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# WILD BIRDS AS EASTERN (EEE) AND WESTERN (WEE) EQUINE ENCEPHALITIS SENTINELS

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*Abstract:* Six species of wild birds were studied for their suitability as sentinels to detect the transmission of EEE and WEE viruses in the Pocomoke Cypress Swamp, Maryland. Blood specimens from birds were tested for virus and for neutralizing antibodies in tubes of primary hamster kidney cell culture. Virus isolations and serological data from bobwhite quail, white-throated sparrows, red-winged blackbirds and English sparrows indicated that transmission of WEE virus began in late July, 1968, and preceded the onset of EEE virus transmission by several weeks and that transmission of both viruses continued into November. Of the species tested, bobwhite quail and white-throated sparrows survived best. Selection and use of wild birds as sentinels are discussed.

## INTRODUCTION

Wild birds, implicated as natural hosts of EEE and WEE in the eastern United States,[5,6,10,13] would seem a logical choice for experimental animals to study natural host - vector relationships, where the amount of virus passed to vectors may depend on species composition, age structure or density of the bird population in a given area. Wild birds, however, have seldom been used in field experiments to investigate EEE or WEE, although the work of Stamm,[12] who detected the transmission of EEE virus by serially bleeding wild yellow - crowned night herons, has indicated that wild birds might be useful as sentinels. Therefore, we attempted to assess the usefulness of wild birds for field experiments designed to detect virus transmission and to determine what characteristics make a species suitable for use as a sentinel. Experiments were conducted in and on the edge of Pocomoke Cypress Swamp, Worcester County, Maryland,[8] where wild birds have been implicated as hosts of EEE and WEE viruses and where both viruses have been isolated repeatedly for several years (Buescher, Yuill and Muul, *unpublished).*

## MATERIALS AND METHODS

Bobwhite quail *(Colinus virginianus)* were purchased when 4-6 weeks old and were maintained indoors until 2 - 2½ months of age, when they were placed in the study area in groups of 10 or less in pens 28 inches long, 12 inches high and 24 inches wide. White-throated sparrows *(Zonotrichia albicollis),* purple grackles *(Quiscalus quiscula),* cowbirds *(Molothrus ater),* red-winged blackbirds *(Agelaius phoeniceus)* and English spar-

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rows *(Passer domesticus)* were captured by mist-net in the vicinity of the swamp, separated by species into groups of 10 or less and maintained in cages measuring 36 inches long, 48 inches high and 18 inches wide.[4]

Blood samples were obtained by jugular venipuncture with syringes moistened with a saline-heparin solution (50 USP units/ml) when birds were introduced into the swamp and at approximately 2-week intervals thereafter. Plasmas were separated by low speed centrifugation and stored at —20 C for use in neutralization tests. Packed cells were stored at —60 C for virus isolation attempts. In addition, a number of dead sentinel birds were collected and stored at —60 C until examined for virus.

#### Neutralization tests

Blood plasmas were heat inactivated (56 C for 30 min) and diluted 1:5 in medium 199 containing 10% inactivated fetal bovine serum (FBS), penicillin (200 units/ml), streptomycin (200 μg/ml) and fungizone (5 μg/ml). Then, an equal volume (0.2 ml) of 10-100 $TCID_{50}$ of either EEE or WEE virus was added to each plasma. Viruses used were EEE m-2449/64 and WEE m-3249/65, both initially obtained as mosquito isolates from the Pocomoke Cypress Swamp. EEE m-2449/64 had been passaged twice in mice prior to one passage in primary hamster kidney cell culture (HK; Grand Island Biological Company, New York), while WEE m-3249/65 was used at the second HK passage level. Plasma-virus mixtures were incubated at 37 C for 30 min prior to inoculation into 2 HK tubes (0.1 ml/tube), which subsequently were incubated at 37 C and examined against control HK tubes for cytopathic effect (CPE). Absence of CPE for 5 days was taken as evidence of antibody in the plasma. Specimens showing CPE in a single tube were retested, if plasma was available, or considered negative.

#### Virus isolation

Packed blood cells were prepared as 20% suspensions in basal medium Eagles' (BME) containing 20% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml) and fungizone (5 μg/ml). Each suspension was inoculated into 2 drained HK tubes (0.1 ml/tube), which were incubated at 37 C for 2 hr and then rinsed with 5% BME-FBS, which also was employed as a maintenance medium. Tubes were observed for at least 5 days. If CPE occurred, passage was made to fresh HK tubes prior to identification of the virus by neutralization with specific EEE or WEE rabbit sera, and a second isolation of the virus was attempted from the original specimen. Thawed bird brains were examined for virus following extraction with needle and syringe. Brains were homogenized in Tenbroeck grinders and centrifuged. The supernatant fluid was inoculated into HK tubes, as above.

## RESULTS

#### Virus isolations

Viruses were recovered from blood samples and from brains of dead sentinels collected between 22 July and 29 October (Table 1). An isolation of WEE was obtained several weeks before the first EEE isolation. Most virus isolations were made from female red-winged blackbirds and female English sparrows. In one English sparrow, EEE virus was isolated from two blood samples taken 10 days apart, and several of the isolations from English sparrows came from birds showing evidence of neutralizing antibody in their sera (Table 1).

#### Detection of antibody

Neutralizing antibodies to EEE or to WEE were not detected in commercially procured quail prior to their exposure in the study area. Antibodies were detected in some birds caught from the wild (Table 2). Neutralizing antibody for EEE virus was found in English sparrows and white-throated sparrows, whereas antibody for WEE virus was found in all the species netted.

Rates of antibody appearance in nonimmune birds were obtained for bobwhite quail, white-throated sparrows, red-winged blackbirds and English sparrows (Table 3). WEE neutralizing antibody appeared in these species between

17 July and 7 August, whereas EEE antibody appeared first in two bobwhite quail bled on 6 August and in the other species shortly thereafter. Most birds produced antibodies to both viruses between 8 August and mid-September. Additional non-immune red-winged blackbirds were placed in the swamp on 26 September and on 15 October. Incidence of antibody in these new birds, together with the occasional appearance of antibody in older sentinels, indicated that EEE and WEE viruses were being transmitted as late as November in 1968 (Table 3).

TABLE 1. Virus isolations made from sentinel birds in 1968.

| Species and number used from 15 July to December | Date and source of isolation | |
|---|---|---|
| | WEE virus | EEE virus |
| Bobwhite quail; | | |
| 41 males and 38 females: | 20 Aug (♂ blood)[a,b] | None |
| White-throated sparrow; | | |
| 22; sexes not identified: | None | 7 Aug (blood)[c]<br>7 Aug (blood)[b] |
| Purple grackle; 3: | None | None |
| Cowbird; 3: | 7 Aug (♂ brain)[a] | None |
| Red-winged blackbird; | | |
| 44 males and 54 females: | 15 Oct (♀ blood)[a,d,e] | 7 Aug (♂ brain)<br>3 Sep (♀ brain)<br>4 Oct (♂ brain)[a]<br>9 Oct (♀ blood)[c]<br>9 Oct (♀ blood)[c]<br>14 Oct (♀ brain)<br>15 Oct (♀ blood)[c,f]<br>15 Oct (♀ blood)[d]<br>29 Oct (♀ blood)[a,c] |
| English sparrow; 40 males, | | |
| 61 females, 7 unknown sex: | 22 Jul (blood) [c]<br>7 Aug (♀ blood)[c]<br>7 Aug (♀ blood)[a,b]<br>7 Aug (♀ blood)[a,c,f]<br>29 Aug (♀ blood)[a,c]<br>1 Sep (♀ brain)[a] | 7 Aug (♂ blood)[c,e,f]<br>13 Aug (♀ brain)[e]<br>19 Aug (♀ blood)[e,f,g]<br>27 Aug (♂ blood)[c]<br>29 Aug (♀ blood)[c,e,f,g]<br>3 Sep (♀ brain)[e]<br>3 Sep (♀ brain)[e]<br>5 Sep (♀ blood)[a,c,e,f]<br>11 Sep (♀ blood) [a,c,e] |

Notes on isolations:

a. Attempt to reisolate virus from original specimen was unsuccessful.
b. Survived 2-weeks or more, subsequently produced antibody to virus isolated.
c. Died soon after date of isolation.
d. Survived 2-weeks or more, but antibody to virus isolated not detected.
e. Possessed neutralizing antibody to the other virus on date of isolation.
f. Possessed neutralizing antibody to the virus isolated on date of isolation.
g. Isolations of 19 Aug and 29 Aug came from the same bird.

TABLE 2. Neutralizing antibody in birds obtained for use as sentinels.

| Species | Date of initial blood samples (1968) | Antibody No. positive/No. tested WEE | EEE |
|---|---|---|---|
| Bobwhite quail | 6 June - 23 Jul | 0/100 | 0/100 |
| White-throated sparrow | 29 Jan - 29 Apr | 2/43 | 3/43 |
| Purple grackle | 22 Apr - 21 May | 2/24 | 0/24 |
| Cowbird | 24 Apr - 21 May | 1/34 | 0/34 |
| Red-winged blackbird | 30 Jan - 21 May | 2/66 | 0/66 |
| | 18 - 26 Sep | 0/57 | 1/57 |
| | 15 Oct | 0/32 | 5/32 |
| English sparrow | 29 Jan - 27 May | 9/45 | 3/45 |
| | 22 - 23 Jul | 0/31 | 0/31 |
| | 19 - 29 Aug | 10/32 | 7/32 |

TABLE 3. Incidence of neutralizing antibody in sentinels.

| Species and date (1968) | Percent non-immune birds developing antibody (Number positive/total birds) WEE antibody | EEE antibody |
|---|---|---|
| Bobwhite quail: | | |
| 5 June - 1 Jul | 0 (0/56) | 0 (0/56) |
| 2 Jul - 16 Jul | 0 (0/74) | 0 (0/74) |
| 17 Jul - 29 Jul | 11 (8/76) | 0 (0/76) |
| 30 Jul - 15 Aug | 28 (20/71) | 27 (21/78) |
| 16 Aug - 4 Sep | 73 (36/49) | 64 (36/56) |
| 5 Sep - 17 Sep | 38 (5/13) | 25 (5/20) |
| 18 Sep - 15 Oct | 43 (3/7) | 53 (8/15) |
| 16 Oct - 29 Oct | 0 (0/4) | 29 (2/7) |
| 30 Oct - 10 Dec | 0 (0/3) | 0 (0/5) |
| White-throated sparrow: | | |
| 28 May - 15 Jul | 0 (0/20) | 0 (0/21) |
| 16 Jul - 7 Aug | 21 (4/19) | 0 (0/20) |
| 8 Aug - 5 Sep | 33 (4/12) | 65 (11/17) |
| 6 Sep - 25 Sep | 33 (2/6) | 0 (0/5) |
| 26 Sep - 24 Oct | 0 (0/2) | 0 (0/5) |
| 25 Oct - 10 Dec | 0 (0/2) | 50 (2/4) |
| Red-winged blackbird: | | |
| 3 Jun - 8 Jul | 0 (0/9) | 0 (0/10) |
| 9 Jul - 23 Jul | 0 (0/5) | 0 (0/5) |
| 24 Jul - 7 Aug | 20 (1/5) | 0 (0/5) |
| 8 Aug - 19 Aug | 67 (2/3) | 25 (1/4) |
| 20 Aug - 15 Oct | 20 (10/49) | 55 (28/51) |
| 16 Oct - 14 Nov | 13 (5/39) | 38 (10/26) |
| 15 Nov - 10 Dec | 5 (1/22) | 22 (2/9) |
| English sparrow: | | |
| 28 May - 24 Jun | 0 (0/16) | 0 (0/19) |
| 25 Jun - 23 Jul | 0 (0/12) | 0 (0/15) |
| 24 Jul - 7 Aug | 22 (5/23) | 0 (0/24) |
| 8 Aug - 11 Sep | 55 (17/31) | 77 (17/22) |
| 12 Sep - 23 Oct | 50 (2/4) | 67 (2/3) |
| 24 Oct - 10 Dec | 100 (1/1) | 0 (0/1) |

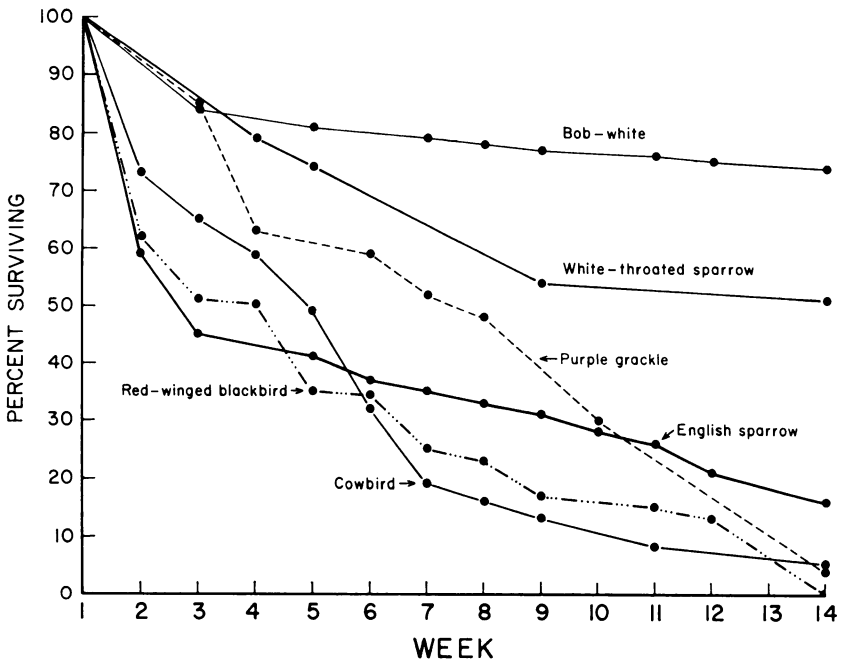


FIGURE 1. Survival of sentinel birds.

Whereas bobwhite quail and white-throated sparrows survived well, other species did not (Fig. 1). Most cowbirds and grackles did not survive to the period of virus transmission, although one cowbird that did survive produced WEE antibody, and one grackle produced EEE antibody. In the case of grackles and red-winged blackbirds, much aggression was noted between individuals, and a considerable number of dead birds were found which appeared to have been pecked to death. Most of the red-winged blackbirds and English sparrows which showed virus in the blood died shortly afterwards (Table 1). However, the frequency of death directly attributable to virus infection could not be determined, as we did not examine all dead birds for virus.

## DISCUSSION

Our study demonstrates that wild birds can be used to: 1) provide proof of EEE and WEE transmission through the isolation of viruses, 2) demonstrate differences in onset of transmission of the two viruses, and 3) monitor the duration of virus transmission to birds in a given area. Duration of virus transmission can be determined from virus isolations or from serological evidence, if the period between infection and the appearance of antibody is known. Neutralizing antibody may appear within 2 weeks after infection with EEE or WEE virus in passerine[14,15] or gallinaceous[2,7] birds. In this study, antibodies appeared in sentinel populations 1 or 2 weeks after the first isolations of virus were made.

A number of considerations determine the appropriateness of a bird species for sentinel use: 1) availability, 2) frequency of pre-existing antibody, 3) survival under study conditions, 4) attractiveness to vectors, and 5) response to infection. A commercial source, such as was used for bobwhite quail, is an excellent means of obtaining sentinels if young birds are available, since problems of pre-existing antibody can be eliminated. The frequency of pre-existing antibody in wild-caught birds probably can be lowered by collecting them well ahead of the expected period of virus transmission or from an area where the viruses do not occur. Species in which antibody has been found probably are attractive to insect vectors, are susceptible to infection and could be used as sentinels if survival is adequate. Survival, in turn, may depend upon many factors, of which intraspecies aggressiveness and food requirements were important in our study. For sentinels, probably granivores are a better choice than insectivorous or omnivorous birds, since proper food is more easily provided.

Bobwhite quail and white-throated sparrows were good sentinels for monitoring transmission of EEE and WEE viruses in our study environment. Survival rates and antibody responses were adequate, and the small number of virus recoveries from these species suggests that their viremias were short term or low level. In contrast, other species were unacceptable as sentinels because of poor survival. English sparrows may be useful as sentinels in studies undertaken in environments where they occur naturally and play a role in the virus ecology[1,3] or where isolation of viruses is important. However, English sparrows appear to have long term and high level viremias,[11] can maintain chronic latent virus infections[11] and might provide continued sources of virus to vectors. Thus, if English sparrows are used as sentinels, they should be exposed to an environment in a manner which prevents them from infecting mosquitoes in the study area. They might be utilized in mosquito traps of some sort.

Wild bird sentinels could be useful in several ways. They could be studied in field enclosures, similar to those of Norris,[9] to determine how changes in density, age structure, sex ratio or other parameters affect rates of virus transmission to an avian population. In addition, some species employed to detect virus transmission may be superior to chickens, which usually are used for this purpose,[3,4] because smaller size permits greater ease of bleeding and less costly maintenance.

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