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Source: Journal of Wildlife Diseases, 45(3) : 817-822

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-45.3.817>

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## Prevalence of Antibodies to West Nile Virus and Other Arboviruses among Crested Caracaras (*Caracara cheriway*) in Florida

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**ABSTRACT:** We documented the antibody prevalence to three arboviruses, St. Louis encephalitis virus (SLEV), eastern equine encephalitis virus (EEEV), and West Nile virus (WNV), in Crested Caracaras (*Caracara cheriway*;  $n=80$ ) in Florida from 2007 to 2008. Antibody prevalence to WNV was higher (9%) than for the other viruses. Most seropositive birds were adults ( $\geq 3$  yr of age), with 55% of adults testing positive for antibodies to at least one virus. Adults were significantly more likely to have antibodies to WNV than nonadults ( $P<0.001$ ). Prevalence of SLEV and EEEV antibodies among Crested Caracaras was 3% for each virus, and three adult caracaras had indistinguishable anti-flavivirus antibodies. The susceptibility of Crested Caracaras to adverse effects of WNV, SLEV, or EEEV infection remains unknown; however, we observed that some free-ranging individuals survived infection and successfully fledged young. Knowledge of arboviral infection among Florida's Crested Caracara, which is both state and federally threatened, is valuable considering increasing pressure on this population from rapid and extensive habitat alterations.

**Key words:** Antibody, Crested Caracara, *Caracara cheriway*, eastern equine encephalitis virus, seroprevalence, St. Louis encephalitis virus, West Nile virus.

Birds are important virus-amplifying hosts for numerous arboviruses (Stamm, 1966), some of which may adversely affect the health of free-ranging birds (McLean and Ubico, 2007). This is a particular concern for state and federally threatened species, such as the Crested Caracara (*Caracara cheriway*, hereafter, caracara; family Falconidae). The caracara is the only caracara species in North America, and a small, isolated population resides in Florida.

Numerous arboviruses circulate within the caracara's Florida range, including West Nile virus (WNV) and St. Louis encephalitis virus (SLEV; both in the family *Flaviviridae*, genus *Flavivirus*), and eastern equine encephalitis virus (EEEV; family *Togaviridae*, Genus *Alphavirus*) (Day and Stark, 1996; Blackmore et al., 2003). To our knowledge, WNV, SLEV, and EEEV infections have not been documented in caracaras. We sampled caracaras in Florida and tested their sera for specific anti-WNV, -SLEV, and -EEEV antibodies to determine antibody prevalence rates and to assess potential age and sex associations with seroprevalence.

We sampled caracaras from April 2007 to August 2008. Flighted caracaras were captured via a phai trap (Beebe and Webster, 1964) modified to accommodate carrion as bait; recently fledged birds were caught by hand. Blood (0.2–0.6 ml) was collected from the ulnar vein of each bird, placed into serum separator tubes, and held at ambient temperature for 1–4 hr before transfer to an iced cooler for 1–4 hr. Thereafter, blood was clarified by centrifugation ( $2,250 \times G$  for 10 min), and sera were stored at  $-20^\circ\text{C}$  until testing, except when shipped on ice to Colorado State University (Fort Collins, Colorado, USA).

Birds were aged based on the coloration of breast feathers; those with breast feathers fully or partially streaked were categorized as "nonadult" (i.e., subadult, juvenile, or fledgling), and birds with fully barred breast feathers were categorized as

TABLE 1. Seroprevalence rates for West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and eastern equine encephalitis virus (EEEV) among 80 Crested Caracaras (*Caracara cheriway*) in south-central Florida (2007–2008).

	<i>n</i>	% Antibody positive			
		WNV	SLEV	Flavivirus <sup>a</sup>	EEEV
Overall	80	16.3	2.5	3.8	2.5
Adults	22	54.5	9.1	13.6	4.5
Nonadults	58	0.0	0.0	0.0	1.7

<sup>a</sup> Flavivirus positive likely represents either anti-WNV antibodies, SLEV antibodies, or both.

“adult” (Wheeler and Clark, 2003). Non-adult birds were  $\leq 2$  yr old, and adults were  $\geq 3$  yr old (Dwyer, unpubl. data). Sex was determined for each bird through DNA analysis (Avian Biotech International, Tallahassee, Florida, USA).

Plaque reduction neutralization test (PRNT), using Vero cell monolayers in six-well plates (Beatty et al., 1995), was used to detect antibodies to WNV, SLEV, and EEEV. Before testing, serum samples were heat inactivated at 56 C for 30 min. Sera were initially tested at a serum dilution of 1:10 in BA-1 medium (M199-Hank’s salts, 1% bovine serum albumin, 350 mg/l sodium bicarbonate, 100 units/ml penicillin, 100  $\mu$ g/ml streptomycin, and 2.5  $\mu$ g/ml amphotericin B in 0.05 M Tris, pH 7.6). The PRNT challenge dose was approximately 100 plaque-forming units of the following strains: WNV NY99-4132 (originally isolated from crow brain in New York, USA), SLEV TBH-28 (originally isolated from human brain in Tampa, Florida, USA), and EEEV FL93 (originally isolated from mosquitoes in Florida). Known anti-WNV and anti-SLEV antibody-positive chicken sera and anti-EEEV horse serum served as positive controls in the respective neutralization assays.

Serum samples with  $\geq 90\%$  neutralization at a 1:10 dilution were considered positive for anti-viral antibodies, and positive samples were serially diluted (twofold) to determine reciprocal endpoint 90% neutralization (PRNT<sub>90</sub>) titers. A fourfold or greater titer of either anti-WNV or anti-SLEV antibodies indicated

that particular virus as the etiologic agent of infection. Sera from three individuals were positive for antibodies to both SLEV and WNV, but we could not demonstrate a fourfold or greater titer of either WNV or SLEV, so these individuals were considered flavivirus positive.

We tested 80 caracaras from 10 counties for antibodies to WNV, SLEV, and EEEV. Sampled birds included 58 nonadults (25 juveniles and 33 subadults) and 22 adults, composed of 47 (59%) females and 33 (41%) males. Fisher’s exact test was used to test for differences in antibody prevalence rates between adults and nonadults.

Antibodies to WNV were detected in seven (9%) of the caracaras. Most (55% [12/22]) of the adults were seropositive for at least one arbovirus, whereas 2% (1/58) of nonadults were seropositive (one juvenile was positive for antibodies to EEEV). Among adults, one (5%) and two individuals (9%) were seropositive for EEEV and SLEV, respectively, whereas three adults (14%) had antibodies to flaviviruses that could not be differentiated as either WNV or SLEV (Table 1). One adult had antibodies to both SLEV and EEEV. Among adults, 66% (7/11) of females and 46% (5/11) of males were positive for antibodies to one or more arboviruses. Serum PRNT<sub>90</sub> antibody titers were from 20 to 80, and seropositive caracaras were captured in six counties (Collier, DeSoto, Hardee, Hendry, Highlands, and Martin; Fig. 1).

Adults were significantly more likely to have anti-WNV antibodies than were

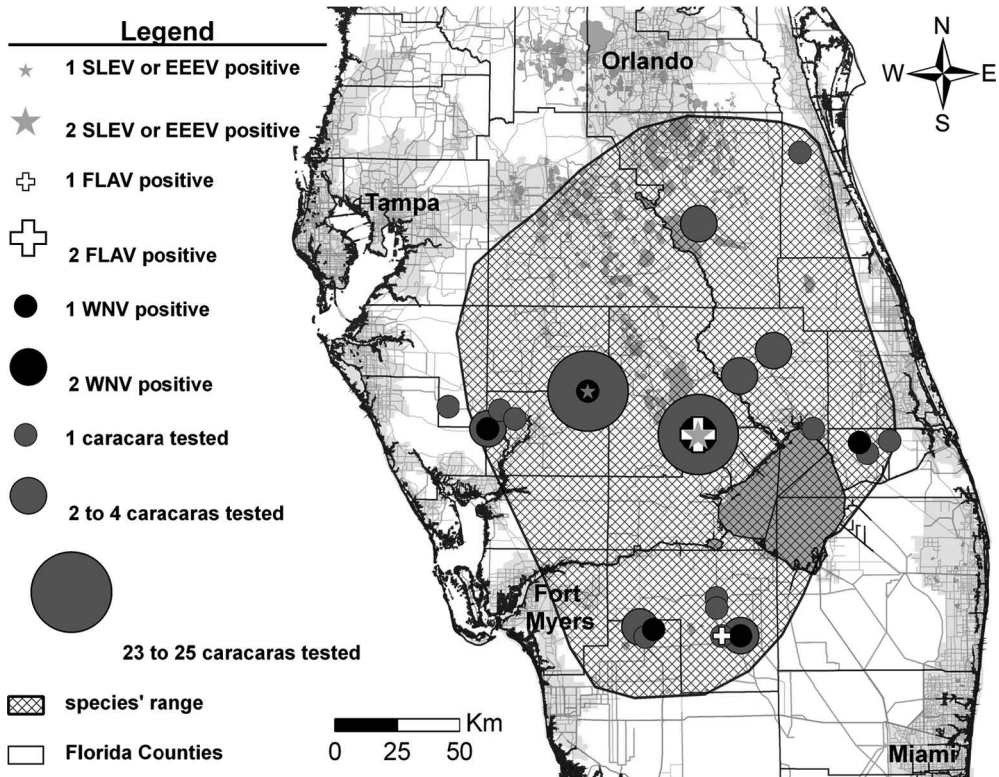


FIGURE 1. Locations of Crested Caracaras (*Caracara cheriway*) when sampled in Florida from 2007 to 2008, including those that tested positive for antibodies to West Nile virus (WNV), St. Louis encephalitis virus (SLEV), eastern equine encephalitis virus (EEEV), and nonspecific flavivirus (FLAV). Seropositive birds were located in Collier, DeSoto, Hardee, Hendry, Highlands, and Martin counties.

nonadults ( $n=80$ ,  $df=1$ ,  $P<0.001$ ), regardless of whether flavivirus antibody-positive birds were considered positive or negative for WNV. We found no difference in arbovirus seroprevalence by sex in adults ( $n=22$ ,  $df=1$ ,  $P=0.669$ ) or across all ages ( $n=80$ ,  $df=1$ ,  $P=1.000$ ). Similarly, there was no difference in WNV seroprevalence by sex in adults ( $n=22$ ,  $df=1$ ,  $P=1.000$ ) or across all ages ( $n=80$ ,  $df=1$ ,  $P=1.000$ ). Seven seropositive adults were providing parental care to dependent fledged young when these adults were sampled. We observed feather mites, lice, or both, and superficial abrasions on the faces of some of the birds sampled, but all birds seemed to be in general good health, were flight capable, and were foraging normally when captured.

West Nile virus was first detected in

Florida in 2001, at which time antibody prevalence among 10 species of free-ranging birds, including passeriforms, columbiforms, and one falconiform (a Red-shouldered Hawk, *Buteo lineatus*), was 10.5% ( $n=152$ ) in Jefferson County, Florida, and Lowndes County, Georgia, USA (Godsey et al., 2005). Both SLEV and EEEV were first reported in Florida in 1952; antibody prevalence rates were 1.3% for EEEV and 0.5% for SLEV ( $n=5,295$ ) among free-ranging birds sampled from 1965 to 1974 (Bigler and Hoff, 1975; Bigler et al., 1976). Arbovirus seroprevalence rates observed among caracaras in south-central Florida in the present study are consistent with these previous studies.

West Nile virus has led to unprecedented mortality in native North American

birds (Marra et al., 2004), whereas fatal EEEV and SLEV infections have been less commonly reported (McLean and Bowen, 1980; Main et al., 1988). Numerous raptors, including members of the family Falconidae, are susceptible to WNV infection and disease, although susceptibility varies among species (Joyner et al., 2006; Saito et al., 2007; Nemeth et al., 2009). Serologic surveys of free-ranging raptors in Wisconsin, California, and Pennsylvania in the USA revealed healthy, free-ranging seropositive individuals (Stout et al., 2005; Hull et al., 2006; Medica et al., 2007). Raptors are potentially exposed to WNV via mosquitoes and oral infection from prey (Nemeth et al., 2006). In our study, caracaras seropositive for WNV, EEEV, SLEV, or a combination, seemed healthy, although individuals that may have died from infection are not accounted for in serologic surveys.

Arboviruses are maintained in enzootic cycles between birds and mosquitoes, and exposure of free-ranging animals depends on many factors, including habitat, season, weather, contact rates between vectors and hosts, and host and vector abundance (Stamm, 1966; Day, 2005). Caracaras in Florida primarily inhabit drained grassland and pasture habitats that are usually adjacent to drainage ditches, where mosquitoes often are abundant (Morrison and Humphrey, 2001; Diuk-Wasser et al., 2006). Breeding caracaras are nonmigratory and live in pairs on defended territories throughout the year. Nonbreeding caracaras are vagrant throughout south-central Florida and aggregate at communal roosts that may consist of >200 individuals during the wet season (approximately late May to October; Dwyer et al., unpubl. data). Transmission rates are potentially high within communal roosts, as evidenced in American crows (*Corvus brachyrhynchos*) in east-central Illinois (Yaremych et al., 2004; Ward et al., 2006). However, we found little evidence of arbovirus infection in caracaras  $\leq 2$  yr of age. Limited evidence for WNV infec-

tion in younger birds could be partially explained by apparently low levels of WNV transmission in counties within our study area in recent years ([http://diseasemaps.usgs.gov/wnv\\_historical.html](http://diseasemaps.usgs.gov/wnv_historical.html)). In addition, longer lived resident animals, such as the Crested Caracara (Morrison, 2003), may be more likely to encounter an infectious mosquito during their lifetime than either shorter lived animals or animals that migrate to regions where mosquito vectors are inactive for part of the year.

In the present study, antibodies to arboviruses were more common in adult caracaras than in younger birds, although the timing of initial infection is unknown. This result is consistent with other studies in which antibody prevalence rates of WNV and other arboviruses were higher among free-ranging adult birds than younger individuals (Garvin et al., 2004; Beveroth et al., 2006). Garvin et al. (2004) hypothesized that young Blue Jays (*Cyanocitta cristata*) may succumb to EEEV infection and therefore may not be represented in seroprevalence studies, and there is evidence that young birds of some species are more susceptible to WNV-associated morbidity and mortality than older birds (Austin et al., 2004; Nemeth and Bowen, 2007; Nemeth et al., 2008). Both adult and juvenile caracaras had relatively high estimated annual survival (89.1 and 69.4%, respectively) before the arrival of WNV to Florida (1994–2000; Morrison, 2003); however, whether infection with WNV or other arboviruses affects survival of this species remains unknown.

We demonstrated that some free-ranging caracaras survive infection with WNV and other arboviruses with no apparent reductions in fecundity. However, Florida's population of caracaras faces a variety of challenges, including geographic isolation and human-induced alterations of nesting and foraging habitat (Morrison and Humphrey, 2001). Changes in land use may affect caracara behavior, as well as mosquito density and distribution,



potentially leading to increased caracara-vector contact, and therefore more opportunities for arbovirus transmission. Further studies are warranted to ascertain whether caracaras suffer negative effects from arboviral infection, and particularly WNV.

This study was funded by the US Fish and Wildlife Service grant agreement 401815G060. This is contribution 123 from the MacArthur Agro-Ecology Research Center of Archbold Biological Station. We are grateful to R. Bowen of Colorado State University for providing laboratory space and supplies and for performing EEEV assays and to D. Forrester for providing comments on the manuscript.

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*Received for publication 29 December 2008.*