ORAL TRANSMISSION OF EASTERN EQUINE ENCEPHALOMYELITIS VIRUS BY A NORTHERN INDIANA STRAIN OF *COQUILLETTIDIA PERTURBANS*[1]

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The ecology of eastern equine encephalomyelitis (EEE) virus in the midwestern USA is poorly understood. Occasional epizootics have accounted for numerous equine deaths as well as several human deaths in southwestern Michigan and northwestern Indiana foci since the early 1940s. While the primary East Coast enzootic vector, *Culiseta melanura* (Coq.) is frequently found in these midwestern foci, probable East Coast epizootic vectors (e.g., *Aedes sollicitans* (Walker)) are rare in the Midwest. Extensive work in southwestern Michigan during the 1980 equine epizootic revealed a probable vector role for *Coquillettidia perturbans* (Walker) based on *Cq. perturbans* biting collections from avians and equines and the most frequent recovery of EEE virus mosquito isolates from *Cs. melanura* and *Cq. perturbans* (D. B. Francy, personal communication). During the 1980–82 epizootic period, *Cq. perturbans* populations were unusually high in northern Indiana in close proximity to the southwestern Michigan-northern Indiana focal area (Craig 1983). More recently, the probable role of *Cq. perturbans* as a vector in East Coast foci has been discussed by Clark et al. (1985). Early work by Chamberlain et al. (1954) had demonstrated a 20% (8/40) rate of transmission of EEE virus to chicks by *Cq. perturbans* (from the southern United States) in the laboratory. In light of the recent epizootics of EEE in our area and the potential role of *Cq. perturbans* as an important epizootic vector, we wanted to investigate the vector competence of a midwestern strain of that mosquito from the EEE virus enzootic area of northern Indiana to evaluate its potential status as a primary vector in epizootic situations.

Field collection of female mosquitoes began in July 1984 with the report of *Cq. perturbans* females taken in light traps by personnel of the St. Joseph County (Indiana) Mosquito Control Project. Newly emerged (scales unrubbed) adult *Cq. perturbans* were collected at Spicer Lake County Park in northwestern St. Joseph County, and orally infected in the laboratory using an artificial membrane feeder (Rutledge et al. 1964, Grimstad et al. 1977). Spicer Lake is located within 1–5 km of EEE virus foci in LaPorte County to the west and within 15–25 km of EEE virus foci in St. Joseph and Elkhart counties in Indiana to the east. Assays of individual mosquitoes for their ability to orally transmit to suckling mice and for the presence of disseminated or non-disseminated infection in adults not transmitting were performed as previously described (Boromisa and Grimstad 1986, Grimstad and Haramis 1983, Grimstad et al. 1985).

We used the NJ/60 strain of EEE virus obtained from the reference bank of the Centers for Disease Control, Fort Collins, CO; this virus stock, isolated from *Cs. melanura*, had undergone 1 mosquito and 4 suckling mouse passages prior to our receiving it (N. Karabatsos, personal communication). The virus was passaged once in suckling mice in our laboratory prior to use in this experiment and titered 10^9 (as seed virus) in African Green monkey kidney (Vero) cell culture. Defibrinated chicken blood was obtained commercially (Colorado Serum Co., Denver, CO). A 1:10 dilution of seed virus in chicken blood (0.5 ml:4.5 ml) was offered in an artificial feeder through a Baudruche membrane (Rutledge et al. 1964) to approximately 250 female *Cq. perturbans* and they were allowed to engorge over a 1 hr period. While most females ingested some blood, only fully engorged females were retained and were then held in an isolation insectary at 20°C, 85% RH, and a 16:8 hr photophase, for 21 days.

We backtitered the blood-virus mixture in Vero cells to determine the infective dose; no antibodies to EEE virus or non-specific inhibitors of neutralization were found in an aliquot of virus-free chicken blood.

Following this extrinsic incubation period, 70 females were selected at random and individual females allowed to refeed on individual 2- to 3-day-old suckling mice (ICR strain, Harlan Sprague-Dawley, Indianapolis, IN). Females that either probed once slightly to those that fully engorged on the suckling mice were im-

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Table 1. Rates of infection and oral transmission of eastern equine encephalomyelitis virus by *Coquillettidia perturbans* collected in northern Indiana.

% (No.) infected[a]			% (No.) transmitting[b]		% (No.) with barriers[c]		
Total	Disseminated	Nondis-seminated	Population	Modified	SGB	MGEB	MGIB
28.6 (20/70)	12.9 (9/70)	15.7 (11/70)	7.1 (5/70)	55.6 (5/9)	44.4 (4/9)	55.0 (11/20)	71.4 (50/70)

[a] Disseminated infections reflect passage of EEE virus beyond the midgut; non-disseminated infections are confined to the midgut only.

[b] The population transmission rate is the number of mosquitoes transmitting of the total refed on suckling mice; the modified transmission rate is the number transmitting of those with disseminated infections. Both rates are presented since various other workers use one or the other only in their reports. The population rate is the most epidemiologically significant, however.

[c] SGB = salivary gland barrier (proportion of mosquitoes with a disseminated infection that failed to orally transmit); MGEB = midgut escape barrier (proportion of mosquitoes with midgut infections that do not have disseminated infections); MGIB = midgut infection barrier (proportion of mosquitoes engorging an infectious bloodmeal but failing to become infected).

mediately frozen at −70°C for further assay. The suckling mice were observed for 7 days for signs of illness, paralysis and death.

Table 1 summarizes the results of this laboratory trial. While 29% (20/70) of the females became infected at the midgut level, only 13% developed disseminated infections after engorging 8 logs of virus in defibrinated chicken blood. Of those with disseminated infections, 56% orally transmitted to suckling mice. However, the population transmission rate (7%) is of greater epidemiologic significance.

This trial demonstrated the ability of a population of *Cq. perturbans* from an Indiana EEE virus enzootic area to become orally infected and transmit virus to a susceptible vertebrate host. While the rate of transmission by the population is low, our results suggest that during epizootic periods this strain would be capable of becoming infected and orally transmitting to susceptible vertebrates. Actually, the low rate of oral transmission by the population fits well with the low number of equine cases reported during epizootic periods when high rates of virus transmission were evident in Michigan avian species (McLean et al. 1985). The low number of Michigan (and northern Indiana) equine cases was probably not related to vaccination status of herds in the area since McLean et al. (1985) noted only 46.5% of equines in the 1980 Michigan epizootic sites had neutralizing antibody to EEE virus (including both naturally acquired and vaccine-induced antibody).

This trial also demonstrated the existence of midgut infection and midgut escape barriers (Hardy et al. 1983) in this species to EEE virus. The salivary gland barrier was of less importance in reducing the potential number of transmitting adults. This pattern of greater numbers of individual mosquitoes with midgut than salivary gland barriers is similar to that seen with La Crosse virus in *Aedes triseriatus* (Say) (Grimstad et al. 1985) and in other mosquito species/virus associations (summarized by Hardy et al. 1983).

It is possible that the extrinsic incubation period we chose was too long or the incubation temperature too cool. However, in 1980–82 the majority of the Michigan equine cases had onset in the late summer when cool evening temperatures were common in that area. In addition, we recognize that feeding via an artificial membrane feeder is less efficient in producing infected mosquitoes than using viremic chicks as has been demonstrated with western equine encephalomyelitis virus (Kramer et al. 1981). The 7% rate of transmission to suckling mice we noted is not significantly different ($0.10 > P > 0.05$; Chi-square 2×2 contingency table analysis with Yates correction, 1 df; Steel and Torrie 1960) from that reported by Chamberlain et al. (1954) using chicks and southern *Cq. perturbans* as noted above. The significant differences ($P < 0.005$) in rates of infection in the two studies (94% vs. 29%) is of considerable interest since it undoubtedly in part reflects geographic variation in susceptibility to EEE virus, a phenomenon noted in previous studies with other arboviruses (summarized by Hardy et al. 1983). While our infection and transmission rates may be underestimated, the potential role of *Cq. perturbans* as a midwestern vector of EEE virus has been established.

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