Reprinted from AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE
Vol. 6, No. 4, July, 1957
Printed in U.S.A.

ISOLATION OF YELLOW FEVER VIRUS FROM *HAEMAGOGUS LUCIFER, H. EQUINUS, H. SPEGAZZINII FALCO, SABETHES CHLOROPTERUS* AND *ANOPHELES NEIVAI* CAPTURED IN PANAMA IN THE FALL OF 1956

ENID DE RODANICHE, PEDRO GALINDO AND CARL M. JOHNSON

Gorgas Memorial Laboratory, Panamá, R. P.

The staff of the Gorgas Memorial Laboratory has been interested for a number of years in the vectors of yellow fever in Panamá. In the fall of 1949 an attempt was made to isolate virus from mosquitoes captured at two locations; Pacora, where human yellow fever deaths were recorded in November and December, 1948 and Buena Vista, where deaths occurred in August and September, 1949. From the end of September, 1949 to the onset of the dry season in early January, 1950, 2,723 mosquiotes were collected from the Pacora station and 1,672 from the Buena Vista station. However, no yellow fever virus was recovered (Rodaniche, 1951). Although no further human cases were recorded from eastern Panamá until the present year, there was reason to suspect the infection had remained endemic there. This view was supported by the finding of occasional juvenile monkeys with high titers of neutralizing antibodies against yellow fever in their blood (Rodaniche, in press). In August, 1956 a new attempt to recover the virus was made.

The first mosquito collecting station was established in August 1956 in the San Blas Territory, in the Sangandi River Valley eight kilometers due south of the Mandinga air strip on the Atlantic side of the Continental Divide. Methods employed in setting up this type of station have been described by Galindo, Trapido and Carpenter (1950). Shortly after collections were begun at Mandinga, the first human yellow fever death in Panamá since the 1948–1951 outbreak was verified. The source of infection was traced to the headwaters of the Pacora River, in the Cerro Azul area, some 60 km. east of the Canal Zone and 20 km. southwest of the Mandinga collecting site on the Pacific side of the Continental Divide. A second station was then established in this area, where collections were made from September 13 to October 9 when they were discontinued for the remainder of the year.

METHODS

Methods employed were essentially the same as those described by Rodaniche and Galindo (1957) in their report on the vectors of yellow fever in Guatemala. Mosquitoes were macerated in 10 per cent rhesus serum saline containing 1,000 Units of penicillin and 1,000 micrograms of streptomycin per cubic centimeter. After centrifugation, the supernatant fluid was used to inoculate groups of 6 young adult white Swiss mice by the intracerebral route. Occasionally groups of infant mice also were used. Yellow fever virus was identified by cross-immunity tests in which mice surviving inoculation with the mosquito viral isolates

were challenged with Theiler's French neuroadapted virus and conversely mice previously immunized against the French strain were challenged with the virus strains recovered from mosquitoes. Mouse protection tests also were conducted using a known positive and known negative monkey serum. In the current investigation mosquitoes were held in the field at atmospheric temperatures for one day before being packed in ice for shipment, a procedure not found feasible in Guatemala. This was done in order to permit digestion of any freshly ingested blood.

RESULTS

In Table 1 data are presented concerning the collections made at the Mandinga station during the 4 month period from August 22 to December 20, 1956. A total of 7,094 mosquitoes comprising 15 different species or species groups were collected and tested. The most abundant species was *Aedes leucocelaenus clarki*, but large numbers of *Haemagogus lucifer*, *spegazzinii falco* and *Sabethes chloropterus* also were found. All four of these species showed heavier density in September than in the succeeding months. Only one isolation of yellow fever virus was made, from a pool of 137 *H. lucifer* collected between September 1 and 6. This strain was recovered initially in mice but later the residue of the original mosquito suspension which had been stored in dry ice was injected subcutaneously in a normal rhesus monkey. The animal did not die but developed fever and showed circulating virus followed by the appearance in its

TABLE 1

Yellow fever virus isolations from mosquitoes captured at the Mandinga station from August 22 to December 20, 1956 and injected into mice

Species of mosquito	Total no. collected	Nos. collected monthly				
		Aug.	Sept.	Oct.	Nov.	Dec.
H. spegazzinii falco	853	86	335	170	137	125
H. equinus	258	19	50	63	74	52
H. lucifer	1,162 (1)	87	540 (1)	237	203	95
A. leucocelaenus clarki	1,707	160	655	485	320	87
S. chloropterus	1,409	162	624	367	158	98
*Sabethes spp.**	654	61	256	179	77	81
Wyeomyia spp	460	178	14	175	55	38
Trichoprosopon magnum	153	40	0	34	41	38
Trichoprosopon spp	155	28	23	104	0	0
Anopheles neivai	142	7	24	16	87	8
Anophelini	46	1	0	14	27	4
Psorophora spp	49	15	0	12	7	15
Aedes spp	12	3	3	0	1	5
Mansonia spp	27	8	0	7	8	4
Culex spp	7	2	0	0	0	5
Total	7,094 (1)	857	2,524	1,863	1,195	655

Number in parenthesis represents virus isolation.

* Not including *S. chloropterus*.

TABLE 2

Yellow fever virus isolations from mosquitoes captured at the Cerro Azul station from September 13 to October 9, 1956 and injected into mice

Species of mosquito	Total no. collected	Nos. collected monthly		Virus isolations
		Sept.	Oct.	
H. spegazzinii falco	56	23	33	1
H. equinus	48	21	27	1
H. lucifer	649	328	321	2
Aedes leucocelaenus clarki	10	7	3	0
S. chloropterus	265	188	77	2
Sabethes spp.	44	28	16	0
Wyeomyia spp.	298	144	154	0
Anopheles neivai	49	4	45	1
Total	1,419	743	676	7

* Not including *S. chloropterus*.

blood of high titers of neutralizing antibody against the French neuroadapted strain.

In Table 2 are listed data concerning the collections made at Cerro Azul during a 26 day period, from September 13 to October 9, 1956, when collections were temporarily suspended. A total of 1,419 mosquitoes were captured and tested, comprising 8 different species or species groups. The most abundant forms were *H. lucifer*, *S. chloropterus* and *Wyeomyia spp.* Seven isolations of yellow fever virus were made from five different species. Virus was recovered once from a pool of 48 *H. equinus*, once from a pool of 56 *H. spegazzinii falco* and once from a pool of 49 *Anopheles neivai*. Each pool included all specimens collected during the 26 day period. Virus was recovered twice from *H. lucifer*, first from a pool of 133 specimens collected from September 9 to 20, and again from a pool of 107 mosquitoes collected between September 21 and 26. Two isolations were also made from *S. chloropterus* pools of 114 and 51 specimens, captured from September 21 to October 2 and from October 5 to 15 respectively. The shortest incubation period observed in mice during this study was 6 days and the longest 14 days with an average of 9.5 days. Two strains showed sufficient initial virulence to kill all mice injected that were not sacrificed for further study. The other five strains required one or more additional intracerebral passages before they produced uniformly fatal infections.

DISCUSSION

H. spegazzinii falco has long been recognized as an important natural vector of yellow fever in South America. The literature concerning this species was recently reviewed by Whitman (1951). Rodaniche and Galindo (1957) reported the first isolations of the virus from *Sabethes chloropterus* and pure pools of *H. equinus* captured in Guatemala in 1956. In the present report the first recoveries of yellow fever virus from pure pools of *H. lucifer* and from *Anopheles neivai*

are described. Boshell and Osorno-Mesa (1944) recovered virus from a mixed pool of *H. spegazzinii falco*, *lucifer* and *equinus* in Columbia but it is not known which species was incriminated. An attempt was made by Galindo, Rodaniche and Trapido (1956) to transmit yellow fever by bite using a Panamanian strain of *H. lucifer*. This experiment gave an unsatisfactory result due to death of all but one of the infected mosquitoes before the end of the incubation period. It was uncertain whether the one remaining mosquito fed or merely probed. However, virus was recovered from a large proportion of the mosquitoes by mouse injection after incubation periods varying from 14 to 30 days. The finding of 3 pools of *H. lucifer* positive for yellow fever, one from the Atlantic side and two from the Pacific side, indicates the importance of this species here. It is very common in the forested areas of eastern Panamá near the Canal Zone (Galindo, Trapido and Carpenter, 1950) and its period of peak incidence seems to bear a direct relationship to the appearance of jungle yellow fever here.

The status of *Anopheles neivai* is more obscure. The present isolation is the first from this genus of mosquito. Davis and Shannon (1931) attempted experimental transmission with two South American species of *Anopheles*, *A. albitarsis* and *tarsimaculatus* but failed to infect monkeys by exposing them to the bites of these anophelines or by injection of suspensions of the ground mosquitoes after an incubation period of 12 to 18 days. Further experimentation is needed to determine whether *A. neivai* can harbor virus for long periods of time and whether it transmits by bite.

It is of interest that naturally infected specimens of *Aedes leucocelaenus clarki*, a geographical race of a recognized South American vector, were not found in the course of this investigation. This was the predominant species at Mandinga where yellow fever activity was apparently minimal. Only 10 specimens were captured at Cerro Azul where virus activity was high, an inadequate sample.

CONCLUSIONS

Yellow fever virus was isolated once from 7,094 mosquitoes captured near Mandinga on the Atlantic side of the Continental Divide east of the Canal. This isolation was made from *H. lucifer*. It was isolated 7 times from 1,419 mosquitoes captured at Cerro Azul on the Pacific side, 60 kilometers east of the Canal Zone. Two recoveries each were made from *H. lucifer* and *S. chloropterus*, and one recovery each from *H. spegazzinii falco*, *H. equinus* and *Anopheles neivai*.

ACKNOWLEDGEMENTS

This investigation was supported by a grant from the Research and Development Division, Office of the Surgeon General, Department of the Army, under contract No. DA-49-007-MD-655. We are indebted to the Public Health Department of the Republic of Panamá for their cooperation in this investigation.

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