PRESENCE OF BUNYAVIRUSES (BUNYAMWERA SEROGROUP) IN SÃO PAULO STATE, BRAZIL

Adélia Hiroko NAGAMORI*
Terezinha Lisieux M.COIMBRA*
Elza da Silva NASSAR*
Luiz Eloy PEREIRA*
Luiza T.M.de SOUZA*
Elza Keiko KIMURA*
Iray Maria ROCCO*

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ABSTRACT: Seven viruses were isolated from sentinel mice, exposed in forested area in the Atlantic Forest region, State of São Paulo, Brazil, from 1974 to 1981. They included five isolates of Maguari, one of Kairi and one of Tucunduba. It was the first time that these viruses were detected in São Paulo State. The isolates were identified by the serological tests: Hemagglutination, Hemagglutination Inhibition, Complement Fixation and Neutralization in suckling mice. The results showed that these viruses belong to Bunyamwera serogroup. As there are reports of human disease caused by viruses belonging to this serogroup further studies are necessary to study ecological aspects involved in the maintenance cycles of these viruses, as well as the possibility of its transmission to human beings in State of São Paulo, Brazil.

DESCRIPTORS: Bunyavirus, Surveillance program, Sentinel mice, Isolation and Identification of viruses.

INTRODUCTION

Bunyamwera (BUN) virus is the prototype for the family Bunyaviridae and the genus *Bunyavirus*. It was first isolated from Aedes species in Central Africa[17] and antibodies to this virus have been detected in vertebrates, including humans in the same area, as well as in South America [9, 13]. In North America, Bunyamwera serogroup viruses, isolated from mosquitoes and vertebrates, have established the geographic distribution and main vectors of Cache Valley, Tensaw, Main Drain and Lokern viruses. Serological surveys point out that some of these viruses are implicated in occasional human infection and occasional CNS infection of large mammals [2, 3, 9].

Maguari (MAG) virus (strain BeAr 7272) was previously isolated from mosquitoes, collected in the Utinga Forest, Pará State, Brazil in 1957 and Argentina [3, 4, 11, 12], subsequently from horses and cattle in Colombia and Argentina, from mosquitoes captured in Trinidad, Colombia and the French Guyana [11], and from various species of mammals (including humans) in Peru, Surinam and Venezuela.[3, 4, 12] Serologic evidence for infection of humans, goat, cattle and equines was observed in Argentina.[10, 18] MAG virus is closely related to Cache Valley virus, which was isolated from a horse with encephalitis in the United States, but it is not responsible for epidemics.[12] It was also obtained from mosquitoes and sentinel hamsters exposed in coastal Ecuador from 1974-1978 [1].

Kairi (KRI) virus (strain TRVL-8900) was first isolated from mosquitoes *Aedes (Och) scapularis* (285 mosquitoes) in Trinidad (Melajo Forest) in 1955, in

* Seção de Vírus Transmitidos por Artrópodos, Serviço de Virologia do Instituto Adolfo Lutz, São Paulo, SP, Brasil

Brazil (Belem-PA) and Colombia (Guayacasira), from other mosquitoes species in Trinidad and Brazil, from monkeys and a rodent in Brazil and from horses in Argentina. Antibodies to KRI virus were found in men and monkeys in Trinidad [5, 11, 21].

Tucunduba virus (unregistered) is mostly transmitted by sabethine mosquitoes and some diurnal species of other tribes. It was active in the region of Altamira (North of Brazil) from 1978 to 1981, in 1983 and 1987 [7]. Tucunduba was obtained from a sick female (18 month-old) with meningoencephalitis who presented fever, headache, diarrhea, vomiting, symptoms and signs of CNS, including paresis and coma. The patient recovered without sequelas.[14] Tucunduba is frequently isolated from pools of Wyeomia mosquitoes, however, its vertebrate host is unknown [20].

The surveillance program on arbovirus activity, conducted between 1974 and 1981 by the Section of Arthropod-Transmitted Viruses (SATV) in the field stations, revealed the presence of some viruses, in the region of Icapara (24° 41'S, 47° 32'W), Iguape county, Ribeira Valley Region and in Guaratuba (23° 45'S, 45° 55'W) just under Casa Grande station at sea level.. They included five isolations of Maguari: SPAn 83375, SPAn 83376, SPAn 83377, SPAn 83390 and SPAn 83391, one of Kairi, SPAn 83389 and one of Tucunduba, SPAn 28719, which is a member of Wyeomya complex.

It was the first time that these viruses were detected in the State of São Paulo, Brazil. These agents have been obtained from human beings, mosquitoes, febrile horses, cattle, birds, monkeys and wild vertebrates in Trinidad, Ecuador, Guyanas, Colombia, Argentina, Panama and in the North of Brazil [3, 4, 11].

Bunyamwera serogroup viruses infect human, domestic and wild large mammals. Serologic surveys have shown evidence for such infections in humans, horses, cattle, goats, dogs, sheep, pigs, caribou, grizzly bears, bison, Dall sheep, moose and deer from Alaska to Argentina [2]. In 1990, in the Brazilian Pantanal, neutralizing antibodies to MAG virus were detected in equine population (two years old) [10].

MATERIALS AND METHODS

Virus isolation

All sentinel mice (a mother and six babies) were exposed inside the forest, for three days, after which they were returned to the laboratory and observed daily for signs of illness: tremors, lethargy and paralysis. Sick mice were tested for virus by inoculating clarified 10% suspensions of their brains intracerebrally in suckling mice and observed daily for two weeks for signs of illness. Subsequently, two passages of brain suspensions of the infected mice were performed and after filtration through a 450 mu Millipore filter, were reinoculated by the intracerebral route (i.c.) into three litters of suckling mice.

Serological techniques for identification and characterization

The isolates were tested by Complement Fixation (CF)[8] for preliminary serologic identification, using a battery of immune mouse ascitic fluids (MIAF) representing recognized viruses in Brazil, including members of Bunyamwera serogroup.

For virus characterization, Sodium desoxycholate sensitivity (SDC) was performed in mice[19]; Hemagglutination (HA) in a pH range from 6,0 to 7,0, and Hemagglutinaton-inhibition (HI) tests were performed according to Casals [6], adapted to microtiter method of Takatsy, modified by Sever[15], and mice Neutralization (N) according to Shope and Sather [16].

RESULTS

All isolated viruses were sensitive to SDC. By the N test showed on table 1 the five isolates of MAG virus were identical to the prototype from Belem, closely related to prototype Bunyamwera and cross-reacted with less intensity with Tensaw virus. They shared CF antigens among themselves, but distinct from Anhembi.

The SP An 83389 virus was identical to prototype KRI from Belem by CF and N tests and cross-reactivity with less intensivity with Bunyamwera (original strain), but distinct from the other 11 members of the serogroup (table 2).

The SP An 28719 virus was identical to Tucunduba virus and cross-reacted considerably with Wyeomyia virus (table 3). The smallest differences were observed with Taiassui, Macaua, Iaco and Anhembi viruses and the greatest differences with Sororoca and Kairi viruses.

TABLE 1

Results of mice N and CF tests with five strains of MAG virus from São Paulo and three known Bunyamwera serogroup

Immune serum \ Antigen or virus	N				CF						
	SPAn 83375	BeAr 7272	SPAn 164102	SPAn 164107	SPAn 83375	SPAn 83376	SPAn 83377	SPAn 83390	SPAn 83391	BeAr 7272	SPAr 2984
SPAn 83375	3.9 *	3.9	> 3.0	3.4	512	128	512	512	512	128	8
SPAn 83376	3.7	3.6	-	-	512	512	512	512	512	128	8
SPAn 83377	3.6	3.6	-	-	512	128	512	512	512	128	8
SPAn 83390	3.7	3.7	-	-	512	128	512	512	512	128	8
SPAn 83391	3.6	3.6	-	-	512	128	512	512	512	128	32
Maguari (Be Ar 7272)	3.7	3.9	> 3.0	-	128	512	128	512	128	128	32
Bunyamwera	3.5	3.6	-	-	-	-	-	-	-	-	-
Tensaw (A9 - 171b)	> 2,0	2.4	> 3.0	-	-	-	-	-	-	-	-

- not tested
* log Neutralization Index

TABLE 2

Results of mice N and CF tests with strain of Kairi (SPAn 83389) and selected Bunyamwera serogroup

Immune serum \ Antigen or virus	N	CF				
	SPAn 83389	SPAn 83389	BeAn 8226	BeAr 671	BeAn 278	SPAn 83375
PAn 83389	3.6 *	64	32	0	0	0
Kairi (BeAr 8226)	3.8	64	64	0	0	0
Bunyamwera (original)	2.0	16	-	-	-	-
Taiassui (BeAn 671)	0	0	-	512	-	-
Tucunduba (BeAr 278)	0.4	0	-	-	256	-
Tucunduba (SPAn 28719)	0.4	0	-	-	-	128
Maguari (SPAn 83375)	1.0	0	-	-	-	-
Wyeomyia (original)	0.5	-	-	-	-	-
Anhembi (SPAr 2984)	0.4	-	-	-	-	-
Macaua (BeAr 306329)	0.5	-	-	-	-	-
Xingu (BeH 388464)	0.8	-	-	-	-	-
Guaroa (BeH 22063)	0.3	-	-	-	-	-
Sororoca (BeAr 32149)	0	-	-	-	-	-
Iaco (BeAr 31206)	0	-	-	-	-	-

- not tested
* log Neutralization Index

TABLE 3

Results of mice N tests withTucunduba (strain SPAn 28719) and selected Bunyamwera serogroup

Virus* / Immune serum	SPAn 28719	BeAr 278
SPAn 28719	3.0	3.5
Tucunduba (BeAr 278)	2.6	4.0
Wyeomyia (original)	2.6	4.3
Taiassui (BeAr 671)	1.6	0.4
Macaua (BeAr 306329)	1.6	1.8
Iaco (BeAr 314206)	1.6	0.7
Sororoca (BeAr 32149)	1.1	-
Kairi (BeAr 8226)	1.1	-
Anhembi (SPAr 2984)	1.6	1.5

- not tested
* log Neutralization Index

DISCUSSION

Bunyamwera complex viruses compraises viruses, subtypes or varieties of one or another virus sharing some epitopes, which are detected in one or another test depending on the set of reagents.

The suckling mouse is considered to be the universal host system for the majority of arbovirus isolations. It is noteworthy that the seven viruses isolated from sentinel mice reflect this susceptibility and suggest the probable enzootic circulation of these viruses with the participation of hematofagous arthropods on the transmission. All five MAG viruses were isolated from the same family of swiss mice, which reveals an intense dissemination of the virus in the studied area. However, human infections have not been detected at the same time in this site.

Except for Tucunduba virus, which was found in the rising region of Guaratuba river in Bertioga county (23°55' S, 46°05'W), the other isolations occurred in the region of Icapara, Iguape county. These two studied areas are situated in the coastal regiòn with a very peculiar vegetation, with predominance of mangrove and sandbank. Despite so many similar environments, these viruses have not been detected in later studies done by SAVT in different sites of São Paulo State, suggesting that Tucunduba virus is restricted to Guaratuba region, while MAG and KRI viruses, to Icapara area.

Two of the MAG virus presented hemaglutinin in pH 6.1 while the other ones after subsequent passages in suckling mice have not showed hemagglutination activity. Thus, those two strains are not included within seroepidemiological surveys of the areas of research, where the surveillance program to arbovirus has been developed, because it is difficult to maintain the hemagglutination activity of those two strains. For this reason, possible human and wild animal infections have not been detected. In 1996, two more strains of MAG virus obtained from blood of birds *Zonotrichia capensis* and *Crotophaga ani* (Santos, umpublished data), in the region of Icapara, demonstrate the existence of a restricted enzootic circulation of the virus in this area. As migratory birds are suspected to be the vertebrate hosts, it is interesting to note that the bird *Crotophaga ani* may be responsible for the geographical distribution of MAG virus since this species of bird travels great distances along South America. This fact was not observed with Tucunduba and KRI viruses suggesting that other specimems of vertebrates could be their hosts. Further research must be done to achieve better evaluation of the epidemiology of those viruses .

The isolations of MAG, KRI and Tucunduba viruses confirm the presence of these viruses in São Paulo State. In the absence of a recognized disease caused by any of these viruses in the State, further studies are necessary to define which vectors and vertebrate hosts are involved in the cycles of these agents in the area, besides serologic surveys to determine the presence of antibodies in humans and livestock.

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PRESENÇA DE BUNYAVIRUS (SOROGRUPO BUNYAMWERA) NO ESTADO DE SÃO PAULO, BRASIL

RESUMO: Sete vírus foram isolados de camundongos sentinela, expostos em área de floresta, na Região da Mata Atlântica, Estado de São Paulo, Brasil, de 1974 a 1981. Estão incluidas cinco amostras de Maguari, uma de Kairi e uma de Tucunduba. Foi a primeira vez que esses vírus foram detectados no Estado de São Paulo. Os isolados foram identificados por testes sorológicos de Hemaglutinação, Inibição de Hemaglutinação, Fixação de Complemento e Neutralização em camundongos lactentes. Os resultados mostraram que esses vírus pertencem ao sorogrupo Bunyamwera. Como existem relatos de doença humana causada por vírus pertencente a esse grupo, conclue-se que é necessário estudar os aspectos ecológicos envolvendo os ciclos de manutenção desses vírus, assim como a possibilidade de sua transmissão em seres humanos no Estado de São Paulo, Brasil.

DESCRITORES: Bunyavirus, Programa de vigilância, Camundongos sentinela, Isolamento e Identificação de vírus

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