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RECENT ADVANCES IN RESEARCH ON THE EASTERN ENCEPHALITIS VIRUS**

Knowledge of the ecology of the Eastern Encephalitis (EE) virus has accumulated rapidly since the agent was first isolated in 1933. The sporadic incidence of infections, lack of direct contact except among pheasants in the same pen, association of outbreaks with swampland areas, and the occurrence of infections following the peak of the mosquito season led to the theory that the virus was transmitted by mosquitoes. Consequently, in early investigations attention was directed to coastal areas and the probability of salt marsh mosquitoes as vectors.

However, in reports of epidemics among pheasants in New Jersey by Beaudette and co-workers,[1-7] the pattern of spread of the virus produced doubt concerning arthropod transmission. Where cases were confined to pheasants in one pen and failed to appear among those in adjacent pens separated only by large mesh chicken wire through which mosquitoes might readily pass, it was obvious that mosquitoes were not responsible for transmission. Similar circumstances were encountered in the course of investigating EE activity among pheasants in Connecticut[39, 41, 48, 50] as well as the occurrence of high mortality at a time when cold weather suppressed adult mosquito activity. However, in the study of daily mortality records it was found that initial deaths occurred during a period of warm weather when mosquito activity was high.

This led to consideration of *primary transmission* into a pheasant flock, probably by an infected mosquito or wild bird, and from the initial case, *secondary contact transmission* that accounted for the rapid transfer of virus from pheasant to pheasant within the flock. The secondary transmission accounted for spectacular mortality which persisted beyond the mosquito season into the cool fall weather. The postulation of the existence of an extra-arthropod secondary type of transmission and the subsequent

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support of this hypothesis by results of controlled field experiments[31, 32, 41, 43, 60] cleared the way for concentration of study on arthropod transmission, but it still left the problem of identifying the arthropod or arthropods involved in the primary spread of the virus from its natural reservoir cycle in nature.

The belief that the EE virus was transmitted primarily by mosquitoes has been supported by an accumulation of evidence in recent years, despite the fact that the virus has been isolated from several other arthropods. Howitt, *et al.*[28] isolated it from mites (*Dermanyssus gallinae*) and chicken lice (*Eumenacanthus stramineus*) collected in Tennessee. The same workers isolated the virus from a mosquito (*Mansonia perturbans*) in Georgia.[29] Chamberlain and co-workers[10] obtained the virus from the mosquito, *Culiseta melanura,* in Louisiana and later from another, *Anopheles crucians,* in the same area.[11] Holden, *et al.*,[27] in New Jersey, recovered virus from three pools of *C. melanura.* Recently, Karstad and co-workers[36] reported isolations from pools of *Anopheles crucians* and *Aedes mitchellae,* and from *Culicoides* species in Georgia. In 1956, during an epidemic in pheasants in New Jersey, four isolations of EE virus were obtained from pools of *C. melanura,*[14] and during the same year five more isolations were obtained from the same species in Massachusetts.[18] This was strong evidence that *C. melanura* was significantly involved in transmission among birds. However, it was suggested in 1959[46] that other species must be considered, because in studies of the mosquito populations at farms in Connecticut where virus activity occurred between 1938 and 1956 (including 20 farms at the time of pheasant and horse deaths) *C. melanura* could be found on only 3 of 25 farms.

Subsequently, the virus was isolated from *Aedes vexans* collected in Connecticut in 1959.[52] However, additional isolations were obtained from *C. melanura* in Massachusetts[24] and from New Jersey,[9] so that at the present time isolations from all other arthropods number less than half those reported from *C. melanura.* From this, Chamberlain, in 1958,[9] postulated that in a "sylvan" cycle of transmission of virus among wild birds, *C. melanura* was primarily involved as the vector. However, in view of the rarity with which this species feeds upon mammals, he suggested that other mosquitoes are probably the vectors during epidemics involving horses and man. This view was supported by results from field studies of mosquitoes involved in the New Jersey epidemic in 1959 when isolations of EE virus were obtained from *Aedes sollicitans, Aedes vexans, Culex restuans,* and *Culex salinarius.*[53]

As attention was directed to mosquitoes other than *C. melanura* that might be involved in maintaining the EE virus in nature, *C. restuans* was of particular interest because it was the only known mosquito capable of transmission among six species found hibernating over winter in Connecticut.[51] No virus was isolated from those collected at farms where virus activity had occurred. In subsequent studies it was concluded that *C. restuans* was unlikely to serve as an overwintering host of the EE virus inasmuch as female mosquitoes that had taken blood were cold-sensitive and unable to survive in experimental hibernation. Those found in hibernation places early in the fall were engorged with plant saps, and when they were experimentally fed only on sugar solutions, they formed fat bodies and underwent successful hibernation.[49]

Among the most significant studies of mosquitoes as hosts of the EE virus is that of Chamberlain and co-workers[11] involving determination of virus-vector relationships. They estimated the infection threshold for a number of mosquito species and classified them according to their transmitting efficiency. Species such as *Psorophora discolor, Aedes triseriatus,* and *A. aegypti* could become infected on blood meals containing small amounts of virus and could transmit with 50 to 90 per cent efficiency. However, for some species of mosquitoes the situation was different. Blood meals containing virus titers as high as 10^8LD_{50} were necessary to infect even a small per cent of *Culex quinquefasciatus, C. salinarius,* and *Anopheles quadrimaculatus*; these transmitted virus at less than five per cent efficiency. Chamberlain and Sudia[13] showed that *Culex tarsalis* was exceptional among *Culex* mosquitoes in that it had an unusually low infection threshold and could transmit the virus from bird to bird. Sudia and co-workers[46] demonstrated that *A. sollicitans* could transmit EE virus from horse to horse when the blood virus titer in the donor animal was only slightly above the average observed in that species.

In addition to their studies on host-virus relationships and biological transmission of EE virus, Chamberlain and Sudia[13] made a very significant study of mechanical transmission. They had observed in transmission experiments utilizing *A. triseriatus* that transmission occurred following infection of the mosquito earlier than expected for usual biological transmission. They subsequently proved the ability of *A. triseriatus* to transmit EE virus mechanically. Ordinary insect pins were infected by running them one-fourth inch under the skin of a viremic chick, and transmission by interrupted feedings of *A. triseriatus* was compared with transmission by jabs of the infected pins. After incubation of 80° F. and 75 per cent relative humidity for up to 70 hours, the individual pins were rejabbed

and mosquitoes induced to reprobe into normal chicks. No significant difference was found between the pins and mosquitoes as mechanical transmitters and transmission by both methods for up to 70 hours was accomplished. By the same method, the investigators found that the stable fly, *Stomoxys calcitrans,* transmitted EE virus up to four hours.

There is a great amount of evidence from studies in the field and in the laboratory supporting the theory that birds are reservoir hosts of EE.[17, 21, 26, 37-39, 45] This area of the subject has received considerable attention from investigators. Kissling and co-workers[39] demonstrated a high incidence of antibody in wild birds, and they found that small birds such as blackbirds, grackles, and English sparrows circulated higher levels of virus in their blood than large birds such as the ibis and egret. Likewise the viremia in small birds persisted longer than in large birds. Stamm[44] lists 24 isolations of EE virus from wild birds in the eastern and Gulf states from 1950 through 1957—the earliest isolation being in March and the latest in September.

In year-round sampling of birds in an endemic area in Louisiana, Kissling and co-workers[38] also isolated EE virus from wild birds as early as March 19th. These workers[40] concluded they could not find support for the hypothesis of annual reintroduction of the virus into the United States by migrating birds from the tropics. In sampling large numbers of resident and migrating birds, they demonstrated that EE activity in birds in Louisiana began as much as two months earlier than the arrival of the principal migration waves of birds from the tropics. They concluded from their studies of migrating and resident wild birds that fresh-water swamps serve as permanent foci for the EE virus in the eastern United States.

Similar conclusions have been drawn from serologic study of large numbers of birds in New Jersey. Kandle[24] has indicated that considerable attention is being directed to wild rodents from which isolations have been obtained throughout the winter months.

No discussion of the advances in the knowledge of EE would be complete without at least some mention of the outstanding developments in virus technology that have occurred since Ten Broeck and Merrill,[47] and Giltner and Shahan[19] originally isolated the virus in 1933 by inoculating a suspension of horse brain into guinea pigs. During the thirty years since that time, there has been an evolution in the techniques utilized for growing the virus. These have included the original use of the guinea pig, the use of both adult and suckling mice, the utilization of embryonated chicken eggs, and inoculation of one-day old chicks. Tissue culture monolayers in fluid medium have also been extensively used; in 1958 and 1959, Porter-

field,[42] and at the same time Henderson and Taylor,[25] pioneered the adaptation of modern tissue culture plaque techniques for study of the EE virus in the laboratory. With these new methods, quantitative as well as qualitative studies are possible.

The geographic distribution of the EE virus has been significantly extended in recent years. While it has long been considered a New World virus, isolations have recently been reported from Thailand, from the Philippines, from Poland, and from the U.S.S.R.[8] It is particularly interesting to note that Casals[8] has reported that by special methods of testing the antigenic relationship between strains of EE virus from different geographical areas it was shown that isolates from North America are distinguishable from South and Central American strains. In addition, he found that isolates from areas remote from the Americas—one from Poland, one from the U.S.S.R., and one from Thailand—are indistinguishable from the strains of virus found in the United States. This is surprising since antigenic variation occurs among the New World strains of virus. Certainly more information and results of field studies abroad are necessary before the significance of the Old World isolations can be determined. It is obvious that with the detection of antigenically distinct strains in North and South America it will soon be possible to better elucidate the role that migrating birds play in distribution of the EE virus.

While wild birds are strongly suspected as disseminating hosts, additional evaluation is necessary before the role they play in the natural history of the virus is determined. Likewise, determination of the role that other animals might play, particularly that of rodents and reptiles, requires more investigation. In 1953, the virus was isolated from the brain of a rat found dead near a pen in Connecticut where pheasants were stricken.[41] In Massachusetts studies during 1960,[20] Hayes showed that three species of snakes and four species of turtles could be experimentally infected. The incubation period was prolonged and the virus remained in the blood of these animals for as long as three weeks. In Georgia during 1960 and 1961, Karstad[35] found 7 of 99 reptiles in the vicinity of the Okefinokee Swamp to be positive for EE antibody. He also reported prolonged periods of viremia and subsequent antibody formation in several species of reptiles following virus inoculation. Craighead and co-workers, in 1962,[16] reported that lizards may serve as hosts of the EE virus in Panama, and that inoculation of some lizard species also resulted in viremia and subsequent antibody formation. In 1964, Hayes and co-workers[23] reported results of studies in Massachusetts from which they concluded that neither small wild mammals nor coldblooded vertebrates

were often infected with the EE virus in nature during a nonepidemic period. Of a considerable number of small mammals, amphibians, and reptiles collected, they found antibody in one eastern cottontail rabbit and in one snapping turtle. However, they observed that experimentally infected snakes and turtles developed a viremia for as long as three weeks. An inoculated garter snake held in an outdoor cage and three spotted turtles held at refrigerator temperature maintained the virus for as long as six months.

From these reports and from the very interesting (but yet unpublished) results of studies by Goldfield and Sussman in New Jersey, referred to by Kandle in 1963,[84] it may be seen that possible overwintering host reservoir animals such as rodents and snakes may provide an ecologic niche in the permanent swamp site for the survival of the EE virus.

It is evident that there are many important questions to be answered. If it were possible it would be extremely useful to predict the year widespread virus activity would occur. It is known from the extensive study of the fluctuation of antibody rates in wild birds that within three years after the last occurrence of virus activity, the disseminating host wild bird population is essentially highly susceptible, setting the stage for recurrence of virus activity. While the occurrence of unusually high mosquito populations has at times coincided with the years in which virus activity was present, a high level of the mosquito population is not essential. However, the proportion of particular potential vector species present in the mosquito population is extremely important. Hayes and Hess[88] have recently presented evidence in support of the theory that a summation of the cumulative rainfall during the late summer and the spring seasons provides an index that may be useful in predicting virus activity the following year.

From experimental work there have come many interesting possibilities that must be explored. For instance, Hurlbut, in 1960, has shown that nonhemophagous insects may harbor the EE virus for considerable periods of time,[80] and it is known that birds may be infected orally. Collins[15] has been able to experimentally infect larvae with encephalitis viruses and has shown that adult mosquitoes from them can subsequently transmit the virus. The question of transovarian passage of the virus from one generation of mosquitoes to the next is not yet answered.

Finally, although the etiologic agent has long been known to be a virus, and an effective vaccine has been developed for protection of horses, there is still no safe and effective vaccine for protecting humans against eastern encephalitis.

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