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EVALUATION OF ENEMAS FOR EXPOSING *AEDES AEGYPTI* TO SUSPENSIONS OF DENGUE-2 VIRUS

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ABSTRACT. To determine if enemas could be used to simulate oral exposure of *Aedes aegypti* to dengue-2 virus, we compared infection rates of mosquitoes administered an enema of a blood suspension containing dengue-2 virus to infection rates in mosquitoes that imbibed the same suspension from a drop of blood. In 1 of 4 experiments infection rates were significantly different. More importantly, during 2 of the 4 trials, 100% of the mosquitoes in the enema treatment group were infected. We speculate that the capillary tube used to administer the enemas abraded the hindgut of mosquitoes in the groups with 100% infection rates and, therefore, virus was inoculated directly into the mosquitoes' hemocoel. We conclude that the method we used for administering enemas cannot be used to simulate oral exposure of *Ae. aegypti* to dengue-2 virus.

Researchers have used enemas in a variety of physiological studies to deliver solutions into the posterior midgut of mosquitoes (Briegel and Lea 1975). For studies on the interaction between mosquitoes and the viruses they transmit, enemas could offer advantages over allowing mosquitoes to imbibe real or artificial virus–blood suspensions. For example, with enemas researchers could deliver precise amounts of a solution, at predetermined times, to many mosquitoes. In addition, they could administer different combinations and sequences of more than one solution. These kinds of experimental scenarios are difficult to control when one is left to rely on the cooperation of mosquitoes to imbibe solutions.

Previous attempts to use enemas to deliver pathogen-infected blood into a mosquito's midgut, however, have sometimes resulted in unusually high infection rates (M. Klowden, unpublished data). Those peculiar results implied that in the process of administering the enemas, the gut barrier is sometimes bypassed and the solution is inoculated directly into the mosquito's hemocoel.

In this study we evaluated whether enemas could be used to deliver a suspension of dengue-2 (DEN-2) virus-infected blood into the midgut of *Aedes aegypti* (Linn.), and thus simulate a natural, oral route of exposure. To do this, we used enemas to deliver part of a DEN-2 virus suspension to mosquitoes and fed the remaining part to a different group of mosquitoes via hanging drops. After extrinsic incubation, infection rates for the 2 groups were compared to determine if results differed between the group given enemas and the group that was exposed orally.

The F_2 generation of *Ae. aegypti* from San Juan, Puerto Rico, was used for our experiments. Larvae were reared in an environmental chamber at 26°C at low densities (200 larvae/28.5 × 24 × 4.5-cm plastic rearing tray). Mosquitoes exposed to virus were held at 30°C, 80% RH and a 12:12 (L:D) photoperiod for 10 days of extrinsic incubation.

The Rexville strain of *Ae. aegypti,* also from San Juan (Scott et al. 1993), was used for amplifying DEN-2 virus and to titer virus–blood suspensions. These mosquitoes were reared as described above but at higher larval densities (400–500 larvae/tray).

We exposed mosquitoes to a DEN-2 virus strain isolated in 1986 from a 5-month-old infant who became ill and died in San Juan, Puerto Rico. Prior to our experiment, this isolate underwent 2 passages in *Toxorhynchites amboinensis* (Doleschall).

To make the virus–blood suspension, we first inoculated *Ae. aegypti* (Rexville strain) with 76 50% mosquito infectious doses (MID_{50}) of DEN-2 virus. After a 10-day incubation at 30°C, inoculated mosquitoes were used to make a virus suspension by grinding 20–25 of them in 0.5 ml of heat-inactivated fetal calf serum. The virus–blood suspension was made by mixing 10 parts of the virus suspension with 9 parts triple-washed human red blood cells and 1 part 50% sucrose solution. Human red blood cells had earlier been drawn from people who were not immune to dengue viruses, mixed with an anticoagulant (ethylenediaminetetraacetic acid), and stored in Alsever's solution at 5°C. Immediately after preparing the virus–blood suspension, we warmed it for 4 min in a 37°C water bath. Half of the suspension was fed to one group of mosquitoes. The other half was placed on wet ice, and within 1 h administered as enemas to a different group of mosquitoes.

Enemas were administered with a glass capillary tube that had been heated and pulled into a narrow tip. Tips of all capillary tubes were placed in a flame to try to eliminate sharp edges.

Using a 30-ml syringe that was connected to the capillary tube, we drew 2 μl of the virus–blood suspension from a calibrated micropipette into the capillary tube. After drawing up the virus–blood suspension, the tip of the tube was immediately dipped in saline to prevent the suspension from drying in the tip and plugging it (Klowden 1981). The tube was then inserted into the anus of a mosquito that had been immobilized by chilling. The suspension was pushed into the mosquito's midgut with air pressure from the syringe. Based on visual estimates of the degree of repletion, a 2-μl enema was approximately the same amount of suspension that *Ae. aegypti* ingested from a hanging drop.

Aedes aegypti were exposed orally to virus by allowing them to imbibe virus–blood suspensions from hanging drops (Gubler and Rosen 1976, Miller et al. 1982). Virus–blood suspensions were presented to mosquitoes as drops on the nylon mesh covering the top of mosquito cages. Only mosquitoes engorged to stage IV or greater on the Pilitt/Jones scale (Pilitt and Jones 1972) were retained.

The mosquito inoculation technique (Rosen and Gubler 1974) in combination with the direct fluorescent antibody technique (Kuberski and Rosen 1977) was used to titer DEN-2 virus in virus–blood suspensions. Titrations were carried out by inoculating 10-fold dilutions into groups of *Ae. aegypti* (Rexville strain). We assayed at least 5 mosquitoes per dilution and used the Kärber method to calculate virus titers (Lennette and Schmidt 1979). Infection status of all experimental mosquitoes was determined by squashing their heads on microscope slides and assaying them with the direct fluorescent antibody test (Kuberski and Rosen 1977). A chi-square test was used with significance at $\alpha = 0.05$ to detect statistical differences between treatments (Sokal and Rohlf 1987).

Infection rates in the first 3 experiments were not significantly different ($P > 0.05$). In the 4th experiment, however, the infection rate of mosquitoes exposed to virus–blood suspensions via enemas was significantly higher than the infection rate of mosquitoes that imbibed a suspension ($P < 0.05$, Table 1). The mean ± SE for virus titers of virus–blood suspensions was $10^{8.2} \pm 10^{0.1}$ MID_{50} per 1.0 ml.

A close examination of the data indicated that enemas were not an acceptable substitute for oral exposure. During 2 of the 4 trials with viremic enemas, 100% of the mosquitoes were infected (Table 1). During 3 years of research on the infection of *Ae. aegypti* with DEN-2 virus, we have observed 100% infection rates only after intrathoracic inoculations. We speculate that during some of the experiments the capillary tube abraded the hindgut during the enema delivery and virus was inoculated directly into the mosquito's hemocoel. Therefore, we conclude that administering virus via our enema protocol is an unreliable method for simulating oral exposure of *Ae. aegypti* with dengue viruses.

Table 1. Dengue-2 virus infection rates of *Aedes aegypti* that either imbibed virus-infected blood from a hanging drop or had it delivered into the midgut by enema.

Experiment	% infected (+/total)	
	Virus–blood suspension imbibed from a hanging drop	Virus–blood suspension administered by enema
1	64 (9/14)	100[1] (12/12)
2	78 (7/9)	56[1] (14/25)
3	65 (20/31)	64[1] (9/14)
4	39 (9/23)	100[2] (18/18)
Mean (total)	58 (45/77)	77 (53/69)

[1] Not significantly different from infection rate of mosquitoes that imbibed the virus–blood suspension from a hanging drop (chi-square test, $P > 0.05$).

[2] Infection rate of mosquitoes administered enemas significantly greater than mosquitoes exposed orally (chi-square, $P < 0.05$).

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