Rev. Inst. Med. trop. São Paulo
20(2):82-86, *março-abril*, 1978

UDC 616.988.2

ISOLATION OF MUCAMBO VIRUS, A MEMBER OF THE VENEZUELAN EQUINE ENCEPHALITIS VIRUS COMPLEX IN THE STATE OF SÃO PAULO, BRASIL

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SUMMARY

During an epidemiological surveillance presently undertaken to observe arboviral activity in certain forested areas, an alphavirus, belonging to the Venezuelan equine encephalitis virus and closely related to Mucambo virus has been isolated. This is the first isolation of a virus from this complex in South Brasil.

The introduction of Mucambo virus in the State of São Paulo was detected both through virus isolation and the presence of neutralizing antibodies in human beings, wild birds and mammals. No clinical cases among human inhabitants or horses being detected, the isolate was considered as an endemic strain of Venezuelan equine encephalitis virus complex.

Mucambo virus activity was recorded in the studied areas up to 1971. The role of wild birds in the virus maintenance cycle and its possible introduction in other areas were discussed.

INTRODUCTION

Arboviruses belonging to "Venezuelan equine encephalitis virus complex" are widely distributed throughout the American continent. Epidemics have occurred in eleven countries and sylvatic foci have also been encountered in several others, including Brasil [1]. In Belém, under forest conditions, a virus, initially identified as Mucambo virus was found to be active [4,8], and some strains isolated in Trinidad and Surinam were also identified as Mucambo [1]. However, further serological studies considered Mucambo virus as an enzootic strain of Venezuelan equine encephalitis virus [10].

Since 1961 a surveillance program on arboviral activity is being conducted in certain forested areas in the State of São Paulo, a number of viruses being isolated. One of them was an alphavirus, closely related to Mucambo virus. This paper deals with this isolation as well as the corresponding serological and epidemiological data obtained.

MATERIALS AND METHODS

Forested areas explored in this study are located in the State of São Paulo, at 23.º S Latitude, in Itapetininga, Casa Grande and Rio Guaratuba and were described elsewhere [7].

Mosquitoes were collected by human bait or CDC miniature light traps and kept alive for 48 hours for digestion of a possible blood meal. They were assorted by species, pooled, suspended in 0.05 M phosphate buffered saline pH 7.6-7.8 and inoculated intracerebrally (IC) in 2-day-old mice.

Wild mammals were trapped, brought alive to the laboratory in São Paulo and killed by cardiac exsanguination. Pools of kidney and heart were inoculated in mice for virus isolation attempts and the serum saved for serology. Carcasses were identified tentativelly and sent to the U.S. National Museum to confirm or correct the identification.

Wild birds were netted with mist nets and bled from the jugular vein or from the heart.

Seção de Vírus Transmitidos por Artrópodos. Instituto Adolfo Lutz, São Paulo, Brasil.

Blood was refrigerated and brought to the laboratory for virus isolation attempts and serology.

Sentinel mice were exposed inside forests, under protective hoods similar to those described by CAUSEY et al. [4].

Hemagglutination-inhibition (HI) tests and complement-fixation (CF) tests were performed according to techniques described elsewhere [2]. Sera for HI tests were extracted with acetone to avoid non-specific inhibitors. Neutralization (N) tests were done in Vero cells. All sera found to inhibit the virus cytopathogenic effect were considered as positive.

Hyperimmune ascitic fluids were prepared against individual viruses in 8 to 12 week old mice according to methods described by TIKASINGH et al. [9].

The standard arbovirus strains used in this research were supplied by the Rockefeller Foundation Virus Laboratories.

RESULTS

A virus, which received the laboratory number An 15600 was isolated from a suckling mouse exposed during 48 hours at Casa Grande field station. The family returned to the São Paulo laboratory on April lst, 1970 and after 5 days of observation one mouse became sick. Its brain was harvested and a suspension inoculated in other mouse families; the isolate was shown to be similar to Mucambo virus by HI and CF testing (Table I).

TABLE I

Identification of An 15600 strain isolated in São Paulo as belonging to «Venezuelan equine encephalitis Virus» close to Mucambo Virus

Virus or Antiserum	An 15600 Antigen or Virus		An 15600 Antibody	
	HI	CF	HI	CF
Mucambo	1280(*)/2560	64/128	1280/2560	128/128
VEE	320/1280	16/256	320/1280	128/128
Pixuna	40/640	16/256	40/1280	8/128
EEE	0/1280	0/256	0/1280	0/128
Mayaro	0/640	0/512	0/1280	8/128
Una	0/80	0/16	0/1280	0/128
WEE	0/160	0/640	0/1280	0/128

(*) Heterologous titer/Homologous titer
VEE: Venezuelan equine encephalitis virus
EEE: Eastern equine encephalitis virus
WEE: Western equine encephalitis virus

Serum survey — Sera from people inhabiting Casa Grande and Itapetininga field stations were tested for neutralizing antibodies against Mucambo virus and 9 out of them (2.3%) were positive. Twenty-seven out of 227 sera from wild birds (10%) and 10 of 79 mammals (12%) were also positive (Table II). All species reacting positively to this virus are shown in Tables III and IV.

The Mucambo reacting sera were further tested for antibodies to Eastern equine encephalitis virus, the other alphavirus known to

TABLE II

Total results of neutralization tests for Mucambo virus with human and wild vertebrate sera collected in selected study areas, State of São Paulo, 1969-1971

Sera	Itapetininga	Casa Grande	Guaratuba
Human	7/288(*)	2/99	—
Wild birds	13/133	4/41	10/103
Wild mammals	4/16	3/39	3/24

(*) = number of positives/number of tested sera

be active in this region [7], no neutralizing antibodies for the latter being found among them.

Mucambo virus had not been isolated from over more than 700 pools of several species of mosquitões or from the wild vertebrates collected from 1969 through 1971.

DISCUSSION

VEE virus was first isolated in 1938 in Venezuela during a severe horse epizootics [3].

TABLE III

Wild birds with Mucambo virus neutralizing antibodies, concerning their behavior in selected study areas of São Paulo, Brasil

Species	Study area	Date	Behavior
Columbigallina talpacoti	Itapetininga	18 Apr. 1968	Summer inhabitant
Stelgidopterix ruficollis	Itapetininga	13 Sep. 1968	Summer inhabitant
Tachyphonus coronatus	Itapetininga	28 Oct. 1968	Permanent resident
Tachyphonus coronatus	Itapetininga	17 Jan. 1969	Permanent resident
Leptotila verreauxi	Itapetininga	10 Feb. 1969	Permanent resident
Vireo chivi	Itapetininga	10 Feb. 1969	Summer inhabitant
Turdus albicollis	Itapetininga	24 Feb. 1969	Permanent resident
Thraupis sayaca	Itapetininga	24 Feb. 1969	Permanent resident
Turdus leucomelas	Itapetininga	3 Mar. 1969	Permanent resident
Turdus rufiventris	Itapetininga	26 Jun. 1969	Permanent resident
Alopochelidon fuccata	Itapetininga	11 Jul. 1969	Summer inhabitant
Myospiza humeralis	Itapetininga	31 Oct. 1969	Permanent resident
Elaenia obscura	Itapetininga	9 Jan. 1970	Summer inhabitant
Chiroxiphia caudata	Casa Grande	3 Aug. 1968	Permanent resident
Thamnophilus caerulescens	Casa Grande	24 Aug. 1968	Permanent resident
Habia rubica	Casa Grande	20 Jun. 1969	Winter inhabitant
Leptopogon amaurocephalus	Casa Grande	20 Jun. 1969	Permanent resident
Thriothorus longirostris	Guaratuba	15 May 1968	Permanent resident
Thraupis cyanoptera	Guaratuba	20 May 1968	Unknown
Lepidocolaptes fuscus	Guaratuba	26 Jun. 1968	Permanent resident
Tanagra pectoralis	Guaratuba	28 Jun. 1968	Winter inhabitant
Manacus manacus	Guaratuba	17 Mar. 1969	Permanent resident
Chlorocerule inda	Guaratuba	17 Mar. 1969	Permanent resident
Ramphocelus brasilius	Guaratuba	17 Mar. 1969	Permanent resident
Turdus albicollis	Guaratuba	30 Apr. 1969	Permanent resident
Coereba flaveola	Guaratuba	4 May 1969	Permanent resident
Platypsaris rufus	Guaratuba	24 Oct. 1969	Summer inhabitant

Subsequently, this virus was found to be widely distributed in the Americas. Outbreaks have occurred from Ica, Peru (14° S latitude) to Texas, USA (28.° N latitude), affecting at least 11 countries [1]. In Brasil, a virus called Mucambo virus, isolated in Belem [4] was initially identified as VEE virus but found sufficiently distinct by serological testing [2]. However, an extensive study carried out by YOUNG & JOHNSON [10] classified Mucambo as an enzootic strain of VEE, i.e., as one of those strains that are maintained in enzootic foci involving wild mosquitoes and rodents, humans and horses being infrequently infected.

The isolation of a virus of the VEE complex and closely related to Mucambo virus in the State of São Paulo represents the first isolation of an agent of that complex at 23°S latitude, enlarging its geographical distribution. As judged by the data obtained, it must have been introduced recently in the areas studied, involving wild birds as reservoirs; this finding is in contrast with the epidemiological pattern exhibited by Mucambo in the North of Bra-

TABLE IV

Wild rodents with Mucambo virus neutralizing antibodies, in selected study areas, State of São Paulo, Brasil

Species	Study area	Date
Cavea aperea	Itapetininga	11 Jul. 1969
Cavea aperea	"	18 Jul. 1969
Oxymycterus sp 1(*)	"	6 Mar. 1970
Nectomys squamipes	"	19 Jun. 1970
Thomazomys (Delomys) sp	Casa Grande	3 Apr. 1970
Thomazomys (Delomys) sp	"	16 Nov. 1970
Rattus norvegicus	"	8 Jan. 1971
Nectomys squamipes	Guaratuba	19 Feb. 1970
Nectomys squamipes	"	10 Jun. 1970
Oxymycterus sp 2	"	9 Dec. 1970

(*) Some of the rodents captured were not identified down to species as they apparently belong to species not described in the State of São Paulo

sil [2,5], where its forest cycle seems to involve primarily wild mammals. However, some other studies [5,9] pointed out the importance of the wild birds in VEE epidemic cycles and their potential role in the introduction of this virus in other areas.

Evidence of infection was obtained among wild bird species that are either summer or winter inhabitants or permanent residents in the São Paulo areas selected for these studies. Summer or Winter temporary residents could be incriminated for introducing the virus, due to their migratory behavior.

The endemic variant of VEE virus isolated in the State of São Paulo did not seem to affect horses in this region, as all equine encephalitis outbreaks were so far found to be caused by Eastern equine encephalitis virus [7].

CAUSEY et al. [4], describing the original Mucambo virus isolation, reported that they were able to isolate this agent from five mild febrile cases. In the State of São Paulo, it was found that inhabitants of the investigated areas had been infected by a virus of the VEE complex, as the serological data showed 2.8% of them to exhibit neutralizing antibodies. Cross-reactions with Eastern equine encephalitis virus — the other alphavirus active in the region — could be excluded; it would thus be inferred that the agent involved was Mucambo virus. However, no overt infectious disease attributed to a Mucambo infection could be detected among inhabitants of Casa Grande or Itapetininga study areas, where an epidemiological surveillance was being conducted at that time. So, if Mucambo virus has caused any human disease it must have been so mild as not to be reported by the local population.

Through wild bird blood testing, Mucambo virus activity could be recorded up to 1971. After that year, antibodies were no longer detected, suggesting that the virus was not able to stablish itself in the area.

It would be important to point out that if an endemic strain of a virus of the VEE complex could be introduced far south off its ecological niches, an epidemic variant would have the same chance to be carried to South Brasil. For this reason, any surveillance program on arboviral activity must include a virus of this complex to detect an eventual virus transmission, or to make a precise diagnosis of an outbreak, due to the importance of this pathogen both for men and domestic animals.

RESUMO

Isolamento do vírus Mucambo, um dos membros do Complexo da Encefalite Equina Venezuelana no Estado de São Paulo, Brasil.

Durante vigilância epidemiológica efetuada em certas áreas florestais do Estado de São Paulo, Brasil, para observar-se a atividade de arbovírus, verificou-se a introdução de um alfavírus, pertencente ao complexo do vírus da Encefalite equina Venezuelana e identificado como sendo amostra do vírus Mucambo, sendo esta a primeira vez que é assinalada a presença de um membro do citado complexo no sul do Brasil. Essa introdução foi detectada por intermédio do isolamento do agente e pela presença de anticorpos na população local, bem como em aves e mamíferos silvestres.

O vírus Mucambo foi considerado como amostra endêmica do complexo viral da Encefalite equina Venezuelana, pois, não foram observados quadros clínicos evidentes em homens e cavalos, devido à infecção pelo vírus em questão.

Discute-se ainda o papel das aves silvestres na introdução de um membro de um complexo viral importante como agente patogênico para o homem e animais domésticos.

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Recebido para publicação em 6/6/1977.