Arboviral Infection in Two Species of Wild Jays (Aves: Corvidae): Evidence for Population Impacts

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ABSTRACT We examined the prevalence of antibodies to three mosquito-borne arboviruses in blue jays, *Cyanocitta cristata*, and Florida scrub-jays, *Aphelocoma coerulescens*, to identify the effects on host survival, the influence of sex and age on infection, and the temporal patterns of antibody prevalence. Blood samples from 306 blue jays and 219 Florida scrub-jays were collected at Archbold Biological Station (Lake Placid, FL) from April 1994 through December 1995. Sera were analyzed for hemagglutination-inhibition antibody to eastern equine encephalitis (EEE) and St. Louis encephalitis (SLE) viruses, and neutralizing antibodies to EEE, Highlands J (HJ), and SLE viruses. Overall, 31.4% of blue jay samples and 22.1% of scrub-jay samples had antibodies to EEE. Antibodies to HJ were detected in slightly >15% of samples in each jay species, and SLE was detected in <3% of the samples in each jay species. A single EEE virus isolation was made from the blood of an 11-d-old scrub-jay nestling. Survival of adult blue jays seropositive to EEE was significantly lower than that of seronegative birds based on resight rates, but infection did not seem to affect survival of adult or juvenile Florida scrub-jays.

KEY WORDS jay, *Cyanocitta cristata*, *Aphelocoma coerulescens*, encephalitis, arbovirus

EASTERN EQUINE ENCEPHALITIS (EEE) virus is a mosquito-borne pathogen that infects wild and domestic animals and occasionally humans. Birds serve as amplification hosts in nature (Scott and Weaver 1989, Gibbs and Tsai 1994). Serological evidence indicates that some individuals of wild avian species commonly infected with EEE survive infection (Crans et al. 1994). However, in eastern North America, the virus has caused mortality of exotic birds, including ring-necked pheasants, *Phasianus colchicus* (Horsfall 1976), and emus, *Dromaius novaehollandiae* (Day and Stark 1996a), as well as captive native species, including whooping cranes, *Grus americana* (Dein et al. 1986). However, EEE-induced mortality has not been reported in free-ranging wild birds. Blue jays, *Cyanocitta cristata*, occur in North America primarily east of the Rocky Mountains. Evidence of EEE infection in blue jays has been reported throughout eastern North America, including Florida (Favorite 1960, Bigler et al. 1975) and New Jersey, where an antibody prevalence of 62% was reported by Crans et al. (1994).

Patterns of annual survival observed in a Florida scrub-jay, *Aphelocoma coerulescens*, population led Woolfenden and Fitzpatrick (1984, 1991) to hypothesize that unidentified epizootics have a major, but intermittent, effect on scrub-jay demography. From 1969 through 1979, annual survival of breeding adult scrub-jays in their study population ranged from 69 to 94% (mean = 82%). Annual survival of juveniles from the same population during the same period ranged from 21 to 44% (mean = 34%). However, from March 1979 to March 1980, only 55% of adults and 1% (1 of 93) of juveniles survived (Woolfenden and Fitzpatrick 1991). The years 1990–1991 and 1998–1999 also were marked by low scrub-jay survival in the same population (G. E. Woolfenden and J. W. Fitzpatrick, unpublished data). Although data on mosquito abundance were not collected and arboviral serosurveys in the scrub-jay population were not attempted during years of greatest juvenile mortality, these periods did correspond with outbreaks of EEE elsewhere in Florida (Day and Stark 1996b, J. F. Day, unpublished data).

As part of a study of the demography of these species, we tested for the effects of arboviral infection on the survival of sympatric blue jays and Florida scrub-jays by monitoring seroprevalence to eastern equine encephalitis (EEE), Highlands J (HJ), and St. Louis encephalitis (SLE) viruses. Also examined were the influence of sex and age on infection as well as temporal patterns of antibody presence.

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Materials and Methods

Study Site. All birds were sampled at Archbold Biological Station (Archbold) (27° 11′ N; 81° 21′ W), Highlands County, Florida, from April 1994 through December 1995. Archbold is a 2,081-ha preserve of near-pristine upland oak scrub, pine flatwoods, and related communities at the southern end of the Lake Wales Ridge in south central Florida (Abrahamson et al. 1984). Archbold is bordered to the east by expansive citrus groves, to the west and south by scattered oak scrub and improved pasture, and to the north by a series of lakes interspersed with oak scrub, pine flatwoods, and bayheads. A small red maple, *Acer rubrum* L. swamp; and several bayheads (Abrahamson et al. 1984) lie 200 m north of the study site. A 36.6-ha sinkhole lake is located to the north of the study site, and many seasonal ponds are scattered throughout the area. Much of the study site consists of low, open oak scrub interspersed with patches of pine flatwoods and grassy swales; the eastern portion consists primarily of mature slash pine, *Pinus elliottii* Englem, and sand pine, *P. clausa* (Chapm.) Vasey, forest and human-modified vegetation (Tarvin and Garvin 2002).

Both blue jays and Florida scrub-jays are year-round residents at Archbold (Tarvin and Woolfenden 1999, Woolfenden and Fitzpatrick 1996). Blue jays have been the focus of intensive study at Archbold since 1991 (Tarvin and Woolfenden 1999, Tarvin and Garvin 2002, Garvin and Greiner 2003). A long-term study of the Florida scrub-jay during which individual birds are censused throughout the year has occurred at Archbold since 1969 (Woolfenden and Fitzpatrick 1984, 1990, 1996).

Field Methods. Blue jays and scrub-jays were captured throughout the study in walk-in treadle traps baited with bread and peanuts, and infrequently with Japanese mist-nets (U.S. Fish and Wildlife banding permit no. 07732-F, University of Florida IACUC #2241). We attempted to capture as many blue jay adults as possible across the study site from January through March and again during June and July. At other times of the year, blue jays were captured irregularly. We also sampled any blue jay nestlings from nests that we could reach. Adult scrub jays were sampled irregularly throughout the study period. All nestling scrub jays that survived to 11 d of age were sampled. Captured birds were bled, banded with numbered U.S. Fish and Wildlife Service aluminum leg bands and a unique combination of colored plastic bands, and released at the capture site. Individuals with a brood patch were identified as females. The sex of many individuals, especially scrub-jays, was determined by behavior after release (Woolfenden and Fitzpatrick 1984). Some blue jays were sexed by laparotomy (Risser 1971). Many scrub-jays were banded as nestlings so their exact age at subsequent captures was known. Other individuals of both species were aged based on plumage characteristics (Dater 1970, Bancroft and Woolfenden 1982).

Blood samples (0.2–0.8 cc) were collected from individuals via jugular venipuncture with a 1-cc syringe rinsed with EDTA. One drop of blood (0.006 ml) was transferred to a cryogenic storage vial (Fisher, Orlando, FL) containing 0.7 ml of biological field diluent (BFD) for later attempts at viral isolation. The BFD contained 90% minimal essential medium with Hanks' salts (Sigma, St. Louis, MO), 10% fetal bovine serum (Intergen, Purchase, NY), 200 U/ml penicillin (Sigma), 200 μg/ml streptomycin (Sigma), 2.5 μg/ml amphotericin B (Sigma), and 50 μg/ml kanamycin (Sigma). Remaining blood was transferred to a vacutainer (Fisher) containing 0.7 ml of BFD for later antibody detection attempts. Samples collected in the field were transported to the laboratory on wet ice.

In the laboratory, samples intended for viral isolation were transferred to liquid nitrogen, and later to an ultralow temperature freezer, and stored at −70 C before shipment on dry ice to the Tampa Branch Laboratory for virus isolation attempts. Blood intended for antibody detection was allowed to clot for 24 h at room temperature and then was centrifuged at $3,400 \times g$ for 30 min to separate blood cells from serum. Resulting sera were placed in cryogenic storage vials and shipped to the Tampa Branch Laboratory for arboviral antibody assay.

Arboviral Serology and Virus Isolation. All sera were first screened for hemagglutination-inhibition antibody (HI) to EEE and SLE viruses. A microadaptation of the HI antibody test of Beaty et al. (1989) was used with a hemagglutinin (HA) prepared from a Florida human EEE isolate (D64–837, Tampa Branch Laboratory) and from a Florida human SLE isolate (TBH-28, Centers for Disease Control and Prevention, Ft. Collins, CO).

Briefly, HA antigens were titrated at optimal pH to an endpoint allowing the addition of 4–8 HA units in a 0.025-ml volume to each aliquot of diluted serum. Twofold serial dilutions of acetone-treated sera starting at 1:10 in 0.4% bovine albumin-borate-saline (Beaty et al. 1989), pH 9.0, were prepared in 96-well disposable microtiter "U" plates by a Microlab AT Plus robotic diluter (Hamilton Co., Reno, NV). Control wells were provided for serum hemagglutinins, antigen, and diluent. Four to 8 units of antigen were added to the serum dilutions, and the mixture was incubated overnight at 4°C. After incubation, 0.05 ml of a standardized goose erythrocyte suspension (Beaty et al. 1989) was added to the serum dilutions and to control wells of test serum, known positive and negative sera, antigen, and diluent. Plates were incubated at 22–24°C for approximately 1 h. When the presence of 4–8 U of HA was confirmed by the antigen titration, the test findings were recorded. A titer of ≥1:10 was regarded as diagnostic of infection with an agent antigenically similar to that used in the test.

Specific antibody confirmation was performed via neutralizing (NT) antibody analysis to EEE, HJ, and SLE viruses. Two NT antibody assay techniques were used. For both tests, a titer of ≥1:10 was regarded as diagnostic of infection with an agent antigenically similar to that used in the test. The first assay was serial virus dilution with undiluted serum (Beaty et al. 1989). The challenge viruses were Florida isolates of SLE

(strain P15), EEE (strain D64-837), and HJ (strain 64A-1519). Before initiation of the serial virus dilution test, serum was heated for 30 min at 56 C. Equal amounts of serum were mixed with appropriate dilutions of virus. Serum-virus mixtures were incubated for 2 h at 37 C and then were transferred to an ice bath for immediate intracerebral inoculation (0.03 ml of each serum–virus mixture) into each of four to six 3-to-4-wk-old laboratory mice. Observation of inoculated animals continued for 14 d. Deaths were recorded and the lethal dose causing mortality in 50% of the test animals (LD_{50}) was determined by the method of Reed and Muench (1938).

The LD_{50} virus dilutions for each series of serum–virus mixtures, along with that of the control, were determined to a single decimal point. A logarithmic LD_{50} was expressed as the exponent of the reciprocal of the endpoint dilution. The log neutralization index of each serum was obtained by subtracting its LD_{50} from that of the control. Indices <1.0 were considered negative, whereas those of 1.0–1.6 were equivocal and reported as negative in the present article, and those ≥ 1.7 were positive.

Our second method of NT antibody assay was the plaque reduction neutralization test (PRNT) described by Olson et al. (1991). Virus stocks used for the PRNT were as follows: EEE strain ME-77132, HJ strain Ct An-B8-74, and SLE strain TNM4-212. Negative control sera were from chickens that were inoculated with diluent and did not have detectable antibody when tested by HI or PRNT. Positive controls were from chickens that were inoculated with the respective virus and had detectable antibody titers to the appropriate virus by HI and PRNT analyses.

Newborn mice (1–3 d old) were used for virus isolation by inoculation with thawed 1:7 mixtures of blood in BFD from field collections. One litter of eight suckling mice was inoculated with each thawed inoculum. Injections in each mouse were 0.015 ml by the intracerebral and 0.03 ml by the intraperitoneal routes. After inoculation, mice were observed daily for at least 14 d. A 1:10 suspension of brain material from sick or dead mice was prepared by homogenization in BFD. The homogenate was centrifuged twice, first at $750 \times g$ for 20 min to remove large debris and then at $3,700 \times g$ for 1 h. The resulting supernatant was passed through a 0.45-μm syringe filter of sterile mixed esters of cellulose that was pretreated with fetal bovine serum to prevent virus adsorption to the filter, and passaged to a second litter of suckling mice. In the event of sickness or death in the second passage mice, brain material was harvested and the NT antibody tests described above were used to confirm the identity of the isolated viral agent. We attempted virus isolation from 261 blue jay and 196 scrub-jay blood samples.

Determination of Antibody Prevalence. All sera samples were considered as independent samples in analyses of infection by age, sex, and season (see below) and were reported as "antibody prevalence" (i.e., the proportion of samples that were antibody positive). We took this approach because several individual jays in our study that were antibody positive upon first capture were antibody negative upon a subsequent recapture (Tables 1 and 2). In at least one case, an individual bird was positive, negative, and then positive again over the course of the sampling period (Table 1). Therefore, an individual bird that did not exhibit detectable antibodies may have been infected before we sampled it, and we therefore cannot conclude that antibody negative birds had never been exposed to the virus.

Age, Sex, and Temporal Distribution. Antibody prevalence was analyzed by age, sex, and season of detection. Birds were divided into four age categories (corresponding North American Banding Codes [Gustafson et al. 1997] in parentheses): 1) nestling (N), 2) hatching-year (L or HY; hereafter, all referred to as HY), 3) second-year (SY), and 4) after second-year (ASY). Nestlings of both species were in the nest for 18–21 d after hatching. SY and ASY birds first captured during autumn could not be accurately distinguished based on plumage characteristics (Bancroft and Woolfenden 1982). These individuals were classified as adults of unknown age (AHY) and were excluded from some analyses. For some analyses, we pooled nestlings and HY birds as "young-of-the-year." Likewise, SY, ASY, and AHY birds were pooled as "adults" for some analyses.

To partition data by season, we identified five seasons that corresponded to shifts in time and energy expenditures of both jay species (Woolfenden and Fitzpatrick 1996, Tarvin and Woolfenden 1999). These seasons were 1) prenesting (February–March; characterized by courtship and high levels of territorial activity), 2) nesting (April–May; characterized by incubation and brooding), 3) postnesting (June–July; characterized by adults caring for nutritionally dependent young), 4) autumn (August–October; characterized by much time spent harvesting and caching acorns), and 5) winter (November–January; characterized by maintenance activities such as eating cached acorns and avoiding predators).

Survival Analysis. We analyzed survival of blue jays in relation to EEE and HJ antibody status by comparing the number of seropositive and seronegative birds sampled from April through July 1994 with the number of these birds that were resighted in 1995. For this analysis, an individual from which at least one antibody-positive sample was collected during the 1994 observation period was considered to have been infected, even if subsequent samples from that same individual were antibody negative. Briefly, birds in 1994 were captured at an array of 54 feeders scattered across the study site and at some nests. In 1995, the same set of 54 feeders was censused for several days over a 2-mo period before the nesting season and again for several days over a 2-mo period after the nesting season. In addition to census observations at feeding stations, we recorded casual observations of marked blue jays at other times during 1995. We excluded birds sampled after July 1994 from the analysis, because antibody prevalence dropped substantially during autumn and winter 1994. We restricted the survival analysis to adult blue jays, because juvenile dispersal

Table 1. Repeated samples of blue jays that exhibited either seroconversion or seroreversion of NT antibodies to EEE, HJ, and SLE

Band no.	Age at first sample	First sampling		Second sampling		Third sampling		Fourth sampling	
		Date	Antibody status	Date	Antibody status	Date	Antibody status	Date	Antibody status
Seroconversions									
EEE									
1142-05981[a]	SY	27 July 1994	−	23 Feb. 1995	+	22 June 1995	−		
1142-05996	SY	17 June 1994	−	20 Feb. 1995	+				
1142-05997[a]		18 June 1994	−	1 Mar. 1995	−	3 Mar. 1995	+		
702-90578[a]	ASY	2 May 1994	−	22 July 1994	+				
872-94698[a,b]	ASY	2 May 1994	−	22 July 1994	−	10 Feb. 1995	+	25 May 1995	−
872-94761	SY	29 July 1994	−	3 Mar. 1995	+				
952-00012[a]	AHY	3 July 1994	−	23 Feb. 1995	+				
952-00016	SY	12 July 1994	−	20 Feb. 1995	+				
952-00020[a]	ASY	18 July 1994	−	14 Feb. 1995	+				
962-62040[a]	ASY	16 May 1994	−	19 June 1995	−	4 Aug. 1995	−		
962-62084	SY	22 July 1994	−	10 Feb. 1995	+				
HJ									
702-90577	ASY	2 May 1994	−	13 July 1994	+				
1142-05997[a]		18 June 1994	−	1 Mar. 1995	−	3 Mar. 1995	+		
SLE									
912-19205	ASY	19 May 1995	−	26 May 1995	+				
Seroreversion									
EEE									
1142-05995	SY	17 June 1994	+	5 July 1994	−				
872-94673[a]	ASY	2 May 1994	+	25 May 1994	−				
872-94694	ASY	10 Mar. 1995	+	6 July 1995	−				
872-94777[a]	ASY	10 Feb. 1995	+	3 Mar. 1995	+	25 May 1995	−		
872-94780	ASY	10 Mar. 1995	+	25 May 1995	−				
952-00044	ASY	15 Feb. 1995	+	1 May 1995	−				
952-00048	ASY	15 Feb. 1995	+	1 May 1995	−	4 Dec. 1995	−		
952-00056[a]	ASY	20 Feb. 1995	+	7 Aug. 1995	−	4 Dec. 1995	−		
952-00059	ASY	20 Feb. 1995	+	19 June 1995	−				
952-00063	ASY	23 Feb. 1995	+	22 June 1995	−				
962-62081[a]	SY	22 July 1994	+	28 July 1995	−				
952-00084	SY	22 Apr. 1995	+	4 Dec. 1995	−	18 Dec. 1995	−		
HJ									
1142-05901		10 May 1994	+	10 Mar. 1995	−	5 July 1995	−		
1142-05981[a]	SY	27 July 1994	+	23 Feb. 1995	+	22 June 1995	−		
702-90578[a]	ASY	2 May 1994	+	22 July 1994	−				
872-94673[a]	ASY	2 May 1994	+	25 May 1994	−				
872-94726[a]	ASY	4 May 1994	+	23 May 1995	−				
872-94777[a]	ASY	10 Feb. 1995	+	3 Mar. 1995	+	25 May 1995	−		
952-00020[a]	ASY	18 July 1994	+	14 Feb. 1995	−				
952-00045	ASY	15 Feb. 1995	+	22 Apr. 1995	−				
952-00050		17 Feb. 1995	+	7 June 1995	−				
952-00056[a]	ASY	20 Feb. 1995	+	7 Aug. 1995	−				
952-00070[a]	ASY	1 Mar. 1995	+	7 July 1995	−	26 July 1995	−	3 Aug. 1995	−
952-00068	ASY	28 Feb. 1995	+	25 July 1995	−				
952-00073[a]	ASY	2 Mar. 1995	+	12 July 1995	−				
952-00075	ASY	3 Mar. 1995	+	19 July 1995	−				
952-00083	ASY	22 Apr. 1995	+	19 Dec. 1995	−				
962-62006	ASY	16 May 1994	+	11 July 1995	−				
962-62007[a]	ASY	7 May 1994	+	13 June 1994	−				
962-62063	SY	10 Apr. 1994	+	29 May 1994	−	1 Feb. 1995	−		
962-62081[a]	SY	22 July 1994	+	28 July 1995	−				
962-62123	ASY	2 May 1994	+	1 June 1995	−				
SLE									
872-94726[a]	ASY	4 May 1994	+	23 May 1995	−				
952-00067	ASY	27 Feb. 1995	+	23 May 1995	−				
Seroconversion-reversion									
HJ									
962-62040[a]	ASY	16 May 1994	−	19 June 1995	+	4 Aug. 1995	−		
962-62088	SY	11 July 1994	−	13 July 1994	+	24 July 1995	−		
872-94698[a,b]	ASY	2 May 1994	−	22 July 1994	+	10 Feb. 1995	−	25 May 1995	−
EEE									
872-94693	ASY	12 July 1994	−	14 Feb. 1995	−	6 July 1995	+	20 July 1995	−
Seroreversion-conversion									
EEE									
952-00070[a]	ASY	1 Mar. 1995	+	7 July 1995	−	26 July 1995	−	3 Aug. 1995	+

[a] These individuals occur in the table more than once.
[b] This individual was sampled a fifth time on 28 July 1995 when it was antibody negative for both EEE and HJ.

Table 2. Repeated samples of Florida scrub-jays that exhibited either seroconversion or seroreversion of NT antibodies to EEE, HJ, and SLE

Band no.	Age at first sample	First sampling		Second sampling		Third sampling	
		Date	Antibody status	Date	Antibody status	Date	Antibody status
Seroconversion							
EEE							
1453-63697[a]	SY	17 July 1994	−	7 Mar. 1995	+		
1453-72883[a]	SY	11 Mar. 1995	−	9 Apr. 1995	+		
HJ							
1453-63697[a]	SY	17 July 1994	−	7 Mar. 1995	+		
1453-63723[a]	SY	2 July 1994	−	20 July 1994	+		
1453-72751	HY	15 July 1994	−	20 July 1994	−	7 Dec. 1994	+
1453-72883[a]	SY	11 Mar. 1995	−	9 Apr. 1995	+		
Seroreversion							
EEE							
1453-63676	AHY	1 June 1995	+	27 Oct. 1995	−		
1453-72836[a]	HY	20 July 1994	+	23 Feb. 1995	−		
1453-78065	AHY	11 Nov. 1994	+	27 Oct. 1995	−		
HJ							
1453-72723[a]	HY	16 July 1994	+	16 Apr. 1995	−		
1453-72836[a]	HY	20 July 1994	+	23 Feb. 1995	−		
SLE							
1453-72836[a]	HY	20 July 1994	+	23 Feb. 1995	−		

[a] These individuals occur in the table more than once.

was high and most nestlings and HY birds were not resighted after dispersal (Tarvin 1998).

Our assessment of survival of seropositive and seronegative scrub-jays differed from that for blue jays, because the scrub-jay population was monitored using different methods (Woolfenden and Fitzpatrick 1984). Most scrub-jay territories in the study site were censused once a month. Unlike blue jays, scrub-jays are sedentary and dispersal distances are generally short. Therefore, disappearance from the study site usually indicates death (Woolfenden and Fitzpatrick 1984), and combining monthly census data with other observations at the study site allowed calculation of the time of death of individual scrub-jays to within several days. We therefore measured scrub-jay survival as the number of days an individual lived after a serum sample was collected. Scrub-jay survival was monitored through 15 October 1999, the date of the October census.

Analysis of scrub-jay survival was restricted to breeders and HY birds. Breeding scrub-jays seldom disperse and the survival of breeding males and females is identical (Woolfenden and Fitzpatrick 1984, 1990), so breeding adults were pooled by sex for analysis. Young scrub-jays begin dispersing after they are ≈1 yr old, but remain within the boundaries of the study site. Both dispersal patterns and survival before breeding differ significantly for male and female scrub-jays (Woolfenden and Fitzpatrick 1984). Therefore, we analyzed the survival of male and female young-of-the-year separately. We restricted analysis of scrub-jay survival to quadrants of the study site referred to as the "demography tract" (Woolfenden and Fitzpatrick 1984), because those birds were censused most frequently. Some scrub-jay individuals were sampled more than once, and survival was measured from the most recent sample date.

Nestling and newly fledged juvenile scrub-jays may die soon after becoming infected with EEE or HJ and such a rapid response to infection may reduce our ability to detect effects of the virus on survival. Considering this, we examined the survival of scrub-jay broods in relation to the presence of arboviral antibodies in siblings. The number of young alive at fledging was determined for scrub-jay broods in 1994 and 1995. In broods for which at least one member survived until the July census of their hatching year when they become nutritionally independent, we measured 1) whether any of the surviving members were EEE or HJ antibody positive, and 2) whether any other brood members had died before nutritional independence.

Statistical Analysis. Most statistical analyses were performed using *G*-tests (likelihood ratio tests; Sokal and Rohlf 1981) on contingency tables. The exclusion of certain avian age groups or seasonal distribution categories from particular analyses precluded the use of log-linear analyses of multilayer contingency tables. *t*-tests were used for scrub-jay survival analysis.

Results

A total of 306 individual blue jays and 219 Florida scrub-jays were captured, uniquely tagged, bled, and released from April 1994 through December 1995. Many individuals were captured and bled more than once (Tables 1 and 2). We tested 379 blue jay serum samples for antibodies to EEE and HJ, and 383 for SLE. We tested 213 Florida scrub-jay serum samples for EEE, 207 for HJ, and 213 for SLE antibodies (Table 3).

Antibodies to EEE were detected in 119 of 379 (31.4%) blue jay and 47 of 213 (22.1%) scrub-jay samples. Antibodies to HJ were detected in 60 of 379 (15.8%) blue jay and 32 of 207 (15.5%) scrub-jay sam-

Table 3. Number of avian sera samples that were analyzed for antibodies to EEE, HJ, and SLE viruses according to year and month of sample collection

Month	Blue jay sera				Florida scrub-jay sera					
	1994		1995		1994			1995		
	EEE/HJ	SLE	EEE/HJ	SLE	EEE	HJ	SLE	EEE	HJ	SLE
Jan.	0	0	1	1	0	0	0	0	0	0
Feb.	0	0	54	56	0	0	0	1	1	1
Mar.	0	0	26	26	0	0	0	5	5	5
April	16	16	9	9	0	0	0	25	25	25
May	43	43	34	34	1	1	1	4	4	4
June	29	29	32	34	0	0	0	5	5	5
July	35	35	52	52	77	77	77	52	52	52
Aug.	4	4	8	8	0	0	0	0	0	0
Sept.	1	1	8	8	0	0	0	1	1	1
Oct.	1	1	0	0	6	4	6	4	4	4
Nov.	0	0	0	0	17	14	17	0	0	0
Dec.	0	0	26	26	6	5	6	9	9	9
Total	129	129	250	254	107	101	107	106	106	106

Individual birds may have been sampled more than once.

ples. Antibodies to SLE were detected in 11 of 383 (2.9%) blue jay and 4 of 213 (1.9%) scrub-jay samples. Antibodies to a single virus were detected in the majority of the positive samples for both avian species. However, antibodies to more than one virus were detected in 36 samples (28 blue jays and eight scrub-jays; Table 4). For most of our statistical analyses, each virus was treated independently. For example, samples that were seropositive for both EEE and HJ were included in analyses of each virus. Because SLE virus infection apparently was rare during our study, we restricted our statistical analyses to EEE and HJ viruses.

Sex and Age of Seropositive Birds. Serum samples collected from males and females were equally likely to be seropositive to EEE and to HJ antibodies in each avian species. Forty-seven of 123 (38.2%) sera from males and 36 of 102 (35.3%) sera from females in blue jays were seropositive to EEE ($G = 0.20$, df = 1, $P = 0.651$), whereas 33 of 123 (26.8%) samples from males and 20 of 102 (19.6%) samples from females were antibody positive to HJ ($G = 1.63$, df = 1, $P = 0.202$). Eight of 28 (28.6%) sera from males and 11 of 33 (33.0%) sera from females in scrub-jays were seropositive to EEE ($G = 0.16$, df = 1, $P = 0.689$), and 3 of 28 (10.7%) sera from males and 4 of 32 (12.5%) sera from females were seropositive to HJ ($G = 0.05$, df = 1, $P = 0.829$).

In blue jays, prevalence of EEE antibody varied significantly among the four age classes when samples from all seasons were combined ($G = 29.30$, df = 3, $P < 0.001$) (Table 5). This pattern potentially could be confounded because the analysis included serum samples collected from adult blue jays from January through March, when jays exhibited the highest EEE antibody prevalence and HY birds do not yet exist (see analysis below). Nonetheless, when restricting the comparison to April through December when all age classes were sampled, only 4 of 56 (7.1%) samples from young-of-the-year (nestlings and HY birds) were antibody positive, whereas 61 of 242 (25.2%) samples from adults were positive ($G = 10.53$, df = 1, $P = 0.001$). This difference did not exist between SY and ASY birds, because sera from 25 of 73 (34.2%) SY birds and 89 of 231 (38.5%) ASY birds were positive (all seasons combined; $G = 0.44$, df = 1, $P = 0.508$).

Similar age-related patterns were observed in blue jay sera positive to HJ virus antibodies (Table 5). Age significantly affected seroprevalence when all samples were included in the analysis ($G = 15.06$, df = 3, $P =$

Table 4. Distribution of single and multiple arboviral infections in blue jays and Florida scrub-jays

Infection status	No. of seropositive samples		Antibody totals
	Blue jays ($n = 379$)	Florida scrub-jays ($n = 213$)	
EEE only	92	39	131
HJ only	34	24	58
SLE only	7	3	10
EEE+HJ	24	7	31
EEE+SLE	2	0	2
SLE+HJ	1	0	1
EEE+HJ+SLE	1	1	2
Total samples	161	74	

Data from 1994 and 1995 combined.

Table 5. Antibody prevalence of EEE and HJ from samples of blue jays of different age classes

Age class	EEE		HJ	
	No. antibody positive/no. of samples	(%)	No. antibody positive/no. of samples	(%)
Nestlings	0/23	(0.0)	2/23	(8.7)
HY	4/33	(12.1)	0/33	(0.0)
SY	25/73	(34.2)	11/73	(15.1)
ASY	89/231	(38.5)	46/231	(19.9)
All birds[a]	119/379	(31.4)	60/379	(15.8)

Data combined for sexes, years, and seasons of capture. Proportions presented in this table represent infection rates calculated across all data, but they are not congruent with certain analyses reported in the text which use subsets of these data. See text for statistical analyses and definition of age codes.

[a] Includes 19 adult birds of unknown age.

Table 6. Antibody prevalence of EEE and HJ in Florida scrub-jays of different age classes

Age class	EEE		HJ	
	No. antibody positive/ no. of samples (%)		No. antibody positive/ no. of samples (%)	
Nestlings	0/2	(0.0)	0/2	(0.0)
HY	24/126	(19.0)	21/122	(17.2)
SY	8/37	(21.6)	7/37	(18.9)
ASY	10/38	(26.3)	4/37	(10.8)
All birds	47/213[a]	(22.1)	32/207[b]	(15.5)

Data combined for sexes, years, and seasons of capture. Proportions presented in this table represent infection rates calculated across all data, but they are not congruent with certain analyses reported in the text which use subsets of these data. See text for statistical analyses and definition of age codes.

[a] Includes 10 adult birds of unknown age.

[b] Includes 9 adult birds of unknown age.

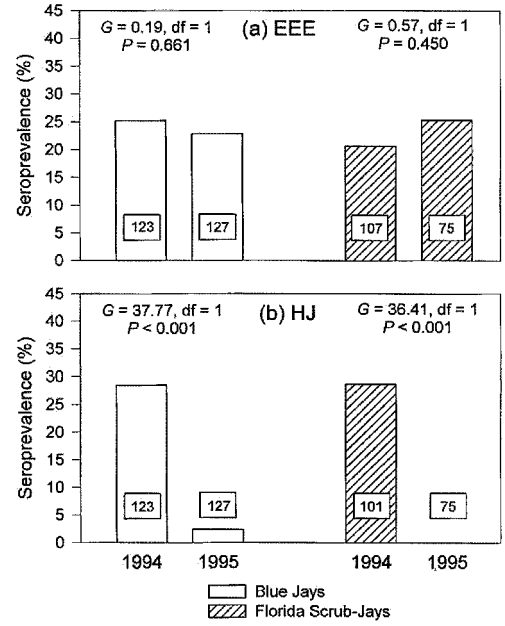

Fig. 1. Effect of sample year on the prevalence of (a) EEE and (b) HJ antibody in blue jay and Florida scrub-jay blood samples (sexes and ages of each species combined). For blue jays, the analyses are restricted to data from April through July of each year because sampling intensity was consistent during those months for both years (see Table 1). For the same reason, the analyses are restricted to the months of May through December for scrub-jays. Sample sizes are in boxes.

0.002). As was the case for EEE, this pattern persisted when the analysis was restricted to seasons when all age classes were present. Two of 54 (3.6%) samples from young-of-the-year were antibody positive, whereas 36 of 242 (14.9%) sera collected from adults were positive ($G = 6.65$, df = 1, $P = 0.010$). Again, the difference did not exist between sera from SY (11 of 73 [15.1%]) and ASY birds (46 of 231 [19.9%]) ($G = 0.89$, df = 1, $P = 0.346$).

In scrub-jays, the prevalence of EEE and HJ antibodies did not differ with age when all data were considered together (EEE, $G = 1.85$, df = 3, $P = 0.605$; HJ, $G = 1.87$, df = 1, $P = 0.600$; Table 6). However, we sampled only two scrub-jay nestlings for antibodies to EEE and HJ, so we repeated the analysis with the nestling age class omitted. Even when data were restricted to samples collected during April through December (when all age classes were present), we detected no significant variation among the three remaining age classes in EEE ($G = 0.50$, df = 2, $P = 0.778$) or HJ ($G = 2.41$, df = 2, $P = 0.299$) antibody prevalence. Similarly, young-of-the-year did not differ significantly from adults in their prevalence to antibodies from either virus (data from April through December: EEE, $G = 1.33$, df = 1, $P = 0.188$; HJ, $G = 0.58$, df = 1, $P = 0.447$). Likewise, we detected no significant difference in prevalence of antibodies to either virus between samples from SY and ASY scrub-jays (data from all seasons combined: EEE, $G = 0.23$, df = 1, $P = 0.634$; HJ, $G = 0.97$, df = 1, $P = 0.324$).

Temporal Distribution of Antibody Presence. Significantly more blue jay sera were positive for EEE and HJ antibodies in 1994 than in 1995 when data from all months were included in the analysis (EEE, $G = 5.06$, df = 1, $P = 0.025$; HJ, $G = 17.78$, df = 1, $P < 0.001$). However, because birds were not sampled before April 1994, and because captures were infrequent and unevenly distributed during autumn months, we reanalyzed the data including only samples collected from April through July, a period during which sampling intensity was similar for both years. This analysis yielded no difference in EEE antibody prevalence between the 2 yr (Fig. 1a). However, significantly more blue jay samples were seropositive to HJ in 1994 than in 1995 (Fig. 1b).

The proportion of EEE-positive samples from Florida scrub-jays did not differ between 1994 (22 of 107; 20.6%) and 1995 (25 of 106; 23.6%) when data from all months were included ($G = 0.28$, df = 1, $P = 0.595$). This pattern remained constant when the analysis was restricted to data collected from May through December when sampling intensity was similar for the 2 yr (Fig. 1a). HJ-positive sera were significantly more common in 1994 than in 1995, regardless of whether samples from all months were included (1994: 29 of 101 [28.7%] were antibody positive; 1995: 3 of 106 [2.8%] were antibody positive; $G = 29.85$, df = 1, $P < 0.001$), or whether the analysis was restricted to samples collected from May through December (Fig. 1b).

The season of capture seemed to affect the presence of both EEE and HJ antibodies in samples collected from adult blue jays (Table 7). Unfortunately, we have data from January through March for only one of the 2 yr (1995), and therefore cannot properly control for annual patterns in seasonal variation. This may not be a problem for the analysis of EEE, because antibody prevalence did not seem to differ between the 2 yr of the study. Prevalence to EEE antibodies was highest in adult blue jay sera during the prenesting season (February and March), and lowest during the winter season (November to January; nestlings and HY birds

Table 7. Effect of season of capture on antibody prevalence of EEE and HJ in adult blue jays

Season of capture	Major avian activity	Inclusive months	EEE		HJ	
			No. antibody positive/ no. of samples (%)		No. antibody positive/ no. of samples (%)	
Prenesting	Courtship	Feb.–Mar.	54/80	(67.5)	22/80	(27.5)
Nesting	Nesting	April–May	19/81	(23.5)	22/81	(27.2)
Postnesting	Dependent young	June–July	39/126	(31.0)	14/126	(11.1)
Autumn	Acorn harvest	Aug.–Oct.	3/11	(27.3)	0/11	(0.0)
Winter	Maintenance	Nov.–Jan.	0/25	(0.0)	0/25	(0.0)

Nestlings and HY jays were excluded, and sexes and years were combined for this table. See text for explanation of season categories and statistical analysis.

omitted from the analysis because they do not occur in all seasons; $G = 62.66$, df = 4, $P < 0.001$; Table 7). The results were qualitatively the same when we performed the analysis for each year separately, although the prenesting season was omitted in the 1994 analysis. Prevalence to HJ antibodies was lowest during autumn and winter seasons ($G = 27.34$, df = 4, $P < 0.001$; Table 7) when the 2 yr were combined. Because HJ antibody prevalence was much higher in 1994 than in 1995, the latter analysis essentially reflects conditions occurring from April 1994 through March 1995.

Small sample sizes precluded statistical analysis of the effect of season on prevalence of EEE and HJ antibodies in samples from SY and ASY scrub-jays. Table 8 presents general patterns of antibody prevalence in sera from scrub-jays across seasons with the 2 yr combined.

Survival Analysis. Forty-three of 62 (69.47%) blue jays negative to EEE in 1994 were resighted in 1995, significantly >10 of 28 (35.7%) EEE-positive blue jays resighted in 1995 ($G = 9.00$, df = 1, $P = 0.003$), indicating a difference in survival rates for these two groups. This pattern held when blue jays also seropositive to either HJ or SLE were culled from the analysis (70.0% of seronegative birds were resighted versus 31.3% of EEE-seropositive birds; $G = 7.09$, df = 1, $P = 0.008$).

Seroprevalence of HJ did not seem to affect adult blue jay survival. Thirty-four of 57 (59.6%) blue jays that were seronegative, and 19 of 33 (57.6%) that were seropositive, in 1994 were resighted in 1995 ($G = 0.04$, df = 1, $P = 0.847$). The pattern remained the same when the comparison was made between birds seropositive only to HJ and those without antibodies to any arbovirus (70.0% of seronegative birds were resighted versus 66.7% of seropositive birds; $G = 0.07$, df = 1, $P = 0.790$).

Neither EEE or HJ infection seemed to affect the survival rate of HY or breeding adult scrub-jays (Table 9). Likewise, scrub-jay broods that had at least one seropositive individual at the time of nutritional independence were no more likely to have lost siblings before sampling than were broods in which none of the surviving individuals was seropositive (Table 10).

Virus isolation attempts were made on 457 blood samples (261 from blue jays and 196 from scrub-jays). A single EEE isolation was made from the blood of an 11 d-old scrub-jay nestling sampled on 26 May 1995. This nestling did not survive to fledging and both nestmates had antibodies to EEE when captured in July 1995. One of these disappeared in August 1995 and the other was alive through the end of the study in December 1995. Fifteen scrub-jay nestlings for which viral isolations were negative between 23 April and 3 June 1995 had antibodies to EEE when sampled on either 20 or 21 July 1995. An additional scrub-jay nestling was negative for SLE via virus isolation on 7 May 1995 and had NT antibodies to SLE on 21 July 1995.

Discussion

Survival of blue jays, but not that of Florida scrub-jays, was significantly reduced by EEE. Although we indexed blue jay survival based on resight rates rather than by confirmed death, we have no reason to believe our survival estimates were confounded by infection-induced dispersal or behavioral inconspicuousness. Even if infection reduced the competitive ability of individual jays instead of killing them, the ultimate result likely would be a substantial reduction in fitness if not eventual death. Moreover, the higher antibody prevalence in adult versus HY blue jays may reflect higher mortality in young birds if EEE is epizootic in

Table 8. Effect of season of capture on antibody prevalence of EEE and HJ in adult Florida scrub-jays

Season of capture	Major avian activity	Inclusive months	EEE		HJ	
			No. antibody positive/ no. of samples (%)		No. antibody positive/ no. of samples (%)	
Prenesting	Courtship	Feb.–Mar.	2/6	(33.3)	1/6	(16.7)
Nesting	Nesting	April–May	5/28	(17.9)	3/28	(10.7)
Postnesting	Dependent young	June–July	9/32	(28.1)	7/32	(21.9)
Autumn	Acorn harvest	Aug.–Oct.	0/5	(0.0)	0/5	(0.0)
Winter	Maintenance	Nov.–Jan.	7/14	(50.0)	0/12	(0.0)

Nestlings and HY jays were excluded, and sexes and years were combined for this table. See text for explanation of season categories and statistical analysis.

Table 9. Postsampling survival of antibody negative and antibody positive Florida scrub-jays

Social and sex status	Virus	Survival (no. days survived/no. days of observation period × 100)						*P*
		Antibody negative			Antibody positive			
		n	Mean % of days	S.E.	*n*	Mean % of days	S.E.	
HY males	EEE	15	75.4	7.17	6	62.3	11.28	0.305
	HJ	14	67.9	7.51	6	75.7	11.50	0.496
HY females	EEE	18	40.3	8.37	3	43.6	28.53	0.821
	HJ	19	36.5	8.02	1	100.0		
Breeding adults (sexes combined)	EEE	19	72.0	7.79	6	68.9	14.50	0.825
	HJ	23	70.0	7.21	1	69.9		

t-tests were performed on arc-sine square-root transformed percentages of the observed time during which the birds survived. Analyses presented in this table are for 1994 and 1995 combined. Analyses based on each year alone yielded qualitatively identical results and are available from K.A.T. (unpublished data).

the blue jay population. Alternatively, because infections in HY birds must be recent it is possible that antibodies were not yet detectable in the young birds at the time of sampling. As a third alternative, we might expect similar age-related patterns of infection if EEE is enzootic, yet infection-induced mortality is low in our study population. Mortality induced by EEE has not been observed in free-ranging native birds, although it has been assumed when avian die-offs have occurred during periods of high EEE activity (Emord and Morris 1984, McLean et al. 1985). Mortality resulting from EEE infections in native species maintained in captivity was reported in whooping cranes in 1984 (Dein et al. 1986), and mortality resulting from naturally occurring EEE infections in exotic species of birds, including the chukar, *Alectoris graeca* (Moulthrop and Gordy 1960); house sparrow, *Passer domesticus* (Byrne et al. 1961); and ring-necked pheasant (Sussman et al. 1958) has been documented. In addition, mortality also has been documented in experimentally infected European starlings, *Sturnus vulgaris* (Komar et al. 1999). Thus, ours is the first documentation of reduced survivorship associated with EEE in a native, free-ranging bird population. Although mounting an immune response may confer immunity to subsequent EEE infections, current and future antibody production may give rise to trade-offs that reduce fitness (Bonneaud et al. 2003).

We detected no effect of EEE virus on survival of either adult or HY Florida scrub-jays. One possible confounding problem is the fact that some antibodies may be maternally inherited (Kissling et al. 1954). If this were the case in the scrub-jays, the presence of antibodies in nestlings or HY birds might not reflect a recent infection. However, our isolation of virus from an 11-d-old nestling indicates that at least some scrub jays were infected in the nest. Moreover, because maternally inherited antibodies only persist for 3–4 wk (Kissling et al. 1954), we doubt that many or any of the antibodies that we detected in the young-of-the-year reflect anything but recent transmission.

We detected no effect of HJ on the survival of either jay species. This result was expected, because this virus has not been associated with mortality in captive or free-ranging species. The infection rate of SLE was too low to allow analysis of its effects on jay survival in our study.

The similar frequency of antibodies to each virus in the two jay species indicates that they either encounter infected vectors at similar frequency or have similar susceptibility to each virus. Unfortunately, because of seasonal variation in sampling between the two species, we were unable to statistically assess the relative difference in antibody prevalence between the two jay species. Although overall antibody prevalence seemed similar for samples collected from both jay species, it is possible that our estimate of prevalence of EEE antibodies in blue jays was low because EEE infections apparently reduced survival in blue jays. If so, the apparent similarity of prevalence in the two species probably is an artifact, and true infection rates are likely to be higher in blue jays. Between-species differences in infection rates could result from variation in exposure to infected vectors caused by environmental or behavioral differences that likely put blue jays in proximity to mosquito vectors. Indeed,

Table 10. Relationship between brood survival and infection status at nutritional independence in Florida scrub-jays

Virus	No. of broods (%)				*G*	df	*P*
	At least one surviving member was antibody positive		No surviving members were antibody positive				
	At least one member died before sampling	No members died before sampling	At least one member died before sampling	No members died before sampling			
EEE	7 (38.9%)	11 (61.1%)	18 (52.9%)	16 (47.1%)	0.937	1	0.333
HJ	4 (36.4%)	7 (63.6%)	20 (50.0%)	20 (50.0%)	0.652	1	0.419

Birds in this table belonged to broods that were observed from hatching through nutritional independence, but they were not sampled for antibodies until nutritional independence. The table compares the proportion of broods that survived intact (i.e., no brood members died before nutritional independence) with the proportion of broods in which at least one member died before nutritional independence in relation to infection status of surviving brood members.

blue jays are more likely to be exposed to *Culiseta melanura* (Coquillett), the enzootic mosquito vector (Bigler et al. 1976, Scott and Weaver 1989), and therefore are more likely to be infected. Florida scrub-jays prefer open and xeric scrub habitat, whereas blue jay habitat preferences include bayhead and red maple swamp where moist and shaded conditions provide better breeding and resting sites for *Cs. melanura.* In addition to, or in combination with, these general habitat differences, microhabitat differences in nesting sites also could account for differences in infection rates. The tendency for blue jays to nest and roost in tree canopies versus that of scrub-jays, which nest and roost in shrubs (Woolfenden and Fitzpatrick 1996, Tarvin and Woolfenden 1999), may put blue jays in proximity to feeding mosquito vectors (Scott and Edman 1991, Crans et al. 1994). Given these differences in nesting sites between the two jay species, we might expect to see differences in nestling infection rates. Immunonaive nestlings likely are exposed to greater mosquito biting pressure than adults because they have large areas of unfeathered skin, and they typically display a lack of defensive behavior toward host-seeking and biting mosquitoes (Blackmore and Dow 1958). Vertical stratification of nest height has been shown to influence probability of infection by arthropod-borne protozoans in birds (Bennett and Fallis 1960, Garvin and Remsen 1997) and similarly may influence prevalence of arboviral infections.

The difference in the effect of EEE on survival in the two jay species is notable given both the close phylogenetic relationship of these species (Espinosa de los Monteros and Cracraft 1997) and their spatial proximity at our study site. Because both species clearly become infected, variation in survivorship may be a result of susceptibility to fatal infection, but we can offer no compelling explanation why susceptibility should be higher in one species than the other.

The results of our study cannot account for the periodic massive die-offs of Florida scrub-jays observed by Woolfenden and Fitzpatrick (1984, 1991), because we did not detect a negative effect of viral infection on scrub-jay survival. However, those die-offs occurred after periods of particularly heavy rainfall when mosquito populations, and therefore transmission rates, may have been especially high. We cannot rule out the possibility that during periods of extremely high transmission, jays may receive multiple inoculations from infected mosquitoes. Furthermore, other factors, such as coinfection with other pathogens or stress may also influence survival. Florida scrub-jays may be more likely to succumb to the effects of infection under such conditions. Perhaps those blue jays that did not survive in our study had received multiple infective bites because they occupied habitat with larger mosquito populations than that of Florida scrub-jays.

Clearly, not all blue jays that were infected with EEE died in our study. Multiple captures of several individuals revealed that antibody positive birds may survive long periods and may be antibody negative upon recapture. Moreover, the lack of EEE antibodies in blue jay samples collected in winter suggests that antibodies had decayed by this time (McLean et al. 1983, Reisen et al. 2003) and that transmission had not occurred recently. Between 27 and 31% of blue jay samples were antibody positive between June and October. If a similar proportion of the birds sampled in winter had been infected during the previous fall or summer, we would expect to have seen approximately six to eight birds with antibodies in the winter unless antibodies had decayed by this time and transmission was no longer occurring. Unfortunately, we caught no birds during the winter that were antibody positive during an earlier sampling, and therefore we have no direct evidence of antibody decay from the fall to winter. Although we have multiple samples from many birds, we were unable to estimate the duration of antibodies with great resolution. Our poor understanding of factors affecting antibody persistence in wild birds (Kuno 2001), as well as the possibility of recrudescence (Crans et al. 1994) and reinfection, limits the inferences we can draw about the impact of arboviruses on avian population dynamics. Nonetheless, our study provides strong evidence that EEE influences survival of free-ranging blue jays.

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References Cited

Abrahamson, W. G., A. F. Johnson, J. N. Layne, and P. A. Peroni. 1984. Vegetation of the Archbold Biological Station, Florida: an example of the southern Lake Wales Ridge. Fla. Sci. 47: 209–250.

Bancroft, G. T., and G. E. Woolfenden. 1982. The molt of scrub jays and blue jays in Florida. Ornithol. Monogr. 29: 1–51.

Beaty, B. J., C. H. Calisher, and R. E. Shope. 1989. Arboviruses, pp. 797–855. *In* N. J. Schmidt and R. W. Emmons [eds.], Diagnostic procedures for viral, rickettsial and chlamydial infections. American Public Health Association, Washington, DC.

Bennett, G. F., and A. M. Fallis. 1960. Blood parasites of birds in Algonquin Park, Canada and a discussion of their transmission. Can. J. Zool. 38: 262–273.

Bigler, W. J., E. Lassing, E. Buff, A. L. Lewis, and G. L. Hoff. 1975. Arbovirus surveillance in Florida: wild vertebrate studies, 1965–1974. J. Wildl. Dis. 11: 348–356.

Bigler, W. J., E. Lassing, E. Buff, E. C. Prather, E. C. Beck, and G. L. Hoff. 1976. Endemic eastern equine encephalomyelitis in Florida: a twenty-year analysis, 1955–1974. Am. J. Trop. Med. Hyg. 25: 884–890.

Blackmore, J. S., and R. P. Dow. 1958. Differential feeding of *Culex tarsalis* on nestling and adult birds. Mosq. News. 18: 15–17.

Bonneaud, C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the cost of mounting an immune response. Am. Nat. 161: 367–379.

Byrne, R. J., F. M. Hetrick, J. E. Scanlon, J. W. Hastings, and L. N. Locke. 1961. Observations on eastern equine encephalitis in Maryland in 1959. J. Am. Vet. Med. Assoc. 139: 661–664.

Crans, W. J., D. F. Caccamise, and J. R. McNelly. 1994. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. J. Med. Entomol. 31: 711–728.

Dater, E. 1970. Dorsal wing coverts of blue jay (*Cyanocitta cristata*) guide to age. Eastern Bird Banding Assoc. News 33: 129.

Day, J. F., and L. M. Stark. 1996a. Eastern equine encephalitis transmission to emus (*Dromaius novaehollandiae*) in Volusia County, Florida: 1992 through 1994. J. Am. Mosq. Control Assoc. 12: 429–436.

Day, J. F., and L. M. Stark. 1996b. Transmission patterns of St. Louis encephalitis and eastern equine encephalitis viruses in Florida: 1978–1993. J. Med. Entomol. 33: 132–139.

Dein, F. J., J. W. Carpenter, G. G. Clark, R. J. Montali, C. L. Crabbs, T. F. Tsai, and D. E. Docherty. 1986. Mortality of captive whooping cranes caused by eastern equine encephalitis virus. J. Am. Vet. Med. Assoc. 189: 1006–1010.

Emord, D. E., and C. D. Morris. 1984. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, USA. VI. Antibody prevalence in wild birds during an interepizootic period. J. Med. Entomol. 4: 395–404.

Espinosa de los Monteros, A., and J. Cracraft. 1997. Intergeneric relationships of the New World jays inferred from cytochrome *b* gene sequences. Condor 99: 490–502.

Favorite, F. G. 1960. Some evidence of local origin of EEE virus in Florida. Mosq. News. 20: 87–92.

Garvin, M. C., and E. C. Greiner. 2003. Epizootiology of *Haemoproteus danilewskyi* Kruse (Haemosporina: Haemoproteidae) in blue jays (*Cyanocitta cristata*) in south-central Florida. J. Wildl. Dis. 39: 1–9.

Garvin, M. C., and J. V. Remsen, Jr. 1997. An alternative hypothesis for heavier parasite loads of brightly colored birds: exposure at the nest. Auk 114: 179–191.

Gibbs, E.P.J., and T. F. Tsai. 1994. Eastern encephalitis, pp. 11–24. *In* G. W. Beran and J. H. Steele [eds.], Handbook of zoonoses. CRC, Boca Raton, FL.

Gustafson, M. E., J. Hildenbrand, and L. Metras. 1997. The North American bird banding manual (electronic version), version 1.0 USGS.

Horsfall, W. R. 1976. Eastern equine encephalomyelitis: summary of literature before 1976. Leafl. No. 5. Department of Entomology, University of Illinois, Urbana, IL.

Kissling, R. E., R. Chamberlain, R. K. Sikes, and M. E. Eidson. 1954. Studies on the North American arthropod-borne encephalitids. III. Eastern equine encephalitis in wild birds. Am. J. Hyg. 60: 251–265.

Komar, N., D. J. Dohm, M. J. Turell, and A. Spielman. 1999. Eastern equine encephalitis virus in birds: relative competence of European starlings (*Sturnus vulgaris*). Am. J. Trop. Med. Hyg. 60: 387–391.

Kuno, G. 2001. Persistence of arboviruses and antiviral antibodies in vertebrate hosts: its occurrence and impacts. Rev. Med. Virol. 11: 165–190.

McLean, R. G., J. Mullenix, J. Kerschner, and J. Hamm. 1983. The house sparrow (*Passer domesticus*) as a sentinel for St. Louis encephalitis virus. Am. J. Trop. Med. Hyg. 32: 1120–1129.

McLean, R. G., G. Frier, G. L. Parham, D. B. Francy, T. P. Monath, E. G. Campos, A. Therrien, J. Kerschner, and C. H. Calisher. 1985. Investigations of the vertebrate hosts of eastern equine encephalitis during an epizootic in Michigan, 1980. Am. J. Trop. Med. Hyg. 34: 1190–1202.

Moulthrop, I. M., and B. A. Gordy. 1960. Eastern equine encephalomyelitis in chukar (*Alectoris graeca*). Avian Dis. 4: 380–383.

Olson, J. G., T. W. Scott, L. H. Lorens, and J. L. Hubbard. 1991. Enzyme immunoassay for detection of antibodies against eastern equine encephalomyelitis virus in chickens. J. Clin. Microbiol. 29: 1457–1461.

Reed, L. J., and H. A. Muench. 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27: 493–497.

Reisen, W. K., R. E. Chiles, E. N. Green, Y. Fang, and F. Mahmood. 2003. Previous infection protects house finches from re-infection with St. Louis encephalitis virus. J. Med. Entomol. 40: 300–305.

Risser, A. C., Jr. 1971. A technique for performing laparotomy on small birds. Condor 73: 376–379.

Scott, T. W., and J. D. Edman. 1991. Effect of host age and arbovirus infection on mosquito attraction and blood-feeding success, pp. 179–204. *In* J. E. Loye and M. Zuk [eds.], Bird–parasite interactions. Oxford University Press, Oxford, England.

Scott, T. W., and S. C. Weaver. 1989. Eastern equine encephalomyelitis virus: epidemiology and evolution of mosquito transmission. Adv. Virus Res. 37: 277–328.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd ed. W. H. Freeman and Company, New York

Sussman, O., D. Cohen, J. E. Gerende, and R. E. Kissling. 1958. Equine encephalitis vaccine studies in pheasants epizootic and pre-epizootic conditions. Ann. N.Y. Acad. Sci. 70: 328–341.

Tarvin, K. A. 1998. The influence of habitat variation on demography of blue jays (*Cyanocitta cristata*) in south-central Florida. Ph.D. dissertation, University of South Florida, Tampa.

Tarvin, K. A., and M. C. Garvin. 2002. Habitat and nesting success of blue jays: importance of scale. Auk 119: 971–983.

Tarvin, K. A., and G. E. Woolfenden. 1999. Blue jay (*Cyanocitta cristata*). *In* A. Poole and F. Gill [eds.], The birds of North America. The Birds of North America, Inc., Philadelphia, PA.

Woolfenden, G. E., and J. W. Fitzpatrick. 1984. The Florida scrub jay: demography of a cooperative-breeding bird. Princeton University Press, Princeton, NJ.

Woolfenden, G. E., and J. W. Fitzpatrick. 1990. Florida scrub jays: a synopsis after 18 years of study, pp. 238–266. *In* P. B. Stacey and W. D. Koenig [eds.], Cooperative breeding in birds: long-term studies of ecology and behavior. Cambridge University Press, Cambridge, MA.

Woolfenden, G. E., and J. W. Fitzpatrick. 1991. Florida Scrub Jay ecology and conservation, pp. 542–565. *In* C. M. Perrins, J. D. Lebreton, and G.J.M. Hirons [eds.], Bird population studies: relevance to conservation and management. Oxford University Press, Oxford, England.

Woolfenden, G. E., and J. W. Fitzpatrick. 1996. Florida scrub-jay (*Aphelocoma coerulescens*). *In* A. Poole and F. Gill [eds.], The birds of North America. The Birds of North America, Inc., Philadelphia, PA.

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