THE ISOLATION OF EASTERN EQUINE ENCEPHALITIS VIRUS FROM *CULEX (MELANOCONION) TAENIOPUS* DYAR AND KNAB IN PANAMA[1]

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Outbreaks of eastern equine encephalitis (EEE) virus among horses occurred in the Republic of Panama in 1946 (Steel and Habel, 1947), 1958 (Murnane *et al.*, 1958) and 1962 (Medina *et al.*, 1965). Numerous strains of this virus have been isolated from fatal cases in horses but no laboratory-confirmed human cases have been reported. Although EEE antibodies have been found in wild lizards in Panama (Craighead *et al.*, 1962), little is known about the natural history of the virus in this country. In an extensive ecological survey in the Almirante area of northwestern Panama, more than 16 types of arboviruses were obtained in 1960–62 (Galindo *et al.*, 1966), none of which were shown to be EEE virus.

This paper reports the isolation, in September 1964, of EEE virus from *Culex (Melanoconion) taeniopus* Dyar and Knab, representing the first isolation of this virus from mosquitoes in Panama.

VIRUS ISOLATION. The virus, designated BTH 3070–5, was isolated from a pool of 30 *Culex (Melanoconion) taeniopus* collected from human bait in Almirante on September 14, 1964. These specimens were shipped on ice by air 2 days later to the laboratory in Panama City and the species identification was done the following morning. The mosquitoes were triturated in 2 ml. of bovalbumin diluent after 2 weeks' storage in a Revco deep-freezer at −65° C. Suspensions were then spun in the refrigerated centrifuge for 30 minutes at 2,500 r.p.m. Aliquots of the supernates were stored in sealed glass ampoules at −65° C.

Three weeks later the contents were inoculated by the intracerebral (i.c.) and intraperitoneal (i.p.) routes into a group of seven 3-day-old Swiss mice. Five of the mice inoculated with the suspension died on the 4th day after inoculation and the other three became sick on the same day. Brain-to-brain passage was made from the sick mice to a group of suckling mice. All of the subinoculated mice became sick or died within 2 days. Stock virus was made from the third passage when all of the infected mice became sick within 26 hours after i.c. inoculation. The titer of a 20 percent infant mouse brain suspension of the virus in adult mice by i.c. route was 10^8 LD_{50}/0.02 ml. The virus was again reisolated from the original mosquito suspension by the hamster kidney tissue culture method.

Blood was taken from the infected suckling mice of the third passage from which stock virus was prepared and hemagglutinating (HA) antigen was extracted from the serum according to the techniques previously described (Clarke and Casals, 1958, Srihongse, 1966). HA titer of 1:320 was obtained when tested within a pH range of 5.75 to 6.0. Sucrose-acetone extracted antigen was prepared from infected mouse brain during the fourth passage and was used in complement-fixation (CF) tests.

VIRUS IDENTIFICATION. Prototype strains of group A arboviruses used for the identification of BTH 3070–5 isolate were obtained from various sources: EEE (3847) isolated from a horse brain at the Government of Panama's Veterinary Laboratory;

[1] This work was supported in part by Grant AI-02984 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

EEE (64-A-11) from the New Jersey State Department of Health; Venezuelan equine encephalitis (VEE, 3880) and Una (BT 1495) from the Middle America Research Unit; Aura (BeAr 10315), Mayaro (BeAr 20290) and Pixuna (BeAr 35645) from the Belem Virus Laboratory; western equine encephalitis (WEE, Fleming) from the Communicable Disease Center.

In the preliminary hemagglutination-inhibition (HI) test, serum HA antigen of BTH 3070–5 reacted with a polyvalent group A serum but not with a group C immune serum. HI tests in Table 1 show a closer relationship of BTH 3070–5 to EEE (3847) than to other group A viruses tested. In CF tests, strain BTH 3070–5 was shown to be indistinguishable from EEE virus (Table 2). Cross-neutralization (NT) tests in adult mice indicated that BTH 3070–5 and EEE (3847) were closely related, if not identical. The EEE (3847) strain was in turn shown by both CF (Table 2) and NT test (Table 3) to be closely related to the New Jersey strain of EEE virus.

Thus, BTH 3070–5 is the first recognized EEE virus recovered from mosquitoes in Panama. The total number of specimens from the same area which were processed for virus isolation during a 12-month period beginning June 1, 1964 includes 12,875 *Culex (Melanoconion)* species and 13,065 other culicines. No other EEE isolates were obtained.

TABLE 1.—Identification of strain BTH 3070–5 by hemagglutination-inhibition test.

Serum	Antigen, 4 units	
	BTH 3070–5	Homologous
BTH 3070–5	160*	..
EEE (3847)	320	320
VEE	20	2,560
WEE	10	2,560
Mayaro	10	2,560
Pixuna	20	2,560
Aura	0	320
Una	20	160
Mucambo	10	160

* Reciprocal of serum titer, 0=less than 1:10.

TABLE 2.—Results of complement-fixation tests of BTH 3070–5 as compared to two different strains of eastern equine encephalitis (EEE) virus.

Serum	Antigen		
	BTH 3070–5	EEE (3847)	EEE (64-A-11)
BTH 3070–5	32/512*	16/256	32/256
EEE (3847)	32/512	32/512	..
EEE (64-A-11)	..	64/512	128/512

* Reciprocal of serum titer/reciprocal of antigen titer.

The mosquito host, *Culex (Melanoconion) taeniopus* Dyar and Knab, has been extensively studied by us in the Almirante area. The species is found primarily in deeply shaded permanent swamps, particularly in the presence of the "silica palm," *Raphia taedigera,* and the "coquillo palm," *Manicaria saccifera.* Adults have a slight preference for the canopy of swamp forests, but will bite in numbers on the ground. The preferred hosts are rodents, but females will frequently attack man and avian hosts. While adults are but rarely taken inside human dwellings, they freely invade the peridomestic habitat near the swamps and bite man in

TABLE 3.—Comparison of BTH 3070–5 and two strains of eastern equine encephalitis virus by mouse neutralization tests.

Serum	Virus		
	BTH 3070–5	EEE (3847)	EEE (64-A-11)
BTH 3070–5	3.5*	3.0	..
EEE (3847)	2.7	3.3	..
EEE (64-A-11)	..	3.3	4.0

* Log_{10} neutralization index, virus-serum mixture was incubated at 37° C. for one hour prior to intracerebral inoculation in young adult mice.

the vicinity of dwellings, as well as on the steps and in the open porches of houses.

Most of the females become active soon after dusk and the peak of biting activity is reached within two hours after dark. Few females seek a blood meal after 10 p.m. The great majority attempt to bite on the lower extremities and they prefer dark to light-skinned humans. While high population densities are only found near the swamps, this species tends to migrate during periods of heavy rains and at such times may be taken biting in fair numbers in well-drained forests at least 7 kilometers away from the nearest known breeding place. In Almirante, *C. taeniopus* has also been found infected with Venezuelan equine encephalitis virus (Grayson and Galindo, 1966) and in Trinidad it was found harboring EEE virus (Downs *et al.*, 1959).

SUMMARY. A strain of eastern equine encephalitis virus, the first to be isolated from mosquitoes in Panama, was obtained from *Culex (Melanoconion) taeniopus* Dyar and Knab collected in the Almirante area in September 1964.

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METHODS FOR DISSECTING MOSQUITOES

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INTRODUCTION. Most of the following methods for dissecting mosquitoes have been employed in this laboratory for a number of years and have proved most useful. The various tools and equipment needed are included. A number of mosquito investigators have written to me about their various special techniques and I have included them here.

TOOLS

1. *Needles.* Sharpened Minuten Nadeln or other fine steel needles or larger insect pins may be inserted into freshly boiled, wooden, applicator sticks (Patton & Evans, 1929); after the sticks dry, the needles are firmly held. Pins vary in quality from brand to brand; many stainless steel pins are not useful. Larsen (personal communication) recommends the "Genuine Brill'd Eys Between" #10 made by James Smith & Sons, London. These are very stiff and sharp.

A large batch of needles in applicator-stick holders can be made up at one time and stored in suitable containers. Long applicator sticks with embedded needles