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SHORT REPORT
Prevalence of neutralizing antibodies against West Nile virus (WNV) in monkeys (*Ateles geoffroyi* and *Alouatta pigra*) and crocodiles (*Crocodylus acutus* and *C. acutus–C. moreletti* hybrids) in Mexico

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SUMMARY

West Nile virus (WNV) is a mosquito-borne neurotropic viral pathogen maintained in an enzootic cycle between mosquitoes (vectors) and birds (natural hosts) with equids, humans, and other vertebrates acting as dead-end hosts. WNV activity in Mexico has been reported in several domestic and wild fauna and in humans, and the virus has been isolated from birds, mosquitoes, and humans. However, no serological studies have been conducted in monkeys, and only two in a limited number of crocodiles (*Crocodylus moreletii*). Here we present data on the prevalence of neutralizing antibodies against WNV in 53 healthy wild monkeys (49 *Ateles geoffroyi* and four *Alouatta pigra*), and 80 semi-captive healthy crocodiles (60 *C. acutus* and 20 *C. acutus–C. moreletti* hybrids) sampled during 2012. None of the monkey sera neutralized WNV, whereas 55% of the crocodile sera presented neutralizing antibodies against WNV. These results can contribute to the design of surveillance programmes in Mexico.

Key words: Crocodiles, monkeys, neutralization, seroprevalence, West Nile virus.

West Nile virus (WNV), a member of the Japanese encephalitis virus serocomplex (family Flaviviridae, genus *Flavivirus*), is a mosquito-borne neurotropic viral pathogen maintained in an enzootic cycle between mosquitoes (vectors) and birds (natural hosts) with equids, humans, and other vertebrates acting as dead-end hosts [1]. WNV was first detected in the Americas in New York in 1999, and since then, the virus has spread across continental United States, north into Canada, and south into Mexico and Central/South America [1]. WNV infection has caused a high mortality in birds and horses and hundreds of human deaths in the United States (http://www.cdc.gov/ncidod/dvbid/westnile/).

The first evidence of WNV activity in Mexico was reported in 2002 when antibodies to WNV were detected in horses in the US border states of Tamaulipas, Coahuila, and Chihuahua, and also in Yucatan State [2]. Since then, viral activity has been reported in several domestic and wild fauna and in humans across the country, and the virus has been isolated from birds, mosquitoes, and humans [2]. However, no serological studies have been conducted in monkeys, and only two in a limited number of

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crocodiles (*Crocodylus moreletii*). Here, data on the prevalence of neutralizing antibodies against WNV in wild monkeys (*Ateles geoffroyi* and *Alouatta pigra*), and semi-captive crocodiles (*C. acutus* and *C. acutus–C. moreletti* hybrids) are presented.

The detection of neutralizing antibodies was undertaken in duplicate using a virus neutralization test (VNT) on Vero cells using twofold serial dilutions of serum (starting from 1:20) and a fixed amount (100 plaque-forming units) of a cell culture passaged New York/1999 (NY99) WNV strain (GenBank accession no. KC407666) [3], as described previously [4]. Titres were calculated as the serum dilution that completely inhibited cytopathic effect. All infectious virus manipulations were performed in biosafety level 3 (BSL-3) facilities. The study was conducted during 2012 at the request of the 'Subdirección de Manejo de Fauna Silvestre, Dirección General de Vida Silvestre, Ministerio del Medio Ambiente y Recursos Naturales de Mexico' (permit no. SGPA/DGVS/03438/12). A χ^2 test was used to determine possible relationships between positive results and the different variables analysed.

A total of 53 monkeys (34% male) were captured and anaesthetized with 10 mg/kg ketamine (Probiomed, Mexico) before sampling. Thirty-nine were adults (>36 months), eight juveniles (12–36 months), and six infants (<12 months). Thirty-one monkeys were from Tekax area (Yucatán State) and 22 from Bacalar area (Quintana Roo State). Both areas are located in the southeastern part of Mexico with similar climate: warm and humid with rainfall in summer and an average annual temperature ranging between 25 °C and 27 °C.

Surveillance of WNV activity is important in order to assess viral expansion and to monitor zoonotic transmission. As the two species of monkeys analysed here (*At. geoffroyi* and *Al. pigra*) are considered as priority species by the 'Species at Risk Conservation Program (PROCER)' of the Commission for Natural Protected Areas (CONANP, http://procer.conanp.gob.mx/), and even though there are no reports of WNV activity in monkeys in Mexico, it seems reasonable to consider them during serosurveillance programmes.

In our study, no neutralization of WNV by sera taken from the monkeys was observed, even though the regions of sampling have an extensive history of WNV circulation [2], thereby suggesting that the two species are unlikely to be susceptible to WNV infection. However, a high infection rate has been previously reported in non-human primate populations: 39·39% in rhesus macaques (*Macaca mulatta*), 20·25% in pigtail macaques (*M. nemestrina*) and 51·36% in baboons (*Papio* spp.) in Louisiana, USA [5]. In contrast, specific antibodies were not detected 2–4 years later in Georgia, USA, in any of the 45 rhesus monkeys, and only in 6·6% of the sooty mangabeys (*Cercocebus atys*) tested [6]. Experimental infections have been reported in several primates, including squirrel monkeys (*Saimiri sciureus*), rhesus monkeys, and cynomolgus monkeys (*M. fasciacularis*), but not all species develop clinical signs of disease; thus confirming the different susceptibility of non-human primates to WNV infection [7]. Additional studies are therefore needed to clearly establish the susceptibility of *At. geoffroyi* and *Al. pigra* to WNV infection.

In comparison, 80 crocodiles (63·75% males), comprising 51 juveniles (0·2–2 m long) and 29 adults (>2 m long), were captured and immobilized, blood samples were taken from the ventral caudal tail vein and the animals released. Animals were sampled in a natural reserve (CIVS, Centro de Investigación de Vida Silvestre, Ministerio Mexicano de Vida Silvestre) sited in Laguna de Chacahua, Oaxaca State. This region is located in the southwestern part of Mexico with a subtropical steppe (low-latitude dry) and high flood risk along with periods of extreme drought, the average annual temperature ranges between 14 °C and 28 °C.

Although the role of reptiles in the life-cycle and epidemiology of WNV has yet to be elucidated, WNV has been shown to be pathogenic for crocodiles [8], which can die 24–48 h after the appearance of clinical signs such as anorexia, tremors, swimming on their sides, spinning in the water, and opisthotonus. Antibodies against WNV have been detected in wild and captive crocodilians (*C. niloticus*) in Israel [9], and in alligators (*Alligator mississippiensis*) in the United States, which have been shown to transmit the virus to non-inoculated tank-mates [10], and presented viral titres in tissues and sera sufficient to infect mosquitoes [10, 11]. Two serological studies in Mexico have previously been undertaken in a limited number of Morelet's crocodiles (*C. moreletii*). The first study demonstrated that 6/7 farmed animals tested presented specific neutralizing antibodies against WNV [12]. In the second study, 35% (22/62) ELISA-positive sera were reported in free-ranging (41%, 13/32) and farmed (30%, 9/30) animals [13].

Our study demonstrated a relatively high prevalence (55%, 44/80) of neutralizing antibodies in the crocodiles tested, with a similar proportion of positive animals in *C. acutus* (53%, 32/60) and *C. acutus–C. moreletti* hybrids (60%, 12/20). Likewise, no

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Table 1. *Crocodile virus neutralization titres (VNT)*

	Species, *n*		Gender, *n*		Age, *n*	
VNT	*Crocodylus acutus*	*C. acutus–C. moreletti* hybrids	Female	Male	Adults	Juvenile
1:20	10	4	3	11	1	13
1:40	8	1	1	8	5	4
1:80	8	1	4	5	2	7
1:160	5	5	7	3	1	9
1:320	0	1	1	0	0	1
1:640	1	0	0	1	0	1

differences in the number of positive animals were observed between males and females, but a higher proportion of positive samples were detected in juveniles (31% *vs.* 68%, $P = 0{\cdot}0012$). Neutralizing antibody titres ranged from 1:20 to 1:640, and two crocodiles demonstrated titres of 1:320 and 1:640. Titres of 1:160 were recorded in 10 animals, 1:40 and 1:80 in nine crocodiles each, and 1:20 for the remaining 14 crocodiles (Table 1). This range of titres is similar to that reported for *C. niloticus* in Israel [9].

In summary, we have determined the prevalence of anti-WNV neutralizing antibodies in crocodiles (*C. acutus* and *C. acutus–C. moreletti* hybrids) and in monkeys (*At. geoffroyi* and *Al. pigra*). While no neutralizing antibodies were detected in monkeys, a relatively high prevalence (55%) was observed in reptiles. These data can contribute to the design of future surveillance programmes in Mexico.

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DECLARATION OF INTEREST

None.

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