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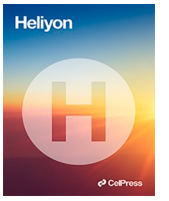

Research article

Development of an Enzyme-Linked Immunosorbent Assay (ELISA) as a tool to detect NS1 of dengue virus serotype 2 in female *Aedes aegypti* eggs for the surveillance of dengue fever transmission

Rocío Argotte-Ramos, Jorge Cime-Castillo, Valeria Vargas, Humberto Lanz-Mendoza, Mario H. Rodriguez **, Maria Carmen Rodriguez *

Centro de Investigación Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, México. Av. Universidad 655, C. P. 62100 Cuernavaca, Morelos, Mexico

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ABSTRACT

Dengue is a significant disease transmitted by *Aedes* mosquitoes in the tropics and subtropics worldwide. The disease is caused by four virus (DENV) serotypes and is transmitted to humans by female *Aedes aegypti* mosquito bites infected with the virus and vertically to their progeny. Current strategies to control dengue transmission focus on the vector. In this study, we describe an indirect Enzyme-Linked Immunosorbent Assay (ELISA), using a monoclonal antibody against the non-structural dengue virus protein 1 (NS1), to detect DENV2 in *Ae. aegypti* eggs. The assay detects NS1 in eggs homogenates with 87.5% sensitivity and 75.0% specificity and it is proposed as a tool for the routine entomovirological surveillance of DENV 2 in field mosquito populations.

1. Introduction

Dengue virus (DENV) causes dengue fever, a major health problem in the tropics and subtropics worldwide. The disease is transmitted to humans by *Aedes* mosquito bites containing DENV and by vertical transmission (VT) from female or male parents to their progeny [1].

Vertical transmission in *Aedes aegypti* has been documented under laboratory conditions [2], as well as in nature [3], and is a mechanism that maintains the virus circulating in nature in the absence of detectable human infections.

DENV vertical transmission has been widely evidenced in larvae and pupae using semi-nested PCR, immunofluorescence assays (IFA) and by RT-PCR [4,5].

Currently, the Mexican public health surveillance system of mosquito vectors is based on the use of mosquito ovitraps [6]. These are monitored weekly by local personnel to obtain information on the number of eggs, which is registered in the Dengue Geographic Integrated System (Dengue-GIS). This system combines ovitraps data with epidemiological data to estimate local dengue risk transmission indexes. Entomovirological surveillance is conducted by DENV detection in a limited number of adults collected in endemic areas. The possibility of detecting DENV in eggs collected in ovitraps would extend the virological surveillance without increasing

* Corresponding author.
** Corresponding author.
E-mail addresses: rsargo@insp.mx (R. Argotte-Ramos), jorge.cime@insp.mx (J. Cime-Castillo), valvar21@yahoo.com.mx (V. Vargas), humberto@insp.mx (H. Lanz-Mendoza), mhenry@insp.mx (M.H. Rodriguez), mrodri@insp.mx (M.C. Rodriguez).

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collection efforts. This surveillance strategy will require easy and affordable methods that could be conducted in local public health laboratories.

Among the non structural proteins of the four DENV serotypes, the Non-Structural Protein 1 (NS1) is highly immunogenic and has been used in assays to detect DENV in adult mosquitoes [7–9], and Enzyme Linked Immunosorbent Assays (ELISA) based on an anti-NS1 monoclonal antibody showed a high level of specificity [10,11], and it is also used as a biomarker to detect viral infection in dengue patients [12–14]. Currently, commercial kits for detecting NS1 protein in serum, are primarily based on ELISA. The assay's sensitivity depends on sampling time, serotype of DENV and the detection kit. Studies have evaluated the sensitivity of these assays and found it to be highest during the first days from fever onset [12].

In this study, we report the development of an ELISA using a monoclonal antibody that recognises the NS1 of the four DENV serotypes [15], to identify this protein in desiccated eggs from *Ae. aegypti* females infected with DENV2.

2. Materials and methods

2.1. Aedes aegypti mosquitoes

Three to five days-old, *Ae. aegypti*, Rockefeller strain from the insectary of Centro de Investigación Sobre Enfermedades Infecciosas-INSP, maintained at constant temperature (28 °C), relative humidity (80%) and a 12:12 light/dark cycle were used. Before and after blood feeding, mosquitoes were fed *ad libitum* with 10% sucrose solution in cottons pads.

2.2. Dengue virus culture and titration

Dengue virus serotype 2 (DENV Serotype 2 New Guinea strain) was cultured in Aag2 cells [16] at 28 °C in supplemented Schneider's *Drosophila* medium (Thermo Fisher Scientific, Rockford, Illinois, USA) for 2 h. Virus titration was performed using a plaque-forming assay immediately before experimental infections, using LLC-MK2 cells [17]. Confluent monolayers of LLC-MK2 cells in a 24-well sterile plate were infected with 0.1 mL of dengue virus, 10-fold dilution series were generated (10^{-3} to 10^{-4}) and incubated for 1 h at 37 °C and 5% CO_2. Each well was supplemented with 500 μL of MEM (minimal essential medium) (Sigma-Aldrich, St. Louis, MO, USA), 500 μL of 2.5% methylcellulose and incubated for seven days at 37 °C and 5% CO_2, until cytopathic effects were observed after staining with 1% crystal violet for 15 min. The virus titer was calculated and expressed as plaque-forming units (pfu) per milliliter.

2.3. Anti-NS1 monoclonal antibody

The anti-NS1 Mab 2B5–C7 from mouse (IgG1), detects NS1 protein of the four serotypes of DENV by Western blot and in excretas of infected DENV2 *Ae. aegypti* mosquitoes, and does not cross react with Zika virus proteins by Western blot [15]. Mab 2B5–C7 was used at 0.490 mg/ml to detect DENV2 in *Ae. aegypti* eggs homogenates.

2.4. Preparation of egg homogenates

Groups of approximately 50 female mosquitoes were offered rabbit blood containing $10^{3.5}$ PFU of DENV2. Mosquitoes were fed for 30 min using membrane glass feeders, connected to a water bath circulator at 37 °C [18]. Partially fed and unfed mosquitoes were discarded and engorged ones were kept in the insectary under controlled conditions. Between day 3–5 post feeding, trays containing water and a strip of filter paper were placed inside mosquito cages to collect eggs. After oviposition, pools of 10 females were analyzed by qPCR to determine DENV prevalence using the methodology described by Rodriguez et al. [15]. Filter paper strips containing approximately 1000–1500 eggs were allowed to dry for at least 24 days and up to two months [19] before used. Desiccated eggs were counted under a stereoscopic microscope (Carton DSZ44) and groups of 100, 200, 300 and 400 eggs were rinsed with PBS pH 7.4 and placed in microtubes containing 600 μL of casein buffer (CB), modified from Sutcliffe et al. [20] containing 0.5% casein, 0.05% Nonidet P-40 (Sigma-Aldrich) on ice (non-ionic Nonidet P-40 was included to assure that the membrane-associated forms of NS1 were released), in Phosphate buffer solution (PBS pH 7.4). Eggs were homogenized for 2 min using a portable, hand-held battery-operated pestle with disposable plastic end, to avoid debris, samples were allowed to sediment for 10 min before use in ELISA.

2.5. ELISA to detect DENV2 NS1 protein

To detect DENV2 NS1 protein in mosquito eggs, 96-well plates NUNCLON™ (NUNC™ Thermo-Fisher Scientific) were coated with 100 μL of 50 mM carbonate buffer, pH 9.5, and 100 μL of each homogenate from 100, 200, 300 and 400 eggs were added. Plates were stored overnight at 4 °C. The buffer was discarded, and wells were blocked with 200 μL of 1% Bovine Serum Albumin (BSA) in PBS, for 1 h at RT. Plate wells were covered with 100 μL of Mab 2B5–C7 (cell supernatant) diluted 1:5 (0.490 mg/mL) in PBS-tween 0.05%, for 1 h at RT. Wells were washed twice using 200 μL of PBS containing 0.05% Tween-20 (Sigma-Aldrich) and incubated with 100 μL of anti-mouse IgG coupled to Horse Radish Peroxidase (HRP) (Zymed, Thermo-Fisher Scientific), diluted 1:5000 in PBS-tween 0.05% for 1 h at RT. Wells were washed twice for 3 min each with PBS-Tween 20 and once with PBS, and developed with 100 μL/per well of *o*-phenylenediamine (OPD) (Sigma-Aldrich) for 15 min. The enzymatic reaction was stopped adding 100 μL of 1 M sulfuric acid. The optical density (OD) readings at wavelength of 492 nm were obtained using a MULTISKAN SkyHigh (Thermo-Fisher Scientific). As a

negative control, we included samples with the same number of eggs coming from uninfected females (fed with rabbit blood without DENV2) and treated under the same experimental conditions; all samples were assayed by quintupled. As positive control we included recombinant protein NS1 (Dengue NS1 ST2 (recombinant), Cat. No. ENZ-PRT104-0100), at concentrations of 0.1–0.6, 0.8, 1 and 2 μg, to make a dose-respond curve and interpolate the cut-off values of the assays. Egg homogenates were considered positive for NS1, when Optical Density (OD) values were above the cut-off value (mean OD of quintupled negative control homogenates) from uninfected mosquitoes, plus 2 standard deviations (SD).

2.6. Prevalence of infection in individual mosquitoes

To estimate the minimum prevalence of DENV2 infection in mosquitoes to detect NS1 in egg homogenates by ELISA, we carried out three independent experiments with groups of 20 mosquitoes. Three days after the mosquitoes were fed with rabbit blood with DENV2 as above, each mosquito was transferred to individual tubes with a wet filter paper at the bottom to collect the oviposited eggs. The prevalence of DENV2 infection was determined by qPCR in individual mosquito that laid eggs. All eggs from each experiment were used to prepare homogenates and analyzed by quintupled samples in ELISA.

2.7. Detection of DENV2 infection in individual mosquitoes by qPCR

Individual mosquitoes from each of the three biological replicates were homogenized with 30Hz (TissueLyser II, Qiagen, Hilden, Alemania) for 1 min at RT and using tungsten beads. Mosquito RNA was extracted using a QIAamp Viral RNA extraction kit from Qiagen, following the manufacturer's protocol. The RNA concentration was measured using a NanoDrop Lite (Thermo Fisher Scientific). Five hundred ng/μL of total RNA obtained from each virus fed mosquito were used for the amplification of DENV2 by qPCR using a QuantiNova probe RT-PCR kit (Qiagen) in a TaqMan assay with specific primers and probes FW: 5′- TAG AGA GCA GAT CTC TGR -3′ RV: 5′- DAY YCC TGC TGT TGG TGG -3′ Probe 5-FAM/CAA TAT GCT GAA ACG CGW GAG AA/3IABkFQ. To design these primers, more than 400 sequences corresponding to the four DENV serotypes were used, the most conserved sequence was selected, which is the 5′ non-coding region and the 5′ end of the capsid gene. These primers were designed using Geneious Prime (Dotmatics), a bioinformatic software for sequence data analysis. To confirm RNA extraction, primers to amplify the endogenous mosquito gene RNase P were used (Bio-Rad, Hercules, California, USA). DENV quantification was conducted using a DENV synthetic control of 350 bp (1.2×10^9 copies) (gBlock™; Integrated DNA Technologies) corresponding to the 3′UTR region and part of the capsid protein, in an absolute quantitative assay. The amplification was done in a Rotor-Gene Q 5plex (Qiagen) thermal cycler.

2.8. Determination of sensitivity, specificity and cut-off value of the assay

To calculate the sensitivity and specificity of the ELISA to detect NS1 protein in mosquito egg homogenates, we analyzed data from eight experiments: two experiments with homogenates with 300 eggs, three experiments with 400 eggs, and three using eggs collected from individual mosquitoes. To determine the best cut-off value of the assay, receiver operating characteristics (ROC) analysis using GraphPad Prism 5.03, was performed [21] and the optimal cut-off value was considered when the sum of sensitivity and specificity was maximized. The sensitivity was considered as the fraction of total confirmed positive (qPCR) samples that were true positives according to the ELISA. The specificity was obtained as the fraction of total confirmed negative (qPCR) samples that were true negatives

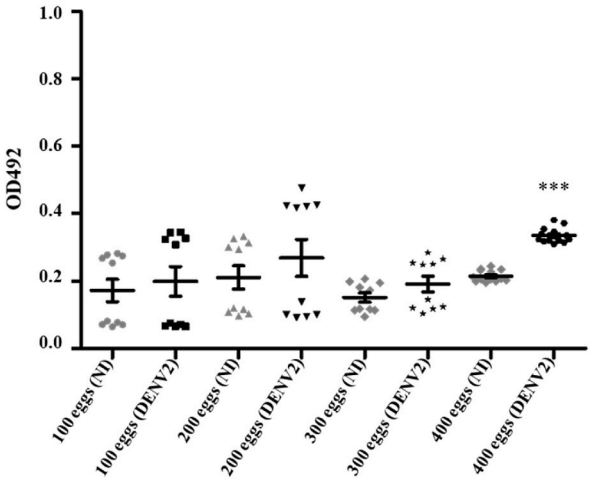

Fig. 1. Detection of NS1 with Mab 2B5–C7 by ELISA. Optical densities (OD492) were obtained from egg homogenates. Data were obtained from two independent experiments for 100, 200 and 300 eggs and three independent experiments for 400 eggs by quintupled samples. Statistical significance (*) was obtained comparing groups of homogenates of DENV2 infected and non-infected eggs (NI) with the same number of eggs using Mann-Whitney *U* test (GraphPad Prism 5.03 software). Significance was assessed at $P \leq 0.05$.

according to the ELISA [22] and calculated as: Sensitivity= (confirmed qPCR positive homogenates/total true positives ELISA homogenates) * 100 and Specificity= (confirmed qPCR negative homogenates/total true negative ELISA homogenates) * 100.

3. Statistics

Statistical comparisons between control groups (egg homogenates from rabbit blood fed mosquitoes (NI)) and treated groups (egg homogenates from mosquitoes fed with DENV2) were conducted using non-parametric Mann-Whitney *U* test. Values of $P \leq 0.05$ were considered significant. Drawing graphs were constructed using GraphPad Prism 5.03. To determine the variability (intra and inter-assay), we calculated the coefficient of variation as: CV% = (standard deviation/mean) * 100.

4. Results

4.1. Detection of NS1 in egg homogenates by ELISA

The NS1 protein was detected using the ELISA in egg homogenates from females with 90% DENV2 infection prevalence Homogenates of 100 eggs from mosquitoes fed with non-infected blood (NI) showed a OD492 mean ± SD of 0.1722 ± 0.0.1047, while the OD492 mean ± SD of homogenates from the same number of eggs from DENV2 mosquitoes fed was 0.1991 ± 0.1377, ($P = 0.578$). OD492 mean ± SD homogenates of 200 NI eggs was 0.2107 ± 0.1086, and that of DENV2 egg homogenates was 0.2689 ± 0.1742 ($P = 0.3153$). OD492 mean ± SD of homogenates of 300 eggs from NI mosquitoes and that of DENV2 infected mosquitoes were 0.1509 ± 0.0433 and 0.1914 ± 0.1558, ($P = 0.06$), respectively. OD492 mean ± SD of homogenates with 400 NI eggs was 0.2148 ± 0.0158, while that of homogenates of DENV2 eggs was 0.3352 ± 0.0207 ($P < 0.0001$) (Fig. 1). The cut-off value calculated with data from 400 eggs with an OD492 was >0.2463 and corresponds to detection of 0.350 μg of NS1 according to the dose-response curve of NS1rec (Fig. 2), this was considered as the detection limit.

4.2. Prevalence of DENV2 infection in individual mosquitoes

A total of 49 individual mosquitoes that laid eggs were analyzed by qPCR to determine prevalence of DENV infection (Table 1). Prevalence and qPCR DENV2 positive females that laid eggs and were NS1 positive by ELISA were: Exp. I, 78.6% prevalence (11 females that laid 80.8% of eggs from the total in ELISA positive homogenate); Exp II. 100% prevalence (16 females that laid 100% of eggs in the homogenate); Exp III, 78.9% prevalence (15 females that laid 76.6% of eggs from the total in the homogenate). Detection of NS1 by ELISA in egg homogenates from the three experiments with mosquitoes fed with rabbit blood (NI) and fed with DENV2, showed a mean OD492 ± SEM of 0.3109 ± 0.0423 and 0.4437 ± 0.0521, respectively ($P < 0.001$) (Fig. 3).

4.3. Cut-off value, sensitivity, and specificity of the ELISA

The optimal cut-off value obtained from ROC curve analysis was >0.3280 and corresponded to 0.433 μg of NS1 protein. Mean OD492 data of eight experiments with eggs coming from non infected (NI) and DENV2 infected female mosquitoes, were used to construct ROC curve (Supplementary Table 1). Analysis data from ROC curve were: AUC = 0.7969; Standard Error = 0.1202; 95% confidence interval = 0.5613 to 1.032; $P = 0.04605$ (Supplementary figure 1). Based on qPCR analysis, sensitivity and specificity values were calculated as 87.5% ((7/8) * 100) and 75.0% ((6/8) * 100), respectively (Supplementary Table 1). The coefficient of variation (CV%) that indicates the variability intra and inter-assay were calculated with two experiments with 300 eggs, three with 400 and three with 1037–1215 eggs (Table 2 and Table 3).

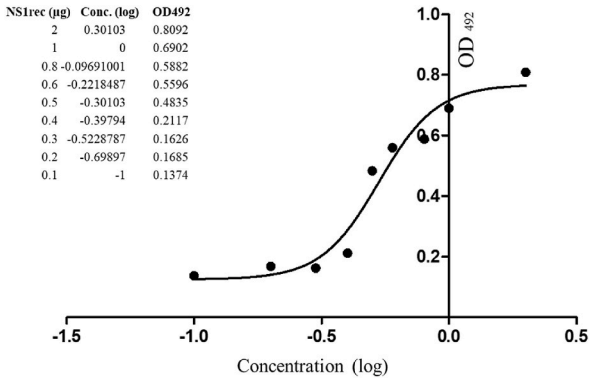

Fig. 2. Dose-response curve of Mab 2B5–C7 detection of NS1 recombinant protein. The concentrations of NS1rec were 0.1–0.8, 1 and 2 μg. Each point represents the mean of three replicates. Interpolating the cut-off value of 0.2463 resulted in a limit of detection of 0.350 μg of NS1 protein.

Table 1
Prevalence infection of individual mosquitoes by qPCR. The prevalence of infection obtained by qPCR (viral copies/μl) and total eggs laid by each mosquito in each experiment were: Exp I (11 qPCR + mosquitoes, prevalence 78.6%); Exp II (16 qPCR^{+} mosquitoes, prevalence 100%); Exp III (15 qPCR^{+} mosquitoes, prevalence 78.9%). Mean number of laid eggs after three experiments was 74 eggs and average prevalence infection was 85.83%. X = mosquitoes that died during the experiment. Neg = negative mosquito by qPCR.

Experiments								
I			II			III		
#Mosquito	DENV qPCR (copies/ul)	#Eggs	#Mosquito	DENV qPCR (copies/ul)	#Eggs	#Mosquito	DENV qPCR (copies/ul)	#Eggs
1	228	79	1	153	62	1	433	75
2	389	73	2	2280	59	2	157	70
3	53.8	81	3	688	82	3	12300	62
4	X	X	4	2260	64	4	373	19
5	Neg	116	5	4400	94	5	154	44
6	X	X	6	323	71	6	259	78
7	Neg	58	7	82.6	81	7	520	59
8	335	55	8	X	X	8	46.2	67
9	23.4	54	9	844	49	9	95.3	60
10	Neg	25	10	2020	67	10	556	81
11	532	79	11	473	64	11	25.3	71
12	395	60	12	214	62	12	330	69
13	3120	83	13	463	74	13	Neg	39
14	X	X	14	196	51	14	Neg	80
15	245	105	15	X	X	15	48.2	89
16	6250	110	16	547	36	16	2330	43
17	8150	59	17	152	66	17	4510	44
18	X	X	18	770	87	18	Neg	**78**
19	X	X	19	X	X	19	X	X
20	X	X	20	X	X	20	Neg	87
Total Eggs		1037			1069			1215
Mean Eggs/Exp.		74			67			81
Mean Eggs					74			
Prevalence/Exp.		78.6%			100%			78.9%
Mean Prevalence					85.83%			

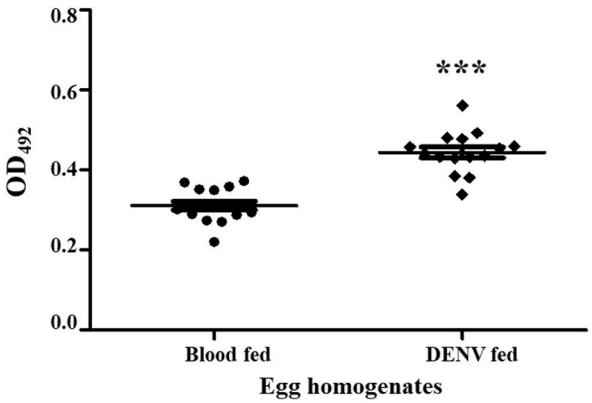

Fig. 3. Detection of NS1 by ELISA in egg homogenates from individual mosquitoes infected with DENV2. Egg homogenates from mosquitoes infected in three independent experiments were analyzed by ELISA; 1057 eggs from 14 mosquitoes; 1069 from 16 mosquitoes and 1215 from 19 mosquitoes infected and confirmed by qPCR. Same number of uninfected eggs in each experiment were used. Mean Optical density (OD_{492}) ± SEM, 0.3110 ± 0.0109 from uninfected and infected egg homogenates 0.4430 ± 0.0520 ($P \leq 0.05$). Mann-Whitney *U* test.

5. Discussion

We developed an ELISA, using a Monoclonal antibody that recognises NS1 of all DENV serotypes [15], to detect DENV serotype 2 infection in *Ae. aegypti* eggs. We propose that this assay could be useful in routine surveillance of dengue transmission in endemic areas.

Currently DENV detection by qPCR remains a costly and time-intensive diagnostic technique, requiring experienced personnel for RNA extraction, with the risk of rapid degradation of RNA and contamination that can lead to false positive amplifications. We present an ELISA assay that can offer a less expensive alternative carried out in a short time to determine DENV2 in eggs from infected *Ae. aegypti* mosquitoes.

Table 2
Variability intra-assay. The coefficient of variation (CV%) indicates the variability in the assays with a different number of eggs. Values represent quintupled samples of egg homogenates by experiment. (NI) non-infected eggs; (DENV2) DENV2 infected eggs.

Experiments	Mean OD492	SD	Eggs NI (CV%)	Mean OD492	SD	Eggs DENV2 (CV%)
300 eggs						
1	0.1111	0.0094	**8.49**	0.1222	0.0149	**12.24**
2	0.1906	0.0137	**7.21**	0.2605	0.0150	**5.77**
400 eggs						
1	0.2076	0.0063	**3.07**	0.3495	0.0148	**4.24**
2	0.2348	0.0058	**2.49**	0.3206	0.0089	**2.78**
3	0.2018	0.0050	**2.49**	0.3353	0.0261	**7.81**
1037–1215 eggs						
I (1037)	0.3602	0.0099	**2.75**	0.4880	0.0474	**9.71**
II (1069)	0.2924	0.0132	**4.52**	0.4444	0.0115	**2.60**
III (1215)	0.2802	0.0370	**13.22**	0.3988	0.0467	**11.71**

Table 3
Variability inter-assay. The coefficient of variation (CV%) indicates the inter-variability between experiments with a different number of eggs in homogenates. Values represent the mean of two or three experiments. (NI) Non-infected eggs; (DENV2) DENV2 infected eggs.

Experiments	Mean OD492	SD	Eggs NI (CV%)	Mean OD492	SD	Eggs DENV2 (CV%)
300 eggs						
(2)	0.1509	0.0433	**28.72**	0.1914	0.0742	**38.76**
400 eggs						
(3)	0.2148	0.0158	**7.35**	0.3352	0.0207	**6.19**
1037–1215 eggs						
(3)	0.311	0.0423	**13.62**	0.4437	0.0521	**11.76**

The high prevalence of mosquitoes' infection obtained in our laboratory (78.6%–90.0%) is different to what is observed in field mosquitoes, where typically 0.1–4.0% prevalence of DENV are observed [23–26]. The results coincide with the vertical transmission of DENV in *Ae. aegypti* females [5,27–30], and are direct evidence of vertical transmission of DENV2 detecting NS1, in the mosquito's first stage of development.

The precision of the assay was determined by the CV% inter- and intra-assay, that showed a CV $< 20\%$ with samples from 400 to 1215 eggs. Which is within the limits of acceptability [31].

The assay with Mab 2B5–C7 detected NS1 in a sample of 400 eggs with 90.0% prevalence (laboratory conditions) but in natural conditions it is expected to require an increase in the number of eggs coming from ovitraps.

In México after an ovitrap is geo-referenced, data on egg counts are entered into the Dengue-GIS to estimate a risk of transmission index combining the ovitrap contents data and the epidemiological surveillance data [6]. In a study carried out in six municipalities in the state of Morelos, Mexico [32] they obtained an average of 32.8 *Ae. aegypti* eggs per ovitrap per week. Considering this average number of eggs per ovitrap and 4% prevalence in the field, we estimated that around 90–100 ovitraps in a 14 day-period would be necessary to obtain the number of eggs for a good performance of an entomovirological surveillance using the NS1 detection test. Undoubtedly, those proportions could change, but one advantage is that egg hatching is no longer required to evidence DENV2. If the virus is detected in any, this will indicate that DENV2 is circulating in the area, and this information will be useful to alert local authorities for a prompt response in applying control actions to prevent dengue transmission.

As indicated before, our ELISA needs further validation in eggs from dengue-infected field-caught mosquitoes. It is our understanding that laboratory conditions may differ when performing the ELISA with field eggs in order to be considered a tool for surveillance of DENV2 circulation. These experiments are currently under consideration.

6. Conclusion

The new ELISA proposed here could detect DENV2 infection, but it has the potentiality to detect all DENV serotypes, in mosquito eggs. Although the specificity of the assay is high, the sensitivity is low, indicating the need for its adaptation to process elevated numbers of eggs for field surveys. Evidently, the assay is not designed to estimate infection intensity, but it could be useful to pinpoint areas of dengue transmission. Field assays in endemic areas are necessary to estimate its application in dengue transmission monitoring.

Ethics statement

Animal procedures and protocols were performed in accordance with the institutional Bioethical Committee of Instituto Nacional de Salud Pública (INSP) (Approval # 1367).

Data availability statement

Data included in article/supplementary material/referenced in article.

CRediT authorship contribution statement

Rocío Argotte-Ramos: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Jorge Cime-Castillo:** Validation, Methodology, Investigation, Data curation. **Valeria Vargas:** Validation, Methodology, Investigation. **Humberto Lanz-Mendoza:** Validation, Supervision, Resources, Investigation, Formal analysis. **Mario H. Rodriguez:** Writing – review & editing, Visualization, Supervision, Resources, Investigation, Formal analysis, Conceptualization. **Maria Carmen Rodriguez:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29329.

References

[1] A.N. Clements, The Biology of Mosquitoes. Volume 3: Transmission of Viruses and Interactions with Bacteria, UK, Cambridge University Press, Cambridge, 2012.
[2] L. Rosen, D.A. Shroyer, R.B. Tesh, J.E. Freier, J.C. Lien, Transovarial transmission of dengue viruses by mosquitoes: Aedes albopictus and Aedes aegypti, Am. J. Trop. Med. Hyg. 32 (5) (1983) 1108–1119, https://doi.org/10.4269/ajtmh.1983.32.1108.
[3] M.M. Khin, K.A. Than, Transovarial transmission of dengue 2 virus by Aedes aegypti in nature, Am. J. Trop. Med. Hyg. 32 (3) (1938) 590–594, https://doi.org/10.4269/ajtmh.1983.32.590.
[4] G. Le Goff, J. Revollo, M. Guerra, M. Cruz, Z. Barja Simon, Y. Roca, J. Vargas Florès, J.P. Hervé, Natural vertical transmission of dengue viruses by Aedes aegypti in Bolivia, Parasite Aug 18 (3) (2011) 277–280, https://doi.org/10.1051/parasite/2011183277.
[5] R. Danis-Lozano, E.E. Díaz-González, I.R. Malo-García, et al., Vertical transmission of dengue virus in Aedes aegypti and its role in the epidemiological persistence of dengue in Central and Southern Mexico, Trop. Med. Int. Health 24 (11) (2019) 1311–1319, https://doi.org/10.1111/tmi.13306.
[6] J.E. Hernández-Ávila, M.H. Rodríguez, R. Santos-Luna, V. Sánchez-Castañeda, S. Román-Pérez, V.H. Ríos-Salgado, J.A. Salas-Sarmiento, Nation-wide, web-based, geographic information system for the integrated surveillance and control of dengue fever in Mexico, PLoS One 8 (8) (2013) e70231, https://doi.org/10.1371/journal.pone.0070231.
[7] C.H. Tan, P.S.J. Wong, M.Z.I. Li, I. Vythilingam, L.C. Ng, Evaluation of the dengue NS1 Ag Strip® for detection of dengue virus antigen in Aedes aegypti (Diptera: Culicidae), Vector Borne Zoonotic Dis. 11 (6) (2011) 789–792, https://doi.org/10.1089/vbz.2010.0028.
[8] N.V. Voge, I. Sánchez-Vargas, C.D. Blair, L. Eisen, B.J. Beaty, Detection of dengue virus NS1 antigen in infected Aedes aegypti using a commercially available kit, Am. J. Trop. Med. Hyg. 88 (2) (2013) 260, 10.4269%2Fajtmh.2012.12-0477.
[9] D.Y. Chao, J.U. Galula, W.F. Shen, B.S. Davis, G.J.J. Chang, Nonstructural protein 1-specific immunoglobulin M and G antibody capture enzyme-linked immunosorbent assays in diagnosis of flaviviral infections in humans, J. Clin. Microbiol. 53 (2) (2015) 557–566, https://doi.org/10.1128/jcm.02735-14.
[10] P.R. Abraham, P.K. N, A. Kumar, Dengue NS1 antigen kit shows high sensitivity for detection of recombinant dengue virus-2 NS1 antigen spiked with Aedes aegypti mosquitoes, Sci. Rep. 11 (1) (2021) 23699, https://doi.org/10.1038/s41598-021-02965-x.
[11] P.R. Abraham, T. Sankari, N.P. Kumar, A. Kumar, Detection of recombinant dengue virus 2 NS1 protein in Aedes aegypti mosquitoes using commercial Dengue NS1 ELISA kit, J. Vector Borne Dis. 59 (1) (2022) 98–101, https://doi.org/10.4103/0972-9062.328975. Jan-Mar.
[12] R. Fisher, Y. Lustig, E.H. Sklan, E. Schwartz, The role of NS1 protein in the diagnosis of flavivirus infections, Viruses 15 (2) (2023) 572, https://doi.org/10.3390/v15020572.
[13] K.I. Matsunaga, M. Kimoto, V.W. Lim, T.L. Thein, S. Vasoo, Y.S. Leo, W. Sun, I. Hirao, Competitive ELISA for a serologic test to detect dengue serotype-specific anti-NS1 IgGs using high-affinity UB-DNA aptamers, Sci. Rep. 11 (1) (2021) 18000, https://doi.org/10.1038/s41598-021-97339-8.
[14] T. Shimelis, A. Mulu, M. Mengesha, A. Alemu, A. Mihret, B.T. Tadesse, A.W. Bartlett, F.W. Belay, G. Schierhout, S. Dittrich, J.A. Crump, S. Vaz Nery, J.M. Kaldor, Detection of dengue virus infection in children presenting with fever in Hawassa, southern Ethiopia, Sci. Rep. 13 (1) (2023) 7997, https://doi.org/10.1038/s41598-023-35143-2.
[15] M.C. Rodriguez, J. Cime-Castillo, R. Argotte-Ramos, V. Vargas, F. Correa-Morales, G. Sánchez-Tejeda, H. Lanz-Mendoza, Detection of NS1 protein from dengue virus in excreta and homogenates of wild-caught Aedes aegypti mosquitoes using monoclonal antibodies, Pathogens and Disease 80 (1) (2022) ftac002, https://doi.org/10.1093/femspd/ftac002.
[16] J. Peleg, Growth of Arboviruses in monolayers from subcultured mosquito embryo cells, Virology 35 (1968) 617–619. https://doi10.1016/0042-6822(68)90293-6.
[17] R.N. Hull, W.R. Cherry, O.J. Tritch, Growth characteristics of monkey kidney cell strains LLC-MK1, LLC-MK2, and LLC-MK2 (NCTC-3196) and their utility in virus research, J. Exp. Med. 115 (5) (1962) 903–918, https://doi.org/10.1084/jem.115.5.903.
[18] R. Danis Lozano, F. Correa Morales, Cría de mosquitos Culicidae y evaluación de insecticidas de uso en salud pública, Primera edición. Instituto Nacional de Salud Pública, Cuernavaca, Morelos. México, 2021.
[19] D.T. Mourya, M.D. Gokhale, A. Basu, P.V. Barde, G.N. Sapkal, V.S. Padbidri, M.M. Gore, Horizontal and vertical transmission of dengue virus type 2 in highly and lowly susceptible strains of Aedes aegypti mosquitoes, Acta Virol. 45 (2) (2001) 67–72. PMID: 11719984.

[20] A.C. Sutcliffe, S.R. Irish, E. Rogier, M. Finney, S. Zohdy, E.M. Dotson, Adaptation of ELISA detection of Plasmodium falciparum and Plasmodium vivax Circumsporozoite proteins in mosquitoes to a multiplex bead-based immunoassay, Malar. J. 20 (1) (2021) 1–13, https://doi.org/10.1186/s12936-021-03910-z.
[21] J. Aliabadi, E. Rakhshandehroo, A. Yektaseresht, Development and evaluation of an indirect ELISA for detection of Teladorsagia circumcincta infection in sheep, BMC Vet. Res. 17 (2021) 326, https://doi.org/10.1186/s12917-021-03042-1.
[22] I. Bosch, A. Reddy, H. de Puig, J.E. Ludert, F. Perdomo-Celis, C.F. Narváez, A. Versiani, D. Fandos, M.L. Nogueira, M. Singla, R. Lodha, G.R. Medigeshi, I. Lorenzana, H.V. Ralde, M. Gélvez-Ramírez, L.A. Villar, M. Hiley, L. Mendoza, N. Salcedo, B.B. Herrera, L. Gehrke, Serotype-specific detection of dengue viruses in a nonstructural protein 1-based enzyme-linked immunosorbent assay validated with a multi-national cohort, PLoS Neglected Trop. Dis. 14 (6) (2020) e0008203, https://doi.org/10.1371/journal.pntd.0008203.
[23] V.T. Chow, Y.C. Chan, R. Yong, et al., Monitoring of dengue viruses in field-caught Aedes aegypti and Aedes albopictus mosquitoes by a type-specific polymerase chain reaction and cycle sequencing, Am. J. Trop. Med. Hyg. 58 (5) (1998) 578–586, https://doi.org/10.4269/ajtmh.1998.58.578.
[24] I.K. Yoon, A. Getis, J. Aldstadt, A.L. Rothman, D. Tannitisupawong, C.J. Koenraadt, T.W. Scott, Fine scale spatiotemporal clustering of dengue virus transmission in children and Aedes aegypti in rural Thai villages, PLoS Neglected Trop. Dis. 6 (7) (2012) e1730, https://doi.org/10.1371/journal.pntd.0001730.
[25] A. Rahayu, U. Saraswati, E. Supriyati, D.A. Kumalawati, R. Hermantara, A. Rovik, E. Arguni, Prevalence and distribution of dengue virus in Aedes aegypti in Yogyakarta city before deployment of wolbachia infected Aedes aegypti, Int. J. Environ. Res. Publ. Health 16 (10) (2019) 1742, https://doi.org/10.3390/ijerph16101742.
[26] P. Nonyong, T. Ekalaksananan, S. Phanthanawiboon, et al., Dengue virus in humans and mosquitoes and their molecular characteristics in northeastern Thailand 2016-2018, PLoS One 16 (9) (2021) e0257460, https://doi.org/10.1371/journal.pone.0257460.
[27] E.A. Buckner, B.W. Alto, L.P. Lounibos, Vertical transmission of Key West dengue-1 virus by Aedes aegypti and Aedes albopictus (Diptera: Culicidae) mosquitoes from Florida, J. Med. Entomol. 50 (6) (2013) 1291–1297, https://doi.org/10.1603/ME13047.
[28] N.E. Martínez, F. Dzul-Manzanilla, C. Gutiérrez-Castro, et al., Natural vertical transmission of dengue-1 virus in Aedes aegypti populations in Acapulco, Mexico, J. Am. Mosq. Control Assoc. 30 (2) (2014) 143–146, https://doi.org/10.2987/14-6402.1.
[29] M. Espinosa, S. Giamperetti, M. Abril, A. Seijo, Vertical Transmission of Dengue Virus in Aedes aegypti Collected in Puerto Iguazú, vol. 56, Revista do Instituto de Medicina Tropical de São Paulo, Misiones, Argentina, 2014, pp. 165–167, https://doi.org/10.1590/S0036-46652014000200013.
[30] C.F. da Costa, R.A. Dos Passos, J.B.P. Lima, et al., Transovarial transmission of DENV in Aedes aegypti in the Amazon basin: a local model of xenomonitoring, Parasites Vectors 10 (2017) 1–9, https://doi.org/10.1186/s13071-017-2194-5.
[31] W. Lexmond, Jv der Mee, F. Ruiter, B. Platzer, G. Stary, E.H. Yen, E. Dehlink, S. Nurko, E. Fiebiger, Development and validation of a standardized ELISA for the detection of soluble Fc-epsilon-RI in human serum, J. Immunol. Methods 373 (1–2) (2011) 192–199, https://doi.org/10.1016/j.jim.2011.08.018.) Oct 28.
[32] Ángel Francisco Betanzos-Reyes, Mario Henry Rodríguez, Martín Romero-Martínez, Eduardo Sesma-Medrano, Hilda Rangel-Flores, René Santos-Luna, Association of dengue fever with Aedes spp. abundance and climatological effects, Salud Publica Mex. 60 (1) (2018) 12–20, https://doi.org/10.21149/8141.