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## WESTERN EQUINE ENCEPHALITIS IN AVIAN POPULATIONS IN NORTH DAKOTA, 1975

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**ABSTRACT:** The involvement of wild birds in western equine encephalitis (WEE) and St. Louis encephalitis (SLE) virus activity in the Red River valley area of North Dakota (USA) during a WEE epidemic was investigated in August 1975. Free-ranging birds were captured with mist nets and nestlings by hand. Virologic and serologic results indicated that a similar rate of WEE virus activity occurred throughout Richland County and between permanent and summer resident birds. The rate of SLE virus activity in the birds of Richland County was lower than for WEE virus, but the SLE antibody prevalence was greater in rural areas than within urban locations. Seven of the nine WEE virus isolations were from nestling birds of four different species; the remaining two from adults of two different species. Overall prevalence of neutralizing (N) antibody against WEE virus was 5% in nestling and 14% in adult birds but was the opposite for N antibody against SLE virus, 17% in nestling and 5% in adult birds. Differences between the two viruses in the presence and persistence of maternal N antibody or differential mortality in nestling birds may have caused the disparity in antibody prevalences.

**Key words:** Wild birds, western equine encephalitis, St. Louis encephalitis, virus isolation, arbovirus, serology, field study.

### INTRODUCTION

Western equine encephalitis (WEE) virus causes clinical disease and death in humans and equines throughout the western United States and Canada. Humans and equines are incidental hosts for the virus, and clinical cases usually occur only sporadically at a low rate in rural areas of the West (Tsai and Monath, 1987). However, dramatic increases in the intensity of WEE virus transmission occur at unpredictable intervals and locations, and major outbreaks have affected large regions. One of the largest epidemics occurred in 1941 in the northern Plains states of the United States and the contiguous Plains area of the western provinces of Canada (Leake, 1941; Eklund, 1946). A smaller in distribution and number of clinical cases WEE outbreak occurred within this region in 1975 (Potter et al., 1977).

The summer amplification of WEE virus involves a bird-mosquito-bird infection cycle with various wild bird species and *Culex tarsalis* mosquitoes; transmission of WEE occurs predominantly in the irrigated areas of western North America

(Hayes, 1981). The collection and testing of wild birds, principally nestling house sparrows (*Passer domesticus*), and vector mosquitoes (*C. tarsalis*) have been utilized to monitor WEE virus activity during the midsummer transmission period in many of the Plains states (Holden et al., 1973b; Hayes, 1981). This surveillance method was sensitive and accurate in determining risk of human infection, retrospectively, in western Texas (Holden et al., 1973b).

Although human St. Louis encephalitis (SLE) cases were not reported in this area in 1975, the largest epidemic year of SLE in the United States occurred throughout the midwestern states (Monath, 1980). Virus activity of SLE in the Plains and midwestern states is most prevalent in urban areas and usually involves *Culex pipiens* complex mosquitoes and peridomestic bird species, such as the house sparrow and various other urban-dwelling species (McLean and Bowen, 1980).

The Red River flooded the valley in eastern North Dakota and northwestern Minnesota in June of 1975, and the valley was declared a disaster area. Standing water

suitable for increased production of the vector mosquito, *C. tarsalis*, covered an extensive area for several weeks (Potter et al., 1977). Equine cases of WEE first appeared in June in Richland County, North Dakota, greatly increased the first of July, and peaked by the end of July, whereas human cases began during the second week of July and peaked during the second week of August. A total of 277 human and 281 equine cases were reported in the northern Red River valley of both North Dakota and Minnesota during 1975 (Potter et al., 1977; Leech et al., 1981). This study was undertaken during the peak of the human cases to determine the bird species involved in the epizootiology of WEE during this outbreak in Richland County.

#### MATERIALS AND METHODS

Richland County (45°56' to 46°38'N, 96°33' to 97°16'W) is in the southeastern corner of North Dakota and is bounded on the east by the Red River. The county has an area of approximately 3,800 km<sup>2</sup> and had a population of 19,289 during 1970. The terrain is relatively flat, and the county is predominantly rural and agricultural producing grains, sugar beets and livestock. Two of the larger urban areas in the county are Wahpeton (population 8,200 in 1975) and Hankinson (population 1,200). The average January temperature is -15 C and July temperature is 21 C; average precipitation is 50 cm/yr.

Birds were captured at 11 rural and two urban (Wahpeton and Hankinson) locations throughout Richland County from 9 to 15 August 1975. Locations were selected to provide representative sampling of the avian population in the eastern part of the county. Free-ranging adult and immature birds were captured with ground-level mist nets. There were 300 net hr (a net hr is one 40 ft (12.2 m) mist net operated for 1 hr of daylight) at the rural sites, 264 within the city of Wahpeton, and 72 in Hankinson. A search was made throughout the area for active nests of selected species of birds, and nestling birds were captured by hand.

The common and scientific names of the birds mentioned in the text and tables are as follows: red-winged blackbird (*Agelaius phoeniceus*), gray catbird (*Dumetella carolinensis*), black-capped chickadee (*Parus atricapillus*), black-billed cuckoo (*Coccyzus erythrophthalmus*), mourning dove (*Zenaidura macroura*), northern flicker (*Colaptes auratus*), Empidonax flycatcher (*Empidonax* sp.), great-crested flycatcher

(*Myiarchus crinitus*), American goldfinch (*Carduelis tristis*), common grackle (*Quiscalus quiscula*), rose-breasted grosbeak (*Pheucticus ludovicianus*), blue jay (*Cyanocitta cristata*), eastern kingbird (*Tyrannus tyrannus*), mallard (*Anas platyrhynchos*), northern oriole (*Icterus galbula*), pigeon (*Columba livia*), American robin (*Turdus migratorius*), yellow-bellied sapsucker (*Sphyrapicus varius*), house sparrow (*Passer domesticus*), Lincoln's sparrow (*Melospiza lincolnii*), song sparrow (*Melospiza melodia*), bank swallow (*Riparia riparia*), barn swallow (*Hirundo rustica*), cliff swallow (*Hirundo pyrrhonota*), brown thrasher (*Toxostoma rufum*), warbling vireo (*Vireo gilvus*), yellow warbler (*Dendroica petechia*), northern waterthrush (*Seiurus noveboracensis*), downy woodpecker (*Picoides pubescens*), hairy woodpecker (*Picoides villosus*), red-headed woodpecker (*Melanerpes erythrocephalus*), and house wren (*Troglodytes aedon*).

Blood was taken from the jugular vein of birds with a 1-ml syringe and 25- to 27-gauge needle. The blood specimen (generally 0.2 ml) was mixed with 0.9 ml of field diluent consisting of cell culture medium 199 (GIBCO Laboratories, Life Technologies, Inc., Grand Island, New York 14072, USA) with antibiotics and 20% heat-inactivated fetal bovine serum. Blood samples were kept on wet ice, allowed to clot, and then centrifuged. The diluted serum was separated and stored in sealed vials at -70 C until tested in the laboratory. Our field procedures were similar to those described elsewhere (Sudia et al., 1970).

For virus isolation, 0.1 ml of each serum specimen was allowed to absorb for 1 hr at 37 C on monolayer cultures of both primary Pekin duck embryo cells (DECC) (Truslow Farms, Chestertown, Maryland 21620, USA) and serially propagated Vero cells (C1008, African green monkey kidney, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA) grown in 25-cm<sup>2</sup> flasks. The cultures were then overlaid with nutrient medium containing 1% Noble agar (Difco Laboratories, P.O. Box 1058A, Detroit, Michigan 48232, USA) and 1:25,000 dilution of neutral red and incubated at 37 C for 7 to 10 days. If plaques were seen, the cell culture was harvested and the supernatant was stored at -70 C until it was injected intracranially into two litters of newborn Swiss albino mice (ICR, specific pathogen-free mouse colony, Centers for Disease Control, Fort Collins, Colorado 80522, USA). The brains from dead or moribund mice were used to prepare a 10% clarified suspension for virus identification by the complement fixation test (Calisher and Maness, 1975).

Serum samples were heat-inactivated at 56 C

TABLE 1. Detection of western equine encephalitis virus and neutralizing antibodies\* in free-ranging birds<sup>b</sup> in Richland County, North Dakota, August 1975.

| Avian species                   | Wahpeton                          |            | Hankinson                         |            | Rural                             |            | Total                             |            |
|---------------------------------|-----------------------------------|------------|-----------------------------------|------------|-----------------------------------|------------|-----------------------------------|------------|
|                                 | Number positive/<br>number tested | % positive | Number positive/<br>number tested | % positive | Number positive/<br>number tested | % positive | Number positive/<br>number tested | % positive |
| <b>Permanent resident</b>       |                                   |            |                                   |            |                                   |            |                                   |            |
| House sparrow                   | 14/91                             | 15         | 12/94                             | 13         | 0/8                               | 0          | 26/193                            | 14         |
| Blue jay                        | 1/1                               |            | —                                 | —          | 1/1                               |            | 2/2                               |            |
| Downy woodpecker                |                                   |            |                                   |            | 1/1                               |            | 1/1                               |            |
| Mallard                         | 1/1                               |            | —                                 | —          | —                                 | —          | 1/1                               |            |
| Other species (3) <sup>c</sup>  | —                                 | —          | —                                 | —          | 0/6                               | 0          | 0/6                               | 0          |
| Subtotal                        | 16/93                             | 17         | 12/94                             | 13         | 2/16                              | 13         | 30/203                            | 15         |
| <b>Summer resident</b>          |                                   |            |                                   |            |                                   |            |                                   |            |
| American robin                  | 3/9                               | 33         | 1/1                               |            | 7/14                              | 50         | 11/24                             | 46         |
| Northern oriole                 | —                                 | —          | —                                 | —          | 2/15                              | 13         | 2/15                              | 13         |
| Barn swallow                    | 0/1                               |            | —                                 | —          | 0/12                              | 0          | 0/13                              | 0          |
| Common grackle                  | 0/7                               | 0          | 0/3                               |            | 0/2                               |            | 0/12                              | 0          |
| Song sparrow                    | 0/7                               | 0          | —                                 | —          | 0/4                               |            | 0/11                              | 0          |
| Mourning dove                   | 0/4 <sup>d</sup>                  |            | 0/1                               |            | 0/1                               |            | 0/6                               | 0          |
| Brown thrasher                  | 0/1                               |            | —                                 | —          | 1/4                               |            | 1/5                               | 20         |
| Other species (13) <sup>e</sup> | 0/12                              | 0          | —                                 | —          | 0/18                              | 0          | 0/30                              | 0          |
| Subtotal                        | 4/41                              | 10         | 0/5                               | 0          | 11/71                             | 16         | 15/117                            | 13         |
| <b>Transient</b>                |                                   |            |                                   |            |                                   |            |                                   |            |
| Lincoln's sparrow               | 0/6 <sup>d</sup>                  | 0          | —                                 | —          | —                                 | —          | 0/6                               | 0          |
| Northern waterthrush            | 0/3                               |            | —                                 | —          | —                                 | —          | 0/3                               |            |
| Subtotal                        | 0/9                               | 0          | —                                 | —          | —                                 | —          | 0/9                               | 0          |
| Total                           | 20/143                            | 14         | 12/99                             | 12         | 13/87                             | 15         | 45/329                            | 14         |

\* &gt;80% plaque reduction of WEE virus in duck embryo cell culture.

<sup>b</sup> Includes immature birds that are flying.<sup>c</sup> Other species include black-capped chickadee, northern flicker, and hairy woodpecker.<sup>d</sup> Western equine encephalitis virus isolated from one of these sampled birds.<sup>e</sup> Other species include gray catbird, Empidonax flycatcher, American goldfinch, black-billed cuckoo, red-headed woodpecker, eastern kingbird, yellow warbler, bank swallow, great-crested flycatcher, house wren, warbling vireo, yellow-bellied sapsucker, rose-breasted grosbeak.

for 30 min and tested for neutralizing (N) antibody against WEE (McMillan strain, human brain, Ontario, Canada, 1941) and SLE (TBH-28 strain, human, Tampa Bay, Florida, USA, 1962) viruses in monolayers of DECC in 25-cm<sup>2</sup> flasks in a plaque-reduction neutralization test (Lindsey et al., 1976; McLean et al., 1983). Equal volumes of serum and virus suspension diluted to contain approximately 100 plaque-forming units were mixed and then incubated at 37°C for 1 hr. A 0.1 ml sample of the mixture was added to DECC, allowed to absorb for 90 min, and then the DECC was overlaid as described above. The DECC cultures with SLE virus received two agar overlays 3 days apart; only the second overlay contained neutral red. Reduction of plaque counts by 80% or more as compared with control cultures was considered pos-

itive for N antibody. The Chi-square ( $\chi^2$ ) test was used to statistically compare the data.

## RESULTS

Three hundred forty-two immature and adult birds of 29 species were captured during the 1 wk sampling period (Table 1). There were 88 flying birds captured with mist nets at the rural sites (0.29 birds captured/net hr), 149 captured in Wahpeton (0.56 birds/net hr), and 105 in Hankinson (1.46 birds/net hr). Significantly more ( $\chi^2 = 41.8$ ,  $P < 0.001$ ) birds were captured at urban sites than at rural sites; however, most of the birds captured within

TABLE 2. Nesting activity of birds in Richland County, North Dakota, during 9 to 15 August 1975.

| Species       | Number of nests examined | Active nests        |        | Nestlings |        |                  |                     |
|---------------|--------------------------|---------------------|--------|-----------|--------|------------------|---------------------|
|               |                          | (Eggs or nestlings) |        | Nests     |        | Mean number/nest | Range of age (days) |
|               |                          | %                   | Number | %         | Number |                  |                     |
| Barn swallow  | 101                      | 39                  | (39)   | 24        | (24)   | 3.2              | 3-17                |
| House sparrow | 21                       | 29                  | (6)    | 19        | (4)    | 3.3              | 2-10                |
| Cliff swallow | 39                       | 31                  | (12)   | 23        | (9)    | 2.2              | 2-18                |
| Pigeon        | 20                       | 65                  | (13)   | 15        | (3)    | 2.0              | 4-9                 |
| Mourning dove | 5                        | 80                  | (4)    | 40        | (2)    | 2.0              | 2-5                 |
| Song sparrow  | 1                        | 100                 | (1)    | 100       | (1)    | 3.0              | 4-5                 |
| Total         | 248                      | 36                  | (90)   | 19        | (47)   | 2.6              | 2-18                |

the two urban centers were house sparrows, particularly in Hankinson where trapping was conducted at a granary that contained feeding flocks. Trapping in Wahpeton was conducted in a city park and at a house sparrow roosting site.

Two hundred forty-eight nests of six species of birds were located and examined, mostly at the rural sites; 36% were active (i.e., contained either eggs or nestlings, Table 2). Blood samples were taken from 109 nestlings (Table 3). More nests of barn swallows were located, and thus more barn swallow nestlings were sampled (Tables 2, 3). Many nests of cliff swallows were beyond reach under bridges. Since house sparrows will occupy abandoned cliff swallow nests (Samuel, 1969), the nests of this species also were not frequently examined under bridges. Less effort was made in searching for nests of mourning doves, song sparrows, and other species. The nestlings of all the species ranged in age from approximately 2 to 18 days.

There were nine isolations of WEE virus, seven from nestlings (Tables 1, 3). The WEE virus prevalence was significantly higher ( $\chi^2 = 11.0$ ,  $P < 0.001$ ) in nestlings (6%) than in older, free-ranging birds (1%). The isolations were scattered widely among avian species and throughout the county. One of the isolations from adult birds was from a transient species (Lincoln sparrow) migrating southward. The three isolations from nestling song sparrows were all birds

from the same nest located within the residential area of Wahpeton on 15 August 1975.

The overall WEE antibody prevalence was 12%. In free-ranging birds, there were no differences in N antibody prevalences between permanent (15%) and summer (13%) resident species ( $\chi^2 = 0.1$ ,  $P < 0.8$ ) and among the rural (15%) and urban (14% and 12%) locations ( $\chi^2 = 0.2$ ,  $P < 0.7$ ) (Table 1). The N antibody prevalence was significantly lower ( $\chi^2 = 5.0$ ,  $P < 0.02$ ) in nestlings (5%) than in older birds (14%). Of the species for which adequate samples were obtained, the American robin had the highest N antibody prevalence (46%), significantly higher than all other species ( $\chi^2 = 3.4$  to 13.6,  $P < 0.05$  to 0.001), followed by the house sparrow (14%) and northern oriole (13%).

No SLE virus was isolated from birds. The overall prevalence of SLE N antibody was 8% (Table 4). In free-ranging birds, the SLE antibody prevalence was significantly less ( $\chi^2 = 13.4$ ,  $P < 0.001$ ) than that for WEE (5% versus 14%), but the antibody prevalence for SLE was significantly higher ( $\chi^2 = 6.7$ ,  $P < 0.01$ ) than for WEE in nestlings (17% versus 5%). Also for SLE, the antibody prevalence was significantly higher ( $\chi^2 = 14.3$ ,  $P < 0.001$ ) in nestlings (17%) than in older birds (5%). For older birds, there was no significant difference in the prevalence of SLE antibody between permanent and summer residents

( $\chi^2 = 0.02$ ,  $P < 0.8$ ) but there was significantly more ( $\chi^2 = 7.6$ ,  $P < 0.01$ ) SLE virus activity in rural areas.

### DISCUSSION

Western equine encephalitis has been endemic in the north-central states, with human cases occurring almost every year since 1934 (Eklund, 1946); 42 cases were reported in North Dakota during the 17 yr period from 1955 to 1971 (McGowan et al., 1973). For three separate years (1941, 1949 and 1953), >100 human cases were reported from North Dakota, and 277 cases occurred in the Red River valley of North Dakota and Minnesota during the 1975 epidemic (Leech et al., 1981). Clinical cases of WEE were widespread in 1975 with equine cases reported in 27 counties in eastern North Dakota and 30 counties in northwestern Minnesota (Potter et al., 1977). Although the sampling of the bird populations in Richland County during August 1975 was limited, the results reflect the dispersed pattern of the WEE epizootic in the region. The serologic and virologic results showed that a similar rate of WEE virus activity occurred throughout the county and between permanent and summer resident species of birds. Also, the virologic results indicated that WEE virus transmission continued in that county for about 8 wk after the onset of the first equine cases. Since equine cases usually follow increased intensity of transmission in the natural bird-mosquito cycle by several weeks, the transmission period in Richland County probably extended for at least 10 wk as it did in Hale County, Texas in 1966–1967 (Holden et al., 1973b). The virus prevalence in nestlings (6%) detected during the week of 9 to 15 August 1975 in Richland County was statistically similar to the virus prevalence in nestling house sparrows in Hale County during the same week in 1966 (6%) and during the end of the transmission period in 1967 (5%) ( $\chi^2 = 0.2$ ,  $P < 0.7$ ). Therefore, virus prevalence in nestling birds also could be used

TABLE 3. Detection of western equine encephalitis virus and neutralizing antibody in nestling birds in Richland County, North Dakota, August 1975.

| Avian species             | Number positive/<br>number tested | % Positive |
|---------------------------|-----------------------------------|------------|
| <b>Permanent resident</b> |                                   |            |
| House sparrow             |                                   |            |
| Virus <sup>a</sup>        | 0/12                              | 0          |
| Antibody <sup>b</sup>     | 1/13                              | 8          |
| Pigeon                    |                                   |            |
| Virus                     | 0/6                               | 0          |
| Antibody                  | 0/4                               |            |
| Subtotal                  |                                   |            |
| Virus                     | 0/18                              | 0          |
| Antibody                  | 1/17                              | 6          |
| <b>Summer resident</b>    |                                   |            |
| Barn swallow              |                                   |            |
| Virus                     | 1/73                              | 1          |
| Antibody                  | 3/63                              | 5          |
| Cliff swallow             |                                   |            |
| Virus                     | 2/11                              | 18         |
| Antibody                  | 0/2                               |            |
| Mourning dove             |                                   |            |
| Virus                     | 1/4                               |            |
| Antibody                  | 0/3                               |            |
| Song sparrow <sup>c</sup> |                                   |            |
| Virus                     | 3/3                               |            |
| Antibody                  | —                                 | —          |
| Subtotal                  |                                   |            |
| Virus                     | 7/91                              | 8          |
| Antibody                  | 4/85                              | 5          |
| <b>Total</b>              |                                   |            |
| Virus                     | 7/109                             | 6          |
| Antibody                  | 5/102                             | 5          |

<sup>a</sup> Western equine encephalitis virus isolated in duck embryo cell culture and identified by complement fixation test.

<sup>b</sup>  $\geq 80\%$  plaque reduction of WEE virus in duck embryo cell culture.

<sup>c</sup> Song sparrow nestlings were collected in Wahpeton; all other nestlings were collected at rural sites.

in locations, such as Richland County, to monitor WEE virus activity and possibly predict the risk to the equine and human populations as it was used, retrospectively, in Hale County, Texas. A critical threshold of virus prevalence in local nestlings to predict increased risk can only be determined after information is obtained on the infection rate during nonepidemic years. Alternatively, serologic monitoring of im-

TABLE 4. Detection of St. Louis encephalitis neutralizing antibodies\* in birds in Richland County, North Dakota, August 1975.

| Avian species                       | Wahpeton                          |            | Hankinson                         |            | Rural                             |            | Total                             |            |
|-------------------------------------|-----------------------------------|------------|-----------------------------------|------------|-----------------------------------|------------|-----------------------------------|------------|
|                                     | Number positive/<br>number tested | % Positive | Number positive/<br>number tested | % Positive | Number positive/<br>number tested | % Positive | Number positive/<br>number tested | % Positive |
| <b>Permanent resident</b>           |                                   |            |                                   |            |                                   |            |                                   |            |
| House sparrow—N <sup>b</sup>        | — <sup>b</sup>                    | —          | —                                 | —          | 3/13                              | 23         | 3/13                              | 23         |
| —Ad                                 | 1/64                              | 2          | 5/98                              | 5          | 1/6                               | 17         | 7/168                             | 4          |
| Pigeon—N                            | —                                 | —          | —                                 | —          | 2/4                               |            | 2/4                               |            |
| Blue jay—Ad                         | 0/1                               | —          | —                                 | —          | 0/1                               |            | 0/2                               |            |
| Downy woodpecker—Ad                 | —                                 | —          | —                                 | —          | 1/1                               |            | 1/1                               |            |
| Mallard—Ad                          | 0/1                               | —          | —                                 | —          | —                                 | —          | 0/1                               |            |
| Other species (3) <sup>c</sup> —Ad  | —                                 | —          | —                                 | —          | 0/6                               | 0          | 0/6                               | 0          |
| Subtotal—N                          | —                                 | —          | —                                 | —          | 5/17                              | 29         | 5/17                              | 29         |
| —Ad                                 | 1/66                              | 2          | 5/98                              | 5          | 2/14                              | 14         | 8/178                             | 5          |
| <b>Summer resident</b>              |                                   |            |                                   |            |                                   |            |                                   |            |
| Barn swallow—N                      | —                                 | —          | —                                 | —          | 12/69                             | 17         | 12/69                             | 17         |
| —Ad                                 | —                                 | —          | —                                 | —          | 1/12                              | 8          | 1/12                              | 8          |
| Cliff swallow—N                     | —                                 | —          | —                                 | —          | 0/9                               | 0          | 0/9                               | 0          |
| —Ad                                 | —                                 | —          | —                                 | —          | 0/1                               |            | 0/1                               |            |
| American robin—Ad                   | 1/8                               | 13         | —                                 | —          | 1/12                              | 8          | 2/20                              | 10         |
| Northern oriole—Ad                  | —                                 | —          | —                                 | —          | 1/14                              | 7          | 1/14                              | 7          |
| Common grackle—Ad                   | 0/6                               | 0          | 0/3                               |            | 0/2                               |            | 0/11                              | 0          |
| Song sparrow—Ad                     | 0/6                               | 0          | —                                 | —          | 0/4                               |            | 0/10                              | 0          |
| Mourning dove—N                     | —                                 | —          | —                                 | —          | 0/3                               |            | 0/3                               |            |
| —Ad                                 | 1/3                               |            | 0/1                               |            | —                                 | —          | 1/4                               |            |
| Other species (14) <sup>d</sup> —Ad | 1/13                              | 8          | —                                 | —          | 0/11                              | 0          | 1/35                              | 3          |
| Subtotal—N                          | —                                 | —          | —                                 | —          | 12/81                             | 15         | 12/81                             | 15         |
| —Ad                                 | 3/36                              | 8          | 0/4                               |            | 3/67                              | 5          | 6/107                             | 6          |
| <b>Transient</b>                    |                                   |            |                                   |            |                                   |            |                                   |            |
| Lincoln's sparrow—Ad                | 0/6                               | 0          | —                                 | —          | —                                 | —          | 0/6                               | 0          |
| Northern waterthrush—Ad             | 0/3                               |            | —                                 | —          | —                                 | —          | 0/3                               |            |
| Subtotal—Ad                         | 0/9                               | 0          | —                                 | —          | —                                 | —          | 0/9                               | 0          |
| Total—N                             | —                                 | —          | —                                 | —          | 17/98                             | 17         | 17/98                             | 17         |
| —Ad                                 | 4/111                             | 4          | 5/102                             | 5          | 5/81                              | 6          | 14/294                            | 5          |

\* ≥ 80% plaque reduction of SLE virus in duck embryo cell culture. No virus was isolated.

<sup>b</sup> N, nestling; Ad, adult including immature that are flying.<sup>c</sup> Other species included black-capped chickadee, northern flicker, and hairy woodpecker.<sup>d</sup> Other species included rose-breasted grosbeak, brown thrasher, gray catbird, Empidonax flycatcher, American goldfinch, black-billed cuckoo, red-headed woodpecker, eastern kingbird, yellow warbler, bank swallow, great-crested flycatcher, warbling vireo, yellow-bellied sapsucker, and house wren (1/1 positive).

mature, free-ranging birds of several, locally-important host species (e.g., American robin and house sparrow) could be used for surveillance if testing is rapid, accurate and sensitive (McLean et al., 1983). In Hale County, nestling house sparrows were thought to be the major am-

plifying hosts of WEE virus every year because of their high prevalences of viremia, because house sparrows comprise over two-thirds of the avian population, and because of their close association with humans and the vector mosquito, *C. tarsalis* (Holden et al., 1973b). However, the

lower infection rates of house sparrows found during this study (14% N antibody), as compared to the significantly higher ( $\chi^2 = 40.2$ ,  $P < 0.001$ ) infection rates in Hale County [56% in 1966 and 45% in 1967 for hemagglutination-inhibition (HI) antibody], would necessitate the use of other bird species instead of or in addition to nestling house sparrows for surveillance. Also, house sparrows comprise a much smaller proportion of the permanent and summer resident birds in Richland County than in Hale County.

The similarity between the N antibody prevalences of permanent and summer resident species indicated that the birds became infected with WEE virus locally. Too few transient species of birds were captured to provide further evidence of local WEE transmission as was found for SLE virus in northern Illinois birds in 1975 (McLean and Bowen, 1980) and eastern equine encephalitis (EEE) virus in Michigan birds in 1980 (McLean et al., 1985). However, the isolation of WEE virus from one of the few transient birds captured provides further evidence of the movement of arboviruses in North America by migratory birds (Stamm and Newman, 1963; Calisher et al., 1971; McLean et al., 1985).

The high prevalence of N antibody and lack of virus isolations in the American robin suggest that this species was probably involved early in the WEE transmission period. The virus isolations from adult and nestling mourning doves at both rural and urban sites indicate the possible importance of this species to the epizootiology of WEE. The multiple infection of the song sparrow nestlings with WEE was not unusual because multiple infections of WEE virus in siblings within a nest and even dual infections with more than one arbovirus have been reported for sibling house sparrow and red-winged blackbird nestlings (Hayes et al., 1967; Holden et al., 1973b). Field and experimental evidence suggested that the multiple infections are a result of mosquito transmission and not

direct bird-to-bird transmission of WEE virus by transovarial transmission from parent to nestling or transfer of virus between nestlings (Holden et al., 1973b). Multiple infections within broods of nestlings could aid in the local amplification of virus transmission.

The age-related data on virus and antibody prevalence indicate current, intense WEE virus activity (high virus prevalence in nestling and relatively high antibody prevalence in adult birds); whereas, SLE virus activity was less intense in the bird populations of Richland County. The fact that we made no SLE virus isolations and that the prevalence of SLE antibody was 8% for all ages of birds, 5% for all free-ranging birds and 4% for house sparrows indicate enzootic virus activity (McLean and Bowen, 1980; McLean et al., 1983). There was no significant difference for the overall prevalence of SLE antibody in adult birds between permanent and summer residents ( $\chi^2 = 0.2$ ,  $P < 0.7$ ), although there was more SLE activity in rural areas ( $\chi^2 = 7.6$ ,  $P < 0.01$ ). The substantially higher prevalence of SLE antibody in nestling birds than adults ( $\chi^2 = 14.3$ ,  $P < 0.001$ ) suggests a differential exposure related to age in the sampled birds or the presence of detectable maternal N antibody in nestlings, particularly for house sparrows and barn swallows. This was not true for WEE because a low prevalence of N antibody and higher prevalence of virus were found in nestlings. Maternal antibody against both SLE and WEE viruses has been detected in nestlings of several avian species (Reeves et al., 1954; Sooter et al., 1954; Bond et al., 1965; Holden et al., 1973a). In laboratory experiments, WEE antibody was not detected in nestlings of WEE-immune house sparrows, and these nestlings were as susceptible to infection with WEE virus as were nestlings of nonimmune house sparrows (Holden et al., 1973a); whereas, maternal N antibody to SLE virus was detected up to 16 days posthatching in house sparrow nestlings from SLE immune adult females. How-



ever, immune nestlings challenged with SLE virus produced viremias of equal or greater duration and magnitude than did controls (Ludwig et al., 1986). This viremic enhancement could further aid in the local amplification of SLE virus transmission. An alternative explanation for the disparity in the antibody prevalence in nestlings against SLE and WEE viruses may be differential mortality. Virus (WEE) has been isolated from the brain of dead house sparrow nestlings in the field (Holden et al., 1973b), and a reduced average survival time in WEE-inoculated nestling house sparrows was observed in a laboratory study (Holden et al., 1973a); whereas, SLE virus does not appear to cause mortality in infected nestling or adult birds (Chamberlain et al., 1957; McLean et al., 1983; Ludwig et al., 1986). Therefore, antibody prevalence in adult birds that survived the infection and in their subsequent offspring (from maternal antibody) would be reduced in avian species that die from the virus as nestlings.

The long history of enzootic and epizootic WEE in this region should stimulate the annual vaccination of equines and the continuation of a surveillance program. The information obtained during this study on the relative abundance of nests and nesting activity as well as on virus and antibody prevalences in various bird species in Richland County should be helpful in selecting sentinel species and in planning future sampling efforts.

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