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Abstract. Clinical and prevalence studies of Chagres virus (Phlebotomus-fever group of arboviruses) infections in Panamanian residents were performed. Clinical cases of Chagres virus infection in three persons are described. Antibody conversions and virus reisolations were demonstrated in all cases. The distribution of Chagres hemagglutination-inhibition antibodies in different regions of Panama were as follows: western region 0.4% of 1,329 samples; central region 4.3% of 2,829; eastern region 2.9% of 276; Perlas Islands 10.9% of 174 (including 17.8% of 107 from San Miguel Island), giving an overall rate of 3.3% in 4,608 samples. These results indicate a widespread distribution of Chagres virus in Panama, with a particularly high rate of antibody prevalence on one of the offshore islands.

Chagres virus (CHG), antigenically classified in the Phlebotomus fever group of arboviruses, was first isolated from the blood sample of a febrile patient during his participation in the jungle warfare training program in the Panama Canal Zone in July 1960.[1,2] Neutralizing antibodies to this virus were also detected in the sera of a few permanent residents in Las Barretas, Coclé Province, Panama during serological surveys. No other reports concerning the occurrence of this virus in man or in animal hosts have appeared. Another new Phlebotomus fever group virus, Punta Toro, was isolated from serum of a febrile patient who had returned from a jungle area of Panama at a Pittsburgh hospital in December 1966.[3] Although these two viruses have not been fully studied, they are related antigenically.

The present communication reports three additional human febrile cases of infection with CHG virus and also presents the results of studies of the prevalence of antibodies among indigenous populations of different geographic regions of the Republic of Panama.

ISOLATION OF VIRUSES

Case 1. (E.F.) A 49-year-old Caucasian female from Las Cumbres, a residential area about 10 miles outside of Panama City, abruptly developed a fever on 5 July 1964. Other symptoms included mild headache with anorexia and nausea which persisted for 3 days. Profuse perspiration was experienced intermittently. Physical examination on the day after onset showed no central nervous system or other signs except a temperature of 102° F. A blood specimen taken on that day showed a white count of 6,450 per mm³. The separated serum was inoculated intracerebrally into seven suckling Swiss mice for virus isolation attempts. Two mice were found dead and two other mice were sick on day 7. Three additional mice died on the 9th day after inoculation. On the second brain-to-brain passage from the two sick mice, all the infants became ill on day 5, and a stock virus was prepared from brain material. Reisolation of the virus was successful from the original serum after 3 weeks storage at −70° C.

Case 2. (E.R.) A Panamanian girl, age 16, with fever was seen by a survey team on 15 July 1964 at Cerro Cama, about 25 miles northwest of Panama city. No other signs and symptoms were recorded. Blood serum obtained the same day was inoculated on 16 July 1964 into suckling mice and hamster kidney tissue cultures (HKTC). Two mice were missing, and two were found dead on the 6th day after inoculation. Three other mice were sick on the same day, and a brain suspension for passage was made from these three mice. On the third passage, all suckling mice were sick 4 days after inoculation, and a virus stock was prepared from their brains. Reisolation of the virus from the original specimen was successful after 2 weeks storage at −70° C. Virus isolation attempts by HKTC failed.

Case 3. (C.J.) A white male physician, 63 years of age became ill with high fever while on an expedition in an area of Darien province close

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to the Colombian border. His symptoms included severe headache, muscular and joint pain, and pain in the eyes. Fever continued for 2 days with intermittent profuse sweating. No sore throat or other signs of upper respiratory infection were noted. The infection subsided without sequelae following a febrile stage of a few days duration. Blood drawn on 5 April 1970, the 1st day of illness, was centrifuged and separated in the field and the serum was kept frozen in liquid nitrogen until shipped to the laboratory 10 days later. This serum was inoculated intracerebrally into a group of seven baby mice and all appeared ill on day 9. Average survival time was shortened to 4.5 days after three brain-to-brain passages in suckling mice with a titer of more than 10^5 LD_{50}/0.02 ml. Vero cell cultures gave negative results when inoculated with the acute phase serum of this patient. However, this cell culture system showed susceptibility to infection with the isolated virus when inoculated with fifth mouse-brain passage material, which had a titer of 4.5 $TCLD_{50}$/0.1 ml. HeP-2 cells showed cytopathogenic effect to a similar titer when inoculated with this virus strain.

VIRUS IDENTIFICATION

Suckling mouse brain antigens, prepared by sucrose-acetone (SA) extraction[4] from the first two isolates (E.F. and E.R.) produced no hemagglutinins (HA) for goose cells at pH 6.0 to 7.0 but were highly reactive in the complement-fixation (CF) tests with homologous immune mouse sera. These two isolates were indistinguishable by cross CF tests. No identification could be made by typing these isolates with the 25 arbovirus reference strains available in our laboratory at that time. Upon receiving an antigen and a hyperimmune ascitic fluid prepared for Chagres virus (Strain J.W. 10)[1] from Dr. Pauline Peralta of the Middle America Research Unit (MARU) on 15 June 1965, cross CF tests showed that our isolated strains were indistinguishable from the CHG virus. Neutralization tests performed in suckling mice confirmed these findings.

SA antigen prepared for the virus isolate of the third case (C.J.) possessed a low-titered HA at pH range of 5.5 to 5.75 after incubation with goose cells at 37° C as previously shown for the J.W. 10 strain.[1] After protamine treatment of this antigen, positive HA with a titer of 1:320 was obtained at room temperature at pH 5.75. The HA of C.J. isolate was also successfully produced by double acetone extraction of infected Vero cell culture fluid, a technique similar to that used for Guama group viruses.[7] Cross hemagglutination-inhibition (HI) tests demonstrated that this isolate (C.J.) is closely related to CHG virus and to the other two isolates mentioned above. In CF tests the C.J. isolate was also shown to be indistinguishable from the J.W. 10 strain of CHG virus.

CF antibody conversions against CHG virus were demonstrated in all three cases reported herein. Similarly, significant rises in antibody titers to CHG virus (as well as to homologous isolates) were observed in HI tests in 2 of the 3 cases tested.

TABLE 1

Chagres hemagglutination inhibiting antibodies in the human population in the Republic of Panama

Area	No. tested	No. positive	% positive
Western Region			
Almirante	559	0	–
Chiriqui	770	5	0.7
Central Region			
Pacific area	1,114	42	3.8
South of Gatun Lake	1,160	47	4.1
Atlantic area and east of Gatun Lake	555	34	6.1
Eastern Region			
Darien	276	8	2.9
Perlas Islands			
San Miguel	107	19	17.8
Sahoga	67	0	–
TOTAL	4,608	155	3.4

SEROLOGICAL SURVEYS

Table 1 shows the results of HI tests among 4,608 people examined. There were no Chagres antibodies detected in 559 samples from Almirante, where several other arboviruses have been isolated from man earlier.[6] Only a small percentage of the population in Chiriqui province, in the central region and in Darien province were tested and a low percentage of positive HI antibodies to CHG was demonstrated. On the other hand, 18% antibody rates were observed in people living on San Miguel, one of the Perlas Islands in the Bay of Panama. Age distribution of subjects

TABLE 2

*Distribution of Chagres hemagglutination-inhibition test results by age of donor and geographic areas**

Age (years)	Chiriqui		Central Region		Darien		San Miguel	
	No. tested	% positive	No. tested	% positive	No. tested	% positive	No. tested	% positive
0–4	7	0	236	2.8	1	0	3	0
5–9	51	0	679	3.3	19	5.5	27	14.8
10–14	181	0	521	3.8	30	0	22	9.9
15–19	93	0	220	3.1	49	0	8	57.0
20–29	136	1.7	406	4.9	86	2.8	13	15.3
30–39	131	1.8	316	5.3	50	6.0	16	23.0
40–49	79	1.4	211	5.6	31	6.7	7	66.0
50–59	50	0	133	9.7	6	0	6	16.6
60 up	36	0	89	7.8	1	0	3	33.3
Unknown	6	0	18	0	3	0	2	0
TOTAL	770	0.6	2,829	4.3	276	2.9	107	17.8

* Areas where negative results were obtained are not included.

tested in these suspected endemic areas is listed in Table 2. No efforts have been made to classify the individuals tested by sex, occupation, etc.

DISCUSSION

The geographic distribution of CHG virus in the three cases reported here indicates that this virus may be present in different areas in Panama. One of these patients lived in an upper-class residential area surrounding a small artificial lake outside of Panama City. A small number of sentinel suckling mice and adult hamsters were exposed in the area, but no virus transmission could be detected. Although outbreaks of another arthropod-borne disease, malaria, occurred in this area recently, no arbovirus infections have been reported there before.

The second strain of CHG virus was isolated from a village south of the Gatun Lake of the Panama Canal. The patient was examined by a medical survey team from the Gorgas Memorial Laboratory (GML) which occasionally visits the villages around this area. Since this region can be reached only with great difficulty, no detailed clinical studies or follow-up of the case was made during her acute stage of illness. The original report of the discovery of CHG virus involved the area north of this large man-made lake.

Field teams from GML have regularly investigated the Darien region of eastern Panama in yellow fever surveillance activities.[7] One of the CHG infections reported here was acquired by a field supervisor during the course of a trip to the Rio Mono-Cerro Quia area in Darien. The isolated virus from this patient (C. J.), designated strain F 764, produced a high titered HA in normal pH ranges tested at room temperature. This property provides the F 764 isolate with a considerable advantage over the original strain, J. W. 10, isolated by MARU investigators.[1] Antigens prepared either from suckling mouse brains or from infected Vero cell culture fluid should be very useful in serological surveys for antibodies to CHG virus.

Results of the serological surveys reported disclose interesting differences in antibody prevalence among different age groups in some of the geographical areas studied. It is apparent that CHG virus infection was highly endemic on San Miguel Island in the Bay of Panama and that it has been prevalent to a lesser degree in the Central Region. However, the data suggest that the virus was present in the highlands of Chiriqui during a period of 20 years prior to the study and not active in that area since that time. It is not known whether the 5 seropositive people had previously lived in a different part of Panama.

Although CHG virus is classified in the Phlebotomus fever group antigenically, there have been no reports of virus isolations from Phlebotomus sandflies or any other insects. Furthermore, the virus has not yet been isolated from wild vertebrates. Thus, nothing is known about the transmission of this virus in nature. The results of our large-scale serological surveys demonstrated high antibody rates among people living in San Miguel of the Perlas Islands. Therefore,

it is possible that ecological studies of CHG virus might be successfully carried out in that area.

SUMMARY

Following the isolation of the prototype strain of Chagres (CHG) virus in Panama in 1960, three additional human infections with this virus have been diagnosed. Two of these febrile subjects were detected in the central part of Panama in 1964; another apparently contracted CHG infection in Darien province of eastern Panama. Virus reisolation attempts were successful and antibody conversions were observed in all three cases.

Serological surveys of some 4,600 people from different geographical areas of Panama showed a low HI antibody rate in human populations. However, a relatively high rate of CHG seropositive subjects was demonstrated in one of the islands in the Bay of Panama.

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