# First evidence of natural vertical transmission of yellow fever virus in *Aedes aegypti*, its epidemic vector

**D. Fontenille[1], M. Diallo[1,2], M. Mondo[2], M. Ndiaye[2] and J. Thonnon[2]** *[1]Laboratoire de Zoologie Médicale, Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM), Institut Pasteur, B.P. 1386, Dakar, Sénégal; [2]Institut Pasteur, B.P. 220, Dakar, Sénégal*

**Abstract**

Entomological investigations were conducted in 1995 in Senegal, following a yellow fever (YF) outbreak. A total of 1125 mosquitoes collected in the field, including males, females and 12–48 h old newly emerged adults reared from wild-caught larvae, were tested for YF virus. Among the 22 species captured, *Aedes aegypti* was the most common. 'Wild' vectors of YF were also captured, including *A. furcifer*, *A. metallicus* and *A. luteocephalus*. In all, 28 YF virus isolations were made: 19 from *A. aegypti* females, including 2 from newly emerged specimens; 5 were obtained from *A. aegypti* males, including one from a pool of newly emerged specimens, 2 from *A. furcifer* females, and one each from a female *A. metallicus* and a female *A. luteocephalus*. The true infection rates (TIRs) were much higher in adult *A. aegypti* than in specimens reared from larvae—8·2% and 31·4% for female and male *A. aegypti* captured on human volunteers, respectively ($P<0·0001$). The TIRs for *A. aegypti* reared from larvae were 1·4% and 0·5% for females and males, respectively ($P>0·05$). This outbreak was an intermediate YF epidemic, involving 4 vector species. Our data provide the first evidence of vertical transmission of YF virus in nature by *A. aegypti*, its main vector to humans, and strongly suggest that vertical transmission played a major role in the spread of the epidemic.

**Keywords**: yellow fever, vertical transmission, *Aedes aegypti*, epidemic, Senegal

## Introduction

Despite a highly effective vaccine, yellow fever (YF) has re-emerged in Africa since the 1980s (MONATH, 1991) due to inefficient vaccination campaigns. During its 'wild' cycle, YF virus is transmitted between monkeys and 'wild' mosquitoes of the genus *Aedes* in Africa and *Haemagogus* and *Sabethes* in tropical America. Human beings are infected with YF virus in Africa during intermediate and urban outbreaks, mainly by *Ae. aegypti*, which is strongly associated with the human environment (MONATH, 1989).

Since the discovery of the transmission of the virus about a century ago by REED (1901), following Finlay's observations, the possibility of vertical transmission from an infected female mosquito to her progeny was under consideration. Natural vertical transmission of YF virus has been observed only once, when CORNET *et al.* (1979) obtained 3 YF isolates from males of the 'wild' *Ae. furcifer–Ae. taylori* group in Senegal. It was only in 1979 that AITKEN *et al.* confirmed the results obtained in 1903 by MARCHOUX & SIMOND (1905), who showed experimentally that the virus could be transmitted from an infected female *Ae. aegypti* to the next generation. Similar experimental transmissions were also made in *Ae. mascarensis* by BEATY *et al.* (1980) and *Haemagogus equinus* by DUTARY & LEDUC (1981). Apart from these experiments, there had been many unsuccessful attempts to confirm vertical transmission (ROSENEAU & GOLDBERGER, 1906; STOKES *et al.*, 1928; PHILIP, 1929; WHITMAN & ANTUNES, 1938). The data we present were obtained during an outbreak of YF in 1995 in Senegal. This epidemic killed at least 46 human beings among an estimated exposed population of 9000. It was quickly stopped, following a prompt immunization campaign conducted by the Senegalese Ministry of Health (THONNON *et al.*, 1996).

Our results demonstrated for the first time that vertical transmission occurs naturally in *Ae. aegypti*, the major vector of YF. The epidemiological implications are discussed.

## Materials and Methods

Entomological surveys were conducted between 28 October 1995 and 11 November 1995, during a YF outbreak in the Koungheul area (14°29 N, 14°59 W) around the dry valley (*vallée fossile*) of the Saloum river, in the Sudanese climatic region of Senegal. Villages are traditionally built and are located in the shrubby savannah. Water is stored indoors and outdoors for domestic use in clay jars and in 200 L barrels. *Erythrocebus patas* monkeys are present in the area. The average annual rainfall was 750 mm in 1994 and 676 mm in 1995. The rainy season lasts from July to October. The last rain preceeding our entomological survey fell on 12 October 1995.

Mosquitoes were collected on immunized human volunteers, during a total of 445 person–hours of capture, and with CDC light traps. They were sorted and pooled by species and sex in the field and stored in liquid nitrogen.

*Aedes* larval development sites were investigated. The Breteau index (total number of containers with larvae of *Ae. aegypti* per 100 houses) and the container index (number of containers in which larvae of *Ae. aegypti* were found per 100 containers) were calculated. Larvae and pupae collected in the field were reared in Dakar in an insectary at 28°C and 80% relative humidity. Specimens from each breeding site were reared separately. Emerged adults were sorted and pooled by species and sex and stored at –70°C 12–48 h after emergence.

Attempts to isolate YF virus were made from mosquito pools. Pools of mosquitoes of the genera *Culex*, *Anopheles* and *Mansonia*, which are not YF vectors, were interspersed among pools of potential vectors as a control of contamination. Two batches of 40 pools were processed each week. The species and origin of each pool were not known by technicians doing the work at the CRORA–WHO collaborative centre at the Pasteur Institute in Dakar. Cultures of *Ae. pseudoscutellaris* continuous cell line AP 61 were inoculated with the pools, and suckling mice were inoculated intracerebrally. With each of the 66 pools of newly emerged mosquitoes, including *Culex* pools, the supernatant of AP 61 cell cultures was sampled at day 7 and subinoculated into another AP 61 cell culture and suckling mice. Ground mosquito pools, cell cultures and suckling mice were handled in a microbiological safety cabinet. Virus identification was made using immune ascitic fluid pools and monoclonal antibodies, following methods described previously (DIGOUTTE *et al.*, 1992). Each time a YF virus isolate was isolated from field-caught males of females, or from newly emerged adults, another inoculation was made from the original suspension for confirmation.

Address for correspondence: D. Fontenille, ORSTOM, B.P. 1386, Dakar, Sénégal.

The minimum infection rate (MIR), which is the minimum percentage of mosquitoes infected with YF, and the true infection rate (TIR), the estimated true proportion of infected mosquitoes, were calculated from pools of different sizes following a maximum likelihood procedure developed by WALTER *et al.* (1980), and using a computer program written by N. Degallier (unpublished data). TIRs were compared using a $Z$ test.

## Results

The numbers of *Aedes* species captured, the number of pools, the number of YF isolates obtained, the MIR and the TIR are shown in the Table. A total of 1125 mosquitoes belonging to 22 species of the genera *Aedes, Aedeomyia, Anopheles, Culex* and *Mansonia* were inoculated. *Ae. aegypti* was the commonest mosquito, represented by the domestic form, formerly known as the aegypti form, which is paler and has white scales on the first abdominal tergite and differs from the 'wild' formosus form found in gallery forests in eastern Senegal. Larval development sites were mainly clay jars, indoors and outdoors, and 200 L barrels situated outdoors. In all, 293 water containers were examined in 13 villages. The average Breteau index was 35 (range 0–135), and 29·7% (range 0–63) of the containers contained larvae or pupae of *Ae. aegypti*. Twenty-eight isolates of YF virus were obtained, all in AP 61 cell cultures; 2 were also isolated by inoculation of suckling mice. Among the 19 isolates obtained from *Ae. aegypti* females, there were 2 isolates from pools of newly emerged specimens, and one of the 5 isolates obtained from *Ae. aegypti* males also originated from a pool of newly emerged specimens. All 3 isolates from newly emerged adults were obtained at the primary inoculation to AP 61 cell cultures.

Seventy-four pools were tested twice, particularly each time an isolate was obtained from males or adults reared from larvae, and all the 13 isolates obtained at the primary isolation were reisolated. The difference between the TIRs of female and male *Ae. aegypti* captured on human volunteers, 8·2% and 31·4% respectively, was significant ($P<0·0001$). The difference between the TIRs of male and female *Ae. aegypti* reared from larvae were not significant. However, the differences between TIRs of males captured on volunteers and newly emerged males, and those of females captured on volunteers and newly emerged females, were significant ($P<0·0001$ and $P<0·01$, respectively).

**Table. Numbers of mosquitoes captured, numbers of yellow fever isolates, and minimum and true yellow fever infection rates; Senegal, 1995**

| Species and sex of *Aedes* | Human bait catches No.[a] | MIR[b] | TIR[c] | Light traps No.[a] | Adults newly emerged from larvae No.[a] | MIR[b] | TIR[c] | Total no. of mosquitoes | Total no. of isolates |
|---|---|---|---|---|---|---|---|---|---|
| *A. aegypti* female | 312 (17/31) | 5·44 [1·72] | 8·17 [1·98] | 0 | 165 (2/30) | 1·33 [0·97] | 1·37 [0·97] | 477 | 19 |
| *A. aegypti* male | 33 (4/6) | 11·76 [8·87] | 31·42 [15·69] | 0 | 189 (1/27) | 0·52 [0·52] | 0·52 [0·52] | 222 | 5 |
| *A. furcifer* female | 19 (2/10) | 10·53 [8·13] | 10·53 [7·67] | 0 | 0 | – | – | 19 | 2 |
| *A. furcifer* male | 2 (0/1) | – | – | 0 | 0 | – | – | 2 | – |
| *A. luteocephalus* female | 1 (1/1) | – | – | 0 | 0 | – | – | 1 | 1 |
| *A. metallicus* female | 1 (1/1) | – | – | 0 | 0 | – | – | 1 | 1 |
| *A. vittatus* male | 5 (0/1) | – | – | 0 | 0 | – | – | 5 | – |
| *A. fowleri* female | 0 | – | – | 1 (0/1) | 0 | – | – | 1 | – |
| *A. sudanensis* female | 0 | – | – | 1 (0/1) | 0 | – | – | 1 | – |
| *A. vexans* female | 0 | – | – | 3 (0/1) | 0 | – | – | 3 | – |
| Other Culicidae | 150 (0/23) | – | – | 209 (0/28) | 34 (0/9) | – | – | 393 | – |
| Total | 523 (25/74) | – | – | 214 (0/31) | 388 (3/66) | – | – | 1125 | 28 |

[a]Numbers of isolates of YF virus obtained/numbers of pools inoculated are shown in parentheses.
[b]Minimum infection rate: minimum number of positive mosquitoes per 100 mosquitoes tested; standard error in brackets.
[c]True infection rate: estimated number of positive mosquitoes per 100 mosquitoes tested, following WALTER *et al.* (1980); standard error in brackets.

## Discussion

The high number of YF virus isolates obtained from mosquitoes during this outbreak may raise doubts about the validity of the results. As many controls as possible were included, and the technicians doing the work were very experienced and worked 'blind', without knowing the identity of the mosquitoes. No isolation was obtained from mosquitoes belonging to the genera *Culex, Anopheles* or *Mansonia*. Mosquito pools from other regions were also processed at the same time and were all negative for YF virus. Human sera and mosquito pools were never processed in the same week; positive human samples were all processed 2 weeks before the first positive mosquito pool was obtained. Confirmation of each isolation was obtained from the original suspension and the possibility of false positive pools resulting from contamination can be rejected.

This outbreak was clearly an intermediate YF epidemic, according to the definitions of CORNET *et al.* (1977) and CORDELLIER (1991). Transmission in the area probably began with 'wild' vectors (*Ae. furcifer, Ae. metallicus* and *Ae. luteocephalus*), which disappeared rapidly after the rainy season. Transmission then continued with *Ae. aegypti*, particularly after the rains when it was the only remaining vector species. While *Ae. aegypti* is known to be the major vector during urban and intermediate outbreaks, vertical transmission to its progeny has never been observed naturally, contrary to dengue 2, 3 and 4 viruses (KHIN & THAN, 1983; HULL *et al.*, 1984; JOSHI *et al.*, 1997). The isolations that we obtained from males captured landing on human beings and from adults reared from larvae demonstrated for the first time that such vertical transmission occurs naturally in *Ae. aegypti*.

The true infection rate was very high in *Ae. aegypti* captured on human bait, and it was significantly higher in males than in females, although only females are haematophagous and so can contract the virus from a viraemic host. If vertical transmission occurs at the same rate in male and female progeny, this observation suggests that it was a major mechanism of YF transmission during this outbreak. In addition, the TIR of 24 newly emerged males was lower than that of males captured on human bait. If we exclude the possibility of venereal transmission from infected females to males (SIMMONS *et al.*, 1931; ROSEN, 1987), the explanation may be that the viral titre of newly emerged adults is too low to permit isolation of the virus from all infected mosquitoes with the laboratory methods we used. Multiplication of the virus to a detectable titre may take a few days, as has been observed with other viruses (HUANG *et al.*, 1992). Experiments conducted by ROSEN *et al.* (1983) on dengue viruses showed that there were more infected F1 adults among *Ae. albopictus* killed 'a relatively long time after emergence as compared with those killed earlier'.

A period of one and a half centuries has elapsed after it was suspected that mosquitoes transmitted YF, and nearly a century after *Ae. aegypti* was demonstrated to be a vector, before vertical transmission by *Ae. aegypti*, shown experimentally in 1905 by MARCHOUX & SIMOND, was observed in nature. This was because only a few studies on vertical transmission were conducted

during outbreaks (AITKEN, 1988), and because the inoculation of samples into new born mice, the classical method for YF virus isolation, was not very sensitive (ROSEN, 1981a). Intrathoracic inoculation of mosquitoes (*Aedes* and *Toxorhynchites*), and inoculation of cultures of *Ae. albopictus* (C6-36) and *Ae. pseudoscutellaris* (AP 61) cell lines, which is much more sensitive and has been used since the 1980s, allowed many more isolations to be made (ROSEN, 1981b; DIGOUTTE *et al.*, 1992). Our data confirmed this, since only 2 isolates were obtained after inoculation of suckling mice among the 28 isolations made by inoculation of AP 61 cell cultures.

It is possible that the Koungheul population of *Ae. aegypti* was very susceptible to the YF virus causing this outbreak, contrary to the 2 Senegalese *Ae. aegypti* colonies previously tested by TABACHNICK *et al.* (1985). It is well known that susceptibility to YF virus varies greatly between different mosquito populations and is under genetic control (WALLIS *et al.*, 1985).

The occurrence of vertical transmission has 2 very important epidemiological implications. The first is that the virus can be transmitted only a few days after the emergence of *Ae. aegypti* females, theoretically at the first blood meal, without being delayed until the viral extrinsic cycle is completed 8 to 12 d later. Transmission in the human population will be more frequent than if there were only horizontal transmission. The second implication is that the YF virus can persist in the area until the next rainy season inside infected eggs laid in peridomestic breeding sites which dry up, such as used tyres and old pots.

Our results are the first evidence of vertical transmission of YF virus in nature by *Ae. aegypti*, its main vector to humans. The vertical transmission rate was very high, contrary to the generally accepted idea suggested by previous experiments or by natural observation of dengue viruses. During this outbreak, vertical transmission probably played a major role in the spread of the epidemic. If the exposed human population has not been promptly vaccinated, many more people would probably have been infected by the virus.

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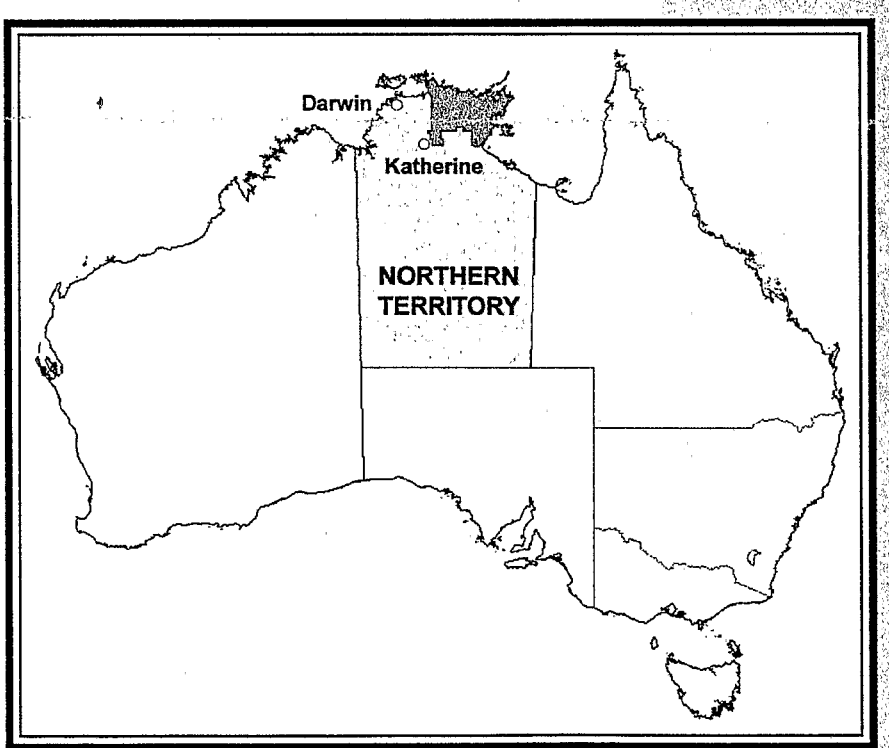


Royal Society of Tropical Medicine and Hygiene,
Manson House, 26 Portland Place,
London, W1N 4EY, UK
Telephone: +44 (0)171 580 2127
Fax: +44 (0)171 436 1389
e-mail: mail@rstmh.org