Restan virus, a new group C arbovirus from Trinidad and Surinam♦

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Eleven viruses belonging to arbovirus group C have so far been described. Six of these were first encountered in Brazil[1-3], four made their debut in Panama[4,5], and one was first found in Trinidad[6].

During 1963 and 1964 seven strains of a group C virus type, which differs from the described prototypes, were isolated in Trinidad[3] and Surinam[4]. We propose calling the agent Restan virus, after a part of Bush Bush island[7], Trinidad, where the first strain was recovered.

MATERIALS AND METHODS

The isolations as well as all further tests were done with groups of seven 2-day-old mice inoculated intracerebrally (i.c.) with 0,02ml amounts. Techniques have been described previously[8].

Complement-fixation (CF) tests were done by a microtechnique adapted from Fulton and Dumbell[9]. The techniques of Clarke and Casals[10] were followed for hemagglutination-inhibition (HI) tests.

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The laboratory rodents were from a colony of *Zygodontomys b. brevicauda* (Allen and Chapman) and *Oryzomys laticeps velutinus* Allen and Chapman maintained at the Trinidad Regional Virus Laboratory (TRVL) by C. Brooke Worth. Both species are native to Bush Bush forest.

RESULTS

Isolations

Trinidadian strains

Three strains of Restan virus have been encountered in Trinidad as of the end of 1965. All were isolated in 1963.

Strain TRVL 51144, the prototype, was isolated from a pool of 13 *Culex* (*Melanoconion*) *portesi* Senevet and Abennenc[11] (referred to as *Culex* sp. nº 9 in earlier TRVL publications) caught in May in Bush Bush forest in a trap baited with sentinel white mice. An attempt to reisolate the virus from the original mosquito suspension after one year's storage in a CO_2-ice box was unsuccessful. No illness was observed in the sentinel mice.

Strain TRVL 52607 also originated from *C. portesi* of Bush Bush forest; these mosquitoes were caught in August. Attempted reisolation of virus from the original mosquito suspension in May 1965 was unsuccessful.

The third strain, TRVL 52699, was isolated and reisolated from serum of a 16-year-old boy living in Arima, a town in North Central Trinidad. During 10-18 August this boy visited the village of Blanchisseuse on the North coast, where on the 17th he fell ill with fever, headache, ague, and, specifically, pain in the back and neck. He was brought to our attention by dr. B. C. Boyd, and a serum specimen taken on the 22nd, when his axillary temperature was 98.4ºF, yielded the virus. The patient recovered uneventfully and was bled again, five days later, on the 27th. No HI or neutralizing antibodies were detected in this second specimen. Since then the boy has refused to be bled.

All three Trinidadian strains killed baby mice inoculated i.c. with the source materials in two to three days. On passage, the average survival time (AST) was shortened to one to two days. The adapted strains killed baby mice inoculated intraperitoneally (i.p.) with an AST of two to three days and wean ling mice inoculated i.c. and i.p. with AST's of three to four and three to six days, respectively. The three strains were indistinguishable in cross CF, HI, and neutralization (N) tests.

Adult hamsters inoculated i.c. or i.p. with TRVL 51144 virus died in about three days postinoculation (p.i.). Adult guinea pigs showed no signs of illness after i.c. or i.p. inoculation of TRVL 51144 virus, and they had neutralizing antibodies to high titers.

Surinam strains

The four strains of Restan virus recovered in Surinam were shown to be indistinguishable from TRVL 51144 virus in N and HI tests. The first three strains were isolated in 1963 from sera of acutely ill Dutch soldiers stationed in Surinam.

Strain D79 was isolated and reisolated from serum of private G. V., collected 16 June 1963. During 5-14 June this soldier took part in a jungle patrol; the conditions under which the men live during these patrols have been described[12]. After return to barracks G. V. fell ill on the l6th with headache, backache, and pain in arms and legs. He felt seriously ill, although his rectal temperature was only 99ºF. After a restless night, he was admitted to the hospital on the 17th with a temperature of 100.4ºF. Within 48 hours after admission all symptoms had disappeared. A serum specimen taken 14 days after the patient's recovery neutralized over 4 logs of homologous virus and showed HI titers of 1:40 with four antigen units of TRVL 51144 virus and of 1:80 with four antigen units of homologous virus. The acute-phase serum was negative in both N and HI tests.

Strains D101 and D102 were isolated from serum specimens of privates G. J. S. and J. A. B., each collected on 14 July 1963. During 7-13 July both men had been on a patrol in the Bush and

Savannah country around Troeliekreek, about 35 miles Southwest of Paramaribo. They were admitted to the hospital on the 14th with fever, headache, and backache. At that time G. J. S. had a temperature of 104.2ºF and a total leukocyte count of 2,650 with 58% lymphocytes. He was discharged free of symptoms on the 15th. On admission J. A. B. had a temperature of 103.5ºF and a total leukocyte count of 4,000 with 52% leukocytes, 5% young leukocytes, 42% lymphocytes, and 1% monocytes. He also had some leukocytes in the urine, which delayed his discharge until the 18th. His temperature had returned to normal on the 15th.

Table 1 – HI an N tests with the TRVL 51144 strain of Restan virus and three Trinidadian group C agents

Antigen	*Zygodontomys* immune serum			
	51144	Oriboca	Caraparu-like	Nepuyo
HI tests (4 units of antigen)				
TRVL 51144	*320**	80	0	40
Oriboca, TRVL 47827	20	*320*	0	0
Caraparu-like, TRVL 34053-1	10	40	*80*	0
N tests				
Virus				
TRVL 51144	*4.0*†	2.5	2.8	1.8
Oriboca	0.1	*3.0*	2.1	1.1
Caraparu-like	1.3	3.0	*6.5*	1.5
Nepuyo, TRVL 18462	0.1	0.1	2.6	*3.1*

* Reciprocal of serum dilution giving complete inhibition of agglutination.
† Log neutralization index.

Convalescent sera of G. J. S. and J. A. B., taken two years after infection, neutralized over 4 logs of D79 virus and 3.5 logs of TRVL 51144 virus. Both sera had HI titers of 1:40 with four antigen units of both these viruses.

The fourth Surinam strain of Restan virus, 28a, was isolated in December 1964 from *Culex* mosquitoes caught in the same general area where G. J. S. and J. A. B. had been on patrol before their illness[13].

Identification

TRVL 51144 virus was not tested for sensitivity to ether or sodium desoxycholate.

A 10% suspension of infected baby-mouse liver in normal saline solution provided a good CF antigen for TRVL 51144 virus, and acetone-extracted baby-mouse liver worked equally well. CF tests readily showed the new agent to be a member of serologic group C.

A hemagglutinating antigen was easily prepared by acetone extraction of infected baby-mouse serum taken 24 hours p.i.; the antigen was most active at 37ºC at a pH of 6.2. TRVL 51144 virus was compared with the three group C serotypes previously recovered in Trinidad in HI and N tests done with immune sera prepared in *Zygodontomys*. The results (Table 1) clearly indicated that it differed from the known Trinidadian group C agents.

Final identification was accomplished at the Belém Virus Laboratory of the Evandro Chagas Institute, Brazil, where TRVL 51144 virus was compared in HI, CF, and N tests Belém prototype strains for seven group C viruses (Table 2)

In HI tests (Table 2) TRVL 51144 virus was more closely related to Marituba and Murutucu than to the other Belém group C agents. The relation with Murutucu was closest, although the two viruses were distinguishable in cross-HI testing. Further HI comparisons with several guinea-pig and mouse immune sera prepared for TRVL 51144, Marituba, and Murutucu viruses confirmed the results shown in Table 2. In another HI test, not shown, TRVL 51144 antigen did not react with immune sera for Ossa and Madrid, group C viruses from Panama[4].

In CF tests (Table 2), TRVL 51144 virus was very closely related to Oriboca and Murutucu viruses and less closely to Marituba and Apeu. It thus had the same CF reaction pattern as Oriboca and Murutucu viruses[2].

In N tests (Table 2), done with guinea-pig immune serum and infected mouse serum as virus source, TRVL 51144, Marituba, and Murutucu viruses were indistinguishable.

Following the precedent of Casals and Whitman[2], who established group C on the basis of the HI reactions of the first five members described, we consider TRVL 51144 virus a distinct entity in group C and propose the name Restan virus.

Table 2 – HI, CF, and N tests with the TRVL 51144 strain of Restan virus and seven Belém group C prototype viruses

(continua)

Antigen	Immune serum (S) or immune ascitic fluid (AF)							
	51144 2 inj. S	Marituba 2 inj. S	Murutucu 4 inj. AF	Oriboca 3 inj. AF	Apeu 2 inj. S	Caraparu 3 inj. AF	Nepuyo 2 inj. S & AF	Itaqui 2 to 3 inj. S
HI tests (4 units of antigen)								
TRVL 51144	160*	160	40	0	0	0	0	0
Marituba, BE An 15	40	1280						
Murutucu, BE An 974	320		320					
Oriboca, BE An 17	<40			320+				
Apeu, BE An 848	40				1280			
Caraparu, BE An 3994	<40					320		
Nepuyo, BE An 10709	<80						320	
Itaqui, BE An 12797	<20							160+
CF tests (2 units of complement)								
TRVL 51144	32+/64+†	16/64+	32+/64+	8/64+	0/0	0/0	0/0	0/0
Marituba	8/64+	32+/64+						
Murutucu	16/64+		16/64+					
Oriboca	32+/64+			16/64+				
Apeu	8/64+				8/64+			

* Reciprocal of serum dilution giving complete inhibition of agglutination. + = endpoint not reached. 0 = negative at 1:10 dilution.

† Reciprocal of serum dilution over reciprocal of antigen dilution giving greater than 50% fixation. 0/0 = negative at 1:8 serum dilution and 1:4 antigen dilution.

Table 2 – HI, CF, and N tests with the TRVL 51144 strain of Restan virus and seven Belém group C prototype viruses

(conclusão)

Antigen	Immune serum (S) or immune ascitic fluid (AF)							
	51144 2 inj. S	Marituba 2 inj. S	Murutucu 4 inj. AF	Oriboca 3 inj. AF	Apeu 2 inj. S	Caraparu 3 inj. AF	Nepuyo 2 inj. S & AF	Itaqui 2 to 3 inj. S
CF tests (2 units of complement)								
Caraparu	0/0					16/64+		
Nepuyo	0/0						16/16	
Itaqui	0/0							32+/16
N tests (Guinea-pig serum, 1 inj.)								
Virus								
TRVL 51144	*5.7*‡	5.9	6.7					
Marituba	4.4	*4.3*	4.3					
Murutucu	4.5	4.0	*5.2*					

‡ Log neutralization index.

Laboratory studies

The two isolations from *Culex portesi* suggested that Restan virus, like the Trinidadian Caraparu-like virus in Bush Bush[14], utilizes rodents as vertebrate hosts. This possibility was tested in the following experiment. Five *Zygodontomys* and five *Oryzomys* from the laboratory colony were each inoculated subcutaneously with 3160 LD_{50} of fourth passage material of Restan virus (TRVL 51144). Three animals of each species were tested for viremia on days one through 4p.i. by methods described previously[15]. As shown in Table 3, all six animals circulated the virus to high titers on days 1-3 p.i., and two *Zygodontomys* still circulated virus on day 4p.i. In all ten inoculated animals HI and CF antibodies appeared, and the 1:3 diluted sera of all ten neutralized at least 126 LD_{50} of Restan virus. These results support the view that rodents may be involved in the natural cycle of the virus.

In an experiment carried out is Paramaribo, Restan strains D101 and D102 were both successfully transmitted from sick to healthy baby mice by *Aedes aegypti* that had been reared from locally collected larvae. The incubation period in the mosquitoes was five to six days.

Table 3 – Virus circulation and antibody development in *Zygodontomys brevicauda* and *Oryzomys laticeps* inoculated subcutaneously with 3160 LD_{50} of 4th-passage material of Restan virus

Animal Nº	Circulating virus titer on day p.i.				Antibodies at 14 days p.i.	
	1	2	3	4	CF	HI
Zy 7381	≥4.8*	≥4.8	≥4.8	2.3	320†	200‡
7382	≥4.8	≥4.8	≥4.8	2.9	320	200
7383	≥4.8	≥4.8	≥4.8	≥0.8	320	200
Or 7386	≥4.8	≥4.8	2.1	≥0.8	80	100
7387	≥4.8	≥4.8	4.3	≥0.8	80	100
7388	3.1	≥4.8	2.8	≥0.8	160	100

* ≥4.8 = serum virus titer $10^{4.8}$ or larger.

† Highest serum dilution giving at least 50% fixation of complement.

‡ Highest serum dilution inhibiting agglutination with four with units of antigen.

Serum survey

Blood specimens from 499 Dutch soldiers who entered Surinam between February 1962 and February 1963 were taken on arrival and again a year later on departure. HI tests were performed on these specimens with Restan, Caraparu-like, and Oriboca antigens. In 17 soldiers antibodies to Restan virus (TRVL 51144) had developed during the year. Four of these 17 had antibodies to Caraparu-like virus (TRVL 34053-1) as well, but the remaining 13 had antibodies only to Restan virus. It has not been established that these antibodies were caused by infection with Restan virus rather than with Marituba or Murutucu.

Of sera from 366 residents of Eastern Trinidad, not more than five showed HI reactions with Restan (TRVL 51144), Oriboca (TRVL 47827), or Caraparu-like (TRVL 34053-1) antigen indicative of possible past infection with Restan or a closely related virus. N tests were not done on these sera.

SUMMARY

Restan virus is a new serotype of arbovirus group C that has been isolated seven times in Trinidad and Surinam during 1963 and 1964. Three strains were recovered from *Culex* spp. and four from acutely ill human beings. The new agent is related most closely to Marituba and Murutucu viruses, from which it is distinguishable only in hemagglutination-inhibition test. It circulates to high titer in laboratory-colonized rodents of the species *Zygodontomys b. brevicauda* and *Oryzomys laticeps velutinus*. In the laboratory it has been transmitted by *Aedes aegypti*. Results of serum surveys are reported.

REFERENCES

1. Causey, O. R., Causey, C. E., Maroja, O. M., and Macedo, D. G., 1961. The isolation of arthropod-borne viruses, including members of two hitherto undescribed serological groups, in the Amazon region of Brazil. *Am. J. Trop. Med. & Hyg., 10:* 227-249.

2. Casals, J., and Whitman, L., 1961. Group C, a new serological group of hitherto undescribed arthropod-borne viruses. Immunological studies. *Am. J. Trop. Med. & Hyg., 10*: 250-258.

3. Shope, R. E., Causey, C. E., and Causey, O. R., 1961. Itaqui virus, a new member of arthropod-borne group C. *Am. J. Trop. Med. & Hyg., 10*: 264-265.

4. de Rodaniche, E., Paes de Andrade, A., and Galindo, P., 1964. Isolation of two antigenically distinct arthropod-borne viruses of group C in Panama. *Am. J. Trop. Med. & Hyg., 13*: 839-343.

5. Srihongse, S., Galindo, P., and Grayson, M. A., 1966. Isolation of group C arboviruses in Panama including two new members, Patois and Zegla. *Am. J. Trop. Med. & Hyg., 15*: 379-384.

6. Spence, L., Anderson, C. R., Aitken, T. H. G., and Downs, W. G., 1966. Nepuyo virus, a new group C agent isolated in Trinidad and Brazil. I. Isolation and properties of the Trinidadian strain. *Am. J. Trop. Med. & Hyg., 15*: 71-74.

7. Downs, W. G., Spence, L., and Aitken, T. H. G., 1962. Studies on the virus of Venezuelan equine encephalomyelitis in Trinidad, W. I. III. Reisolation of virus. *Am. J. Trop. Med. & Hyg., 11*: 841-843.

8. Spence, L., Anderson, C. R., Aitken, T. H. G., and Downs, W. G., 1962. Bimiti virus, a new agent isolated from Trinidadian mosquitoes. *Am. J. Trop. Med. & Hyg., 11*: 414-417.

9. Fulton, F., and Dumbell, K. R., 1948. The serological comparison of strains of influenza virus. *J. Gen. Microbiol., 3*: 97-111.

10. Clarke, D. H., and Casals, J., 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am. J. Trop. Med. & Hyg., 7*: 561-573.

11. Aitken, T. H. G., and Galindo, P., 1966. On the identity of *Culex* (Melanoconion) *portesi* Senevet & Abonnenc 1941 (Diptera, Culicidae). *Proc. Ent. Soc. Wash., 68*: 198-208.

12. Karbaat, J., Jonkers, A. H., and Spence, L., 1964. Arbovirus infections in Dutch military personnel stationed in Surinam: A preliminary study. *Trop. Geogr. Med., 4:* 370-376.

13. de Haas, R. A., Central Laboratorium, Paramaribo, 1965. Personal communication.

14. Jonkers, A. H., Spence, L., Downs, W. G., and Worth, C. B., 1964. Laboratory studies with wild rodents and viruses native to Trinidad. II. Studies with the Trinidad Caraparu-like agent TRVL 34053-1. *Am. J. Trop. Med. & Hyg., 13*: 728-733.

15. Jonkers, A. H., Spence, L., and Olivier, O. *Laboratory studies with wild rodents and viruses native to Trinidad*. III. Studies with three Guama group viruses. In preparation.

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