PREVALENCE OF HEMAGGLUTINATION-INHIBITION AND NEUTRALIZING ANTIBODIES TO ARBOVIRUSES IN HORSES OF JAVA

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Abstract. A study was conducted to measure the prevalence of hemagglutination-inhibition (HI) and neutralizing antibodies against two arboviruses (Chikungunya and Japanese encephalitis virus) in horses of Java, Indonesia. Blood specimens were collected from a sample of 112 horses at two stables: Pulo Mas, a racing track-horse complex, located in a residential area in North Jakarta, and Pamulang, a riding school, located in a rural environment of West Jaya. Sera were tested by the HI assay and plaque reduction neutralization test. JEV antibodies were detected by HI in 58 (52%) of the horses, while only 11 (10%) had Chikungunya antibodies by HI. The proportion of Pamulang horses infected with JEV (66%) was significantly higher than found among Pulo Mas horses (40%) screened ($p < 0.01$). Of the 58 horses with JEV antibodies by HI, 52 (90%) were found to have specific neutralization antibodies to JEV. HI and neutralization tests on horse sera indicated that the risk to alphavirus infections was minimal in horses surveyed from Java. However, there was a high risk of JEV infection among the same population.

INTRODUCTION

Arboviruses are transmitted from infected to susceptible vertebrates by arthropods, and are responsible for causing apparant and inapparant infections in human and animals (Bres, 1986). There are more than 500 viruses in this group which are divided into 5 viral families: Togaviridae (Alphaviruses), Flaviviridae, Bunyaviridae, Reoviridae and Rhabdoviridae (Miller, 1993). Viruses can be serologically distinguished between the two families, although there is often serologic crossreactivity. The hemagglutination-inhibition (HI) assay is the standard serology used in detecting antibody to arboviruses. While HI can differentiate between viral families, it often can not differentiate between members within a family such as Japanese encephalitis virus (JEV) and dengue virus. More specific assays, such as the neutralization test, are needed to differentiate between viral family members.

Evidence exists for the presence of several arboviruses in Indonesia. Alphaviruses such as Chikungunya (Chik) and Ross river have been detected serologically in animals on the island of Lombok (Olson *et al*, 1983). Serological evidence for flaviviruses (*eg* Sepik, Kunjin, Murray valley encephalitis, Zika, and JEV) has also been found in animals on Lombok. JEV, the most important mosquito-borne human pathogen in countries such as Japan, China and Korea, has been isolated from the sera of sentinel pigs and from *Culex* mosquito collected at pig- raising areas in West Java (Van Peenen *et al*, 1975). The only study of Indonesian horses reported HI and neutralizing antibodies against alpha and flaviviruses in horses on Lombok. Horses were reported to have antibodies to JEV and Zika as measured by HI. In addition, neutralizing antibodies against JEV, Kunjin and Sepik were detected in those horses.

Many of these viruses pose a threat to Indonesians, yet little is known about the role of horses in the transmission cycle since there has been only one such investigation from 10 years ago. The present study was designed to measure the prevalence of antibodies against arboviruses in horses from Java, in order to assess the threat to horses and determine their potential role in arbovirus transmission.

MATERIAL AND METHODS

Samples were collected from horses at two stables. Pulo Mas is a racing track-horse complex, located in a residential area in North Jakarta. Pamulang is a riding school, located in West Java,

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approximately 10 km from South Jakarta in a rural environment.

Horses were divided into 2 groups: locally bred horses, which included all horses born in Indonesia, and imported horses which were all thoroughbreds (Thb). Of the 112 horses tested, 89 horses were locally bred and 23 were imported from various countries. At Pulo Mas, only five (9%) of 62 horses were imported, but 18 (36%) of 50 Pamulang horses were imported.

Blood samples were collected in November 1989. Each blood sample was allowed to clot at 4°C, centrifuged at 1,500 rpm for 10 minutes, serum separated and stored at -20°C until assayed.

Sera were tested by the HI assay for antibodies to JEV and Chik using the standard procedures of Clark and Casals (Clark and Casals, 1958). Modified for microtechnique (Sever, 1962). Horses with antibody titers of 1 : 10 or greater were considered to be positive. Sera having antibody to JEV as detected by HI were tested by the plaque reduction neutralization test (PRNT) similar to previously procedures (Earley *et al*, 1967). Briefly, serial fourfold dilutions of serum were incubated with 50 plaque-forming units (pfu) of JEV for 1 hour at 37°C in a 5% CO_2 environment. Residual virus was quantitated by adsorbing 50 µL of each dilution onto BHK-21 (Baby hampster kidney) cells in 24-well plates (2 wells/dilution) for 1 hour. The plates were then overlaid with carboxymethylcellulose (Sigma). After 72 hours, the cells were stained using napthol blue black solution (0, 1%) and an 80% reduction in the number of plaques was used as the end point for neutralization titers. A titer of 1 : 10 or greater was considered positive.

RESULTS

The prevalence of antibody against the two arboviruses as measured by HI is shown in Table 1. Overall, antibodies were demonstrated in 58 out of 112 (52%) horses screened for JEV and in 11 (10%) against Chik. The proportion of Pamulang horses infected with JEV (66%) was significantly higher than found among Pulo Mas horses (44%) screened ($p < 0.01$). However, there was no significant difference ($p > 0.05$) in the proportion of Pulo Mas horses (8%) infected with Chik compared with Pamulang horses (12%).

The reciprocal titer of all horses with antibodies ranged from 10 to 40. The only exception was one Pamulang horse with a titer of 80 to JEV (Table 2). The geometric mean HI reciprocal titer for JEV was 15.4. In addition, although the number of horses with antibodies to Chik was small, the reciprocal geometric mean titer of those horses to Chik (18.7) was comparable to the mean titer for JEV.

As shown in Table 3, regardless of whether locally bred or imported as thoroughbreds, there was no significant ($p > 0.05$) difference in JEV antibody prevalence between horses screened from Pulo Mas and Pamulang. Similarly, the prevalence of Chik did not vary significantly by location (Pulo Mas and Pamulang), among either imported or locally bred horses.

The prevalence of antibodies to JEV among imported horses screened from Pulo Mas (100%) was significantly higher than for those locally bred (35%) ($p < 0.05$). In contrast, locally bred horses at Pamulang were more likely to have antibodies (75%)

Table 1

Prevalence of HI antibody to JEV and Chik in horses screened in Indonesia.

	Pulo Mas	Pamulang
JEV	25/62* (40%)	33/50 (66*)#
Chik	5/62 (8%)	6/50 (10%)

* Number of horses antibody positive/Number of horses tested

# $p < 0.01$

Table 2

Reciprocal HI titer of horses at Pulo Mas and Pamulang.

HI titer	JEV	Chik
< 10	54*	101
10	29	3
20	23	6
40	5	2
80	1	0

* Number of horses with the respective titer

Table 3

Prevalence of HI antibody to JEV and Chik in imported and locally bred horses.

	Imported		Local	
	Pulo Mas	Pamulang	Pulo Mas	Pamulang
JEV	5/5* (100 %)	9/18 (50 %)	20/57 (35 %)	24/32 (75 %)
Chik	0/5 (0 %)	5/18 (28 %)	5/57 (9 %)	0/32 (0 %)

* Number of horses antibody positive/Number of horses tested

Table 4

Prevalence of HI antibody to JEV and Chik in horses at Pulo Mas and Pamulang.

	Pulo Mas		Pamulang	
	Imported	Local	Imported	Local
JEV	5/5* (100 %)#	20/57 (35 %)	9/18 (50 %)	24/32 (75 %)# #
Chik	0/5 (0 %)	5/57 (9 %)	5/18 (28 %)	0/32 (0 %)

* Number of horses antibody positive/Number of horses tested
# $p < 0.05$
# # $p < 0.001$

than imported thoroughbreds (50%) ($p < 0.001$). Interestingly, all of Pamulang horses which had antibodies to Chik were imported horses (Table 4).

The age distribution of horses with HI antibodies to JEV and Chik are shown in Fig 1 and Fig 2. The imported horses at Pulo Mas, all with antibody to JEV, were in the 6 years and older age groups. At Pamulang, significant differences ($p < 0.05$) in prevalence values by age were detected in the proportion of locally bred and imported horses in the 3-5 years old category for both JEV (75% *vs* 29%) and Chik antibody (43% *vs* 0%).

Sera with antibody to JEV as measured by HI were further analyzed by PRNT in order to more

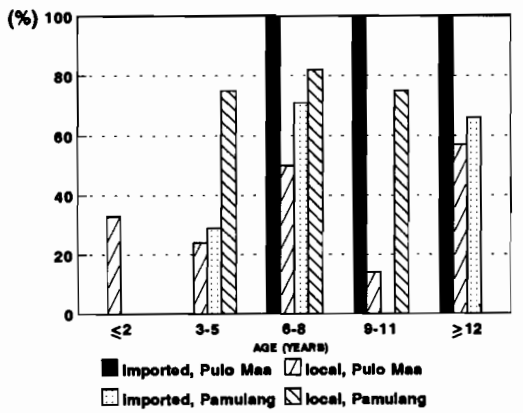

Fig 1–Age distribution of horses with HIV antibody to JEV, by location and thoroughbreed status.

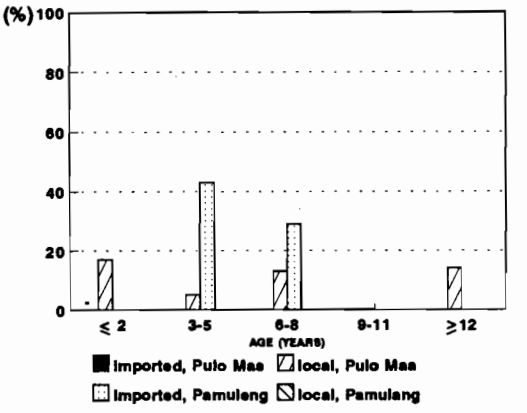

Fig 2–Age distribution of horses with HI antibody to Chik, by location and thoroughbreed status.

accurately determine the number of horses with true JEV titers. Of the 58 horses with JEV antibodies by HI, 52 (90%) were found to have specific neutralization antibodies to JEV. The geometric mean neutralizing titer for JEV was 75.6. The six horses which were negative by the neutralization assay, all had HI reciprocal titers of 10.

DISCUSSION

Arboviruses are known to cause illness in both humans and domesticated animals. This report describes the prevalence in horses of antibodies to two arboviruses known to occur in Indonesia: Chik and JEV. Although Chik is not known to cause illness in horses, other serologically crossreactive alphaviruses, *eg* Getah virus, are known to cause disease in horses (Kono, 1986). Getah virus causes fever, rash and/or edema of the limbs in horses. Unfortunately, specific serological tests for those viruses are not presently available in Indonesia.

In this study, only 10% of horses had antibodies to Chik. The fact that all of the imported horses at Pamulang had antibody to Chik, but none of locally bred horses, suggests acquired infection resulted from exposure before they were brought to Indonesia. The lack of antibodies to alphaviruses indicates that the risk to horses of infection with such viruses is minimal in Java, and therefore veterinarians should not give them high priority on a differential list for unknown illness. In addition, health officials should not consider horses as a likely reservoir for human alphavirus infections.

Conversely, the study found that over 50% of the horses had antibodies to JEV. High seroprevalence rates among horses have also been reported from other countries like Thailand and Japan (Burke and Leake, 1986). The signs/symptoms associated with JEV infection in horses include high temperature, excitement followed by apathy, depression and spastic or flaccid paralysis of the legs. The pathological changes are meningitis and encephalitis (Merchant and Pecker, 1969; Gould *et al*, 1964).

Overall, the proportion of horses with JEV antibodies in Pamulang was significantly higher than those stabled at Pulo Mas. The rural location of the stable at Pamulang and its near proximity to a rice field, also identified as a breeding site of *Culex* mosquitos, contrasted to the more urban setting of the Pulo Mas stable. This finding suggests measured "risk" associated with the more rural setting of Pamulang.

Statistically higher JEV antibody prevalence was found among locally bred horses at Pamulang in the 3-5 year age group compared with their imported counterparts. This finding may reflect greater viral (JEV) exposure among younger horses locally bred at Pamulang. No age specific differences were noted between the two populations age > 5 years.

This report documents, for the first time, the prevalence of antibody against JEV in horses from Java. Previously, JEV has been confirmed through serology and virus isolation in pigs from Indonesia (Van Peenen *et al*, 1974, 1975; Koesharjono *et al*, 1974) and only recently has infection been documented in Javanese cattle (Tan *et al*, 1993). Human clinical JEV infections have also been documented for the first time in Bali (Jennings *et al*, 1974). It remains doubtful that horses serve as representative hosts in the transmission of JEV to human. However, veterinarians should consider JEV infection in horses presenting with encephalitis signs/symptoms and their usefulness as amplifying hosts in general JEV surveillance effect.

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