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Antibodies to West Nile Virus in Wild and Farmed Crocodiles in Southeastern Mexico

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Abstract

Surveillance for evidence of West Nile virus (WNV) infection in Morelet's crocodiles (*Crocodylus moreletii*) was conducted in Campeche State, Mexico, in 2007. Sera from 62 crocodiles (32 free-ranging and 30 captive) were assayed for antibodies to WNV by epitope-blocking enzyme-linked immunosorbent assay. Antibodies to WNV were detected in 13 (41%) wild and nine (30%) captive crocodiles, and the overall antibody prevalence was 35%. Although evidence of WNV infection in captive crocodiles has been reported in Mexico, we provide the first evidence of WNV exposure in wild crocodiles in Mexico.

Keywords

Crocodile; flavivirus; Mexico; surveillance; West Nile virus

West Nile virus (WNV; *Flaviviridae: Flavivirus*) is maintained in nature in an enzootic transmission cycle primarily involving *Culex* spp. mosquitoes and birds (Colpitts et al., 2012). The virus was first detected in the Western Hemisphere in 1999 during an outbreak of human, equine, and avian encephalitis in New York City (Lanciotti et al., 1999; Nash et al., 2001). Since then, WNV has dispersed across the Western Hemisphere and is found throughout the United States, Mexico, Canada, and the Caribbean, in addition to parts of Central and South America (Blitvich, 2008; Kramer et al., 2008).

Several reptile species are susceptible to WNV infection (Nir et al., 1969; Klenk and Komar, 2003; Miller et al., 2003; Steinman et al., 2003, 2006; Jacobson et al., 2005; Farfan-Ale et

al., 2006). The virus was responsible for significant mortality in farmed American alligators (*Alligator mississippiensis*) in Georgia during separate outbreaks in 2001 and 2002 (Miller et al., 2003; Jacobson et al., 2005). Approximately 1,250 of the 10,000 alligators housed at the farm died, and the consumption of infected horsemeat was implicated as the source of the outbreaks. A high prevalence (86%) of antibody to WNV was detected in healthy farmed Morelet's crocodiles (*Crocodylus moreletii*) in Ciudad del Carmen, southwestern Campeche State, Mexico in 2004 (Farfan-Ale et al., 2006). However, the crocodiles had travel histories and might have been infected with WNV in the neighboring states of Yucatán or Tabasco. A high WNV antibody prevalence (70%) was also detected in healthy farmed Nile crocodiles (*Crocodylus niloticus*) in Israel in 2002 (Steinman et al., 2003). Because the sample populations in the Mexican and Israeli surveys were small ($n=7$ and 20, respectively) and comprised solely captive animals, larger studies are needed to provide more precise estimates of WNV antibody prevalence in crocodiles.

To determine the prevalence of antibody to WNV in wild and captive crocodiles in Campeche State, we collected blood from 62 Morelet's crocodiles (32 free-ranging and 30 captive) in four study sites in the northwest of the state, March to September 2007 (Fig. 1; Los Petenes Biosphere Reserve, Site A: 19°55'63.4"N, 90°27'53.0"W; Site B: 20°37'00.3"N, 90°26'59.9"W; Site C: 19°56'69.0"N, 90°22'48.2"W; D (Campeche City): 19°51'59.8"N, 90°31'25.5"W). Wild crocodiles were captured at three sites in the coastal wetlands of Los Petenes Biosphere Reserve. The reserve is 382,000 ha comprised primarily of forest islands, islets, and mangroves. Crocodiles were captured at night using a spotlight and headlamps following published methods (Padilla et al., 2011). Crocodiles 80 cm long were captured and manually restrained using handheld aluminum poles with stainless steel cable and crocodiles <80 cm were captured by hand. Captive crocodiles were sampled at Centro de Estudios Tecnológicos del Mar No 2 (CETMAR), a technologic high school located in Campeche City. At the time of sampling, approximately 300 crocodiles were housed at CETMAR. Hatchlings are housed indoors under artificial light, whereas juveniles, subadults, and adults are housed in outdoor enclosures with natural light. Crocodiles are fed once a week, and their diet consists exclusively of fish.

The sex of each crocodile was determined by digital palpation of genital organs in the cloacae. Crocodiles were classified as hatchlings (0–50 cm), juveniles (51–100 cm), subadults (101–150 cm), and adults (>150 cm; Platt and Thorbjarnarson, 2000). Whole blood was collected from the caudal or supravertebral vein using a sterile syringe and 22- or 23-gauge needle. Blood samples were dispensed into Vacutainer tubes (Becton Dickinson, Toluca, Mexico), placed on wet ice in an insulated container, and transported to the laboratory. Samples were microfuged for 10 min at 10,000 × G and 4 C, and supernatants were collected and stored at –80 C. All crocodiles appeared healthy at sampling. None of the captive crocodiles had a history of WNV-like illness and all were born and reared at CETMAR.

Sera were analyzed by epitope-blocking enzyme-linked immunosorbent assay (ELISA) using the WNV-specific monoclonal antibody (MAb) 3.1112G (Chemicon International, Temecula, California, USA) as described (Blitvich et al., 2003a, b). Coating antigen was prepared from WNV-infected *Aedes albopictus* (C6/36) cell cultures. The ability of the test sera to block the binding of the MAb to WNV antigen was compared with the blocking ability of control serum without antibody to flaviviruses. Data were expressed as relative percentages and inhibition values 30% were considered antibody positive. Previous studies have demonstrated a 100% concordance between the blocking ELISA and plaque reduction neutralization test when used to analyze sera from a small number of crocodiles for WNV-specific antibodies (Farfan-Ale et al., 2006).

Antibodies to WNV were detected by blocking ELISA in 22 (35%) crocodiles (Table 1). Antibody prevalences for WNV in free-ranging and captive crocodiles were 30% ($n=32$) and 41% ($n=30$), respectively ($P=0.434$; Fisher's exact test; and SAS version 9.2, SAS Institute, Cary, North Carolina, USA). West Nile virus antibody prevalences for free-ranging and captive crocodiles of the same developmental stage were not significantly different (data not shown).

To the best of our knowledge, this is the first evidence of WNV exposure in free-ranging crocodilians. Sera from 30 caimans (*Caiman crocodilus yacare*) in Brazil in 2009 were assayed for evidence of WNV infection by blocking ELISA and reverse transcriptase-PCR, but all were negative (Pauvolid-Correa et al., 2011). Because the caimans were temporally and spatially associated with horses (*Equus ferus caballus*) antibody positive for WNV, their findings were not due to the absence of WNV in the area. One possible explanation for these contrasting findings is that caimans are less susceptible than Morelet's crocodiles to WNV infection. Alternatively, mosquito vectors may consider Morelet's crocodiles to be a preferable source of blood. Although serologic or molecular assays have not been performed to identify the blood meals of mosquitoes temporally and spatially associated with Morelet's crocodiles or caimans, observational studies revealed that 80% of mosquitoes that fed on caimans in Brazil were *Culex (Melanoconion) theobaldi* and *Mansonia* spp. (Viana et al., 2010). These species are not considered major vectors of WNV. Alligator blood was detected in six mosquito species, including *Culex quinquefasciatus* and *Culex nigripalpus* (which play an important role in WNV transmission), during an investigation at alligator farms in Louisiana, USA, in 2004–2006 (Unlu et al., 2010). At one farm, 13 of 126 (10.3%) engorged *C. quinquefasciatus* contained alligator blood. Alligator blood was also detected in seven of 13 (54%) mosquitoes during an investigation at an alligator farm in Florida in 2003 (Rodrigues and Maruniak, 2006).

Our data demonstrate that WNV infects wild and captive Morelet's crocodiles in Mexico. Investigations of the potential of this crocodilian species to serve as reservoir hosts of WNV are needed. More specifically, research is needed to determine the duration and magnitude of viremia, clinical signs, and mortality rates (if any) in crocodiles experimentally challenged with WNV. Such research is particularly important considering that other species of ectothermic vertebrates (e.g., American alligators and lake frogs [*Rana ridibunda*]), develop WNV viremia of sufficient magnitude to infect susceptible mosquitoes (Kostiukov et al., 1986; Klenk et al., 2004; Jacobson et al., 2005).

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LITERATURE CITED

- Blitvich BJ. Transmission dynamics and changing epidemiology of West Nile virus. *Anim Health Res Rev.* 2008; 9:71–86. [PubMed: 18348742]
- Blitvich BJ, Marlenee NL, Hall RA, Calisher CH, Bowen RA, Roehrig JT, Komar N, Langevin SA, Beaty BJ. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. *J Clin Microbiol.* 2003a; 41:1041–1047. [PubMed: 12624027]
- Blitvich BJ, Bowen RA, Marlenee NL, Hall RA, Bunning ML, Beaty BJ. Epitope-blocking enzyme-linked immunosorbent assays for detection of West Nile virus antibodies in domestic mammals. *J Clin Microbiol.* 2003b; 41:2676–2679. [PubMed: 12791902]
- Colpitts TM, Conway MJ, Montgomery RR, Fikrig E. West Nile virus: Biology, transmission, and human infection. *Clin Microbiol Rev.* 2012; 25:635–648. [PubMed: 23034323]

- Farfan-Ale JA, Blitvich BJ, Marlenee NL, Lorono-Pino MA, Puerto-Manzano F, Garcia-Rejon JE, Rosado-Paredes EP, Flores-Flores LF, Ortega-Salazar A, Chavez-Medina J, et al. Antibodies to West Nile virus in asymptomatic mammals, birds, and reptiles in the Yucatan Peninsula of Mexico. *Am J Trop Med Hyg.* 2006; 74:908–914. [PubMed: 16687701]
- Jacobson ER, Ginn PE, Troutman JM, Farina L, Stark L, Klenk K, Burkhalter KL, Komar N. West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. *J Wildl Dis.* 2005; 41:96–106. [PubMed: 15827215]
- Klenk K, Komar N. Poor replication of West Nile virus (New York 1999 strain) in three reptilian and one amphibian species. *Am J Trop Med Hyg.* 2003; 69:260–262. [PubMed: 14628941]
- Klenk K, Snow J, Morgan K, Bowen R, Stephens M, Foster F, Gordy P, Beckett S, Komar N, Gubler D, Bunning M. Alligators as West Nile virus amplifiers. *Emerg Infect Dis.* 2004; 10:2150–2155. [PubMed: 15663852]
- Kostiukov MA, Alekseev AN, Bulychev VP, Gordeeva ZE. Experimental evidence for infection of *Culex pipiens* L. mosquitoes by West Nile fever virus from *Rana ridibunda* Pallas and its transmission by bites. *Med Parazitol (Mosk).* 1986; 6:76–78. [PubMed: 3029556]
- Kramer LD, Styer LM, Ebel GD. A global perspective on the epidemiology of West Nile virus. *Annu Rev Entomol.* 2008; 53:61–81. [PubMed: 17645411]
- Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science.* 1999; 286:2333–2337. [PubMed: 10600742]
- Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol.* 2000; 38:4066–4071. [PubMed: 11060069]
- Miller DL, Mauel MJ, Baldwin C, Burtle G, Ingram D, Hines ME 2nd, Frazier KS. West Nile virus in farmed alligators. *Emerg Infect Dis.* 2003; 9:794–799. [PubMed: 12890319]
- Nash D, Mostashari F, Fine A, Miller J, O’Leary D, Murray K, Huang A, Rosenberg A, Greenberg A, Sherman M, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med.* 2001; 344:1807–1814. [PubMed: 11407341]
- Nir Y, Lasowski Y, Avivi A, Cgoldwasser R. Survey for antibodies to arboviruses in the serum of various animals in Israel during 1965–1966. *Am J Trop Med Hyg.* 1969; 18:416–422. [PubMed: 4889830]
- Padilla SE, Weber M, Jacobson ER. Hematologic and plasma biochemical reference intervals for Morelet’s crocodiles (*Crocodylus moreletii*) in the northern wetlands of Campeche, Mexico. *J Wildl Dis.* 2011; 47:511–522. [PubMed: 21719816]
- Pauvolid-Correa A, Morales MA, Levis S, Figueiredo LT, Couto-Lima D, Campos Z, Nogueira MF, da Silva EE, Nogueira RM, Schatzmayr HG. Neutralising antibodies for West Nile virus in horses from Brazilian Pantanal. *Mem Inst Oswaldo Cruz.* 2011; 106:467–474. [PubMed: 21739036]
- Platt S, Thorbjarnarson J. Population status and conservation of Morelet’s crocodile (*Crocodylus moreletii*) in northern Belize. *Biol Conserv.* 2000; 96:13–20.
- Rodrigues SC, Maruniak JE. Blood meal identification from mosquitoes collected at a commercial alligator farm. *J Am Mosq Control Assoc.* 2006; 22:557–560. [PubMed: 17067063]
- Steinman A, Banet-Noach C, Tal S, Levi O, Simanov L, Perk S, Malkinson M, Shpigel N. West Nile virus infection in crocodiles. *Emerg Infect Dis.* 2003; 9:887–889. [PubMed: 12899140]
- Steinman A, Banet-Noach C, Simanov L, Grinfeld N, Aizenberg Z, Levi O, Lahav D, Malkinson M, Perk S, Shpigel NY. Experimental infection of common garter snakes (*Thamnophis sirtalis*) with West Nile virus. *Vector Borne Zoonotic Dis.* 2006; 6:361–368. [PubMed: 17187570]
- Unlu I, Kramer WL, Roy AF, Foil LD. Detection of West Nile virus RNA in mosquitoes and identification of mosquito blood meals collected at alligator farms in Louisiana. *J Med Entomol.* 2010; 47:625–633. [PubMed: 20695278]
- Viana LA, Soares P, Paiva F, Lourenco-De-Oliveira R. Caiman-biting mosquitoes and the natural vectors of *Hepatozoon caimani* in Brazil. *J Med Entomol.* 2010; 47:670–676. [PubMed: 20695284]

Figure 1.

Study sites in Campeche State, Mexico, where Morelet's crocodiles (*Crocodylus moreletii*) were sampled and tested for antibody to West Nile virus. Three sites (A, B, C) are within the northern wetlands of Los Petenes Biosphere Reserve and one (D) is within the Centro de Estudios Tecnológicos del Mar No 2. The box within the inset denotes the three Mexican states comprising the Yucatan Peninsula: Campeche, Yucatán, and Quintana Roo.

Table 1

Numbers of free-ranging and captive Morelet's crocodiles (*Crocodylus moreletii*) in Campeche State, Mexico, 2007, with antibodies to West Nile virus according to developmental stage.

Habitat	No. positive/total (%)			
	Yearling	Juvenile	Subadult	Adult
Wild	1/2 (50)	6/10 (60)	4/11 (36)	2/9 (22)
Captive	0/0	2/4 (50)	5/13 (39)	2/13 (15)
Total	1/2 (50)	8/14 (57)	9/24 (38)	4/22 (18)
				22/62 (36)