

CIRCULATION OF EASTERN EQUINE ENCEPHALITIS, WESTERN EQUINE ENCEPHALITIS, ILHÉUS, MAGUARI AND TACAIUMA VIRUSES IN EQUINES OF THE BRAZILIAN PANTANAL, SOUTH AMERICA.

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SUMMARY

Neutralizing antibodies to EEE (6.7%), WEE (1.2%), ILH (26.6%), MAG (28.2%) and TCM (15.7%) viruses were found in sera of 432 equines of the Brazilian Pantanal, area where undiagnosed horse deaths are frequently observed.

A 4-fold rise in CF titer to EEE virus was detected in acute and convalescent sera of an encephalitis horse sacrificed in 1992. Antibodies to EEE, ILH, MAG and TCM viruses were detected in horses less than 2 years old indicating recent circulation of these viruses in the Pantanal. The evidence of recent equine encephalitis associated with rising CF titer to EEE warrants a more intensive study with attempts to isolate virus from horses with clinical manifestations of encephalitis.

KEY WORDS: Arboviruses; Pantanal region; Equine encephalitis; Serological survey in equines.

INTRODUCTION

The Pantanal (meaning swamp) is an area of 139.111 km², ranging in altitude from 80 to 130m above sea level, located in the heartland of South America, between 15° and 22°S, and 55° and 61°W. It is composed of territories belonging to Brazil, Bolivia and Paraguay¹. The area receives alluvial sediment of several rivers of the upper Paraguay river basin, with an associated large variation in the soil type. The rich fauna consists of about 658 species of birds, 80 species of mammals, 50 species of reptiles and 230 species of fishes².

Extensive cattle farms of 10,000 to 200,000 hectares occupy the majority of this flood plain. It is populated by 6,799,526 cattle, 89,379 buffaloes and 119,222 equines*. The Pantanal can be considered one of the most important livestock production centers in Brazil, Bolivia and Paraguay⁸.

Official data on incidence of arbovirus equine encephalomyelitis are not available. Frequent

epizootics of undiagnosed horse deaths in this area with ecological conditions favoring vector transmission raise the possibility that arbovirus infections are responsible.

The results of a survey of equine sera from farms located in the Brazilian territory are presented here and are consistent with arboviral disease.

MATERIALS AND METHODS

Area

The Brazilian Pantanal consists of 9 sub-areas: Cáceres, Poconé, Barão de Melgaço, Paiaguás, Nhecolândia, Abobral, Aquidauana, Miranda e Nabileque¹ (Figure 1).

Sera were collected from equines on 5 farms. Alegria (F₁), Campo Dora (F₂), Ipanema (F₃), Porto Alegre (F₄) farms are located in Nhecolândia Pantanal and Santo Antonio de Jacadigo (F₅) farm is in Nabileque Pantanal.

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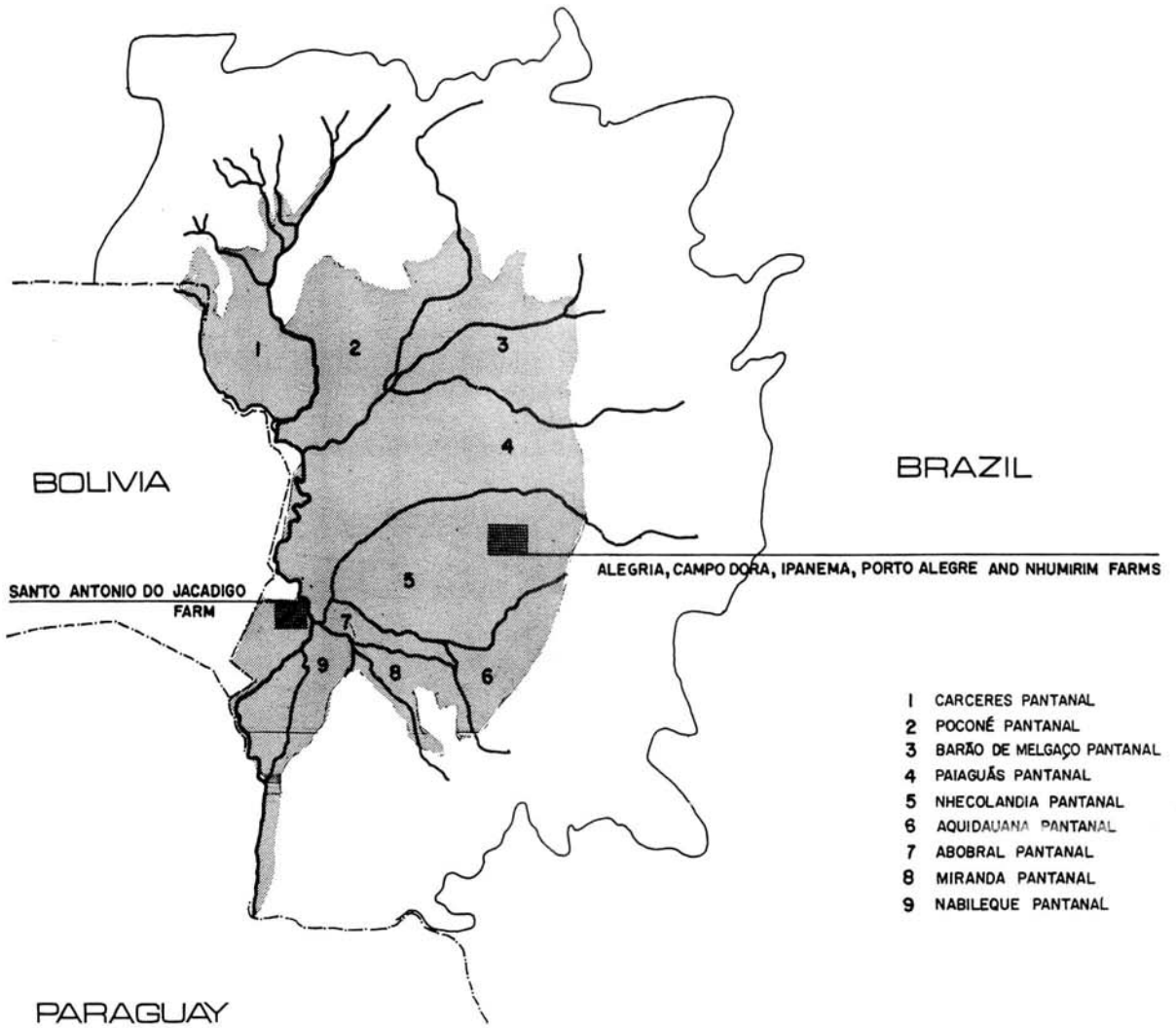
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* Data from the Department of Agriculture of Mato Grosso do Sul State, Brazil.

FIGURE 1

LOCALIZATION OF THE INVESTIGATED FARMS
IN THE PANTANAL REGION



SOUTH AMERICA

UPPER PARAGUAY RIVER BASIN

PANTANAL REGION

PARAGUAY RIVER

PACIFIC OCEAN

ATLANTIC OCEAN



Serological survey

Sera of 432 equines from the five farms were stored at 20°C. They were tested in the laboratory of Evandro Chagas Institute by hemagglutination-inhibition (HI) according to the procedure described by SHOPE⁷, using antigens of eastern equine encephalitis (EEE), western equine encephalitis (WEE), Mucambo (MUC) and Mayaro of the alphaviruses; Ilhéus (ILH), S. Louis encephalitis, Caciporé and Rocio of the flaviviruses and Caraparú, Catu, Guaroa, Maguari (MAG), Oropouche and Tacaiuma (TCM) of the bunyaviruses.

Sera positive in the HI test were tested for neutralizing antibody by the technique described by CASALS³.

Paired acute and convalescent sera were collected on April 27 and May 11, 1992 from a horse

of Nhumirim farm, located in Nhecolândia Pantanal. This animal had neurologic disease with uncoordination and unsteady walking in circles. The onset of the horse illness was on April 26. After 20 days of the disease the animal was sacrificed and necropsied. The paired sera were tested by HI and neutralization (N) tests to the same antigens used in the survey. The complement-fixation (CF) test³ was performed to EEE, ILH, MAG and MUC antigens and an attempt was made to isolate virus from the acute serum by intracerebral inoculation of suckling mice.

RESULTS

HI and N antibodies were detected to EEE, WEE, ILH, MAG and TCM viruses. Neutralizing antibody prevalence was to EEE 6.7%; WEE, 1.2%; ILH, 26.6%; MAG, 28.2% and TCM, 15.7%, (Table 1).

TABELA 1

Prevalence of neutralizing antibodies to EEE, WEE, ILH, MAG and TCM viruses in equines of five farms, Pantanal region, South America, 1992

FARM	EEE	WEE	ILH	MAG	TCM
F1	15/134(11.2)*	0/134(0.0)	46/134(34.3)	49/134(36.6)	27/134(20.1)
F2	2/114(1.7)	0/114(0.0)	17/114(14.9)	27/114(23.7)	17/114(14.9)
F3	2/57(3.5)	0/57(0.0)	10/57(17.5)	19/57(33.3)	12/57(21.1)
F4	1/50(2.0)	1/50(2.0)	19/50(38.0)	5/50(10.0)	2/50(4.0)
F5	9/77(11.7)	4/77(5.2)	23/77(29.9)	22/77(28.6)	10/77(13.0)
Total	29/432(6.7)	5/432(1.2)	115/432(26.6)	122/432(28.2)	68/432(15.7)

* n° positive/n° tested (%). positive = log neutralization index > 1.8

Viruses: EEE — Eastern equine encephalitis (Be An 7526)
 WEE — Western equine encephalitis (Be An 70.100)
 ILH — Ilhéus (Be H 7445)
 MAG — Maguari (Be Ar 7272)
 TCM — Tacaiuma (Be An 73)

Reactivity to EEE, ILH, MAG and TCM was observed in all farms, but reactivity to WEE was found only at the Porto Alegre farm in Nhecolândia Pantanal and at Santo Antonio do Jacadigo farm, near Bolivia.

Ten horses less than two years old from the Alegria, Campo Dora, Ipanema and Porto Alegre farms reacted with EEE, ILH, TCM or MAG antigens, indicative of recent circulation of these viruses.

Paired sera from the horse presenting with signs of encephalitis showed a 4-fold rise in antibody titer by CF test to EEE antigen and a rise in log neutralization index to EEE virus (Table 2). This horse also had HI antibodies to EEE (1:40) ILH (1:40) MUC (1:20) and MAG (1:20) antigens. No virus was isolated from the acute phase serum.

At the postmortem the horse had congestion of the leptomeninges with focal hemorrhage and calcification areas of neuronal necrosis in the gray

matter, mainly near the hemorrhagic area in the meninges and edema and perivascular inflammatory infiltrates of mononuclear cells in the white matter.

Table 2
Results of FC and N tests in paired serum samples of one horse with clinical diagnosis of encephalitis; Pantanal, 1992

Test	Virus		EEE		ILH		MAG		MUC							
	S1	S2	Control negative	Control positive	S1	S2	Control negative	Control positive	S1	S2	Control negative	Control positive	S1	S2	Control negative	Control positive
CF	32	128	< 8	> 32	< 8	< 8	< 8	> 32	< 8	NT	< 8	> 16	< 8	NT	< 8	> 16
N	3.2*	3.9	< 0.9	5.2	NT	NT	NT	NT	2.8	1.7	< 1.7	2.9	2.1	2.1	< 1.7	4.7

S1 e S2 — paired serum samples collected in 4-27-92 and 5-11-92

CF — complement fixation

N — neutralization

* — log neutralization index

NT — not tested

DISCUSSION

There is serological evidence implicating EEE virus as a recent cause of equine encephalitis in the Brazilian Pantanal and evidence of past infection with WEE virus. The ecological aspects of the region with fresh water swamps is similar to those of the swamp areas of North America near the Great Lakes and Atlantic seacoast where EEE virus is prevalent.

The geographic proximity to northern Argentina affected since 1908 by periodic epizootics of eastern and western equine encephalitis⁵ suggest that Pantanal may be an important focus of virus maintenance and possibly the source of introduction of virus into other epizootic areas.

Antibody to Mucambo virus, a sub-type of Venezuelan equine encephalomyelitis (VEE) complex, was detected in the horse with encephalitis. It is also important to verify in further investigations the possible circulation of other sub-types such as sub-types which was recovered from mosquitoes in Argentina in 1980⁵ and from mosquitoes and one bat in São Paulo State, Brazil, in 1978².

The significance of antibodies to MAG, ILH and TCM viruses in horses is not known. MAG

virus is closely related to Cache Valley virus which was isolated from a horse with encephalitis in the United States⁵, but it is not associated with epidemics. MAG virus has been isolated previously from mosquitoes in northern Brazil and Argentina^{4,5} and from equines and cattle in Columbia and French Guiana⁴. A high prevalence of MAG antibodies was previously observed in humans, horses and birds in Argentina⁶. Horses from Argentina had shown 80% prevalence of N antibodies to MAG virus⁵, without implicating this agent in epidemics of encephalitis.

Tacaiuma virus has been isolated from mosquitoes and a febrile person in northern Brazil⁴. Antibodies were detected in people and horses in Brazil and Argentina^{4,6}, in people in French Guiana and from bats, rodents and birds in Brazil⁴.

Since Ilhéus virus was first isolated in Brazil from mosquitoes⁴ it has also been recovered from mosquitoes, birds, sentinel monkey and man in Brazil and other countries of South and Central America⁴. HI antibodies were previously detected in horses in Brazil⁹. Neither TCM nor ILH viruses are known to cause equine encephalitis.

Although the cause of recurrent equine encephalitis in the Pantanal has not yet been con-

firmed, the evidence given herein of recent equine encephalitis associated with rising titers of antibody to EEE virus warrant a more intensive study with attempts to isolate the virus from sick horses.

There is no information about arbovirus encephalitis in residents of the Pantanal, which may reflect an insensitive surveillance of human disease in the area.

RESUMO

Circulação dos vírus das encefalites equina do Leste e Oeste, Ilhéus, Maguari e Tacaiuma em equinos da região do Pantanal brasileiro, América do Sul.

Em um inquérito sorológico realizado em 432 equinos da região do Pantanal, território brasileiro, foram detectados anticorpos neutralizantes para os vírus EEE (6,7%), WEE (1,2%), ILH (26,6%), MAG (28,2%) e TCM (15,7%). Detectou-se também em um cavalo com um quadro clínico de encefalite um aumento de 4 vezes no título de anticorpos fixadores de complemento para EEE em soros coletados nas fases aguda e convalescente da doença. Cavalos com menos de 2 anos de idade apresentaram anticorpos neutralizantes para EEE, ILH, MAG e TCM, indicando circulação recente desses vírus no Pantanal.

Os achados justificam intensificar esses estudos na região com tentativas de isolamento de vírus de animais apresentando manifestações clínicas de encefalite.

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