Occurrence of Pacora virus (PAC: Bunyaviridae: *Bunyavirus* - like) in Brazilian Amazonia: New findings

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The Pacora virus (PAC: Bunyaviridae: *Bunyavirus* - like), was known only through some isolates obtained from mosquitoes (*Culex dunni*) in Panama, and two isolates obtained in the Brazilian Amazon region from the sylvatic birds *Phlegopsis nigromaculata* and *Automolus ochrolaemus*. In order to add to the knowledge of the arboviruses' cycles, mosquitoes were collected in the Zoobotanical Park of Macapá (Amapá State), from August, 18th to September, 11th, in areas of rain forest and savannah. The collections were done at both ground and canopy levels in the forest and at ground level in the savannah, with human bait and light traps. 5642 mosquitoes have been collected, of which 5580 (or 184 pools) have been inoculated intracerebrally in newborn mice. Two strains of PAC virus have been isolated from unidentified *Culex* mosquitoes. This result is the first report of PAC virus in Amapá State. The virus has also been isolated for the first time from mosquitoes in Brazil. Thus, the presence of the complete cycle of sylvatic transmission of this agent is confirmed in Brazilian Amazonia. The ecological data available for the hosts show that the virus circulates at the lower level in the rain forest (0-5 m) with mostly nocturnal mosquitoes as vectors.

*O vírus PAC era conhecido apenas por alguns isolamentos obtidos no Panamá, a partir de mosquitos (**Culex dunni**) e de dois isolamentos obtidos na Amazônia brasileira a partir de aves (**Phlegopsis nigromaculata** e **Automolus ochrolaemus**). Com objetivo de aprimorar os conhecimentos sobre o ciclo silvestre de transmissão dos arbovírus foram realizadas coletas de mosquitos no Parque Zoobotânico de Macapá (AP), no período de 18/08 à 11/09/92, em uma área de floresta de terra firme e em uma área de savana. As coletas foram realizadas a nível do solo e da copa da floresta, e somente a nível do solo na savana, utilizando-se simultaneamente iscas humana e luminosa. De um total de 5640 mosquitos coletados, 5580 (184 lotes) foram inoculados intracerebralmente em camundongos recém-nascidos. Dois isolamentos do vírus PAC foram obtidos de dois lotes constituídos de 50 mosquitos indeterminados do gênero **Culex**. Além do primeiro registro da presença do vírus PAC no Estado do Amapá, os presentes achados confirmam a existência do ciclo completo de transmissão do vírus na Amazônia brasileira. Os dados ecológicos disponíveis indicam que este vírus circula dentro dos estratos baixos (0-5 m) na floresta de terra firme, transmitido por mosquitos noturnos.*

The Pacora virus (PAC: Bunyaviridae: *Bunyavirus* - like), distantly related to the antigenic group Simbu (1), is known by some strains isolated from mosquitoes of the species *Culex dunni* collected in Panama (2), and by two isolations made from wild birds (*Phlegopsis nigromaculata*, Formicariidae and *Automolus ochrolaemus*, Furnariidae) collected in Brazil (3). These species of birds are dwelling preferentially in *terra firme* forests, at low level (0-5 m) (2) and *Culex* mosquitoes

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are generally nocturnal and ornithophilous in habits (4). However, the existence of the transmission cycle of PAC virus in Brazil had not been definitely established.

As a part of a wider study entitled "A comparative study of the seasonality of mosquitoes (Diptera, Culicidae) and isolation attempts of arboviruses in the rain forest and savannah of Amapá State, Brazil", this paper will present new data about the cycle of this arbovirus.

The study has been conducted in the Zoobotanical Garden, located in the district of Fazendinha, and situated on the road Macapá - Santana, 10 km from Macapá (5). The climate is classified as tropical humid, with a mean annual precipitation of 2500 mm and a mean annual temperature of about 27°C (6,7). The area consists of a patch of old secondary rain forest bordered by an open area of savannah (8).

Adult mosquitoes have been collected simultaneously in the savannah and the forest, and in the latter at ground level and in the canopy. Anthropophilous species have been collected as they were landing on human bait and others with light traps (9,10). The samples have been conserved and processed following standard entomological (11) and virological methods (12).

From August, 18 to September, 11, 1992 a total of 5642 mosquitoes, accounting for at least 36 species and 13 genera, have been collected. Of these, 5580 (184 pools) have been inoculated intracerebrally in newborn mice (Table 1). Two strains of PAC virus (13) have been isolated from the 36 pools (1722 species) made of unidentified *Culex* mosquitoes. Despite the fact that the latter represented 30.8% of the total, the minimum infection rate (MIR) for PAC virus was fairly low (0.11%; 2/1722), suggesting a low level of transmission.

Since PAC virus is recorded for the first time in Amapá State and from mosquitoes in Brazil, its complete sylvatic cycle is confirmed to occur in Brazilian Amazonia. As suggested by the ecological preferences of the known hosts, this virus has probably a bird-*Culex* cycle, mainly at ground level in the forest and nocturnal, following the habits of its mosquito vectors which are nocturnal and ornithophilous in habits (4).

At the present time, it is difficult to speculate about the causes of the disjunct geographical distribution of this virus, which has been found only in Central America and Amazonia. However, as some authors have already noted, arboviruses with mosquito-bird cycles are in general widely distributed (2,14,15), probably due to the migrating habits of some of their hosts (16,17,18). Among others, Saint Louis

Table 1 - Mosquitoes collected in the Zoobotanical Park, Macapá, Amapá State, Brazil, August, 18 - September, 11, 1992, and inoculated for arbovirus isolation; the positive species are in boldface.

Species	*Collected*	*Inoculated*	*Pools*
Culicidae			
Anophelinae			
An. (Anopheles) sp.	10	5	1
An. (Nyssorhynchus) sp.	210	208	6
An. (Nys.) braziliensis	830	829	21
An. (Nys.) nuneztovari	12	5	1
An. (Nys.) oswaldoi	3		
An. (Nys.) triannulatus	670	670	17
Culicinae			
Aedeomyiini			
Aedeomyia (Aedeomyia) squamipennis	515	514	19
Aedini			
Ae. (Ochlerotatus) scapularis	26	24	2
Ae. (Och.) serratus	69	67	4
Psorophora (Grabhamia) cingulata	2		
Ps. (Janthinosoma) albipes	108	104	4
Ps. (Jan.) ferox	400	398	17
Culicini			
***Culex* sp.**	**1722**	**1722**	**36**
Cx. (Culex) sp.	13	13	1
Cx. (Cux.) corniger	1		
Cx. (Cux.) coronator	33	28	2
Cx. (Cux.) declarator	17	17	2
Cx. (Melanoconion) sp.	22	17	1
Cx. (Mel.) adamesi	1		
Cx. (Mel.) pedroi	6		
Cx. (Mel.) spissipes	18	18	2
Cx. (Mel.) ocellatus	1		
Cx. (Mel.) portesi	1		
Cx. (Mel.) vomerifer	1		
Mansoniini			
Cq. (Rhynchotaenia) nigricans	1		
Cq. (Rhy.) venezuelensis	310	310	10
Mansonia (Mansonia) sp.	95	95	4
Ma. (Man.) humeralis	6	6	1
Ma. (Man.) amazonensis	2		
Ma. (Man.) pseudotitillans	20	20	1
Ma. (Man.) titillans	313	313	14
Sabethini			
Limatus sp.	1	1	1
Li. durhamii	4	1	1
Phoniomyia sp.	9	6	1
Sabethes (Sabethes) belisarioi	5	5	1
Sa. (Sab.) cyaneus	1	1	1
Sa. (Sab.) quasicyaneus	9	9	1
Sa. (Sab.) tarsopus	1	1	1
Sa. (Sabethoides) glaucodaemon	12	12	1
Trichoprosopon digitatum	17	17	3
Wyeomyia sp.	57	57	3
Uranotaeniini			
Uranotaenia sp.	76	76	3
Ur. hystera	12	11	1
Total	5642	5580	184

Encephalitis (SLE), Eastern and Western Equine Encephalitis (EEE and WEE, respectively), Ilheus (ILH) and Gamboa (GAM), and Una (UNA) viruses have been found from North America (or at least Central America) to Argentina (19,20) and may also be found in the Guianas and Brazil. Turlock virus (TUR) is found from North America to Brazil (21). Thus it may well be possible that, although not being so extensively distributed, the PAC virus has been dispersed by its avian hosts. ■

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5. Altitude: 14 m above see level and geographic coordinates: 00°02'S, 51°03'W
6. According to the Koppen's classification, the Af climate is characterized by *(i)* the drier month with 60 mm or more rain, and *(ii)* the annual average of the maximum and minimum temperatures being 31°C and 23°C, respectively; the drier and wetter quarters are September-November and March-May, respectively; annual mean air relative humidity is 85% and the mean annual sun time is 2200 hours
7. Sudam 1984 *Atlas climatológico da Amazônia brasileira*. Projeto de Hidrologia e Climatologia da Amazônia. Publ. 39, Belém
8. Its extension is about one km wide by two km depth between the road and a lake in the savannah
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10. In the forest, at ground level and on a platform at 15 m, and in the savannah at ground level, the human baited collecting sessions were conducted from 10:00 h to 13:00 h and from 16:00 h to 19:00 h; at each location, a "CDC miniature light-trap" was operating from 18:00 h to 6:00 h
11. In the field, the samples were conditioned in glass tubes for storage in liquid nitrogen (-196°C); in the laboratory, the sorting of the mosquitoes was done above a chilling table (-20°C) and monospecific pools of 50 specimens each were conserved in a freezer (-70°C) until further processing
12. After being ground, each pool was diluted in bovine albumin (0.75%) with antibiotics, centrifuged at 2000 rpm for 10 min; the supernatant was then inoculated intracerebrally into 6 newborn mice (0.02 ml/animal), which were observed daily during three weeks for any sign of disease; if this was the case, further passages and/or serological tests were done with the brain of the sick animals. The identification of the strains was done by the complement fixation test, according to Fulton and Dumbell (1946 *J Gen Microbiol* 3: 97-111), and using ascitic fluids hyperimune against known Amazonian arboviruses
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Seasonal shift in lizard diet: The seasonality in food resources affecting the diet of *Liolaemus lutzae* (Tropiduridae)

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The diet of the tropidurid lizard *Liolaemus lutzae* was studied on a seasonal basis in the tropical habitat of the Restinga da Barra de Maricá, Maricá County, Rio de Janeiro State, southeastern Brazil. The diet composition of the lizard was compared between the wet (summer) and dry season (winter) and related to environmental availability of food resources. The data showed that in both seasons the diet was composed of animal and plant items but differed significantly in terms of proportions consumed (Kolmogorov-Smirnov test, $D_{max} = 0.268$; $p < 0.001$). The abundance of arthropods in the environment in the dry season ($x = 2.1$ arthropods/m^2) was considerably lower being only 39.7% of that measured during the wet season ($x = 5.4$ arthropods/m^2). However in both seasons the proportions of categories of arthropods consumed were significantly related to their availability in the environment (Spearman rank correlation, $r_s = 0.87$; $N = 13$; $p < 0.001$ for dry season; $r_s = 0.62$; $N = 18$; $p < 0.01$ for wet season). The proportion of *Ipomoea litorallis* flowers consumed by the lizards was significantly related to the availability of flowers in the environment ($r_s = 0.83$; $N = 12$; $p < 0.001$) indicating that, in the same way as observed for arthropods, they are consumed oportunistically. The consumption of leaves was higher during the dry season compared to the wet season (ANOVA, $F = 9.34$; $p < 0.001$). The food niche breadth of *Liolaemus lutzae* during the dry season ($B = 3.78$) was 68% broader than that of the wet season ($B = 2.25$). The data indicate that *Liolaemus lutzae* has a generalized diet that shifts seasonally and that in both seasons the lizard tends to consume arthropods in similar proportions to those found in the environment but with a slight variation in the level of prey selectiveness. The flowers of *I. litorallis* and leaves of plants of the area may play an important role not only as a food resource but also as a source of water.

*A composição da dieta do lagarto tropidurídeo **Liolaemus lutzae** foi estudada em um caráter sazonal na Restinga da Barra de Maricá, Município de Maricá, Rio de Janeiro, sudeste do Brasil. A dieta do lagarto foi comparada entre as estações de chuva (verão) e seca (inverno), sendo relacionada com a disponibilidade de recursos alimentares no ambiente. Os dados mostraram que em ambas as estações a dieta estava composta por itens de origem animal e vegetal mas diferindo significativamente em termos das proporções consumidas (Kolmogorov-Smirnov test, $D_{max} = 0.268$; $p < 0.001$). A abundância de artrópodos no ambiente na estação seca ($x = 2,1$ artrópodos/m^2) era apenas 39.7% daquela medida na estação de chuvas ($x = 5,4$ artrópodos/m^2). Contudo, em ambas as estações as proporções de categorias de artrópodos consumidos estavam significativamente relacionadas à sua abundância no ambiente (correlação por postos de Spearman, $r_s = 0.87$; $N = 13$; $p < 0.001$ para o inverno; $r_s = 0.62$; $N = 18$; $p < 0.01$ para o verão). A proporção de flores da planta **Ipomoea litorallis** consumidas pelos lagartos estava relacionada com a sua disponibilidade no ambiente ($r_s = 0.83$; $N = 12$; $p < 0.001$), indicando que, da mesma forma ao observado para os artrópodos as flores são consumidas oportunisticamente. O consumo de folhas foi maior durante a estação seca (ANOVA, $F = 9.34$; $p < 0.001$). A largura relativa do nicho alimentar de **Liolaemus lutzae** durante a estação seca ($B = 3,78$) foi 68% maior do que a estimada para a estação de chuvas ($B = 2,25$). Os dados indicam que **Liolaemus lutzae** possui uma dieta generalizada que muda sazonalmente e que em ambas as estações este lagarto tende a consumir os artrópodos em proporções similares àquelas deste grupo no ambiente, mas com uma pequena variação no nível de seletividade em relação às presas. As flores de **I. litorallis** são consumidas oportunisti-camente e as folhas das plantas ingeridas provavelmente possuem um papel importante não apenas como recurso alimentar, mas também como fonte de água.*

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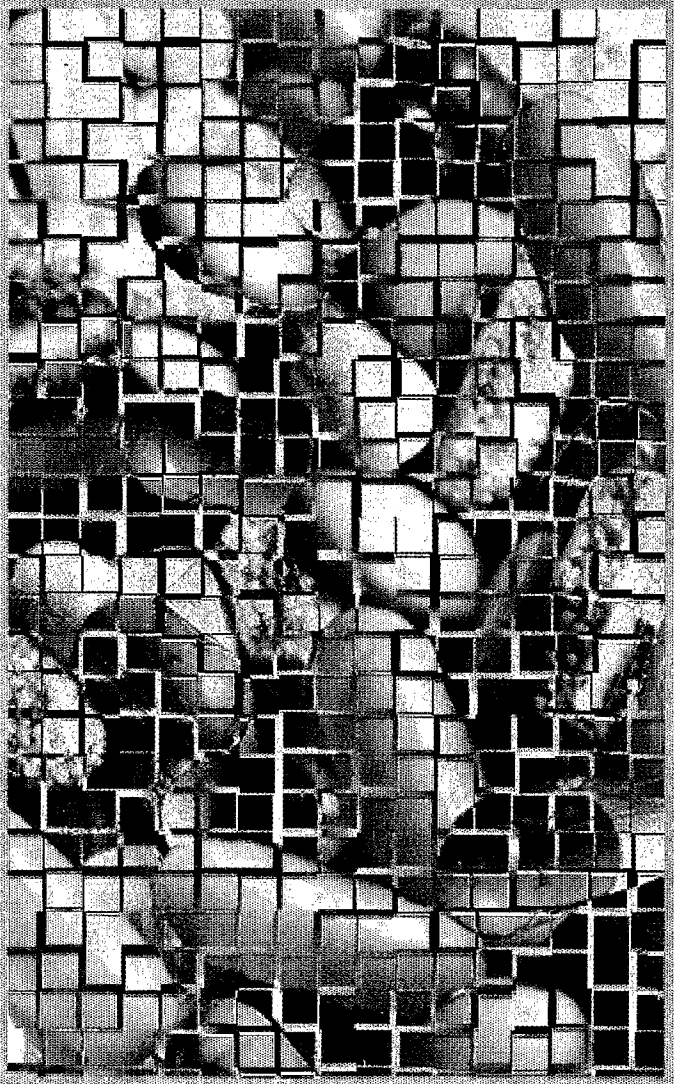

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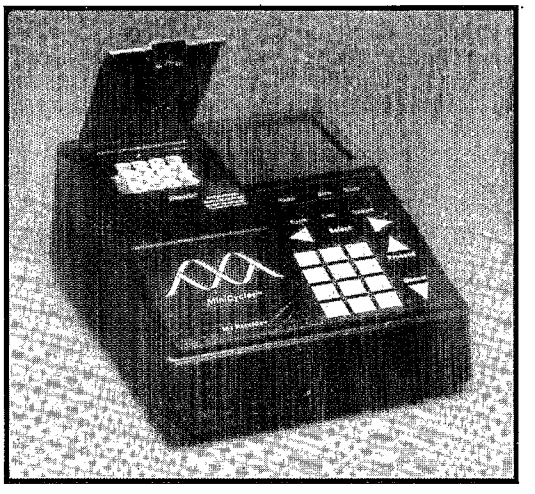

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