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ISOLATION OF YELLOW FEVER VIRUS FROM *HAEMAGOGUS MESODENTATUS*, *H. EQUINUS* AND *SABETHES CHLOROPTERUS* CAPTURED IN GUATEMALA IN 1956

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A wave of sylvan yellow fever has been progressing steadily northward in Central America in recent years. The first cases since 1905 were identified in Panama in the late fall of 1948 (Herrera *et al.*, 1949). Since that time, outbreaks have appeared successively in Costa Rica, Nicaragua and Honduras. Data concerning these outbreaks have been reviewed by Soper (1955). Near the beginning of the year 1956 the northward sweep reached Guatemala, and arrangements were made by the Pan American Sanitary Bureau with the Gorgas Memorial Laboratory to attempt virus isolations from mosquitoes captured in areas showing yellow fever activity. The staff of the Gorgas Memorial Laboratory has been interested in the problem of the Central American vectors of the disease for a number of years. Trapido and Galindo (1955) and Galindo and Trapido (1956) have shown that the principal South American vectors, *Haemagogus spegazzinii* together with its subspecies *falco*, and *Aedes leucocelaenus*, are scarce or do not occur at all in certain areas of Central America where sylvan yellow fever outbreaks have appeared. In 1949, an attempt was made by one of the authors (Rodaniche, 1951) to recover yellow fever virus from mosquitoes captured in the Pacora-Buena Vista sector of Panama with negative results. A similar attempt was made again in 1954 in connection with an outbreak of sylvan yellow fever in Honduras. *Haemagogus* captures were small, however, and findings again were negative for yellow fever virus (Rodaniche, 1956). During the recent Guatemalan outbreak, Drs. J. Boshell Manrique and George Bevier, who were engaged in field epidemiological studies there for the P.A.S.B., arranged to ship mosquitoes to the Gorgas Memorial Laboratory for possible virus isolations. Collecting stations were established by the P.A.S.B. in the Motagua valley of Guatemala in two principal zones; Rio Blanco, where simian deaths were first noted in February 1956, and El Tipon, where epizootic activity was first observed in May of the same year. Shipments from Rio Blanco were begun in March, and from Tipon in May, and have been continued up to the present time (October) from both locations. Details of the epidemiological studies conducted by the Pan American Sanitary Bureau are to be reported by Drs. Boshell and Bevier. In the present report, we shall deal only with the yellow fever virus isolations effected in the Gorgas Memorial Laboratory.

The overwhelming majority of diptera received were Culicidae. Exceptions are noted in Table 1. Shipments were made by plane, the mosquitoes being stored in screw-capped bottles lined with plaster of Paris and contained within plastic bags in ice-filled thermos jugs. The time elapsing between capture and receipt at the Laboratory never exceeded 4 days and the mosquitoes frequently

TABLE 1

Yellow fever virus isolations from diptera collected in the Motagua Valley, Guatemala, from March to October, 1956*

Species	Number of specimens received	Number of virus isolations
Haemagogus equinus	1,668	3
Haemagogus mesodentatus	4,021	14
Sabethes chloropterus	3,141	4
Sabethes cyaneus	163	0
Sabethes tarsopus	73	0
Sabethes spp.†	30	0
Aedes angustivittatus	18	0
Aedes scapularis	2	0
Aedes serratus	1	0
Aedes terrens	9	0
Aedes (Ochlerotatus)	2	0
Psorophora cingulata	2	0
Psorophora ferox	7	0
Psorophora lutzii	369	0
Trichoprosopon leucopus	1	0
Trichoprosopon magnum	1	0
Trichoprosopon spp.	24	0
Wyeomyia	289	0
Mansonia indubitans	2	0
Mansonia titillans	1	0
Anopheles eiseni	1	0
Anopheles neivai	1	0
Anopheles punctimacula	1	0
Chagasia spp.	21	0
Chagasia bathanus	15	0
Simulium sp.	10	0
Phlebotomus sp.	1	0
Culicoides sp.	1	0
Total	9,893	21

* Captures were made by Drs. J. Boshell-Manrique and G. Bevier of the P.A.S.B.
† Not including *Sabethes chloropterus*.

were still active on arrival. They were classified according to species and any obviously engorged specimens were discarded. However, a number of mosquitoes were found to contain small to moderate quantities of fresh blood on trituration. It was not considered practical under field conditions to maintain the mosquitoes for 12 or more hours at environmental temperatures in order that any recent blood meal might be digested before storing them in ice for shipping.

MATERIALS AND METHODS

In the virus section of the laboratory, the mosquitoes either were used at once for mouse inoculation or were stored in sealed tubes in dry ice until such time as inoculation into mice was feasible. Each mosquito pool was triturated in a sterile mortar and then emulsified in 10 per cent rhesus-serum saline con-

taining 1,000 units of potassium penicillin G and 1,000 micrograms of dihydrostreptomycin per cubic centimeter. In general, one cc. of diluent was used per 50 mosquitoes and roughly proportionate quantities for larger or smaller pools. However, 0.5 cc. was considered minimal.

The number of mosquitoes used as inoculum for each mouse group, varied from one for the rarer species, to 163 in the largest pool employed. Size of pools was governed by several factors; the number of specimens of each species or species group received, the place of capture and the time of capture. At the beginning of the study, the more abundant species were pooled in groups of approximately 50. Smaller pools were preferred as giving more epidemiological information. Later in the course of the study, larger pools were made wherever feasible to conserve time and materials.

After centrifugation at 1,500 r.p.m. for 10 minutes at a temperature of 4°C., the supernatant solution was injected intracerebrally into groups of 6 white Swiss mice, each mouse receiving 0.03 cc. Young adult mice 21 to 30 days of age were usually employed, as sufficient infant mice were not available. Infant mice were occasionally used for special procedures. Mice were kept under daily observation for a period of 30 days. Any showing suspicious symptoms were sacrificed and examined postmortem for macroscopic pathology. Their brains were removed aseptically and impression smears stained with Giemsa and cultures on blood agar plates and in thioglycollate broth were prepared. Then the brains were suspended in physiological saline solution for subinoculation in mice.

IDENTIFICATION OF YELLOW FEVER VIRUS

Identification of yellow fever virus was based on cross-immunity and mouse-protection tests. For cross-immunity tests, mice surviving original inoculation with mosquito suspensions or successive subinoculations, were challenged with 10,000 LD/50 of French neurotropic yellow fever virus. Results were considered positive if these mice remained healthy after all controls had died. In reverse cross-immunity tests, virus recovered in mice from infected mosquitoes was inoculated intracerebrally into groups of 6 mice previously immunized against the French neurotropic yellow fever virus and into 6 normal controls. Results were considered positive if all the yellow-fever-immune mice remained healthy after the normal mice had sickened or died. In addition, an intraperitoneal mouse protection test employing a known yellow-fever-immune and a known negative monkey serum, according to the technique developed by Sawyer and Lloyd (1931), was conducted for each virus strain isolated. Survival of at least 5 of the 6 mice receiving the immune-serum virus mixture and death of at least 5 of the 6 mice injected with the negative serum-virus mixture after an incubation period of at least 4 days was interpreted as a positive reaction.

For the first isolation of yellow fever virus from *Haemagogus mesodentatus*, a night monkey and a marmoset, previously tested and found free of antibodies to yellow fever, were inoculated subcutaneously with first mouse passage virus. Circulating virus was recovered from the blood of these monkeys on the fourth

day after injection. The animals died on the sixth and ninth days after inoculation respectively, and the diagnosis of yellow fever was confirmed by Dr. Carl M. Johnson by histopathological examination.

RESULTS

A total of 9,893 diptera were used for virus isolation attempts during the course of these studies. The great majority (8,848) belonged to the species *Haemagogus mesodentatus* with 4,021 specimens, *H. equinus* with 1,686, and *Sabethes chloropterus* with 3,141 (Table 1). Yellow fever virus was recovered 14 times from pools of *Haemagogus mesodentatus*, 3 times from *H. equinus* and 4 times from *Sabethes chloropterus*. No virus isolations were made from any of the other species.

Pools of *H. mesodentatus* from which yellow fever virus was recovered varied from 35 to 163 specimens with an average of 77. Eleven isolations were made from 2,412 specimens received from Rio Blanco from March to May and 3 isolations from 1,609 specimens received from El Tipon in June and July. Pools of *H. equinus* yielding virus contained 31, 51, and 136 specimens respectively. One isolation was made from a pool of this species captured at Rio Blanco in March and April and one from a pool captured in the same area in May. The third isolation was from specimens captured in El Tipon in June. A total of 328 *H. equinus* were obtained from Rio Blanco and 1,358 from El Tipon. All isolations from *Sabethes chloropterus* were made from specimens captured in May, three being from pools of 78, 122, and 122 mosquitoes respectively, from Rio Blanco where a total of 2,527 specimens was captured, and one from a pool of 47 specimens from El Tipon where 614 specimens were obtained.

The average incubation period in mice on primary isolation was 13 days, the shortest 7 days, and the longest 25 days. Virus isolates showed somewhat variable virulence for mice on primary isolation, some requiring several intracerebral passages before death of all animals in the inoculated groups ensued.

One group of mice inoculated with the pool of 78 *Sabethes chloropterus* yielded a mixture of two viruses. Yellow fever virus was recovered in pure state by inoculation of a night monkey and a marmoset both of which showed circulating virus on the fourth day. The other component of the mixture has not yet been identified. Two other neurotropic viruses, shown by immunological studies not to be yellow fever virus, also were recovered in the course of these studies.

DISCUSSION

Whitman (1951) has reviewed work incriminating *H. spegazzinii* and its subspecies *falco* as the principal vectors of sylvan yellow fever in Brazil and Colombia respectively. Yellow fever virus recently was isolated repeatedly in Trinidad from pools of *Haemagogus spegazzinii* by Downs, Aitken and Anderson (1955), this being the only species of the genus recognized by them on the island. The present report includes the first isolations of yellow fever virus from *Haemagogus mesodentatus*, *Sabethes chloropterus* and pure pools of *H. equinus*. In a

few instances, the mosquitoes contained recently ingested blood and the presence of virus may have been due to this factor.

Galindo, Rodaniche and Trapido (1956) in laboratory transmission experiments found that a high proportion of *H. mesodentatus*, together with its subspecies *gorgasi*, harbor virus and that this species readily transmits by bite. This finding, together with the frequent isolations from naturally infected specimens both during and after simian epizootic activity in Guatemala, clearly demonstrates the importance of this species as a natural vector.

The capacity of South American strains of *H. equinus* to transmit yellow fever virus by bite was originally demonstrated by Waddell and Taylor (1945). In the previously cited report, Galindo *et al.* (1956) showed that Central American strains also possess this capacity. The present work represents the first isolations of virus from pure pools of wild-caught *H. equinus*. However, Boshell and Osorno-Mesa (1944) isolated virus from a mixed pool of *Haemagogus* containing *equinus, spegazzinii falco* and *lucifer*.

Virus was isolated also for the first time from *Sabethes chloropterus*, although it had previously been recovered once by Shannon, Whitman and Franco (1938) from a mixed pool of sabethines including Sabethoides, Limatus, Wyeomyia and Trichoprosopon. One successful transmission by bite was obtained with colonized *Sabethes chloropterus* by Galindo and co-workers (1956), but virus was never recovered from specimens inoculated into mice. It is possible that there may be individual variations within the species to account for this discrepancy. At any rate, the status of *S. chloropterus* as a vector of yellow fever merits further investigation.

CONCLUSIONS

Isolation of yellow fever virus from naturally infected *Haemagogus mesodentatus, H. equinus* and *Sabethes chloropterus* captured in Guatemala is reported. The frequency of isolation from *H. mesodentatus* indicates that this species is epidemiologically the most important in that area of the three species mentioned.

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