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A Large Scale Laboratory Cage Trial of *Aedes* Densonucleosis Virus (AeDNV)

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ABSTRACT *Aedes aegypti* (L.) (Diptera: Culicidae) the primary vector of dengue viruses (DENV1–4), oviposit in and around human dwellings, including sites difficult to locate, making control of this mosquito challenging. We explored the efficacy and sustainability of *Aedes* Densonucleosis Virus (AeDNV) as a biocontrol agent for *Ae. aegypti* in and among oviposition sites in large laboratory cages (>92 m^3) as a prelude to field trials. Select cages were seeded with AeDNV in a single oviposition site (OPS) with unseeded OPSs established at varied distances. Quantitative real-time polymerase chain reaction was used to track dispersal and accumulation of AeDNV among OPSs. All eggs were collected weekly from each cage and counted. We asked: (1) Is AeDNV dispersed over varying distances and can it accumulate and persist in novel OPSs? (2) Are egg densities reduced in AeDNV treated populations? AeDNV was dispersed to and sustained in novel OPSs. Virus accumulation in OPSs was positively correlated with egg densities and proximity to the initial infection source affected the timing of dispersal and maintenance of viral titers. AeDNV did not significantly reduce *Ae. aegypti* egg densities. The current study documents that adult female *Ae. aegypti* oviposition behavior leads to successful viral dispersal from treated to novel containers in large-scale cages; however, the AeDNV titers reached were not sufficient to reduce egg densities.

KEY WORDS *Aedes aegypti*, *Aedes* densonucleosis virus, biological control, virus accumulation and dispersal, female oviposition behavior

The geographical area in which Dengue viruses (DENV1–4) are endemic has expanded in the last two decades and novel methods of reducing DENV transmission are needed (Gubler 2004, Mackenzie et al. 2004). A primary target in the reduction of DENV transmission is via control of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes, the primary vector of DENV. Because *Ae. aegypti* oviposit in and around human dwellings, oviposition sites are often hidden or difficult to reach. These sites include the many small containers (discarded cans, cartons, bike tires, evaporative air conditioners, cemetery vases, ant traps, and so forth) located around households where DENV transmission occurs. In addition, skip oviposition behavior of *Ae. aegypti* females, in which a single female disperses her eggs among several sites, has been viewed as an obstacle to effective control (Reiter 2007). Reiter (2007) expressed the need for control efforts that either exploit or negate skip-oviposition behavior.

AeDNV is a mosquito-specific densonucleosis virus in the family Parvoviridae that was first isolated from a laboratory colony of *Ae. aegypti* (Lebedeva et al. 1972). It replicates in the nuclei of mosquito cells (Buchatsky 1989, Carlson et al. 2006). Larvae of *Ae. aegypti* become infected through their anal papillae in the aquatic larval habitat. Infected larvae excrete virus into the larval habitat (Ledermann et al. 2004) until they either pupate or die. AeDNV titers of 10^8 genome equivalents (geq)/ml cause infection in mosquito larvae (Ledermann et al. 2004); however, in the lab, negligible amounts ($<10^2$ geq/ml) of virus can also result in larval infection (unpublished data). Larval mortality is dosage dependent with >50% mortality occurring at viral titers $\geq 10^{10}$ geq/ml (Ledermann et al. 2004). Some larvae survive initial infection, pupate and eclose as infected adults. Infected females vertically transmit AeDNV and as a result transmit it to larval habitats during oviposition (Suchman et al. 2006, Carlson et al. 2006) and diuresis (Suchman et al. 2009). AeDNV is capable of surviving periodic drying and in preliminary experiments where we allowed containers with water inoculated with 10^{10} geq/ml to evaporate, the viral load decreased by only one log (unpublished data).

Previous small scale studies demonstrating the potential of AeDNV as a mosquito biocontrol agent have also shown that AeDNV causes significant larval mortality (Buchatsky et al. 1987, Buchatsky 1989, Ledermann et al. 2004), reduces adult lifespan and daily survival (Suchman et al. 2006), and reduces female fecundity (Suchman et al. 2006). Limited field trials

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using a patented preparation of AeDNV have also been shown to reduce populations of *Ae. aegypti* (Buchatsky et al. 1987, Buchatsky 1989).

In the current study, we used AeDNV to treat mosquitoes in large population cages (>92 m^3) to asses whether to proceed to field trials. A unique design to our population cages enabled us to explore the transfer of AeDNV among various oviposition sites by exploiting the oviposition behavior of adult female *Ae. aegypti.* We also assessed whether egg densities were reduced in AeDNV treated cages.

Materials and Methods

Generation of a Genetically Diverse Laboratory Strain (GDLS) of *Ae. aegypti.* We created a genetically diverse target population from the southern coastal region of Chiapas, Mexico. We collected *Ae. aegypti* larvae and pupae from 10 geographically separate locations in that region; Pijijiapan (15° 41′ N, 93° 12′ W), Mapastepec (15° 26′ N, 92° 54′ W), Escuintla (15° 19′ N, 93° 39′ W), Huixtla (15° 08′ N, 92° 28′ W), Huehuetan (15° 01′ N, 92° 22′ W), Mazatán (14° 52′ N, 92° 26′ W), Rio Florido (15° 51′ N, 92° 20′ W), Puerto Chiapas (14° 33′ N, 92° 15′ W), Ciudad Hidalgo (14° 40′ N, 92° 09′ W), and Motozintla (15° 22′ N, 92° 14′ W). We reared the field collected larvae and pupae to adults in the laboratory where we maintained each population separately. We then collected and stored F_1 eggs from each population. We maintained all subsequent generations separately.

We combined all ten strains for each experiment. We hatched F_2 eggs from each of the geographic populations in separate larval rearing pans (10 total). Upon pupation, we collected ≈50 pupae from each of the ten populations and combined them into a single emergence container in a 0.3 m^3 cage. Adults were allowed to mate, blood-feed, and lay eggs over the course of a month. The eggs collected and stored from this mating constituted a GDLS.

The justification for this approach is that in theory, allele frequencies in any one collection will drift through time and gradually genetic diversity will be lost at a rate proportional to the effective population size (Fisher 1930, Wright 1931). However, if the initial collections are maintained as separate strains then allele frequencies and diversity among all strains should be preserved. This approach assumes no laboratory adaptation. However, we acknowledge that parallel adaptations among all separately maintained strains are highly likely and we are in the process of formally testing this prediction.

Mosquito Rearing. The caged populations of mosquitoes were reared and maintained under a 12:12 LD photoperiod at 27°C ± 2°C and 82% ± 3% humidity. Larvae were fed ground Brewer's yeast tablets ad libitum. Adult mosquitoes were provided with raisins and were allowed to feed once a week on restrained mice. OPSs were available constantly and the inner surface of each was lined with filter paper (Blotting Paper 3MM, Life Science Products, Inc., Frederick, CO) for egg collection. The filter papers were collected twice between feedings to prevent hatching and were placed in plastic bags awaiting counting. Eggs were added back weekly as describe below.

Population Cages and Experimental Design. Experiments to study the efficacy of AeDNV as a biocontrol agent for *Ae. aegypti* mosquitoes were conducted in six large cages between 92–122 m^3 in volume. Each cage was accessed through a series of three sleeves, each opening to a shelf at a different height (Fig. 1A). Each shelf held three OPSs which were glass Pyrex containers (Fig. 1A). All OPSs were filled with 600 ml tap water and dissolved 500 mg Brewer's yeast tablets (Country Life, Hauppauge, NY) as a larval food source at the initiation of the experiment. Water was added weekly to each container to maintain a 600 ml level.

One of the goals of this experiment was to determine the ability of AeDNV to be transferred from an AeDNV-seeded OPS to novel OPSs at various distances by infected adults. Therefore, in two of the treatment cages (seeded-infected; Fig. 1B, C) we seeded only site 1A (Fig. 1D) with 10^{11} geq/ml of AeDNV in 600 ml tap water. We used 10^{11} geq/ml of AeDNV as our inoculation titer because it has been shown to cause >75% larval mortality (Ledermann et al. 2004). The uninfected cages were open to seeded-infected cages (Fig. 1E, F) via a 3.8 cm. diameter hole (Figs. 1G) and the distance between the OPSs in the seeded-infected cage and those in the novel-infected cage through the opening was ≈5 m. Two cages served as control cages (Fig. 1H, I).

We established populations by adding 300 GDLS eggs on filter paper to site 1A in each of the cages at weeks 0, 1, 2, and 3 for a total of 1,200 GDLS eggs. This ensured that a sufficient number of mosquitoes were infected at the beginning of the study. The adult mosquitoes began to oviposit on filter paper which lined the OPSs by week 3 after which eggs laid by existing females were returned.

Eggs were returned to treated cages at a rate proportional to egg densities in the control cage and proportional to where they were laid. A return rate of 300 eggs per week was held constant in the control cages. For example, if a control cage produced 6,000 eggs one week and we returned 300 to that cage, then we returned 5% (300/6000) of the eggs. If a treated cage in the same week produced 600 eggs then we would return 30 eggs (600 * 0.05) or if, conversely, a treated cage the same week produced 60,000 eggs then we would return 3,000 eggs. With this methodology, when egg densities were the same in treated and control cages we returned equal numbers of eggs to each. If egg densities were lower in treated cages then we returned fewer eggs to the treated cage and if egg densities were greater in treated cages (if, e.g., there was a greater survival of larvae due to density dependence) then we returned more eggs to the treated cage. If we were to simply return 300 eggs each week to the treated cages, we would have artificially increased the rate of recruitment when egg densities in treated cages were low and conversely artificially decreased the recruitment rate when egg densities from treated cages were high.

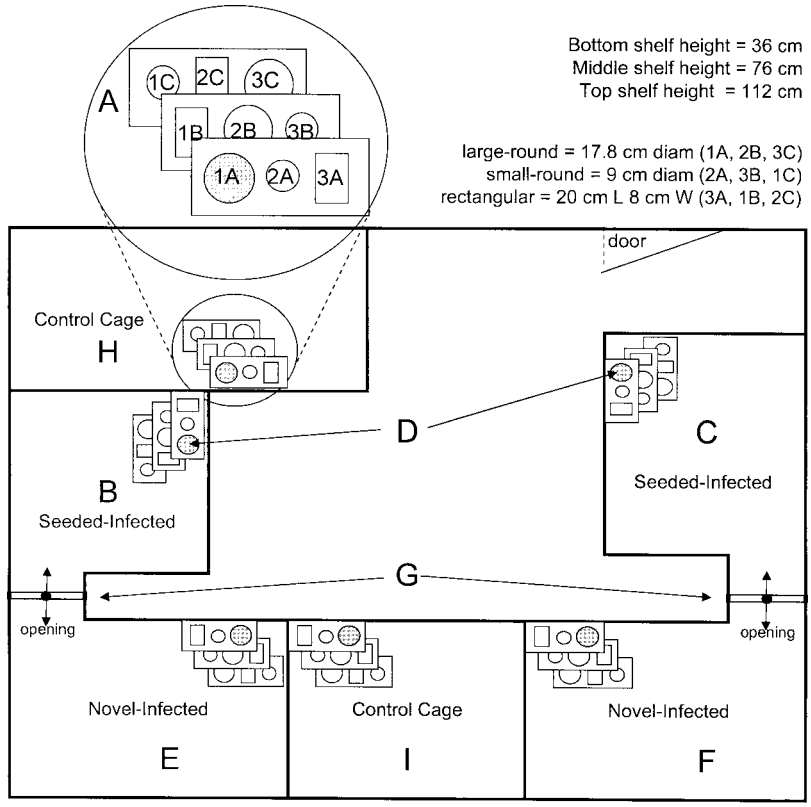

Fig. 1. Experimental design and set-up of large cages. All six cages were between 92–122 m^3 in volume. Seeded-infected cages (B and C) each contained one of nine OPS (D) that was seeded with 10^{11} geq/ml of AeDNV. These seeded-infected cages were opened to the novel-infected cages (E and F) via a 3.8 cm diameter hole (G). The novel-infected cages did not receive virus. Two cages (H and I) served as controls.

Data Collection. The nature and design of this experiment (large cages with limited access, infected water sources, large adult populations, and a 25-wk trial) restricted the types of data we were able to collect. First, we did not remove any OPSs from the cages as the risk of viral contamination from infected water sources to uninfected areas was high. Therefore, we were unable to take data on larvae or pupae such as mortality or infection rates. Second, the limited access to the cages as well as the large adult densities made data collection on adults difficult. We were not able to take data on adult mortality or longevity because we did not have physical access to dead adults inside the cages. In addition, at the time of the experiment we did not have an effective means of taking sufficient nondestructive adult samples to assess infection rates or population size. Therefore, we collected only those data in which we did not destroy or permanently remove any larvae, pupae, or adults as well as those data that did not require the removal of OPSs from the cages.

We monitored changes in egg densities in AeDNV-treated populations relative to the untreated controls. Egg densities were determined by counting the number of eggs laid on filter papers weekly. To assess the accumulation and persistence of AeDNV in the OPSs, we collected 1 ml water samples every 2 wk from all OPSs in each treatment cage. Water samples were also taken from site 1A in control cages to ensure that contamination had not occurred. All water samples were taken from within the cages by accessing the OPSs through sleeves. We performed quantitative real-time PCR analysis (RT-PCR) on the water samples to assess viral titers. We followed the RT-PCR procedures of Ledermann et al. (2004) but used the Bio-Rad iQ5 Mulitcolor Real-Time PCR Detection System (Hercules, CA). We compared all titers quantified from OPSs in treatment cages to two negative controls (sterile water and water samples from OPSs in control cages) to ensure that any virus detected in treatment cages was not an artifact.

We conducted a linear regression of AeDNV virus titer in OPSs (seeded-infected cages only) each week versus the corresponding egg density 2 wk before the virus sampling, to assess whether egg densities affected AeDNV accumulation. The lag-time incorporated in this analysis assumes that eggs laid one week would theoretically take ~2 wk to affect the AeDNV titers detected via larval excretion of the virus. This takes into account the time of embryo development once an egg is laid (3–4 d) and the development of the larvae (6–7 d).

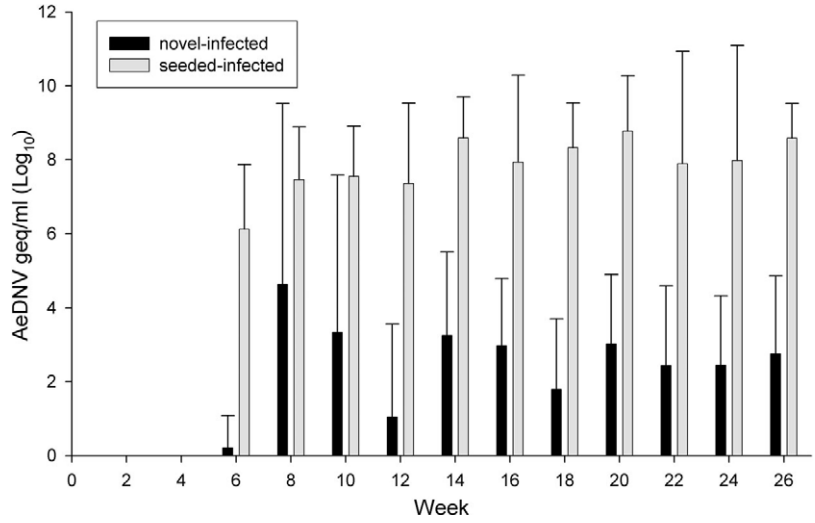

Fig. 2. The average weekly titer ($\log_{10}$) in OPS ± SD in seeded-infected and novel-infected cages, $n = 18$. There was a significant difference between treatments at each time point ($P < 0.05$) based on paired t-test at each time point.

Relationship Between Egg and Adult Densities. In an attempt to address our limitations of our system for assessing population reduction, we conducted a separate experiment in the same cages after the completion of the AeDNV cage trial. Because egg densities alone are not a sensitive measurement of adult population size, we set up an experiment to determine if a correlation existed between egg densities and adult female densities in this caged system. We established six populations of GDLS mosquitoes as described previously without AeDNV. In addition, eggs were added back at 200 eggs per week rather than 300 eggs per week, OPSs were emptied and cleaned weekly and cages were not opened to each other. This allowed us six complete replicates. We collected and counted eggs weekly. On week 3 we began taking adult samples. Adults were sampled by placing a BG-Sentinel Mosquito Trap (Biogents AG, Regensburg, Germany) in each cage for 1 hr weekly. The adults were counted and sexed on cold plates then returned to cages. The adults experienced between 5–25% mortality using these methods. We conducted several linear regressions to find the best-fit model within a biological framework in which female adult numbers could be predicted from egg numbers.

Results

Virus Dispersal and Accumulation. In both seeded-infected cages the titer of the water in the initially

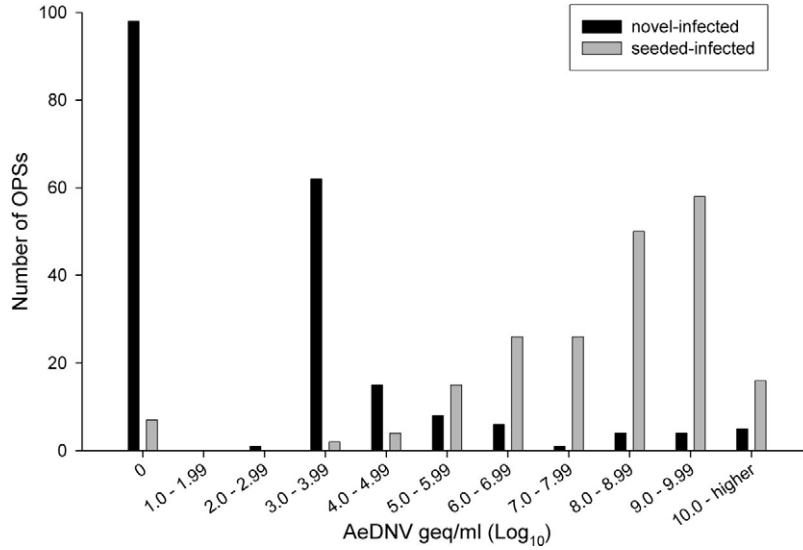

Fig. 3. Frequency distribution of AeDNV titers ($\log_{10}$ geq/ml) in OPSs in novel-infected and seeded-infected cages.

Table 1. Summary statistics of AeDNV titers in novel OPSs over weeks 6–26 in seeded-infected cages (B and C) and weeks 8–26 in novel-infected cages (E and F)

Treatment	Cage	N	Mean	SD	Median	Range
Seeded infected	B	88	1.30×10^9	2.17×10^9	3.78×10^8	3.00×10^3–1.07×10^{10}
	C	88	5.71×10^9	4.89×10^{10}	7.81×10^7	0.00–4.59×10^{11}
Novel infected	E	90	4.62×10^8	2.39×10^9	1.84×10^3	0.00–1.90×10^{10}
	F	90	9.02×10^9	7.49×10^{10}	2.26×10^3	0.00–7.09×10^{11}

Titers are reported in geq/ml.

seeded OPS remained at $\approx 10^{10}$ geq/ml of AeDNV over the course of the study and by week 6, 2 wk after initial blood feed, virus had been dispersed to all OPSs. The average weekly titer of OPSs in the seeded-infected treatment group remained above 10^6 geq/ml (Fig. 2) and the most frequent titer among the OPSs over the course of the trial was 10^9 geq/ml (Fig. 3). At least one novel OPS had reached 10^9 geq/ml of virus by week 10 in both seeded-infected cages and one OPS reached $\geq 10^{10}$ geq/ml. The average titer in novel OPSs in each seeded-infected cage over the course of the study was $\approx 10^9$ geq/ml with the median at $\approx 10^8$ and 10^7 geq/ml in cages B and C, respectively (Table 1).

In the novel-infected cages only one OPS had detectable virus (10^3 geq/ml) by week 6. It was not until week 8 that additional OPSs had detectable virus (six of nine OPSs in cage E and four of nine OPSs in cage F). We conducted paired *t*-tests for each week and found that the average titer of OPSs each week in the novel-infected group was significantly lower ($P < 0.001$) than those from the seeded-infected group (Fig. 2). The majority of OPSs in the novel-infected cages throughout the course of the trial did not have detectable viral titers (Fig. 3). Titers in three OPSs from the novel-infected cages did however reach 10^{10} geq/ml in week 8 and 10 but dropped off significantly after that time and remained at between 10^4 and 10^6 geq/ml. The average titer in OPSs in novel infected cages E and F over the course of the study was $\approx 10^8$ and 10^9 geq/ml, respectively (Table 1). However, the data were skewed and therefore the median (10^3 geq/ml) more accurately predicts the OPS titers (Table 1).

We conducted a linear regression analyses to determine if there was a relationship between AeDNV titers and egg densities. We found that there was a positive and significant effect of egg densities on AeDNV accumulation (Fig. 4).

Egg Densities. A repeated measures analysis with autoregressive errors was performed (PROC MIXED in SAS 9.1, SAS Institute, 2003) on log-transformed egg densities in each cage among treatment groups over the course of 25 wk (Table 2). There were two time points (weeks 22 and 23) in which egg densities were significantly higher in control cages than in the novel-infected cages ($P = 0.0498$) and seeded-infected cages ($P = 0.0486$), respectively (Fig. 5). There appeared to be a drop in egg densities in the seeded-infected and novel-infected cages around week 15. The corresponding number of OPSs containing titers $\geq 10^{10}$ geq/ml was at its greatest with three OPSs available in the seeded-infected cages only (Fig. 5). Also, near the end of the study, the control cages appeared to have higher egg densities than either of the treatment groups. However, when we analyzed these data using a basic two-period repeated measures (PROC MIXED in SAS 9.1, SAS Institute, 2003) in which we compared

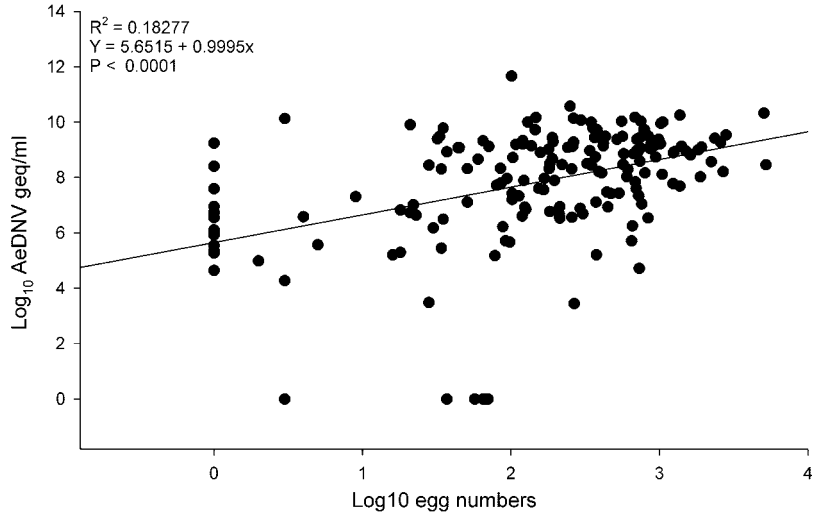

Fig. 4. Linear regression of AeDNV titers ($\log_{10}$ geq/ml) in oviposition sites each week on egg counts ($\log_{10}$) in the same OPSs the previous week.

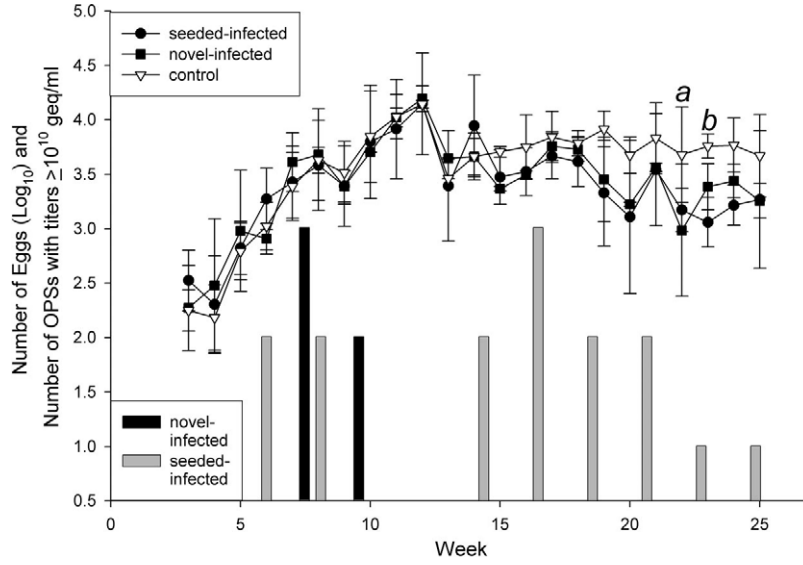

Fig. 5. Egg densities in control, novel-infected, and seeded-infected cages with corresponding the frequency distribution of OPSs with AeDNV titers $\geq 10^{10}$ geq/ml over the course of 25 wk. Average number of eggs produced/treatment ($\log_{10}$) ± SD $n = 2$ for each treatment. (a) A significant difference between control and novel-infected. (b) A significant difference between control and seeded-infected ($P \leq 0.05$).

the average egg density in the first 5 wk and the average egg density in the last 5 wk, the difference among the treatment groups within each period was not significant (Fig. 6).

Relationship Between Egg and Adult Densities. In the secondary experiment assessing egg and adult densities, we conducted three linear regression analyses (PROC REG in SAS 9.2, SAS Institute, 2007) in which we compared adult female samples collected 1 wk with eggs collected 1 wk prior ($r^2 = 0.1679$, $P = 0.0008$), 2 wk prior ($r^2 = 0.1123$, $P = 0.0068$), and the sum of eggs collected both 1 and 2 wk prior ($r^2 = 0.2427$, $P = 0.0002$). A correlation analysis (PROC CORR in SAS 9.2) confirmed that eggs collected 1 wk prior were not correlated with eggs collected 2 wk before adults ($r = 0.1586$, $P = 0.2106$). Although all models showed that there was a significant relationship, the best fit model within a biological framework was the latter in which adult female densities were best predicted by the sum of eggs collected 1 and 2 wk prior (Table 3).

Discussion

The goal of this study was twofold. First, we wanted to confirm that AeDNV could be successfully dispersed to and maintained in novel OPSs by exploiting the behavior of adult females. Second, we aimed to test if AeDNV was capable of reducing *Ae. aegypti* egg densities by using an experimental design simulating an optimal field situation in which OPSs were unperturbed and adult females had guaranteed access to at least one OPS containing $\geq 10^{10}$ geq/ml AeDNV.

We found that AeDNV was dispersed, accumulated and persisted in novel OPSs in this caged system. Virus accumulation in OPSs is positively correlated with egg densities indicating that OPSs containing relatively

Table 2. ANOVA table from the repeated measures analysis with autoregressive errors on log-transformed egg densities in each cage among treatment groups over the course of 25 wk (PROC MIXED SAS 9.1)

Effect	Num DF	Den DF	*F* Value	Pr > *F*
Treatment	2	3.09	0.52	0.6372
Week	22	48.9	11.75	<0.0001
Treatment*week	44	40.6	0.89	0.6550

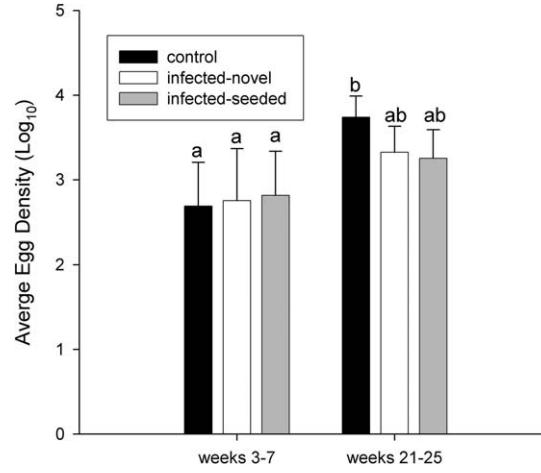

Fig. 6. Average egg density ($\log_{10}$) ± SD among treatment groups in the first 5 wk and last 5 wk of the trial. Bars with the same letter were not significantly different based on a basic two-period repeated measures analysis of variance (ANOVA) ($P < 0.05$).

Table 3. Summary statistics and ANOVA table from the regression analysis of adult females on eggs over the course of 9 wk

Variable	df	Parameter estimate	SE	*t*-value	Pr > t	N	Mean	SD
Intercept	1	27.48929	15.80221	1.74	0.0870			
Eggs wk 1	1	0.00514	0.00159	3.24	0.0019	64	7,262	3,453
Eggs wk 2	1	0.00381	0.00155	2.46	0.0169	64	7,365	3,380
Females						64	93.0781	47.5346

Female numbers were based on weekly BG-Sentinel Mosquito Trap sampling. Egg numbers were based on total eggs collected 1 and 2 wk before the week in which adult females were collected.

high egg densities generate an equally high number of infected larvae and thus result in greater virus accumulation. The initially seeded OPS was an important source of AeDNV as its proximity to novel OPSs affected the timing of dispersal and maintenance of viral titers in the novel OPSs. OPSs in the seeded-infected cages contained virus 2 wk before those in the novel-infected cages and were able to maintain higher viral titers for the duration of the study. The median titers in novel OPSs in the seeded-infected cage were between 10^7 to 10^8 geq/ml whereas the median titer in those novel OPSs in the novel-infected caged was 10^3 geq/ml. These results expand on and are consistent with the small-scale evaluation by Suchman et al. (2009) of AeDNV dispersal to novel containers where novel containers immediately adjacent to seeded containers reached titers no $>10^8$ geq/ml.

Adult female *Ae. aegypti* behavior was an influential factor in viral dispersal to novel sites. It is apparent by the presence of AeDNV in the novel-infected OPSs that at least some infected females migrated to the novel-infected cage and oviposited in these containers, however, this dispersal was neither consistent nor frequent enough to build up and sustain high viral titers. One possible explanation is that the movement of females from one cage to the next was passive and therefore limited by distance ($\approx$5 m) and the small size of the opening (3.8 cm). However, this may have been a result of active female behavior in that females emerging from OPSs near the initial infection source may not have searched for additional OPSs because OPSs were available nearby. Edman et al. (1995) reported that *Ae. aegypti* dispersal is driven by site availability. They found that the female recapture rate was greater in houses where OPSs had been added than in those where they had been removed, indicating that females tended not to disperse when sufficient sites were available. Finally, viral titers $\geq 10^{10}$ geq/ml may not have been reached in novel OPSs because the rate at which water in novel OPSs was infected, especially those in the novel-infected cages, may have not been fast enough to overcome the viral decay rate of one log per 4 d (Suchman et al. 2009).

We were unable to detect a significant difference in egg densities between the controls and treatment groups although there was a trend near the end of the 25 wk period showing more eggs in the control cages than in treatment cages. However, differences in the average egg densities among the treatment groups during the first 5 wk of the trial and those among the treatment groups during the last 5 wk of the trial were both not significant. The apparent decrease in egg densities in both seeded-infected and novel-infected cages around week 15 corresponded to a slight increase (from 2 to 3) in the availability of OPSs containing viral titers $\geq 10^{10}$ geq/ml in the seeded cage only. This small increase most likely does not explain the small decrease in egg densities as the increase was found in only one of the seeded-infected cages.

Unfortunately egg production is not a sensitive indicator of population reduction and therefore a significant decrease in population size among the treatment groups may have been missed. For example, we may not have seen a significant decrease in egg densities in the treatment cages because of factors that affect and are a result of density-dependent larval mortality. Ledermann et al. (2004) and Suchman et al. (2006) reported that not only was mortality higher in infected larvae, but that eggs from infected females had a reduced hatch rate. Therefore, the mortality from AeDNV may have replaced mortality from larval competition and therefore caused no net loss to the population. This compensatory mortality could pose as a limitation to biological control efforts of container breeding mosquitoes such as *Ae. aegypti* (Washburn 1995, Juliano 2007) and could potentially lead to a net gain in terms of adult size, fecundity, and longevity due to this release from competition (Renshaw et al. 1994, Mahmood et al. 1997, Blackmore and Lord 2000, Reiskind and Lounibos 2009). However, in the secondary experiment in which we compared egg and adult densities, we found a significant positive correlation between the two. Therefore, we would have expected to detect significant differences in egg densities between control and treated group if in fact adult populations were significantly reduced.

This experiment was designed to provide optimal and consistent conditions (large initial infected population, small containers d $\leq$20 cm, close proximity to infection source, unperturbed) for the spread, accumulation, and maintenance of AeDNV yet we found that virus dispersed to novel OPSs was not maintained at titers sufficient to reduce egg densities. This trial was conducted to determine whether to proceed to field trials. As field conditions such as rain, wind, OPS type, and size, as well as human use of OPSs are likely to have a greater degree of variability, successful *Ae. aegypti* population reduction may be limited with virus accumulation to titers $\geq 10^{10}$ geq/ml being the greatest obstacle. Therefore, choice of initial infection site as well as size, availability, and proximity of novel OPSs to the infection source would need to be considered

as they may affect AeDNV accumulation and dispersal by females. Future large-scale studies should attempt to tease apart the limits of virus dispersal and the role of female oviposition behavior, as well as to find ways to more accurately address how adult densities, mortality, and longevity are affected by AeDNV.

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