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Domestic Dogs as Sentinels for West Nile Virus but not *Aedes*-borne Flaviviruses, Mexico

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We tested 294 domestic pet dogs in Mexico for neutralizing antibodies for mosquito-borne flaviviruses. We found high (42.6%) exposure to West Nile virus in Reynosa (northern Mexico) and low (1.2%) exposure in Tuxtla Gutierrez (southern Mexico) but very limited exposure to *Aedes*-borne flaviviruses. Domestic dogs may be useful sentinels for West Nile virus.

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Mosquito-transmitted viruses represent substantial health burdens across the Americas. Despite the broad geographic ranges of *Aedes* spp. and *Culex* spp. mosquitoes, the endemicity of human arboviral diseases is incongruent with these vector distributions (*1*,*2*). Animal sentinels may therefore be useful for signaling areas of virus transmission and human risk, especially in resource-poor settings where human diseases may be underreported. Although *Ae. aegypti* mosquitoes have been considered to feed predominantly on humans and *Cx. quinquefasciatus* mosquitoes on birds, our recent work studying host feeding patterns in southern Texas, USA (*3*), and northern Mexico (*4*) has documented substantial feeding on dogs for both species, presenting a novel opportunity to evaluate dogs for possible sentinel surveillance. Because dogs are ubiquitous and share the domestic environment with humans, tracking their exposures might provide evidence for understanding human risk and a sensitive indicator of geographic variation for mosquito-borne disease risk. We aimed to estimate domestic dog exposure to Zika virus (ZIKV), dengue virus 1 (DENV-1) and DENV-2, and West Nile virus (WNV) in northern and southern Mexico based on the presence and quantity of specific neutralizing antibodies as a proxy for human risk.

During 2018–2019, we sampled pet dogs from 3 residential areas in the city of Tuxtla Gutierrez, Chiapas, in southern Mexico and 8 neighborhoods in the city of Reynosa, Tamaulipas, in northern Mexico (Figure). We initially screened serum or plasma samples at a 1:10 dilution, then further tested those that neutralized PFUs by ≥90% in duplicates at serial 2-fold dilutions that ranged from 1:10 to 1:320 to determine 90% endpoint titers (Appendix, https://wwwnc.cdc.gov/EID/article/28/5/21-1879-App1.pdf).

We tested blood samples from 294 pet dogs (predominantly mixed breeds, chihuahuas, and pit bulls). Canine exposure to WNV was widespread, and we found a higher prevalence of neutralizing antibodies to WNV in dogs from Reynosa (72/169, 42.6%) than in those from Tuxtla Gutierrez (1/87, 1.2%; Appendix). In contrast, only 2 (0.7%) dogs from Tuxtla Gutierrez had neutralizing antibodies for ZIKV exposure, showing endpoint titers of 40 and 10. However, the dog with a ZIKV titer of 40 also had a 90% plaque-reduction neutralization test titer of 20 for WNV; we could not screen the dog with a ZIKV titer of 10 for other viruses because of low sample volume. A single dog from Tuxtla Gutierrez had a low titer monotypic reaction for DENV-2, the only evidence of exposure to an *Aedes*-borne flavivirus (Appendix). A sample size analysis indicated that the level of sampling we conducted supports 95% confidence that true prevalence of neutralizing antibodies in these canine populations did not exceed 1% for each of these *Aedes*-borne flaviviruses.

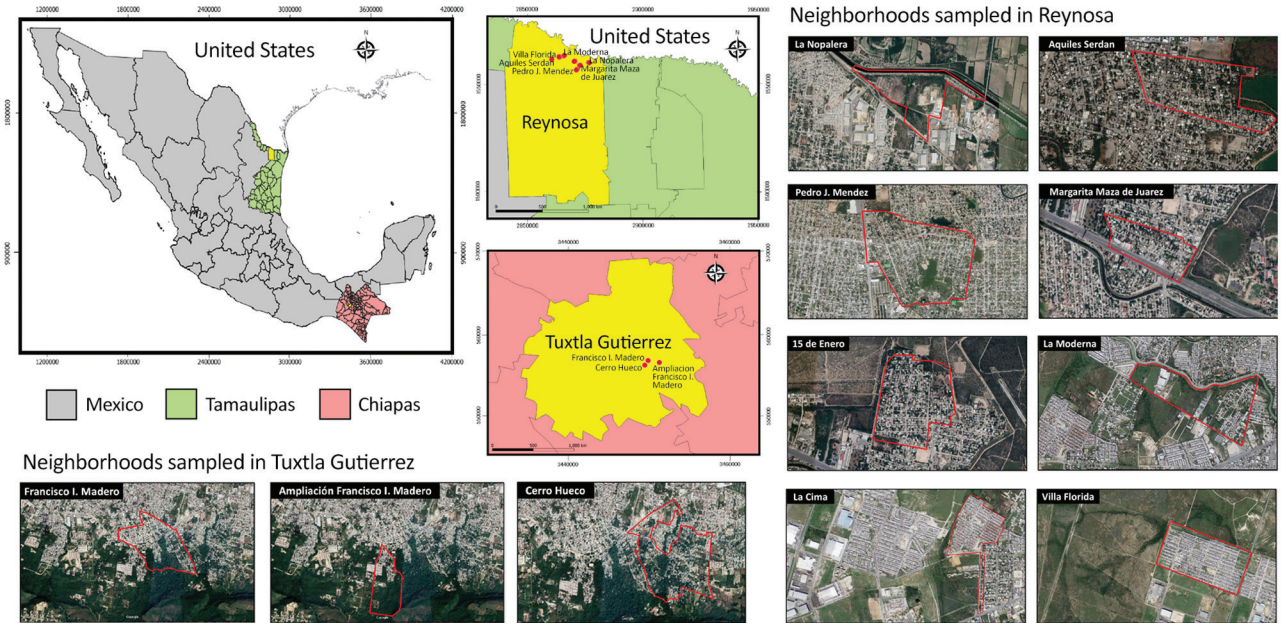

Figure. Sampling locations in Tuxtla Gutierrez, Chiapas, and Reynosa, Tamaulipas, Mexico, for study of neutralizing antibodies for mosquito-borne flaviviruses in domestic dogs. Map was created using QGIS 3.18.2 (https://qgis.org/en/site) with public domain map data from Instituto Nacional de Estadística, Geografía e Informatica (National Institute of Statistics, Geography, and Computer Science [INEGI]; https://www.inegi.org.mx/app/mapas) and satellite images from Google Maps (https://www.google.com.mx/maps).

Our data suggested substantial WNV enzootic activity in Reynosa and corroborated prior observations of high use of dogs as blood meal hosts by *Cx. quinquefasciatus* mosquitoes. Despite detecting neutralizing antibodies for WNV in 42.6% of dogs from Reynosa, the number of reported human WNV cases in Mexico has remained low (*5*), suggesting that transmission occurs among domestic animals but either humans have not been infected or cases have not been reported. Texas has a high number of reported human WNV cases (Texas Department of State Health Services, https://dshs.texas.gov/idcu/disease/arboviral/westNile/#stats). The lower reported numbers of WNV cases in Mexico might be in part because of the high seroprevalence of antibodies for other flaviviruses, which have been shown to protect against severe clinical infection from WNV, thus leading to reduced testing (*6*). Low WNV seroprevalence among dogs in Tuxtla Gutierrez might reflect a larger diversity of vertebrates with lower WNV competence, fed upon by *Culex* mosquitoes in the study area.

The relative lack of canine exposure to *Aedes*-borne flaviviruses suggests not an absence of these viruses circulating in these communities but that dogs are likely insensitive sentinels of the viruses' transmission in Mexico. In Chiapas, 7,972 human cases of dengue and 763 cases of Zika had been reported during 2016–2020 (*7*,*8*). Considering the timing of our sampling and the ages of the dogs, we expect that ≈75% of sampled dogs were living in these communities during DENV and ZIKV transmission activity. In the state of Tamaulipas, there were 3,988 human cases of dengue (*7*) and 733 cases of Zika during 2016–2020 (*8*). Given recent quantification that >50% of *Ae. aegypti* in southern Texas and northern Mexico feed on dogs (*3*,*4*), our serologic data suggest that either the probability of virus spillover into dogs is low or that, although dogs are susceptible to infection, neutralizing antibodies developed weakly or waned rapidly (*9*).

Our study suggests substantial WNV enzootic activity in Reynosa, Mexico and corroborates observations that *Cx. quinquefasciatus* mosquitoes, a primary vector of WNV, use high numbers of dogs for blood meals. Therefore, domestic pet dogs may be useful sentinels of WNV transmission, as previously suggested in other regions (*10*).

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Viral Hepatitis E Outbreaks in Refugees and Internally Displaced Populations, sub-Saharan Africa, 2010–2020

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Hepatitis E virus is a common cause of acute viral hepatitis. We analyzed reports of hepatitis E outbreaks among forcibly displaced populations in sub-Saharan Africa during 2010–2020. Twelve independent outbreaks occurred, and >30,000 cases were reported. Transmission was attributed to poor sanitation and overcrowding.

Hepatitis E virus (HEV) is a common etiology of acute viral hepatitis worldwide (*1*). Large-scale, often protracted outbreaks caused by HEV infection in refugee and internally displaced person (IDP) settlements and camps have occurred (*1*), particularly in sub-Saharan Africa, a region with nearly one third of the global forcibly displaced population (*2*). Previous epidemiologic studies of HEV infections in forcibly displaced persons have focused on singular events (*3*,*4*). The objective of this study was to identify trends in HEV outbreaks among forcibly displaced populations in sub-Saharan Africa.

We conducted a focused review of all English-language curated reports posted on ProMED-mail (ProMED) during 2010–2020 concerning HEV in forcibly displaced populations in sub-Saharan Africa. ProMED uses formal and informal disease surveillance mechanisms to rapidly report emerging disease events in animals, humans, and plants globally (*5*). It has been validated as a rapid and accurate tool for determining and describing global outbreaks. We verified all reports via PubMed, ReliefWeb, the UN High Commission for Refugees, World Health Organization (WHO), and references secondarily collected from ProMED. We used the keyword "hepatitis E" in applicable search engines for reports published during 2010–2020. We included records documenting "refugee(s) and/or asylum seeker(s) and/or internally displaced person(s)" in sub-Saharan Africa as defined by the World Bank (*6*). We considered outbreaks unique on the basis of date and location of cases. When screening ProMED reports, we used the most recent report pertaining to an outbreak. In cases where discrepancies existed between data sources reporting on the same outbreak, we retained the higher number of case counts. Three independent investigators (A.D., B.L., and A.M.) manually reviewed the databases.

Twelve hepatitis E outbreaks among forcibly displaced persons resulting in a total of >30,000 suspected or confirmed cases of acute HEV and ≥610 deaths were reported during 2010–2020 (Appendix Table, https://wwwnc.cdc.gov/EID/article/28/5/21-2546-App1.pdf). Outbreaks occurred in Sudan, South Sudan, Ethiopia, Chad, Niger, Namibia, Burkina Faso, Kenya, and Nigeria (Figure). One outbreak in displaced persons in South Sudan's Bentiu camp for

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Appendix

Description of Study Areas in Northern and Southern Mexico

The city of Reynosa, Tamaulipas, Mexico, with >600,000 residents, covers ≈3,100 km^2 adjacent to the US border across from McAllen, Texas. The municipality of Tuxtla Gutierrez in Chiapas, Mexico covers an area of 412 km^2 and has a population of ≈600,000 residents according to the 2020 population census. The ecologic park there, El Zapotal, contains Zoológico Miguél Álvarez del Toro, a zoo housing fauna endemic to Chiapas.

Methods

Dog Blood Sample Collection

Over 2 different time periods, we collected blood samples from dogs in 2 regions of Mexico. Oversight was performed by the Texas A&M University Institutional Animal Use and Care Committee and the institutional review board of El Colegio de la Frontera Sur.

During December 2018, we sampled pet dogs from 3 residential areas adjacent to El Zapotal, Tuxtla Gutierrez, recruited by door-to-door home visits. During March–October 2019, we collected samples in 8 neighborhoods in Reynosa (Figure). In both study locations, rabies vaccinations for dogs were offered as an incentive for participating pet owners. Owners provided written informed consent and data on dogs' age, sex, and breed. Many owners reported their pets as mongrels when breeds were mixed or unknown.

We collected blood by cephalic, jugular, or medial saphenous venipuncture into tubes containing a clot activator for serum or EDTA (ethylenediaminetetraacetic acid) as an anticoagulant for plasma samples (Becton, Dickinson, and Company; https://www.bd.com). All samples were kept cold until laboratory processing. We spun blood samples and stored aliquots

of serum, clot, whole blood, plasma, and erythrocytes at −80°C for 1–3 mo until shipping them to Texas A&M University for analysis. The Centers for Disease Control and Prevention (CDC) and the US Department of Agriculture issued import permits.

Virus Propagation and Titration

All work involving infectious viruses was performed in a biosafety level 2 facility. We tested all propagates by real-time reverse transcription PCR for ZIKV (*1*), DENV-1 through -4 (*2*), and WNV (*3*) to confirm viral identity. We inoculated virus stocks in T-25 flasks with Vero CCL-81 (American Type Culture Collection; https://www.atcc.org) cultures for virus propagation. When we observed cytopathic effect, we harvested viral suspensions, then centrifuged, filtered, aliquoted, and stored them at −80°C. We then titrated virus strains in Vero cells by plaque assay (*4*).

Plaque Reduction Neutralization Testing

We heat-inactivated serum and plasma samples at 56°C for 30 min and then following standard protocols (*5*) to test them by 90% plaque reduction neutralization testing ($PRNT_{90}$) for their ability to neutralize plaque formation by DENV-1, DENV-2, ZIKV, and WNV. We used the mouse hyperimmune ascitic fluids of all 4 viruses as positive controls.

We initially screened serum and plasma samples at a 1:10 dilution and further tested those that neutralized PFUs by ≥90% in duplicates at serial 2-fold dilutions ranging from 1:10 to 1:320 to determine 90% endpoint titers. We considered serum samples seropositive in a monotypic reaction when a serum dilution in duplicate of ≥1:20 reduced the formation of PFUs ≥90% in only 1 of the 4 flaviviruses tested. We also considered serum samples seropositive in a heterologous reaction when it reduced ≥90% of the formation of plaques of a flavivirus and the reciprocal neutralizing antibody titer was ≥4-fold greater than what was observed for the other 3 tested flaviviruses (*6*). Serum and plasma samples that had $PRNT_{90}$ titers of 10, in either monotypic or heterotypic reactions, or that we could not test for all flaviviruses were considered inconclusive. We considered undetermined those serum samples that presented PRNT titer ≥20 for >1 flavivirus and presented titer difference <4-fold greater for any flavivirus. We considered seronegative those serum samples with PRNT titers <10 for all 4 flaviviruses (*7*).

Statistical Analysis

We calculated seroprevalence for each virus by dividing the total number of confirmed positives by the total number of samples tested for neutralizing antibodies to that particular virus. For WNV testing, some serum samples had insufficient volume to confirm the endpoint titer after screening positive at 1:10. Accordingly, we applied the same percentage of confirmed positive samples to those unconfirmed samples to enable seroprevalence estimation. Because of the large number of dogs in our sample set that tested negative, we performed a post hoc sample size analysis to estimate the maximum number of dogs expected to be seropositive for a virus based on the number of dogs in our study that tested negative. The formula we used was

$$D = [1-(1-a)^{1/n}][N-(n-1)/2],$$

where D is the expected number of seropositive dogs, a is confidence, N is total number of dogs, and n is the subset of dogs tested (*8*).

Results

Of the 256 dogs we tested for WNV, 88 (34.4%) showed antibody titers ≥10. We performed endpoint titers for 83 samples and considered the remaining 5 inconclusive because they had insufficient volume for testing. From the 83 samples fully tested, we confirmed 69 (83.1%) positive for WNV. Applying this 83.1% proportion to the 5 samples that had a titer ≥10 but insufficient volume to confirm a titer ≥20 added 4 WNV-positive dogs, all from Reynosa, to the total. We estimated that 73/256 (28.5%) were positive for WNV antibodies. Among the remaining 183 samples, 11 were negative for all 4 viruses, 2 were inconclusive, and 1 was seropositive for an undetermined flavivirus. Of the 69 confirmed positive, 14 (20.3%) had $PRNT_{90}$ titers ≥320. Among the 69 dogs confirmed positive plus the 4 dogs estimated to be positive for WNV from among the samples with insufficient volume, seroprevalence was significantly higher among dogs from Reynosa 72/169 (42.6%) than dogs from Tuxtla Gutierrez 1/87 (1.2%) ($\chi^2 = 46.41$, $p < 0.001$).

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WNV (yellow fever virus 17D/WNV Flamingo 383–99) and mouse hyperimmune ascitic fluids (MHIAF) used as positive controls.

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Appendix Table. Demographics and test results of dogs in study of dogs as sentinels of West Nile virus*

Study ID	Age, y/Sex	Breed	Neighborhood	City	ZIKV, titers	DENV-1, titers	DENV-2, titers	WNV, titers	Result
1	Unk/Unk	Unk	REY	REY	<10	<10	<10	40	WNV
6	1.5/F	Mix	LN	REY	<10	<10	<10	80	WNV
13†	3/M	Chihuahua	LN	REY	<10	<10	<10	80	WNV
14†	1/M	Chihuahua	LN	REY	<10	<10	<10	80	WNV
15	3/M	Schnauzer	LN	REY	<10	<10	<10	80	WNV
16	2/M	Poodle	LN	REY	<10	<10	<10	40	WNV
17	3/M	Mix	LN	REY	<10	<10	<10	20	WNV
18	3/F	Mix	LN	REY	<10	<10	<10	80	WNV
21	1/F	Mix	LN	REY	<10	<10	<10	40	WNV
22	1/M	Labrador	LN	REY	<10	<10	<10	80	WNV
34	5/M	Mix	AS	REY	<10	<10	<10	160	WNV
39‡	5/F	Chihuahua	AS	REY	<10	<10	<10	≥320	WNV
42‡	5/M	Chihuahua	AS	REY	<10	<10	<10	40	WNV
45	4/M	Mix	AS	REY	<10	<10	<10	160	WNV
47§	2/F	Mix	PJM	REY	<10	<10	<10	160	WNV
48§	3/F	Mix	PJM	REY	<10	<10	<10	40	WNV
51§	7/F	Mix	PJM	REY	<10	<10	<10	40	WNV
53	1.5/M	Bulldog	PJM	REY	<10	<10	<10	160	WNV
63	3/M	Mix	PJM	REY	<10	<10	<10	≥320	WNV
65	4/F	Pitbull	PJM	REY	<10	<10	<10	80	WNV
66¶	2/F	Mix	MMJ	REY	<10	<10	<10	160	WNV
67¶	2/F	Mix	MMJ	REY	<10	<10	<10	160	WNV
68¶	6/M	Mix	MMJ	REY	<10	<10	<10	40	WNV
69#	Unk/M	Mix	MMJ	REY	<10	<10	<10	160	WNV
70**	3/F	Mix	MMJ	REY	<10	<10	<10	160	WNV
72#	4/F	Mix	MMJ	REY	<10	<10	<10	80	WNV
73**	2/M	Mix	MMJ	REY	<10	<10	<10	80	WNV
74	2/F	Mix	MMJ	REY	<10	<10	<10	≥320	WNV
76††	1.5/M	Pitbull	15DE	REY	<10	<10	<10	160	WNV
78††	4/M	Mix	15DE	REY	<10	<10	<10	80	WNV
79	2/F	Mix	15DE	REY	<10	<10	<10	40	WNV
82	2/M	Mix	15DE	REY	<10	<10	<10	≥320	WNV
83	5/M	Mix	15DE	REY	<10	<10	<10	40	WNV
84	1/M	Mix	15DE	REY	<10	<10	<10	80	WNV
85	9/M	Mix	15DE	REY	<10	<10	<10	160	WNV
92	1/M	Mix	15DE	REY	<10	<10	<10	≥320	WNV
93‡‡	3/M	Mix	15DE	REY	<10	<10	<10	80	WNV
94‡‡	10/M	Mix	15DE	REY	<10	<10	<10	≥320	WNV
102	2/M	Pitbull	LM	REY	<10	<10	<10	40	WNV
104	3/M	Chihuahua	LM	REY	<10	<10	<10	≥320	WNV
108	5/F	Border collie	LM	REY	<10	<10	<10	≥320	WNV
112	2/F	Mix	LM	REY	<10	<10	<10	≥320	WNV
114	4/F	French bulldog	LM	REY	<10	10	<10	<10	Inconclusive
127§§	10/M	Pug	LC	REY	<10	<10	<10	80	WNV
128	3/M	Labrador	LC	REY	<10	<10	<10	10	Inconclusive
129§§	3/F	Pug	LC	REY	<10	<10	<10	≥320	WNV
131	4/F	Mix	LC	REY	<10	<10	<10	40	WNV
137	3/F	Pomeranian	LC	REY	<10	<10	<10	160	WNV
141	2.5/M	Chihuahua	LC	REY	<10	<10	<10	80	WNV
142	6/M	Chihuahua	LC	REY	<10	<10	<10	160	WNV
145	2/M	Chihuahua	LC	REY	<10	10	<10	≥320	WNV
148	5/M	Dachshund	LC	REY	<10	<10	<10	160	WNV
152	2/M	Schnauzer	LC	REY	<10	<10	<10	80	WNV
156	3/F	Mix	LC	REY	<10	<10	<10	80	WNV
157	4/F	Chihuahua	LC	REY	<10	<10	<10	80	WNV
159	4/F	Mix	LC	REY	<10	<10	<10	≥320	WNV
170	10/F	Mix	VF	REY	<10	<10	<10	20	WNV
171	1.5/M	Pitbull	VF	REY	<10	<10	<10	≥320	WNV
172	2/M	Pitbull	VF	REY	<10	<10	<10	20	WNV
178	4/M	Chihuahua	VF	REY	<10	<10	<10	80	WNV
183	2/M	German shepherd	VF	REY	<10	<10	<10	20	WNV
184	7/M	Mix	VF	REY	<10	<10	<10	40	WNV
187	2/M	Mix	VF	REY	<10	<10	<10	80	WNV
194	4/F	Chihuahua	VF	REY	<10	<10	<10	80	WNV
196¶¶	2/F	Mix	VF	REY	<10	<10	<10	160	WNV
197¶¶	14/M	Chihuahua	VF	REY	<10	<10	<10	80	WNV
202	10/F	Mix	VF	REY	<10	<10	<10	160	WNV

Study ID	Age, y/Sex	Breed	Neighborhood	City	ZIKV, titers	DENV-1, titers	DENV-2, titers	WNV, titers	Result
209	5/F	Mix	VF	REY	<10	<10	<10	40	WNV
212	1/F	Chihuahua	VF	REY	<10	<10	<10	160	WNV
214	1/F	Chihuahua	VF	REY	<10	<10	<10	≥320	WNV
241	2/M	Mix	FIM	TGZ	40	<10	<10	20	Und. flavivirus
246	2/F	Pug	FIM	TGZ	10	NT	NT	NT	Inconclusive
278	1/F	Chihuahua	FIM	TGZ	<10	<10	20	<10	DENV-2
313	3/M	Basset hound	CH	TGZ	<10	<10	<10	160	WNV

*15DE, 15 de Enero; AS, Aquiles Serdan; CH, Cerro Hueco; DENV, Dengue virus; FIM, Francisco I. Madero; LC, La Cima; LM, La Moderna; LN, La Nopalera; MMJ, Margarita Maza de Juarez; NT, not tested; PJM, Pedro J. Mendez; REY, Reynosa; TGZ, Tuxtla Gutiérrez; Ukn, unknown; Und, undetermined; VF, Villa Florida; WNV, West Nile virus; ZIKV, Zika virus

†–¶¶ Dogs had same owner.