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

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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 Aedes furcifer, Aedes metallicus and Aedes luteocephalus. In all, 28 YF virus isolations were made: 19 from A. aegypti females, including 3 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<0.001). 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 Senegal 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 Sababia in tropical America. Human beings are infected with YF virus in Africa during intermediate and urban outbreaks, mainly by A. 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" Aedes furcifer—Ae. taylori group in Senegal. It was only in 1979 that ATKEN et al. confirmed the results obtained in 1903 by MARCOUX & SIMONE (1905), who showed experimentally that the virus could be transmitted from an infected female A. aegypti to the next generation. Similar experimental transmissions were also made in Aedes maculipennis by BEATTY et al. (1980) and Haemagogus apipunie 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 (THONNION et al., 1996).

Our results demonstrated for the first time that vertical transmission occurs naturally in A. 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 (valide 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 preceding 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 Aedes aegypti per 100 houses) and the container index (number of containers in which larvae of Aedes 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 induce YF virus were made from mosquito pools. Pools of mosquitoes of the genera Culex, Anopheles and Mansonia, which are not YF vectors, were interpersed 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 Aedes pseudocostalis continuous cell line AP 61 were inoculated with the pools, and sucking 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 sucking mice. Ground mosquito pools, cell cultures and sucking 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.
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. (1990), and using a computer program written by N. Degliort (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 32 species of the genera Aedes, Aedomyia, Anopholes, Culex and Mansonia were inoculated. Aedes 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' formae, and is known to be the major vector during urban and intermural outbreaks. While Aedes aegypti is the only remaining vector species. While Aedes 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 Aedes aegypti.

The true infection rate was very high in Aedes aegypti captured on human bait, and it was significantly higher in males than in females, although only females are hematophagous and so can contract the virus from a viremic 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 emerged 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 Aedes aegypti was demonstrated to be a vector, before vertical transmission by Aedes aegypti, was observed experimentally by Marion and Smith in 1905; MARION, was observed in nature. This was because only a few studies on vertical transmission were conducted.

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

<table>
<thead>
<tr>
<th>Species and sex of Aedes</th>
<th>Human bait catches</th>
<th>Light traps</th>
<th>Adults newly emerged from larvae</th>
<th>Total no. of pools inoculated</th>
<th>Total no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aedes aegypti female</td>
<td>213/17(131)</td>
<td>9-4/1-72</td>
<td>8-17-1-98</td>
<td>0</td>
<td>165(230)</td>
</tr>
<tr>
<td>Aedes aegypti male</td>
<td>32(4/6)</td>
<td>1-76-0-87</td>
<td>31-42-3-69</td>
<td>0</td>
<td>189(127)</td>
</tr>
<tr>
<td>Aedes furcifer female</td>
<td>19(0/10)</td>
<td>10-52-0-13</td>
<td>10-33-0-77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aedes furcifer male</td>
<td>2(0/1)</td>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Aedes albopictus female</td>
<td>1(1/7)</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aedes albopictus male</td>
<td>0(0/1)</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aedes senowolfi female</td>
<td>0(0/1)</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aedes senowolfi male</td>
<td>0(0/1)</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aedes africanus</td>
<td>150(0/23)</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>523(2574)</td>
<td></td>
<td></td>
<td>214(0/35)</td>
<td>388(3/60)</td>
</tr>
</tbody>
</table>

aNumber of isolates of YF virus obtained/numbers of pools inoculated are shown in parentheses.
bMinimum infection rate: minimum number of positive mosquitoes per 100 mosquitoes tested; standard error in brackets.
cTrue 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 weeks 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 Cordeillier (1991). Transmission in the
VERTICAL TRANSMISSION OF YELLOW FEVER VIRUS IN Aedes aegypti


Received 11 February 1997; revised 19 May 1997; accepted for publication 20 May 1997.