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Short Communication

Identification of Antibodies against Dengue Virus in Domestic Dogs: A Potential Reservoir at Home?

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ABSTRACT

Background: Mosquitoes are efficient vectors of pathogenic arboviruses of importance to man because they remain infected throughout their life without developing the infection. These vector-borne diseases affect humans and also can affect animals.

Objective: To evaluate the presence of anti-dengue antibodies in domestic dogs from a rural area of Veracruz, Mexico.

Materials and Methodology: Serum samples were analyzed for dengue virus infection using three different ELISA tests (Panbio- Dengue IgG, Platelia Dengue NS1 antigen, in-house system anti-rNS3, and western blot assay.

Results: An overall seroprevalence of 53.2% was obtained and based on the identification of NS1 protein, 29.8% infected dogs were found.

Conclusions: The dog is capable of inducing a humoral immune response on proteins of DENV, which requires furtherinvestigation of the role of the dog as a host in DENV infection.

Keywords: Antibodies; NS1; NS3; Dengue virus; Domestic dogs; Reservoir

BACKGROUND

Dengue is an endemic infectious disease in tropical countries. This infection can be caused by any of the dengue virus serotypes (DENV1-4), and it is transmitted through the bite of female mosquitoes *Aedes aegypti* and *A. albopictus* [1,2]. The objective of this study was to analyze the presence of anti-dengue antibodies in dogs' owners of an endemic area of the state of Veracruz; Mexico using two commercial kits and an "in-house" ELISA assay using a mixture of NS3 recombinant proteins from the four serotypes.

METHODS

Owned dogs were analyzed in this study, belonging to communities Teotepec (18°26'51'' N, 95°04'55'' W) and located at a height of 360° masl, and El Jobo (18°27'39'' N, 95°04'58'' W) located at 400° masl in central Veracruz, Mexico (Figure 1).

A blood sample (7 mL) from the saphenous vein of owned dogs was collected with the consent and in the presence of their owners. The samples were centrifuged at 73.6 gm for 15 min to separate the blood serum. The sera were stored at -20°C until use. Serum samples were analyzed for dengue virus infection using three different tests, including Panbio Dengue IgG Capture ELISA (Panbio Diagnostics, Brisbane, Australia), Platelia Dengue NS1 antigen capture ELISA (Bio-Rad) detection, and "in-house" system (anti-rNS3). The commercial kits were used according to the providers instructions with a slight modification for the Panbio test, peroxidase-labeled goat anti-dog IgG antibody (Pierce, Rockford, IL, USA) was added at a 1:8,000 dilution in PBS/0.05% as a detection antibody. An indirect ELISA method previously characterized [3]. The cutoff (0.21) for this assay was established using the average obtained from a sample of 25 apparently healthy human sera plus two Standard Deviations (SDs). Positive samples were defined as samples with absorbance greater than two SDs above the mean of the negative control. Western Blot (WB) assay according to a previously described protocol [3]. The concordance analysis between the techniques used was carried out with the Kappa index.

RESULTS

A total of 47 sera of owner dogs of the villages of Teotepec and El Jobo, Veracruz were analyzed and tested with three ELISA tests, two to determine the presence of antibodies against dengue antigens and one test to identify viral antigen (Figure 2).

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A total of 17 (36.2%) samples were negative with all the tests, while 30 (63.8%) were reactive with at least one test. However, discordance among tests was very high, with a total of at least 24/47 (51.0%) samples with discordant ELISA results. Only one sample (2.1%) was reactive with all three tests, there were an additional twelve samples (25.5%) presenting reactivity in two ELISA tests, although with different combinations of tests (Table 1).

Nineteen samples (40.2%) were reactive with the Panbio Dengue IgG Capture ELISA, fourteen samples (29.8%) were reactive

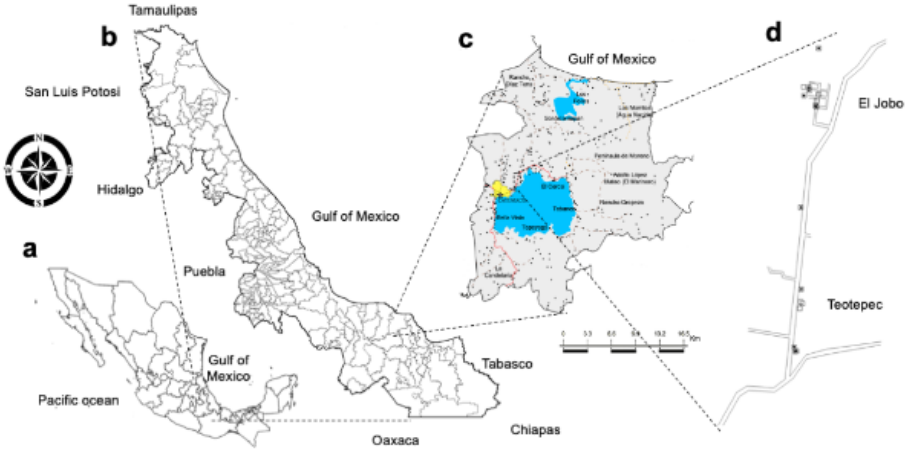

Figure 1: *Geographical location of Teotepec.*

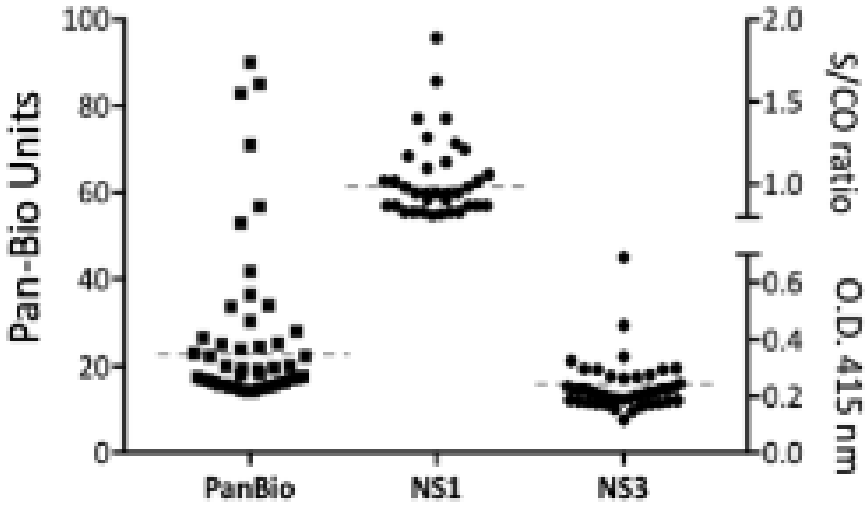

Figure 2: *Graph of ELISA test results.*

Table 1: *Reactivity of samples against different test ELISA.*

Group	Panbio Dengue IgG Capture ELISA	Platelia Dengue NS1 antigen capture ELISA	In-house ELISA test (anti-rNS3)	Number of reactive samples (%)
1	R	R	R	1 (2.1%)
2	R	N	R	6 (12.8%)
3	N	R	R	4 (8.5%)
4	R	R	N	6 (12.85%)
5	R	N	N	19 (40.4%)
6	N	R	N	14 (29.8%)
7	N	N	R	11 (23.4%)
8	N	N	N	17 (36.2%)
Total[a]	19 (40.4%)	14 (29.85)	11 (23.4%)	47 (100%)

Note: The sum of the values and percentages (a) of the last row does not reflect the total number of samples, but refers to the samples which were reactive for each test

Groups 1: Samples reactive for more than two tests

Groups 2-4: Samples reactive for two tests

Groups 5-7: Samples reactive for one test

Group 8: Sample negative for all test

R: Reactive, N: Negative

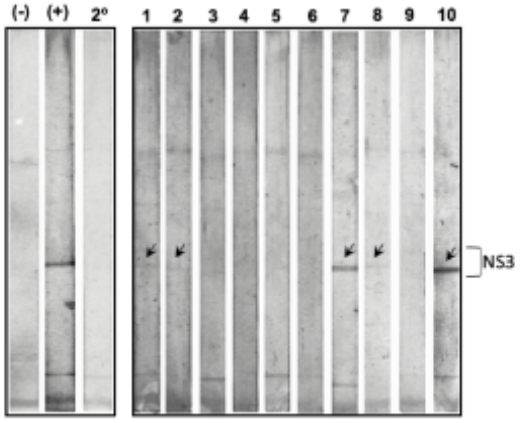

Figure 3: *Sera were assayed against the recombinant NS3 protein by Western Blot assays.*

with the Platelia Dengue NS1 antigen capture ELISA, and eleven samples (23.4%) were reactive with the in-house system test (anti-rNS3). Six samples (12.8%) were reactive with the Panbio Dengue IgG Capture ELISA test, and the Platelia Dengue NS1 antigen capture ELISA assay (Table 1), another six samples (12.8%) were reactive with Panbio Dengue IgG Capture ELISA test, and the in-house ELISA test (anti-rNS3), and four samples (8.5%) were reactive with Panbio Dengue IgG Capture ELISA test, and the in-house ELISA test (anti-rNS3) (Table 1).

On the other hand, the concordance index between the tests used was analyzed, a poor degree of concordance was observed between the Panbio Dengue IgG Capture ELISA test and the Platelia Dengue NS1 antigen capture ELISA assay (K=0.031), The degree of agreement between the Platelia Dengue NS1 antigen capture ELISA assay and the in-house ELISA test (anti-rNS3) (K=0.002) was poor. The degree of agreement between Panbio Dengue IgG Capture ELISA test and the in-house ELISA test (anti-rNS3) was poor (K=0.147).

According to the identification of antibodies using the Panbio Dengue IgG Capture ELISA test, a seroprevalence of 40.4% (19/47, 95% CI; 39.2-41.6) was obtained, but if we use the in-house ELISA test (anti-rNS3), a seroprevalence of 23.4% (11/47, 95% CI; 22.7-25.1) was obtained. Based on the combination of ELISA tests (Panbio and anti-rNS3), a total of 6/47 (12.7% CI95%; 10.4-15.0) samples were positive, obtaining an overall seroprevalence of 53.2%. Based on the identification of NS1 protein, 14/47 (29.8%, 95% CI; 29.4-30.2) infected dogs were found. On the other hand, the sera were assayed against the recombinant NS3 protein by WB assays. Of the 11 samples that were positive for NS3 by ELISA they were analyzed by western blot and a total of 5 samples showed recognition of the protein rNS3 with different intensity in the protein recognition (Figure 3).

DISCUSSION

A. aegypti and *A. albopictus* play an important role as vectors for the transmission of viral and parasitic diseases, especially in countries with tropical and subtropical weather. These vector-borne diseases affect humans and also can affect animals. It is noted that those mosquitoes are efficient vectors of pathogenic arboviruses of importance to man because they remain infected throughout their life without developing the infection [4,5]. The process of transmission of some pathogens depends on the participation of a reservoir that allows its survival, most of these pathogens that affect humans have a zoonotic origin [6,7]. In this work, we reported that a group of dogs living in an endemic area for DENV infection showed high seropositivity for specific virus antigens, a reactivity of sera was observed against NS3 recombinant protein of all four serotypes and proteins of a virus total extract, therefore, the presence of IgG antibodies against viral proteins (NS3 and E) strongly suggests that the dogs were previously exposed to the virus and the presence of NS1 may indicate active DENV infections. In a previous study, we observed that the ELISA assay using the recombinant protein rNS3 has an excellent agreement compared with screening for NS1 antigen (Bio-Rad), that is, all samples that were positive for NS1 antigen have antibodies against the protein rNS3 [3]. In addition to that, the finding of antibody by testing WB, although at a very low title, suggests that viral proteins are being processed and presented by the immune system of the dog, indicating that the dog is susceptible to infection by DENV. To date only no-human primates are known as a reservoir for dengue virus [8], however, there are reports that after the human, the dog is the second vertebrate host to the mosquito *A. aegypti* [9]. Some studies on the feeding sources of mosquitoes revealed that *A. aegypti* and *A. albopictus* can feed on the blood of domestic dogs [10], and recently it was found in a dengue-endemic area that domestic dogs are naturally infected [11].

Finally, it is not known if the dog can resolve the infection or maintain in an asymptomatic form, the results of this study showed that the dog's immune system is capable of processing and presenting viral antigens and generating a humoral immune response against DENV proteins, however, the role of the dog as host and/or reservoir of dengue infection requires further investigation [12].

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