Journal of the American Mosquito Control Association, 15(4):475–478, 1999

AEDES ALBOPICTUS FROM ALBANIA: A POTENTIAL VECTOR OF DENGUE VIRUSES

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ABSTRACT. *Aedes albopictus* collected in Durazzo, the main port of Albania, were tested for oral susceptibility to dengue type 2 virus and their infection rates were compared to those of an *Aedes aegypti* strain (Paea) and another strain of *Ae. albopictus* (Tananarive). Infection rates for the Albanian *Ae. albopictus* were dose dependent, ranging from 38.9 ± 13.6% to 85.1% with the titer of the meal increasing from $10^{8.1}$ to $10^{9.1}$ 50% mosquito infectious doses (MID_{50})/ml. The percentage of infected females was lower for the *Ae. albopictus* Durazzo strain than for the *Ae. aegypti* Paea strain: 38.9 ± 13.6% compared with 92.4 ± 4.9% for a meal of $10^{8.1}$ MID_{50}/ml, respectively. However, the difference was less when the titer of the meal was increased: 85.1% compared with 100% for a meal of $10^{9.1}$ MID_{50}/ml, respectively. The infection rate was also lower for the Durazzo strain than for the Tananarive strain of *Ae. albopictus.* The degree of viral replication in infected females was not significantly different in the 3 strains tested and we were able to demonstrate the ability of females from the Durazzo strain to transmit the virus in the course of a blood meal. Our results lead us to conclude that *Ae. albopictus* from Albania could serve as a vector for dengue virus.

KEY WORDS *Aedes albopictus,* Albania, dengue type 2 virus, oral susceptibility, transmission

INTRODUCTION

Albania was the 1st European country to report the introduction of *Aedes albopictus* (Skuse). Mosquitoes were detected in August 1979 in the small town of Laç. In subsequent surveys, *Ae. albopictus* was found in 5 other towns on the northern coastal plain from Tirana and Durrës (Durazzo) to Shkodër (Scutari). The mosquito was also present at a dump for discarded tires in Xibrakë and was then considered as established in Albania. Larvae were mostly found in tires, as in Laç and Shkodër, but could also be recovered from water containers. The species was probably introduced from the People's Republic of China via commercial trade in 1974–75 (Adhami and Murati 1987, Adhami and Reiter 1998) in Durrës (Durazzo), the only infested seaport and the principal point of entry for goods from China.

Soon afterwards, *Ae. albopictus* was 1st reported in Italy, near Genoa (Sabatini et al. 1990), and then 1 year later in the city of Padua (Dalla Pozza and Majori 1992). Subsequently, it spread throughout northern and central Italy (Knudsen et al. 1996). The Albanian and Italian infestations did not have the same origin because the *Ae. albopictus* found in Italy seemed to have come from North America (Dalla Poza et al. 1994).

Other European countries are at risk of being infested with *Ae. albopictus.* This species has demonstrated an ability to adapt to a wide variety of larval habitats and has a greater tolerance to low temperatures than does *Aedes aegypti* (L.) The potential area of repartition of *Ae. albopictus* is much wider than that of *Ae. aegypti,* which was eradicated from the Mediterranean basin in the 1950s (Rodhain 1995).

The introduction of *Ae. albopictus* to the Mediterranean basin is a threat to public health because this species is a well-known experimental vector for several pathogenic microoorganisms (Mitchell 1995, Rodhain 1995). *Aedes albopictus* is a natural vector of dengue and can transmit the virus vertically to its progeny (Rosen et al. 1983).

Dengue has been present in Europe in the past, especially in Greece, where 1 of the largest outbreaks of dengue ever recorded took place in Athens and the neighboring areas in 1927–28, when one million cases and about 1,000 deaths occurred (Rosen 1986). After the eradication of *Ae. aegypti* from the Mediterranean basin, only imported cases of dengue were reported. The recent introduction of *Ae. albopictus* raises the possibility of renewed transmission of dengue. The purpose of the present study was to determine the oral susceptibility of *Ae. albopictus* females from Albania to dengue type 2 virus and their ability to transmit that virus.

MATERIALS AND METHODS

Mosquitoes: The *Ae. albopictus* mosquitoes were collected as 3rd- and 4th-stage larvae in June 1996 in Durazzo, the main port of Albania, in a small barrel containing rainwater. These field-collected mosquitoes (F_0 generation) were maintained in the laboratory at 28 ± 1°C with 80% relative humidity and a 16:8 h light:dark photoperiod. Adults were given 10% sucrose solution and females were allowed to feed on beef blood to produce eggs. The eggs representing the F_1 progeny were sent to the Pasteur Institute (Paris, France) in July 1996 and the strain has been maintained since that time in our laboratory.

The Paea strain of *Ae. aegypti* provided by the Louis Malarde Institute (Tahiti, French Polynesia)

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and reared in Paris since 1994 was used for comparative purposes. The Tananarive strain of *Ae. albopictus* provided by the Pasteur Institute of Madagascar and reared in Paris since 1988 was also used, in 1 trial, for comparative purposes.

Virus: A dengue type 2 virus strain, provided by L. Rosen, was isolated from a human serum collected in Bangkok (Thailand) in 1974. This virus had been passed only in different mosquito species *(Toxorhynchites amboinensis, Ae. albopictus,* and *Ae. aegypti)* by intrathoracic inoculation (Rosen and Gubler 1974). Viral stocks were produced by inoculating *Ae. albopictus* cells of the C6/36 clone (Igarashi 1978) with triturated infected mosquitoes. The mosquito cells were maintained at 28°C on RPMI-1640 medium supplemented with nonessential amino acids, penicillin, streptomycin, and 10% heat-inactivated (56°C for 30 min) fetal calf serum. The percentage of infected cells was monitored during the incubation period by the indirect fluorescent antibody (IFA) assay (Kuberski and Rosen 1977). When 100% of the cells were infected, the supernatant fluid was collected, and the pH was adjusted to 7.5 with 10% sodium bicarbonate. The virus stock was divided into aliquots and stored at −80°C until used. Titration of the virus stock was carried out in *Ae. aegypti* (Paea strain) by intrathoracically inoculating serial dilutions of the supernatant. Mosquito infection was detected by an IFA assay on head squashes after 14 days of incubation at 28 ± 1°C. Titers were calculated by the 50% endpoint method (Reed and Muench 1938) and expressed as 50% mosquito infectious doses (MID_{50}) per milliter.

Oral infection of mosquitoes: The oral susceptibility of females was tested by a feeding protocol described elsewhere (Vazeille-Falcoz et al., 1999). Briefly, 5- to 7-day-old females were deprived of sucrose solution 24 h before the infectious meal and then allowed to feed for 20 min through a chicken skin membrane covering an apparatus containing the feeding mixture maintained at 37°C. The infectious meal consisted of ⅔ washed rabbit erythrocytes, ⅓ virus suspension, and adenosine triphosphate (as a phagostimulant) at a final concentration of 5×10^{-3} M. Only fully engorged females were transfered to small cardboard containers and maintained at 28 ± 1°C for 12 or 14 days. Surviving females were killed and tested for the presence of dengue virus by an IFA assay on head squashes.

Titration of virus content: Titration of virus in bodies of infected mosquitoes was carried out on 8 *Ae. albopictus* from the Durazzo strain (F_6), 8 *Ae. albopictus* from the Tananarive strain, and 8 *Ae. aegypti* from the Paea strain. Each body was ground in 0.4 ml of RPMI-1640 medium supplemented with penicillin, streptomycin, and 10% heat-inactivated (56°C for 30 min) fetal calf serum. The supernatant fluid, recovered after a 20-min centrifugation at 500 × *g*, was filtered through membranes with pores 220 nm in diameter. Titration of triturated bodies was carried out in *Ae. aegypti* (Paea strain) as for virus stock titration.

Transmission experiment: We used a transmission method derived from that described by Gubler and Rosen (1976a). Surviving *Ae. albopictus* females from the Durazzo strain were pooled in a single cage 12 days after the infective artificial meal. They were deprived from food for 4 h and then allowed to feed on a drop of whole rabbit blood. This drop was left for 30 min on the mesh top of the cage and then frozen at −80°C and replaced by a new drop to avoid inactivation of any virus present. All the mosquitoes were allowed to feed on 7 successive drops that were pooled at the end of the experiment. All females were killed at the end of the meal. The gut of each female was dissected to check for the presence of blood and an IFA assay was performed on a head squash to check for the presence of dengue infection. Thus, we were able to determine the number of infected females that fed on the blood drops.

The whole blood was injected intrathoracically in *Ae. aegypti* (Paea strain) to try to detect virus. However, because of the toxicity of the whole rabbit blood for the inoculated mosquitoes, the inoculum was subsequently diluted and the incubation period was reduced from 14 to 7 days at 28°C. Surviving females were killed and tested for the presence of dengue virus by an IFA assay on head squashes.

Statistical analysis: Variations in the titer of body contents of surviving infected females at 14 days postinfection were compared using the Kruskal–Wallis test (Simstat software, Pentraeth, Anglesey, Wales, UK).

RESULTS

Oral infection of mosquitoes

As shown in Table 1, we were able to orally infect *Ae. albopictus* females from the Durazzo strain with dengue type 2 virus. The infection rates were dose dependent, ranging from 38.9 ± 13.6 (mean value and standard deviation for 4 assays) for a meal of an average of $10^{8.1}$ MID_{50}/ml to 85.1% (value for 1 assay) for a meal of $10^{9.1}$ MID_{50}/ml. However, the percentage of infected females was lower for the *Ae. albopictus* Durazzo strain than for the *Ae. aegypti* Paea strain: 38.9 ± 13.6% versus 92.4 ± 4.9%, with a meal of an average of $10^{8.1}$ MID_{50}/ml, respectively. This difference was reduced when the titer of the meal was increased, 85.1% versus 100% for a meal of $10^{9.1}$ MID_{50}/ml. When another strain of *Ae. albopictus* (the Tananarive strain) was included as a control, the percentage of infected females was also lower for the Durazzo strain (38.9 ± 13.6%) than for the Tananarive strain (88.6%).

Titration of body content

The results of individual body titration are shown in Table 2. The mean values and standard devia-

Table 1. Infection rates of *Aedes albopictus* and *Aedes aegypti* females orally infected with different amounts of dengue type 2 virus. Immunofluorescent antibody assay performed 12 or 14 days postinfection.[1]

Titer of the meal (MID_{50}/ml)	% of infected females (*n*)		
	Aedes albopictus Durazzo strain	*Aedes aegypti* Paea strain	*Aedes albopictus* Tananarive strain
$10^{8.1}$	38.9 ± 13.6[2] (89)	92.4 ± 4.9[2] (255)	88.6 (44)
$10^{9.1}$	85.1 (47)[3]	100 (56)[3]	NT

[1] MID_{50}/ml, 50% mosquito infectious doses per milliliter; *n*, no. of females; NT, not tested.
[2] Mean value ± standard deviation for 4 assays.
[3] Immunofluorescent antibody assay performed 12 days after infection instead of 14 days.

tions expressed in log of the MID_{50}/ml were 6.4 ± 0.7 for the *Ae. albopictus* Durazzo strain, 7.1 ± 0.4 for the *Ae. albopictus* Tananarive strain, and 6.6 ± 0.9 for the *Ae. aegypti* Paea strain. These 3 values were not significantly different when compared by the Kruskal–Wallis test ($P = 0.203$).

Transmission experiment

Out of the 47 females involved in the transmission experiment, 17 fed on the whole rabbit blood and out of these 17 females, 15 were infected, that is, had a positive head squash the day of the transmission experiment. Of the 15 infected females that fed on the whole rabbit blood, 3 were fully engorged, 6 had a small blood meal in their midgut, 4 had a very small quantity of blood in their midgut, and 3 had only some traces of blood in their foregut or their midgut.

In the 1st attempt to demonstrate virus in the whole rabbit blood, the mortality rate of the inoculated mosquitoes was 75% (15/20) at day 7 with undiluted material. The next attempt was done with dilutions of the blood and the incubation period was reduced to 7 days. The infection rates observed with the whole rabbit blood were 100% (5/5) for the undiluted inoculum, 92.3% (50/54) for a 1:20 dilution, and 30% (15/50) for a 1:80 dilution. Toxicity was still observed with the diluted material because mortality rates were 37.9% (33/87) for a 1:20 dilution and 20.6% (13/63) for a 1:80 dilution, 7 days postinoculation.

Table 2. Virus content of bodies of 8 females of each strain (*Aedes albopictus* or *Aedes aegypti*) infected orally with dengue type 2 virus. Titration performed 12 days postinfection.

	Titer of body content of individual females (log MID_{50}/ml[1])		
	Aedes albopictus Durazzo strain (F_6)	*Aedes albopictus* Tananarive strain	*Aedes aegypti* Paea strain
	8	7.5	6.2
	7.7	6.9	7.1
	7.1	6.9	5
	5.8	6.9	6.8
	6.2	7.1	6.7
	5.4	7.8	6.7
	6	7.2	6.3
	6.4	6.4	6
Mean value ± SD	6.4 ± 0.7	7.1 ± 0.4	6.6 ± 0.9

[1] MID_{50}/ml, 50% mosquito infectious doses per milliliter.

DISCUSSION

Analysis of our experimental results shows that *Ae. albopictus,* Durazzo strain, from Albania can be infected orally with dengue type 2 virus. The percentage of females infected was lower than that of another strain of *Ae. albopictus* from Madagascar, the Tananarive strain, and also lower than that of a strain of *Ae. aegypti* from French Polynesia, the Paea strain. However, the latter difference was reduced when the number of ingested viral particles was increased. For dengue viruses, as well as for many other arboviruses, oral infection of mosquitoes with an artificial meal is well known to require a much higher number of infectious particles than that necessary when feeding on a viremic host (Rodhain and Rosen 1998). Consequently, the high virus titer required to artificially infect the *Ae. albopictus* from Albania might not be required when feeding on a natural host. Furthermore, the oral susceptibility of the vector is not the only factor involved in an outbreak. The virulence of the viral strain, the immune background of the human population, the density of the vector, and the frequency of vector–host contacts must also be taken in consideration (Gubler 1998). Another important factor is that *Ae. albopictus* can transmit dengue virus to its progeny at a higher rate than does *Ae. aegypti* (Rosen et al. 1983).

The orally infected females from the Durazzo strain replicated the virus to a titer similar to that observed for another strain of *Ae. albopictus* from Madagascar and for a strain of *Ae. aegypti* from French Polynesia, where this mosquito is the main vector during dengue outbreaks (Rosen 1967). Thus, when females of the Durazzo strain are infected orally, they are able to replicate the viral particles to the same extent as a more orally susceptible strain. Gubler and Rosen (1976b) had already observed this type of result in other strains

of *Ae. albopictus*. Finally, we were able to demonstrate the ability of the orally infected females of the Durazzo strain to transmit the virus in the course of an artificial blood meal.

These results, along with the fact that *Ae. albopictus* does not have to compete with *Ae. aegypti* in the Mediterranean basin lead us to conclude that in the case of an introduction of a dengue virus in this area, *Ae. albopictus* from Albania would be an adequate vector. This mosquito would probably be able to transmit any of the 4 serotypes of dengue because if a species or a strain of mosquito is orally susceptible to 1 type of dengue virus, it is also known to be susceptible to the 3 other types (Gubler and Rosen 1976b). The European human populations are largely nonimmune to all 4 serotypes of dengue virus. Health authorities should be aware of this potential threat and initiate or intensify the control of *Ae. albopictus* in Europe to avoid the infestation of other areas and keep existing populations under control.

ACKNOWLEDGMENT

We thank Nadia Monnier for rearing the mosquitoes and Leon Rosen for reading the manuscript.

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