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Short Communication

West Nile and St. Louis Encephalitis Viruses Antibodies Surveillance in Captive and Free-Ranging Birds of Prey from Argentina

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Abstract: We evaluated the prevalence of WNV and SLEV neutralizing antibodies in captive and free-ranging raptors from Argentina by plaque-reduction neutralization test. Eighty plasma samples from 12 species were analyzed. Only one captive adult Crowned Eagle (*Harpyhaliaetus coronatus*) was WNV seropositive (prevalence: 1.25%; antibody titer of 1:80). Two captive Crowned Eagles were SLEV seropositive (prevalence: 2.50%; antibody titers: 1:80 and 1:40).These findings expand the geographic distribution of WNV and SLEV and confirm their activity in central and northeastern Argentina. West Nile virus activity in Argentina may represent a potential threat to Crowned Eagles and other endangered raptors in this country.

Keywords: West Nile virus, Saint Louis encephalitis virus, neutralizing antibodies, raptors, Argentina

Since its arrival to North America in 1999 and subsequent dissemination through Central and South America (Nash et al. 2001; Komar and Clark 2006; Diaz et al. 2008; Petersen and Hayes 2008; Hunsperger et al. 2009), concern has been raised about the possible effect of West Nile virus (WNV; Flavivirus, Flaviviridae) on immunologically naïve and endangered Neotropical birds populations (Saggese 2007; Blitvich 2008; Bataille et al. 2009). Raptors (Orders Accipitriformes, Falconiformes, and Estrigiformes) are particularly susceptible to WNV infection (Wünschmann et al. 2005; Nemeth et al. 2009; Hull et al. 2010). High morbidity and mortality of raptors has been reported in the USA, Canada, and Europe (Gancz et al. 2004; Saito et al. 2007; Höfle et al. 2008; Nemeth et al. 2007, 2009). Different studies conducted in experimentally inoculated birds and also on free-ranging raptors show a gradient of no obvious illness, subclinical, clinical manifestation, and death across raptors infected with WNV (Nemeth et al. 2006; Ellis et al. 2007; Jiménez-Clavero et al. 2008; Ziegler et al. 2013).

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Currently, 136 species of diurnal and nocturnal birds of prey inhabit South America and 18.3% of them are considered threatened or near-threatened (BirdLife 2013). Combined with other well-recognized threats, avian pathogens may have a negative impact on wild raptor populations (Newton 2002; Saggese 2007). Those species with limited geographic distribution, reduced natural numbers, fragmented populations, and low heterozygosis are highly susceptible to the deleterious effects of emerging diseases, like WNV (Newton 2002; Saggese 2007). Threatened and non-threatened species of raptors maintained in zoos and rehabilitation centers for ex situ conservation programs may be at risk as well (Saggese 2007).

WNV activity was detected in several central and northern Argentinian provinces, evidenced by the detection of neutralizing antibodies (PRNTAb), in a large mosaic of avian species, starting in the early 2000s (Diaz et al. 2008, 2011). Although no human encephalitis outbreak by WNV was reported, sporadic febrile human cases were observed. Beyond these few pieces of information, data surrounding distribution and impact of WNV in South America are scarce (Komar and Clark 2006; Morales et al. 2006; Petersen and Hayes 2008; Diaz et al. 2008).

Another flavivirus co-circulating and re-emerging in the American continent is St. Louis encephalitis virus (SLEV) (Spinsanti et al. 2009), which is broadly distributed in subtropical and temperate areas of Argentina (Díaz et al. 2013). Although birds are not affected by SLEV infection, they have an essential role in the transmission and maintenance of this virus (Reisen 2003).

The presence of WNV activity in Argentina may represent a considerable potential threat to both free-ranging and captive birds (Saggese 2007). However, the pattern of WNV and SLEV infection in Argentinean raptors remains basically unknown. In this study we evaluated the prevalence of WNV and SLEV antibodies in captive and free-ranging birds of prey from Argentina.

Sampling was conducted between March 2007 and April 2010 at selected zoos and wildlife rehabilitation centers and free-ranging raptors located in different geographic areas of Argentina (Table 1).

Plasma samples were tested for specific SLEV and WNV antibodies by plaque-reduction neutralization test (PRNT) using autochthonous viral strains (WNV ArE/729906 or SLEV CbaAr-4005). The etiologic agent responsible for the infection was identified in serum diluted at least 1/10 following Diaz et al. (2008).

A total of 80 plasma samples from 12 species of Accipitriformes, 5 species of Falconiformes, and 4 species of Estrigiformes were analyzed. Only monotypic immunological reactions were detected, no cross-reaction between viruses was observed. Only one sample was WNV seropositive (prevalence: 1.25%; 1/80), an adult Crowned Eagle (antibody titer: 1:80) (Table 1) held captive in Casa Grande, Córdoba in January 2008 (Fig. 1).

Two Crowned Eagles, one from Güira Oga, Misiones province and another one from Buenos Aires city, had detectable NTAb against SLEV (titers: 1:80 and 1:40, respectively). Overall, the prevalence of SLEV NTAb was 2.50% (2/80).

The adult Crowned Eagle WNV seropositive was a captive bird housed with other birds of prey in an open-air aviary located in a rural-suburban environment of the Dry Chaco Eco region. Interestingly, a few days before sampling, an adult Crowned Eagle housed in the same aviary was found dead, although necropsy and additional post-mortem investigation were not pursued by the zoo. This finding confirms exposure in an endangered species, expands the geographic distribution of WNV, and confirms its activity within Cordoba province (Diaz et al. 2008). Cordoba city, 81 km away, was the nearest site of WNV activity previously reported in raptors: an American kestrel (*Falco sparverius*) had been previously found seropositive, with a bird community seroprevalence of 1.1% between January 2005 and June 2006 (Díaz et al. 2011), 2 years before our result reported here. At the moment, the others birds of prey seropositive for WNV in Argentina come from another American kestrel and a Rufous-thighed Hawk (*Accipiter erythronemius*), from a rural area of Northeastern Cordoba province and from an urban site of Tucuman province, respectively (Díaz et al. 2011).

The apparent absence of WNV activity in sampled raptors could be a result of the limited sampling size in some sites. However, a relatively large number of raptors tested in Misiones (33 individuals/17 species) were also negative. The lack of WNV seropositive birds in Misiones province is surprising considering recent reports of WNV activity in the closer Corrientes and Chaco provinces, where free-ranging black howler monkeys (*Alouatta caraya*) had a seroprevalence of 19.81% in 2010 (Morales et al. 2011). Furthermore, this lack of exposure could indicate a high susceptibility to future infection and a potential conservation risk for endangered captive and free-ranging raptors inhabiting Misiones. Güira Oga is home to one of

Table 1. Seroprevalence and titers of WNV and SLEV for different raptors from Argentina

Species	Prevalence		Location/date[2]
	WNV[1]	SLEV[1]	
Accipitridae	1/41	2/41	
Crowned Eagle *Harpyhaliaetus coronatus*	1/22	2/22	Algarrobo del Aguila (N), La Pampa. January 2007; Mendoza city[3] (J, 2A), Mendoza. March 2007; San Rafael[3] (2A), Mendoza. March 2007; Casa Grande[3] (A), Córdoba. January 2008; Jagüel del Monte (2N), La Pampa. February 2008; Güira Oga[3] (2A), Iguazú, Misiones. March 2008; Belén (A), Catamarca. April 2008; Chamical (J), La Rioja. October 2008; Buenos Aires city[3] (4 J, A). May 2007, April 2008, October 2008 and April 2009; Lavalle (3N, A), Mendoza. January 2007-2009, November 2009
Solitary Eagle *Harpyhaliaetus solitarius*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Savanna Hawk *Buteogallus meridionalis*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Black-chested Buzzard-eagle *Geranoaetus melanoleucus*	0/5	0/5	Pilcaniyeu (4N), Rio Negro. December 2010
Short-tailed Hawk *Buteo brachyurus*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Black-and-white Hawk-eagle *Spizaetus melanoleucus*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Ornate Hawk-eagle *Spizaetus ornatus*	0/3	0/3	Güira Oga[3], Iguazú (3A), Misiones. March 2008
Black Hawk-eagle *Spizaetus tyrannus*	0/1	0/1	Güira Oga[3], Iguazú, Misiones. March 2008
Plumbeous Kite *Ictinia plumbea*	0/4	0/4	Güira Oga[3], Iguazú (4A), Misiones. March 2008
American Swallow-tailed Kite *Elanoides forficatus*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Roadside Hawk *Rupornis magnirostris*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Falconidae	0/25	0/25	
Southern Caracara *Caracara plancus*	0/15	0/15	Deseado (N), Santa Cruz. November 2010; Güer Aike (14N), Santa Cruz. November and December 2010
Collared Forest-falcon *Micrastur semitorquatus*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Peregrine Falcon *Falco peregrinus cassini*	0/7	0/7	Güer Aike (3N), Santa Cruz. December 2009. Mártires (1N), Paso de Indios (3N), Chubut. November 2010

Table 1. continued

Species	Prevalence		Location/date[2]
	WNV[1]	SLEV[1]	
Aplomado Falcon *Falco femoralis*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Bat Falcon *Falco rufigularis*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Tytonidae	0/2	0/2	
Barn Owl *Tyto alba*	0/2	0/2	Güira Oga[3], Iguazú (A), Misiones. March 2008
Strigidae	0/12	0/12	
Burrowing Owl *Speotyto cunicularia*	0/2	0/2	Güira Oga[3], Iguazú (2A), Misiones. March 2008
Tropical Screech-owl *Megascops choliba*	0/9	0/9	Güira Oga[3], Iguazú (9A), Misiones. March 2008
Stygian Owl *Asio stygius*	0/1	0/1	Güira Oga[3], Iguazú (A), Misiones. March 2008
Total	1/80	2/80	

[1]PNRT antibodies titers $\geq$1/10 are positive infection for WNV and SLEV.
[2]Sampled site/department, sampled size and age (*N* nestling, *J* juvenile, *A* adult), Province. Month Year.
[3]Indicates captive birds.

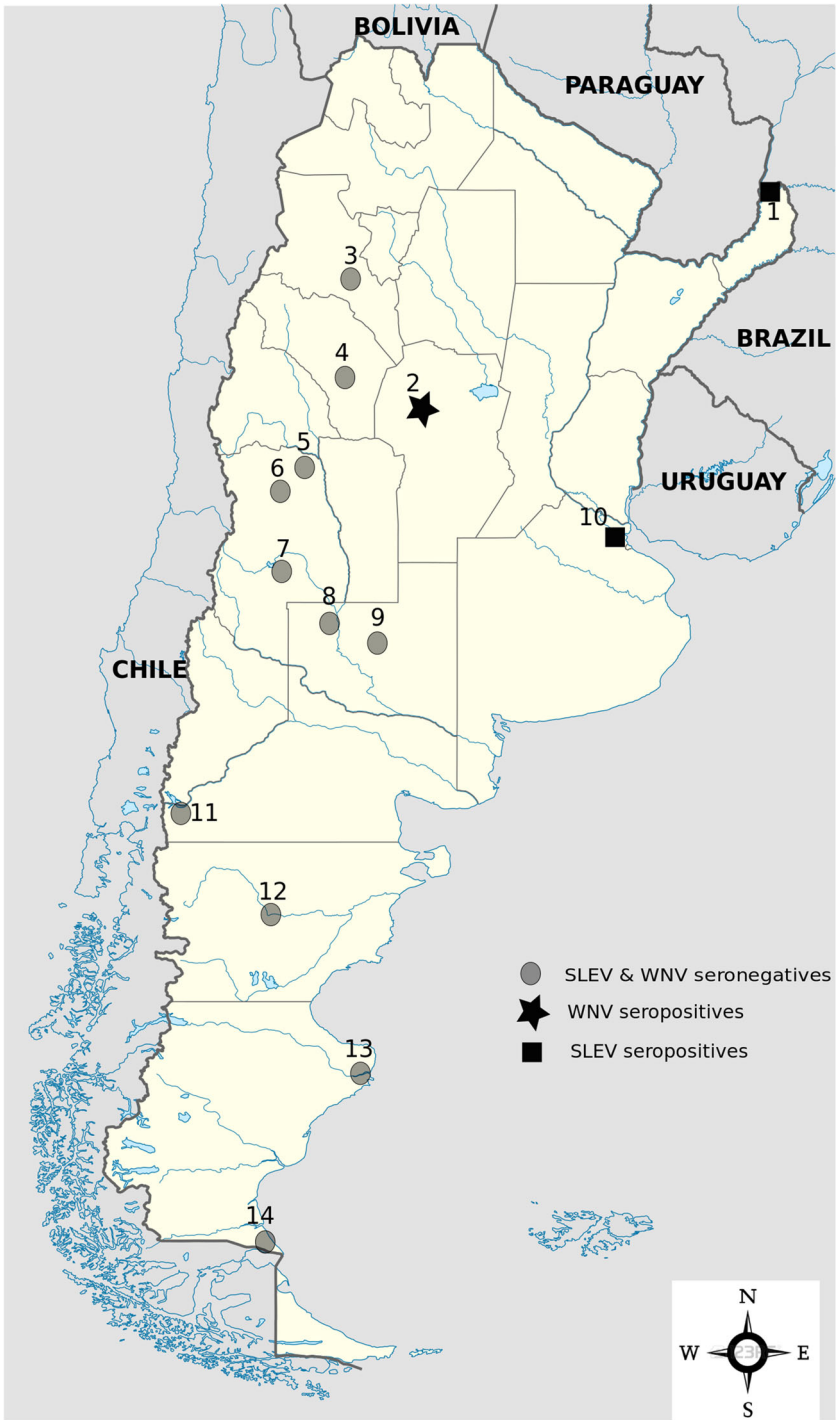

Figure 1. Spatial distribution of WNV and SLEV seropositive and seronegative raptors in Argentina, 2007–2010.

the largest captive collections of forest eagles in Argentina and these birds have a high value for ex situ conservation programs.

Contrasting with the WNV activity in USA and Canada, no mortality events have been reported in Neotropical birds (Komar and Clark 2006; Díaz et al. 2011). Reasons for this difference are not clearly understood, but circulation of bird-attenuated viral strains, avian host WNV infection resistant, co-circulation of antigenically related flaviviruses, and dilution effect of viral activity in a richer mosquito and avian community have been suggested (Gubler 2007; Swaddle and Calos 2008; Keesing et al. 2010). Moreover, lack of a proper surveillance system could likely encrypt the actual WNV activity in the region (Petersen and Hayes 2008).

One adult Crowned Eagle SLEV seropositive was a captive female in Güira Oga for more than 12 years and

sampled in March 2008. Based on its antibody titer of 1:80 we suspect that SLEV infection was recently acquired. Hodara et al. (1991) described SLEV seropositive people when studying prevalence of several arthropod-borne diseases in Misiones province, but technical limitations of their work precluded conclusions about the exact identity of the specific antibodies (a non-specific hemagglutination inhibition test was used by these investigators). Therefore, this is the first confirmation of SLEV activity in this province.

The other SLEV positive Crowned Eagle was a juvenile found in San Juan province (western Argentina) and moved to Buenos Aires for rehabilitation 2 months before the sampling date (April 2009). The low titer (1:40) and the history of recent movement suggests two potential scenarios for the infection origin. Between January and May 2009, a SLEV case was registered in Buenos Aires (López et al. 2011) suggesting infection of this bird may have occurred during this time. Alternatively, if this eagle was infected in San Juan before its arrival in Buenos Aires, this would demonstrate viral circulation at least 2 years previous to a recent urban outbreak (López et al. 2011). Unfortunately, no other birds were investigated for SLEV NTAb.

Recently, the use of zoo collections and rescue centers has been advocated for disease surveillance (Nemeth et al. 2007; Pultorak et al. 2011). In Argentina, human health surveillance programs are designed to report encephalitis and fever syndromes with infrequent etiological confirmation, although prophylactic measures are rarely implemented as result. Raptors, and other species of birds, are highly susceptible to WNV and SLEV infection (Wünschmann et al. 2005; Nemeth et al. 2009; Hull et al. 2010) mounting an antibody response to detectable levels by serological tests such as ELISA and PRNT (Nemeth et al. 2007). Zoo and rehabilitation centers house a large number of birds from different orders including raptors, parrots, waterfowl, etc. that could constitute an excellent, sensitive, and operative source of biomedical samples for WNV and SLEV activity surveillance (Nemeth et al. 2007; Pultorak et al. 2011).

Our data confirm the endemic circulation of WNV in the province of Cordoba, according with previous evidence and suggest a wide geographic distribution of SLEV and WNV in Argentina (Diaz et al. 2008). Our limited sample size did not allow confirming the absence of viral activity in raptors at locations where only seronegative birds were found, potentially underestimating the real infection status of raptors at the present time. However, this represents the first screening for flavivirus serological status in birds of prey at a large spatial scale in Argentina and South America.

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