Dengue Serotypes Circulating in *Aedes aegypti* and Humans in a Poor or Peripheral Neighborhood at Reynosa, Mexico

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Abstract. Eleven entomological variables were correlated with households seropositive (Y = 1) or seronegative (Y = 0) for dengue virus depending on the percentage of seropositive to total participants per household. DENV serotypes were identified in mosquito vectors. Three entomologic (spring, summer, and fall) and two serologic surveys (spring and fall) were done in 77 households at Reynosa, Mexico in 2014. Numbers of water-filled containers, larvae, and pupae were counted per household, and adult *Aedes aegypti* (L.) were collected with a backpack aspirator after landing on humans. In spring and fall, incidence of IgG and IgM dengue antibodies was determined per household and DENV serotypes in mosquitoes collected indoors. The households were infested with 1,573 female and 1,142 male *Ae. aegypti*. About 56 and 46% of 322 residents were participants in spring and fall, respectively, resulting in 117 and 95 positive cases for IgG/IgM in 68 and 62% of seropositive households. Recent transmission occurred in 8% of households with three IgM-positive participants and three IgG seroconversions, and all seropositive samples were DENV-2. A multivariate logistic regression indicated host-seeking females were the only explanatory variable linked to 48 seropositive households in fall, with 85% (289) clumped in 24 households. Host-seeking female mosquitoes also were correlated linearly with the number of IgG/IgM cases per household where an increase in 50 mosquitoes matched one IgG/IgM case. DENV-1 was detected in one pool of *Ae. aegypti*, and in another of *Aedes albopictus* Skuse. DENV-2 was amplified (10^7 RNA copies per milliliter) in one pool of 10 male *Ae. aegypti*, and at 3.40 log 10^4 in one male *Ae. albopictus*. DENV-1 and DENV-2 circulating in humans and mosquitoes were determined, with vertical cycles in both species of mosquito vector.

Introduction

Half the population of the world is at risk of being infected by one or more of four dengue virus (DENV) serotypes (Bhatt et al. 2013). There are no effective vaccines or antiviral drugs, while global trade, apathy to remove infested containers, and vector resistance to insecticides resulted in a disease pandemic (Soo et al. 2016). Since the beginning of dengue surveillance in 1982, multiple aspects of the mosquito-borne disease have been studied in humans and vectors in Mexico (Briseño-García et el. 1996, Dantés et al. 2014. Four DENV serotypes currently occur in the USA-

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Mexico border area seldom surveyed for dengue. In the 2005 dengue outbreak in the binational areas of Matamoros-Brownsville and Reynosa-McAllen in northeastern Mexico, viral serotypes DENV-2, DENV-3, and DENV-4 were isolated from humans, including mixed infections of DENV-2 and DENV-3 (CDC 1996, Rawlings et al. 1998). At Matamoros, two epidemiological surveys found breeding sites, lack of air-conditioning or drainage, and low family income linked with IgG-positive households (Brunkard et al. 2004) while human cases were forecast by temperature and rainfall (Brunkard et al. 2008). In 2017, four DENV serotypes were detected in patients at city hospitals (Requena-Castro et al. 2017).

Only three entomologic surveys have been published: American scientists collected mosquitoes at Reynosa from October through November 1995, with 847 *Aedes aegypti* (L.), 1,033 *Aedes albopictus* Skuse, and 420 *Aedes* spp., and only the DENV-2 serotype was found in two pools of *Ae. aegypti* (without mentioning collection type or gender of mosquitoes) (CDC 1996). Concurrently, Mexican entomologists with backpack aspirators and CDC traps collected 2,651 *Ae. aegypti* (1,051 females and 1,600 males) and 2,986 *Ae. albopictus* (2,339 females and 647 males) and found only one pool of 89 *Ae. albopictus* formed by 10 males positive for DENV-2 and DENV-3 confirmed by RT-PCR (Ibáñez-Bernal et al. 1995). At Cd. Juarez, Chihuahua, DENV-2 and DENV-3 occurred in female *Ae. aegypti* in CDC traps (without mentioning the number of mosquitoes collected) (De la Mora-Covarrubias et al. 2010).

Dengue has not been studied at the USA-Mexico border despite movement of people crossing international bridges and intense migration from Central America. Therefore, we surveyed for dengue virus (DENV) serotypes circulating in immatures, resting, and host-seeking *Ae. aegypti* and IgG and IgM incidence in residents of a small isolated neighborhood at the north side of Reynosa, 2 km from the USA-Mexico border wall.

Materials and Methods

The study was at Nuevo Amanecer, at Reynosa, Mexico (19° 14' 39.91" W and 26° 3' 16.2" N). The city is 33 m above sea level and has a population of 700,000. Climate is hot-dry with 22°C mean annual temperature and a dry season of 40 days in July-August known as "canicula" with daily temperatures of 40-42°C. Conversely, winter encompasses at least 20 days in December–January with minimum temperatures of 0-5°C (INEGI 2014). Dengue is endemic in the study area. Nuevo Amanecer was selected as the study area after the State Health Secretariate (Gloria L. Doria-Cobos, personal communication) reported 16 fever cases and five hemorrhagic cases in 2013. The survey was done in an eight-block experimental area where 77 households of similar size with houses of similar construction materials were selected depending on the willingness of the owners to participate in the study. Three entomological and two serological surveys were done in selected households.

Three entomological surveys were done during each 3-week period in March-April, July-August, and October-November 2014. During every survey, each of the 77 households was inspected once. All potential larval containers were counted, and the presence of water was scored. Larvae collected from containers were counted and returned to the containers, but pupae were placed in 500-ml vials and transported to a local laboratory for rearing. Adults were confined in screened cages containing 10% sucrose solution and reared under standard insectary conditions (28 + 2°C, 85% relative humidity, and 12:12 light:dark hours). Emerged adults were later sorted by sex into pools of different sizes from one to 10 mosquitoes each and stored at -70°C until tested for DENV.

A backpack aspirator was used to capture resting males and females indoors and outdoors. Mosquitoes resting indoors were put into 50-ml vials, transported to a local laboratory, killed by cold, identified, and stored in liquid nitrogen at -70°C until tested for DENV. Percentage of male and female mosquitoes were calculated from human-landed collections. One person sat on the open porch of each household, and mosquitoes of both genders attracted to his exposed legs were caught using a mouth aspirator for 30 minutes each hour from 1700 to 1900 hours. Human-landing collected mosquitoes were counted and released at the porch to avoid reducing abundance of *Ae. aegypti*, because preliminary surveys resulted in less abundance. On each day, four households were sampled and thus all 77 households were sampled every 3 weeks. The mosquitoes were identified morphologically using taxonomic keys (Darsie and Ward 2005).

At each of the 77 households, the residents were asked to voluntarily participate in the serological surveys. Two cross-sectional surveys were done from 1 March through 15 April and from 1 October through 15 November 2014. Each participant provided a 5-ml blood sample by venipuncture using tubes without anticoagulant to obtain sera. Blood samples were transported at 4°C to a local laboratory where serum was separated and stored at -80°C until further analysis.

ELISA kits were used for qualitative detection of IgM- and IgG-specific DENV antibodies in human serum samples using dengue-derived recombinant antigen (DENRA serotypes 1-4). The assay was done according to manufacturer instructions (InBios International, Seattle, WA; DENV Detect™ IgM Capture ELISA Kit; and DENV Detect™ IgG ELISA Kit, Catalog No. DDGS-R). To determine the diagnostic performance of the ELISA, the immune status index or cut-off value was calculated by dividing the OD mean value of DENRA by that of NCA. A cut-off value ≥2.84 indicated the sample(s) was positive, while ≤1.65 indicated negative sample(s). Any equivocal results (samples with ISR between 1.65 and 2.84) were repeated (until a clear result was obtained) before reported as positive or negative.

Originally, the DENV to extract viral RNA for checks was isolated at the Laboratory of Arbovirology of the Centro Regional de Investigación "Dr Hideyo Noguchi" at the University of Yucatán. The viruses were obtained from patients who signed informed consent to participate in a study approved by the Institutional Bioethics Committee of IPN, Mexico. Viruses were obtained during outbreaks in the Yucatan Peninsula from October 2007 to January 2009 and donated to our group by Dr. María Alba Loroño-Pino. DENV viral RNA for each serotype (DENV-1 Yuc18494; DENV-2 Yuc17438; DENV-3 Yuc18603; DENV-4 Yuc18571) was purified by a QIAmp viral RNA kit (Qiagen, Hilden, Germany) according to manufacturer instructions. After RNA extraction for each serotype, a specific fragment from the 3´ UTR region was amplified by RT-PCR using primers previously described (Johnson et al. 2005). The purified PCR products were cleaned with the gel extraction kit and amplicons cloned (Sambrook et al. 1989) in the vector following manufacturer instructions. Positive clones were confirmed by PCR and sequencing of successful clones was by Eurofins Genomics Company (https://www.eurofinsgenomics.eu/en/custom-dna-sequencing.aspx).

Real-Time-PCR (TaqMan) to estimate viral DENV RNA was used on all serum samples identified as positive by IgM ELISA following manufacturer instructions (Eurofins Genomics Company). Details of protocols for genomic RNA purification, serotype specific primers and probe sequences, and RT-PCR conditions were

published by Johnson (2005) and Honório et al. (2009). DENV virus genome copies per microliter were determined by standard curves from serial dilutions (1:10) of each individual serotype-cloned fragment. Briefly, 1µg per µl of each viral RNA genome was used for serial dilutions (1:10) and used in triplicate by the Real-Time PCR System to generate standard curves following manufacturer instructions (Qiagen, Hilden, Germany). The cycle threshold value of each experimental detection for DENV was interpolated in the equation for the specific serotype, and the genome copy number was determined.

Indoor-resting mosquitoes collected by backpack aspirator and those reared from pupae to adults were used to detect viral RNA. Female and male whole-body mosquitoes were put in batches ranging from one to 10 per pool into 1.5-ml Eppendorf tubes, macerated with a polypropylene pestle, and centrifuged at 3,500 rpm for 15 minutes at 4°C to remove debris. The homogenate (supernatant) was put into a new Eppendorf tube and stored at -70°C, then 140 µl of the homogenate and 560 µl viral lysis buffer were analyzed, and the viral RNA kit was used to isolate viral RNA that was stored at -70°C until analysis. A single step RT-PCR was used to amplify a 500-bp fragment of RNA viral genome that encodes the structural E protein of DENV using RT-PCR following manufacturer instructions (Qiagen, Hilden, Germany). Details of protocols for genomic RNA purification, primer sequences, RT-PCR conditions, and detection of PCR products by 1% agarose gel were published by Lanciotti et al. (1992). Positive and negative DENV check samples were run in parallel.

To identify predictors influencing the number of households positive for DENV antibody-positive participants in the two serologic surveys, two multivariate logistic regressions (spring and fall) were done with PROC LOGISTIC (SAS 2012) where the response variable was binary with Y = 1 (when the ratio of IgG/IgM positive to total participants per household was ≥0.5 or otherwise Y = 0). Each analysis used 11 predictors: variable amount of shade which was binary (X = 1 if shady area ≥50% of the back and front yards; otherwise X = 0), and 10 entomologic ones: total number of positive containers, larvae, pupae, and adults captured indoors or outdoors and in human-landed collections separated by sex; both analyses used the serological and entomological data of March and October. Significance of model goodness-of-fit was tested by a -2 Likelihood ratio χ^2 test, with significance of individual explanatory variables by the Pearson χ^2 test ($P < 0.05$) (Stroup 2011). With data from October, linear regression was done with the number of IgG/IgM-positive participants per household as the response variable on the number of host-seeking females.

All procedures involving use of humans for mosquito collections were reviewed and approved by the Bioethics Committee of the Escuela Nacional de Medicina y Homeopatia of the Instituto Politécnico Nacional (México City) as ENMH-CB-061-2013. Written informed consent was obtained from all human-landing collectors. Written informed consent was provided by all adult blood donors (>18 years), and by a parent or guardian for children. All data from blood donors were anonymous.

Results

From the 77 households, in total, 4,352 containers with water were documented, which were 1,478, 1,592, and 1,282 from the first, second, and third sampling periods, respectively. Most (>80%) containers were discarded tires, buckets, pots, bottles, and cans/plastics (<250-ml plastic/metal vessels). Only 12 water-filled containers did not harbor larvae and were excluded from analysis. Only 3% (n = 118) of the containers were positive for larvae or pupae of *Ae. aegypti*, with

38, 49, and 31 from the three sampling periods, respectively. The few containers were very productive, and 29,588 larvae and 1,314 pupae were collected, averaging 250.74 and 11.13 per container, respectively. The number of pupae was almost 4% of the total, with an average of 5.68 pupae per household (1,314 per 231 total).

Overall, 2,715 adult *Ae. aegypti* (1,573 females and 1,142 males) were collected during the study, representing 543 (21%), 1,123 (41%), and 1,049 (39%) for the first, second, and third sampling periods, respectively. Most adults were caught by human-landed collection (1,527 or 56%), followed by resting outdoors (953 or 35%), or resting indoors (235 or 9%). Percentages of female and male *Ae. aegypti* collected resting indoors were similar (~50%) but females were dominant over males in human-landed collections (78% = 1,197 of 1,527), while mostly males were caught (27% = 257 of 953) in outdoor-resting collections.

Overall, 181 of 322 residents of the 77 households consented and donated blood for the study. One hundred seventeen in the first sero-survey were IgG-positive (three IgM-positive and 65 seronegative) and 95 in the second sero-survey were IgG-positive (three IgM-positive and 58 seronegative). Independent of the antibody, the seropositive rate per household was 0.68 (52 of 77) and 0.62 (48 of 77) in March and October, respectively, with an average of 65% (Table 1).

Nineteen percent (35 of 181) of participants in March withdrew from the study and were replaced in October by other members of the same family in the same household. The replacement rate was 7% (12 of 181); therefore, we did not obtain an estimate for seroprevalence rate. The IgM antibody was detected only in three paired blood samples of three female participants (households 13-, 17-, and 60-year old) and for the serotype DENV-2 (3% = three of 77). Likewise, three IgG seroconversions (a seropositive participant who was seronegative with the first blood sample) in households 13, 17, and 60 (one 31-year old female and two males: 35- and 48-year old) also were detected for DENV-2. Thus, the overall recent (IgM cases plus seroconversions) transmission rate per household was 8% (six of 77).

Logistic regression analysis for the relationship between entomological data and incidence of IgG/IgM-positive households was significant only in October. When compared with an empty model, the whole model provided a significantly better fit to the data set ($\beta = 0$), and the regression coefficient of at least one of the explanatory variables was nonzero (-2 log likelihood $\chi^2 = 19.92$, df = 11, $P = 0.04$). The final model had only a significant predictor: human-landing collected females of fall (estimate = 0.2322, standard error = 0.11, Wald $\chi^2 = 4.008$, $P = 0.04$); the odds ratio of these human-seeking female mosquitoes was estimated at 1.26 (95% upper CI = 1.583 and lower CI = 1.005) which is the exponentiated coefficient of the predictor. Thus, in the fall, the presence of one human-seeking female (from a total of 339) raised the odds of an IgG/IgM-positive household by 1.26 at the point when the means of the other covariates were constant (Hosmer and Lemeshow 2002).

The 85% (289 of 339) of human-seeking females were captured in 50% (24) of the seropositive households while only 50 mosquitoes were in 27% (eight) of seronegative households. The spatial distribution of the mosquitoes was clustered, with the number varying from zero to 48 per household, and the number of households free of mosquitoes was similar in seropositive (24) and seronegative ones (21) (Fig. 1). In seropositive households, 27% (78 of 289) of human-seeking females were captured in only two households, while in seronegative ones, 62% (31 of 50) of human-seeking females also were found in two households.

Table 1. Number of Residents, Participants, Seronegative and Seropositive Participants for IgG and IgM[1] Dengue Antibodies per Household (HH) in 77 Households Examined in Two Serological Surveys (March and October) in 2014, in a neighborhood at Reynosa, Mexico. The response variable was binary depending of the ratio of number of seropositive BD to total BD per household: a positive household (Y = 1) was when the ratio ≥0.50, otherwise Y = 0.

		March					October			
HH	Residents	Participants	No. seronegative	No. seropositive	Positive (1) or negative (0) HH	Increase (+) or decrease (-) of participants[1, 2] March-October	Participants	No. seronegative	No. seropositive	Positive (1) or negative (0) HH
1	5	4	1	4	1		4	1	4	1
2	1	1	0	1	1		1	0	1	1
3	4	3	1	2	1		3	1	2	1
4	5	3	3	0	0		3	3	0	0
5	5	5	4	1	0		5	3	2	0
6	4	1	0	1	1	+	2	0	2	1
7	4	3	1	2	1		3	1	2	1
8	3	1	0	1	1	+	2	1	1	1
9	3	2	1	1	1		2	1	1	1
10	6	4	2	2	1		4	2	2	1
11	6	5	3	2	0	-	4	2	2	1
12	4	3	1	2	1		3	1	2	1
13	4	2	0	2	1		2	0	2	1
14	6	3	1	2	1	+	5	2	3	1
15	6	3	0	3	1		3	0	3	1
16	4	4	4	0	0		4	0	0	0
17	2	1	0	1	1		1	0	1	1
18	4	3	1	2	1		3	1	2	1
19	4	3	1	2	1	+	4	1	3	1
20	3	1	0	1	1		1	0	1	1
21	1	1	0	1	1		1	0	1	1
22	5	2	0	2	1		2	1	1	1
23	3	2	0	2	1		2	0	2	1
24	4	2	0	2	1		1	0	1	1
25	3	1	0	1	1	+	2	0	2	1
26	2	2	0	2	1	-	0	0	0	0
27	2	2	0	2	1	-	1	0	1	1
28	5	3	0	3	1		3	0	3	1
29	7	3	2	1	0	-	2	0	2	1
30	4	3	1	2	1	+	4	1	3	1
31	5	4	1	4	1	-	3	1	3	1
32	5	0	0	0	0		0	0	0	0
33	3	0	0	0	0		0	0	0	0
34	4	0	0	0	0		0	0	0	0
35	6	4	3	1	0		4	2	2	1
36	5	1	0	1	1	-	0	0	0	0
37	1	1	0	1	1		1	0	1	1
38	4	2	1	1	1	+	3	1	2	1
39	5	0	0	0	0		0	0	0	0
40	4	1	1	0	0		1	1	0	0
41	3	3	0	3	1		3	0	3	1
42	4	3	1	1	0		3	1	2	1
43	5	5	2	3	1	-	0	0	0	0
44	1	1	0	1	1		1	0	1	1
45	4	3	1	2	1	-	2	0	2	1
46	4	3	1	2	1		3	1	2	1
47	4	2	0	2	1	-	0	0	0	0
48	7	2	0	2	1		2	0	2	1
49	4	1	0	1	1	-	0	0	0	0
50	3	2	0	2	1		2	1	1	1
51	5	4	3	1	0		4	3	1	0
52	6	2	0	2	1		2	0	2	1
53	3	3	0	3	1		3	0	3	1
54	4	1	0	1	1		1	0	1	1
55	4	4	3	1	0		4	3	1	0
56	4	3	3	0	0		3	3	0	0
57	5	1	0	1	1		1	0	1	1
58	3	2	0	2	1		1	0	1	1
59	3	2	0	2	1		2	0	2	1
60	3	2	0	2	1	-	0	0	0	0

61	2	1	0	1	1	-	0	0	0	0
62	9	5	3	2	0	+	8	5	0	0
63	7	6	0	6	1	-	1	0	1	1
64	4	0	0	0	0		0	0	0	1
65	6	3	2	1	0		3	2	1	0
66	6	3	0	3	1	-	2	0	2	0
67	3	2	2	0	0	-	1	1	0	0
68	6	4	3	1	0		4	3	1	0
69	3	0	0	0	0		0	0	0	0
70	1	3	0	3	1	+	0	0	0	0
71	7	5	3	2	0	+	2	2	0	0
72	3	0	0	0	0		0	0	0	0
73	5	0	0	0	0		0	0	0	0
74	4	2	1	1	1		2	1	1	1
75	2	2	0	2	1		2	0	2	1
76	4	1	1	0	0	-	2	1	1	1
77	10	6	3	3	1		5	4	1	0
Total	322	181	65	117			158	58	95	

Seropositive participant rate	0.36 (65/181)	0.37 58/158
Seropositive HH rate	0.68 52/77	0.62 48/77

[1]Only three IgM-positive participants (one per household) were detected in three paired samples.
[2]There was a 19% rejection (35 of 181) in participants to donor blood in October in the same households.
[3]The replacement rate of participants in October that comprised other volunteers (relatives) in the same household was 7% (12 of 181).

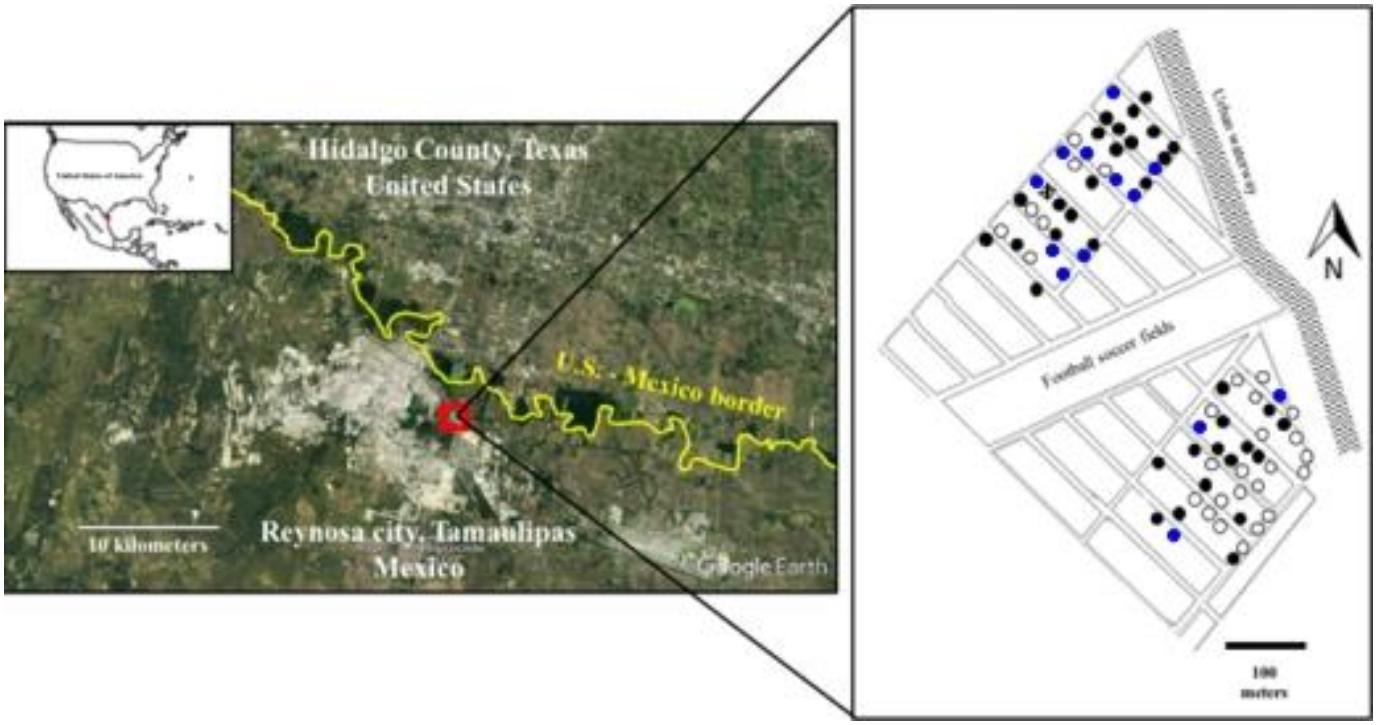

Fig. 1. Map of the neighborhood surveyed at the US-Mexico border; seropositive (solid black circles) and seronegative households (white circles); and seropositive households where host-seeking female mosquitoes in fall were >10 (solid blue circles). X denotes a seronegative household with >10 host-seeking female mosquitoes.

Linear regression was used to determine the influence of human-seeking female mosquitoes on the number of participants positive for IgG/IgM per household as the response variable; the relationship was small (R = 0.31) but significant (F = 5.59, df = 1, P < 0.05). Although the determination coefficient (R^2 = 0.10) indicated only 10% of the variation in the response variable was explained by variation in the number of human-seeking females per household, a slope of 0.02 meant an increase of 50 human-seeking females was correlated with an increase in one IgG/IgM-positive participant per household in the cluster of households surveyed (Fig. 2).

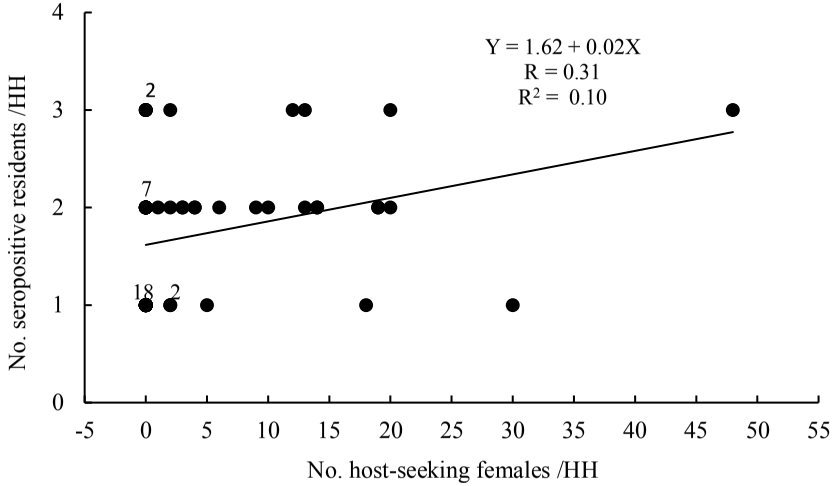

Fig. 2. Regression of number of IgG/IgM-positive blood donors with number of host-seeking female *Ae. aegypti* as the response variable collected per household in 48 seropositive households in a dengue-endemic setting at Reynosa, Mexico, in fall 2014.

In total, 955 mosquitoes (235 indoor-resting specimens and 720 adults reared from pupae) were tested for DENV in 633 pools (each pool contained one to 10 mosquitoes). Only three pools were infected and collected from IgG-positive households, with an overall prevalence of 0.47% (three of 633). Of the three positive pools, two were positive for DENV-2 (three indoor-resting females, 3.49 x 10^4 RNA copies per milliliter, 1 July and 10 pupae-emerged males, 1.28 x 10^7 RNA copies per milliliter, 5 September), and the third pool was positive for DENV-1 (a single resting male, 2.71 x 10^4 RNA copies per milliliter, 14 August). Therefore, vertical transmission was detected at a rate of 0.31% (two of 633). DENV-1 was detected at 3.40 log 10^4 in one male *Ae. albopictus*.

Discussion

Entomological estimates used household as the sample unit with a total of 77 households which is within the recommended size for dengue surveys (WHO 1997). Overall, wild *Ae. aegypti* were not abundant. Our estimated average [1,197 of 231 (in the three samplings)] of five human-seeking female *Ae. aegypti* per household is in accordance with the range of five to 10 females per household in Puerto Rico (Newton and Reiter 1992), but smaller than 20 females per household reported in a dengue-endemic tropical country such as Thailand (Yasuno and Ton 1970).

The household as the unit that was the response variable used to generate the binary variable for the study is an epidemiologically relative measure because 13 and nine seropositive participants were in 10 and seven seronegative households in spring and fall; nonetheless, it permitted detecting that households with more old/recent infections (≥50%) were also those with most human-seeking female mosquitoes. Incidence of dengue is clustered in cities and households (Mammen et al. 2008, García-Rejón et al. 2011, Raghwani et al. 2011). The main factor resulting in fine-scale clustering is short flight range (~100 m) of *Ae. aegypti* females and dispersal by a few infectious females at short time intervals (Morrison et al. 2010, Scott and Morrison 2010). We have evidence that at least nine infectious female mosquitoes were putatively circulating among the 77 households: three females were DENV-positive in one pool, the three females connected with IgG seroconversions of fall, and three females responsible for IgM-positive cases. It is pertinent to emphasize that human-seeking females were abundant in six households where we found the three IgM cases (13, 4, and 12 human-seeking females) and three seroconversions (14, 30, and 0 human-seeking females). Regarding DENV in male *Ae. aegypti*, researchers reported an alternating sequence of horizontal and vector transmission evolved in arboviruses to survive periods of adverse conditions (Buckner et al. 2013, Ebert 2013, Lequime and Lambrechts 2014). However, it also is necessary to document when DENV begins amplification until it reaches a viral load in which vector transmission is possible (Grunnill and Boots 2016). Joshi et al. (2002) reported that, in *Ae. aegypti*, vector transmission varied from 2 to 10% in males and 3 to 20% in females of the progeny of each generation through seven generations. Minimum infection rate was observed to range from 2.7 in November to 28 in June, confirming the greatest infection rate in males occurred during the dry season (Arunachalam et al. 2008). In Thailand, vector transmission in monthly samples of *Ae. aegypti* varied from 0 to 24 of 1,000 mosquitoes, increasing gradually until June, 2 months before the peak in human cases of dengue (Thongrungkiat et al. 2011).

In our study, the vector transmission rate of DENV-1 and DENV-2 was low (0.47%) and detected in the second part of 2014 but included one 10-male pool very infected by 1.23 x 10^7 RNA copies per milliliter of DENV-2. Perhaps the load resulted from amplification that increased gradually through spring-summer; however, it is within the range (10^5–10^7 RNA copies per female) reported as infectious to humans (Nguyen et al. 2013). The connection between vector transmission and horizontal infections was shown when sisters of infected males transmitted DENV to laboratory mice (Mourya et al. 2001, Lequime et al. 2016).

In conclusion, we showed that most dengue infections were old (68% per household) with a few new ones (8% per household) occurring in a stable pattern where DENV-1 and DENV-2 circulated vertically and horizontally, and viral amplification reached infectious loads until vector generations in August-September. Summing our results with literature reports, we concluded four DENV serotypes circulate at the USA-Mexico border, and we recommend indoor interventions against adult *Ae. aegypti* in August-September to stop viral amplification and transmission.

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First Report of *Hepatozoon* spp.[1] In a Dog at the Paso del Norte Region, US-Mexico

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Abstract. Canine hepatozoonosis is a tick-borne disease caused by apicomplexan hemoparasites of the genus *Hepatozoon* spp. (Apicomplexa: Hepatozoidae). We report a clinical case of hepatozoonosis in a domestic Siberian husky dog, *Canis lupus familiaris* (Linnaeus). The dog was a male of 2 years remitted to the University Veterinary Hospital because it appeared lame in the hind limbs. During clinical evaluation it presented ataxia, loss of proprioception in both hind limbs, hyperreflexia of the right hind limb, and hyporeflexia of the left hind limb. Intervertebral disc disease was ruled out with radiographic and magnetic resonance imaging. Serological tests for infectious diseases such as ehrlichiosis, anaplasmosis (SNAP 4Dx Plus® test, IDEXX Laboratories), and leptospirosis (SNAP® Lepto Test, IDEXX Laboratories) were negative. Four biopsy samples of the biceps femoris muscle were taken. Histopathological diagnosis reported "onion skin" cysts 200 to 300 µm, suggestive of *Hepatozoon americanum.* Clinical confirmation of *Hepatozoon* spp. at the border of Mexico with Texas and New Mexico could impact differential diagnosis of neuromuscular disease in the area, especially in dogs where the causes of muscular pain and weakness and lameness are non-diagnostic.

Canine hepatozoonosis is a disease transmitted by ticks infected with protozoa of the Hepatozoidae family. Unlike other tick-borne diseases, infection does not occur through blood, but when a dog, *Canis lupus familiaris* (Linnaeus), swallows a tick. To date, dogs can be infected by two tick-borne *Hepatozoon* spp.: *H. canis* and *H. americanum.* Although phylogenetically related, the two species differ in a variety of aspects that include clinical signs, life cycles, and the tick that acts as the definitive host and transmitter. In Europe, Africa, and Asia, *H. canis* is the prevalent species that causes infection. In contrast, dogs in the southern USA have been infected with *H. americanum,* while in Central and South America both *Hepatozoon* species occur.

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