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ARBOVIRUSES IN WATER BIRDS (CICONIIFORMES, PELECANIFORMES) FROM FLORIDA

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ABSTRACT: Sera from 360 ciconiform and pelecaniform birds collected in Florida (USA) from 1974 to 1990 were tested for serum neutralizing (SN) antibodies to eastern equine encephalitis (EEE), St. Louis encephalitis (SLE), and Everglades (EVE) viruses. Serum neutralizing antibodies to EEE virus were detected in 2%, to SLE virus in 7%, and to EVE virus in none of the samples. Pelecaniform birds (16%) had a higher antibody prevalence ($P < 0.02$) for SLE virus than did ciconiform birds (5%). Virus could not be isolated from 67 samples. Nestling birds with SN antibodies to both EEE and SLE viruses were found in both fresh water and marine colonies. Antibodies were more prevalent in adult and fledged juvenile birds than in nestlings.

Key words: Ciconiiformes, Pelecaniformes, arbovirus, eastern equine encephalitis virus, St. Louis encephalitis virus, Florida.

INTRODUCTION

Antibodies to arboviruses have been reported in ciconiform and pelecaniform birds (Kissling et al., 1954, 1955; Stamm, 1958; Buescher et al., 1959; Scherer et al., 1959, 1976; Favorite, 1960; Rodaniche and Galindo, 1961; Ventura, 1965; Bigler et al., 1967; Jennings et al., 1969; Campillo-Sainz, 1969; Dickerman et al., 1976; McLean et al., 1979; Aguirre et al., 1991, 1992). St. Louis encephalitis (SLE) virus has been isolated from ciconiform (Ranzenhofer et al., 1957; Dickerman et al., 1972) and pelecaniform birds (Spence, 1980) in tropical America. Because of their close association with habitats important for mosquito breeding, migration over great distances, and post-breeding dispersal, water birds should be considered as potentially important hosts in epizootics of these diseases.

Antibody to eastern equine encephalitis (EEE), SLE, Everglades (EVE) (formerly Venezuelan equine encephalitis (VEE)), and Highlands J (HJ) viruses, have been identified in free-ranging Florida (USA) birds and mammals (Henderson et al., 1962; Chamberlain et al., 1969; Wellings et al., 1972; Bigler et al., 1975, 1976); however, water birds have been tested infrequently in those studies. Highlands J virus is the eastern form of western equine encephalitis (WEE) which now is distinguished from the WEE found in the western USA (Calisher et al., 1988).

Serum neutralizing antibodies to EEE virus were detected in five of 16 ciconiforms and one of five pelecaniforms and to HJ virus in one of 16 ciconiforms and none of five pelecaniforms in north-central Florida in 1958 (Favorite, 1960). Hemagglutination inhibition (HI) antibody against EEE virus was detected in four (5%) of 85 cattle egrets (*Bubulcus ibis*) in 1965 (Bigler et al., 1967), against SLE virus in one (0.5%) of 187 herons (ardeids), against EEE virus in four (2%) of 187 ardeids, and against WEE (HJ) virus in one (1%) of 109 ardeids, and EVE in none of 92 ardeids from 1965 to 1976 (Bigler et al., 1975). After an SLE epizootic in the Tampa Bay, Florida, area in 1962 all of five cattle egrets, two little blue herons (*Egretta caerulea*), and one wood stork (*Mycteria americana*) had HI antibody to SLE virus (Jennings et al., 1969). Bigler et al. (1976) reported EEE virus to be enzootic in Florida fresh water swamps with foci in the central peninsula and in the panhandle. Eastern equine encephalitis and SLE virus both have been isolated from mosquitoes collected in the Tampa Bay

area (Wellings et al., 1972), and the Florida Everglades (Chamberlain et al., 1969). Both ciconiform and pelecaniform blood were identified in meals of *Culiseta melanura*, the principal enzootic mosquito vector of EEE virus from Florida (Edman et al., 1972). *Culex nigripalpus*, the principal vector of SLE virus in Florida (Sudia and Chamberlain, 1964) readily feeds on ciconiforms in experimental situations (Edman et al., 1974). We could find no report of arboviral isolations from Florida ciconiform or pelecaniform birds. Although EEE virus is enzootic in Florida, the role that water birds might play in the epizootiology of this and other arboviruses in Florida is not known. Our objective was to determine the prevalence of antibodies against EEE, SLE and EVE viruses in water birds from peninsular Florida, especially nestling birds from southern Florida.

METHODS

Serum samples were collected in Florida as a part of two separate studies. In the first, samples were collected during 1974, and 1987 to 1990, as part of a survey of diseases of ciconiforms (n = 228, all ages) and included Alachua, Marion, Levy, Pinellas, Palm Beach, Collier, Dade, and Monroe Counties, and Lake Okeechobee and Florida Bay (25° to 30°N, 80°10′ to 83°05′W). Nestlings were easily captured while still in the nest. In addition, nestlings, juveniles and adults found dead in colonies and along roadsides or that died within 3 days of arrival at a rehabilitation center were used. The second group of birds all were sampled alive as part of SLE virus surveillance in Lee County in 1978; these included 90 nestlings ciconiforms, anhingas (*Anhinga anhinga*) and double-crested cormorants (*Phalacrocorax auritus*) captured on their nests at Hemp Key (26°36′N, 82°10′W) and Sanibel Island (26°27′N, 82°05′W), and 42 adult and juvenile brown pelicans (*Pelecanus occidentalis*) from a rehabilitation center on Captiva Island (26°32′N, 82°12′W).

The birds were distributed throughout peninsular Florida (Fig. 1). Members of 13 species were tested (Table 1). Most (n = 316) of the samples were from nestlings and the remainder (n = 44) were from adults and fledged juvenile birds. Both freshwater (n = 83 birds) and marine sites (mangrove islands in Florida Bay, and an island off the west coast of Florida) (n = 277 birds) were sampled. Samples were distributed

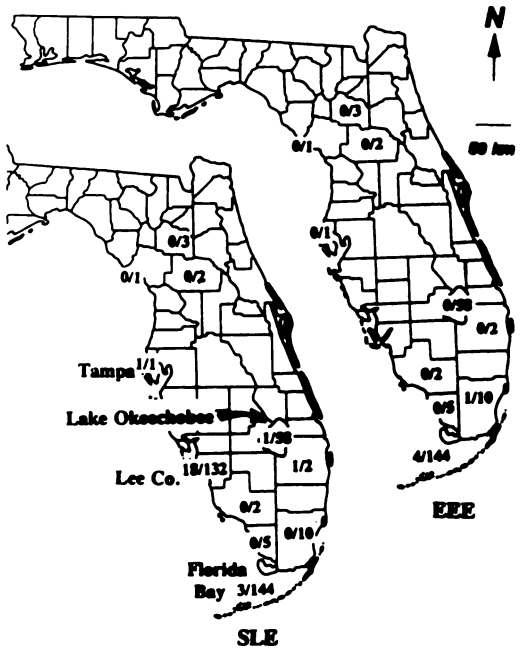

FIGURE 1. Collection locations for water birds tested for St. Louis encephalitis (SLE) and eastern equine encephalitis (EEE) virus antibodies in Florida (number seropositive/number tested).

annually as follows: 1974 (n = 1), 1978 (132), 1987 (2), 1988 (68), 1989 (65), 1990 (92). Blood was collected from the jugular or brachial veins of 244 live birds and from the heart of 116 dead birds. Nestling age was estimated for those birds sampled during 1987 to 1990 by recording the hatch date for the individual bird, or by measuring bill length and comparing birds of known age of the same species. Dead birds were examined, tissues were preserved in 10% neutral buffered formalin, and embedded in paraffin; 5 μ thick sections were stained with hematoxylin and eosin.

Serum was separated by centrifugation of clotted whole blood and stored frozen at −70 C until analyzed. All samples from 1974 and 1987 to 1990 were tested for SN antibodies to EEE, SLE, and EVE viruses. Samples from 1978 were tested for SLE virus SN antibodies only. Viral isolation was attempted on the 1987 to 1990 serum samples with adequate quantity available (n = 67) by placing 0.1 ml of each serum sample onto a monolayer culture of a continuous cell line of Vero cells (American Type Culture Collection, Rockland, Maryland, USA) grown in six-well plastic plates (McLean et al., 1985b). The inocula were allowed to absorb for 1 hr at 37 C and were then overlaid with M199 nutrient medium (Gibco, BRL, Life Technolo-

TABLE 1. Prevalence of neutralizing antibody against eastern equine encephalitis (EEE) and St. Louis encephalitis (SLE) viruses in 13 species of water birds sampled in Florida, 1974 to 1990.

Species	EEE		SLE	
	Number positive/ number tested	Prevalence (%)	Number positive/ number tested	Prevalence (%)
Ciconiiformes				
Great blue heron (*Ardea herodias*)	3/89	3	3/91	3
Roseate spoonbill (*Ajaia ajaja*)	1/44	2	0/44	0
Great egret (*Casmerodius albus*)	1/55	2	2/60	3
Green-backed heron (*Butorides striatus*)	NT[a]	—[b]	4/22	18
White ibis (*Eudocimus albus*)	0/12	0	1/12	8
Little blue heron (*Egretta caerulea*)	0/10	0	0/10	0
Tricolored heron (*Egretta tricolor*)	0/9	—	2/19	10
Snowy egret (*Egretta thula*)	0/4	—	2/33	6
Black-crowned night-heron (*Nycticorax nycticorax*)	0/4	—	1/10	10
Wood stork (*Mycteria americana*)	0/1	—	0/1	—
Ciconiiformes subtotal	5/228[c]	2	15/302	5
Pelecaniformes				
Brown pelican (*Pelecanus occidentalis*)	NT	—	4/42	10
Anhinga (*Anhinga anhinga*)	NT	—	3/3	—
Double-crested cormorant (*Phalacrocorax auritus*)	NT	—	2/13	15
Pelecaniformes subtotal	NT	—	9/58	16
Total	5/228[c]	2	24/360	7

[a] NT, not tested.
[b] Prevalence is not calculated for samples of fewer than 10 birds.
[c] Not all serum samples were evaluated for EEE antibodies.

gies Inc., Grand Island, New York, USA) containing 1% Noble agar (Difco Laboratories Inc., Detroit, Michigan, USA) and 1:25,000 neutral red (Gibco, BRL, Life Technologies Inc.) and incubated at 37 C in 5% CO_2 for 10 days or until plaques were observed.

For antibody tests, heat-inactivated (56 C for 30 min) serum specimens were tested for SN antibody against SLE (TBH-28 strain), EEE (NJO/60 strain), and EVE (FE3-7C strain) viruses by the plaque-reduction neutralization test in Vero cell culture in 6-well plastic plates (McLean et al., 1983, 1985a, b). Equal 0.1 ml volumes of serum were mixed with each virus diluted to contain 100 to 200 plaque-forming units. The mixtures were incubated overnight at 4 C and then 0.1 ml of each was added to Vero cell cultures and allowed to absorb for 1 hr. The inoculated cultures were overlaid with agar medium with neutral red for EEE and EVE viruses and without neutral red for SLE virus. The plates inoculated with SLE virus received a second overlay containing agar, nutrient medium and neutral red after 6 days. Inoculated cultures were held at 37 C in 5% CO_2 until plaques were counted. Serum was considered positive for SN antibody if plaque counts were reduced by ≥80% compared with positive and negative controls.

Chi-square tests of prevalence data at $P < 0.05$ were used to determine significant differences between groups of birds (Siegel, 1956).

RESULTS

Five birds of three species had SN antibodies for EEE virus, and 24 birds of 10 species had SN antibodies to SLE virus (Table 1). No birds had SN antibody to EVE virus. No viruses were isolated from any of the 67 sera tested. Anhingas (three of three), green-backed herons (18%), and double-crested cormorants (15%), had the highest prevalence of SLE virus antibodies. Antibodies against SLE virus were significantly ($P < 0.02$) more prevalent in pelecaniform birds than in ciconiform birds.

Eastern equine encephalitis virus antibodies were significantly ($P < 0.02$) more

prevalent in adult and fledged juvenile birds (7%) than in nestlings (1%). However, prevalences were not significantly different for SLE viral antibodies between adults and juveniles (7%) and nestlings (5%). The youngest birds with SN antibodies were a great egret with SLE viral antibodies at 8 to 16 days of age, and a roseate spoonbill with EEE virus antibodies at 12 to 20 days of age.

No significant differences were found in SN antibody prevalences between birds collected at saltwater sites (SLE = 8%; EEE = 3%) versus those collected at freshwater sites (SLE = 4%; EEE = 1%). Both SLE and EEE virus antibodies were found in nestlings that had not left their natal marine island in Florida Bay; EEE virus antibodies were observed in one roseate spoonbill at 12 to 20 days old, while SLE viral antibodies occurred in one great blue heron 1.5 to 2 months old, and one great egret at 8 to 16 days old.

Eastern equine encephalitis virus SN antibodies were detected only during 1988 (6%) and 1989 (2%). St. Louis encephalitis virus SN antibodies were detected only during 1978 (14%), 1989 (5%) and 1990 (3%).

There were no significant differences in SN antibody prevalences between birds sampled dead (EEE, 3%; SLE, 5%) and birds sampled alive (EEE, 2%; SLE, 7%). No consistent cause of death or pathology was found in dead birds with SN antibodies, nor were lesions suggestive of arboviral infections observed.

DISCUSSION

Pelecaniform birds sampled in this study in 1978 in Lee County on the southwest coast of Florida had a higher ($P < 0.01$) antibody prevalence (16%) for SLE virus than either the total sample of ciconiforms (5%) or than the ciconiforms tested during 1978 in the same area (12%). The sampling of the Lee County birds occurred the year following a SLE epidemic involving human cases in 1977 (Nelson et al., 1983). Thus it is possible that the high prevalence observed was the result of previous exposure of adult birds and maternal antibody passed to the nestling birds. Ciconiform birds in this study had a higher prevalence of EEE and SLE virus antibodies, than Florida birds in general in 1965 to 1974 (SLE = 0.5%, EEE = 1.3%; Bigler et al., 1975) but less than found in 1960 and 1961 (SLE = 7.0%, EEE = 10.3%; Henderson et al., 1962). In New Jersey (USA) where EEE virus also is epizootic, antibody prevalences in glossy ibis (*Plegadis falcinellus*) were greater (14%) than in ciconiform species in this study (W. J. Crans, pers. comm.). Because of the year to year fluctuations in prevalences observed both in this study and in comparison with others it is unlikely that short term antibody prevalence studies will elucidate the relative importance of water birds in the epizootiology of EEE and SLE viral epizootics. However, prevalences in both ciconiform and pelecaniform birds have been high enough at times to suggest that they might be important in the maintenance, distribution and epizootics of these viruses.

We did not test for antibodies against HJ virus. They were detected in one little blue heron in central Florida in 1958 (Favorite, 1960). Among other avian species, Favorite (1960) detected HJ (WEE) virus antibodies in 1% of birds in central Florida in 1958, and Henderson et al. (1962) detected them in both 1960 (23%) and 1961 (3%). Further work is necessary to understand the prevalence of HJ virus antibodies in Florida water birds.

Sparsely feathered and immobile nestlings may be particularly susceptible to exposure to mosquito-borne viruses (Blackmore and Dow, 1958; Kale et al., 1972) and post-fledging dispersal may be important in movement of the viruses out of enzootic foci. Based on the detection of antibody to SLE virus among nestling ciconiforms >15 days old and still confined to their nests on marine islands, we propose that transmission does occur in the marine environment of Florida Bay. Buescher et al. (1959) found that maternal antibody to

Japanese encephalitis virus was virtually gone by 15 days of age. The mangrove islands on which these birds nest have freshwater pools that occur periodically in times of heavy rainfall.

Bigler et al. (1975) found no evidence for EEE virus in birds or mammals tested south of Lake Okeechobee; equine cases are less common in this area of the state (Wilson et al., 1986). Our findings constitute the first report of birds with antibodies against EEE virus south of that lake, and also for birds in marine environments; however, the possibility that exposure occurred elsewhere, and that nestlings had passively acquired antibodies was not ruled out.

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