Surveillance for Yellow Fever Virus in Eastern Senegal During 1993

MOUMOUNI TRAORÉ-LAMIZANA,[1] DIDIER FONTENILLE,[1] HERVÉ G. ZELLER,[2] MIREILLE MONDO,[2] MAWLOUTH DIALLO,[1] FRANÇOIS ADAM,[1] MARCEL EYRAUD,[1] ABDOURAHMANE MAIGA,[2] AND JEAN-PIERRE DIGOUTTE[2]

J. Med. Entomol. 33(5): 760–765 (1996)

ABSTRACT During the 1993 rainy season, 15,806 mosquitoes, including 14,304 *Aedes* ssp., were collected and tested for virus infection in 702 and 547 pools, respectively. *Aedes furcifer* (Edwards) was the most abundant species collected througout the survey period. Yellow fever (YF) virus was detected in 187 pools: *Ae. furcifer* (123 isolates), *Ae. taylori* (Edwards) (41 isolates), and *Ae. luteocephalus* (Newstead) (23 isolates). A high prevalence of immunoglobulin (IgG) antibodies was found in human and simian populations. Results clearly indicated that increased sylvatic YF activity in eastern Senegal has the increased the risk of YF transmission among rural populations in West Africa. Our results showed that a minimal survey period may be effective in detecting the circulation of YF in the Kedougou area.

KEY WORDS *Aedes*, epidemics, yellow fever, Senegal, surveillance, forecasting

YELLOW FEVER (YF) virus was endemic in Senegal until the beginning of this century, when the discovery of the vectorial role of the yellow fever mosquito, *Aedes aegypti* (L.), in urban epidemics by Finlay (1881) made prevention through mosquito control possible. Failure to sustain source reduction control against *Ae. aegypti* led to an epidemic in Dakar in 1927 (Breteau 1954), after which vector control was renewed. During the 1927 epidemic, Mathis et al. (1928) isolated the YF virus which was attenuated in 1940 to create the Dakar strain vaccine. However, neurotropism by this vaccine in children <10 yr of age led to the termination of quadrennial vaccination in 1960. Following the epidemic which occurred in Diourbel in 1965 (Chambon et al. 1967), a search for the area of virus maintenance was undertaken in Senegal.

After it was shown that YF antibodies were present in young children in the Kedougou area (Robin et al. 1972) and that there was a sylvatic cycle of YF virus transmission in eastern Senegal (Taufflieb et al. 1973), research was carried out in West Africa to understand the development of sylvatic cycles and the genesis of epidemics. An arbovirus surveillance program was established in 1972 in the Kedougou region to forecast epidemics. This program documented the recurrence of epizootics at 4- to 6-yr cycles by the isolation of virus from mosquitoes and the detection of antibodies in simian and human sera during the rainy season. We present here surveillance results for 1993.

Materials and Methods

Study Area. The Kedougou area (Fig. 1) at 12° 11′ W, 12° 33′ N is situated in the extreme southeast of Senegal between Guinea in the south and Mali in the east. The area is hilly and contrasts with the low and flat plain, which constitutes the rest of Senegal. The hills represent the last spur of the Fouta Djalon mountains. Forest gallery generally occupies valleys. The region belongs to the sudano–guinean phytogeographic domain and is crossed by the Gambia River, which is fed by many seasonal tributaries draining the Fouta Djalon (Adam 1965). Rainfall from 1931 to 1990 averaged 1,256 mm during the rainy season from May to October. In 1993 the rains started in May, peaking in September (Fig. 2) with a total precipitation of 1,110.7 mm. Mean temperature ranged from 23.5°C in January to 33.0°C in April. Human population density is low with 2.5 inhabitants per square kilometer living in small, dispersed agricultural villages. Stations and sampling periods for adult mosquitoes were selected based on previous studies (Taufflieb et al. 1973, Cornet et al. 1978). The main mosquito collection site (Pk 10) was located 10 km north of Kedougou (Fig. 1) in forest gallery and included stations at ground level, the forest gallery with 3 platforms 6 m high and 1 platform 10 m high, and the savanna area. Four villages studied were Ngari (situated 9 km northeast of Kedougou), Kenioto (3 km northeast of Kedougou), Silling (13 km west of Kedougou), and Bandafassi (20 km west of Kedougou).

Mosquito Collection and Processing. Mosquitoes were collected from human bait[3] on 8 con-

[1] Laboratoire ORSTOM (Institut Français de Recherche Scientifique pour le Developpement en Cooperation) de Zoologie Medicale, P.O. Box 1386, Dakar, Senegal.

[2] Laboratoire des Arbovirus, Institut Pasteur, P.O. Box 220, Dakar, Senegal.

[3] Collectors were immunized against YF and protocols were approved by Comité Consultatif National d'Etique pour les Sciences de la Vie et de la Santé.

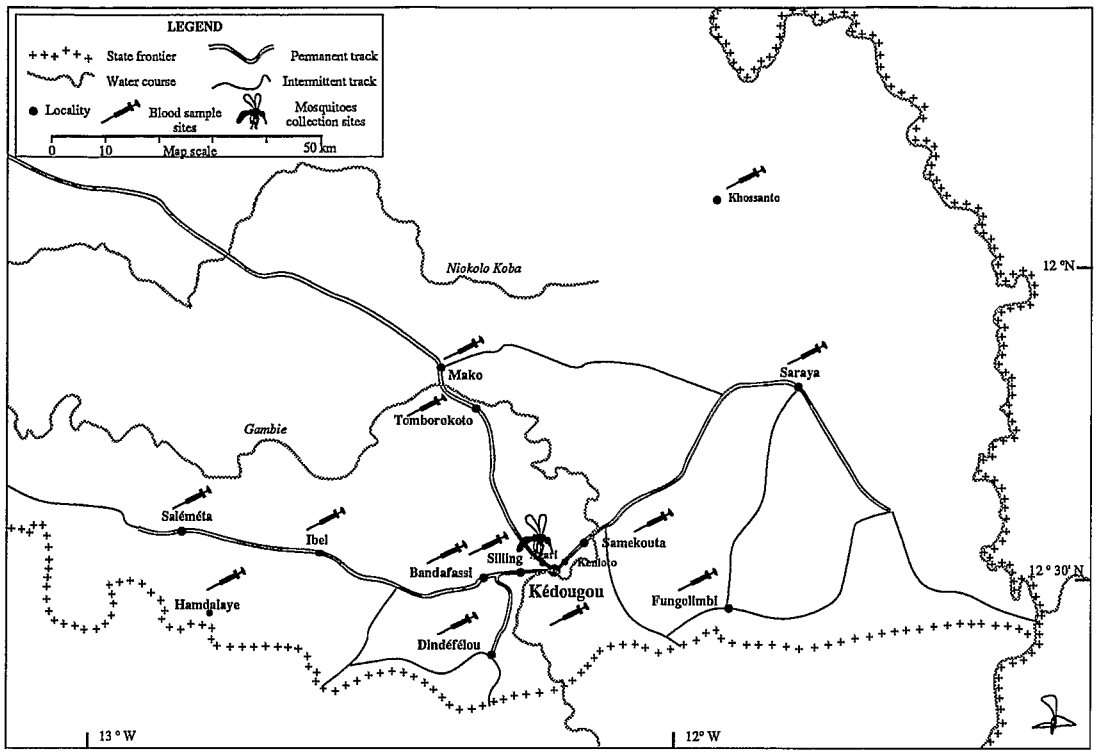

Fig. 1. Map of village sites where serological surveys and mosquitoes collection carried out.

secutive days in July, October, and November. Twenty-four volunteers (18 in 6 groups at Pk10 and 6 in 3 groups in the villages) collected mosquitoes from 1730 to 2030 hours using methods described by Traore-Lamizana et al. (1994). Mosquitoes were killed by quick freezing at −18°C, and then pooled on a chill table by species, location, and date (≤50 specimens per pool). Pools were stored in liquid nitrogen in the field and then at −70°C in the laboratory in Dakar before testing. Each pool was ground in 1 ml Leibovitz 15 medium containing 5% fetal calf sera, centrifuged, and the supernatant inoculated into AP 61 (*Aedes pseudoscutellaris* Theobald) or Vero cells as described previously (Digoutte et al. 1992). Cells were incubated at 28°C (AP61) or 37°C (Vero), and cytopathogenic effects recorded daily. Within 10 d, slides were prepared for immunofluorescence assay (IFA) against 7 immune ascitic grouping fluids for most of the African mosquito-borne arboviruses. Monoclonal YF virus antibodies were used for type determination (Digoutte et al. 1992). Other viruses were identified by complement fixation and seroneutralization tests by intracerebral inoculation into suckling mice.

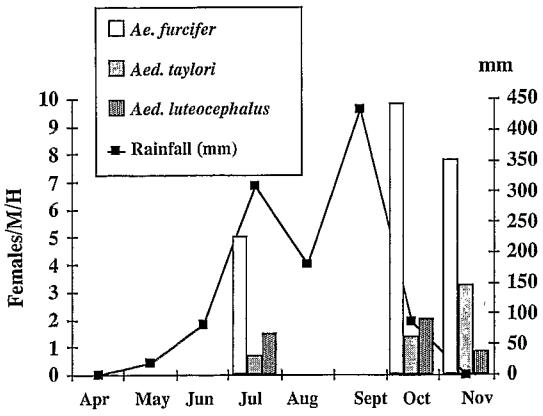

Fig. 2. Mosquito abundance (females per person per hour) and rainfall in Kedougou area, 1993.

Human and Monkey Blood Samples. Serological surveys were carried out in the villages of Fongolimbi, Samekouta, Saraya, Mako, Dindefelou, Khossanto, Silling, Hamdalaye, Bandafassi, Salemata, Tomborokoto, and Ibel in November 1993 (Fig. 1).

Blood samples also were taken in January and February 1994 from *Cercopithecus œthiops, Erytrocebus patas,* and *Papio papio* monkeys living in forest galleries and frequently visiting villages and fields in the Kedougou region.

Human and monkey blood samples were centrifuged within 4 h of collection and sera stored in liquid nitrogen in the field, and then at −70°C in Dakar before serological testing and virus isolation attemps. Positive IgG captured by YF antigen was

Table 1. *Aedes* collected in the Kedougou area in 1993, pools tested and yellow fever virus isolations

Genus	Species	Sex	July		Oct.–Nov.		
			No.	Pools	No.	Pools	
				No.		No.	+
Aedes							
(*Diceromyia*)	*furcifer*		1,908	61	6,782	171	123
		Male			130	11	
	taylori		248	12	1,652	67	39
		Male	19	4	74	10	
	furcifer-taylori		13	1			
(*Stegomyia*)	*luteocephalus*		563	18	1,149	54	25
	aegypti		25	7	31	11	
	metallicus		1	1	32	12	
	unilineatus		5	3	2	2	
	africanus		1	1			
	cozi				1	1	
	neoafricanus				6	1	
(*Aedimorphus*)	*vittatus*		612	25	105	14	
		Male	328	11	1	1	
	dalzieli		10	5	890	32	
	fowleri		55	4	3	2	
	argenteopunctatus		5	3	65	7	
	minutus		45	6	2	2	
Aedes spp.			8	6	85	18	
Anopheles spp.			74	13	220	44	
Culex ssp.			6	3	151	20	
Mansonia spp.					1,051	38	
Total			3,926	184	12,432	518	187

+, Pool was positive for YF.

detected by capture mouse immune ascitic fluids by enzyme immunoassay (EIA) (Lhuillier and Sarthou 1983). All human and monkey sera were tested for yellow fever virus specific antigen at the WHO collaborative Center for Reaseach on Arboviruses in Dakar. The positivity background was calculated following the methods of Lhuillier et al. (1985), based on the kinetics of yellow fever IgM.

Results

Overall, 16,358 mosquitoes in 4 genera and 39 species were collected in 1993 and processed in 702 pools (Table 1). *Aedes* mosquitoes represented 91% of all mosquitoes collected; 73% of the *Aedes* were *Diceromyia*, 12% *Stegomyia*, and 14% *Aedimorphus*.

Most *Diceromyia* (82%) were *Ae. furcifer* (Edwards) and 18% were *Ae. taylori* (Edwards). *Ae. luteocephalus* (Newstead) comprised 94.3% of *Stegomyia*, followed by *Ae. aegypti* 3.1%; other species were rare. *Ae. taylori* was the least abundant in July (0.7 females per person per hour) but increased in October and November (1.7 and 3.2). *Ae. furcifer* was the most abundant human biting species (5.0 females per person per hour in July, 9.8 in October, and 7.8 in November). *Ae. luteocephalus* followed the same pattern (1.8 in July, 2.0 in October, and 0.8 in November).

In total, 187 YF isolates were obtained during the October–November period from *Ae. furcifer*, *Ae. taylori*, and *Ae. luteocephalus* (Table 1). These 3 species comprised 58% of the total pools tested. YF virus was isolated from 72, 58, and 46% pools of *Ae. furcifer*, *Ae. taylori*, and *Ae. luteocephalus*, respectively, collected in October and November. No viruses were found in July. The minimal field infection rate for the entire study was 14.2 per 1,000 females tested for *Ae. furcifer*, 20.5 for *Ae. taylori*, and 14.6 for *Ae. luteocephalus*. YF isolates were recovered in October and November from *Ae. furcifer* (123 isolates) collected at all sites, and from *Ae. taylori* (39 isolates) and *Ae. luteocephalus* (25 isolates) at Pk 10 sites. In Ngari village, 9 of 12 pools collected in October and November were positive; minimal field infection rate was 19.7. The man biting rate by *Ae. furcifer* during these 2 mo totalled 2,310. Four pools containing both Zika and YF viruses were collected in October from *Ae. furcifer* (2 isolates) and from *Ae. luteocephalus* (2 isolates) at sites 2, 4, 5, and Ngari. Overall, 3,820 adults from

Table 2. Human serologic results

Age	No.	YF IgG%
0–5	5	80.0
6–10	83	51.8
11–15	60	71.6
16–20	31	77.4
21–30	74	90.5
31–40	71	84.5
41–50	49	89.8
>50	38	84.2
Total	411	77.1

Table 3. Simian serologic results

	C. aethiops		*E. patas*		*P. papio*	
Age	No.	Yf IgG	No.	YF IgG	No.	YF IgG
Baby	7	5	0	0	2	2
Young	19	19	5	5	3	3
Adult	14	13	3	1	0	0
Total, %	40	37 92.5	8	6 75.0	5	5 100%

3 other genera in 293 pools were processed for virus with negative results for yellow fever virus.

Serological Results. Of 411 human sera tested from 4 villages (Saraya, Silling, Bandafassi, and Fongolimbi), 317 were positive for IgG against YF virus; none were IgM positive (Table 2). Among 53 monkeys collected, 48 were positive for IgG, and 1 young *C. aethiops* male was positive for IgM (Table 3).

Discussion

During this short-term arbovirus survey at Kedougou, no collection were made in August and September. The 1st collections were made in July, and the 2nd in October, after virus amplification. Minimal field infection rates increased by 26% from October to November for *Ae. luteocephalus,* and 25% for *Ae. furcifer,* and decreased by 25% for *Ae. taylori. Ae. furcifer* and *Ae. luteocephalus* abundance decreased rapidly, whereas *Ae. taylori* abundance continued to increase from October to November. Like Cornet et al. (1978), we confirmed that *Ae. luteocephalus* disappeared more quickly after the rains than *Diceromyia* ssp. *Ae. furcifer* human biting rates clearly were greater than those of *Ae. taylori* at Ngari and Silling villages. *Ae. furcifer* was abundant in these villages and at the savanna site in October and November.

All YF virus isolations were made from *Ae. furcifer, Ae. taylori,* and *Ae. luteocephalus.* These species were incriminated previously as vectors in the YF virus sylvatic cycle in this region (Cornet et al. 1979a, b). No isolations were made from *Ae. aegypti* or *Ae. metallicus* (Edwards). Virus was not isolated in July, indicating the absence or low level of virus circulation at the beginning of the rainy season. Viral amplification was rapid, and YF was isolated from 73% of the mosquitoes pooled at the end of the rainy season. *Ae. furcifer* because of its elevated biting rates and its high infection rate was considered to be the main vector.

At the end of the rainy season, YF virus was found at all the villages surveyed, some of them 15 km apart, indicating that the epizootic had a wide distribution. *Ae. furcifer* was the only species abundantly biting humans in the villages. There, the biting rate of infected, but not necessarily infective, mosquitoes was ≥45 per person per hour during early evenings. It is probable that every person in the study villages received several virus inoculations during the 1993 rainy season. However, clinical cases of YF were not reported by the hospitals and dispensaries in the region. Lack of human cases was attributed to the elevated antibody rates in the village population. The presence of IgG in humans indicated a previous vaccination or infection. Desgrées du Loû and Pison (1994), in a

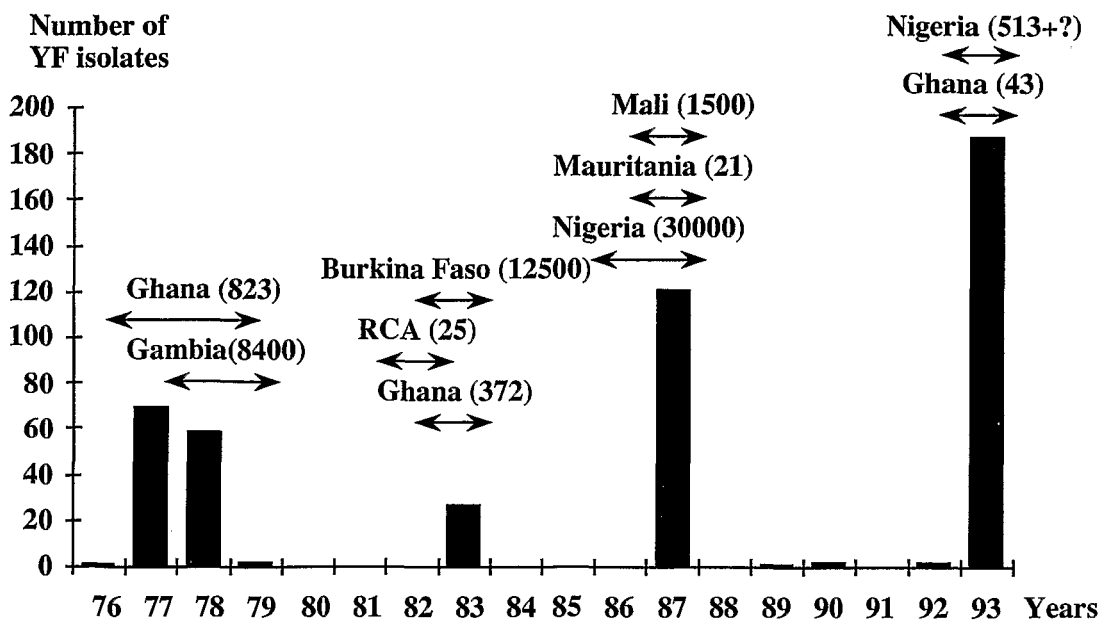

Fig. 3. Yellow fever isolates from mosquitoes collected in southeastern Senegal plotted with the duration of YF epidemics and human cases number in West Africa (1976–1993).

survey of vaccine coverage in the Kedougou area, found that 91% of 0- to 10-y-old children were vaccinated in the village of Bandafassi, and in the area only 74% of person aged ≥15 y were vaccinated at least once. In monkeys, IgG indicated previous contact with the flavivirus, whereas IgM indicated recent infection with YF virus (e.g., the young *C. aethiops*).

Natural vertical transmission of YF virus has been demonstrated previously in eastern Senegal by virus isolation from pools of *Ae. furcifer–taylori* males (Cornet et al. 1979b). However, 2 strains of virus were isolated from *Ae. taylori* in November 1992, indicating the transmission of YF virus during the year before the present epizootic. YF virus was not isolated from 25 pools of males collected in November 1993. Similarly, virus was not isolated from 266 mosquito pools collected in July 1994; but 7 pools were positive for YF in October and November (data not shown). These data indicate that the rate of vertical transmission probably was low both before and after the 1993 epizootic.

The year 1993 was particularly wet with 1,107 mm of rain, as compared with 879 mm in 1992. Nevertheless, because the rainfall was greater before July 1992 (504.7 mm) than during July 1993 (253.1 mm), mosquito catches in July 1992 were 2 times greater for *Ae. furcifer* females, 5 times greater for *Ae. taylori,* and 2 times greater for *Ae. luteocephalus* compared with 1993 (data not shown). In contrast during October and November 1993, greater catches of *Ae. furcifer, Ae. taylori,* and *Ae. luteocephalus* (20, 2, and 3 times, respectively) were made per day compared with those from October and November 1992. Many authors have noted the positive relationship between increased rainfall and mosquito abundance, especially for species such as *Ae. luteocephalus* whose larval container habitats are filled by rain water. Rickenbach et al. (1971) and Cordellier and Geoffroy (1972) noted that the female abundance lagged 2 mo behind increases a rainfall. Mondet and Montagne (1993) showed that the role of rainfall is complex and is based on daily (not monthly) quantity, temporary distribution, and their total.

Arbovirus activity has been monitored routinely at Kedougou since 1970, based principally on mosquito catches and testing blood samples from monkeys. Results obtained from 1970 to 1980 indicated that October and November were the 2 mo having highest mosquito infection rates. Since 1989, surveillance has emphasized mosquito collections during 10 d in July, October, and November and a serological survey of the human population in November. A striking correlation exists between increased YF virus isolations during this period from mosquitoes captured in Senegal and YF epidemics in humans in western Africa (Fig. 3).

We conclude that an increase of yellow fever activity in the sylvatic cycle in eastern Senegal may forecast epidemics in surrounding West African countries. The Kedougou emergence zone may serve as an indicator for West Africa. Early detection of elevated sylvatic circulation of YF virus may provide sufficient time to prevent urban YF epidemics through vaccination campaigns.

Acknowledgments

We thank M. Diallo, S. Ba, and G. Biankhinche for their technical assistance, the Father Cousseau of Kedougou Catholic Mission for his active cooperation, his assistance and help for this study by his great knowledge of the population of Kedougou areas. This work was supported by the Institut Français de Recherche Scientifique pour le Developpement en Cooperation (ORSTOM) and by the Institut Pasteur of Dakar.

References Cited

Adam, J. G. 1965. Généralités sur la flore et la végétation du Sénégal. Etud. Senegal. 9: 155–214.

Breteau, H. 1954. La fièvre jaune en Afrique occidentale française. Un aspect de la mèdecine préventive massive. Bull. WHO 11: 453–481.

Chambon, L., I. Wone, P. Bres, M. Cornet, C. Ly, A. Michel, A. Lacan, Y. Robin, B. E. Henderson, K. H. Williams, R. Caiman, D. Lambert, M. Rey, I. Diop Mar, J. L. Oudart, G. Causse, H. Ba, M. Martin, and J. C. Artus. 1967. Une épidémie de fièvre jaune au Sénégal en 1965. L'épidémie humaine. Bull. WHO 36: 113–150.

Cordellier, R., and B. Geoffroy. 1972. Observations sur les vecteurs potentiels de fièvre jaune en République Centrafricaine. Cah. O.R.S.T.O.M. Ser. Entomol. Med. Parasitol. 10: 127–144.

Cornet, M., Y. Robin, C. Hannoun, B. Corniou, P. Bres, and G. Causse. 1968. Une épidémie de fièvre jaune au Sénégal en 1965. Recherches épidémiologiques. Bull. WHO 39: 845–858.

Cornet, M., R. Chateau, M. Valade, P. L. Dieng, M. Raymond, and A. Laurand. 1978. Données bioécologiques sur les vecteurs potentiels du virus amaril au Sénégal oriental. Rôle des différentes espèces dans la transmission du virus. Cah. ORSTOM. Ser. Entomol. Med. Parasitol. 16: 315–341.

Cornet, M., Y. Robin, R. Chateau, G. Heme, C. Adam, M. Valade, G. Le Gonidec. C. Jan, J. Renaudet, P. L. Dieng, J. F. Bangoura, and A. Roland. 1979A. Isolements d'arbovirus au Sénégal oriental à partir de moustiques (1972–1977) et notes sur l'épidémiologie des virus transmis par les *Aedes* en particulier du virus amaril. Cah. ORSTOM Ser. Entomol. Med. Parasitol. 17: 149–163.

Cornet, M., Y. Robin, G. Heme, C. Adam, J. Renaudet, M. Valade, and M. Eyraud. 1979B. Une poussée épizootique de fièvre jaune selvatique au Sénégal oriental. Isolement du virus de lots de moustiques adultes males et femelles. Med. Maladie Infect. 9: 63–66.

Desgrées du Loû, A., and G. Pison. 1994. Barriers to universal child immunization in rural Senegal 5 years after the accelerated Expanded Programme on Immunization. Bull. WHO 72: 751–759.

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

Finley, C. L. 1881. El mosquito hypoteticamente considerado como agente de transmision de la fiebre amarilla. Anales de la reale Academia de ciencas de la Habana, 18: 147–169.

Lhuillier, M., and J. L. Sarthou. 1983. CHROM-ELISA: a new technique for rapid identification of arboviruses. Ann. Virol. 13E: 339–347.

Lhuillier, M., J. L. Sarthou, R. Cordellier, G. M. Gershy-Damet, N. Monteny, B. Bouchite, and P. Calen. 1985. Epidémie rurale de la fièvre jaune avec transmission interhumaine en Côte d'Ivoire en 1982. Bull. WHO 63: 527–536.

Mathis, C., A. W. Sellards, and J. Laigret. 1928. Sensibilité du *Macaca rhesus* au virus de la fièvre jaune. C. R. Acad. Sci. (Paris) 188: 604–606.

Mondet, B., and L. Montagne. 1993. Presence en Côte d'Ivoire, variations saisonnières et statut épidémiologique d'*Aedes (Stegomyia) usambara* Mattingly, 1953 (Diptera: Culicidae). Ann. Soc. Entomol. Fr. 29: 261–267.

Rickenbach, A., L. Ferrara, M. Germain, J. P. Eouzan, and J. P. Button. 1971. Quelques données sur la biologie de trois vecteurs potentiels de fièvre jaune *Aedes (Stegomyia) africanus* (Theo.), *Ae. (S.) simpsoni* (Theo.) et *Ae. (S.) aegypti* (L.) dans la région de Yaounde (Cameroun). Cah. ORSTOM Ser. Entomol. Med. Parasitol. 9: 285–299.

Robin, Y., R. Taufflieb, and M. Cornet. 1972. Passé, présent et devenir possible de l'épidémiologie de la fièvre jaune et de sa surveillance en Afrique de l'Ouest francophone. Cah. ORSTOM Ser. Entomol. Med. Parasitol. 10: 95–98.

Taufflieb, R., M. Cornet, G. Le Gonidec, and Y. Robin. 1973. Un foyer selvatique de fièvre jaune au Sénégal oriental. Cah. ORSTOM Ser. Entomol. Med. Parasitol. 11: 211–220.

Traoré-Lamizana, M., H. Zeller, E. Monlun, M. Mondo, J. P. Hervy, F. Adam, and J. P. Digoutte. 1994. Dengue 2 outbreak in Southeastern Senegal during 1990: virus isolations from mosquitoes (Diptera:Culicidae). J. Med. Entomol. 31: 623–627.

Received for publication 9 June 1995; accepted 6 March 1996.