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THE BLOOD-FEEDING HABITS OF *AEDES SOLLICITANS* (WALKER) IN RELATION TO EASTERN EQUINE ENCEPHALITIS VIRUS IN COASTAL AREAS OF NEW JERSEY I. HOST SELECTION IN NATURE DETERMINED BY PRECIPITIN TESTS ON WILD-CAUGHT SPECIMENS[1]

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ABSTRACT: Precipitin tests were conducted on 1735 wild-caught *Aedes sollicitans* (Walker) collected from upland and salt marsh habitats in coastal areas of New Jersey from 1962-1984. Results showed that 98.2 percent of the blood meals came from mammalian hosts. Deer represented the host most often fed upon in both upland and salt marsh habitats. Small mammals (primarily rabbits and rodents) represented 8.4-13.9 percent of the overall mammalian feedings. Birds accounted for 1.4 percent of the blood meals identified during the investigation. In upland areas, passerine and gallinaceous birds were fed upon most frequently, but in salt marsh areas virtually all of the blood meals were derived from ciconiiforms. The significance of these findings is discussed in relation to the epidemiology of eastern equine encephalitis virus.

INTRODUCTION

The salt marsh mosquito, *Aedes sollicitans* (Walker), is common along the eastern coast of the United States and creates considerable nuisance through most of its range (Carpenter and LaCasse 1955). *Aedes sollicitans* is also an extremely efficient vector of eastern equine encephalitis (EEE) virus (Chamberlain et al. 1954) and has been implicated as a probable vector to humans along the New Jersey coast (Kandle 1960, Hayes et al. 1962, Goldfield et al. 1966). Crans (1977) reviewed the literature in this regard and observed that *Ae. sollicitans* met each of the criteria outlined by Sudia et al. (1975) for documentation of vector status. Since that time, additional evidence has been obtained to show that *Ae. sollicitans* does acquire EEE virus during epizootic periods in coastal areas of New Jersey where human cases of the disease have been most common (Crans et al. 1986).

Wild birds serve as the primary hosts of EEE virus (Stamm et al. 1962, Stamm 1963) and small birds in the order Passeriformes are known to circulate high virus titers (Kissling et al. 1954) that can infect a variety of mosquito species, including *Ae. sollicitans* (Chamberlain et al. 1954). To acquire EEE virus, *Ae. sollicitans* would be expected to feed on birds during their viremic period. Available data, however, suggest that *Ae. sollicitans* is a mammal feeding species and only rarely feeds on birds in nature (Thompson et al. 1963, Edman and Downe 1964, Schaefer and Steelman 1969, Edman 1971).

In 1962, a long-term study was initiated to investigate the blood-feeding habits of *Ae. sollicitans* in areas of New Jersey where EEE was known to be enzootic. Initial work focused on the collection of blooded specimens for precipitin testing. Later work focused on behavior related to the blood-meal patterns that emerged. This paper and those that follow in the series examine factors that influence avian feeding by *Ae. sollicitans* and relate that information to the epidemiology of EEE virus and its potential transfer to humans.

MATERIALS AND METHODS

The mosquitoes tested in this investigation were collected in coastal areas of southern New Jersey from 1962-1984. Two major types of habitats were sampled. In upland areas, mosquitoes were collected from grassy fields and their woodland borders within 1 km of salt marsh habitat. Mist net records from the study sites

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showed that more than 80 species of passerine birds frequented the habitat during the season (Crans and Caccamise in prep.). In tidal areas, mosquitoes were collected from stands of the salt marsh grasses, *Distichilis spicata* and *Spartina patens* that bordered upland forest. A variety of salt marsh wading birds foraged in the open water on the marsh and some utilized the upland edge as a roosting site. Commonly observed birds included: glossy ibis *(Plegadis falcinellus)*, black-crowned night heron *(Nycticorax nycticorax)*, little blue heron *(Florida caerulea)*, great blue heron *(Ardea herodius)*, snowy egret *(Egretta thula)*, and great egret *(Casmerodius albus)*.

The majority of mosquitoes tested in these studies were collected by sweeping vegetation with an insect collection net or by vacuuming vegetation with a power aspirator. Samples were frozen in the field on dry ice and later separated from debris by hand. In upland areas, a small portion of the test series were taken from light trap and resting box collections .

The abdominal contents of any specimens containing evidence of a blood meal were expressed onto filter paper in the laboratory by rolling each abdomen with a clean glass rod. The filter spots were coded and stored for future testing by precipitin techniques.

Preparation of Antisera

Antisera used for the identification of mosquito blood meals were prepared both in rabbits and chickens. New Zealand-white rabbits were used primarily for the production of screening antisera to identify the blood meal with a broad host category. White leghorn roosters were used for the production of more specific antisera, including antisera to rabbit and to the avian orders.

Rabbits were presensitized with a single 1.0 ml injection of antigen approximately one month prior to the initial injection series. Thereafter, each rabbit received four 1.0 ml subcutaneous injections on alternate days with antigen that was diluted with five parts 0.9 percent normal saline. Seven days after the last injection in the series, the rabbits were trial bled from the ear and the serum fraction was tested for titer. Rabbits that yielded a titer below 1:5000 for homologous sera were reinjected with a second full series. Rabbits with sufficient titer were bled from the ear and reused for antisera production at a later date.

Chickens received two intravenous injections of 2.5 ml undiluted serum with two days between injections. Seven days after the last injection the birds were exsanguinated from the heart. Antisera prepared in chickens were brought to an NaCl concentration of 8.0 percent in keeping with the conditions of maximum precipitation described by Goodman et al. (1951).

Blood drawn from the test animals was transferred directly to 50 ml test tubes and allowed to separate overnight. The following day, the serum fraction was decanted and the clots were centrifuged at least once to recover as much serum as possible. Before storage, 0.1 percent sodium azide was added to the sample. The antisera were then bottled in 5 ml portions and refrigerated at approximately 40°C. Each of the antisera used in the test series was periodically screened for heterologous reaction with routine tests against the serum of animals included in the test pattern. If heterophilic reactions were noted, antisera were adsorbed with heterologous sera. When adsorption resulted in a drop in titer below 1:5000, the antiserum was discarded.

Blood Meal Identification

For blood meal identification, each blood spot previously expressed onto filter paper was cut from the paper and extracted overnight in 0.3 ml of 0.9 percent physiological saline. The specimens were tested for host by the agar gel diffusion method described by Crans (1969). All blood spots were screened first for broad category of host, using the polyvalent antisera prepared in rabbits, and then tested for specific host, using the antisera prepared in chickens.

RESULTS

A total of 1,735 blood meals reacted to one or more of the antisera used in the test series. TABLE 1 lists the results obtained from the initial screenings with the polyvalent antisera prepared in rabbits. Data show that 98.2 percent of specimens contained mammalian blood and only 1.4 percent contained blood from an avian source. Blood from reptiles and amphibians was detected in some of the specimens but represented less than 1 percent of the total. No significant differences in the broad pattern of blood feeding were found when the data were analyzed by year, month, site or collection method.

TABLE 2 lists the mammalian hosts for those specimens that gave a clear reaction to a specific host category with the specific antisera prepared in chickens. Multiple feedings have been counted once for each host. Data show that *Ae. sollicitans* fed most frequently on large mammals with deer being the predominant host in both habitats. Rabbits and rodents represent the most frequently fed upon small mammals. Rodent blood was more common in the specimens collected from the salt marsh, possibly reflecting the large rodent populations known to be present on the salt marshes of New Jersey (Bosenberg 1979). Data show that some of the specimens captured on the salt marsh obtained their blood meals at more inland sites. Although cattle were present about 1

TABLE 1. Vertebrate Class of the blood meal hosts of *Aedes sollicitans* collected in New Jersey from 1962 to 1984.

Vertebrate Class	Number Reacting	Percent of Total
Mammal	1,703	98.2
Avian	25	1.4
Reptile	5	0.3
Amphibian	2	0.1

TABLE 2. A comparison of the mammalian hosts detected in *Aedes sollicitans* from upland and salt marsh habitats of New Jersey.

Host	Upland	Salt Marsh
Deer	894	306
Human	79	1
Horse	23	3
Cow	13	85
Pig	8	0
Total Large Mammal	1,017 (91.6%)	395 (86.1%)
Canine	17	0
Rabbit	58	28
Rodent	18	32
Raccoon	0	4
Total Small Mammal	93 (8.4%)	64 (13.9%)
Total Specimens	1,110	459

km from the collection site, horses were not kept at any of the local farms in the area.

TABLE 3 lists the avian blood meals identified in this study by Order. In upland areas, the specimens most frequently tested positive for passerine and gallinaceous avian hosts. On the salt marsh, virtually all of the specimens tested positive for members of the heron group, the dominant bird in that habitat.

DISCUSSION

The data presented in this study support the findings of other investigators who have shown that *Ae. sollicitans* is strongly oriented toward mammalian hosts (Crans 1964, Edman and Downe 1964, Schaefer and Steelman 1969, Edman 1971, Magnarelli 1977). Avian blood meals appear to be rare in *Ae. sollicitans*

TABLE 3. A comparison of the avian hosts detected in *Aedes sollicitans* from upland and salt marsh habitats of New Jersey.

Order	Upland	Salt Marsh
Passeriformes	4	0
Galliformes	3	0
Anseriformes	1	0
Ciconiiformes	0	5
Ciconiiformes/ Passeriformes*	0	3
Galliformes/ Passeriformes*	9	0

*Samples reacted to both antisera.

and neither geographical location nor habitat appear to change that fact. The number of avian feedings on the salt marsh did not significantly differ from those in upland collections. Thompson et al. (1963) and Schaefer and Steelman (1969) collected *Ae. sollicitans* from more southern salt marsh habitats and obtained similar results.

Few studies have identified avian blood meals to Order, but Edman (1971) did show that *Culex* species that were oriented toward avian hosts fed largely on Ciconiiformes in a brackish habitat in Florida. In our study, all of the avian feedings detected in *Ae. sollicitans* collected from salt marsh habitat contained the blood of Ciconiiformes indicating that a small percentage of the host seeking population does take advantage of a relatively abundant avian blood meal source on the salt marsh - upland ecotone. Kissling et al. (1954) found eight species of herons with antibody to EEE in a coastal area of Louisiana where EEE was known to be enzootic. To date, no one has further investigated the role of Ciconiiformes as a possible disseminating agent for the encephalitis viruses in salt marsh areas.

Edman et al. (1974) showed that many of the wading birds engage in anti-mosquito behavior and, thereby, discourage blood feeding by host seeking mosquitoes. Tolerant wading species, however, also exist and the black-crowned night heron *(Nycticorax nycticorax)*, green heron *(Butorides striatus)*, great egret *(Casmerodius albus)*, and great blue heron *(Ardea herodius)* may have special epidemiological importance because of their mosquito tolerance. The great blue heron and little blue heron *(Florida caerulea)* were very common in the areas where *Ae. sollicitans* were collected in our study and were observed to move from the salt marsh to adjacent cedar swamps where *Culiseta melanura*, the enzoootic vector of EEE, occurred in large numbers. Crans et al. (1986) collected two pools of *Ae. sollicitans* that were infected with EEE virus from this general area during an epizootic in 1982.

The results of this investigation show that *Ae. sollicitans* is strongly oriented to mammalian hosts. Mammals constitute the overwhelming majority of blood meal hosts in field collected specimens but avian hosts are occasionally fed upon in upland as well as estuarine habitats. Since virus has been isolated from *Ae. sollicitans* during epizootic periods, the occasional infectious specimen detected during arbovirus surveillance is most likely the result of this species' limited contact with birds. Most of the data on avian sources of EEE amplification have focused on Passerine species (Stamm 1963, Emord and Morris 1984). Our data suggest that nonpasserine bird species should be more closely investigated as a possible reservoir for EEE virus especially in coastal areas where large potential vector populations emanate from salt marsh habitat.

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