

## Arboviruses in Serra Norte, Carajás region, Pará, Brazil

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This report outlines studies carried out from 1982 to 1987 in Serra Norte, Carajás region, Pará State, to ascertain the existence of known or new arbovirus types in the area, especially those of medical interest, and to gather information about their natural patterns of activity. These studies have included both serological tests and attempts to isolate viruses from haematophagous insects, wild animals and man. A total of 184,884 haematophagous insects were collected and blood and tissue samples were obtained from 1,726 wild vertebrates caught in the area. Attempts to isolate viruses in suckling mice yielded 21 strains, including 15 different serological types, of which 7 have been shown to constitute new types, so far found only in the Brazilian Amazonian region, and two of them have already been confirmed to be new types to the world. Serological studies performed on 2,680 sera (1,380 humans and 1,300 wild animals) against selected arboviruses pointed out the circulation of several of these agents in the investigated area. The Carajás region therefore, maintains established ecosystems which are rich in arbovirus fauna, and exploration of these natural niches must be done carefully and rationally to avoid breaking out the current foci of infestation maintained in the forest. Further studies are necessary to obtain a better understanding of these agents in this region and of the threats that they may pose to the health of humans and livestock.

*Este relato descreve estudos efetuados no período de 1982 a 1987, em Serra Norte, região dos Carajás, Estado do Pará, para averiguar a existência de conhecidos ou novos tipos de arbovírus na área, especialmente, aqueles de interesse médico, e colher informações acerca de seus padrões naturais de atividades. Esses estudos incluíram testes sorológicos e tentativas para isolamento de vírus a partir de insetos hematofagos, animais silvestres e humanos. Um total de 184.884 insetos hematofagos foram coletados. Sangue e vísceras foram obtidos de 1.726 vertebrados silvestres capturados na área. As tentativas para isolar vírus em camundongos recém-nascidos forneceram 21 amostras, incluindo 15 tipos sorológicos diferentes; destes, demonstrou-se que sete eram tipos*

*novos, encontrados somente na Amazônia Brasileira, e dois deles já foram confirmados ser em tipos novos para o mundo. Estudos sorológicos executados em 2.680 soros (1.380 humanos e 1.300 animais silvestres) contra arbovírus selecionados indicaram a circulação de diversos desses agentes na área investigada. A região dos Carajás, por conseguinte, mantém ecossistemas estabelecidos que são ricos em arbovírus. Assim, a exploração desses nichos naturais deve ser feita cuidadosa e racionalmente para evitar a rutura do focos correntes de infestação mantidos na floresta. Estudos ulteriores são necessários para obter um melhor conhecimento desses agentes nessa região e os danos que eles possam causar à saúde humana e de animais domésticos.*

The arboviruses are a heterogeneous group of viruses with one common ecological characteristic: they all have the capacity to reproduce in the cells of haematophagous arthropods and to be transmitted from them to susceptible vertebrate hosts. They constitute the largest known group of viruses with 535 members registered in 1991 in the most recent supplement to the *International catalogue of arboviruses 1985 including certain other viruses of vertebrates* (1).

Arbovirus studies conducted in many areas of the Bra-

zilian Amazon region have revealed the presence of a large number of these agents (2). As the relentless pressures of human populations (such as agrovillas, mining exploration and dams) impinge upon the forests of the Amazon, ecosystems new to man are established. These can be considered the current foci of infestation. In 1982, one of the new areas selected for study was the Serra Norte region (3,4) which is part of a group of hills that make up the Serra dos Carajás, where a large iron mining project is being carried out (Fig. 1). Until then, however, apart from some sporadic and limited serological enquiries, practically nothing was known about the autochthonous arboviruses of this region. Some deductions could, however, be made from the results of studies

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Figure 1. Carajás region.

carried out in adjacent areas, as well as on the basis of knowledge acquired in other parts of Amazonia (5).

The sites studied are shown in Figure 2. Details of the techniques used in collecting material (6,7,8), virus isolation and identification (9), and serological testing (10,11) are described elsewhere.

The work carried out during the five years of investigation has led to the isolation of 21 strains, obtained from all the types of arthropods examined (mosquitoes, sandflies and midges) and also from vertebrates (Table 1). *Lutzomyia* sp. accounted for 14 viral isolates; *Psorophora albipes* for one; Ceratopogonidae sp. for two; Formicariidae birds for three and a *Proechimys guyannensis* rodent for one.

A total of 17 virus isolations (7 from the Changuinola (CGL) group, 6 from the Vesicular Stomatitis Virus (VSV) group, 2 of them Bunyaviridae ungrouped and 2 ungrouped-unclassified) were obtained from 184,884 haematophagous arthropods (146,284 females and 38,600 males), representing at least 70 different species and distributed in 2,278 pools.

A total of 1,726 wild vertebrates were captured and examined for either HI antibodies or attempts for virus isolation (or both). This number of vertebrates was made up of 1,253 birds (15 families, 62 species), 214 rodents (4 families, 15 species), 133 marsupials (1 family, 10 species), 29 primates (2 families, 5 species), 72 chiroptera and 25 other miscellaneous animals. The viscera of these animals yielded four arbovirus strains. Three of them were isolated from birds — Eastern Equine Encephalitis (EEE) from Formicariidae species, St. Louis Encephalitis (SLE) from *Conopophaga aurita*, another Formicariid, and Turlock (TUR) from *Myrmoborus myotherinus*, also a Formicariid. The other, Icoaraci (ICO), was isolated from a *Proechimys* gr. *guyannensis* rodent.

These isolations have been found to comprise 15 different serological types, of which 7 (Carajás, Marabá, Tapirapé, Arataú, Itacaiúnas, Buritirama and Para-

uapebas) have been shown to constitute new types, so far found only in the Brazilian Amazonian region, and two of them (Carajás and Marabá) have already been confirmed to be new types to the world (12).

No virus strains were isolated from the 55 blood samples obtained from febrile patients.

Arbovirus antibody surveys carried out on 1,380 human sera have shown a reasonable level of immunity against several agents which induce human febrile illness. The great majority of the individuals tested were male, aged 13-58. Haemagglutination-inhibition (HI) antibodies against flaviviruses were the most prevalent (44.2%), probably due to vaccination against the yellow fever (YF) virus, although specific antibodies to Ilhéus (ILH), at a level of 0.4%, were also found. Among the group A arboviruses, Mayaro (MAY) prompted by far the largest number of positive reactions (4.6%). However, a few positive reactions (1.2%) to the Mucambo (MUC) virus, a member of the Venezuelan Equine Encephalitis (VEE) complex, were also obtained, and in rare instances positive reactions to the EEE virus were detected (0.4% of the cases). Antibodies to the Oropouche (ORO) virus, an agent previously implicated in several epidemics occurring within the Brazilian

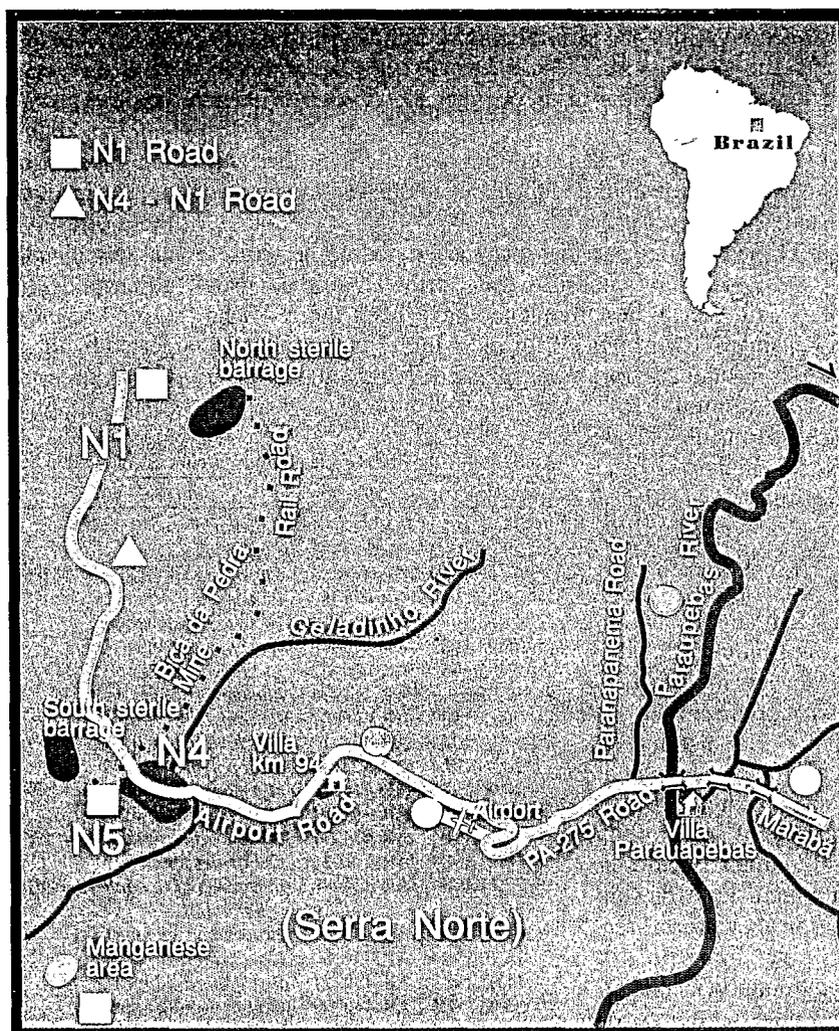


Figure 2. Map of Serra Norte, showing the location of the different sites studied.

Amazon region, were found in 5.7% of the individuals. A low prevalence of antibodies to some other arboviruses was also found: Guaroa (GRO) 1.8%; Caraparu (CAR) 0.9%; Itaporanga (ITP) 0.8%; Tacaiuma (TCM) 0.5% and Western Equine Encephalitis (WEE) 0.3%. Second samples were taken from 58 individuals and 8 serological conversions were observed in these sera: YF (2), MAY (4), MUC (1) and Group B (1).

Concerning the distribution of HI antibodies in the wild animals, the birds (827 plasmas tested) showed varying rates of immunity: ORO 4.6%, WEE 1.6%, Group B 1.0%, TUR 0.8%, MAY 0.7%, EEE 0.4% and SLE 0.4%. Birds of the family Formicariidae (ant eaters) showed a higher incidence of antibodies than other bird families.

Primates had a high level of immunity to MAY and lower levels of antibodies against YF and ORO. Only 4 of the marsupials gave positive results for the group B viruses, and just one *Didelphis marsupialis* was found to be positive for the group A viruses (EEE and WEE).

A few rodents showed HI antibodies against the ICO virus and the CAR virus. It is interesting to note that one edentate, *Dasyus novemcinctus*, was found to contain neutralizing antibodies against the Carajás virus, a *Proechimys* gr. *longicaudatus* rodent was found to contain neutralizing antibodies against the Marabá virus, and another rodent (*Proechimys* sp.) had neutralizing antibodies against the Santarém (STM) virus. These animals thus became suspected of involvement in the maintenance cycles of these agents.

Serum samples taken from the bats and the other animals which were captured failed to react against the viruses tested.

These data indicate that many arthropod-borne viruses are widely disseminated in Serra Norte. The viruses isolated with the highest frequency have been those of the VSV and CGL groups. With one single exception (AR 439971, obtained from *Psorophora albipes*) all of these were isolated from phlebotomine sandflies, which confirms the important role of these diptera as arthropod vectors of these agents.

The VSV serogroup, family Rhabdoviridae, genus *Vesiculovirus*, currently consists of 13 distinct virus types which have been isolated from a variety of arthropods and animals (including humans), and for this reason they are of veterinary and public health importance (13).

The CGL serogroup viruses, family

Reoviridae, genus *Orbivirus*, represent viruses so far isolated only in Central and South America and they have been associated with phlebotomine sandflies in nature. Between 1960 and 1980, a total of 178 CGL serogroup viruses were recovered from biting insects and mammals in tropical rainforest regions of Brazil, Colombia and Panama. In a recent study, 24 of these viruses were selected as representative specimens and their antigenic, biological and chemical properties were examined. Twelve of these viruses were shown to be distinct by both the neutralization test (NT) and by polyacrylamide gel electrophoresis (PAGE). This study clearly shows that a great many more Changuinola serotypes may exist (14).

The isolations of the Itacaiúnas and Buritirama serotypes from insects of the Ceratopogonidae family constitute the only isolations in Brazil of arboviruses (apart from ORO) from these arthropods. Out of 60 genera which have been assigned to this family, only four are known to feed on man, as indeed on any other vertebrate, and *Culicoides* is by far the most important genus with respect to both human and animal health. These diminutive insects avidly seek out human blood during the day, and especially at sunset, biting individuals both inside houses and outside. They reproduce in decomposing organic vegetable matter, such as, for example, cacao skins and cut banana tree trunks. The midge is an important vector of several severe viral diseases in domestic animals, such as blue tongue, bovine ephemeral fever and African horsesickness (15).

In addition, the isolations of the SLE and EEE viruses, together with the presence of antibodies against these agents in birds, confirm the presence, in Serra Norte, of these viruses, which are responsible for serious neurological complications in humans and, in the case of EEE, in equines also, the latter many times affected epizootically (16). The presence of the WEE virus in the area was suggested by the detection of specific antibodies to this virus in the serum samples of several birds.

Table 1 — Arboviruses isolated from specimens collected in Serra Norte.

Date	Strain	Source	Place	Antigenic group	Type
28.01.1983	AR 411391	<i>Lutzomyia</i> species	Parapanema road	VSV	Carajás
29.01.1983	AR 411415	<i>Lutzomyia</i> species	N4 - N1 road	VSV	Carajás
04.02.1983	AR 411453	<i>Lutzomyia</i> species	N4 - N1 road	VSV	Carajás
04.02.1983	AR 411459	<i>Lutzomyia</i> species	N4 - N1 road	VSV	Marabá
25.04.1983	AR 414556	Formicariidae species	Airport road	A	EEE
20.05.1983	AR 415948	<i>Lutzomyia</i> species	N4 - N1 road	Changuinola	Altamira
20.05.1983	AR 415962	<i>Lutzomyia</i> species	N4 - N1 road	Changuinola	Parauapebas
20.05.1983	AR 415974	<i>Lutzomyia</i> species	N4 - N1 road	Changuinola	Parauapebas
26.05.1983	AR 416001	<i>Lutzomyia</i> species	N4 - N1 road	VSV	Carajás
24-28.01.1984	AR 421710	<i>Psychodopygus carrerai</i>	N5 - N1 road	Ungrouped	Santarém
20.05.1984	AR 423728	<i>Conopophaga aurita</i>	PA 275, Viveiro	B	SLE
20.05.1984	AR 423732	<i>Myrmoborus myotherinus</i>	PA 275, Viveiro	Turlock	Turlock
22.10.1984	AR 434201	<i>Psychodopygus davisi</i>	N4 - N1 road	Changuinola	Jamanxi
24.10.1984	AR 434091	<i>Lutzomyia dasipodogeton</i>	N4 - N1 road	VSV	Marabá
18.03.1984	AR 427036	<i>Culicoides</i> species	N4 - N1 road	Ungrouped	Itacaiúnas
27.11.1984	AR 434948	<i>Lutzomyia (Trichophoromyia) ubiquilatis</i>	N4 - N1 road	Changuinola	Aratau
09.02.1985	AR 432757	<i>Proechimys guyanensis</i>	Manganese area	Phlebotomus	Icoaraci
24.10.1984	AR 434080	<i>Lutzomyia dasipodogeton</i>	N1 road	Changuinola	Tapiropé
11.03.1985	AR 439971	<i>Psorophora albipes</i>	Parauapebas road	Changuinola	Canindé
06.03.1985	AR 440009	Ceratopogonidae species	N1 road	Ungrouped	Buritirama
24-25.11.1987	AR 472083	Flebotomineo nº 7	N1 road	Bunyaviridae	Santarém

The TUR virus is an agent which typically circulates in a bird-*Culex*-bird cycle. In Brazilian Amazonia, it has been isolated sporadically from at least 11 different species of birds as well as from mosquitoes (17).

The finding that 4.6% of wild birds had antibodies to the ORO virus confirms that these animals, and in particular those belonging to the family Formicariidae, are naturally infected by this agent, which indicates that they may be important hosts.

The occurrence of YF antibodies in monkeys, suggesting circulation of the YF virus in the jungle, constitutes a strong reason for continued vaccination of settlers to protect them against this agent. Despite the proven efficacy of the available vaccine, the YF virus continues to be the arbovirus responsible for most human deaths in Brazil.

In order to obtain a more thorough understanding of the arboviruses of the Serra Norte region and the threats that they may pose to the health of humans and livestock, a better knowledge of the new isolates is needed to evaluate the pathogenicity and distribution of these agents in the area. A knowledge of disease foci on the site could allow effective management of a potentially serious problem. The pathology associated with known arboviruses, such as the EEE, SLE, ILH, MAY and ORO viruses, warrants further study so as to clarify the levels of risk to human health.

The application of new molecular techniques, such as recombinant DNA methodology, monoclonal antibodies, and enzyme immunoassays, to questions concerning host-virus and host-vector interactions is exciting. To determine the epidemiological relevance of recent developments in molecular virology, collaborative studies will be needed between experts on the molecular and ecological aspects of arbovirus transmission. Interdisciplinary studies like these are the most efficient way of achieving significant improvements in our understanding and, ultimately, in our control of these important diseases.

In any event, studies of the viral fauna of the Amazon can only help to define the present and potential medical problems of the area. ■

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7. Vertebrates were caught by various methods including trapping, shooting and mist netting. Serum and plasma were maintained at -20°C. Blood coagulate and sediment, after treatment with heparin, were preserved in liquid nitrogen. Visceral fragments were also kept in liquid nitrogen. In the case of bats, the salivary glands were also removed
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18. *Acknowledgments*: The work in Serra Norte (Carajás Project) was carried out under an agreement signed between the Instituto Evandro Chagas (SESP Foundation) and the Companhia Vale do Rio Doce (CVRD), enabling research on tropical diseases in the area. The agreement lasted until 1987, and during this period a large team of specialists including ORSTOM medical entomologists (Agreement ORSTOM/CNPq/SESP Foundation) contributed to the collection of data and samples necessary for the development of the research. We thank Miguel Leite Raposo de Oliveira for linguistic revision of the manuscript.