Eastern Equine Encephalomyelitis Virus in Relation to the Avian Community of a Coastal Cedar Swamp

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ABSTRACT Eastern equine encephalomyelitis virus (EEEV) is perpetuated in eastern North America in a mosquito–wild bird maintenance cycle that involves *Culiseta melanura* (Coquillett) as the principal enzootic vector and passerine birds as the primary amplifying hosts. We examined the role of birds in the EEEV cycle at a site in southern New Jersey where EEEV cycles annually at high levels. Birds and mosquitoes were sampled during three epiornitics and one season of limited virus activity. We examined antibody prevalence in birds in relation to eight physical and natural history characteristics. Our goal was to compare EEEV cycling in *C. melanura* and the primary avian hosts better to understand the mechanisms that initiate annual epiornitics. Antibody prevalence was highest in the Blue Jay (62%), Wood Thrush (60%), and Tufted Titmouse (44%). Resident status of birds was the natural history characteristic most closely linked to participation in the EEEV cycle. Species spending the greatest amount of time at our study site (permanent residents, summer residents) had the highest antibody rates. We captured viremic birds as early as 25 May, 51 d before we first detected virus in *C. melanura*. We recaptured 10 after hatching year adults and one hatching year (HY) bird that seroconverted before we detected virus in *C. melanura*. We also found EEEV antibody in 15 HY birds up to 31 d before we isolated EEEV from *C. melanura*. We provide evidence that a cryptic cycle develops weeks before epiornitic cycling is detected in *C. melanura* by traditional laboratory techniques, indicating that the early season cycle is initiated by the recrudescence of latent virus in previously infected birds.

KEY WORDS *Culiseta melanura*, eastern equine encephalomyelitis, bird

EASTERN EQUINE ENCEPHALOMYELITIS VIRUS (EEEV) is perpetuated in eastern North America in a maintenance cycle that involves *Culiseta melanura* (Coquillett) as the principal enzootic vector and passerine birds as the primary amplifying hosts (Morris 1988, Scott & Weaver 1989). In some years, EEEV reaches epizootic proportions and produces clinical disease in horses, humans, and exotic birds such as pheasants and chukar partridges. *C. melanura* is an avian feeder (Crans 1964, Edman et al. 1972) and is probably not responsible for the transmission of EEEV to either humans or horses. The mosquito vectors that are responsible for the epizootic phase of the cycle vary among geographic areas, and the epidemiology of the nonavian transmission cycles is unclear.

Two rather distinct epidemiological cycles for EEEV have been investigated in northeastern portions of North America. An inland cycle occurs sporadically in upland Red Maple swamps (*Acer rubrum* L.) and the surrounding wet woodlands (Grady et al. 1978, Morris et al. 1980), and also in swamp habitats in Michigan (McLean et al. 1985). A coastal cycle occurs annually from New Jersey to Maryland where Atlantic White Cedar swamps (*Chamaecyparis thyoides* [L.]) drain into salt-marsh habitat (Chamberlain 1958, Altman et al. 1967). The inland cycle involves *C. melanura* as the epiornitic vector and *Aedes vexans* (Meigen) (Hayes et al. 1962), *Aedes canadensis* (Theobald) (F. Krenick, personal communication), or *Coquillettidia perturbans* (Walker) (Crans & Schulze 1986) as the principal vectors to mammalian hosts. The coastal cycle involves *C. melanura* as the epiornitic vector and *Aedes sollicitans* (Walker) as the primary epizootic and epidemic vector (Crans 1977, Crans et al. 1986).

Stamm (1963) listed 51 species of birds that were either naturally or experimentally susceptible to EEEV. Since that time, numerous bird species have been added to that list, and data clearly show that EEEV cycles in wild birds and *C. melanura* over a wide geographic area in the eastern United States under a wide variety of epidemiological circumstances.

Emord & Morris (1984) examined the relationships between antibody levels in wild birds and virus isolations from *C. melanura* in upstate New York during a 3-yr interepizootic period. Results showed that antibody levels to EEEV quickly

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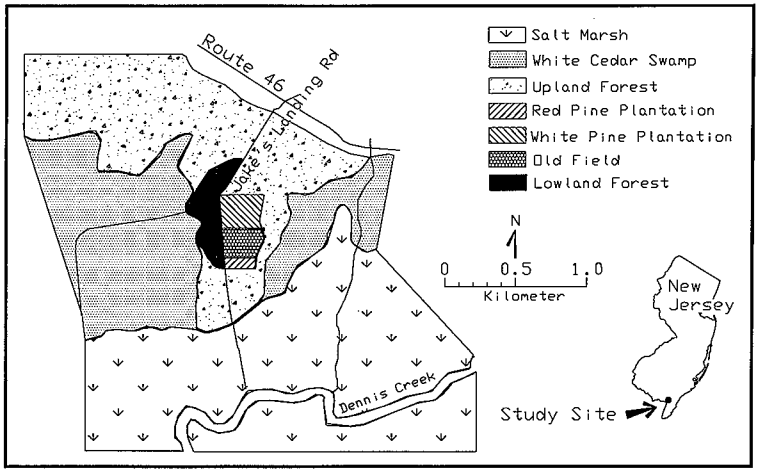

Fig. 1. Map of the study site showing major habitat types.

disappeared from the passerine population in the absence of active transmission and that the arrival of migrating birds in the spring failed to reintroduce virus at levels that reinitiated epiornitic transmission.

In this study, we examined EEEV activity in the avian community and *C. melanura* in a coastal area of southern New Jersey where virus circulates in wild birds and mosquitoes nearly every year. In this paper, we show that avian participation in the EEEV cycle is related directly to the amount of time birds spend at our study site, and that EEEV appears in birds before it is evident in *C. melanura*. We also provide evidence for a cryptic EEEV cycle that develops in early spring through a process of recrudescence of latent virus in previously infected birds.

Materials and Methods

Study Site. The study was conducted in a portion of Belleplain State Forest 2.8 km southwest of Dennisville, Cape May County, NJ (Fig. 1). The study site consisted of a 1.3-km peninsula of upland oak-pine forest that extended directly into a salt marsh dominated by *Spartina alterniflora* Loisel. A large Atlantic White Cedar swamp bordered both sides of the peninsula and drained directly onto the salt marsh. The acid waters of the cedar swamp provided extensive breeding habitat for *C. melanura*. Dennis Creek, a sizable tidal creek, passed within 0.5 km of the upland habitat and provided drainage across 4.0 km of marshland to Delaware Bay. Two large salt-hay farms, 1.2 km to the south and 2.5 km to the northwest, provided extensive *Spartina patens* (Alt.) Muho habitat where large populations of *A. sollicitans* occurred.

The gradation of the peninsula from upland to salt marsh provided five distinct habitats within the study site: (1) an upland forest consisting primarily of White Oak (*Quercus alba* L.), Black Oak (*Quercus velutina* Lam.), and Pitch Pine (*Pinus rigida* Mill.); (2) a lowland deciduous forest composed of Red Maple, Sweetgum (*Liquidambar styraciflua* L.), Sourgum (*Nyssa sylvatica* Marsh.), Sassafras (*Sassafras albidum* [Nutt.]), Persimmon (*Diospyros virginiana* L.), and American Holly (*Ilex opaca* Ait.); (3) a mature White Pine (*Pinus strobus* L.) plantation; (4) a dying Red Pine (*Pinus resinosa* Ait.) plantation; and (5) a 1-ha old field.

Mosquito Collections. We have collected and tested mosquitoes from our study site in Belleplain State Forest annually since 1975. For this study, we analyzed results of virus isolations from 1979 to 1984, bracketing our sample interval for birds (1980–1983) by 1 yr. We sampled a minimum of 25 resting boxes (Burbutis & Jobbins 1958) placed within White Pine plantations at the study site once weekly from 1 June to 15 October. All specimens were frozen on dry ice at the collection site and transported to the laboratory, where they were stored at −70°C until they were sorted to species on a chill table and pooled into lots of ≤50 females.

Avian Capture and Bleeding Methods. We captured birds with 9–12 20-m Japanese mist nets within the various habitats listed above. We operated nets from dawn to 1500 hours 3 d each week from 18 June to 5 November in 1980, 16 April–20 October 1981, 11 May–20 October 1982, and 11 May–23 August 1983. In 1983 we terminated bird collections in August because an EEEV outbreak in another part of the state required our attention (Crans & Schulze 1986). Birds were identified to species, and age and sex were determined whenever possible (U.S. Fish and Wildlife Service and Canadian Wildlife Service 1977, Wood & Beimborn 1981). From 1981 to 1983, birds were fitted with Fish and Wildlife Service bird bands to permit identification of recaptures. Our age classifications were as follows: after hatching year (AHY), a bird hatched in any year before its year of capture; hatching year (HY), a bird hatched in the year of its capture; local (L), a HY bird incapable of sustained flight, thus known to have hatched at our study site.

Birds were bled from the jugular vein (Kerlin 1964) using a 1-cm^3 tuberculin syringe and a 26-g needle. Blood sample size ranged from 0.05 to 0.7 cm^3 depending on the size of the bird, and samples were diluted with a bird blood diluent (Sudia et al. 1972). After bleeding, the birds were returned to their holding cages for ≈15 min and then released.

Avian Breeding and Wintering Ranges. We grouped birds into categories representing breeding and wintering ranges. The breeding range categories grouped species according to likely natal site. Similarly, the wintering range categories grouped species according to the geographic area where they likely spend the winter (Pough 1949, Peterson 1980). Neither of the groupings necessarily represents species characteristics, but rather describes likely ranges for the individual birds captured at our study site. For breeding range we identified seven categories (Fig. 2). The New Jersey-only category (NJ-only) includes species that are permanent residents at our study site. New Jersey to northeastern United States (NJ-to-NE) includes species with breeding ranges extending northward from our study area to the Canadian border. Northeastern United States (NE-US) includes species that nest in New England. New Jersey to southeastern United States (NJ-to-SE) represents migratory species having southern New Jersey as the northern limit of their breeding range. Northeastern United States to Canada (NE-to-Can) includes species with breeding ranges extending from New England into Canada. Canada only (Can-only) includes species with breeding ranges north of the United States border. The final category, New Jersey to Canada (NJ-to-Can), is inclusive.

Blood Processing. Fresh blood samples were held at ambient temperature for 15 min and then refrigerated at 4°C until transported to the laboratory. Samples then were further diluted with bird blood diluent to ≤1:10. Samples then were centrifuged for 15 min at 2,500 rpm in a refrigerated centrifuge (10°C). Extracted serum and the cell samples then were frozen at −70°C until virus isolation and antibody testing could be performed.

Sucrose-acetone extracted mouse-brain antigens (Ten Broeck strain of EEEV) and 10% EEEV infected mouse brain stocks in 20% newborn calf serum were prepared for hemagglutination-inhibition (HI) and plaque reduction neutralization (PRNT) tests. The materials also were used as controls in EEEV isolation and identification procedures. Reagents were prepared before the transfer of field samples to the laboratory. This minimized the potential for contamination and ensured consistency among tests throughout each field season. Ampoules were wet, frozen, and stored at −70°C.

Hemagglutination-Inhibition Tests. Bird sera were tested for HI antibody by initially screening 1:10 and 1:20 dilutions of sera with 4 to 8 hemagglutinating units of EEEV antigens (Clarke & Casals 1958). All HI tests were done in microtiter plates; lipid inhibitors were removed from the sera by acetone extraction. Positive samples were titered in subsequent tests.

Plaque Reduction Neutralization Tests. HI-positive sera together with an equal number of HI-negative sera were tested by PRNT by the methods of Main et al. (1988). These tests were conducted by incubating equal volumes of avian sera diluted at 1:10 and 120–160 plaque-forming units of 10^{th} suckling mouse brain passage of the Ten Broeck strain of EEEV for 1 h at 37°C. The assay was performed in Vero cells in 24-well Co-star wells (Schmidt 1979). Ninety-percent endpoints were selected as criteria for positive samples.

Virus Isolation Attempts. Samples of bird blood were screened for the presence of virus in microtiter plates by inoculating 0.1 ml of a 1:10 dilution of Vero cells with 1:10 dilutions of the cell serum residue left after the majority of the serum had been removed for serologic tests (Schmidt 1979). Positive samples were titered, and the isolates were identified by one-way neutralization tests using the Ten Broeck strain of EEEV.

We tested *C. melanura* in White Leghorn chicks <12 h old by the methods described by Chamberlain et al. (1954b). The brain suspensions from chicks showing symptoms were inoculated into fresh chicks, suckling mice, and duck embryo tissue culture. Virus identification was made by direct fluorescent antibody from the tissue culture preparations.

Data Reduction. Data were analyzed using SAS for personal computers (SAS Institute 1987). Seasonal patterns of antibody prevalence were evaluated by blocking samples into 2-wk inter-

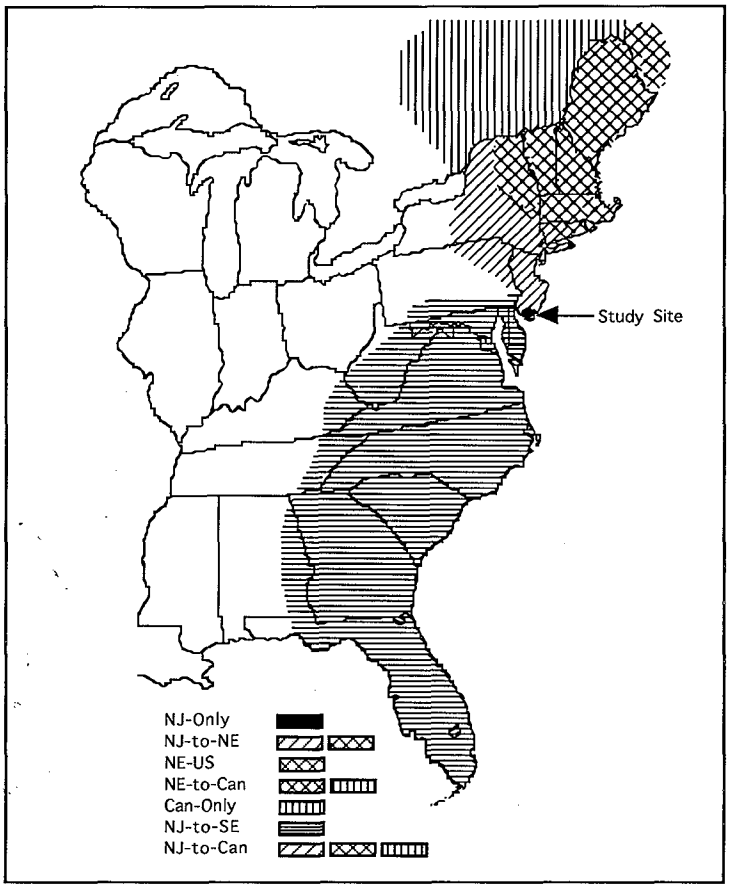

Fig. 2. Map of breeding range categories for birds captured at our study site. Each species was categorized according to its overall breeding range in relation to the geographic location of our study site.

vals and using the mean of each block to represent prevalence in that time interval. Differences in antibody prevalence among avian characteristics were tested using chi-square contingency tests ($P < 0.05$). Expected values were calculated based on the null hypothesis that categories based on avian characteristics all had the same infectivity (Zar 1974). Virus isolations from mosquitoes were expressed as minimum infection rates (MIRs) calculated as isolations per 1,000 specimens tested. MIRs were calculated monthly as well as for the entire season.

Results

Virus Isolations from Mosquitoes. From 1979 to 1984 we collected a total of 48,510 *C. mela-*

nura that we processed in 1,010 pools. From these we obtained 104 isolations of EEEV. All isolations from *C. melanura* and the other species were made from July to October. Over the 6-yr period, we never isolated virus in June despite testing 8,192 *C. melanura* in 174 pools and 3,375 specimens of other early season mosquito species. Dates of first virus isolations from *C. melanura* in the 6-yr period were 15, 17, and 23 July and 1, 2, and 29 August (Fig. 3).

Monthly averages for MIRs varied during periods of active transmission from 0.38 to 5.6. The 3 yr with July onsets included the highest MIRs (3.9–5.6) of our study. However, in 1980 and 1984, when we first detected active transmission in August, we also recorded relatively high MIRs (5.1 and 4.9, respectively). Virus activity peaked in September or October every year except 1983, when only one isolation was made over the entire season. In October 1984 the New Jersey State Department of Health made the October collections and reported two isolations from *C. melanura*, without indicating the number of mosquitoes tested (Fig. 3). As a result, the MIR presented for October 1984 was estimated from average sample sizes for the previous 5 yr.

Virus Isolations from Birds. Overall, 19 of 1,848 (1.03%) birds were viremic at the time of capture. In 1980, the seasonal pattern of EEEV-positive birds paralleled the pattern of virus isolations from *C. melanura* (i.e., viremic birds were not detected until the virus was isolated from mosquitoes [Fig. 4]). In 1981 and 1982, however, six viremic birds were collected 7–51 d before isolations were made from mosquitoes. The early avian viremias were distributed among six different bird species, and all but one were in AHY birds. The Gray Catbird in Fig. 4 was a recapture that had been bled the previous year. On 13 May 1981 this bird had a PRNT titer of 1:20 to EEEV. On 8 June 1982 the bird was viremic.

Antibody Prevalence. From 1980 to 1983 we tested 69 different bird species for antibody to EEEV, of which 68% of the species (47) and 27% of the total individuals (494) were antibody-positive (Table 1). There were general similarities in the seasonal patterns of antibody prevalence in most years (Fig. 5). The percentage of positive birds generally started at relatively high levels in spring, but decreased in either May or June. Antibody levels then increased a second time as the season progressed, peaking again in late summer. The early season pattern in 1981 was somewhat different because fewer positive birds were collected in May, although late-summer patterns were more typical.

We found considerable variation among species in the percentage positive for EEEV antibody (Table 1). Excluding species with small sample sizes (<10), highest percentages of individuals positive for EEEV antibody were in

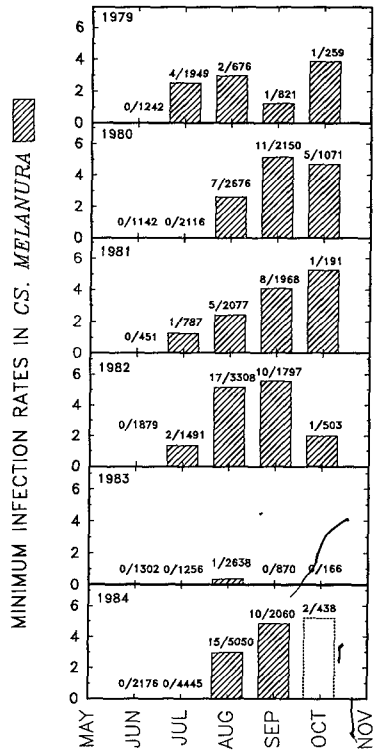

Fig. 3. Histograms representing minimum infection rates (virus isolations per 1,000 mosquitoes tested) by month for the years 1979–1984. Values above each bar represent number of positive pools/total number of mosquitoes tested. The October 1984 histogram is an estimate. Two isolations were obtained, but the number of mosquitoes tested was not known. We estimated the number of mosquitoes tested by averaging the number of mosquitoes collected in October for the preceding 5 yr.

the Blue Jay (61.9%, n = 42), followed by Wood Thrush (59.9%, n = 177), Tufted Titmouse (44.2%, n = 77), and Carolina Chickadee (38.9%, n = 162). Yellow-rumped Warblers were collected most frequently (n = 297), yet only 5.7% were seropositive. Similarly, only 6.8% of 74 samples of Common Grackles were positive.

During May our blood samples came entirely from AHY birds. L and HY birds first appeared at

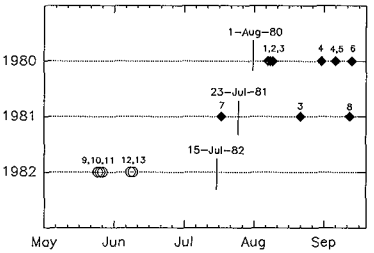

Fig. 4. Dates of capture for birds that were captured with an active viremia. Numbers above points indicate species as follows: 1, Blue Jay; 2, Wood Thrush; 3, Ovenbird; 4, American Robin; 5, American Redstart; 6, Black-and-White Warbler; 7, Tufted Titmouse; 8, Common Yellowthroat; 9, Pine Warbler; 10, Yellow-billed Cuckoo; 11, Carolina Chickadee; 12, Gray Catbird; 13, Red-winged Blackbird. Open symbols are AHY birds; filled symbols are HY birds.

our study site in early June, and by mid-July they composed an average of 40–50% of our samples (Fig. 6). A total of 226 young of the year birds (27.7%) had antibody to EEEV at the time of their capture. The date of capture for 15 of these birds (6.6%) preceded the date of earliest virus detection in mosquitoes (Fig. 7). On 24 June 1980, we captured a fledgling Wood Thrush with antibody to EEEV 31 d before the earliest virus detection in mosquitoes (on 1 August). In 1981 and 1982 we captured 4 L and 10 HY birds with antibody to EEEV 9–30 d before virus detection in mosquitoes (Common Yellowthroat, Wood Thrush, Brown Creeper, American Robin, Hairy Woodpecker, Downy Woodpecker, Gray Catbird, three Carolina Chickadees, four Tufted Titmice). In 1983 virus activity was minimal, with only one virus isolation from mosquitoes, on 29 August. We bled 54 HY birds through 23 August and found none with antibody to EEEV.

In 1981 and 1982 we recaptured 20 and 9 individuals, respectively, that seroconverted at the study site (Fig. 8). A total of 12 of these birds seroconverted before the dates we first detected virus in mosquitoes. In 1983 we recaptured 40 birds, but none showed evidence of seroconversion.

Antibody Prevalence in Relation to Avian Characteristics. We examined patterns of antibody prevalence in relation to eight physical and natural history characteristics of the bird species at our study site. Using χ^2 contingency tests, we failed to detect significant differences between the proportion of individuals positive for EEEV antibody and age ($\chi^2 = 1.2$, df = 1, $P = 0.27$), sex ($\chi^2 = 0.04$, df = 1, $P = 0.84$), and number of broods produced per year ($\chi^2 = 2.65$, df = 1, $P = 0.103$). Relationships were significant for resident status, nest habitat, breeding range, wintering range, and body size as indicated by the band size recommended by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977) (Fig. 9).

We recognized five classes of resident status (Fig. 10A). Permanent and summer residents both had higher than expected percentages positive for EEEV antibody (Fig. 9). They made up 19 and 44%, respectively, of the sample population but accounted for 26 and 56% of the individuals positive for EEEV antibody. Migrants and winter residents had lower than expected values.

Summer residents and partial migrants were the only categories of resident status that showed significant differences among species ($\chi^2 = 100.0$, df = 25, $P < 0.001$; $\chi^2 = 48.3$, df = 11, $P < 0.001$, respectively). In both categories the species with the highest percentage of individuals positive for EEEV antibody was appreciably higher than the next highest species (Fig. 11). For summer residents, Wood Thrush had antibody levels 1.6-fold higher than the next highest species (Ovenbird), and for partial migrants Blue Jays were 2.2-fold higher than Pine Warblers. In both cases the frequency of individuals positive was well above the expected values calculated in the χ^2 contingency tables (Wood Thrush: 106 observed, 60.1 expected; Blue Jay: 26 observed, 10.3 expected).

No significant differences were found in antibody levels among migratory species ($\chi^2 = 18.5$, df = 17, $P = 0.36$). Antibody levels for all migratory species averaged 9.1%. Some winter residents tested positive for EEEV antibody, although the antibody rates were low (5.4%). Sample sizes for individual species were small, with the exception of the Yellow-rumped Warbler (297 individuals). Nonetheless, all other winter residents had very low frequencies of individuals positive for EEEV antibody. Frequency of Red-breasted Nuthatch individuals positive for EEEV antibody was 8%, followed by Yellow-rumped Warbler (6%). The remaining winter residents all tested negative.

We recognized six classes of nest habitat (Fig. 10B). Birds that nested within 1–3 m of the ground and cavity nesters showed higher than expected percentages of individuals positive for EEEV antibody (Fig. 9). They made up 27 and 20%, respectively, of the sample population but accounted for 39 and 31% of the individuals positive for EEEV antibody. The category for birds that nested at variable heights had only one species with an adequate sample size (Yellow-rumped Warbler), thus we excluded that category from analysis.

Within each class of nest habitat, EEEV antibody differed significantly among species for birds nesting on the ground ($\chi^2 = 37.8$, df = 20, $P < 0.009$), 1–3 m above the ground (low) ($\chi^2 = 71.7$, df = 14, $P < 0.001$), 3–7 m high (medium)

Table 1. Species of birds tested, their physical and natural history characteristics, sample sizes, and percentage positive for HI antibodies for EEEV

Species	Band size	Resident status[a]	Wintering range[b]	Nest site[c]	Breeding range[d]	Sample size by year[e] 1980 (Tot/+)	1981 (Tot/+)	1982 (Tot/+)	1983 (Tot/+)	Total no. sampled	% Positive for EEE
American Redstart (*Setophaga ruticilla* (Linnaeus))	0	SR	MA	MED	NJCA	4/ 1	8/ 1	12/ 3	0/ 0	24	20.8
American Robin (*Turdus migratorius* (Linnaeus))	2	SR	SE	LOW	NJCA	14/ 3	3/ 0	5/ 04	1/ 0	23	30.4
Black-and-White Warbler (*Mniotilta varia* (Linnaeus))	0	SR	SE	GRD	NJCA	28/ 2	21/ 3	21/ 5	5/ 1	75	14.7
Black-throated Blue Warbler (*Dendroica caerulescens* (Gmelin))	0	MG	MA	GRD	NECA	13/ 0	10/ 3	5/ 1	0/ 0	28	14.3
Blue Jay (*Cyanocitta cristata* (Linnaeus))	3	PM	SE	MED	NJCA	12/ 3	16/1 2	14/ 11	0/ 0	42	61.9
Brown Creeper (*Certhia familiaris* (Linnaeus))	0	PM	SE	CAV	NJCA	9/ 0	6/ 3	0/ 0	0/ 0	15	20.0
Brown Thrasher (*Toxostoma rufum* (Linnaeus))	2	SR	SE	LOW	NJNE	3/ 1	3/ 2	3/ 1	1/ 1	10	50.0
Brown-headed Cowbird (*Molothrus ater* (Boddaert))	1B	PM	SE	MED	NJCA	0/ 0	6/ 1	2/ 1	2/ 0	10	20.0
Canada Warbler (*Wilsonia canadensis* (Linnaeus))	*0*	*MG*	*SA*	*VAR*	*NECA*	*0/ 0*	*2/ 0*	*4/ 1*	*0/ 0*	*6*	16.7
Cardinal (*Richmondena cardinalis* (Linnaeus))	1A	PR	NE	LOW	NJON	14/ 4	26/ 9	15/ 8	7/ 1	62	38.5
Carolina Chickadee (*Parus carolinensis* Audobon))	0	PR	NE	CAV	NJON	34/ 6	78/ 30	47/ 27	3/ 1	162	38.9
Carolina Wren (*Tryothorus ludovicianus* (Latham))	1	PR	NE	GRD	NJON	1/ 1	4/ 1	3/ 2	2/ 0	10	40.0
Common Grackle (Quiscalus quiscula (Linnaeus))	3	PM	SE	MED	NJCA	18/ 3	36/ 0	19/ 2	1/ 0	74	6.8
Dark-eyed Junco (*Junco hyemalis* (Linnaeus))	0	WR	NE	GRD	NECA	5/ 0	0/ 0	3/ 0	0/ 0	8	0.0
Downy Woodpecker (*Dendrocopus pubescens* (Linnaeus))	1B	PR	NE	CAV	NJON	9/ 0	9/ 0	6/ 02	0/ 0	24	8.3
Eastern Wood Pewee (*Contopus virens* (Linnaeus))	0	SR	MA	MED	NJCA	4/ 0	0/ 0	3/ 2	0/ 0	7	28.6
Gray Catbird (*Dumetella carolinensis* (Linnaeus))	1A	SR	SE	LOW	NJCA	15/ 3	50/ 12	38/ 24	16/ 1	119	33.6
Gray-cheeked Thrush (*Hylocichla minima* (Lafresnaye))	1B	MG	MA	VAR	NECA	1/ 0	1/ 0	1/ 0	1/ 0	4	0.0
Great Crested Flycatcher (*Myiarchus crinitus* (Linnaeus))	1A	SR	MA	CAV	NJCA	6/ 0	5/ 0	8/ 02	2/ 0	21	9.5
Hairy Woodpecker (*Dendrocopos villosus* (Linnaeus))	2	PR	NE	CAV	NJON	2/ 0	6/ 02	5/ 03	1/ 0	14	35.7
Hermit Thrush (*Hylocichla guttata* (Pallas))	1B	MG	SE	GRD	NECA	15/ 0	1/ 0	1/ 0	1/ 0	18	0
House Wren (*Troglodytes aedon* Vieillot)	0	SR	SE	CAV	NJCA	*2/ 0*	*1/ 0*	*2/ 1*	*0/ 0*	*5*	20.0
Indigo Bunting (*Passerina cyanea* (Linnaeus))	1	SR	MA	GRD	NJCA	0/ 0	11/ 1	3/ 0	0/ 0	14	7.1
Magnolia Warbler (*Dendroica magnolia* (Wilson))	0	MG	MA	MED	NECA	1/ 0	1/ 0	4/ 2	0/ 0	6	33.3
Nashville Warbler (*Vermivora ruficapilla* (Wilson))	0	MG	MA	GRD	NECA	3/ 0	0/ 0	1/ 1	0/ 0	4	25.0
Northern Waterthrush (Seiurus noveboracensis (Gmelin))	1	MG	MA	CAV	NECA	4/ 0	6/ 0	8/ 0	0/ 0	18	0.0
Northern Flicker (*Colaptes auratus* (Linnaeus))	3	PM	SE	CAV	NJCA	8/ 0	8/ 03	5/ 02	0/ 0	21	23.8
Ovenbird (*Seiurus aurocapillus* (Linnaeus))	1	SR	SE	GRD	NJCA	34/ 6	64/ 22	61/ 35	16/ 2	175	37.1
Pine Warbler (*Dendroica pinus* (Wilson))	0	PM	SE	HIH	NJNE	9/ 0	17/ 2	10/ 6	3/ 3	39	28.2
Prairie Warbler (*Dendroica discolor* (Vieillot))	0	SR	SE	LOW	NJNE	1/ 0	0/ 0	1/ 1	3/ 1	5	40.0
Prothonotary Warbler (*Protonotaria citrea* (Boddaert))	0	SR	MA	CAV	NJSE	1/ 0	1/ 0	2/ 1	0/ 0	4	25.0
Red-breasted Nuthatch (*Sitta canadensis* Linnaeus)	0	WR	NE	CAV	NECA	3/ 1	10/ 0	0/ 0	0/ 0	13	7.7
Red-eyed Vireo (*Vireo olivaeus* (Linnaeus))	1	SR	SA	LOW	NJCA	1/ 0	13/ 2	2/ 1	3/ 1	19	21.0
Red-winged Blackbird (*Agelaius phoeniceus* (Linnaeus))	1A	PM	SE	LOW	NJCA	2/ 0	5/ 0	3/ 1	0/ 0	10	10.0
Ruby-crowned Kinglet (*Regulus calendula* (Linnaeus))	0	MG	SE	HIH	CAON	5/ 0	1/ 0	1/ 0	0/ 0	7	0.0
Rufous-sided Towhee (*Pipilo erythrophthalmus* (Linnaeus))	1A	PM	SE	LOW	NJNE	4/ 1	3/ 0	0/ 0	1/ 0	8	12.5
Scarlet Tanager (*Piranga olivacea* (Gmelin))	1B	SR	SA	HIH	NJCA	2/ 0	5/ 1	0/ 0	2/ 0	9	11.1
Swainson's Thrush (*Hylocichla ustulata* (Nuttall))	1B	MG	MA	LOW	NECA	5/ 0	7/ 0	6/ 0	3/ 0	21	0.0
Tufted Titmouse (*Parus bicolor* (Linnaeus))	1B	PR	NE	CAV	NJON	13/ 3	38/ 17	26/ 14	0/ 0	77	44.2
Veery (*Hylocichla fuscescens* (Stephens))	1B	MG	MA	GRD	NEUS	0/ 0	5/ 0	9/ 3	4/ 0	18	16.7
White-eyed Vireo (*Vireo griseus* (Boddaert))	0	SR	SE	LOW	NJSE	3/ 2	7/ 0	10/ 2	3/ 1	25	20.0
White-throated Sparrow (*Zonotrichia albicollis* (Gmelin))	1B	WR	NE	GRD	NECA	0/ 0	1/ 0	2/ 0	4/ 0	7	0.0
Wood Thrush (*Hylocichla mustelina* (Gmelin))	1A	SR	MA	LOW	NJNE	35/19	69/ 38	49/ 37	24/12	177	59.9
Worm-eating Warbler (*Helmitheros vermivorus* (Gmelin))	0	MG	MA	GRD	NEUS	0/ 0	3/ 0	1/ 0	1/ 0	5	0.0
Yellow-billed Cuckoo (*Coccyzus americanus* (Linnaeus))	1	SR	SA	LOW	NJCA	5/ 0	5/ 0	10/ 2	1/ 0	21	9.5
Yellow-rumped Warbler (*Dendroica coronata* (Linnaeus))	0	WR	NE	VAR	NECA	212/ 0	24/ 1	61/ 16	0/ 0	297	5.7
Yellow-throated Warbler (*Dendroica dominica* (Linnaeus))	0	SR	SE	HIH	NJSE	1/ 0	0/ 0	1/ 0	2/ 0	4	0.0
Common Yellowthroad (*Geothlypis trichas* (Linnaeus))	0	SR	SE	GRD	NJCA	12/ 0	13/ 4	19/ 7	8/ 3	52	26.9
Other species[f]						4/ 0	12/ 2	8/ 2	7/ 2	31	19.4
Totals						579/59	621/172	520/233	128/30	1848	27%

[a] Resident status: SR, summer resident; PM, partial migrant; MG, migrant; PB, permanent resident; WR, winter resident.
[b] Wintering range: SA, South America; MA, Middle America; SE, southeastern United States; NE, northeastern United States.
[c] Nest site: GRD, on or near the ground; LOW, 1–3 m; MED, 3–7 m; HIH, >7 m; CAV, cavity nests; VAR, variable locations.
[d] Breeding range: NJNE, southern New Jersey to northeastern United States; NJCA, southern New Jersey to Canada; NJON, southern New Jersey only; NECA, northeastern United States to Canada; NEUS, largely from northeastern United States; NJSE, southern New Jersey to southern United States; CAON, Canada only.
[e] Sample size by year: within each year values are total number of birds sampled/number of birds positive for HI antibody for EEEV.
[f] Other species: includes the 20 species with sample sizes ≤3 specimens .

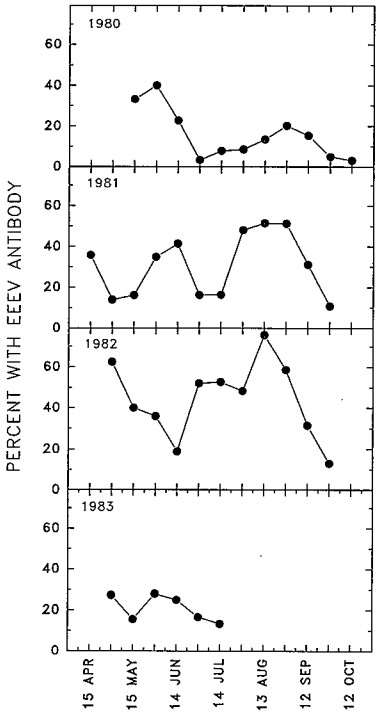

Fig. 5. Antibody prevalence in birds captured at our study site in southern New Jersey. Values represent 15-d averages during the mosquito seasons of 1980–1983.

(χ^2 = 44.8, df = 9, $P < 0.001$), and cavity nesters (χ^2 = 35.3, df = 11, $P < 0.001$). We found no significant differences in antibody levels for the high nest category (χ^2 = 6.0, df = 5, P = 0.304). Highest antibody rates were near 60% for species in the low and medium categories, but only near 40% for species nesting on the ground and in cavities (Fig. 12).

We recognized seven categories of breeding range (Fig. 10C). Species that bred in New Jersey and those with breeding ranges extending from our study site north to the Canadian border (categories NJ-only, NJ-to-NE) showed higher than expected percentages of individuals positive for EEEV antibody (Fig. 9). They made up 19 and 13%, respectively, of the sample population but accounted for 27 and 26% of the individuals positive for EEEV antibody. Species breeding from New England into Canada (NE-to-Can) had appreciably lower than expected levels of EEEV antibody. The remaining four groups had antibody levels near expected values. Variation among species within each category of breeding range differed significantly only among the species in categories NJ-to-NE (χ^2 = 24.1, df = 8, $P < 0.001$) and NJ-to-Can (χ^2 = 79.0, df = 25, $P < 0.001$). In both categories, values among species ranged from 10 to 60% positive (Fig. 13).

Wintering Range. We recognized four classes of winter range (Fig. 10D). Birds wintering in Middle America were the only group with appreciably higher than expected antibody levels (Fig. 9). They made up 20% of the sample population but accounted for 27% of the individuals positive for EEEV antibody. Birds wintering in New Jersey only and South America both had lower than expected values.

Positivity rates for EEEV antibody varied significantly among species in three of the four categories of wintering range: Middle America (χ^2 = 101.6, df = 24, $P < 0.001$), NJ-to-SE US (χ^2 = 74.8, df = 24, $P < 0.001$), NJ-only (χ^2 = 114.8, df = 12, $P < 0.001$). Of all the species wintering in Middle America, only the Wood Thrush showed a high proportion of individuals positive for EEEV antibody (Fig. 14). Species in the NJ-to-SE US category decreased more or less uniformly from a high of 60% to ≈10%. In the NJ-only category, five of eight species had antibody levels near 40%, whereas the remaining three were <10% positive.

We recognized six size classes of birds based on the band sizes (increasing size: 0, 1, 1B, 1A, 2, 3) recommended by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977) (Fig. 10E). We found no pattern in antibody levels according to size. Birds with a band size of 1A were the only group to show appreciably higher than expected proportions of individuals positive for EEEV antibody (Fig. 9). They made up 22% of the sample population but accounted for 35% of the individuals positive for EEEV antibody. Birds with band size 0 had antibody levels considerably lower than expected. They made up 44% of the sample population, but accounted for only 30% of the positives.

Within each size category, variation among species differed significantly for band size: 0 (χ^2 = 101.7, df = 32, $P < 0.001$), 1 (χ^2 = 17.8, df = 7, $P < 0.013$), 1B (χ^2 = 38.9, df = 11, $P < 0.001$), 1A (χ^2 = 44.1, df = 6, $P < 0.001$), and 3 (χ^2 = 42.4, df = 3, $P < 0.001$). We found no apparent pattern in antibody responses among species within size category 2.

Discussion

The EEEV Cycle in Southern New Jersey. In our 6-yr study we isolated EEEV from *C. mela-*

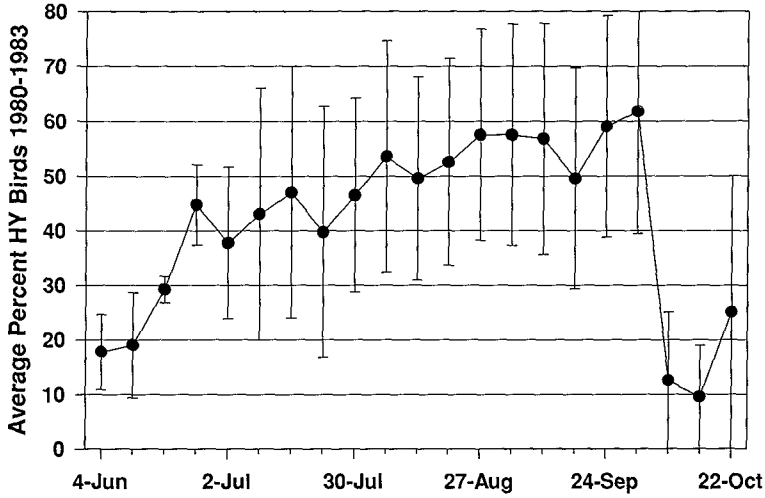

Fig. 6. Mean percentage of HY birds in the samples taken in 1980–1983 for antibody determination. Vertical lines are standard errors.

nura every year. In five of those years, the MIRs in *C. melanura* peaked between 3.9 and 5.6. The consistent occurrence and high level of EEEV transmission exposed the avifauna at our coastal site on a yearly basis. EEEV activity typically began in July or August and persisted through October, exposing birds from every category of resident status to infection.

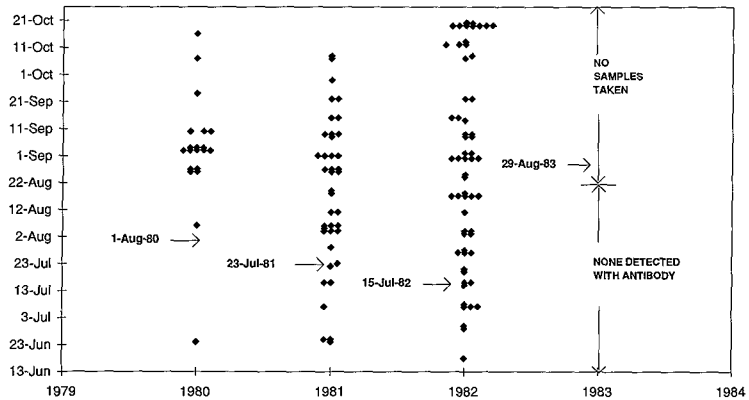

Fig. 7. HY birds captured with antibody to EEEV in relation to first isolations in *C. melanura* (horizontal arrows) from 1980 to 1983.

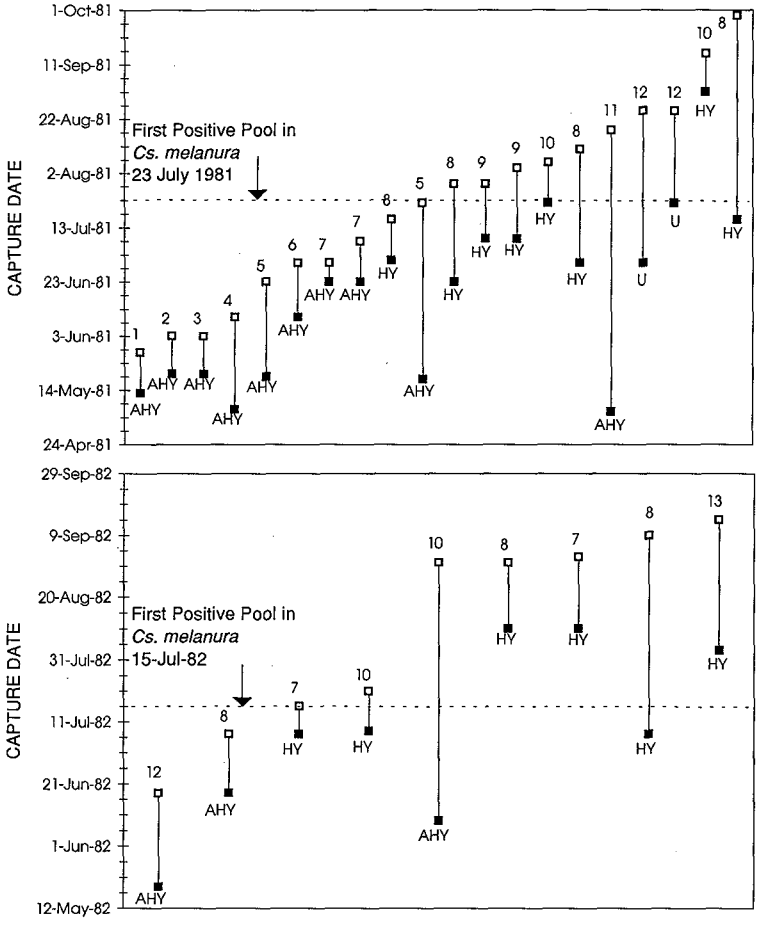

Fig. 8. Dates in 1981 (top) and 1982 (bottom) of capture for birds (residents, summer residents) that seroconverted at the study site in relation to the first isolations of EEEV from *C. melanura*. Solid squares indicate last capture date when negative for EEEV antibody; open squares indicate first capture date when positive for EEEV antibody. Dashed lines indicate dates of first isolations in *C. melanura*. Letters below solid squares signify age of bird. Numbers above open squares indicate the following species: 1, Brown Thrasher; 2, Wood Thrush; 3, Black-and-White Warbler; 4, Scarlet Tanager; 5, Red-eyed Vireo; 6, Indigo Bunting; 7, Tufted Titmouse; 8, Northern Cardinal; 9, Ovenbird; 10, Brown-headed Cowbird; 11, Carolina Chickadee; 12, Hairy Woodpecker.

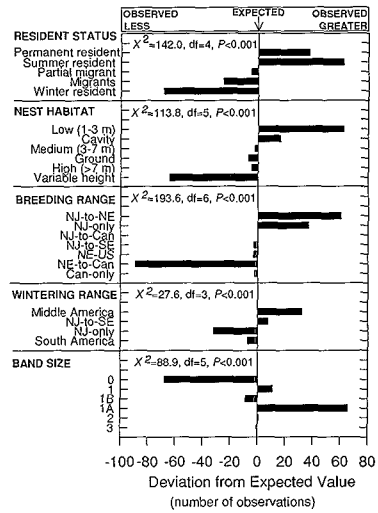

Fig. 9. χ^2 contingency tests for avian natural history characteristics. Bars to the right of zero indicate observed levels for antibody prevalence greater than expected; bars to the left of zero indicate observed levels of antibody prevalence less than expected.

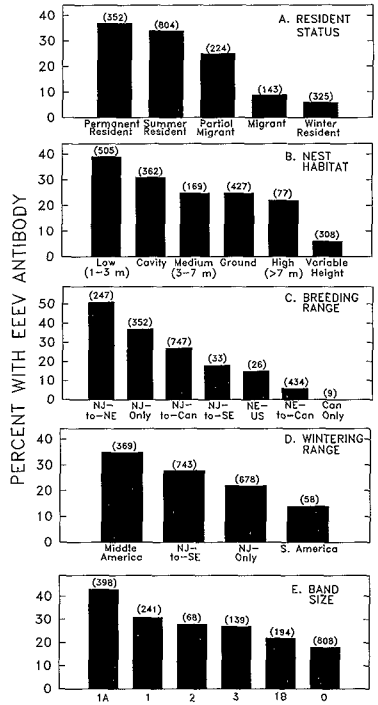

Fig. 10. EEEV antibody prevalence according to five avian natural history characteristics. Number above each bar is sample size.

The seasonal cycle was initiated by permanent residents and summer residents that nested at the study site in spring and amplified by HY birds when they entered the local populations in spring and early summer. In late summer and early fall, the cycle was perpetuated by migrants and winter residents when they returned to our study area from their breeding areas. The high antibody prevalence we found at our study site was probably the result of annual reinfection of locally nesting birds. This is in direct contrast to the inland pattern studied by Emord & Morris (1984) in upstate New York. In that area, EEEV appeared sporadically, with long interepizootic periods characterized by declining antibody titers in birds. Antibody rates at our study site were much higher than those reported from Massachusetts (Main et al. 1988), about equal to those reported from Maryland (Dalrymple et al. 1972), and somewhat lower than levels found in Alabama (Stamm 1963) and Michigan (McLean et al. 1985).

At our study site we collected six viremic birds that predated the first detection of virus in mosquitoes. Only one specimen was a HY bird, a Tufted Titmouse captured in 1981 just 7 d before we detected EEEV in *C. melanura*. Because we collected mosquitoes only once per week, this bird probably was infected by the onset of the *C. melanura* cycle that year. The remaining birds, all captured in 1982, were viremic 37–51 d before virus was detected in *C. melanura*.

The early season viremias in birds indicate that some form of virus activity takes place in nature before detection of virus in the mosquito cycle that is documented regularly by virus surveillance programs. Other investigators have attributed early season seroconversions in birds to virus transmission by mosquitoes (Feemster et al. 1958, Daniels et al. 1962). We have conducted virus surveillance at our study site continuously since 1975 and have never detected virus in mosquitoes before July. Emord & Morris (1984) be-

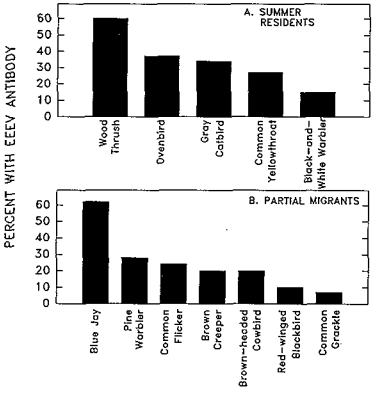

Fig. 11. EEEV antibody prevalence for categories of residence status that yielded significant χ^2 contingency tests in comparisons among species.

lieved that early season virus activity in birds was the result of chronic infections, because the titer levels they observed were lower than would be expected from a recent infection. The early season viremias we detected in May and June may well have developed through recrudescence of latent virus from a previous infection, because they all occurred in local birds that had nested at the study site during at least 1 yr of virus cycling.

In most years the seasonal pattern of antibody prevalence in birds began at moderate to high levels in early spring, declined from mid-June to mid-July, and increased again later in the summer. The antibody-positive individuals early in the season were permanent resident and summer resident AHY birds that presumably were infected by EEEV in previous years. As the young produced by these birds fledged and entered the general population, the proportion of individuals positive for EEEV antibody declined (i.e., mid-June to mid-July). In mid-June HY birds made up <50% of our samples, but by the second week of July they composed >80%. The increase in antibody prevalence in late summer occurred when HY birds became infected and developed

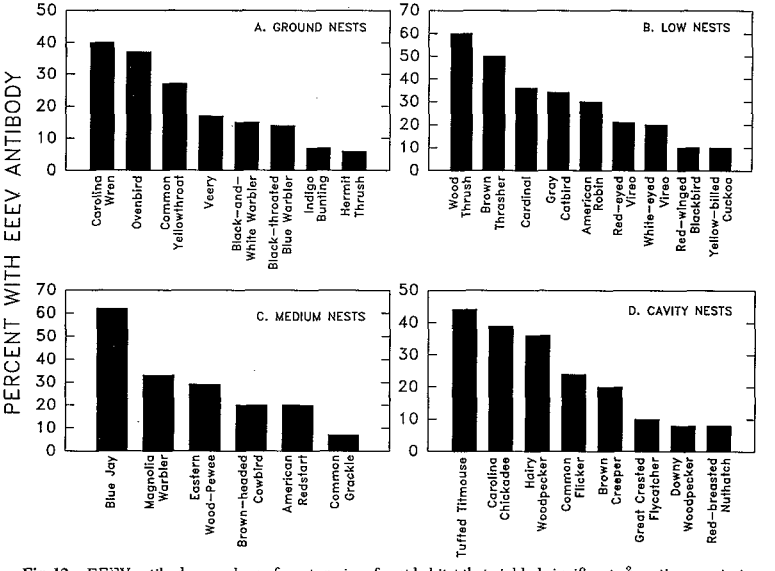

Fig. 12. EEEV antibody prevalence for categories of nest habitat that yielded significant χ^2 contingency tests in comparisons among species.

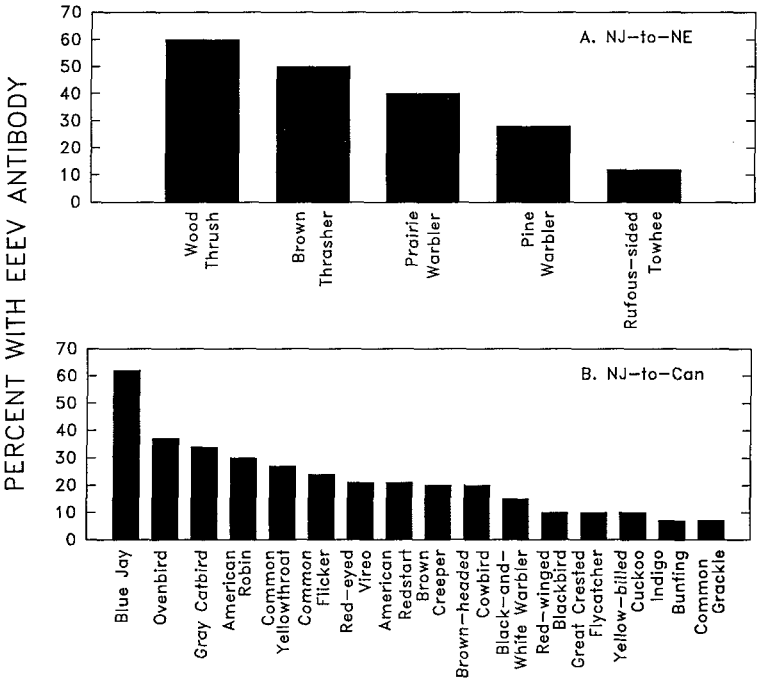

Fig. 13. EEEV antibody prevalence for categories of breeding range that yielded significant χ^2 contingency tests in comparisons among species.

antibody. The late-season decline was attributed to dilution by uninfected migrants from the north that passed through the study area from the south and uninfected winter residents that took up residence at the study site in early fall. This generalized seasonal pattern differs markedly from the interepizootic pattern examined by Emord & Morris (1984) in upstate New York and by McLean et al. (1986) in Michigan, but is similar to that reported from a freshwater swamp in Maryland (Dalrymple et al. 1972).

In 3 of 4 yr of our study, we detected antibody in HY birds a month or more before we detected EEEV in *C. melanura*. Kissling et al. (1954) and Reeves et al. (1954) showed that maternal antibody could be transferred from previously infected adults to their offspring. The antibody we found in HY birds before detection of virus in *C. melanura* does not appear to be maternal antibody. Kissling et al. (1954) showed that maternal antibody persists only ≈3–4 wk. The HY birds we captured in mist nets were probably much older than that and, therefore, would have lost all traces of maternal antibody at the time of capture. In addition, if the source of early season antibody in HY birds were maternal in origin, we would expect the highest levels in years following major epiornitics. The 1982 season yielded the highest MIRs of our investigation, yet the following year we detected no early season antibody in HY birds. Early season development of antibody in HY birds may be an indicator that EEEV will be active later in the season, because we detected EEEV antibody in HY birds only in the epiornitic years (1980, 1981, 1982). In 1983,

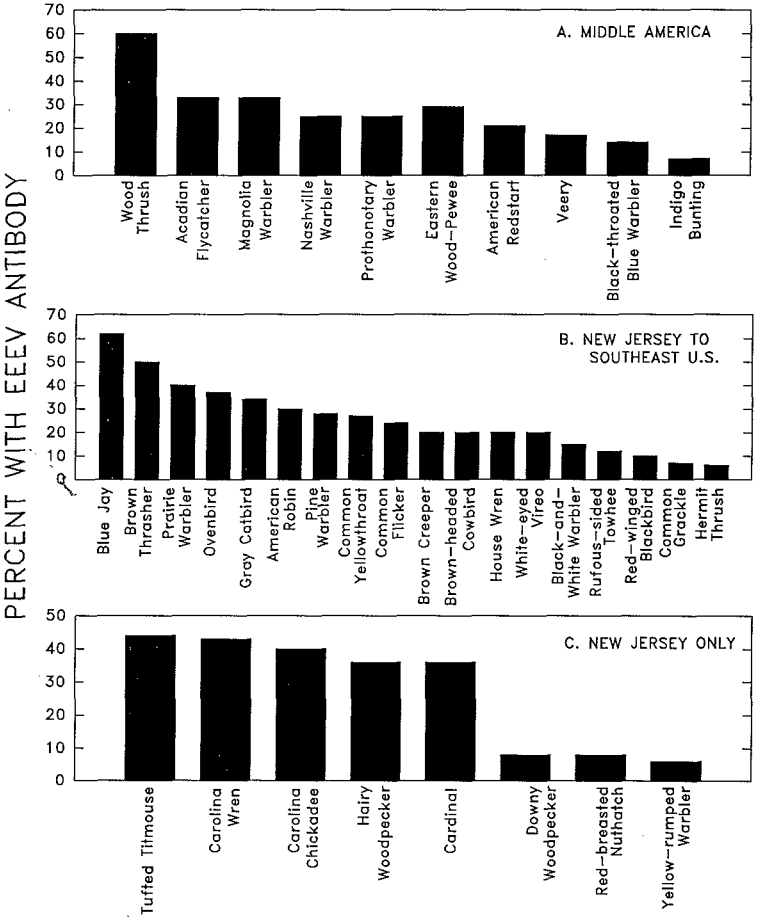

Fig. 14. EEEV antibody prevalence for categories of wintering range that yielded significant χ^2 contingency tests in comparisons among species.

virus activity was extremely limited, and we detected no antibody in HY birds that year.

We recaptured 29 birds that seroconverted. Of those, 10 birds showed evidence of seroconversion 8 to 56 d before we detected virus in *C. melanura*. Antibody titers from all our early season seroconversions were low (≤1:80) indicating that the titer changes were not the result of new infections. Our results support the hypothesis that the early season seroconversions resulted from latent infections that recrudesced between captures.

Antibody Prevalence in Relation to Avian Natural History Characteristics. We examined antibody prevalence in relation to avian natural history characteristics to identify factors that influence involvement of birds in the EEEV cycle. Characteristics were considered to be potentially important when all species sharing a characteristic showed similar levels of antibody prevalence (i.e., no significant difference detected in antibody prevalence among species). A natural history characteristic may make the risk of infection either more likely or less likely. Conversely, when individual species within a group sharing a characteristic showed significant differences in antibody prevalence, we concluded that the characteristic was not related to involvement in the EEEV cycle.

To apply this logic to our results, we examined the characteristics for which we detected no significant differences in comparisons among species. For example, we found high antibody prevalence for permanent residents and low prevalence for migrants and winter residents. It appears, therefore, that permanent residents have an increased likelihood for involvement in the EEEV cycle, whereas being a migrant or winter resident mitigates against involvement. Comparisons among species within groups were significantly different for both summer residents and partial migrants, indicating that some species made contact with EEEV, whereas others did not. The Blue Jay, a partial migrant, and the Wood Thrush, a summer resident, each showed far higher antibody levels than other species in their respective categories. Blue Jays are considered to be partial migrants, because in many geographic areas a portion of the population leaves the area during winter (Pough 1949). However, our study site in southern New Jersey represents an intermediate zone where a high percentage of the population behaves like permanent residents, remaining throughout the winter.

High antibody prevalence among permanent and summer residents indicates that time in the study area increases the risk of EEEV infection. A similar pattern was reported for an endemic focus in Maryland (Dalrymple et al. 1972). High prevalence in Wood Thrush, a summer resident, indicates that presence during the mosquito season is fundamental to involvement in the EEEV cycle. Wood Thrushes depart in mid-September and are absent until the third week of April, but they still had the second highest antibody prevalence among the birds we examined.

Four of the five categories for which we had valid tests showed significant differences among species for nesting habitat, indicating that nesting habitat probably did not influence contact with EEEV. We did not detect significant differences among species in the high nest category. However, this category had only three species with vastly different sample sizes. Antibody prevalence in the high nest category ranged from 0% in the Ruby-crowned Kinglet to 28% in the Pine Warbler. In this case the lack of significant differences appears more related to sample characteristics than to the influence of nest height.

The three breeding range categories that included New Jersey and areas to the north (NJ-to-NE, NJ-only, NJ-to-Can) had the highest levels of antibody prevalence. Of these, only the NJ-only category failed to show significant differences among species. This indicates that species breeding at the study site had a uniformly high probability of contracting EEEV. This reinforces the result we obtained for resident status because both parameters indicate that birds closely linked to the study area respond uniformly regarding involvement in the EEEV cycle. The lowest antibody prevalence occurred in the species (e.g., Black-throated Blue Warbler, White-throated Sparrow, Ruby-crowned Kinglet) that nested near the northern limit of EEEV activity (NE-to-Can, Can-only), where the virus appears only sporadically, providing little opportunity for repeated infection.

The two breeding range categories that showed significant differences among species had levels of antibody prevalence that ranged from a high of 62% in the Blue Jay to a low of 6.8% in the Common Grackle. Such variation among species indicates that for these species there are additional factors influencing involvement in the EEEV cycle. For example, many of these individuals probably bred at northern locations where EEEV was not endemic. The Blue Jay had antibody levels much higher than other species in the NJ-to-Can category, but most of the Blue Jays we sampled were local birds. Other species in the NJ-to-Can category included local populations that became mixed with migrants from the north later in the season. During each fall migration, we captured large numbers of unbanded HY birds (e.g., Ovenbird, American Robin, American Redstart) that clearly arrived en masse from breeding areas to the north. Their sudden appearance convinced us that they were recent arrivals that diluted the local populations, resulting in lower levels of antibody prevalence for these species at that time.

Comparisons were significantly different among species for all the categories of wintering range except South America. This indicates that, for most species, location of the wintering site had little to do with the risk of involvement in the EEEV cycle. Because infection rates for species in the South America category were uniformly low, there may be some negative relationship between wintering in South America and involvement in the EEEV cycle.

Body size was not related to contact with EEEV. Every size category but one showed significant differences among species, indicating wide variation among species within each size

category. The one category that failed to show significance (size 2) had the smallest sample size (68 individuals) and was composed of only four species (American Robin, Brown Thrasher, Hairy Woodpecker, Yellow-billed Cuckoo).

Initialization of the EEEV Cycle at Endemic Foci. Despite decades of research, the mechanisms that initiate EEEV cycling remain an enigma (Morris 1988, Scott 1988, Reisen 1990). Scott (1988) and Scott & Weaver (1989) dismissed several explanations as either improbable or unsupported: transovarial transmission, overwintering of infected adult mosquitoes, airblown movement of infected vectors from southern foci, and the introduction of tropical forms of EEEV by migrating birds. The remaining three alternatives include (1) annual reintroduction by migrating birds from active foci in the southern United States; (2) an unknown nonmosquito vector; and (3) recrudescence of virus in birds with chronic, latent infections. Morris (1988) offered an additional possibility (4) based on the active transmission of an avirulent phase of EEEV by *C. melanura*. Morris speculated that the avirulent EEEV was passed on to birds, where it changed to a virulent form in response to the variations in avian physiology that occur in early summer following the breeding season.

Our results indicate that the annual introduction of EEEV into our study site was not likely by migrating birds from foci in the southern United States. Resident status was the only avian natural history characteristic we evaluated that showed strong linkage to the EEEV cycle. Although antibody prevalence was high for summer residents that pass through the southern United States, it was extremely low in migratory species that followed the same migratory route but breed north of our study area. Moreover, winter residents from our study site that did not pass through the southern United States showed antibody levels similar to those of the migrants, indicating that exposure to EEEV for both groups was similar.

Our data on viremic birds further discount the probability that virus was introduced by migrating birds that became infected in the southern United States. Virus activity peaked in 1982 after 3 yr of documented epiornitic cycling at our study site. In that year we captured five viremic birds in late May and early June. All were AHY birds that most likely were hatched at our study site and therefore were exposed to one or more of the three epiornitics we documented between 1979 and 1981. If the early season viremias were imported from the south, they would have occurred soon after the arrival of partial migrants and summer residents. Pine Warblers and Redwinged Blackbirds arrive in March or early April. The Yellow-billed Cuckoo arrives in late April or early May. The Carolina Chickadee, a permanent resident, probably never left the study site. Only the Catbird arrived late enough to import virus from southern foci. However, the catbird was a recapture that had antibody from a previous infection, suggesting the possibility that recrudescence of latent infection rather than acquisition of the virus in the southern United States was responsible for the viremia.

It generally is accepted that the EEEV cycle is initiated when virus first appears in *C. melanura*. However, we observed three events that suggest that EEEV may be active in a cryptic cycle weeks before epiornitic outbreaks. Before the detection of EEEV in *C. melanura*, we found that some local birds became viremic, that seroconversions occurred in recaptured birds, and that HY birds developed antibody. None of the mechanisms proposed to explain the initiation of EEEV cycling can explain these early season observations of virus activity.

Cryptic Cycle for EEEV Early in the Season. We propose that a cryptic transmission cycle of EEEV develops in early spring, weeks before epiornitic cycling is detected in *C. melanura* by traditional laboratory techniques (e.g., suckling mice, chick embryo, 12-h-old chicks [Chamberlain et al. 1954]). The cryptic cycle begins with recrudescence of latent virus in previously infected AHY birds. In the early spring, reactivation of latent virus may result from changes in the physiological state of local birds (i.e., summer residents, residents) related to factors such as stress of migration, establishment of territory, or other breeding activities. Because early season viremias were uncommon, we believe that few birds become viremic at any one time. However, before detection of EEEV in *C. melanura* there is a small but constant segment of the local bird population with circulating virus.

Cool nighttime temperatures, low populations of *C. melanura*, and high antibody prevalence in AHY birds prevent the virus from reaching epiornitic levels during May and early June. In addition, the viremia titer in recrudescing AHY birds may not be high enough to infect *C. melanura* that have a relatively high oral infection threshold for EEEV (Schaeffer & Arnold 1954). As the local nestlings fledge, they represent a subpopulation of susceptible hosts that form an increasing portion of the bird population. The increase of susceptible HY birds in the population sets the stage for amplification of the virus that recrudesces in AHY birds. However, epiornitic cycling requires an influx of newly emerged *C. melanura*. If recrudescing virus is acquired by *C. melanura* females that have already completed one or more gonotrophic cycles, it is unlikely that these females will live long enough for the virus to replicate and be transmitted to other hosts. In some years the virus can become epiornitic by early July, when conditions are favorable for *C. melanura*. If vector populations are limited by unfavorable conditions during that

critical period, the virus cycle is either postponed until the August emergence or eliminated entirely.

Our early season observations of virus activity provide support for a cryptic EEEV cycle. Recrudescence of latent virus could have caused the early season viremias as well as the early season seroconversions that occurred in birds at our study site. All five of the viremias in May and June were in AHY birds that were present at the study site during at least one prior epiornitic. As a result, these birds could have harbored a latent infection that recrudesced during the breeding season. We were able to document one example of a viremia in a previously infected bird. On 13 May 1981 we captured an AHY catbird with a PRNT titer of 1:20 to EEEV. This bird was viremic when recaptured on 8 June 1982.

All the early season seroconversions we detected occurred in resident AHY birds. As a result, each lived through at least one documented epiornitic at our study site. The absence of antibody in these birds in the initial bleeding may have resulted from reversion after a prior infection. Emord & Morris (1984) showed that antibody levels progressively declined in the bird population in the absence of mosquito transmission, and Main et al. (1988) showed that neutralizing antibody was ephemeral in many species of previously infected birds. Recrudescence from latent virus may have been responsible for the seroconversions we detected during May and June. If those birds had undetectable antibody titers from a prior infection but recrudesced between captures, they would have tested negative on the first bleeding and positive on the second.

The antibody we found in early season samples from HY birds could not be the direct result of recrudescence. However, if infections recrudesced in the parents, the virus could have been transmitted to the nestlings by either mosquitoes or ectoparasitic arthropods. Durden et al. (1993) recently showed that chicken mites *Dermanyssus gallinae* (De Geer) could acquire EEEV in the laboratory from viremic chicks and transmit the infection to naive birds. This suggests that nonculicid arthropods could be involved in an early season cycle, provided that virus titers from recrudescing birds are high enough to infect mites. Morris (1988) offered another possible explanation based on a cycle involving an avirulent form of EEEV. In his hypothesis, an avirulent form of the virus is transmitted to birds by *C. melanura* in the early spring. Presence of EEEV would go undetected by traditional laboratory techniques so long as it remained in its avirulent phase. The virus is not evident until it is transformed to its virulent form in response to changes that take place in birds after the nesting season. We detected early season antibody in HY birds only in years when epiornitic cycling was documented later in the season. If Morris's hypothesis is correct, this antibody could have been induced in HY birds by avirulent EEEV.

We have proposed a cryptic cycle for EEEV that provides an explanation for aspects of the EEEV cycle that have remained an enigma to date. Among these are mechanisms to explain the source of EEEV that initiates the cycle each season, why the cycle is delayed until July or August, and early season seroconversions in birds that have been interpreted as early season arthropod transmission of virus.

Considerable additional information is required to validate the mechanisms we have proposed. If recrudescence is the source of virus each spring, latent virus must be detected in AHY birds captured before mosquito activity. If the virus titers in recrudescing birds are below the infection threshold for *C. melanura*, low levels of virus should be detected in freshly fed *C. melanura* captured early in the season. If the antibody we detected in HY birds originated from infections in recrudescing parents, a mechanism for transfer to the young must be shown. New and emerging technologies like the polymerase chain reaction have the specificity and sensitivity to address these and related questions regarding the epidemiology of EEEV.

Acknowledgments

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References Cited

Altman, R. M., M. Goldfield & O. Sussman. 1967. The impact of vector-borne viral diseases in the Middle Atlantic States. Med. Clin. North Am. 51: 661–671.

Burbutis, P. P. & D. M. Jobbins. 1958. Studies on the use of a diurnal resting box for the collection of *Cs. melanura* (Coq.). Bull. Brooklyn Entomol. Soc. 411: 53–58.

Chamberlain, R. W. 1958. Vector relationships of the arthropod-borne encephalitides in North America. Ann. N.Y. Acad. Sci. 70: 312–319.

Chamberlain, R. W., R. K. Sikes & R. E. Kissling. 1954. Use of chicks in eastern and western equine encephalitis studies. J. Immunol. 73: 106–114.

Clarke, D. H. & J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. 7: 561–573.

Crans, W. J. 1964. Continued host preference studies with New Jersey mosquitoes, 1963. Proc. N.J. Mosq. Exterm. Assoc. 51: 50–58.

1977. The status of *Aedes sollicitans* as an epidemic vector of eastern equine encephalitis in New Jersey. Mosq. News 37: 85–89.

Crans, W. J. & T. L. Schulze. 1986. Evidence incriminating *Coquillettidia perturbans* (Diptera: Culicidae) as an epizootic vector of eastern equine encephalitis. I. Isolation of EEE virus from *Cq. perturbans* during an epizootic among horses in New Jersey. Bull. Soc. Vector Ecol. 11: 178–184.

Crans, W. J., L. J. McCuiston & T. L. Schulze. 1986. Evidence incriminating *Coquillettidia perturbans* (Diptera: Culicidae) as an epizootic vector of eastern equine encephalitis. II. Ecological investigations following an inland epizootic in New Jersey. Bull. Soc. Vector Ecol. 11: 185–190.

Dalrymple, J. M., O. P. Young, B. F. Eldridge & P. K. Russell. 1972. Ecology of arboviruses in a Maryland freshwater swamp. III. Vertebrate hosts. Am. J. Epidemiol. 96: 129–140.

Daniels, J. B., G. Edall, N. J. Fiumara, K. F. Girard, R. O. Hayes, R. A. MacCready, F. R. Philbrook & R. E. Wheeler. 1962. Continuing investigation and study of eastern equine encephalitis and related virus diseases in Massachusetts in 1961. Appendix of Special Report, Commonwealth of Massachusetts, House, No. 3465.

Durden, L. A., K. J. Linthicum & T. P. Monath. 1993. Laboratory transmission of eastern equine encephalomyelitis virus to chickens by chicken mites (Acari: Dermanyssidae). J. Med. Entomol. 30: 281–285.

Edman, J. D., L. A. Webber & H. W. Kale. 1972. Host-feeding patterns of Florida mosquitoes. II. *Culiseta*. J. Med. Entomol. 9: 429–434.

Emord, D. E. & 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. 21: 395–404.

Feemster, R. F., R. E. Wheeler, J. B. Daniels, H. D. Rose, M. Schaeffer, R. E. Kissling, R. O. Hayes, E. R. Alexander & W. A. Murray. 1958. Field and laboratory studies on equine encephalitis. N. Engl. J. Med. 259: 107–113.

Grady, G. F., H. K. Maxfield, S. W. Hildreth, R. J. Timperi, Jr., R. F. Gilfilean, B. J. Rosenau, D. B. Francy, C. H. Calisher, L. C. Marcus & M. A. Madoff. 1978. Eastern equine encephalitis in Massachusetts, 1957–1976. Am. J. Epidemiol. 107: 170–178.

Hayes, R. O., L. D. Beadle, A. D. Hess, O. Sussman & M. J. Bonese. 1962. Entomological aspects of the 1959 outbreak of eastern encephalitis in New Jersey. Am. J. Trop. Med. Hyg. 11: 115–121.

Kerlin, R. E. 1964. Venipuncture of small birds. J. Am. Vet. Med. Assoc. 144: 870–874.

Kissling, R. E., M. E. Eidson & D. D. Stamm. 1954. Transfer of maternal neutralizing antibodies against eastern equine encephalomyelitis virus in birds. J. Infect. Dis. 95: 179–181.

Main, A. J., K. S. Anderson, H. K. Maxfield, B. Rosenau & C. Oliver. 1988. Duration of *Alphavirus* neutralizing antibody in naturally infected birds. Am. J. Trop. Med. Hyg. 38: 208–217.

McLean, R. G., G. Frier, G. L. Parham, D. B. Francy, T. P. Monath, E. G. Campos, A. Therrien, J. Kerschner & 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.

Morris, C. D. 1988. Eastern equine encephalomyelitis, pp. 1–19. *In* T. P. Monath [ed.], Arboviruses: epidemiology and ecology, vol. 3. CRC, Boca Raton, FL.

Morris, C. D., M. E. Corey, D. E. Emord & J. J. Howard. 1980. Epizootiology of eastern equine encephalomyelitis virus in upstate New York, USA. I. Introduction, demography and natural environment of an endemic focus. J. Med. Entomol. 17: 442–452.

Peterson, R. T. 1980. A field guide to the birds east of the rockies. Houghton Mifflin, Boston, MA.

Pough, R. H. 1949. Audubon land bird guide. Doubleday, Garden City, NY.

Reeves, W. C., J. M. Sturgeon, E. M. French & B. Brookaman. 1954. Transovarian transmission of neutralizing substances to western equine and St. Louis viruses by avian hosts. J. Infect. Dis. 95: 168–178.

Reisen, W. K. 1990. North American mosquito-borne arboviruses: questions of persistence and amplification. Bull. Soc. Vector Ecol. 15: 11–21.

SAS Institute. 1987. SAS for personal computers, version 6.04. SAS Institute, Cary, NC.

Schaeffer, M. & E. H. Arnold. 1954. Studies on the North American arthropod-borne encephalitides. I. Introduction. Contributions of newer field-laboratory approaches. Am. J. Hyg. 60: 231–236.

Schmidt, N. J. 1979. Cell culture techniques for diagnostic virology, pp. 65–139. *In* E. H. Lennette & N. J. Schmidt [eds.], Diagnostic procedures for viruses, Rickettsiae, and Chlamydiae. American Public Health Association, Washington, DC.

Scott, T. W. 1988. Vertebrate host ecology, pp. 257–280. *In* T. P. Monath [ed.], Arboviruses: epidemiology and ecology, vol 1. CRC, Boca Raton, FL.

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

Stamm, D. D. 1963. Susceptibility of bird populations to eastern, western and St. Louis encephalitis viruses, pp. 591–603. *In* Proceedings, 13th International Ornithology Congress, vol. I. American Ornithologists Union, Baton Rouge, LA.

Sudia, W. D., R. D. Lord & R. O. Hayes. 1972. Collection and processing of vertebrate specimens for arbovirus studies. U.S. Department of Health, Education and Welfare, Public Health Service, National Communicable Disease Center, Atlanta, GA.

U.S. Fish and Wildlife Service and Canadian Wildlife Service. 1977. North American bird banding manual, vol. II, bird banding techniques. Canadian Wildlife Service, Ottawa, Canada.

Wood, M. & D. Beimborn. 1981. A bird-bander's guide to determination of age and sex of selected species. Afton Press, Afton, MN.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.

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