SHORT PAPER

Serosurvey for hepatitis A in neotropical primates in southeast Brazil

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Abstract

Background Hepatitis A virus (HAV) is the cause of a zoonotic disease, which has only humans and non-humans primates as its natural hosts.
Methods The seroprevalence of antibodies anti-HAV in wild and captive neotropical primates were investigated.
Results 4.9% (18/369) were positive for antibodies anti-HAV, in captivity.
Conclusion Implications for health managements are discussed.

Introduction

The hepatitis A is a zoonotic disease caused by a RNA virus (Hepatitis A virus – HAV), classified as a *Hepatovirus*, Picornaviridae Family. The natural hosts for this virus are humans and non-human primates (NHP) [1, 14].

There is just one serotype of HAV, but several strains are known, which are divided in seven genotypes. The genotypes I, II, and VII have exclusively human strains, while the genotypes IV, V, and VI have exclusively simian strains. The genotype III has both human and NHP strains [11]. Although the HAV has a worldwide distribution, the antigenic difference between the strains is minimal, [2, 13] which enables the use of human diagnostic test for NHP samples [16].

Once HAV has a fecal–oral infection, sanitation has an important role in the disease's epidemiology [8, 21]. The physiopathology is poorly understood, but is accepted that the virus replicates in the liver and is eliminated with the feces [11, 21].

Non-human primates represent a natural reservoir of virus [21], and Old World primates (OWP) and New World primates (NWP) can be infected with the HAV [4–6, 9, 12, 18, 19, 21]. In NHP, the diseases are usually asymptomatic; but when clinical disease occurs, the signals are unspecific and vary from mild to fatal outcome [11, 21].

Diagnosis is made by serological detection of specific anti-HAV antibodies, or by viral antigen detection in blood or feces during the acute phase of the disease [21]. The presence of IgM anti-HAV indicates acute infection or early convalescence. In contrast, IgG anti-HAV is found in early phases of the infection, reaching peak levels during the convalescence and remaining detectable for decades [11].

This study investigated the occurrence of anti-HAV antibodies in NWP from *in situ* and *ex situ* at southeast region of Brazil.

Methods

Serum samples from 419 NWP (364 *ex situ*; 55 *in situ*) of 32 species were tested. All free-ranging animals were from Presidente Epitaceo and Anaurilandia municipalities (22°07′S, 52°30′W), southeast of Brazil.

Blood samples were processed and serum samples were maintained during a variable period of time at −20°C and then transferred to −70°C until tested.

All samples were tested for IgM anti-HAV and total anti-HAV (IgM and IgG) antibodies with enzyme-linked immunosorbent assay (ELISA).

For the IgM anti-HAV test, two different ELISA capture kits were used: an in-house IgM anti-HAV ELISA, developed by the Viral Hepatitis Reference Center – FIOCRUZ, and a commercial kit Bioelisa HAV IgM (Biokit® S.A., Barcelona, Spain).

For the total anti-HAV test, three different ELISA competition kits were used. First, 288 samples were tested with an in-house total anti-HAV ELISA manufactured by the Viral Hepatitis Reference Center – FIOCRUZ. From these samples, those who had an indeterminate result were retested with the commercial kit Hepanostika® HAV Antibody (Organon Teknika BV, Boxtel, the Netherlands). The remaining samples (n = 131) were tested with the commercial kit Bioelisa HAV (Biokit® S.A., Barcelona, Spain), and those who had an indeterminate result were retested in duplicate with the same kit.

All tests were performed according to the manufacturer's procedures recommendations, with positive and negative controls in each batch.

The procedures adopted were approved by the Bioethic Commission of the School of Veterinary Medicine and Animal Sciences of University of São Paulo (protocol number 120/2002), and in full compliance with specific federal permits issued by the Brazilian Ministry of Environment (IBAMA, process number 02027/003259/0248).

Results

From the 419 NWP tested, 45.6% (191/419) were Callithrichidae, 38.4% (161/419) Cebidae, 15.5% (65/419) Atelidae, 0.25% (1/419) Pithecidae, and 0.25% (1/419) Aotidae (Table 1).

All samples were negative for IgM anti-HAV and therefore the genotyping was not possible.

Regarding the total anti-HAV test, 5.2% (22/419; 95% exact CI: 3.5–7.8%) of the captive animals were positive (Table 2). All samples from free-ranging animals were negative for total anti-HAV.

Discussion

The percent (5.2%) of positive animals for total anti-HAV found in our work is remarkably below than from other studies, where 33% to 95% of positives

Table 1 Distribution of animals in relationship to the species. The numbers in parenthesis represent positive animals for total anti-hepatitis A virus. CAPT, captivity; FRE, free-ranging. Total N = 419. Brazil, 2013

Species[1]	Condition	n	Species[1]	Condition	n
Alouatta belzebul	CAPT	1	*Cebus albifrons*	CAPT	1
Alouatta caraya	CAPT/FRE	5/33	*Lagothrix lagothricha*	CAPT	4 (1)
Alouatta fusca	CAPT	4	*Leontophitecus chrysometas*	CAPT	43
Alouatta sp.	CAPT	4	*Lentophitecus chrysopygus*	CAPT	25 (2)
Aotus sp.	CAPT	1	*Leontophitecus rosalia*	CAPT	4
Ateles marginatus	CAPT	6	*Leontophitecus sp.*	CAPT	2
Ateles paniscus	CAPT	7	*Mico humeralifer*	CAPT	4
Ateles belzebul	CAPT	1 (1)	*Mico metanurus*	CAPT	2
Callicebus personatus	CAPT	1	*Saguinus bicolor*	CAPT	10
Callimico goeldii	CAPT	3	*Saguinus martinsi*	CAPT	2
Callithrix argentata	CAPT	2	*Saguinus midas*	CAPT	3 (2)
Callithrix aurita	CAPT	1	*Saguinus niger*	CAPT	4
Callithrix geoffroyi	CAPT	17 (1)	*Saguinus mystax*	CAPT	1 (1)
Callithrix jacchus	CAPT	29 (2)	*Saguinus sp.*	CAPT	4
Callitrhix kuhlii	CAPT	2	*Saimiri sciureus*	CAPT	6
Callithrix penicillata	CAPT	28	*Sapajus sp.*	CAPT/FRE	131 (11)/22
Callithrix sp.	CAPT	5	*Sapajus xanthostemos*	CAPT	1 (1)

[1]Primate taxonomy according to Paglia et al. [15]

Table 2 Positive animals (n = 22) with regard to the gender and family. Brazil, 2013

Animals	Positive animals, %
Males	54.5 (12/22)
Females	45.5 (10/22)
Callitrichidae	36.4 (8/22)
Cebidae	54.5 (12/22)
Atelidae	9.1 (2/22)

were found for captive animals [1, 4, 10, 12, 17, 19] and 22% to 37% tested positive for wild animals [5, 6].

One important factor to justify the low prevalence of anti-HAV antibodies in our study regards the species studied. There are some simian strains of HAV that are specific for OWP, what favors the virus circulation in certain species. On the other hand, until now, just one strain of HAV, the PA21, was isolated from NWP, but this strain was also isolated from humans, indicating that it has no significant species specificity [3, 21].

As NWP can be infected with human strains of HAV, we expected that animals with higher contact with humans would have greater possibility to present anti-HAV antibodies. When we compare the free-ranging animals, with those kept in captivity, we note that this hypothesis may be acceptable because the incidence of positive animals was only seen in *ex situ* animals that have frequently contact with people (visitors, keepers, technicians etc.).

According to the present results, one may assume that the virus does not circulate among the free-ranging NWP population studied. These results are important in regions where the environment is changing. In addition, researchers are concerned with the animal's movement. Eventually, wild animals originated from rescue programs are destined for different locations, returning to nature through reintroductions, or sent to captivities in contact with people. Therefore, it can be considered a source of infection to a place where it did not occur before [7, 20].

Observing the positive captivity animals for total anti-HAV in this study, we can consider that the virus circulates in this population and we can see that 54.5% (12/22; 95% exact CI: 32.2%-75.6%) belong to the Cebidae family. It could be explained due to the biology of the animals belonging to this family. The Cebidae, especially those of the *Cebus* and *Sapajus* genus kept in captivity, have the habit to go to the ground and therefore could have easier contact with contaminated food or objects.

Finally, this is the first study where animals of the *Leontopihtecus* genus were found to have anti-HAV antibodies, and it could be important for the conservation programs involving these animals.

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