

## RELATIONSHIP BETWEEN THE PREVALENCE OF ANTIBODIES TO ARBOVIRUS AND HEPATITIS B VIRUS IN THE VALE DO RIBEIRA REGION, BRAZIL.

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### SUMMARY

280 students, between 6 and 14 years old, residents in the Iguape county, southern coast of the State of São Paulo, were studied in order to identify the existence of a possible association between the prevalence of specific antibodies to the hepatitis B virus and the exposure to haematophagous mosquitoes, evaluated indirectly through the prevalence of antibodies to 17 arboviruses isolated in Brazil.

The children were from 4 areas with different topographical characteristics: 89 of the children were from the urban zone of the town of Iguape, 89 were from the peri-urban zone, 30 were from the rural area with extensive banana plantations, and 72 were from the jungle zone. Previous studies had shown significantly higher prevalence of antibodies to different arboviruses in the cultivated zone and the jungle zone, when compared to the urban and peri-urban zones of Iguape. The detection of antibodies to the HBV surface antigen (HBs Ag) was done through the radioimmunoassay (Ausab, Abbott Laboratory). The cases considered positive were confirmed through the presence of anti-core HBV antibodies (anti HBc EIA Roche).

A significantly higher prevalence of anti HBV antibodies was observed in children from the jungle zone (26/72 = 36.1%) when compared to those from the urban zone (5/89 = 5.6%), peri-urban (6/89 = 6.7%) or from the cultivated zone (0/30 = 0%).

The result suggest the existence of a common factor in the dissemination of the arboviruses and the hepatitis B virus, supporting the hypothesis that mosquitoes may play an important role in the HBV transmission in tropical forested region.

**KEY WORDS:** Hepatitis B transmission; Hepatitis B seroepidemiology. Arbovirus and Hepatitis B

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### INTRODUCTION

The higher prevalence of hepatitis B virus (HBV) markers in human populations in tropical regions<sup>3, 34</sup> suggested that, along with conventional transmission mechanisms observed in

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other parts of the world, additional modes of transmission might be present in the tropics.

The high prevalence in some of these regions of diseases transmitted by haematophagous arthropods and the low frequency of blood transfusion or injections among their inhabitants, raised the first suspicion of HBV transmission via mosquitoes and other insects as reported by BLUMBERG et al.<sup>4</sup>. Detection of HB Ags in various species of mosquitoes and other haematophagous arthropods captured in tropical regions<sup>6, 8, 17, 27, 35, 40, 41, 42</sup> and even urban areas of the United States<sup>13</sup>, has somehow corroborated this hypothesis. Experimental studies showed that when various species of mosquitoes<sup>2, 6, 7, 28, 30, 31, 38</sup> and other haematophagous arthropods<sup>12, 26, 31, 37, 39, 40</sup> were fed blood containing HBs Ag, it was possible to detect this marker in mosquitoes for approximately up to 4 days, in most of the studies, and for periods up to several weeks in the other arthropods. It was observed, however, that the HBs Ag disappears once the blood is fully digested, indicating that no viral replication occurred in the animal<sup>6, 7, 31</sup>.

According to these results, infections of HBV via arthropods would be the result of mechanical transmission. In this mode of transmission, which was already been described for several types of plant and animal viruses, such as the myxoma virus in rabbits and certain arboviruses<sup>11</sup>, the virus would remain viable in the structure of the sucking apparatus of the arthropods and might be transmitted passively from a contaminated host to a susceptible one, of the refeeding time for the mosquito, or when it completed a feeding which had been interrupted<sup>5</sup>. As is the case with certain viruses such as the Venezuelan equine encephalitis<sup>11</sup> or Rift Valley fever viruses which reach very high concentration in blood; the likelihood of mechanical transmission of HBV, which is highly infectious and highly resistant to inactivation<sup>36</sup>, appears possible.

This mechanical transmission should occur more frequently in tropical regions, where there is intensive the activity of haematophagous arthropods.

A seroepidemiologic survey to HBV, carried out in São Paulo State, Brazil<sup>29</sup>, demonstrated

an unusually high prevalence of anti-HBs among children living in a camping area near Serra do Mar, a rural area near the coastal region of the State. The study showed a prevalence of 31.2% (10/32) among children whose age ranged from 1 to 5 years, and 48.7% (19/39) among those in the 6 to 11 years group. These children were of low socioeconomic status. However, among the adult population of low socioeconomic status living in the urban area of the city of São Paulo, the prevalence of anti-HBs was only 14.58%<sup>29</sup>, suggesting that factors other than the socioeconomic status determined, the high prevalence of immunological markers to HBV in this locality.

In order to test the hypothesis that wild culicidae might be involved in HBV transmission, a seroepidemiologic survey to the HBV was conducted in a region with similar ecological characteristics, namely the Vale do Ribeira, which is also included in the so called Serra do Mar System. This area is characterized by an abundant Culicidae fauna<sup>15, 16</sup>. Since 1977 the Department of Epidemiology of the Public Health School of the University of São Paulo, in collaboration with Evandro Chagas Institute, has conducted seroepidemiological surveys for arboviruses in the human population. The prevalence of arbovirus antibodies should thus provided an indirect indicator of the risk of infections transmitted by mosquitoes. The prevalence of immunological markers of the HBV should be correlated with that of arbovirus antibodies.

## MATERIAL AND METHODS

**THE AREA.** The serological survey was carried out in Iguape, one of the counties of the Vale do Ribeira, located in the coastal area of the State of São Paulo, southeast Brazil, in a region still extensively covered by forests.

In 1976 an epidemic of arbovirus encephalitis resulted in a morbidity index of 717.5/100.000 inhabitants in Iguape<sup>21</sup>. Seroepidemiological studies showed that arbovirus as belonging to the **Togaviridae** and **Flaviviridae** families, pathogenic for human<sup>22-25</sup>, are circulating in the region.

Within the limits of the county, four areas with different topographical and phytological

characteristics, were studied. These areas, have been described in details previously<sup>25</sup>. They are briefly:

a) Urban Zone: including Iguape, a town located in the coast and a health resort in a large neighbouring island, which presents urban structure;

b) Peri-Urban Zone: Rocio District, in the outskirts of Iguape, with rural characteristics, poor houses sparsely located among the vegetation; grass and small bushes predominate.

c) Cultivated Zone: in the coastal plains, with a few areas of residual forest. Extensive planting of bananas, and, more recently, of vegetables and grain, is carried out.

d) Jungle Zone: still extensively covered by forests, made up of tall trees, dense foliage and high degree of heterogeneity, where the utilization of land agricultural and cattle-raising purposes is still limited.

#### CASE STUDY

In the 1981-1982 period, blood samples were collected for an arbovirus seroepidemiological study from 337 students (6 to 14 years old) from Iguape county. These children proved to have different prevalences of HI antibodies to 17 arbovirus isolated in Brazil, according to the locality where they lived. Among these 337 sera, preserved at -20°C, 280 presented an enough amount that permitted the research of HBV immunological markers. The 280 children resided in different areas: 89 of them lived in the Urban Zone; 89 in the Periurban Zone, 30 in the Cultivated Zone and 72 in the Jungle Zone. The majority of the children (70.1%) had lived all their lives in the place at which they were residing at the time of the study. Only 2.8% had lived there for a period of less than one year. The 280 children belonged to 233 different families. In 33 families two or more children were examined.

Socioeconomic status was estimated on the basis of the father's profession, and proved to be low, with no significant difference between the areas of residences, since 71(30.5%) of the fathers were agricultural workers, 58(24.9%) were

manual laborers, 27(11.6%) were fishermen, 27(11.6%) were low level public servants, 13(5.6%) were clerks in commercial establishments, and 4(1.7%) were elementary schoolteachers. In 33 cases (14.2%) this information was not available. As to sex, 51.7% (135/261) were male and 48.3 (126/261) were female. In 19 cases, this information was not available.

Anti-HBs Ag was determined by radioimmunoassay (Ausab-Abbott Laboratories).

The positive sera were tested to anti-HBc antibodies by an immunoenzymatic technique (Roche Laboratory). To ensure accuracy in the results, only cases where both markers were present were considered positive. In schoolchildren from the Cultivated Zone anti-HBc antibodies were measured in all sera.

#### RESULTS

Of the 280 sera studied, 37 (13.2%) were positive for anti-HBs. A prevalence of 27/71 (38.0%) was presented by children of farm workers, 3/27 (11.1%) by children of fishermen and 7/135 (5.2%) by children whose fathers worked at others activities. As to sex there was no significant difference ( $p > 0.05$ ) between prevalence in male (12.6%) and female (15.1%). In one of the positive cases, this information was not available. As to age distribution, no statistically significant difference ( $p > 0.05$ ) was observed in the prevalence of anti-HBs Ag antibodies among the groups of 6 to 8 years (11.8%), 9 to 11 years (13.3%) and 12 to 14 years (16.0%).

The prevalence rates of anti-HBs Ag and arbovirus antibodies according to the four different geographic areas considered may be seen in Table 1. In the Jungle area, the rate of sera with positive anti-HBs was 36.1% (26/72), significantly higher than other zones ( $p < 0.001$ ). In the three localities of this Jungle area, prevalence was 36% (9/25) in the Pavia district, 37.8% (14/37) in the Pé da Serra district, and 30% (3/10) in the Despraiado district.

None of the cases from the Bananal Zone presented anti-HBc or anti-HBs antibodies.

When we only considered the families where no more than one child was studied, the anti-

HBs prevalences were of 26.7% in the Jungle Zone, 8.2% in the Periurban Zone and 6.1% in Urban Zone showing a significant difference between Jungle Zone and Urban and Periurban Zones ( $\chi^2 = 10.5050$   $p < 0.01$ ).

## DISCUSSION

There are significant differences in the prevalence of HBs antibodies according to the geographic area considered. The prevalence of

TABLE 1  
Prevalence of HBs Ag antibodies (anti-Ags HB) and HI antibodies against 17 arbovirus in school children, aged 6 to 14 years, for different areas of Vale do Ribeira, São Paulo, Brazil.

Geographic area	Nº tested	Nº positive (%) Anti-HBs Ag	Nº positive (%) Anti-arbovirus
Urban Zone (Iguape and Ilha Comprida)	89	5 ( 5.6% )	2 ( 2.2% )
Periurban Zone (Rocio)	89	6 ( 6.7% )	3 ( 3.4% )
Cultivated Zone	30	0 ( 0.0% )	7 (23.3% )
Jungle Zone	72	26 (36.1% )	13 (18.0% )
Total	280	37 (13.2% )	25 ( 8.9% )

$\chi^2 = 36.2883$   $p < 0.001$

36.1% in children from 6 to 4 years old, the vast majority of whom (73%) were children of farmers who lived in the Jungle Zone, is unusual, both in relation to children from other areas of the present study and in relation to the rates observed in the adult population of low socioeconomic status in the city of São Paulo, where the prevalence of anti-HBs antibodies was 14.58%<sup>29</sup>. The rates were similar to those of high prevalence (48.7%) observed in 6 to 11 year-old children living in the Serra do Mar region<sup>29</sup>, also in the southern coast of São Paulo State, which presents climatic and phytological conditions similar to those of the Vale do Ribeira.

In the present study, the children had a homogeneous socioeconomic status. Also, there were no significant differences as to sex and age of those infected by HBV ( $p > 0.05$ ). Thus, it is probable that a factor linked to the geographic area could be influencing the larger dissemination of the HBV in the Jungle Zone. The fact that similar rates were found in three different localities of the Jungle Zone somehow removes the possi-

bility of a local epidemic of infection by HBV, since these populations have almost no contact at all among themselves. Besides even we exclude 33 families where two or more children were studied (15 from Jungle zone), the prevalence of positive sera to anti-HBs antibodies remains significantly higher in the Jungle Zone than other areas.

Previous study, carried out among these very same children, also detected a clear pattern in the prevalence of HI antibodies to 17 arboviruses in Brazil, depending on the geographical area under consideration<sup>25</sup>. The prevalence of arbovirus antibodies was significantly higher among those living in the rural zone (Jungle and Cultivated Zones) than observed among children living in the Urban and Peri-Urban Zones<sup>25</sup>.

The results suggest the existence of a common factor in the dissemination of VHB and of the arboviruses in forested areas, one of the possibilities being that of transmission by means

of anthropophilic mosquitoes, extremely abundant in that region<sup>15, 16</sup>.

Nevertheless, the transmission mechanisms for both viruses would have totally different characteristics. Thus, in relation to the arboviruses, there is a continuous maintenance cycle of these agents in the forest, with participation of wild vertebrates, especially mammals and birds, and of haematophagous mosquitoes in which the virus multiplies. When humans enter this ecosystem, they face a high probability of becoming infected. The HBV, on the other hand, would be mechanically transmitted by haematophagous arthropods; this requires, besides the high density of these arthropods, exogenous introduction of the virus by infected humans. This might occur in one area and not in another, even though the second area might be just a few kilometers away from the first, due to the fact that this transmission depends on the degree of dispersion of the mechanical vectors. Furthermore, there are no known animal reservoirs to HBV except man. The association between the prevalence of arbovirus antibodies and anti-HBs, which seem to be directly proportional (the higher the prevalence of anti-arbovirus antibodies, the higher the prevalence of anti-HBs Ag antibodies), is sharply interrupted in the Cultivated Zone, the very place of highest prevalence of arbovirus antibodies.

A reasonable explanation to this discrepancy (0% of anti-HBs and 23,3% of arbovirus antibodies) would be the total absence of HBV carriers in the Cultivated Zone. The total absence of HBV markers among children in this zone corroborates this hypothesis. Should HBV be introduced in this locality, the expectation would be for an infection prevalence comparable to that of Jungle Zone since in the open areas of the extraforest environment of Iguape there is a high frequency of different species of Culicidae<sup>15, 16</sup>. Additional research directed to this area becomes necessary. In the Urban and Peri-Urban Zones of the town of the Iguape, the epidemiological behavior would be equivalent to that observed in the Urban Zone of the city of São Paulo.

Other studies have attempted to establish a correlation between HBV markers and the presence of mosquitoes<sup>1, 14, 20, 32</sup>; the results are con-

flicting. However, the different conditions under which these studies were carried out and the different techniques employed for the detection of HBV immunological markers make the comparison of the results difficult.

Unusually high prevalences of HBV immunological markers have been consistently observed among Indian tribes in the western Amazon area, varying, in the adult individuals from 60,7% to 100%<sup>9, 10, 18, 19</sup>. As for inhabitants of the rural zone in the Vale do Ribeira these populations live in relative isolation, in close contact with the forest. High rates of infection by arbovirus, such as Mayaro, have also been observed in the Amazon region<sup>33</sup>. Even if there is a wide gap between the Indians of the Amazon regions and the inhabitants of the Vale do Ribeira area, both populations have something in common: they both live in close contact with the forest environment and its fauna. The present study seems to support the hypothesis that mosquitoes may play an important role in the transmission of hepatitis B in the tropical forested regions. However, further research becomes necessary to shed further light on the matter.

## RESUMO

### Relação entre prevalência de anticorpos para arbovirus e virus de hepatite B na região do Vale do Ribeira, Brasil.

280 escolares de 6 a 14 anos de idade, residentes em Iguape, Vale do Ribeira, sudeste do Estado de São Paulo, foram estudados com o objetivo de verificar possível associação entre a prevalência de anticorpos para hepatite B e a exposição a mosquitos hematófagos, avaliada indiretamente pela prevalência de anticorpos de arbovirus. As crianças eram originárias de 4 áreas com características topográficas e fitográficas diferentes: 89 residiam em área urbana, 89 em área periurbana, 30 em área de cultivo extensivo de banana e hortaliças e 72 em área florestal. Estudos prévios mostraram significante maior prevalência de anticorpos de arbovirus nas áreas cultivada e florestal do que nas áreas urbana e periurbana. A pesquisa de anti-HBs foi feita por radioimunoensaio (Ausab, Laboratório Abbott) e de anti-HBc por ensaio imunoenzimático (Roche). Observou-se que os escolares residentes

na área florestal apresentaram mais alta prevalência (26/72 = 36.1%) de anticorpos para hepatite B de que os residentes nas áreas urbana (5/89 = 5.6%), periurbana (6/89 = 6.7%) e cultivada (0/30 = 0%). Os resultados sugerem a existência de fator comum na transmissão de arboviroses e de hepatite B, apoiando a hipótese que nas regiões tropicais com presença de mata mosquitos possam desempenhar importante papel na transmissão da hepatite B.

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