

Prevalence of antibodies to equine alphaviruses in the State of Pará, Brazil

Prevalência de anticorpos para alphavirus em equinos do estado do Pará, Brasil

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ABSTRACT: The State of Pará comprises 26% of Brazilian Amazon region, where a large diversity of arboviruses has been described. This study sought to assess the prevalence and distribution of hemagglutination inhibition (HI) antibodies against antigens of four alphaviruses (Togaviridae: *Alphavirus*) from the species: *Eastern equine encephalitis* (EEEV), *Western equine encephalitis* (WEEV), *Mayaro virus* (MAYV), and *Mucambo virus* (MUCV) in 753 serum samples of horses in Pará State, Brazil. All investigated arboviruses were detected and indicate that horses are susceptible to these alphaviruses, and show evidences of their active circulation in farm animals in the Brazilian Amazon.

KEYWORDS: Eastern equine encephalitis virus; Western equine encephalitis virus; Mayaro virus; Mucambo virus; hemagglutination inhibition test.

RESUMO: O estado do Pará corresponde a 26% da Amazônia brasileira, onde uma grande diversidade de arbovírus foi descrita. Este estudo procurou avaliar a prevalência e a distribuição de anticorpos inibidores da hemaglutinação (IH) contra antígenos de quatro alphavirus (Togaviridae: *Alphavirus*), das espécies: *Vírus da encefalite equina do leste* (EEEV), *Vírus da encefalite equina do oeste* (WEEV), *Vírus mayaro* (MAYV) e *Vírus mucambo* (MUCV), de 753 amostras de soro de equinos no estado do Pará, Brasil. Todos os arbovírus pesquisados foram detectados, indicando que os equinos são suscetíveis a esses *Alphavirus* e mostrando evidências de sua circulação ativa em animais de fazenda na Amazônia brasileira.

PALAVRAS-CHAVE: Vírus da encefalite equina do leste; Vírus da encefalite equina do oeste; Vírus mayaro; Vírus mucambo; teste da inibição da hemaglutinação.

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The criteria by RODRIGUES et al. (2010) for positivity were chosen. Positive reactions were classified as either monotypic reactions (MR; reaction against only one antigen or reaction that was at least four-fold stronger against one antigen compared to all other antigens of the same viral genus), heterotypic reactions (HR; reactions against two or more viruses exhibiting similar titers or the predominance of a given antigen was less than four-fold than the others), or total reactions (TR; monotypic reactions plus heterotypic reactions).

All horses presented high serologic prevalence to the four alphaviruses used in this study. The prevalences for TR, HR, and MR are presented in Table 1. The table shows that all four viruses belonged to the genus *Alphavirus*, all of which have already been isolated from arthropods and/or wild vertebrates in Brazil and even from humans in the Western hemisphere, and only EEEV and WEEV have been proven to cause disease in horses.

In the Brazilian Amazon, EEEV and WEEV have been isolated from birds, horses, and mosquitoes in the suburbs of Belém and Southern Pará State, Brazil. Antibodies against these arboviruses have also been found, with a low prevalence, in wild mammals (SHOPE et al., 1966) and birds (DÉGALLIER et al., 1992). The results of this study indicate a broad distribution of these arboviruses in the Brazilian Amazon. It should be emphasized that there is a high prevalence of HI antibodies against EEEV and WEEV in unvaccinated horses, which indicates frequent exposure of these animals to mosquitoes that carry these arboviruses.

Despite the lack of reports on clinical disease in horses in Pará State, Brazil, only one epizootic episode by EEEV has been reported in the city of Bragança in 1962 (CAUSEY et al., 1962), and another in Marajó Island recently (CAMPOS et al., 2013); however, there are no reported cases of encephalitis-like human disease in the Amazon region caused by these arboviruses (WOODALL, 2004). Although EEEV causes neurological illness in human beings in the North America (WEAVER; REISEN, 2010), only two fatal cases had been reported in South America, with one being in Brazil, in the state of the Bahia (ALICE, 1956), and the other one, in Trinidad and Tobago (CORNIUO et al., 1972). AGUILAR et al.

(2007) believe that the low incidence of severe cases of human encephalitis in South America is probably due to low infectivity and/or non-virulence of the isolated strains of EEEV that circulates in this region.

Some epizootic strains of VEEV may be transmitted from horse-to-mosquitoes-to-human, and the epidemic transmission cycle of VEEV involves horses that serve as the main source of the virus, thus being a source of infection to new hematophagous mosquitoes (STOTT, 2003). The MUCV subtype III of VEEV (YOUNG; JOHNSON, 1969) was isolated in Amazonia, and it was associated with sporadic self-limited febrile syndromes in humans in Brazil, Trinidad and Tobago, Surinam, and French Guiana.

The MUCV subtype was isolated in Southeastern Brazil, close to the Ribeira Valley, in the State of São Paulo (LOPES; SACHETTA, 1978); however, there are no reports of disease in domestic animals. Nevertheless, a 10-month-old equine experimentally infected via intramuscular route with high doses of MUCV has developed viremia, which lasted for 3 days, with fever 24 hours later, and persistent leucopenia (SHOPE et al., 1964). Additionally, IVERSSON et al. (1993) found anti-MUCV antibodies in an equine exhibiting encephalitis-like illness in the Brazilian Pantanal.

MAYV was first isolated in Trinidad and Tobago in 1954 (ANDERSON et al., 1957) and is frequently isolated in northern South America from humans, wild vertebrates (mainly monkeys), and mosquitoes. Outbreaks in humans caused by this rash febrile disease virus have been frequently reported in Amazon (AZEVEDO et al., 2009). In this study, the prevalence of HI antibodies against MAYV was low. There are no reports of disease caused by this virus in domestic animals; however, the clinical syndrome showed by humans presents symptoms that may not be perceived in horses.

The results of this study lead us to the following conclusions: these are preliminary evidences of active alphavirus infections in horses in the Brazilian Amazon; these animals must have been exposed to many thousands of mosquito bites in locations that sometimes serve as transmission foci for those viruses, although there was no evidence that horses are frequently exposed to arboviruses; the results obtained in this study showed evidence of such condition, which may represent a public health risk to humans, who are susceptible to developing diseases caused by alphaviruses; and finally, the horses showed higher prevalence of antibodies in TR and HR, suggesting that these animals can detect the circulation of such alphaviruses.

Table 1. Prevalence of HI antibodies against antigens of four alphaviruses in horses sera with heterotypic reactions (HR), monotypic reactions (MR), and total reactions (TR) obtained in State of Pará, Brazil.

Virus	Horses					
	HR	MR	TR	%HR	%MR	%TR
EEEV	153	74	227	20.31	9.82	30.14
WEEV	148	24	172	19.65	3.18	22.84
MAYV	36	04	40	4.78	0.53	5.31
MUCV	54	09	63	7.17	1.19	8.36
Total	391	111	502	51.92	14.74	66.66

EEEV: Eastern equine encephalitis virus, WEEV: Western equine encephalitis virus, MAYV: Mayaro virus, MUCV: Mucambo virus.

ETHICS COMMITTEE AND BIOSECURITY

This study was approved by the Animal Research Ethics Committee (CEPAN) of the Evandro Chagas Institute (IEC) (protocol 054/2009 CEPAN/IEC).

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