Am. J. Trop. Med. Hyg., 62(1), 2000, pp. 151–156
Copyright © 2000 by The American Society of Tropical Medicine and Hygiene

VERTICAL TRANSMISSION OF THE YELLOW FEVER VIRUS BY *AEDES AEGYPTI* (DIPTERA, CULICIDAE): DYNAMICS OF INFECTION IN F_1 ADULT PROGENY OF ORALLY INFECTED FEMALES

MAWLOUTH DIALLO, JOCELYN THONNON, AND DIDIER FONTENILLE

Institut Pasteur, Dakar, Senegal; Laboratoire de Zoologie Médicale, Institut de Recherche pour le Développement, Institut Pasteur, Dakar, Senegal

Abstract. Vertical transmission of yellow fever virus from orally infected females to their progeny was experimentally demonstrated in 2 *Aedes aegypti* colonies from the Dakar and Koungheul regions in Senegal. A total of 10,530 F_1 adult mosquito progeny were tested. The overall vertical transmission rate was 0.97%, with no significant difference between the Dakar and Koungheul colonies. The infection rates were significantly higher in females (1.15%) than in males (0.74%) in both colonies. The virus was not isolated from the progeny of the first oviposition cycle (OVC1). The true infection rates were 0.27% and 1.99%, respectively, for the OVC2 and OVC3 progeny in the Dakar colony, and 1.1% and 1.48%, respectively, for the OVC2 and OVC3 progeny in the Koungheul colony. The infection rates increased with extrinsic incubation in both male and female offspring of the 2 colonies, reaching 5.2% in 20-day-old OVC3 female progeny in the Dakar colony. The epidemiologic consequences of these results are discussed.

In 1995, a yellow fever epidemic occurred in Koungheul, Senegal.[1] Entomologic surveys provided evidence of vertical transmission of the yellow fever virus by its mosquito vector, *Aedes aegypti.* The virus was isolated from wild males and recently emerged adults from larvae collected in the field.[2] Vertical transmission of yellow fever virus has already been proved experimentally in *Ae. aegypti,*[3,4] and 3 strains of this virus have been isolated from male *Ae. furcifer-taylori* mosquitoes.[5] The capacity of other mosquito species to vertically transmit yellow fever virus has also been shown experimentally in *Ae. mascarensis* and *Haemagogus equinus.*[6,7]

The studies conducted in Koungheul on *Aedes* mosquitoes collected in the field had shown that the infection rate in recently emerged adults was lower than that in males caught on humans. This observation suggested that the viral titer in emerging adults infected by vertical transmission was low and hard to detect with conventional isolation methods, whereas the older mosquitoes had a higher titer. The objective of this study was to study the dynamics of viral infection in emerging adult mosquitoes vertically infected, using the same isolation methods as those used during the Koungheul epidemic.

MATERIALS AND METHODS

Mosquitoes. Two geographic strains of *Ae. aegypti* from Dakar, and Koungheul, Senegal were used. These colonies were reared at 27 ± 1°C and a relative humidity of 70–80% with a 12-hr photoperiod. Adult mosquitoes were regularly fed on guinea pig blood or 10% sugar and larval stages were provided with Tetra Baby Fish Food® (TetraWerke, Melle, Germany).

Virus. The viral strain used was ArD 114891. It was isolated from a pool of 10 *Ae. aegypti* females caught on humans in the village of Koung-Koung during the epidemic that occurred in Koungheul, Senegal in 1995. This strain was passed twice on AP61 cell lines (*Ae. pseudoscutellaris*) and 3 times on suckling mice. The viral stock that we used in our experiments was prepared with triturated, infected suckling mice brains. It was titrated by inoculation of serial 10-fold dilutions into suckling mice. The titer was calculated by the method of Reed and Muench.[8] The titer obtained was 8.5 log 50% lethal doses (LD_{50}s)/0.02 ml.

Oral infection of mosquitoes. Fertilized, 3–5-day-old female mosquitoes that had never taken a blood meal were used. The F_{10} generation mosquitoes from the Dakar colony and F_4 generation mosquitoes from the Koungheul colony were used. Selected females were placed in cylindrical cardboard cages and starved for 24 hr before the infectious meal. The mosquitoes were then allowed to feed on an infectious meal through a chicken skin membrane according to the artificial feeding method described by Rutledge and others.[9] The infectious meal consisted of two-thirds washed rabbit erythrocytes and one-third viral suspension. Adenosine triphosphate was added at a final concentration of 5×10^{-3} M as a phagostimulant. The virus-blood suspension was left on the cage for 1 hr.

Experimental procedure. At the end of their meal, the mosquitoes were cold-anesthetized, sorted according to their state of stomacal repletion, and placed in groups in rearing cages. Only fully engorged females were preserved and maintained at 27 ± 1°C and a relative humidity of 70–80% for extrinsic incubation of the virus. An ovitrap was placed in the cage and withdrawn at the end of each oviposition cycle (OVC). After each oviposition, a guinea pig was placed on top of the cage to allow the mosquitoes to have a blood meal and to achieve a new gonotrophic cycle.

After 20 days of incubation and after achieving 3 oviposition cycles, surviving mosquitoes were killed and stored at −70°C until virus assays. They were then individually triturated in Leibovitz 15 medium containing antibiotics (Fungizone® [Gibco-BRL, Paisley, Scotland] and streptomycin) and 30% fetal calf serum. The suspension was centrifuged at 8,500 × *g* for 30 min at 4°C. The supernatant was filtered and inoculated into AP61 cell culture lines to test for the presence of yellow fever virus.

Eggs obtained from each oviposition cycle were kept separate from each other and wet 24 hr to ensure embryonic development. They were then dried at ambient temperature before being hatched in water. The larvae were reared at

Fonds Documentaire IRD
010022260

Fonds Documentaire IRD
Cote : B* 22260 Ex : 1

TABLE 1

True infection rates of the F_1 adults progeny of orally infected *Aedes aegypti* females from Dakar, Senegal according to the sex and the gonotrophic cycle

Ovipositions		Sex	Number of tested mosquitoes	Number of tested pools	Number of positive pools	TIR*
1		Males	460	46	0	–
(2–4)†		Females	540	54	0	–
	Subtotal		1,000	100	0	–
2		Males	680	68	1	0.15 (0.15)
(6–9)†		Females	1,170	117	4	0.35 (0.17)
	Subtotal		1,850	185	5	0.27 (0.12)
3		Males	970	97	14	1.55 (0.41)
(14–18)†		Females	1,340	134	28	2.32 (0.43)
	Subtotal		2,310	231	42	1.99 (0.30)
	Total males		2,110	211	15	0.73 (0.19)
	Total females		3,050	305	32	1.10 (0.19)
	Total		5,160	516	47	0.95 (0.14)

* TIR = true infection rate (estimated number of positive mosquitoes per 100 mosquitoes tested) according to Chiang and Reeves.[11] Values in parentheses are standard errors.
† Days after an infectious meal.

30°C. After emergence, the F_1 adults were reared in the same experimental conditions as their parents and fed only on 10% sucrose. Specimens that emerged on the same day were put in the same cage.

At variable periods of time after emergence (days 0, 2, 5, 10, 15, and 20), mosquito progeny were cold-killed and pooled by sex. Pools of 10 individuals were formed and stored at −70°C until assayed for virus. Mosquito pools were triturated and inoculated into AP61 cell culture lines for virologic testing using the same methods as those used for field studies.[2] Yellow fever virus was detected by indirect immunofluorescence using immune ascites following methods described by Digoutte and others.[10]

Methods of analysis. The true infection rate (TIR, number of positive mosquitoes per 100 mosquitoes tested) was calculated with pools of same size using the methods of Chiang and Reeves[11] and Walter and others.[12] Because estimation of infection by the TIR is superior or at least equal to that by the minimum infection rate, we have used the TIR. Computation and analysis of the TIRs was done for progeny of both infected and uninfected female parents. The effect of sex, colony and OVC on the TIR was compared using the chi-square test with P values <0.05 considered statistically significant.

RESULTS

For the 2 *Ae. aegypti* populations, 10,530 F_1 progeny of infected females were tested for infection by yellow fever virus. Yellow fever virus was found associated with 98 of the 1,053 mosquito pools constituted.

The true infection rate was 0.97 ± 0.09% (±SE) for all mosquitoes tested. Of the 4,580 male offspring tested, the vertical transmission rate was 0.74 ± 0.13%. Of the 5,950 female offspring tested, the vertical transmission rate was 1.15 ± 0.14%. The difference in the infection rate between males and females was statistically significant ($\chi^2 = 4.33$, $P = 0.037$).

***Aedes aegypti* colony from Dakar.** The first OVC eggs of infected females were obtained between days 2 and 4 after the infectious meal, the second OVC eggs were obtained between days 6 and 9, and the third OVC eggs were obtained between days 14 and 18. Of 91 females that survived after 3 OVCs, 74 (81.3%) were infected with yellow fever virus.

A total of 5,160 F_1 adult progeny (2,110 males and 3,050 females) were tested. Yellow fever virus was identified in 47 of the 516 pools of F_1 adult progeny. Among the pools of infected mosquito progeny, 31.9% were obtained from pools of male mosquitoes and 68.1% were obtained from pools of female mosquitoes (Table 1). The infection rate of all tested pools was estimated to be 0.95%. No significant difference in the infection rate was observed between males and females ($\chi^2 = 1.86$, $P = 0.17$).

Table 1 also shows the infection rates obtained in adult progeny according to the oviposition cycle. Vertical transmission was not observed in the OVC1 progeny. In the OVC2 progeny, virus was isolated only from 2 mosquito pools made on the day of emergence and from 3 mosquito pools made 20 days after emergence. The true infection rate was 0.27% in the OVC2 progeny and 1.99% in the OVC3 progeny. However, there was no significant difference between males and females in both cases. A significant difference was observed between the true infection rate of the OVC2 progeny and that of the OVC3 progeny ($\chi^2 = 25.13$, $P < 0.0001$).

The infection rates obtained varied according to the age of the mosquitoes. The dynamics of virus infection were observed only in F_1 adults of the third gonotrophic cycle (Figure 1). Up to 10 days after emergence, the infection rates were low (<2%). Beyond 10 days after emergence, the infection rates were higher with maximums of 6.71 ± 6.6% and 5.25 ± 2.3% for 20-day-old males and females, respectively. There was a positive correlation between the infection rates and the age of the mosquitoes after emergence in both male and female progeny ($r^2 = 0.862$, $F = 18.76$, $P = 0.023$ and $r^2 = 0.905$, $F = 38.05$, $P = 0.0035$ for females and males, respectively).

***Aedes aegypti* colony from Koungheul.** The first OVC eggs of infected females were obtained between days 2 and 6 after the infection meal, the second OVC eggs were ob-

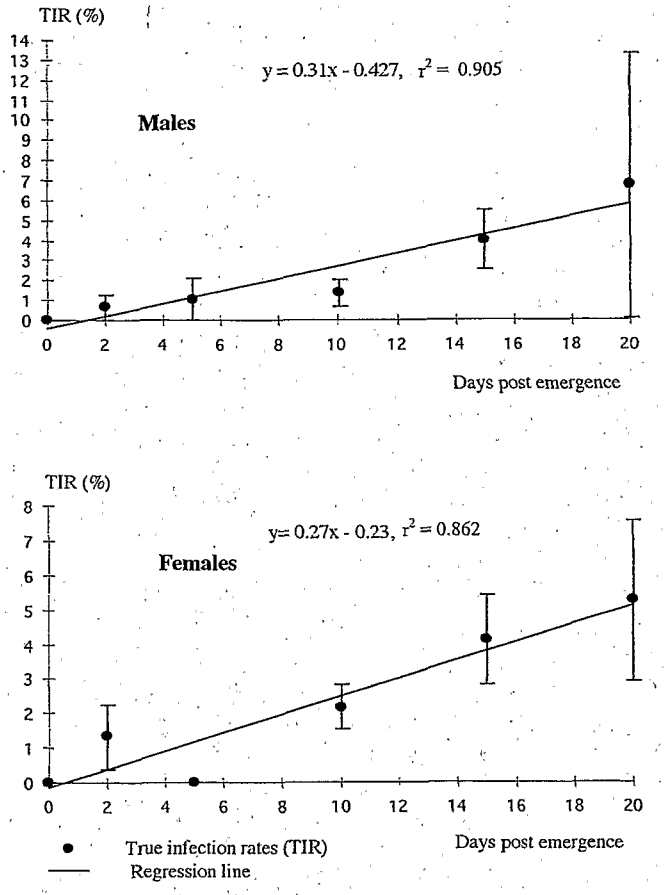

FIGURE 1. Correlation between true infection rates and age of the progeny of the third oviposition cycle of an *Aedes aegypti* colony from Dakar, Senegal orally infected with yellow fever virus.

tained between days 8 and 12, and the third OVC eggs were obtained between days 14 and 20. Of 115 females that survived after 3 OVCs, 103 (89.56%) were infected with yellow fever virus.

A total of 5,370 F_1 adult progeny (2,470 males and 2,900 females) were tested (Table 2). Among the 537 pools made, 51 were infected with yellow fever virus. The overall vertical transmission rate was estimated to be 0.99%. No significant difference was observed between the infection rates of males and females ($\chi^2 = 2.08$, $P = 0.15$).

Vertical transmission was not detected in the OVC1 progeny. The vertical transmission rate was 1.1% in the OVC2 progeny and 1.48% in the OVC3 progeny. No significant difference was observed between infection rates of the OVC2 and OVC3 progeny ($\chi^2 = 1.37$, $P = 0.24$). Similarly, the difference in infection rates between males and females were not significant ($\chi^2 = 1.75$, $P = 0.18$ and $\chi^2 = 1.34$, $P = 0.25$ for the OVC2 and OVC3 progeny, respectively).

Figures 2 and 3 show yellow fever virus infection in the progeny of females infected according to their age after emergence. It was observed that regardless of the stage of the gonotrophic cycle and the sex of the mosquito, a positive correlation was obtained between the vertical transmission rate and the age of the mosquitoes after emergence ($r^2 = 0.958$, $F = 91.16$, $P = 0.0007$ and $r^2 = 0.915$, $F = 43.14$, $P = 0.0028$ in males and females, respectively, of the OVC2 progeny and $r^2 = 0.705$, $F = 9.54$, $P = 0.037$ and $r^2 = 0.698$, $F = 9.24$, $P = 0.038$ in males and females, respectively, of the OVC3 progeny).

Comparison between the 2 colonies of *Ae. aegypti*. Comparative analysis of the results showed no significant differences between the infection rates of parent females of the 2 *Ae. aegypti* populations from Dakar and Koungheul orally infected with yellow fever virus ($\chi^2 = 2.86$, $P = 0.091$). This was also true for the vertical transmission rates obtained from the OVC3 progeny, which were similar in the 2 colonies ($\chi^2 = 1.46$, $P = 0.228$). The only difference observed was in the infection rates of the OVC2 progeny, which were significantly higher in the *Ae. aegypti* colony from Koungheul ($\chi^2 = 9.675$, $P = 0.002$).

The highest infection rates were obtained with the Dakar colony on day 20. These rates were 6.71% in males and 5.25% in females (Figure 1). The maximum infection rate in the Koungheul colony was 3.13%. Except for the OVC3 female progeny of the Koungheul colony, the highest replication rates were obtained from the tenth day onward after emergence.

TABLE 2

True infection rates of the F_1 adults progeny of orally infected *Aedes aegypti* females from Koungheul, Senegal according to the sex and the gonotrophic cycle

Ovipositions		Sex	Number of tested mosquitoes	Number of tested pools	Number of positive pools	TIR*
1		Males	500	50	0	–
(2–6)†		Females	580	58	0	–
	Subtotal		1,080	108	0	–
2		Males	1,140	114	9	0.82 (0.27)
(8–12)†		Females	1,340	134	17	1.35 (0.32)
	Subtotal		2,480	248	26	1.10 (0.21)
3		Males	830	83	9	1.14 (0.38)
(14–20)†		Females	980	98	16	1.76 (0.44)
	Subtotal		1,810	181	25	1.48 (0.29)
	Total males		2,470	247	18	0.80 (0.18)
	Total females		2,900	290	33	1.20 (0.21)
	Total		5,370	537	51	0.99 (0.14)

* TIR = true infection rate (estimated number of positive mosquitoes per 100 mosquitoes tested) according to Chiang and Reeves.[11] Values in parentheses are standard errors.
† Days after an infectious meal.

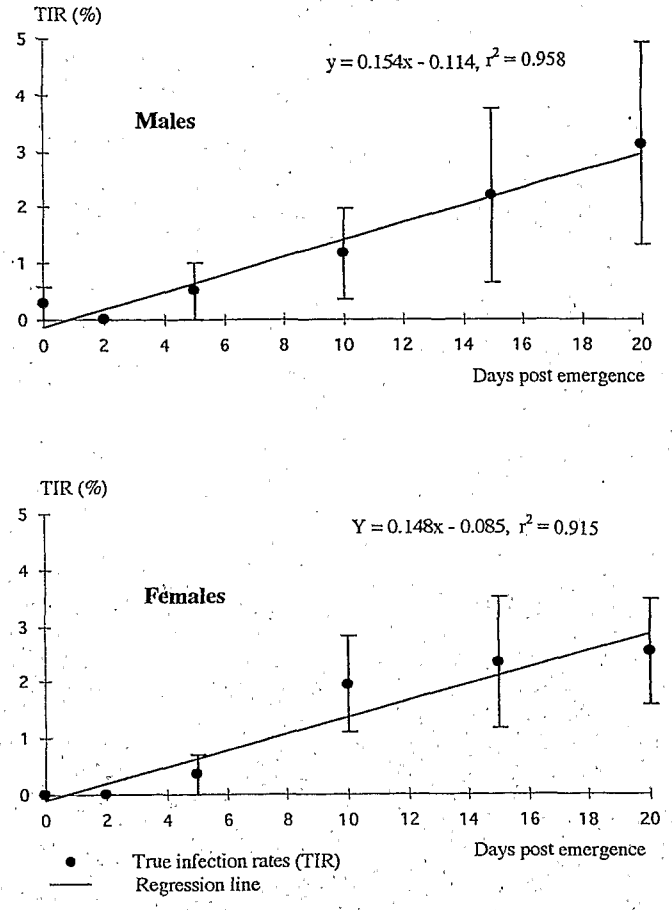

FIGURE 2. Correlation between true infection rates and age of the progeny of the second oviposition cycle of an *Aedes aegypti* colony from Koungheul, Senegal orally infected with yellow fever virus.

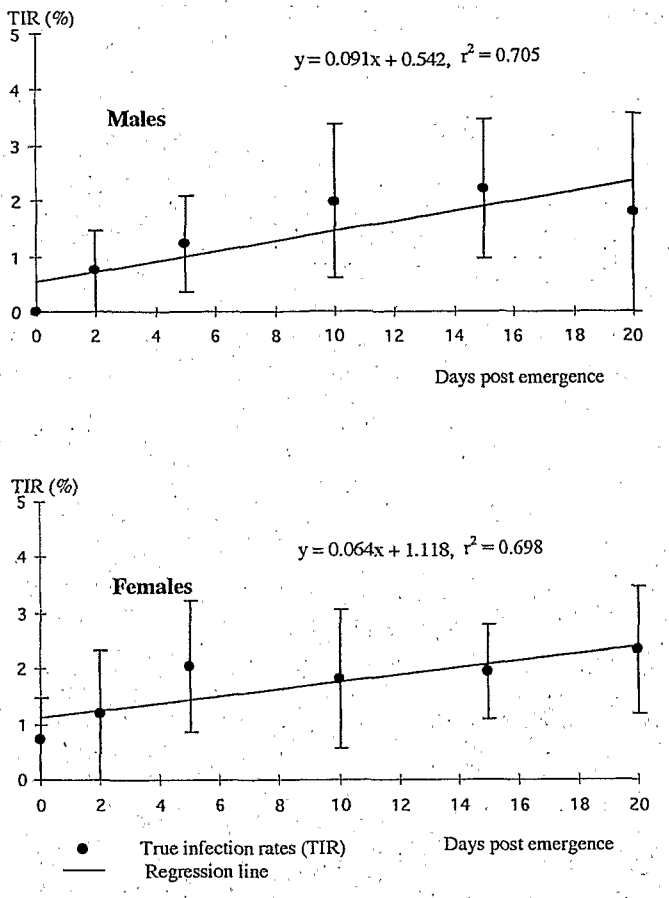

FIGURE 3. Correlation between true infection rates and age of the progeny of the third oviposition cycle of an *Aedes aegypti* colony from Koungheul, Senegal orally infected with yellow fever virus.

DISCUSSION

The results presented show that orally infected *Ae. aegypti* populations from Dakar and Koungheul can transmit yellow fever virus vertically. The infection rates obtained with orally infected females show that the *Ae. aegypti* colonies used were highly susceptible to yellow fever virus, in contrast to what Tabachnick and others observed with 2 sylvan Senegalese colonies of *Ae. aegypti.*[13] This species is present in Senegal in 2 forms: a wild form, *Ae. aegypti formosus,* which has never been found associated with yellow fever virus, and a domestic form, *Ae. aegypti aegypti,* which is a proven vector of this virus.[2]

The vertical transmission rates were relatively high compared with results obtained by other investigators.[4,6] However, they were lower than those obtained in nature with adult males caught on humans (31.42 ± 15.7%).[2] *A priori,* the method of infecting the female parents could not account for these differences since it has been shown with dengue virus[14] and San Angelo virus and Kunjin virus[15] that the infection rates in progeny showed very little difference regardless of whether the female mothers were infected orally or intrathoracically. The geographic origins of the *Ae. aegypti* colonies used could partly explain the differences in infection rates obtained by the various investigators. Indeed, the susceptibility to arboviruses depends on the geographic origin of the mosquito populations and is under genetic control,[16,17] although Beaty and others[6] noted only minor differences in vertical transmission compared with *Ae. aegypti* colonies from Africa, Asia, and North America (Santo Domingo).

The results show that the vertical transmission rate was not significantly different between the 2 colonies ($\chi^2 = 0.04$, $P = 0.845$). A difference was observed only in OVC2 progeny, which were significantly more infected in the Koungheul colony. It was also observed that the vertical transmission rates were higher in females than in males. The venereal transmission of infected males to females previously demonstrated[18,19] could account for these differences. In addition to females that have inherited the virus from their parents, it would also include female progeny that do not carry the virus at their emergence but become contaminated while mating with infected males.

Vertical transmission experiments conducted earlier with yellow fever viruses and dengue 1 virus showed that the vertical transmission rates decreased with the successive gonotrophic cycles of infected mosquitoes.[6,14] In contrast, our study has shown an increasing TIR dependent on the successive gonotrophic cycles. The highest infection rates we obtained were observed in the last gonotrophic cycles. However, a comparative analysis of our results with those

of previous studies[4,6] showed that vertical transmission takes place in the same time period and would depend more on the time of infection of parental female genitalia than on the oviposition cycle. A hypothesis that would account for this difference would be only speculative because the transmission mechanisms are still unclear. For yellow fever virus, the vertical transmission mechanism has never been previously studied. However, it has been shown with 3 other flaviviruses that infection was not transovarian, but occurred in mature eggs, in contrast to what was observed by other investigators, in which follicular epithelium, nurse cells, and oocytes were infected.[20] In addition, these contradictory results were also observed in the La Crosse virus–*Ae. triseriatus* model. Using this model, Watts and others[21] demonstrated the vertical transmission of the La Crosse virus by *Ae. triseriatus,* but subsequent studies did not isolate virus from the OVC1 larvae.[22]

The absence of vertical transmission in adults emerging from the first gonotrophic cycle could be related to the dynamics of virus replication in the genitalia of the orally infected females. For yellow fever virus, the first oviposition cycle probably occurred before ovaries were infected. The low infection rates of individuals emerging from the second cycle achieved 6–9 days after the infectious meal in the *Ae. aegypti* populations from Dakar could be explained by an initial infection in the ovaries at the time of oviposition. An earlier, complete ovarian infection would account for the higher infection rate observed in individuals emerging from the same oviposition cycle in the Koungheul colony.

The gradual multiplication of the virus in the mosquitoes that were infected by vertical transmission explains the increase in infection rates with time, which has previously been suggested by Rosen and others with dengue 1 virus and *Ae. albopictus*[14] and by our own field observations.[2] Even if the virus was not found in some of the mosquito pools that had just emerged, it is possible that these mosquitoes were infected. The negative results obtained from these mosquitoes could be due to a low virus titer in the pools and/or to inadequate sensitivity of our isolation method. Although the rates of vertical transmission we obtained are sometimes very high, they were below the infection rates obtained with adult males caught in the field. The highest infection rates we obtained were 6.71% and 5.25% for males and females, respectively, from the Dakar colony at 20 days post-emergence. If our results showed an increase in infection rates during the period of mosquito development, they would not by themselves account for the high vertical transmission rates obtained during the Koungheul epidemic. High rates of transovarian transmission with the dengue 3 virus has been observed in *Ae. aegypti* females collected in the field and pooled 3–4 days after emergence from larval stages.[23] In addition to the age of the emerging mosquitoes, several other factors would have contributed to the high levels of infection recorded in nature. Most of the males caught on humans were caught while they attempted to mate with females in search of a blood meal. These fortuitous catches were not representative of the male populations and they therefore brought about a sampling bias that was the basis of the high infection rates obtained in nature. Environmental factors could also have influenced the vertical transmission rates since it has been shown that temperature affects vectorial competence as well as the vertical transmission rates of infected females.[24,25]

The results presented allow us to partly explain our field observations during the 1995 Koungheul epidemic when the TIR were very much higher in males captured than in recently emerged males. Our results show that with the conventional method of virus isolation on AP61 cell cultures, it would have been judicious to inoculate the F_1 adults several days after emergence instead of 24–48 hr afterwards as we did. More sensitive methods, such as the polymerase chain reaction or inoculation of mosquitoes, would have probably detected more positive pools. When contaminated at birth, the mosquitoes can transmit the virus earlier than during a conventional horizontal transmission requiring the completion of the extrinsic cycle of the virus. The older the mosquito, the higher will be the rate of transmission.

Acknowledgments: We thank C. Mathiot, F. Rodhain, A. A. Sall, and L. Lochouarn for improving the manuscript with their comments. We also thank A. Fall, C. Fortez, M. Ndiaye, R. Sylla, and A. Thiaw for technical assistance.

Financial support: This work was supported by the Institut Pasteur de Dakar and by the Institut de Recherche pour le Développement (IRD, formerly Institut Français de Recherche Scientifique pour le Développement en Coopération [ORSTOM]).

Authors' addresses: Mawlouth Diallo, Laboratoire de Zoologie Médicale, Institut de Recherche pour le Développement (IRD, formerly ORSTOM), Institut Pasteur, BP 220, Dakar, Senegal. Jocelyn Thonnon, Institut Pasteur de la Guyane, BP 6010, 97 306 Cayenne, French Guiana. Didier Fontenille, Laboratoire IRD d'Entomologie Médicale, Organisation de Coordination pour la Luttre Contre Endémies en Afrique Centrale, BP 288 Yaounde, Cameroon.

Reprint requests: Mawlouth Diallo, Laboratoire de Zoologie Médicale, Institut de Recherche pour le Développement (IRD, formerly ORSTOM), Institut Pasteur, BP 220, Dakar, Senegal.

REFERENCES

1. Thonnon J, Fontenille D, Tall A, Dialto M, Renaudineau Y, Baudez B, Raphenon G, 1998. Re-emergence of yellow fever in Senegal in 1995. *Am J Trop Med Hyg 59:* 108–114.
2. Fontenille D, Diallo M, Mondo M, Ndlaye M, Thonnon J, 1997. First evidence of natural vertical tranmission of yellow fever virus in *Aedes aegypti,* its epidemic vector. *Trans R Soc Trop Med Hyg 91:* 533–535.
3. Marchoux E, Simmond PL, 1905. La transmission héréditaire du virus de la fièvre jaune chez le *Stegomyia fasciata. C R Soc Biol (Paris) 59:* 259–260.
4. Aitken THG, Tesh RB, Beaty BJ, Rosen L, 1979. Transovarial transmission of yellow fever virus by mosquitoes *(Aedes aegypti). Am J Trop Med Hyg 28:* 119–121.
5. Cornet M, Robin Y, Heme G, Adam C, Renaudet J, Valade M, Eyraud M, 1979. Une pousée épizootique de fièvre jaune selvatique au Sénégal Oriental. Isolement du virus de lots de moustiques adultes mâles et femelles. *Med Mal Infectieuses 9:* 63–66.
6. Beaty BJ, Tesh RB, Aitken THG, 1980. Transovarial transmission of yellow fever virus in *Stegomyia* mosquitoes. *Am J Trop Med Hyg 29:* 125–132.
7. Dutary BE, Leduc JW, 1981. Transovarial transmission of yellow fever virus by a svlvatic *vector Haemagogus equinus. Trans R Soc Trop Med Hyg 75:* 128.
8. Reed LJ, Muench H, 1938. A simple method of estimating fifty percent endpoints. *Am J Hyg 27:* 493–497.
9. Rutledge LC, Ward RA, Gould DJ, 1964. Studies on the feeding response of mosquitoes to nutritive solutions in a new membrane feeder. *Mosq News 14:* 407–419.

10. Digoutte JP, Calvo-Wilson MA, Mondo M, Traord-Lamizana M, Adam F, 1992. Continuous cell lines and immune ascite fluid pools in arbovirus detection. *Res Virol 143:* 417–422.
11. Chiang CL, Reeves W, 1962. Statistical estimation of virus infection rates in mosquitoes vector populations. *Am J Hyg 75:* 377–391.
12. Walter SD, Hildreth SW, Beaty BJ, 1980. Estimation of infection rates in populations of organisms using pools of variable size. *Am J Epidemiol 20:* 124–128.
13. Tabachnick WJ, Wallis GP, Aitken THG, Miller BR, Amato GD, Lorenz L, Powel JR, Beaty BJ, 1985. Oral infection of *Aedes aegypti* with yellow fever virus: geographic variation and genetic considerations. *Am J Trop Med Hyg 34:* 1219–1224.
14. Rosen L, Shroyer DA, Tesh RB, Freier JE, Lien JC, 1983. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti. Am J Trop Med Hyg 32:* 1108–1119.
15. Tesh RB, 1980. Experimental studies on the transovarial transmission of Kunjin and San Angelo viruses in mosquitoes. *Am J Trop Med Hyg 29:* 657–666.
16. Wallis GP, Aitken THG, Beaty BJ, Lorenz L, Amato GD, Tabachnick WJ, 1985. Selection for susceptibility and refractoriness of *Aedes aegypti* to oral infection with yellow fever virus. *Am J Trop Med Hyg 34:* 1225–1231.
17. Gubler DJ, Rosen L, 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses. *Am J Trop Med Hyg 25:* 318–325.
18. Simmons JS, St. John JH, Holt RL, Reynolds FHK, 1931. The possible transfer of dengue virus from infected to normal mosquitoes during copulation. *Am J Trop Med Hyg 11:* 199–216.
19. Rosen L, 1987. Sexual transmission of dengue viruses by *Aedes albopictus. Am J Trop Med Hyg 37:* 398–402.
20. Chandler U, Blair CD, Beaty BJ, 1998. La Crosse virus infection of *Aedes triseriatus* (Diptera: Culicidae) ovaries before dissemination of virus from the midgut. *J Med Entomol 35:* 567–572.
21. Watts DM, Pantuwatana S, DeFoliart GR, Yuill TM, Thompson WH, 1973. Transovarial transmission of La Cross virus (California encephalitis group) in the mosquito *Aedes triseriatus. Science 182:* 1140–1141.
22. Miller BR, DeFoliart GR, Yuill TM, 1979. *Aedes triseriatus* and La Crosse virus: lack of infection in eggs of the first ovarian cycle following oral infection of females. *Am J Trop Med Hyg 28:* 897–901.
23. Joshi V, Singhi M, Chaudhary RC, 1997. Transovarial transmission of dengue 3 virus by *Aedes aegypti. Trans R Soc Trop Med Hyg 90:* 643–644.
24. Watts DM, Burke DS, Harrison BA, Whitmire RE, Nisalak A, 1987. Effect of temperature on the vector efficiency of *Aedes aegypti* for dengue 2 virus. *Am J Trop Med Hyg 36:* 143–152.
25. Hardy JL, Rosen L, Kramer LD, Presser SB, Shrover D A, Turell MJ, 1980. Effect of rearing temperature on transovarial transmission of St. Louis encephalitis virus in mosquitoes. *Am J Trop Med Hyg 29:* 963–968.

VOLUME 62 JANUARY 2000 NUMBER 1

The American Journal of TROPICAL MEDICINE & HYGIENE

PM 86
14 AVR. 2000
Santé

OFFICIAL JOURNAL OF
ICAN SOCIETY OF TROPICAL MEDICINE AND HYGIENE

ISSN 0002-9637