VIRUSES ISOLATED FROM PANAMANIAN SLOTHS*

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Abstract. Seven virus strains were isolated in Vero cells from whole blood samples from 80 wild-caught sloths, Bradypus variegatus and Choloepus hoffmanni, from Central Panamá. Four strains of at least two different serotypes are related to Changuinola virus; two of these were associated with prolonged or recrudescent viremias. One strain is an antigenic subtype of Punta Toro virus, and another, described here as Bradypus-4 virus, is a new, antigenically ungrouped virus. A second new virus from sloths, Utiva virus, forms an antigenic complex within the Simbu serogroup with Utina and Pintupo viruses. Tests on sequential plasma samples from radio-marked free-ranging sloths and from recently captured animals maintained in captivity showed that both species develop neutralizing antibodies following naturally acquired virus infections. Antibodies against the Changuinola and Simbu serogroup viruses are widespread in both sloth species and are especially prevalent in Choloepus, but are virtually absent in all other wild vertebrate species tested.

Tree sloths are arboreal herbivorous mammals native to New World tropical forests. They are limited to two genera: Choloepus (two-toed sloths) and Bradypus (three-toed sloths), with each genus assigned to a separate family. Other papers in this series have referred to sloths' high natural population density, wide distribution, low and variable metabolic rates, remarkable susceptibility to experimental arbovirus infections, and natural infection by flaviviruses, especially St. Louis encephalitis (SLE) virus. 

Apart from flaviviruses, sloths in previous studies have yielded isolates of four different arboviruses, all from the family Bunyaviridae and all from Brazil. These include three agents of the genus Bunyavirus from three-toed sloths Bradypus tridactylus: Oropouche and Utina viruses of antigenic serogroup Simbu, and Murutucu virus of serogroup C. The two-toed sloth Choloepus didactylus has yielded Anhanga virus (genus Phlebovirus). During studies of flavivirus infections of Panamanian sloths at the Gorgas Memorial Laboratory, several non-flavivirus agents were recovered. This paper describes these viruses, of which two appear to be new, and reports the development and prevalence of antibodies against these viruses in sloths and other wild Panamanian vertebrates. These findings are discussed for their relevance to some tropical arbovirus cycles and to the unusual behavior of viruses in sloths.

MATERIALS AND METHODS

Animals and study areas

Two-toed sloths Choloepus hoffmanni and three-toed sloths Bradypus variegatus were captured in five areas of Central Panamá. Age determination, blood sampling and localities of capture have all been described previously. Briefly, sloths from Aguaclata and Chilibre villages, located in secondary forest interspersed with primary forest patches, were brought to the Gorgas Memorial Laboratory (GML) where they were bled for virus isolation and antibody detection, and then maintained in mosquito-free quarters for experiments. Sloths from Majé, El Llano-Carti road camp, and Cerro Azul, all located in primary forest, were bled, marked and released.

In addition, a number of sloths at Cerro Azul were equipped with radio transmitters between 16 September 1974 and 15 August 1975; these animals were periodically recaptured for serial blood samples. Small radio transmitters were attached to collars and fitted to sloth necks as described by

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Montgomery et al.³ Thirty-two radio-marked *Bradypus* were followed for an average of 111 days each (minimum 29, maximum 289 days); of these, 23 were native Cerro Azul sloths, and nine were imported from Aguacate, and the El Llanocartí area. Two mother-baby pairs of radio-marked *Choloepus* were followed during September, October, and November for 49 and 50 days, respectively. Four other *Bradypus* and one other *Choloepus* were captured and bled only once. From the 36 *Bradypus*, 110 blood samples were tested for virus isolation and 122 plasmas were tested for antibodies. The five *Choloepus* from Cerro Azul yielded a total of four samples for virus isolation and 11 for serology. Thirty-one wild mammals other than sloths were trapped or shot at Cerro Azul.

At Majé, wild vertebrates of species other than sloths were either netted or trapped routinely by the GML Bayano River Program or rescued from river impoundment flooding by the International Society for the Protection of Animals. Blood samples were taken as described previously.³

**Virus isolation**

Whole heparinized venous sloth blood was divided into two aliquots. On field trips these were frozen within 2 hours in liquid nitrogen; in the laboratory they were stored immediately at −60°C in electric freezers. Subsequent isolation attempts were done in tube cultures of Vero cells.⁵ Each tube was inoculated with 0.1 ml of whole blood, either undiluted or at 1:10 in phosphate-buffered saline, with 0.5% gelatin; two tubes were used per dilution. The second as yet unthawed whole blood aliquot was saved for reisolation attempts. Tubes showing cytopathic effect (CPE) were frozen, thawed, and centrifuged at 10⁴ × g for 30 min. The supernate was passed again into Vero cells to confirm viral etiology of CPE.

Special procedures have been described for the recovery and titration of Changuinola serogroup virus strain Pan An 341275 from the blood of *Choloepus* no. 20 in the presence of circulating SLE virus.¹

**Preparation of immune reagents**

Crude 20% saline suspensions of the brains of infected mice or hamsters were used as immunizing antigens. Hamster immune serum was produced against the Phlebotomus fever serogroup strain Pan An 53038 as described previously,⁶ except that doses were at weekly intervals and serum was collected 3 weeks after the final inoculation. Hyperimmune ascitic fluid (MAF) against *Bradypus*-4 virus was made in a mouse which had been inoculated intracerebrally (i.c) as a neonate, and had recovered from illness occurring 9–11 days after inoculation. Intraperitoneal (ip) inoculations of infected mouse brain in Freund’s complete adjuvant (FCA) on days 21 and 37, and of sarcoma 180 TG cells on day 38, were followed by harvest of ascitic fluid on day 47.

Plaque-purified Simbu serogroup isolates were passed four times in suckling mice ic for adaptation. Intravenous (iv) inoculation of 3-- to 4-week-old mice was followed by ip administration of infected brain in FCA on days 7, 14 and 24, and of sarcoma cells on day 22. Immunization of mice against unpurified Utive virus was similar except that additional iv inoculations accompanied the ip shots. Schedules for immunization against Utive virus which did not include iv inoculation failed to stimulate satisfactory levels of neutralizing antibody.

Changuinola serogroup viruses did not produce satisfactory neutralizing antibody levels by any schedule.

**Sero logic identification of viruses**

Infected hamster or mouse brain extracts were sucrose-acetone extracted¹⁰ and sonicated to prepare antigens for micro-adaptations of the complement fixation (CF)¹¹ and hemagglutination-inhibition (HI)¹⁰ tests. Vero cell supernate harvests prepared by freezing, thawing, and centrifuging at 10⁴ × g were used as antigens for plaque-reduction neutralization (PRN) tests.¹² Isolates were characterized antigenically at GML by one or more of the CF, HI, and PRN tests, with the following reagents: 1) NIH arbovirus grouping immune reagents; 2) viruses and hyperimmune hamster sera and MAF from this and previous studies;² ³ convalescent plasmas from sloths yielding virus isolates; and 4) prototype viruses and MAF from the Yale Arbovirus Research Unit (YARU). The reference Utinga virus used was the prototype strain BeAn 84785, received from YARU as second passage mouse brain, and passed up to three times further in mice or Vero cells to make antigens. The reference Pintupu virus was prototype
strain PanAr 517.18 We are indebted to Dr. Robert Shope for extensive CF tests of some isolates at YARU, and to Mr. Richard Kinney and Dr. Charles Calisher for CF, HI, and PRN tests of Simbu serogroup viruses at the Centers for Disease Control Laboratory in Fort Collins.

**Characterization of Bradypus-4 virus**

Chemical and physical characterization tests used tissue culture harvests of Bradypus-4 virus at the third to fifth Vero passage level. For lipid solvent sensitivity, this agent, and control viruses polio 2 and vesicular stomatitis-Indiana (VSV) viruses, were incubated at room temperature as a mixture of 0.05 ml CHCl₃ and 1.0 ml stock virus preparation. A duplicate set of control tubes was not treated with CHCl₃. After a 30 min incubation, all samples were centrifuged at 1,500 rpm for 5 min, and the aqueous fractions were assayed in Vero cell tube cultures to estimate tissue culture median infectious doses (TCID₅₀).

To determine viral nucleic acid type, serial 10-fold dilutions of Bradypus-4, vaccinia and VSV viruses were inoculated into two sets of Vero cell tubes, of which one contained 10⁻⁵ M 3-bromodeoxyuridine (BUDR) in the maintenance medium. Tubes were observed for CPE for calculation of TCID₅₀ titers.

Bradypus-4 virus was passaged sequentially through prewetted Millipore filters of 0.45 and 0.22 μm average pore size. Samples taken prior to filtration and after each filtration step were assayed for plaques in Vero cells using an overlay of 1% Noble agar in Eagle's Minimal Essential Medium, with final concentrations of 25 mM HEPES, 5 mM tricine, 2% fetal calf serum, 0.01% DEAE-dextran, 0.12% NaHCO₃, and 4 mM NaOH. Plates were incubated at 35°C in 5% CO₂, neutral red was added on day 6, and plaques were observed days 7–10 after inoculation.

The sensitivity of different cell culture types to Bradypus-4 virus was tested at the Virology Laboratory of INCIENSA, Tres Ríos, Costa Rica, by the senior author. Tube cultures of Hep2, BGM, WI38, BHK-21, Vero and primary human amnion cells were adsorbed for one hour with 0.1 ml containing approximately 200 Vero TCID₅₀ of Bradypus-4 virus, then incubated at 37°C. Cultures were frozen at −85°C when 75% of the cells showed CPE, then thawed, centrifuged for 30 min at 1,600 × g and assayed in Vero cell cultures.

**Plaque purification**

Simbu serogroup viruses were plaque-purified on Vero cell monolayers in 9.6 cm² or 1.7 cm² wells using the overlay of Tesh et al.⁹ Plaques were picked through Pasteur pipettes, and amplified by a passage in Vero cell tube cultures. After three purification-amplification cycles, the final amplification passage was used as antigen in the PRN test, and also serially passed 4 four times in newborn mice to make immunizing, CF, and HI test antigens of purified viruses.

**Serologic survey**

Plasmas or sera from sloths and other animals were heat inactivated at 56°C for 30 min. They were tested for PRN antibody at 1:8 and higher dilutions against 30–100 plaque-forming units of challenge virus, using Vero cells in procedures described by Tesh et al.¹⁰ Several individual samples had positive titers of 1:8 against a wide variety of unrelated viruses, suggesting non-specific inhibition at this dilution. Therefore, 90% plaque reduction by serum or plasma diluted at least 1:16 was considered positive.

**RESULTS**

**Virus isolates**

Six virus strains were isolated from 79 newly-captured sloths tested (Table 1). Five of these isolates represent three different antigenic groups: Chiquinola (CGL), Simbu and Phlebotomus fever, of the Orbivirus, Bunyavirus, and Phlebo-virus taxa, respectively. One of the isolates is antigenically ungrouped. The ungrouped agent and the Simbu serogroup virus appear to be new viruses. An additional CGL-related strain was isolated in 1975 during yellow fever virus experiments at GML.⁶ Denominator values for negative sloths are not available from this yellow fever study, and we report here only data on the animal infected with the CGL-related virus.

**CGL serogroup viruses**

Four sloths yielded viruses indistinguishable by CF test from CGL virus (Table 1). Two of the host animals were radio-marked Cerro Azul Bry-pus, and two (one Bradypus and one Choloepus)
Table 1

<table>
<thead>
<tr>
<th>Locality</th>
<th>Species</th>
<th>No. isolated/no. animals tested</th>
<th>Date</th>
<th>Strain</th>
<th>Arbovirus antigenic group</th>
<th>Virus type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerro Azul</td>
<td>Bradypus</td>
<td>4/36</td>
<td>1 Dec 1974</td>
<td>Pan An 53038</td>
<td>Phlebotomus fever</td>
<td>Punta Toro Complex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22 May 1975</td>
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<td>Changuinola</td>
<td>Changuinola Complex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 Jul 1975</td>
<td>Pan An 53061</td>
<td>Changuinola</td>
<td>Changuinola Complex</td>
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<td></td>
<td></td>
<td></td>
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<td>Pan An 48878</td>
<td>Simbu</td>
<td>Utive*</td>
</tr>
<tr>
<td>Chilibre</td>
<td>Bradypus</td>
<td>1/7</td>
<td>9 Feb 1976</td>
<td>Pan An 648052</td>
<td>Ungrouped</td>
<td>Bradypus-4*</td>
</tr>
<tr>
<td></td>
<td>Choleopus</td>
<td>0/5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aguacate</td>
<td>Bradypus</td>
<td>1/9†</td>
<td>Jan 1975</td>
<td>Pan An 307566</td>
<td>Changuinola</td>
<td>Changuinola Complex</td>
</tr>
<tr>
<td></td>
<td>Choleopus</td>
<td>1/1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ancon, Gorgona,</td>
<td>Bradypus</td>
<td>0/1</td>
<td>2 Dec 1976</td>
<td>Pan An 341275</td>
<td>Changuinola</td>
<td>Changuinola Complex</td>
</tr>
<tr>
<td>Juanina,</td>
<td>Choleopus</td>
<td>0/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arraiwan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Bradypus</td>
<td>6/16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Choleopus</td>
<td>1/17</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* New virus.
† Changuinola complex strain Pan An 307566 was isolated from the blood of an Aguacate Bradypus experimentally infected with yellow fever virus. Further denominator data on negative sloths are not available from the yellow fever study.

had been captured at Aguacate for use in flavivirus infection experiments.\(^4\,^6\) The four strains were all reisolated, and all four host sloths developed convalescent phase neutralizing antibodies.

Though closely related by CF test, the four strains were separable into two different serotypes by PRN tests. These tests used paired acute and convalescent phase plasmas of host sloths as immune reagents, because of difficulty in stimulating usable antibody levels in conventional laboratory animals. The three strains from B. variegatus were closely related to each other but were different from the fourth strain, isolated from C. hoffmanni.

To determine the natural vertebrate host range of CGL-related viruses, plasmas from 61 birds of 36 species and 337 mammals of 28 species were collected at Majé, and tested for PRN antibodies against an isolate from a Cerro Azul Bradypus. Antibody was detected almost exclusively in C. hoffmanni sloths (Table 2). Eighteen of 54 plasmas from this species were positive, in contrast to only two of 83 primate plasmas, and none of the plasmas of the remaining species.

Antibody against the CGL-related virus was widespread in Central Panamá in both sloth species (Table 3). Antibody was more prevalent in C. hoffmanni than in B. variegatus, and increased in prevalence with age.

There was no obvious seasonality of CGL-related virus infections in sloths. Seroconversions of free-ranging radio-marked sloths at Cerro Azul occurred in September-October late in the rainy season (one infant Choleopus), as well as during the January-May dry season (two adult Bradypus) and early in the rainy season in May-July (three adult Bradypus). In addition, CGL-related viruses were isolated in December and January from one sloth of each species originally captured in Aguacate and kept in the laboratory.

The CGL-related viremia patterns of these two captive sloths are noteworthy. Viremia was detected in the B. variegatus at 38 and 42 days after capture and housing in laboratory conditions free of biting insects. This animal had been inoculated with YF virus\(^6\) 3 days before the first day of detected CGL viremia. The second captive sloth (Choleopus no. 20) was viremic when bled the day after arrival in the laboratory, immediately before inoculation with SLE virus. This naturally-acquired CGL-related virus was detectable in blood for at least 28 days. It circulated concurrently with experimental SLE virus, and also with high levels of neutralizing antibodies against two other CGL-
Table 2
Prevalence of plaque-reduction neutralizing antibodies against three sloth viruses in wild vertebrates captured at Majé from June 1974 to August 1976

<table>
<thead>
<tr>
<th>Species</th>
<th>No. positive/no. tested against virus* (strain)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uve (Pan 148878)</td>
</tr>
<tr>
<td>Birds (36 species)</td>
<td>0/61</td>
</tr>
<tr>
<td>Opossums (3 species)†</td>
<td>0/18</td>
</tr>
<tr>
<td>Bats (7 species)†</td>
<td>0/20</td>
</tr>
<tr>
<td>Primates:</td>
<td></td>
</tr>
<tr>
<td>A louatta villosus</td>
<td>0/14</td>
</tr>
<tr>
<td>A catarinensis</td>
<td>1/27</td>
</tr>
<tr>
<td>Cebus capucinus</td>
<td>0/2</td>
</tr>
<tr>
<td>Saginus Geoffroyi</td>
<td>0/40</td>
</tr>
<tr>
<td>Carnivores:</td>
<td></td>
</tr>
<tr>
<td>Nasua nasua</td>
<td>0/5</td>
</tr>
<tr>
<td>Potos flavus</td>
<td>0/7</td>
</tr>
<tr>
<td>Rodents:</td>
<td></td>
</tr>
<tr>
<td>Four species†</td>
<td>0/48</td>
</tr>
<tr>
<td>Dasyprocta punctata</td>
<td>0/8</td>
</tr>
<tr>
<td>Coendou rothschildi</td>
<td>0/1</td>
</tr>
<tr>
<td>Rabbits:</td>
<td></td>
</tr>
<tr>
<td>Sylvilagus brasiliensis</td>
<td>0/15</td>
</tr>
<tr>
<td>Xenarthrans:</td>
<td></td>
</tr>
<tr>
<td>Three species†</td>
<td>0/20</td>
</tr>
<tr>
<td>Bradypus variegatus</td>
<td>3/58</td>
</tr>
<tr>
<td>Choloepus highmanni</td>
<td>23/54</td>
</tr>
</tbody>
</table>

* CGL, Chanagaua, PT, Punta Tora.
† Mammalian species (number of individuals tested) uniformly negative to all three viruses: Opossums, Didelphis marsupialis (14), Myoproechus nesnadi (2), Marmosa sp. (2), bats, Arthus jamaicensis (6), S. notatus (6), Cynops perspicillatus (6), Tomatea hedinae (1), Vampyrus symphurus (1), C. calurus (1), Creotethys bicolored (1), rodents: Agouti para (2), Proechimys sc穿越niosus (22), S. graciosus (11), S. mystax (13), xenarthrans: Cebus capucinus (1), Dasyproctu novemcinctus (5), Tamandua tetradactyla (14).

tests, the sloth strain was tested against immune reagents for all eight Old World and 15 New World phleboviruses recognized in 1975, including all known Panamanian phleboviruses. Reaction was noted only against Punta Toro virus and a closely related strain VP-366G. Two-way PRN tests against viruses of the Punta Toro complex showed the sloth virus to be closely related or identical to Punta Toro virus, as well as VP-366G and VP-419T viruses from Panamanian sandflies, but distinct from Buenaventura virus (Table 5). These four Phlebotomus fever serogroup arboviruses are members of an incompletely characterized antigenic complex which includes about 40 Panamanian sandfly isolates. CF tests using hamster sera showed a one-way cross between the sloth agent and antisera against Punta Toro and Buenaventura viruses, indicating it to be a subtype of Punta Toro virus.

PRN antibodies against this Punta Toro-complex agent occur in sloths from all areas examined, but are also found in a wide variety of other mammal species (Tables 2, 3). Antibody was more prevalent in Choloepus than in Bradypus, and was not detected in known subadult sloths.

This virus may be only seasonally active in sloths. The original virus isolate, and seroconversions in two other Cerro Azul radio-marked sloths, were all clustered in November and December 1974, at the end of the rainy season. During this period of virus activity, none of 31 Cerro Azul wild animals of 13 species other than sloths had detectable PRN antibody against the Punta Toro-like virus, including 14 howler monkeys Alouatta villosus.

Simbu serogroup virus

Strain Pan An 48878 was isolated from whole blood of an adult Bradypus variegatus sloth, bled 2 July 1975 at Cerro Azul. Reisolation attempts were unsuccessful, and the host animal died before it could be recaptured to detect seroconversion. By CF reaction, strain Pan An 48878 is a member of the Simbu serogroup of bunyaviruses. PRN and HI tests show that it is an apparently new virus, assigned the name Utive (UTIV) after a village and river near Cerro Azul.

Complement-fixture tests at YARU and GML showed that UTIV virus is closely related or identical to Utinga (UTI) virus from a Brazilian Bradypus tridactylus sloth. Both UTI and UTIV are related strains from sloths (Table 4). These high heterologous PRN antibody levels did not change appreciably, although the titer against the homologous strain eventually increased 16-fold. The CF antibody titer against the homologous virus remained constant in Choloepus no. 20 throughout the period of observation.

Phlebotomus fever serogroup virus

Strain Pan An 53038 was isolated and reisolated from a radio-marked Bradypus bled at Cerro Azul on 1 December 1974. The viremic titer of this animal was 1.5 log10 Vero TCID50/ml. The host animal subsequently developed homologous neutralizing antibody. In one-way screening PRN
Table 3

Plaque-reduction neutralizing antibodies against three viruses in sloths from Panama

<table>
<thead>
<tr>
<th>Locality</th>
<th>Age</th>
<th>UTIV</th>
<th>CGL-complex</th>
<th>PT complex</th>
<th>UTIV</th>
<th>CGL-complex</th>
<th>PT-complex</th>
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</thead>
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<tr>
<td>Majé</td>
<td>Adult</td>
<td>16/32 (50)§</td>
<td>13/32 (42)</td>
<td>17/32 (53)</td>
<td>3/33 (9)</td>
<td>0/33</td>
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<tr>
<td></td>
<td>Young</td>
<td>4/9 (44)</td>
<td>0/9</td>
<td>0/9</td>
<td>0/21</td>
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</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>5/13 (38)</td>
<td>5/13 (38)</td>
<td>6/13 (46)</td>
<td>2/4 (50)</td>
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</tr>
<tr>
<td>Cerro Azul</td>
<td>Adult</td>
<td>1/3 (33)</td>
<td>3/3 (100)</td>
<td>0/3</td>
<td>3/36 (8)</td>
<td>8/36 (22)</td>
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<tr>
<td></td>
<td>Young</td>
<td>0/2</td>
<td>1/2 (50)</td>
<td>0/2</td>
<td>NT§</td>
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<td>NT</td>
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<tr>
<td>El Llano-Cartí</td>
<td>Adult</td>
<td>0/3</td>
<td>3/3 (100)</td>
<td>0/3</td>
<td>0/10</td>
<td>1/10 (10)</td>
<td>2/10 (20)</td>
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<td>NT</td>
<td>10/65 (15)</td>
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<tr>
<td>All localities</td>
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<td>30/57 (53)</td>
<td>17/38 (45)</td>
<td>6/79 (8)</td>
<td>19/144 (13)</td>
<td>11/79 (14)</td>
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<td>6/13 (46)</td>
<td>2/4 (50)</td>
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</tr>
<tr>
<td>Total</td>
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<td>38/87 (44)</td>
<td>23/62 (37)</td>
<td>8/106 (8)</td>
<td>19/171 (11)</td>
<td>11/106 (10)</td>
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</tbody>
</table>

* UTIV, Utive virus (strain Pan Ar 49878); CGL, Changuinola (strain Pan An 307566 against plasma from El Aguacate only, strain Pan An 56663 against all others); PT, Punta Tosba (strain Pan An 53038).
1 Adult Bradypus (forearm >160 mm) are at least two years old. Choloepus probably reach adult size (forearm >170 mm) slightly later.
2 Number positive/number tested (percent positive). Positive defined as >90% plaque reduction by plasma diluted 1:36.
3 NT, not tested.

Table 4

Viremia and plaque-reduction neutralizing (PRN) antibody responses of Choloepus No. 26 naturally infected by Changuinola (CGL)-complex strain Pan An 341275 and experimentally infected with SLE virus

<table>
<thead>
<tr>
<th>Day after SLE virus inoculation</th>
<th>Detectable SLE viremia</th>
<th>Pan An 341275</th>
<th>Pan An 341275</th>
<th>Pan An 59663</th>
<th>Pan An 507366</th>
<th>PRN titer against CGL-complex strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>-</td>
<td>-</td>
<td>0.5*</td>
<td>&lt;0.5*</td>
<td>8.2</td>
<td>512</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>1.8</td>
<td>8</td>
<td>512</td>
</tr>
<tr>
<td>28</td>
<td>NT§</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>128</td>
<td>&gt;512</td>
</tr>
<tr>
<td>35</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>128</td>
<td>128</td>
</tr>
</tbody>
</table>

* Virus detection in suckling mice, by ic inoculation of 0.02 ml undiluted whole blood and at 10-fold blood dilutions.
1 Log2 Vero cell medium infectious doses per ml whole blood.
2 Reciprocal of weakest plasma dilution reducing plaque numbers by at least 90%.
3 NT, not tested.

indistinguishable by CF test from Pintupo (PIN) virus, strain Pan Ar 517, isolated from Panamanian Culicoides diabolicus-complex biting midges. Further CF tests by Kinney and Calisher have placed UTI and UTIV viruses in an antigenic complex of the Simbu serogroup with Orpouche and Facey’s Paddock viruses.

Plaque reduction neutralization and HI tests by Kinney and Calisher showed that UTIV virus is distinct from UTI and other Simbu group agents. These tests were extended at GML to include PIN virus, which was plaque-purified because the prototype strain was mixed in the original Culicoides pool with a strain of UTI virus. UTIV virus was also plaque-purified for parallel testing. By both HI and PRN tests at GML, UTI, UTIV and PIN viruses were clearly distinct from each other (Tables 6 and 7). No antigenic difference was seen between purified and unpurified UTIV virus preparations.

Utive, UTI, and PIN viruses behaved similarly in Vero cell cultures, all producing plaques by 5 days under a gum tragacanth overlay. However, UTIV virus is less mouse pathogenic than its sister
viruses. Mouse-adapted PIN and UTI viruses produce fatal illness in suckling mice beginning 3-4 days after ic inoculation, in contrast with 6 days for mouse-adapted UTIV virus. Intraperitoneal inoculation of PIN and UTI virus stimulate HI and PRN antibody in weanling mice without the iv doses needed to stimulate UTIV antibody. In addition, UTIV virus causes no illness or antibody in suckling hamsters inoculated iv, nor in day-old chicks inoculated iv and intramuscularly.

In Central Panamá, PRN antibody against UTIV virus is essentially confined to sloths. At Majé, of 61 birds and 225 mammals other than sloths, only a single monkey had detectable antibody against UTIV virus (Tables 2, 3). All 61 negative bird plasmas and 193 of the negative mammal plasmas were also tested for PRN antibodies against unpurified UTI and/or PIN viruses; the latter reagent contained a minor proportion of UTI-type plaques. None was positive against the other serotypes. In contrast, of 50 sloth plasmas tested against all three serotypes, 29 were positive against either or both UTI and PIN viruses, including 16 which were negative against UTIV.

Ungrouped virus

An adult male Bradypus variegatus captured near Chilibre was brought to GMI on 7 February 1976. On 9 February, a whole blood sample was taken from which an apparently new virus was isolated in Vero cell culture. Four days later, the animal was inoculated with SLE virus; the next whole blood sample, taken 3 days after SLE virus

---

**Table 5**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Bradypus-103 pre-bleed</th>
<th>Bradypus-103 post-bleed</th>
<th>Punta Toro</th>
<th>Buena Ventura</th>
<th>VP-419T</th>
<th>VP-366G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan An 53038</td>
<td>&lt;8*</td>
<td>8–16</td>
<td>32</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>32</td>
</tr>
<tr>
<td>Punta Toro</td>
<td>&lt;8</td>
<td>1,024</td>
<td>3,000</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>32</td>
</tr>
<tr>
<td>Buena Ventura</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>128</td>
<td>&lt;16</td>
<td>&lt;16</td>
<td>16</td>
</tr>
<tr>
<td>VP-419T</td>
<td>&lt;8</td>
<td>8</td>
<td>24</td>
<td>NT</td>
<td>32</td>
<td>&lt;16</td>
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<tr>
<td>VP-366G</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;16</td>
<td>NT</td>
<td>&lt;16</td>
<td>32</td>
</tr>
</tbody>
</table>

* Pan An 53038 pre-bleed plasma from host sloth 40 days before date of detected viremia; post-bleed plasma taken 38 days after date of detected viremia. Punta Toro, VP-419 and VP-366: hyperimmune hamster sera. Buena Ventura: hyperimmune mouse ascitic fluid.

† Reciprocal of weakest antibody dilution causing 50% plaque inhibition.

---

**Table 6**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Utinga</th>
<th>Pintupo (P)*</th>
<th>Utinga</th>
<th>Pintupo (P)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utinga</td>
<td>128†</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Pintupo (P)</td>
<td>8</td>
<td>1,024</td>
<td>NT‡</td>
<td>32</td>
</tr>
<tr>
<td>Utinga</td>
<td>32</td>
<td>32</td>
<td>1,024</td>
<td>1,024</td>
</tr>
<tr>
<td>Utinga</td>
<td>32</td>
<td>64</td>
<td>512</td>
<td>1,024</td>
</tr>
</tbody>
</table>

* (P) indicates plaque purification of test antigen and immunizing virus.
† Reciprocal of weakest ascitic fluid dilution inhibiting 90% of 40–50 plaques after overnight incubation at 4°C.
‡ NT, not tested.

**Table 7**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Utinga</th>
<th>Pintupo (P)*</th>
<th>Utinga</th>
<th>Pintupo (P)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utinga</td>
<td>40†</td>
<td>20</td>
<td>&lt;10</td>
<td>20</td>
</tr>
<tr>
<td>Pintupo (P)</td>
<td>10</td>
<td>160</td>
<td>NT‡</td>
<td>20</td>
</tr>
<tr>
<td>Utinga</td>
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<td>NT</td>
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<td>80</td>
</tr>
<tr>
<td>Utinga</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>160</td>
</tr>
</tbody>
</table>

* (P) indicates plaque-purification of test antigen and immunizing virus.
† Reciprocal of weakest ascitic fluid dilution completely inhibiting 4–8 units of antigen, at pH 5.1–6.4.
‡ NT, not tested.
inoculation, also contained the new virus. The agent was reisolated from both bloods, but was not isolated from 12 subsequent blood samples taken during the following 35 days. We propose the name Bradykus-4 for the new virus, after its host animal.

Plaque reduction neutralization and CF antibodies against Bradykus-4 virus were detected in plasma taken 45 days after the first isolate was obtained but not at 29 days. PRN titers of at least 1:512 and CF titers of at least 1:16 were recorded from days 45 and 94 after first Bradykus-4 virus appearance; on day 149, the PRN titer remained at 1:512, but CF antibody was undetectable (titer < 1:4). The delayed and protracted experimental SLE viremia in this animal is described elsewhere; it was not detectable until 17 days after SLE virus inoculation.

Bradykus-4 virus is probably an RNA virus with a lipid-containing envelope. The infectious titer was reduced by at least 1,000-fold by chloroform treatment, but by only 0.5 log_{10} TCID_{50} by BUVR. The effect of these treatments on control viruses was consistent with their nucleic acid and envelope types. Bradykus-4 virus passed a filter of 0.22 μm pore size. On primary isolation it caused focal CPE proceeding to complete cell destruction in Vero cells but no illness in mice inoculated ic. After one or two passages in Vero cells, the virus caused lethargy or prostration in some but not all newborn mice; many ill mice eventually recovered. After four mouse passages, Bradykus-4 virus consistently killed newborn mice beginning day 7 after ic inoculation. The virus caused no illness in suckling hamsters inoculated ic. After three passages in suckling mice or Vero cells, titers of tissue culture fluids and mouse brain suspensions were no higher than 4.5 log_{10} TCID_{50}/0.1 ml. Bradykus-4 virus also caused complete degeneration of the following cell cultures: HeLa, BHK-21, WI38, BGM, and primary human amnion, growing to approximately equal titers in these lines and Vero cells. A sucrose-acetone extracted, sonicated suckling mouse brain antigen failed to agglutinate goose red blood cells at pH 5.8–7.0. Human type O red blood cells did not adsorb to infected Vero cell monolayers.

In CF tests at GML, Bradykus-4 antigen did not react with a Changuinola virus antiserum which fixed complement with four other CGL-related sloth isolates, nor with NIH grouping ascitic fluids for arbovirus serogroups A, B, C, Simbu, Bunyamwera, Capim, California, Anopheles A, Anopheles B, and Turlock. A Bradykus-4 mouse hyperimmune ascitic fluid titering 1:64 failed to fix complement at GML with antigens of the following Phlebotomus fever serogroup viruses: Aquacate, Anhanga, Arumowot, BeAr 100049, Bujaru, Cacao, Caimito, Candiru, Chargas, Chilibre, Frijoles, Icoaraci, Itaparanga, Karimabad, Nigue, Pacui, Punta Toro, Salehabad, Sandfly Fever (Naples), Sandfly Fever (Sicilian) and SudAn 754-61. At YARU, CF tests by Dr. Robert Shope showed no reaction between Bradykus-4 hyperimmune ascitic fluid and the antigens of 216 different arbovirus and other animal viruses. HI tests by Dr. Shope showed no inhibition by a 1:10 dilution of Bradykus-4 antisera against 4–8 units of the following virus antigens: Aino, Anopheles B, Arumowot, AUS R7949, Bahig, BeAn 84381, BeAn 141106, Belmont, Bhavan, BFS 5002, Buttonwillow, CoAr 3624, Gambou, Germiston, Gordil, Grand Arbaud, Guaroa, Gumbo Limbo, Hazara, and I 47.

**DISCUSSION**

These preliminary results on natural virus infections of sloths suggest further studies for at least three reasons. First, they help clarify the natural cycles of some poorly-understood tropical viruses. Second, they emphasize the unusual patterns of virus infections in sloths, already observed in experimental studies. Third, they include two natural relatively simple systems for the study of viral genetic reassortment.

First, sloths seem to be the principal vertebrate hosts of viruses of the CGL and Utinga antigenic complexes. In both cases, sloths have provided most or all of the isolates from vertebrate sources. Only two CGL-group strains have been recovered from vertebrates other than sloths, in contrast to our four sloth isolates and a fifth, “Jari” virus, from a Brazilian Choleopsis didactylus (personal communication, F. P. Pinheiro and Travassos da Rosa, A. P. A. and J. F., Instituto Evandro Chagas, Belem, Brazil). Also, antibody against both CGL and Utinga complex viruses occurs almost exclusively in sloths; indeed, difficulties in raising antibody against UTV and CGL-related viruses in a variety of laboratory animals may reflect the specialization of these viruses for sloths. Finally, all of our isolations have been from blood, an important consideration for an arbovirus. A viremic amplifying host may be especially important for the maintenance of CGL-related viruses. In Pan-
amá, these viruses are principally found in phlebotomine sandfly species known to feed on sloths,8,16,17 but are not transmitted transovari-
ally as readily as other viruses found in these insects.8-18 Sloths with natural CGL viremias as protracted as that of Choloepus 20 might serve as ef-
cient infection sources for sandfly vectors, and merit further study.

In contrast to the CGL and Utinga complex viruses, strain Pan An 53038 is not necessarily restricted to sloths. Although it is the only Punta Toro-like virus to have been isolated from a Pan-
amanian vertebrate, antibody against this or another closely related virus was detected in sev-
eral other forest mammal species. Nothing is known of the natural cycle of Bradypus-4 virus.

Second, some sloths have unusual and unpredic-
table patterns of virus circulation. Of the CGL-
related strains, Pan An 307566 appeared in the
blood of a sloth which had been maintained for
38 days under conditions precluding insect or con-
tact virus transmission; Pan An 341275 virus was
detectable in the host animal's blood for at least
28 days, and was shown to circulate simulta-
neously with SLE virus for thirteen days. CGL
virus is an orbivirus, and similar prolonged and
recrudescence viremias have been described for
other viruses in this genus.19-21 The simultaneous
circulation of a CGL group virus with SLE virus in
Choloepus number 20 is intriguing. In contrast,
Bradypus-4 virus probably did not circulate si-
taneously with SLE virus, which remained undetected in the host animal until 2 weeks after
the last date of observed Bradypus-4 viremia.

Further studies defining and clarifying the reasons
for long and unpredictable viremias in sloths might
explain the frequency with which viruses are iso-
lated from sloth blood in this and other3 studies.
This high frequency is difficult to explain except
by either 1) unusually long viremias,4 or 2) recru-
descent viremia of sequestered virus, stimulated
by stress of capture and handling or by another
superimposed virus infection. Both possible
mechanisms were observed in this study, and could
reconcile the high number of apparently valid iso-
lates from Bradypus sloths with the relatively low
prevalence of antibodies in this genus, a pattern
also observed for Oropouche virus in Brazilian
Bradypus.22

Third, sloths and their CGL and Simbu sero-
group viruses might be a suitable system in which
to study antigenic plasticity and proposed genetic
reassortment in nature. The viruses belong to an-
tigenic complexes, in which viruses share either
identical internal (CF) antigens or external (HI
and neutralization) antigens, but not both. This
type of complex is common among orbiviruses23,24
and bunyaviruses,25,26 and is often marked by
transmission of several related serotypes simulta-
neously in the same area under apparently ide-
tical ecological circumstances. This antigenic
plasticity may result from genetic reassort-
ment,27,28 which has been demonstrated in vi-
tro.29-30 For genome segments to reassort, two dif-
f erent virus types must infect the same cell. Our
results suggest that conditions for this event are
optimal in sloths, because of 1) their exceptionally
long viremias, 2) evidence for chronic or recrud-
descence infection, and 3) the demonstration of two
simultaneous viremic infections in the same ani-
mal.

Whatever the genetic mechanism, some way to
induce antigenic change in sloth-specific viruses is
probably necessary in nature, because sloths are
long-lived, with a slow turnover rate.31 Since sloths
with PRN antibody are resistant to challenge with
at least one homologous virus,4 sloth populations
might become naturally immunized by a single
antigenic type, requiring reinfection by a changed
serotype to ensure continued virus transmission.
Choloepus no. 20 demonstrates circulation of a
CGL-related virus in the presence of antibody to a
second, related serotype.

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provided virus strain Pan An 307566 and infor-
mation on its isolation and host animal.

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