Supplementary files
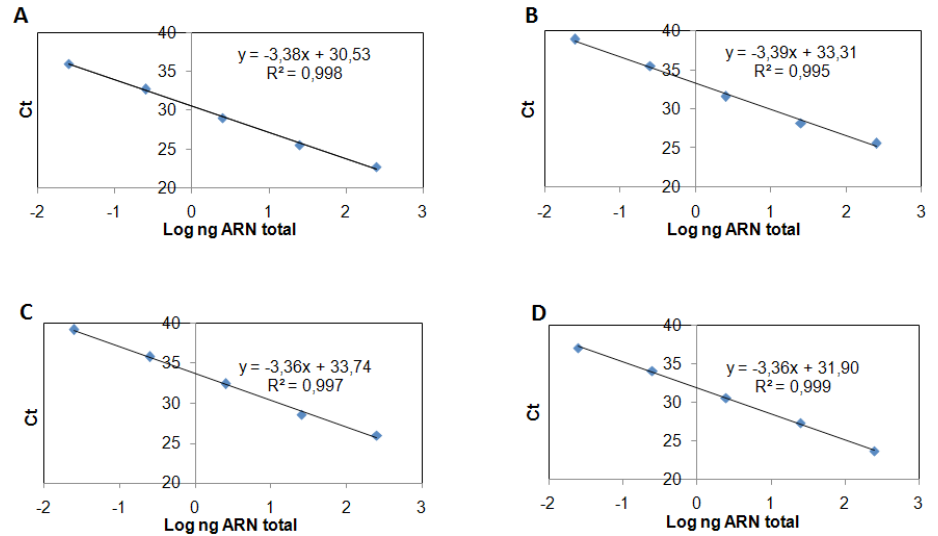

The amplification efficiencies obtained for the genes after applying the equation were:
A) *α-tubulin* gene, 97.3%
B) *defensin A* gene, 97.2%
C) *cecropin A* gene, 98.5%

Figure 1S. Efficiency of gene amplification. qPCR amplification efficiencies of *α-tubulin*, *defensin A*, and *cecropin A* genes. Each gene DNA was diluted in serial 10-fold ranges and the cycle threshold (Ct) value at each dilution was measured. The Ct represents the average of two independent experiments done by triplicate. Then, a curve was obtained for: a) *α-tubulin*, b) *defensin* A or c) *cecropin* A gene from which qPCR efficiencies (E) were assessed. The slopes of the curves were used to calculate E according to the equation: E = 10(-1/slope) - 1 × 100, where E = 100 corresponds to a 100% efficiency.
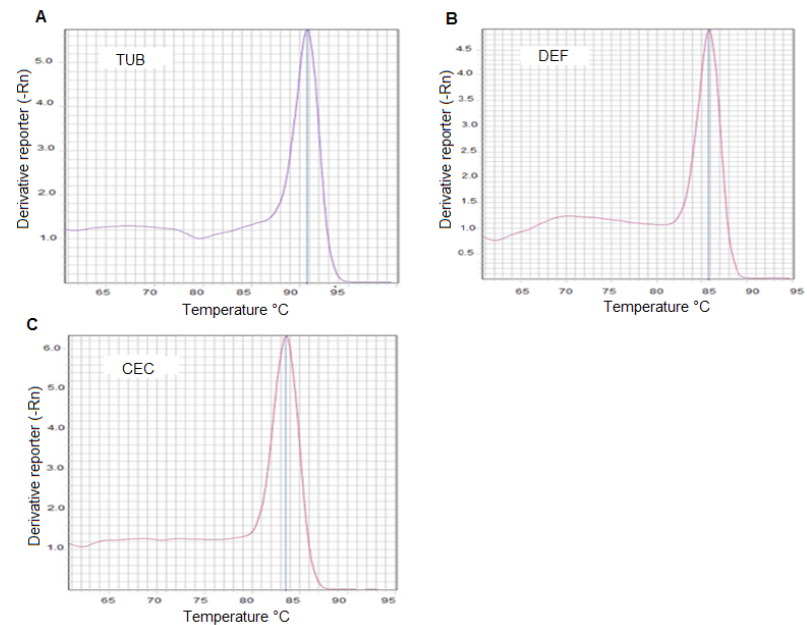

Figure 2S. DNA qPCR melting curve analysis for the detection of gene specificity. Three fragments, the first from the *α-tubulin* gene, the second from the *defensin A*, and the last from the *cecropin A* gene were synthesized by qPCR using specific primers for each gene. The resulting products were subjected to post-PCR melt analysis. Only one peak was detected with primers for A) *α-tubulin* gene; B) *defensin A* gene or C) *cecropin A* gene.
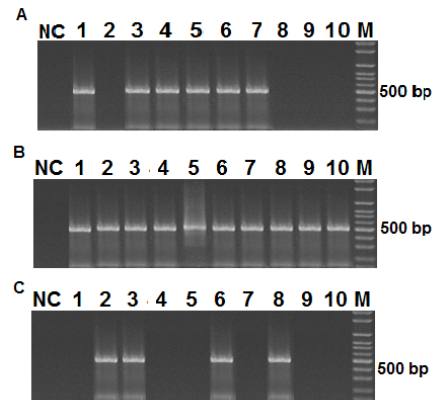
Figure 3S. Infection, Dissemination and Potential Transmission of DENV-1 in *Aedes aegypti* mosquitoes. Mosquitoes (50) were infected with DENV-1 (1.6 x 105 PFU/ml) and after 15 days, RNA was extracted from different tissues of individual mosquitoes and visualized using nested PCR followed by electrophoresis on a 2% agarose gel and SYBR Green staining. The 482 bp band corresponds to the DENV-1 amplification product from abdomens (A), wings and legs (B), and heads (C).

Repeatability

Table 1S. Repeatability of qPCR assay for *defensin A* gene in DEN 1-infected *Aedes aegypti* mosquitoes

Sample	Replicated runs (Ct)			Mean	SD	Repeatability	% CV
	1	2	3				
Calibrator	25.6	26.8	27.1	26.5	0.8	0.03	3.0
5 h	31.5	32.4	31.6	31.8	0.5	0.02	1.5
24 h	30.5	31.2	30.6	30.8	0.4	0.01	1.2
10 d	31.8	31.5	31.8	31.7	0.2	0.01	0.5
15 d	32.6	31.3	32.6	32.2	0.8	0.02	2.3

SD: standard deviation; CV: coefficient of variance

Table 2S. Repeatability of qPCR assay for *cecropin A* gene in DEN 1-infected *Aedes aegypti* mosquitoes

Sample	Replicated runs (Ct)			Mean	SD	Repeatability (CV)	% CV
	1	2	3				
Calibrator	23	24	22	23.0	1.0	0.04	4.3
5 h	26.9	27.5	27.4	27.3	0.3	0.01	1.2
24 h	29.2	29.5	29.2	29.3	0.2	0.01	0.6
10 d	26.9	26.7	26.4	26.7	0.3	0.01	0.9
15 d	28.7	28.8	28.8	28.8	0.1	0.00	0.2

SD: standard deviation; CV: coefficient of variance

Table 3S. Repeatability of qPCR assay for *defensin A* gene in Escherichia coli-infected *Aedes aegypti* mosquitoes

Sample	Replicated runs (Ct)			Mean	SD	Repeatability (CV)	% CV
	1	2	3				
Calibrator	27	27.2	26.8	27.0	0.2	0.01	0.7
5 h	25.4	25.7	25.4	25.5	0.2	0.01	0.7
24 h	27.9	27.1	27.5	27.5	0.4	0.01	1.5
10 d	25.9	26.9	26.6	26.5	0.5	0.02	1.9
15 d	22.8	23.1	22.8	22.9	0.2	0.01	0.8

SD: standard deviation; CV: coefficient of variance

Table 4S. Repeatability of qPCR assay for *cecropin A* gene in
Escherichia coli-infected *Aedes aegypti* mosquitoes

Sample	Replicated runs (Ct)			Mean	SD	Repeatability (CV)	% CV
	1	2	3				
Calibrator	26	26	25	25.7	0.6	0.02	2.2
5 h	24	25	24	24.3	0.6	0.02	2.4
24 h	25.1	25.9	25.8	25.6	0.4	0.02	1.7
10 d	24.8	25.3	25.3	25.1	0.3	0.01	1.1
15 d	23.9	24	24.4	24.2	0.4	0.01	1.5

SD: standard deviation; CV: coefficient of variance

For all tables, the repeatability was calculated as the percent coefficient of
variance (%CV) of Ct triplicate values of a sample within a single experiment.

Reproducibility

Table 5S. Reproducibility of qPCR assay for *defensin A* gene in DEN
1-infected *Aedes aegypti* mosquitoes

Sample	Run (% CV)			Mean	SD	* Reproducibility run 1 ~ run 2 ~ run 3 (% CV)
	1	2	3			
Calibrator	3.0	2.8	2.9	2.9	0.10	0.034
5 h	1.5	1.4	1.5	1.5	0.08	0.051
24 h	1.2	1.4	1.2	1.3	0.11	0.084
10 d	0.5	0.4	0.5	0.5	0.08	0.155
15 d	2.3	2.2	2.3	2.3	0.07	0.030

SD: standard deviation; CV: coefficient of variance; CV: coefficient of variance

Table 6S. Reproducibility of qPCR assay for *cecropin A* gene in DEN
1-infected *Ae. aegypti* mosquitoes

Sample	Run (% CV)			Mean	SD	* Reproducibility run 1 ~ run 2 ~ run 3 (% CV)
	1	2	3			
Calibrator	4.3	4.3	4.2	4.3	0.08	0.018
5 h	1.2	1.3	1.3	1.3	0.07	0.055
24 h	0.6	0.5	0.6	0.6	0.06	0.098
10 d	0.9	0.8	0.8	0.8	0.08	0.098
15 d	0.2	0.2	0.1	0.2	0.06	0.347

CV: coefficient of variance; SD: Standard deviation

Table 7S. Reproducibility of qPCR assay for *defensin A* gene in Escherichia
coli-infected *Ae. aegypti* mosquitoes

Sample	Run (% CV)			Mean	SD	* Reproducibility run 1 ~ run 2 ~ run 3 (% CV)
	1	2	3			
Calibrator	0.7	0.9	0.7	0.8	0.11	0.135
5 h	0.7	0.8	0.7	0.7	0.06	0.089
24 h	1.5	1.4	1.6	1.5	0.10	0.070
10 d	1.9	1.9	1.8	1.9	0.07	0.038
15 d	0.8	0.9	0.8	0.8	0.07	0.090

CV: coefficient of variance; SD: Standard deviation

Table 8S. Reproducibility of qPCR assay for *cecropin A* gene in Escherichia
coli-infected *Aedes aegypti* mosquitoes

Sample	Run (% CV)			Mean	SD	* Reproducibility run 1 ~ run 2 ~ run 3 (% CV)
	1	2	3			
Calibrator	2.2	2.1	2.2	2.2	0.08	0.035
5 h	2.4	2.6	2.4	2.5	0.12	0.051
24 h	1.7	1.7	1.6	1.7	0.06	0.035
10 d	1.1	1.2	1.2	1.2	0.03	0.025
15 d	1.5	1.3	1.5	1.4	0.11	0.075

CV: coefficient of variance; SD: Standard deviation

For all tables, the reproducibility was calculated as the percent coefficient of variance
(%CV) of Ct triplicate values of a sample between assays.

Table 9S. Rates of infection, dissemination and potential transmission of the DENV-1 in Ae. aegypti mosquitoes

Infection (%)[a]	Dissemination (%)[b]	P. transmission (%)[c]	
70 (35/50)	100 (35/35)	45.7 (16/35)	(p<0,01)*

[a] Rate of infection: Number with DENV virus-positive abdomens/number tested

[b] Rate of dissemination: Number with DENV virus-positive legs and wings/number with DEN virus-positive abdomens

[c] Rate of potential transmission: Number with DENV virus-positive heads/number with DEN virus-positive abdomens

* Open Epi Versión 3.03 (Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com, updated 2013/04/06, accessed 2020/04/03) was used for statistical analysis