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An epizootic among the monkeys of the north coast of Honduras was reported beginning in December of 1953 and continuing into the late summer of 1954. The cause was assumed to be yellow fever and in two instances pathological confirmation of this diagnosis was obtained. Similar epizootics have occurred in other Central American countries in recent years (Elton, 1952; Yellow Fever Conference, 1955). Full details concerning this epizootic may be found in the report of Trapido and Galindo, 1955, and need not be repeated here.

Plans were made to capture and test mosquitoes from the affected area for yellow fever virus with a view to the identification of a possible natural vector. All mosquito collections were carried out by Drs. H. Trapido and P. Galindo, who have described methods of capture and location of the collecting sites in the report previously cited. Collections were made in August and early September of 1954. The mosquitoes were identified in the live state, pooled by species or species groups and shipped frozen in dry ice to the Gorgas Memorial Laboratory in Panama.

A total of 10,309 mosquitoes were received (See Table 1), the overwhelming majority (10,089) from the La Masica collecting station where a dead monkey with pathological changes characteristic of yellow fever was found on August 6. The remaining 220 specimens were captured at La Masica Arriba (208) and Lancetilla (12). A total of 15 different species or species groups were included, the two largest being the *Psorophora ferox* group with 5,657 specimens and *Trichoprosopon magnus* with 1,393 specimens. The only known natural vector of yellow fever received was *Haemagogus spegazzinii falco*, of which 14 specimens captured at La Masica Arriba were submitted for study.

The discrepancies between the numbers of mosquitoes given here and those reported in the paper describing the field work (Trapido and Galindo, 1955) are due to two factors: a small group of mosquitoes collected on the day before the routine collections were begun on August 8th was not included in the summary contained in the Trapido and Galindo paper; and small groups of mosquitoes of species not considered of possible significance in transmission, though received at the field laboratory alive, were not frozen for inoculation.

METHODS

The mosquitoes were triturated in groups of 50 or less with 10 per cent inactivated rhesus serum-saline which had been tested and found free of antibodies against yellow fever. Two cubic centimeters of the diluent were used for 50 mosquitoes and proportionately smaller amounts for smaller groups. The resultant suspension was then centrifuged at 5,000 r.p.m. during 15 minutes at

TABLE 1

Classification of mosquitoes collected in Honduras for virus studies

Place of Origin	Species	Total Number Received
Lancetilla	*Sabethes chloropterus*	12
La Masica Arriba	*Haemagogus spegazzinii falco*	14
	Psorophora cingulata	13
	Trichoprosopon magnus	100
	Trichoprosopon spp.	18
	Wyeomyia spp.	48
	Sabethes chloropterus	15
La Masica	*Haemagogus equinus*	498
	Aedes serratus group	162
	Aedes angustivittatus	21
	Aedes (Ochlerotatus) spp.	918
	Mansonia spp.	710
	Psorophora ferox group	5,657
	Trichoprosopon magnus	1,393
	Trichoprosopon spp	16
	Wyeomyia spp.	55
	Sabethes cyaneus-tarsopus	77
	Sabethes chloropterus	235
	Sabethini	343
	Chagasia bathanus	4
	Total	10,309

Aedes (Ochlerotatus) spp. includes: *A. serratus*, *A. tormentor*, *A. hastatus*, *A. oligopistus* and *A. angustivittatus*.

Psorophora ferox group includes: *P. ferox*, *P. lutzii* and *P. varipes*.

Mansonia spp. includes: *M. indubitans*, *M. titillans*, *M. venezuelensis*, and *M. nigricans*.

Sabethini includes: *Wyeomyia (Wyeomyia)* spp., *Wyeomyia (Dendromyia)* spp., *Trichoprosopon longipes*, *T. espini*, *T. fluviatilis*, *Limatus durhamii* and *Limatus asulleptus*.

a temperature of 5°C. To that part of the supernatant fluid destined for intracerebral mouse injection was added a concentration of 600 Units of penicillin and 600 micrograms of streptomycin per cubic centimeter, in order to control the bacterial contamination. Antibiotics were not added to the part destined for monkey inoculation.

Six young adult Swiss white mice were injected intracerebrally per specimen. The mice were kept under close daily observation for a period of 30 days. Any mice found dead, or sacrificed when showing signs of illness, were autopsied and their brains removed for direct smears, cultures and subinoculations in mice. Mice surviving throughout the observation period were challenged for immunity by the intracerebral injection of 100 LD_{50} of French neurotropic virus.

Rhesus monkeys, *Macaca mulatta*, were used for attempted virus isolation from four species or species groups: *Haemagogus equinus*, *Trichoprosopon magnus*, *Aedes (Ochlerotatus)* spp. and *Mansonia* spp. These species were selected since

epidemiological evidence seemed to incriminate them most strongly. Monkeys were inoculated subcutaneously with the pooled residual supernatant solutions of the centrifuged mosquito suspensions after the part destined for mouse inoculation had been separated. Monkeys were kept under observation for a period of one month and their temperatures were taken twice daily. At the end of 2, 4 and 6 week intervals after inoculation the monkeys were bled and their serums tested for yellow fever antibodies. Both the intracerebral and intraperitoneal mouse protection test techniques were employed.

RESULTS

No yellow fever virus was recovered from any of the 10,309 mosquitoes examined either in mice or in rhesus monkeys. Mice failed to show immunity when challenged at the end of their observation period with French neurotropic virus and the monkeys failed to develop neutralizing antibodies in their blood.

PSOROPHORA VIRUS

Although yellow fever virus was not recovered during this study, an unidentified neurotropic virus was isolated in mice from 50 specimens of a group of *Psorophora* captured at La Masica on September 2, 1954. The group included *Psorophora ferox*, *P. lutzii* and *P. varipes*. This virus has been maintained for 15 continuous passages to date in mice. No other susceptible animal has as yet been found. Cross-immunity experiments in mice and protection tests using known positive antiserums have demonstrated that this is not a strain of yellow fever virus. The following evidence indicates that it is not a spontaneous virus of our stock mice. Spontaneous infection with a similar virus has never been observed in our stock mice in the course of extensive experimentation. All mice inoculated with passage virus have developed the disease indicating the absence of immunity in the stock. Reinoculation of mice with the residue from the original mosquito suspension after 20 days of storage at −15°C. resulted in a second isolation of the same virus. Further studies of this agent are being conducted and will be published later.

CONCLUSIONS

Yellow fever virus was not recovered from 10,309 mosquitoes captured in Honduras in the late summer and early fall of 1954.

A neurotropic virus immunologically distinct from yellow fever was isolated in mice from one group of 50 *Psorophora*.

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