

## The isolation of virus from natural and sentinel hosts in the Amazon valley\*

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During the past six years the Belém Virus Laboratory has been engaged in investigation of the viruses which are transmitted to vertebrate hosts by arthropods<sup>1</sup>. A total of more than 1600 isolates was obtained in Belém during the period between November 1954 and the end of 1960. The techniques for isolation and sources of virus provide the subject of this paper.

### METHODS

At the Belém Laboratory in 1954, all original material was inoculated into two groups of infant and one group of adult mice. It was soon discovered that infant mice were succumbing to agents which produced no symptoms in adult mice. As isolations increased and space became inadequate, adults were eliminated from the primary inoculations, and finally, as confidence was gained in the recognition of sick mice, reliance was placed on one group of infants, two to four days of age. Younger animals were prone to succumb to some viruses in less than 24 hours, and older litters showed varying degrees of resistance. The practice was thus soon established of using infant mice exclusively, for all isolation attempts. When it is necessary to make inoculations in the field, because of distance from the Laboratory or inadequacy of refrigeration, infant mice can be provided by taking or sending the pregnant females in various appropriate stages of gestation. All filtrable agents obtained by the injection of infant mice are studied.

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Needless to say, there have been some isolates which have proven not to be arboviruses. By the end of 1960, these amounted to about 10 per cent of the total infections studied and to about 5 per cent of the total strains isolated.

Serum from vertebrates for inoculation of mice is used as obtained, or is diluted in 0.75 per cent buffered bovine albumen (BA). Cebus monkey serum is toxic for mice and must be diluted 1:5 or 1:10. All tissues from sacrificed animals, including mouse brain, are prepared at a 10 per cent dilution in BA diluent. Arthropods for virus isolation and tissues from dead animals are triturated in the diluents to which has been added 1200 units of penicilin and 10mg of streptomycin per milliliter. Tissue suspensions are centrifuged for 15 minutes at 2000rpm and arthropod suspensions are centrifuged for 30 to 45 minutes at 3000rpm. Whenever possible the supernatant is kept in the minus 60°C freezer for attempts at reisolation. All isolated viruses are preserved as 20 per cent brain emulsion or whole serum, desiccated from the frozen state.

#### SOURCES

The usual four principal sources for arbovirus isolation have been explored in several study areas over the years. These are:

- 1) the human and domestic animal fever cases;
- 2) trapped wild animals, birds and reptiles;
- 3) the arthropods collected on human and animal bait;  
and
- 4) sentinel animals (principally monkeys and infant mice)

#### HUMAN AND ANIMAL FEBRILE CASES

An epidemic affords the most favorable condition for the isolation of virus from the vertebrate host. When an endemic state exists,

the chance of finding the febrile case is much reduced. However, by searching out and observing the itinerant or immigrant workers in such an area, viral activity may be detected that otherwise would pass unobserved. In the Belém Laboratory, among 95 isolations from man in six and a half years, 32 were made in three epidemics, one of which was due to Yellow fever<sup>2</sup>, another to Mayaro<sup>3</sup>, and the last to Oropouche virus<sup>4</sup>. The remaining 63 isolates represent 14 different viral types from persons associated with forest who were immigrants from other regions.

An epizootic of EEE involving horses on the Bragança pastures is reported elsewhere<sup>5</sup>.

#### WILD ANIMALS

Arboviruses were isolated from 28 wild mammals collected in the forest study areas between November 1954 and the end of 1960. Some of these doubtless were participants in epizootics at the time. This was probably the case in 1959, when Bussuquara was isolated from the blood of three trapped wild rodents (*Proechimys*), from mosquitoes (*Culex (Melanoconion)* sp.) and from sentinel mice, between May 10 and June 12 in a study area 5km from Belém. One isolation of virus (Guama) has been made from a group of fledgling wrens among a total of more than 3500 birds tested.

#### ARTHROPODS

During the six year period ending in 1960, 57 arboviruses were isolated from 274,000 forest mosquitoes, collected on human bait or with sentinel mice. This gives an overall average of at least one infected mosquito for each 4800 inoculated into infant mice. In epizootic conditions, as when horses were dying of EEE, the ratio was at least one in 800, and positive pools probably contained more than one infected mosquito. The large number of *Haemagogus* pools positive for Mayaro and of *Psorophora* pools positive for Ilheus in collections at the Brazilia highway study area during the past few months, seems to indicate that epizootics are occurring in this area among as yet unknown hosts.

### SENTINEL ANIMALS

The sentinel *Cebus* monkey was first used in December 1954. It was captured on seacoast islands and exposed in the Amazon forests near Belém. Its susceptibility to the viruses of this region was shown by semi-weekly bleedings in which circulating virus was detected by mouse inoculation and antibodies were demonstrated to the respective agents isolated from the blood.

In mid 1957 the sentinel mouse group, consisting of mother and six infants was introduced into the same study area. It was found that, after exposure for 24 hours, a group might yield one to seven strains of the same virus, or that different members of the group might yield two or three separate viruses, or that a single individual might even yield two viruses. This duplication of isolates which might conceivably result from a single arthropod in interrupted feedings, led to a revision in the reporting of isolations for statistical study, to make the data comparable to isolations from other vertebrates. The sentinel animal isolations are now reported as infections. All multiple identical strains are counted as one infection, and each different type of virus isolated from a group is of course counted as a different infection. In seasons of high transmission rates, the number of infections exceeds the number of positive mouse groups because of more frequent double infections.

A comparison of the samples processed during 1960 to show the rate of arbovirus isolation from each source indicates that in the areas studied the sentinel infant mouse affords the most efficient means for revealing arbovirus activity (Table 1). Two hundred and eight samples from sick vertebrates, human and animal, yielded six isolations for a rate of 2.9 per cent positives. A total of 594 samples from wild mammals gave ten viruses for a rate of 1.7 per cent. Arthropod vectors, inoculated as pools, showed 19 positives, or a rate of 0.6 per cent. Sentinel mice with 253 infections (which involved 744 isolations) from 1,743 groups gave a rate of 14.5 per cent which is exactly five times the rate for sick vertebrates.

The virus types isolated from each source are listed in Table 2. Those isolated only from indicated sources in 1960 are underlined.

It is seen from this listing that 15 different types were isolated from the sentinel mouse, eight of which were found only in these sentinels in 1960, and would have been missed had the mouse group not been exposed and studied. On the other hand, each of the sources explored also revealed agents not picked up by other sources during the year. Five of the 13 arthropod isolation were found only in arthropods, three of the seven wild animal isolates were found exclusively in wild mammals, and two of the four human isolates were picked up only in human sera during this year. Thus, if any one of these sources had been omitted, some part of the picture would have been missing. Even so, there is no assurance, nor even indication, that the picture as it stands is complete. Much remains to be discovered about the ecology of arboviroses in the Amazon region.

#### SUMÁRIO

Uma revisão dos seis anos de atividade do Laboratório de Vírus de Belém, mostra que, na área estudada, o camundongo infante sentinela proporciona o meio mais eficiente para constatar a atividade de arbovírus. Tôdas as fontes naturais, todavia, incluindo vertebrados doentes, animais silvestres e artrópodos vetores contribuem para um compreensivo reconhecimento dos vírus presentes na região.

#### SUMMARY

A review of six years' activity in the Belém Virus Laboratory shows that in the area studied the sentinel infant mouse affords the most efficient means for revealing arborvirus activity. All natural sources, however, including sick vertebrates, wild animals and arthropod vectors contribute to a comprehensive knowledge of the viruses present in a region.

Table 1 – Origin and number of samples studied in 1960, and the number and percent of virus isolations obtained from each source

	Number samples		Virus isolation		Per cent positive	
Sick Vertebrate		208		6		2.9
Human	126		4		3.2	
Domestic animal	92		2		2.0	
Wild Animal		1,499		10		0.7
Mammal	594		10		1.7	
Sentinel Animal		1,965		745		37.9 (12.9)
Monkey	222		1		0.5	
Mouse group	1,743		253*		14.5	
Arthropod Vector		3,094		19		0.6
Mosquito (Pools)	2,890		18		0.6	
Other "	240		1		0.5	

\* Number mouse groups infected.

Table 2 – Source of virus isolation in 1960 to show kind and number of types obtained from each source

				Sentinel		
	Human	Domestic animal	Wild animal	Monkey	Mouse	Arthropod
	Guaroa* H 22511 Oriboca Catu	EEE	Capim AN 24232 AN 24262 Bussuquara Murutucu Guamá Oropouche	Yellow fever	Marituba Apeú Caraparu Itaqui Moju AN 20525 Guajará AN 20076 EEE St. Louis Oriboca Guamá Catu Murutucu Guará Catu AN 7722	Mayaro Aura Una Wyeomyia Tacaiuma EEE Bussuquara St. Louis Oriboca Guamá Catu AN 7722 Oropouche
Total						
Number types	4	1	7	1	15	13
Only from this source	2	0	3	1	8	5

\* Virus underlined not isolated from other sources in 1960.

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