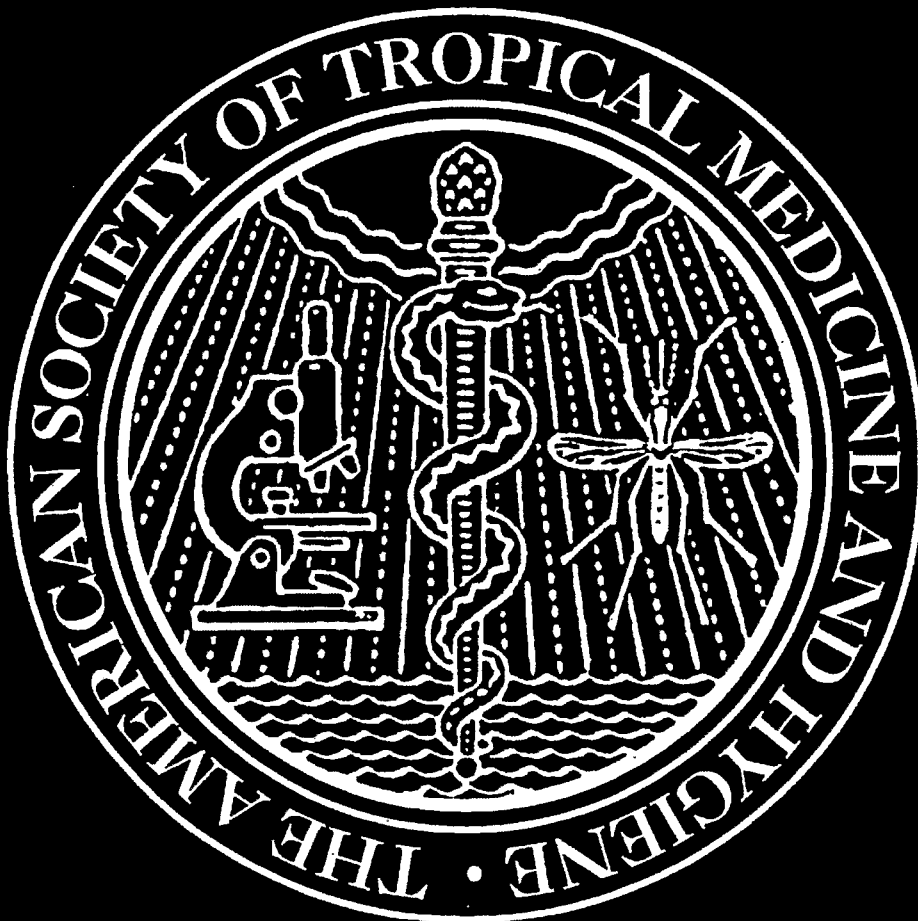
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