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PREVALENCE OF ANTIBODIES TO DENGUE VIRUS, HEPADNAVIRUS AND ROTAVIRUS IN NON-HUMAN PRIMATES

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ABSTRACT. The aim of this study was to determine the prevalence of several viral antibodies in non-human primates from two zoological gardens from Venezuela. Only two out of 66 sera were positive for antibodies to dengue virus by hemagglutination inhibition. Six out of 62 sera exhibited antibodies against Hepatitis B virus (HBV) core antigen. Viral DNA was detected by nested PCR in one positive serum and in three negative without serological evidence of HBV infection. Sequence analysis showed the circulation of HBV genotype F, predominant in Venezuela. Antibodies against rotavirus antigens were found in 87% (20/23) of Old World primates and in 50% (13/26) of New World primates. Both the prevalence of antibodies and the mean O.D. value by ELISA were significantly lower in New World primate sera. These results suggest that non-human primates do not seem to represent an important reservoir for dengue virus infection, highly endemic in Venezuela. Anthropozoonotic infection of HBV seems to occur among primates not only from the Old but also from the New World, reinforcing the importance of vaccination of species at risk. This study also suggests a lower frequency of infection by rotavirus of non-human primates from the New World, compared to primates from the Old World. **Key words:** monkeys, virus, zoonosis, flavivirus, hepatitis B virus, rotavirus, New World.

SEROPREVALENCIA DE VIRUS DENGUE, HEPADNAVIRUS Y ROTAVIRUS EN PRIMATES NO HUMANOS

RESUMEN. En este estudio se determinó la prevalencia de anticuerpos contra varios virus en primates no humanos de dos parques zoológicos de Venezuela. Sólo dos de 66 sueros fueron positivos, por inhibición de la hemaglutinación, para anticuerpos contra virus dengue. Seis de 62 sueros presentaron anticuerpos contra la cápside del virus de la hepatitis B virus (VHB). Se detectó el ADN viral, mediante PCR en dos rondas, en uno de éstos y en tres sueros sin evidencia serológica de infección por VHB. El análisis de la secuencia mostró la circulación del VHB genotipo F, predominante en Venezuela. Un 87% (20/23) de los sueros de primates del Viejo Mundo y un 50% (13/26) de los del Nuevo Mundo mostraron anticuerpos contra antígenos de rotavirus. Tanto la prevalencia de anticuerpos como los valores promedio de D.O. por ELISA fueron significativamente menores en sueros de primates del Nuevo Mundo. Los primates no humanos no parecen jugar un papel importante como reservorio de la infección por virus dengue, altamente prevalente en el país. La infección por cepas humanas del VHB en primates sugiere infección antroponótica y la importancia de vacunar las especies a riesgo. Los resultados sugieren igualmente una menor frecuencia de infección por rotavirus en primates del Nuevo Mundo. **Palabras claves:** monos, virus, zoonosis, flavivirus, virus de la hepatitis B , rotavirus, Nuevo Mundo.

INTRODUCTION

Non-human primates are the closest mammalian relative to humans and can harbour pathogens that might infect humans through zoonotic transmission. Close contacts of human with non-human primates in some rural communities and in zoological gardens might represent a public health problem. The best example of this threat is the origin of HIV-1 and HIV-2, for whom the occurrence of at least seven zoonotic transmissions have been proposed[9]. In addition, this risk needs to be assessed if considering these animals as candidates for xenotransplantation. On the other hand, many non-human primates are endangered species that might be at risk of disease acquisition by anthropozoonotic transmission.

Flaviviruses belong to the *Flaviviridae* family and include important human pathogens like dengue, yellow fever, Japanese, Saint Louis and tick-borne encephalitis, and West Nile viruses. Most of the members of the Flavivirus genus are transmitted by arthropods [19]. Non-human primates have been described as reservoir or may be susceptible to infection by several flaviviruses [5, 11, 18, 21, 23, 25, 26, 30]. In the Old World, non-human primates have been described as reservoir of Yellow fever and dengue viruses, while monkeys have not been described in the New World as reservoir for dengue virus, although a systematic survey has not been performed [25].

Around 2 billion persons have been infected by hepatitis B virus (HBV) and around 350 millions of them are chronic carriers [31]. A significant group of these chronic carriers may progress to cirrhosis and hepatocellular carcinoma. HBV is a DNA virus and the prototype member of the family *Hepadnaviridae*, where eight human genotypes (A-H) and several simian genotypes closely related to human isolates have been described [1,24]. Hepadnavirus indigenous to woolly monkey (WMHBV) is the only new world monkey hepadnavirus described so far, and is more closely related to American genotypes of the HBV (F and H) than to other human HBV genotypes (A, B, C, D, E and G)[15]. HBV genotype F is the most divergent of the human HBV genotypes, is autochthonous to South America and highly predominant in Venezuela [3].

Rotavirus is the most common cause of acute non-bacterial gastroenteritis in humans. Rotavirus infects nearly all children by 5 years of age and leads to an estimated 2 million hospitalizations and over 400,000 deaths worldwide each year [14]. More than 10 serotypes and genotypes of rotavirus have been described[14]. Two rotavirus strains, RRV and SA11, were originally isolated

from monkeys and are strains widely used in many laboratories throughout the world. However, the role of rotavirus in diarrheal disease in non-human primates has not been well established.

The aim of this study was to determine the prevalence of infection or exposure to these important viral agents in non-human primates from two zoological gardens from Venezuela and to assess their implications in animal health and conservation.

MATERIALS AND METHODS

Primate sera were collected for routine clinical analysis in two zoological gardens from Venezuela: Parque Zoológico Bararida, Barquisimeto (n= 41), and Parque Zoológico El Pinar, Caracas (n=13). The sera collection included new world monkeys: *Cebus apella*, *Lagothurix lagothricha*, *Alouatta seniculus*, *Ateles belzebuth*, *Aotus lemurinus* and *Saimiri* (squirrel monkey) and old world monkeys: *Cercopithecus*, *Chlorocebus aethiops*, *Papio hamadryas*, *Pan troglodytes* and a prosimian *Lemur catta*. Simians from the genus *Alouatta* are free-living in the Bararida Park. In addition, eight sera from *Allouatta* seniculus were obtained from free-living animals from Guri dick, Venezuela, and four sera from *Cebus apella* from Argentina.

Antibodies to dengue viruses were determined by hemagglutination inhibition, using dengue virus 2 Infected mouse brain extract as antigen, at an optimal pH for hemagglutination of 6.4 [4]. Human sera positive and negative for anti-dengue antibodies were used as control.

Antibodies to HBV core antigen were determined by commercial immunoassays (DIMA Diagnostika, C.A., Guarenas, Edo. Miranda, Venezuela). Sera were also tested by nested PCR for the presence of HBV DNA, using highly conserved primers from the core region, as previously described [8]. HBV DNA was retested in a second serum of HBV DNA positive primates in order to confirm the viremia.

For sequence analysis, purified PCR fragments were sequenced using dye terminator labelling method (ABI PRISM™ Dye terminator Cycle Sequencing ready reaction Kit; Perkin Elmer; Foster; CA) with 377 DNA sequencer (Applied Biosystems, Foster, CA). Both strands of DNA were sequenced. Nucleotide alignments and phylogenetic analyses were performed using DNAMAN Version 5.2.2. (Lynnon Bio Soft, Canada). Phylogenetic trees were obtained using the Neighbor Joining Method (100 bootstrap replicas). Genetic distances were evaluated with Kimura 2 parameters corrections. Nucleotide sequence data have been deposited into the GenBank database under the accession numbers AY861665- AY861668.

Total immunoglobulin antibodies to rotavirus were determined by ELISA, using purified 5 µg/ml rotavirus double shelled particles from OSU strain, as described previously [22]. Since the inner capsid protein VP6 shares common epitopes among the different strains of group A rotavirus, this ELISA ensures the detection of antibodies against almost any strain of the most abundant gorup of rotavirus [14].

Statistical significance was assessed by the chi square test with Yate's correction, according to a computerized Epi Info program, version 5.01b (Centers for Disease Control and Prevention, Atlanta, GA, USA). Mean O.D. were compared using Student's T test (http://www.physics.csbsju.edu).

RESULTS

In order to evaluate the exposure to dengue viruses, rotaviruses and hepadnaviruses, sera from non-human

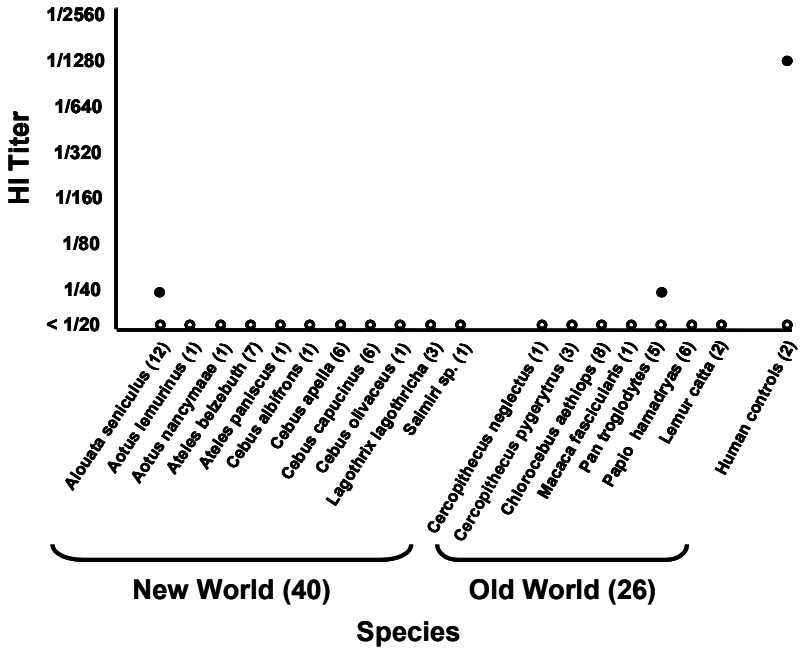

Figure 1. Antibodies to dengue virus, determined by hemagglutination inhibition. Black circles represent negative sera and black filled circles positive ones.

primates were tested for the presence of antibodies against these viral pathogens. A total of 66 sera were screened for antibodies against dengue virus by hemagglutination inhibition. Only two sera exhibited titres of 1/160, one from *Alouatta seniculus* and one from *Pan troglodytes*. These titres were significantly lower than the one found in the human control sera (1/1280) (Figure 1).

Six out of 62 sera exhibited antibodies against HBV core antigen. HBV DNA was detected in one of these six sera. In parallel, 46 sera were tested for the presence of HBV DNA, irrespective of their anti-HBc status. Three anti-HBc negative sera, exhibited HBV DNA, which could be amplified by using highly conserved primers from the core region, for a total of four primate sera positive for HBV DNA. HBV DNA was amplified for the surface antigen region in one out of these four sera. None of these primate sera, positive for HBV markers, exhibited signs of hepatitis infection. Sequence analysis showed that all the HBV non-human primates isolates were very closely related to human Venezuelan strains of genotype F, clades II and III (Figure 2).

A total of 45 sera were tested for anti-rotavirus antibodies by ELISA. Anti-rotavirus antibodies were found in 16/19 (84%) primates from the Old World, compared to 13/26 (50%) primates from the New World, being this difference significant (p= 0.04) (Figure 3). The mean O.D. obtained with the positive sera from New World primates (0.381) was also lower than the one obtained with positive sera from Old World primates (0.897, p<0.001) or with human positive controls (1.061), suggesting lower titers of antibodies in the former. In order to verify that the anti-human conjugate used in the ELISA test was less efficient for recognizing antibodies present in the sera of New World primates compared to the sera of the Old World, sera were directly absorbed to the ELISA plates and then the anti-human conjugate was added to the wells. As it can be seen in Figure 4, although the O.D. observed at different dilutions of non-human primate sera were lower than the ones observed with human sera, similar O.D. were observed when using sera from Old World primates or New World primates, particularly at the 1/200 dilution, which was the dilution used for testing anti-rotavirus antibodies.

DISCUSSION

Dengue virus infection is highly endemic in Venezuela since 1989. However, the non-human primates sera analyzed from two different cities from Venezuela did not exhibit evidence of exposure to this virus. These results

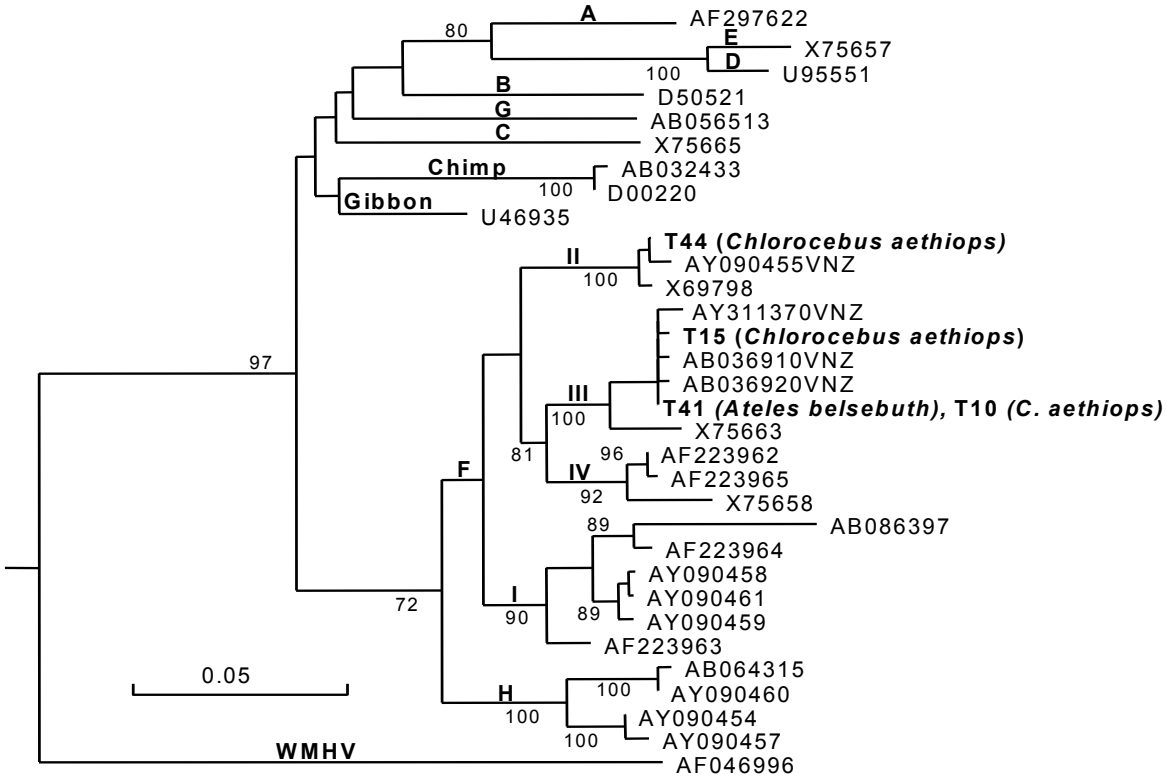

Figure 2: Phylogenetic analysis of HBV primate DNA in the core region (324 nt), according to the Neighbor Joining Method (100 bootstrap replicas). Genetic distances were evaluated with Kimura 2 parameters corrections. Bootstrap values over 70% are shown in the tree for the branching in genotypes. The scale represents the number of substitutions/site/100 bases. Letters in bold indicate genotype. Names in bold correspond to primate isolates, while the other isolates are named according to their accession number in the GenBank. Roman numbers in bold in the phylogenetic trees refer to the HBV genotype F clades. VNZ: Venezuelan isolates.

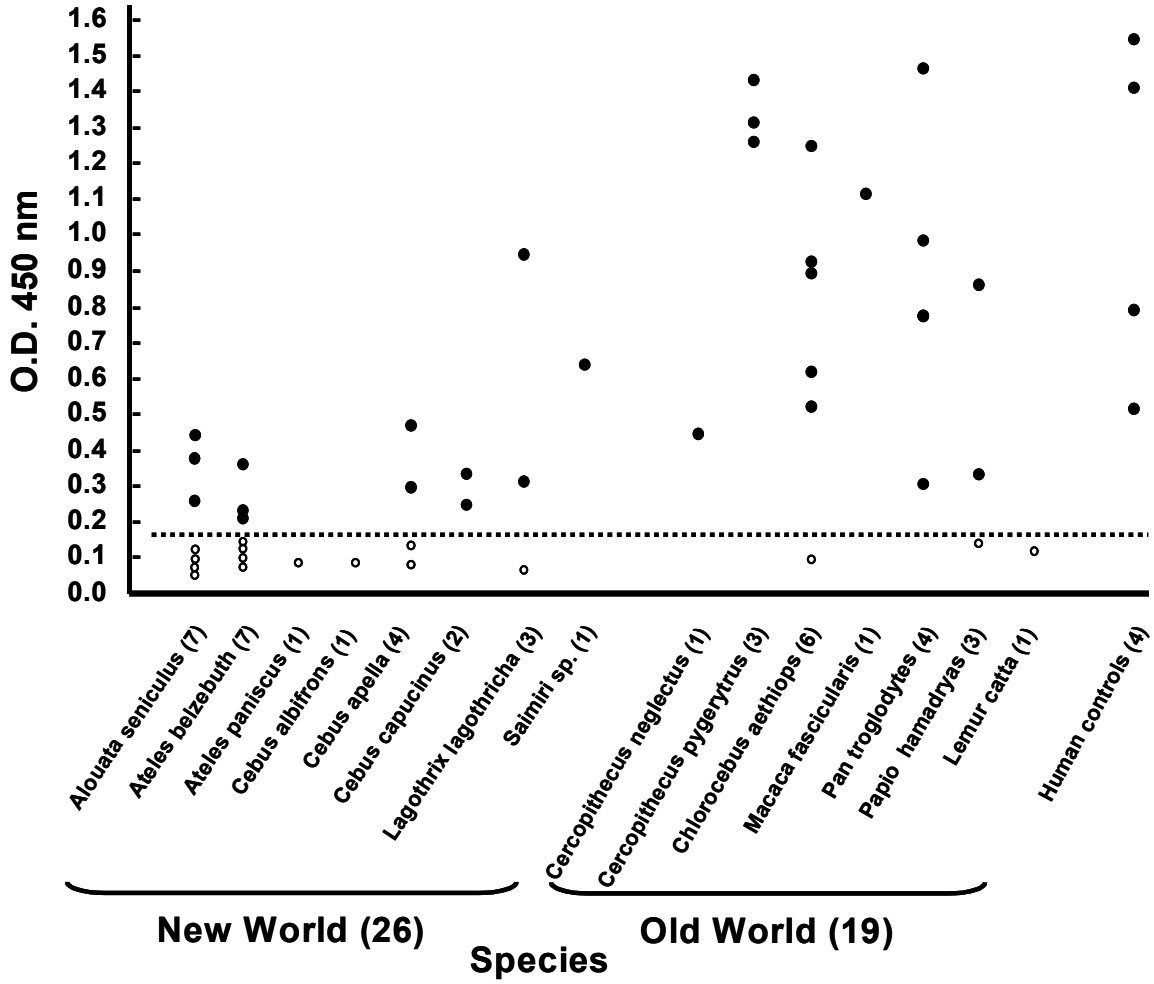

Figure 3. Antibodies to rotavirus, determined by ELISA. Black circles represent negative sera and black filled circles positive ones.

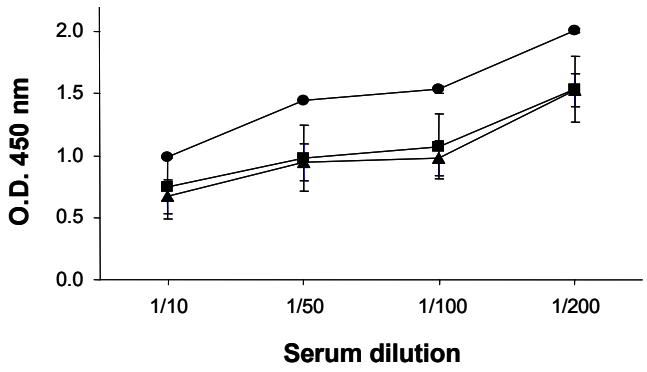

Figure 4: Evaluation of the efficiency of anti-human conjugate for detecting non human primate antibodies. Different dilutions of sera were directly absorbed to the ELISA plates and then the anti-human conjugate was added to the wells. ▲ : sera from New World primates (mean of n=6). ■: sera from Old World primates (mean of n=10). ● : human control sera (mean of n=2). A prozone effect was observed with low dilutions of sera.

are in agreement with the notion that no non-human primate reservoir has so far been described for dengue viruses in the New World [25]. A sylvan cycle has been proposed in Asia and Africa, with distinct dengue virus strains and arthropods, and non-human primates as reservoir [29]. Due to the cross reactivity described for the hemagglutination inhibition test used in this study, it is expected that antibodies to yellow fever virus might be detected by this assay. *Alouatta* spp. is a known reservoir for yellow fever virus in Venezuela. However, infected monkeys are always located in specific geographic paths. This situation might explain why no antibodies were found in sera of Alouatta monkeys obtained from other locations of Venezuela. *Alouatta caraya* primates have shown evidence of exposure to Saint Louis encephalitis virus in Argentina, but not to dengue or yellow fever viruses [5]. These results suggest that monkeys do not seem to represent an important reservoir for dengue virus infections in urban locations in Venezuela, even in a situation of high endemicity. This situation is in contrast to the one observed for West Nile virus in North America, for which evidence

of exposure was found in 36% of captive monkeys in areas with circulation of West Nile virus [23].

HBV infection has been described in non-human primates, particularly among apes, being very less frequent in monkeys [10, 24, 27]. Non-human primates can be infected by human or simian HBV genotypes, being the former probably an anthropozoonotic event. One of the first *Hepadnaviruses* indigenous to non-human primates completely characterized was the Woolly monkey Hepatitis B Virus (WMHBV), which infects a monkey from the New World and is phylogenetically distant to all human and other primates HBV genotypes [15]. *Chlorocebus aethiops* is one of the few species of monkeys which has been shown to be carriers of HBV infection, while Spider monkeys (*Ateles* spp) are closely related to Woolly monkeys and are susceptible to the WMHBV infection [16]. The two isolates sequenced in our study from these 2 species of monkeys were related to genotype F, specifically to the 2 clades inside genotype F which circulates in Venezuela [6]. Three out of the 4 HBV amplified products obtained in this work were from sera negative for anticore antibodies. In all cases HBV DNA was only detected by highly conserved primers in nested PCR. Altogether these results suggest that these monkeys were suffering an anthropozoonotic occult infection, which usually occurs with low levels of viremia [8]. It might be speculated that these non-human primate species are not as permissive for sustaining HBV replication as humans, explaining the low level of viremia and the failure in many of them to produce an evident anticore response. Alternatively, HBV infection may frequently course in non-human primates with atypical serological markers. Persistence of low levels of HBV DNA, even in the presence of neutralizing anti-HBsAg antibodies, has been described in apes [10,17].

HBV genotypes F and H are the most divergent of the human genotypes and segregates separately from the other human genotypes, when simian HBV genotypes are included in the phylogenetic tree (Figure 2) [7]. Experimental transmission of human HBV genotypes A and D to baboons resulted in low level of viremia for both genotypes[2]. It is not known how efficiently non-human primates can be infected with HBV genotype F. The relatively high frequence of anthropozoonotic HBV infection among these captive monkeys suggest that HBV genotype F is readily transmitted to monkeys, although probably with a low efficiency of replication.

The work of Otsyula et al. in 1996 showed by the first time serological evidence of rotavirus infection in some non-human primates from the Old World but not in black mangabeys and black-and-white colobus [20]. Antibodies to rotavirus were found in the sera of all except 3 of the primate species analyzed in our study. For these 3 species only one serum sample was tested (*Ateles paniscus*, *Cebus albifrons* and *Lemur catta*; Figure 3). Differences in the serological test might explain these results. The possibility that New World non-human primates were less susceptible to rotavirus infection was suggested by Jiang et al. [12], in a reduced number of samples and testing only one species. This observation is confirmed in our study in terms of lower prevalence and lower O.D. values by ELISA. It is not know however if New World primates might be infected with antigenically divergent types of rotavirus, reducing the affinity of recognition of the antigen used in the ELISA. However, this hypothesis seems quite improbable, since VP6, the immunodominant protein present in the antigen used in our ELISA, harbours conserved domains which are broadly recognized by antibodies elicited by infection with different serotypes and genotypes of group A rotavirus [14].

Several examples are already known of differences in susceptibility to viruses between non-human primates form the Old and the New World. In some of these examples co-evolution between hosts and pathogens might be playing a role, like for retroviruses and herpesviruses[13, 28]. Similar differences seem to occur also for rotavirus infection among non-human primates.

In conclusion, these results suggest that non-human primates do not seem to represent an important reservoir for dengue virus infection, highly endemic in Venezuela. Anthropozoonotic infection of HBV seems to occur among primates not only from the Old but also from the New World, reinforcing the importance of vaccination of species at risk and of staff attending these animals. This study also suggests a lower frequency of infection by rotavirus of non-human primates from the New World, compared to primates from the Old World. These results warrant further studies in free living organisms to confirm the absence of a non human primate reservoir for dengue virus and the lower susceptibility to rotavirus in primates form the New World.

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REFERENCES

1. **Arauz-Ruiz, P., Norder, H., Robertson, B.H.** and **Magnius, L.O.** Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J. Gen. Virol.,* **83:**2059-2073, 2002.

2. **Baptista, M., Kramvis, A., Jammeh, S., Naicker, J., Galpin J.S., Kew, M.C.** Follow up of infection of chacma baboons with inoculum containing A and non-A genotypes of hepatitis B virus. *World. J. Gastroenterol.,* **9:**731-735, 2003.

3. **Blitz, L., Pujol, F.H., Swenson, P.D., Porto, L., Atencio, R., Araujo, M., Costa, L., Callejas-Monsalve, D., Torres, J.R., Fields, H.A., Lambert, S., Van Geyt, C., Norder, H., Magnius, L.O., Echevarría, J.M., Stuyver, L.** Antigenic diversity of hepatitis B virus strains of genotype F in Amerindians and other population groups from Venezuela. *J. Clin. Microbiol.,* **36:**648-651, 1998

4. Clarke, D., Casals, J. Techniques for Hemagglutination and Hemagglutination-Inhibition with Arthropod-Borne Viruses. *Amer. J. Trop. Med. Hyg.,* **7:**561-573, 1958.

5. Contigiani, M.S., Fernandez, C., Spinsanti, L.I., Diaz, G.E. Prevalence of Flavivirus antibodies in *Alouatta caraya* primate autochthonous of Argentina. *Medicina (B Aires),* **60**:348-350, 2000.

6. Devesa, M., Rodríguez, C., León, G., Liprandi, F., Pujol, F.H. Clade analysis and surface antigen polymorphism of hepatitis B virus American genotypes. *J. Med. Virol.,* **72:**377-384, 2004.

7. Grethe, S., Heckel, J.O., Rietschel, W., Hufert, F.T. Molecular epidemiology of hepatitis B virus variants in nonhuman primates. *J. Virol.,* **74:**5377-5381, 2000.

8. Gutierrez, C., Devesa, M., Loureiro, C.L., Leon, G., Liprandi, F., Pujol, F.H. Molecular and serological evaluation of surface antigen negative hepatitis B virus infection in blood donors from Venezuela. *J. Med. Virol.,* **73:**200-207, 2004.

9. Hahn, B.H., Shaw, G.M., De Cock, K.M., Sharp, P.M. AIDS as a zoonosis: scientific and public health implications. *Science,* **287:**607-614, 2000

10. Heckel, J.O., Rietschel, W., Hufert, F.T. Prevalence of hepatitis B virus infections in nonhuman primates. *J. Med. Primatol.,* **30:**14-19, 2001.

11. Inoue, S., Morita, K., Matias, R.R., Tuplano, J.V., Resuello, R.R., Candelario, J.R., Cruz, D.J., Mapua, C.A., Hasebe, F., Igarashi, A., Natividad, F.F. Distribution of three arbovirus antibodies among monkeys (Macaca fascicularis) in the Philippines. *J. Med. Primatol.,* **32:**89-94, 2003.

12. Jiang, B., McClure, H.M., Fankhauser, R.L., Monroe, S.S., Glass, R.I. Prevalence of rotavirus and norovirus antibodies in non-human primates. *J. Med. Primatol.,* **33:**30-33, 2004.

13. Kalter, S.S., Heberling, R.L., Cooke, A.W., Barry, J.D., Tian, P.Y., Northam, W.J. Viral infections of nonhuman primates. *Lab. Anim. Sci.,* **47:**461-467, 1997.

14. Kapikian, AZ, Hoshino, Y., Chanock, R.M. Rotavirus. In: *Fields Virology.* **Knipe** and **Howley (eds).** Lippincott Williams and Wilkins, Philadelphia, PA, 2002, pp 1787-1833.

15. Lanford, R.E., Chavez, D., Brasky, K.M., Burns, R.B. 3rd, Rico-Hesse, R. Isolation of a hepadnavirus from the woolly monkey, a New World primate. *Proc. Natl. Acad. Sci. USA,* **95:**5757-5761, 1998.

16. Lanford, R.E., Chavez, D., Barrera, A., Brasky, K.M. An infectious clone of woolly monkey hepatitis B virus. *J. Virol.,* **77:**7814-7819, 2003.

17. Makuwa, M., Souquiere, S., Telfer, P., Leroy, E., Bourry, O., Rouquet, P., Clifford, S., Wickings, E.J., Roques, P., Simon, F. Occurrence of hepatitis viruses in wild-born non-human primates: a 3 year (1998-2001) epidemiological survey in Gabon. *J. Med. Primatol.,* **32:**307-314, 2003.

18. Monath, T.P. Yellow fever: an update. *Lancet Infect. Dis.,* **1:**11-20, 2001.

19. Monath, T.P., Tsai, T.F. Flaviviruses. In: *Clinical Virology.* **Richman, D., Whitley, R.** and **Hayden, F. (eds).** Churchill Livingstone Inc., 1997, pp 1133-1181.

20. Otsyula, M., Yee, J., Suleman, M., Tarara, R., Martins, J., Woods, P., Glass, R., Jennings, M. Rotavirus infection in African, non-human primates. *Ann. Trop. Med. Parasitol.,* **90:**659-661, 1996.

21. Peiris, J.S., Dittus, W.P., Ratnayake, C.B. Seroepidemiology of dengue and other arboviruses in a natural population of toque macaques (Macaca sinica) at Polonnaruwa, Sri Lanka. *J. Med. Primatol.,* **22:**240-245, 1993.

22. Pujol, F.H., Vásquez, G., Rojas, A.M., Fuenmayor, M.E., Loureiro, C.L., Pérez-Schael, I., Estes, M.K., Liprandi F. Norwalk virus infection in Venezuela. *Ann. Trop. Med. Parasitol.,* **92:**205-211, 1998.

23. Ratterree, M.S., da Rosa, A.P., Bohm, R.P. Jr., Cogswell, F.B., Phillippi, K.M., Caillouet, K., Schwanberger, S., Shope, R.E., Tesh, R.B. West Nile virus infection in nonhuman primate breeding colony, concurrent with human epidemic, southern Louisiana. *Emerg. Infect. Dis.,* **9:**1388-1394, 2003.

24. Robertson, B.H., Margolis, H.S. Primate hepatitis B viruses - genetic diversity, geography and evolution. *Rev. Med. Virol.,* **12:**133-141, 2002.

25. Rodhain, F. The role of monkeys in the biology of dengue and yellow fever. *Comp. Immunol. Microbiol. Infect. Dis.,* **14:**9-19, 1991.

26. de Silva, A.M., Dittus, W.P., Amerasinghe, P.H., Amerasinghe, F.P. Serologic evidence for an epizootic dengue virus infecting toque macaques (Macaca sinica) at Polonnaruwa, Sri Lanka. *Am. J. Trop. Med. Hyg.,* **60:**300-306, 1999.

27. Starkman, S.E., MacDonald, D.M., Lewis, J.C., Holmes, E.C., Simmonds, P. Geographic and species association of hepatitis B virus genotypes in non-human primates. *Virology,* **314:**381-393, 2003.

28. Vogel, T.U., Evans, D.T., Urvater, J.A., O'Connor, D.H., Hughes, A.L., Watkins, D.I. Major histocompatibility complex class I genes in primates: co-evolution with pathogens. *Immunol. Rev.,* **167:**327-337, 1999.

29. Wang, E., Ni, H., Xu, R., Barrett, A.D., Watowich, S.J., Gubler, D.J., Weaver, S.C. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *J. Virol.,* **74:**3227-3234, 2000.

30. Wolfe, N.D., Kilbourn, A.M., Karesh, W.B., Rahman, H.A., Bosi, E.J., Cropp, B.C., Andau, M., Spielman, A., Gubler, D.J. Sylvatic transmission of arboviruses among Bornean orangutans. *Am. J. Trop. Med. Hyg.,* **64:**310-316, 2001.

31. Zuckerman, A.J., Zuckerman, J.N. Current topics in hepatitis B. *J. Infection,* **41:**130-136, 2000.

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