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# A MOSQUITO DENSOVIRUS INFECTING *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* FROM THAILAND

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*Abstract.* A previously undescribed mosquito densovirus was detected in colonies of *Aedes aegypti* and *Ae. albopictus* from Thailand, using a polymerase chain reaction (PCR)–based assay. Phylogenetic analysis of this virus showed it to be most closely related to *A*DNV isolated from Russian *Ae. aegypti*. Both *Aedes* species were susceptible to oral infection with the Thai-strain virus. Larval mortality for *Ae. albopictus* was higher (82%) than for *Ae. aegypti* (51%). *Aedes aegypti* were able to transmit the virus vertically to a high (58%) proportion of $G_1$ progeny, and the virus was maintained persistently for up to six generations. A PCR survey of adult *Ae. aegypti* and *Ae. albopictus* in Thailand indicated that only *Ae. aegypti* are infected in the field, with an overall prevalence of 44%. Densovirus infection in adult *Ae. aegypti* showed distinct seasonal variation. The Thai strain densovirus may play a role in structuring *Ae. albopictus* and *Ae. aegypti* populations in nature.

Insect densoviruses are small, autonomous, non-enveloped DNA viruses that belong to the family Parvoviridae. They are characterized by the presence of inverted terminal repeat sequences and the separate encapsidation of complementary single-stranded DNA. Two subgroups of insect densoviruses have been recognized on the basis of lepidopteran isolates.[1] Subgroup I densoviruses have a 6-kb genome, code their structural and nonstructural proteins from separate strands, and infect all insect cells except those of the midgut. Subgroup II densoviruses have a smaller genome, approximately 4.9 kb, code all their proteins from one strand, and infect only insect midgut cells. Mosquito densoviruses do not easily fit into this classification scheme, and as such form a third subgroup. They are characterized as having a 4-kb genome and infecting all tissues of their insect hosts.[2] While Subgroup I and II densoviruses typically encapsidate both plus and minus DNA strands with equal frequency, some mosquito densoviruses encapsidate primarily minus strands.[3,4]

To date, five mosquito densoviruses have been described. The first, *A*DNV, was isolated from *Aedes aegypti* larvae,[3,5] while the second, *Aa*PV, was recovered from an *Ae. albopictus* (C6/36) cell line.[4,6,7] Genomic sequence data have been obtained for both these densoviruses. A third was observed by electron microscopy of an *Ae. pseudoscutellaris* (AP-61) cell line.[8] More recently, two additional mosquito densoviruses (*Ta*DNV and *He*DNV) were detected using a polymerase chain reaction (PCR) assay in infected *Toxorhynchites amboinensis* (TRA-284) and *Haemagogus equinus* (GML-HE-12) cell lines, respectively.[2] The PCR products from both viruses were sequenced, and infection was further confirmed by electron microscopy and indirect fluorescent antibody tests.

Mosquito densoviruses are believed to be maintained in nature primarily by horizontal transmission, although transovarial and venereal transmission have also been recorded.[2,9] These viruses are often pathogenic to their arthropod hosts. *A*DNV was found to efficiently kill larval *Ae. aegypti,* as well as *Ae. caspius,* and to a lesser extent *Culex pipiens.*[10] *Aa*PV is highly pathogenic for *Ae. aegypti,* killing up to 95% of larvae after oral infection.[4,11] Although *Ta*DNV appeared nonpathogenic in experiments with *Aedes* and *Culex,* up to 10% of *Ae. aegypti* larvae died after being orally infected with *He*DNV.[2]

In this paper, we describe a sixth mosquito densovirus, designated *ATh*DNV, isolated from adult *Ae. albopictus* and *Ae. aegypti* mosquitoes from Thailand. We report on the infectivity and pathogenicity of this virus in *Ae. aegypti* and *Ae. albopictus,* and its prevalence in natural populations of both species. In addition, we describe its vertical transmission and persistence in an *Ae. aegypti* colony.

## MATERIALS AND METHODS

**Virus source.** The Thai strain densovirus was detected in a colony of *Ae. albopictus* that originated from larvae collected in 1992 from Krabi Province in southern Thailand, and had been in the laboratory for 5 generations. The virus was also detected in a colony of *Ae. aegypti* that was started with adult mosquitoes collected in 1993 from Chachoengsao Province in eastern Thailand, and had been in the same laboratory for 3 generations. Infected larvae from the *Ae. aegypti* colony were used as the source of virus in the investigations of infectivity, pathogenicity, and vertical transmission. Although it is not known how these colonies first acquired their infections, we subsequently refer to them as naturally infected to distinguish from mosquitoes that we infected experimentally.

**Mosquitoes used in experimental infection studies.** Three colonies of *Aedes* mosquitoes were used for investigations on virus infectivity and pathogenicity. Two uninfected *Ae. aegypti* colonies originating from geographically distant (> 800 km) populations were established; one from larval collections made in Chachoengsao Province in 1994 (colony CHA), and the second from egg collections made in Songkhla Province, southern Thailand in 1995 (colony SON). In addition, an uninfected *Ae. albopictus* colony was founded with adult females collected in Chachoengsao Province in 1994. To ensure that newly colonized mosquitoes were not infected with densovirus, field-collected females were allowed to oviposit in individual vials, then were killed and screened by a PCR after oviposition. Offspring of densovirus-negative mothers were maintained together and raised through 4 generations, then tested again by the PCR before being used in the experiments.

**Detection of mosquito densoviruses by the PCR.** Mosquitoes were screened with a PCR assay using primers designed from the densovirus open reading frame 3.[2] Crude DNA extractions were performed by homogenizing an individual adult or larval mosquito in 100 μl of STE buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0), using the methods of O'Neill and others.[12] One microliter of the supernatant was used as DNA template in the PCR. Densovirus-infected *Ae. albopictus* C6/36 cell line DNA was used as a positive control. Viral DNA was PCR amplified in 20-μl reaction volumes: 2 μl of 10× buffer (Promega, Madison, WI), 2 μl of 25mM $MgCl_2$, 0.5 μl of dNTPs (10 mM each), 0.5 μl of each primer (20 mM each), and 1 unit of *Taq* DNA polymerase (Promega). The PCR thermal profile was 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min per cycle for 35 cycles. The PCR products were run on a 1% agarose gel with a 1-kb ladder (Gibco/BRL, Gaithersburg, MD) to determine the presence and size of amplified DNA. Samples that yielded products of the expected size (350 basepairs) were scored as positive. The Thai strain densovirus was initially detected by PCR screening of adult mosquitoes from two colonies of *Aedes.* To further confirm the presence of densovirus in these colonies, amplified products were digested with *Ava* II since a *Ava* II restriction site was always present in all known mosquito densoviruses. Digestion with *Ava* II yields products of approximately 110 and 240 basepairs.

**Phylogenetic analysis of *ATh*DNV fragments.** To characterize the genome of the Thai densovirus, DNA samples from the naturally infected *Ae. albopictus* and *Ae. aegypti* colonies were amplified and sequenced. The PCR products were purified using spin columns (Wizard PCR Preps; Promega) and sequenced on an ABI377 automated sequencer (Perkin Elmer, Norwalk, CT). Resulting sequences were manually aligned with other described mosquito densovirus sequences and subjected to maximum parsimony analysis using the heuristic search algorithm of PAUP version 3.0.[13] One hundred bootstrap replications were carried out. The number of nucleotide substitutions was estimated using the two-parameter method[14] with PHYLIP version 3.5c.[15]

**Susceptibility to *ATh*DNV and pathogenicity for *Ae. aegypti* and *Ae. albopictus.*** Pools of 50 first-instar larvae from the uninfected *Ae. aegypti* (CHA) and *Ae. albopictus* colonies were placed in separate autoclaved rearing trays with 1 liter of distilled water, with 2 replicates for each species. Approximately 20 freshly dead larvae from the naturally infected *Ae. aegypti* colony were confirmed infected by PCR-screening of larval heads, then ground and added to each rearing tray as a source of densovirus. Mosquito larvae were reared to adulthood under insectary conditions, and fed on autoclaved fish food pellets until pupation. Surviving adults were killed and assayed by the PCR to compare the densovirus infection rate in adult *Ae. aegypti* and *Ae. albopictus* mosquitoes.

**Susceptibility to infection between two populations of *Ae. aegypti.*** Pools of 100 first-instar larvae from each of the two uninfected *Ae. aegypti* colonies were placed in separate autoclaved rearing trays with 1 liter of distilled water, with 2 replicates for each population. Approximately 20 PCR-positive dead larvae from the naturally infected *Ae. aegypti* colony were ground and added to each rearing tray as a source of densovirus. Mosquito larvae were reared to adulthood as previously described. Surviving adults were assayed by the PCR to compare susceptibility to the Thai strain densovirus between two geographically distant *Ae. aegypti* populations.

**Vertical transmission and persistence of *ATh*DNV in *Ae. aegypti.*** Adult female *Ae. aegypti* from the naturally infected colony were used to establish 14 infected female lines. Parent generation ($G_0$) females were bloodfed and individually isolated for egg-laying. After oviposition, females were killed and tested by the PCR to confirm infection status. The $G_1$ eggs from each densovirus-positive female were hatched and reared separately in autoclaved trays as described above. Dead larvae and pupae were removed daily. Adult $G_1$ offspring from each infected female line were maintained in separate cages and allowed to mate with their siblings. One week after emergence, $G_1$ females were blood fed and allowed to oviposit on common egg paper set up in each cage, then screened by the PCR for the presence of densovirus. Successive generations from each female line were maintained together in the same manner for 7 generations, with at least 10 offspring from each female-line generation being tested for densovirus.

**Prevalence of *ATh*DNV in natural *Ae. aegypti* and *Ae. albopictus* populations.** Adult *Ae. albopictus* and *Ae. aegypti* from 11 provinces throughout Thailand were collected using mosquito landing catches and attractant resting boxes,[16,17] from January to June 1997. Specimens were identified using morphologic keys,[18,19] and stored at −70°C until DNA was extracted. Mosquitoes were screened by the PCR for the presence of densovirus as previously described. In addition, seasonal variation in densovirus infection prevalence in *Ae. aegypti* was investigated. Mosquitoes were collected from 12 randomly selected houses in Village 2, Plaeng Yao District, Chachoengsao Province from April to September 1995, using attractant resting boxes. Specimens were identified using morphologic keys and stored at −70°C until screened by the PCR for the presence of densovirus.

**Statistical analysis.** The significance of differences in densovirus infection frequency between mosquito groups was evaluated with chi-square and Mann-Whitney U tests as indicated, using Statistix Version 4.1 (Analytical Software, Tallahassee, FL). Filial infection rates among successive generations were compared by one-way analysis of variance (ANOVA), with proportions arcsin transformed. Spearman's rank correlation coefficient was used to investigate the association between vertical transmission and mortality rates. Probability values $< 0.05$ were considered significant.

## RESULTS

**Detection and characterization of *ATh*DNV from *Ae. albopictus* and *Ae. aegypti.*** The PCR screening using densovirus specific primers revealed the presence of the viral genome in laboratory colonies of *Ae. albopictus* and *Ae. aegypti.* Products of the expected size (350 basepairs) were amplified from both species. Amplified products could be digested with *Ava* II to fragments approximately 110 and 240 basepairs in size, further confirming their identity as densovirus DNA. The densovirus sequences obtained from both infected *Aedes* colonies were identical at the DNA lev-

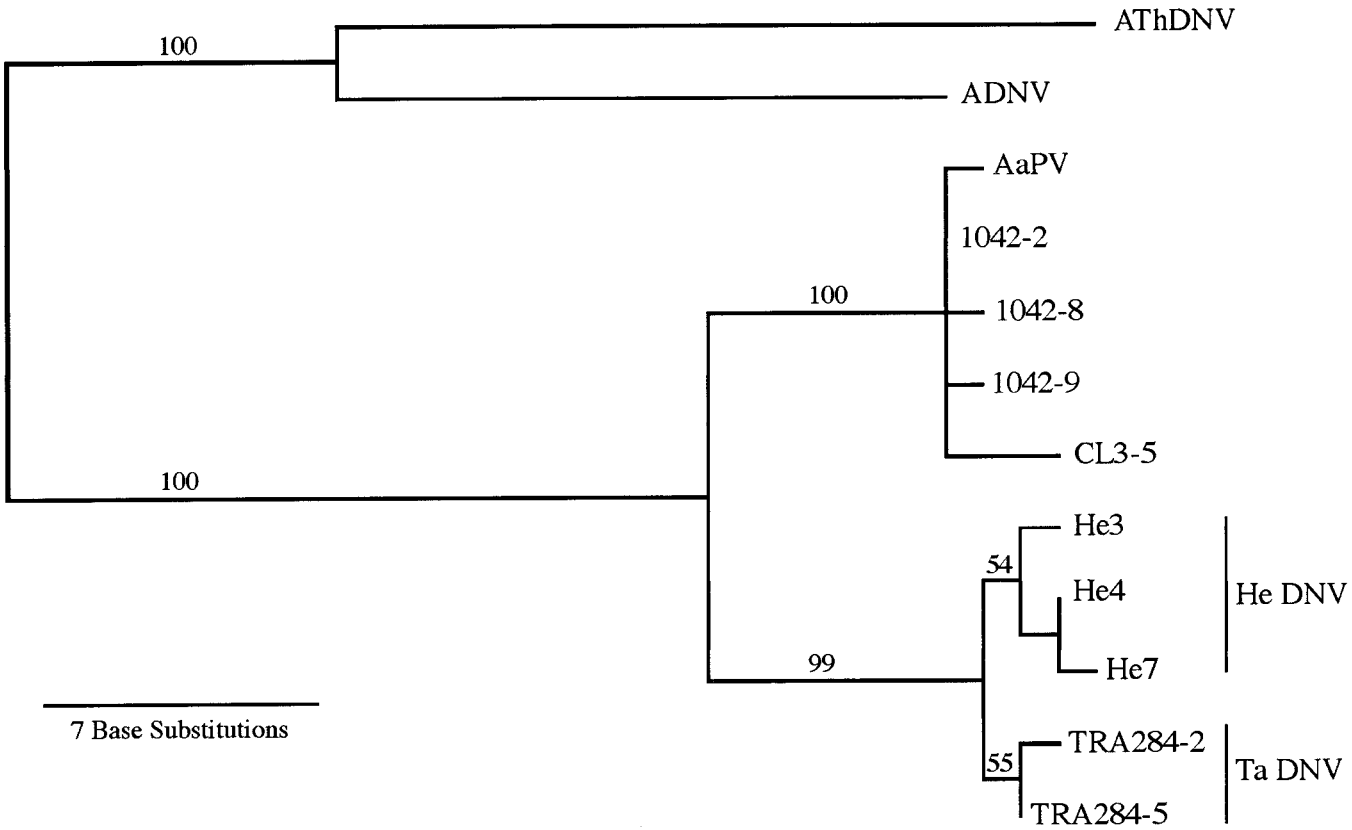


FIGURE 1. Most parsimonious unrooted tree generated from heuristic search algorithm of PAUP version 3.0 based on 301 basepairs of sequence data, showing the relationship of *ATh*DNV with other described mosquito densoviruses. Numbers above the branches refer to bootstrap values for 100 replicates. Source of nucleotide sequence data is as follows: *A*DNV from *Aedes aegypti* (GenBank accession no. M37899); *Aa*PV from an *Ae. albopictus* cell line (GenBank accession no. X74945); 1042-2, 1042-8, and 1042-9 from an *Ae. aegypti* cell line;[2] CL3-5 from a *Culex theileri* cell line;[2] He3, He4, He7 from a *Haemagogus equinus* cell line;[2] and TRA284-2 and TRA284-5 from a *Toxorhynchites amboinensis* cell line.[2]

el. These sequences could be easily aligned with those of other known densoviruses. The Thai densovirus most closely resembled *A*DNV,[3] with a 13.3% divergence at the DNA level (Kimura 2-parameter distance, transition:transversion ratio of 1:2). Divergence from other known mosquito densoviruses ranged between 20.9% and 22.3%. Maximum parsimony analysis generated one most parsimonious tree (Figure 1) and upheld the results of the nucleotide similarity data. Bootstrap values supported all major branches of the phylogenetic tree, which separated mosquito densoviruses into three main groups.

***Aedes* susceptibility to infection with *ATh*DNV.** Eighty percent of surviving *Ae. aegypti* adults (20 of 25) were PCR positive for densovirus after first-instar larval infection with *ATh*DNV (Table 1). In contrast, 33.3% (6 of 18) of surviving *Ae. albopictus* adults appeared infected. Larval mortality in *Ae. albopictus* was 82% (82 of 100) compared with 51% (51 of 100) in *Ae. aegypti*, suggesting that the virus may be more pathogenic to *Ae. albopictus*.

TABLE 1
First-instar oral infectivity of *ATh*DNV for mosquitoes as measured by positive polymerase chain reaction amplification

| Mosquito species | Infection in surviving adults | Mortality |
|---|---|---|
| *Aedes aegypti* | 80.0% (20/25) | 51.0% (51/100) |
| *Ae. albopictus* | 33.3% (6/18) | 82.0% (82/100) |

Both colonies of *Ae. aegypti* appeared equally susceptible to horizontal infection with the virus (Table 2), with 79.4% (54 of 68) of surviving adults from the Chachoengsao colony and 71.0% (71 of 100) from the Songkhla colony being infected ($\chi^2 = 1.5$, degrees of freedom [df] = 1, $P > 0.22$). Overall infection frequencies did not vary between males and females ($\chi^2 = 0.10$, df = 1, $P > 0.78$). Eighty percent of females (28 of 35) and 78.8% (26 of 33) of males from the Chachoengsao colony were positive for densovirus infection, compared with 71.1% (59 of 83) of females and 70.6% (12 of 17) of males from the Songkhla colony.

**Vertical transmission and persistence of *ATh*DNV in *Ae. aegypti*.** All 14 female lines transmitted the infection to at least some of their $G_1$ progeny. Mean adult survival in the first generation was 68.7% (167 of 243), with an average of 57.5% (96 of 167) of surviving adults being infected (Table 3). Filial infection rates in adult G1 progeny were positively associated with mortality in the same generation; this relationship was marginally significant ($r = 0.44$, $n = 14$, $P = 0.11$, by the Spearman rank correlation coefficient). Four female lines did not produce surviving offspring beyond the second generation. Densovirus infection was detected for 4

TABLE 2
Oral infectivity of *ATh*DNV for *Aedes aegypti* as measured by positive polymerase chain reaction amplification in surviving adults

| Mosquito population | Total adults infected | Females infected | Males infected |
|---|---|---|---|
| Chachoengsao | 79.4% (54/68) | 80.0% (28/35) | 78.8% (26/33) |
| Songkhla | 71.0% (71/100) | 71.1% (59/83) | 70.6% (12/17) |
| Total | 74.4% (125/168) | 73.7% (87/118) | 76.0% (38/50) |

generations in all 10 of the remaining lines, for 5 generations in 7 lines, and for 6 generations in 4 lines. Mean filial infection rates were not significantly different among the first 4 generations, with an average of 52.8% (391 of 740) of surviving adults being infected ($F_{3,86} = 0.62$, $P = 0.61$; by ANOVA, proportions arcsin transformed). Within those female lines that continued to maintain the virus beyond the fourth generation, adult infection rates decreased to 32.7% (51 of 156) in the fifth and sixth generations. No infected adult offspring were observed by the seventh generation in any of the female lines tested. Filial infection rates showed no significant sex bias, with an average of 42.7% (234 of 548) of females and 42.3% (208 of 492) of males being PCR-positive for densovirus ($P = 0.59$, by the Mann-Whitney U test).

**Prevalence of *ATh*DNV in natural *Ae. aegypti* and *Ae. albopictus* populations.** A total of 97 field-caught adult *Ae. aegypti* and 79 adult *Ae. albopictus* from 11 provinces throughout Thailand were screened by the PCR for densovirus (Table 4). Infection prevalence in *Ae. aegypti* was 44.3% (43 of 97). In contrast, none of the *Ae. albopictus* tested were positive for densovirus infection. To investigate seasonal variation in infection prevalence, *Ae. aegypti* adults were collected monthly from attractant resting boxes in houses in Village 2, Chachoengsao Province from April to September 1995. An average of 22.4% (189 of 844) of *Ae. aegypti* tested positive for densovirus infection by the PCR assay (Table 5). Median infection prevalence was not significantly different between males (134 of 571) and females (55 of 273) ($P = 0.26$, by the Mann-Whitney U test). Significant heterogeneity in monthly infection frequency was detected ($\chi^2 = 54.28$, df = 5, $P < 0.001$), with the most PCR-positive mosquitoes recorded in July (108 of 308) and the fewest in August (2 of 31). Furthermore, no females tested positive for densovirus during the months of August (0 of 7) and September (0 of 39), when overall prevalence was the lowest.

## DISCUSSION

We have detected a Thai strain densovirus in *Aedes* mosquitoes that appears to be distinct from other described mosquito densoviruses. Phylogenetic analysis shows it to be most closely related to the *A*DNV described by Afanasiev and others,[3] with 86.7% similarity on the DNA level, based on Kimura 2-parameter distances. In addition, the Thai densovirus appears to be ecologically distinct from known mosquito densoviruses, with high rates of vertical transmission and persistence over several generations. As such, we propose a new designation, *ATh*DNV, for this densovirus strain.

*ATh*DNV was initially detected in an *Ae. albopictus* colony that had been maintained in the laboratory for 5 generations. The identical virus was also detected in an *Ae. aegypti* colony that was maintained at the same facility. Our data indicate that densovirus infection is rare in natural populations of adult *Ae. albopictus* in Thailand. In contrast, infection is widespread and common in adult field populations of *Ae. aegypti*. It is likely that the *ATh*DNV infection initially detected in the laboratory colony of *Ae. albopictus* was acquired by chance contamination with *Ae. aegypti*. Several explanations may account for the difference in infection prevalence between these two species in the field. *Aedes albopictus* may have less contact with the virus as a result of its larval habitat utilisation. Alternatively, infection prevalence in adult *Ae. albopictus* may be low as a result of high larval-stage mortality (Table 1), with few infected individuals surviving to adulthood.

*Aedes aegypti* and *Ae. albopictus* both appear to be susceptible to oral infection with *ATh*DNV. More than 80% of surviving *Ae. aegypti* adults were PCR positive for densovirus after first-instar infection with the virus. The infection rate in surviving *Ae. albopictus* adults was much lower, approximately 30%. Our data suggest that *ATh*DNV may be more pathogenic for *Ae. albopictus*, with more than 80% of the larvae dying before pupation, compared with a 50% lar-

TABLE 3
Vertical transmission and persistence of *ATh*DNV in naturally infected *Aedes aegypti*, measured by polymerase chain reaction

| Generation | No. of female lines tested | No. of female lines transmitting *Ath*DNV | Infection in surviving adult progeny | | |
|---|---|---|---|---|---|
| | | | Total | Female | Male |
| 1 | 14 | 14 | 57.5% (96/167) | 59.8% (55/92) | 54.7% (41/75) |
| 2 | 11 | 11 | 48.3% (87/180) | 55.4% (41/74) | 43.4% (46/106) |
| 3 | 10 | 10 | 52.2% (130/249) | 48.1% (77/160) | 59.6% (53/89) |
| 4 | 10 | 10 | 54.2% (78/144) | 56.9% (41/72) | 51.4% (37/72) |
| 5 | 10 | 7 | 20.8% (30/144) | 13.9% (10/72) | 27.8% (20/72) |
| 6 | 7 | 4 | 19.4% (21/108) | 18.5% (10/54) | 20.4% (11/54) |
| 7 | 4 | 0 | 0% (0/48) | 0% (0/24) | 0% (0/24) |
| Total | | | 42.5% (442/1040) | 42.7% (234/548) | 42.3% (208/492) |

TABLE 4

Densovirus infection prevalence in natural populations of *Aedes aegypti* and *Ae. albopictus* in Thailand, based on a polymerase chain reaction

| Location (Province) | *Ae. aegypti* | *Ae. albopictus* |
|---|---|---|
| Chaiyaphum | 17.6% (3/17) | 0.0% (0/11) |
| Kanchanaburi | 33.3% (2/6) | 0.0% (0/16) |
| Lumpang | 33.3% (1/3) | 0.0% (0/3) |
| Lumphun | 50.0% (2/4) | 0.0% (0/3) |
| Nakhon Ratchasima | 38.9% (7/18) | 0.0% (0/5) |
| Phitsanulok | 50.0% (2/4) | 0.0% (0/2) |
| Phrae | 50.0% (2/4) | 0.0% (0/4) |
| Satun | 50.0% (5/10) | 0.0% (0/5) |
| Songkhla | 71.4% (10/14) | 0.0% (0/10) |
| Surat Thani | 100.0% (5/5) | 0.0% (0/10) |
| Ubon Ratchathani | 33.3% (4/12) | 0.0% (0/10) |
| Total | 44.3% (43/97) | 0.0% (0/79) |

val mortality in experimentally infected *Ae. aegypti.* However, since viral titer was not estimated in our study, it is possible that differences in mortality between the two species were a result of differences in inoculating dose. High levels of pathogenicity have also been observed with *Aa*PV. Barreau and others[11] noted up to 90% mortality of *Ae. aegypti* when ground infected larvae were added to the water in which they were reared. It is clear that the Thai strain densovirus can also be transmitted with relatively low host mortality, as indicated by its maintenance in the *Aedes* colonies where it was originally detected. Larval mortality in these naturally infected colonies appears to be on the order of 30% (Kittayapong P, unpublished data). Presumably these colonies encounter much lower virus concentrations under normal rearing conditions than in our experimental inoculum.

The two populations of *Ae. aegypti* showed a similar susceptibility to oral infection with *ATh*DNV. Overall infection frequency in surviving adults was 74%, and showed no significant sex bias. In their experiments with *Aa*PV, Barreau and others[11] recorded infection rates up to 32% in *Ae. aegypti* adults that had been exposed to the virus as first instar larvae. O'Neill and others[2] found 21% of adult *Ae. aegypti* to be PCR-positive for densovirus after first-instar infection with *He*DNV. These infection rates in adult *Ae. aegypti* are less than half of those we report for the Thai densovirus strain. Our observations may be explained by a lower pathogenicity of *ATh*DNV for *Ae. aegypti* or an enhanced susceptibility of *Ae. aegypti* to *ATh*DNV.

Vertical transmission has been reported with *A*DNV and *Aa*PV in *Ae. aegypti,* and with *He*DNV in *Ae. albopictus.*[2,9] O'Neill and others[2] found *He*DNV to be vertically transmitted to 20% of adult offspring of experimentally infected *Ae. albopictus* mothers; however, the virus was not transmitted in other mosquito species, including *Ae. aegypti.* Barreau and others[9] showed that vertical transmission of *Aa*PV in experimentally infected *Ae. aegypti* depended upon virus titer in the infected mother. Females with low virus titers produced no infected adult progeny, while those with higher titers transmitted the virus to approximately 5–7% of their adult progeny. Vertical transmission did not persist beyond the second generation. Our study of naturally infected *Ae. aegypti* found a much higher rate of vertical transmission, with 57.5% of surviving adult progeny being infected in the first generation. All infected females transmitted the virus to at least some of their $G_1$ offspring. Densovirus infection was maintained for 4 generations in 10 female lines, while transmission to adult progeny was recorded until generation 6 in 4 lines. Filial infection rates in first-generation adult progeny were positively associated with mortality in the same generation. These observations are consistent with those of Barreau and others,[9] who noted that females that transmitted the virus to their adult offspring also exhibited high mortality rates in their progeny. However, we also found virus transmission to $G_1$ progeny in three female lines with mortality rates of less than 10%, suggesting a low virus titer in the mother. Our data indicate the Thai strain densovirus can be persistently maintained over several generations, and may be vertically transmitted by mothers with low virus titers. These findings suggest that vertical transmission may play a more important role in the maintenance of *ATh*DNV in nature than it does in other mosquito densoviruses.

We found the prevalence of densovirus infection in field populations of adult *Ae. aegypti* to vary seasonally, ranging from 6.5% to 35.1% over a six-month period. Furthermore, during August and September, when overall prevalence was the lowest, no females (n = 46) tested positive for densovirus. Despite apparently high rates of vertical transmission, horizontal transmission clearly must play a large role in the persistence of *ATh*DNV in the field. The relative importance of horizontal and vertical transmission may vary seasonally, in response to variables such as rainfall that may influence the amount of densovirus present in the environment.

Considering that *ATh*DNV appears capable of being maintained in field populations of *Ae. aegypti* and that under laboratory conditions it appears more virulent for *Ae. albopictus,* it is possible that this virus may be involved in structuring *Ae. aegypti* and *Ae. albopictus* populations in nature. Interestingly, *Ae. albopictus* has been commonly reported to

TABLE 5

Seasonal variation in densovirus infection in natural populations of adult *Aedes aegypti* from Chachoengsao province, as measured by positive polymerase chain reaction amplification

| Month | Total infected | Females infected | Males infected |
|---|---|---|---|
| April | 22.4% (13/58) | 20.0% (3/15) | 23.3% (10/43) |
| May | 21.6% (19/88) | 11.6% (5/43) | 31.1% (14/45) |
| Jun | 16.3% (36/221) | 20.3% (16/79) | 14.1% (20/142) |
| July | 35.1% (108/308) | 34.4% (31/90) | 35.3% (77/218) |
| August | 6.5% (2/31) | 0% (0/7) | 8.3% (2/24) |
| September | 8.0% (11/138) | 0% (0/39) | 11/1% (11/99) |
| Total | 22.4 (189/844) | 20.1% (55/273) | 23.5% (134/571) |

outcompete *Ae. aegypti* in the United States, and in certain areas may be replacing it.[20–22] It has been proposed that a Gregarine parasite may be mediating competition between the two mosquito species in North America.[23–25] However, similar observations have not been reported in Thailand, where *Ae. aegypti* numbers have remained high even in areas where the two species share larval habitats.[26–28] The observed differential mortality between *Ae. albopictus* and *Ae. aegypti* when infected with *ATh*DNV, together with the widespread occurrence of this virus in natural *Ae. aegypti* populations in Thailand, suggests that the virus may play a role in providing a competitive advantage to *Ae. aegypti*. Further field studies are needed to determine to what extent *ATh*DNV influences competitive outcomes between these two species.

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