

Original Article

Prevalence of Antibody against Hepatitis E Virus in Various Species of Non-Human Primates: Evidence of Widespread Infection in Japanese Monkeys (*Macaca fuscata*)

Makoto Hirano, Xin Ding, Huy Thien-Tuan Tran, Tian-Cheng Li¹, Naokazu Takeda¹, Tetsutaro Sata, Shin Nakamura² and Kenji Abe*

Department of Pathology and ¹Department of Virology II, National Institute of Infectious Diseases, Tokyo 162-8640 and

²Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Aichi 484-8506, Japan

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SUMMARY: We screened 495 serum samples from 20 species of non-human primates for the antibody against hepatitis E virus (HEV). Anti-HEV IgG was detected in 84 of 232 (36.2%) Japanese monkeys, 2 of 19 (10.5%) cynomolgus monkeys, 3 of 83 (3.6%) rhesus monkeys, and 1 of 1 (100%) Taiwanese monkey, respectively. These results suggest that HEV is circulating among monkeys belonging to the genus *macaca*. A high prevalence of anti-HEV IgG was observed in Japanese macaques (*M. fuscata*) despite the fact that Japan is non-endemic for hepatitis E. It is possible that HEV can be transmitted from Japanese macaques to humans. Further, the rate of antibody positivity was found to increase with age in Japanese macaques. Seropositive macaques were found throughout Japan, but the seroprevalence rate differed among geographic regions.

INTRODUCTION

Hepatitis E virus (HEV) is the etiologic agent of hepatitis E, previously called enterically transmitted non-A, non-B hepatitis. HEV is thought to spread via the fecal-oral route, and outbreaks of hepatitis E are attributed to water contaminated with HEV. Areas in which hepatitis E is endemic are confined to developing nations where the majority of people lack an adequate water supply (1). Hepatitis E is not endemic in industrialized nations, though the prevalence of anti-HEV antibody among healthy adults has been found to be 1-3% in the United States (2) and 3-4% in Japan (3). In Japan, less than 10 cases of hepatitis E are reported annually, and most of these cases have usually been associated with travel to endemic areas. However, several recent studies have suggested the existence of HEV strains indigenous to Japan, based on findings that several HEV strains were recovered from Japanese patients with acute hepatic failure, none of whom had traveled abroad (4,5). The route of HEV infection in those patients is still unknown. It has been suggested that swine (6,7) and rodents (8-10) are reservoirs of HEV, but the exact role of domestic and wild animals in the transmission of HEV to humans remains unclear. Arankalle et al. suggested, based on a recombinant antigen-based enzyme-linked immunosorbent assay (ELISA), that three species of Old World monkeys (*Macaca mullata*, *M. radiata*, and *Presbytes entellus*) are reservoirs of HEV in India (11). In order to screen other candidate non-human primates as HEV reservoirs, we conducted a seroepidemiological survey for the anti-HEV antibody using serum samples from various species of hominoids and monkeys.

MATERIALS AND METHODS

Serum samples: We collected 495 serum samples from 20 species of non-human primates, including hominoids (98 chimpanzees, 9 orangutans, and 7 pileated gibbons), Old World monkeys (232 Japanese monkeys, 83 rhesus monkeys, 19 cynomolgus monkeys, 7 hamadryas baboons, 5 stump-tailed monkeys, 4 pig-tailed monkeys, 3 Assamese monkeys, 2 bonnet monkeys, 2 green monkeys, 2 patas monkeys, and 1 Taiwanese monkey), New World monkeys (9 tufted capuchins, 6 owl monkeys, 1 white-fronted capuchin, and 1 squirrel monkey) and prosimii (2 ring-tailed lemurs and 2 greater galagos), as listed in Table 1. Male and female Japanese monkeys numbered 50 and 177, respectively. All monkeys were housed in an indoor-outdoor facility in Kyoto University Primate Research Institute and were maintained according to the guidelines of that institution. All chimpanzees used in this study were born in West Africa and imported to Japan in 1979. We used serum samples of hominoids obtained at quarantine immediately after their arrival in Japan. None had been previously inoculated with human serum, any blood products, or any hepatitis viruses. The serum samples of non-human primates were kept at -40°C or below until testing.

ELISA: A recombinant open reading frame (ORF) 2 protein of HEV expressed by a recombinant baculovirus was used as the antigen in ELISA, as previously described (3,12). In brief, sera of non-human primates were diluted at 1:200 and added to assay plates coated with the recombinant HEV ORF2 protein. Horseradish peroxidase (HRP)-conjugated goat anti-human IgM at 1:2,000 dilution (Cappel, Durham, N.C., USA) was used to detect antigen-bound primate IgM. HRP-conjugated goat anti-human IgG at 1:10,000 dilution (Cappel) and anti-monkey IgG at 1:1,000 dilution (EY Laboratories, San Mateo, Calif., USA) were used to detect hominoid and monkey IgGs, respectively. Human serum known to be positive for both anti-HEV IgG and IgM was included in every assay plate as

*Corresponding author: Mailing address: Department of Pathology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan. Tel: +81-3-5285-1111 ext. 2624, Fax: +81-3-5285-1189, E-mail: kenjiabe@nih.go.jp

Table 1. Prevalence of anti-HEV IgG in various species of non-human primates

	Species	Place of capture	<i>n</i>	Anti-HEV IgG (%)
Hominoids	Chimpanzee (<i>Pan troglodytes</i>)	West Africa	98	0
	Orangutan (<i>Pongo pygmaeus</i>)	Indonesia	9	0
	Pileated gibbon (<i>Hylobates pileatus</i>)	Southeast Asia	7	0
Old World monkeys	Japanese monkey (<i>Macaca fuscata</i>)	Japan	232	84 (36.2)
	Rhesus monkey (<i>Macaca mulatta</i>)	Southeast Asia	83	3 (3.6)
	Cynomolgus monkey (<i>Macaca fascicularis</i>)	Southeast Asia	19	2 (10.5)
	Hamadryas baboon (<i>Papio hamadryas</i>)	Northeast Africa	7	0
	Stump-tailed monkey (<i>Macaca arctoides</i>)	Southeast Asia	5	0
	Pig-tailed monkey (<i>Macaca nemestrina</i>)	Southeast Asia	4	0
	Assamese monkey (<i>Macaca assamensis</i>)	India	3	0
	Bonnet monkey (<i>Macaca radiata</i>)	India	2	0
	Green monkey (<i>Chlorocebus aethiops</i>)	Central Africa	2	0
	Patas monkey (<i>Erythrocebus patas</i>)	Central Africa	2	0
Taiwanese monkey (<i>Macaca cyclopis</i>)	Taiwan	1	1 (100.0)	
New World monkeys	Tufted capuchin (<i>Cebus apella</i>)	Brazil	9	0
	Owl monkey (<i>Aotus trivirgatus</i>)	Brazil, Venezuela	6	0
	White-fronted capuchin (<i>Cebus albifrons</i>)	Brazil, Colombia	1	0
	Squirrel monkey (<i>Saimiri sciureus</i>)	Brazil, Venezuela	1	0
Prosimii	Ring-tailed lemur (<i>Lemur catta</i>)	Madagascar	2	0
	Greater galago (<i>Otolemur crassicaudatus</i>)	South Africa	2	0

a positive control. Anti-HEV antibody titers of serum samples were expressed as OD₄₉₂ values. The cut-off value was set at 0.2 of OD₄₉₂, based on the fact that the mean+3SD values for human sera known to be negative for both anti-HEV IgG and IgM never exceeded 0.2 in the abovementioned assays for non-human primates.

Statistical analyses: Statistical analyses were performed by the chi-square test or Fisher's exact test. A difference with a *P* value of <0.05 was considered significant.

RESULTS

Anti-HEV IgG titers of serum samples from 360 Old World monkeys were determined by a recombinant antigen-based ELISA. The distribution of OD₄₉₂ values is shown in Fig. 1. When the cut-off value was set at 0.2 as described in Materials and Methods, the majority (281 of 360) of titers of monkey sera were negative; that is, they fell below this cut-off value. The mean and SD for these negative samples were 0.063 and 0.029, respectively. Thus, the cut-off value at 0.2 of OD₄₉₂ corresponds to 4.7 SD above the mean for the negative samples.

Eighty-four serum samples of 232 (36.2%) Japanese monkeys, 3 of 83 (3.6%) rhesus monkeys, 2 of 19 (10.5%) cynomolgus monkeys, and 1 of 1 (100%) Taiwanese monkey were positive for anti-HEV IgG when tested by ELISA (Table 1). These results indicated that HEV is circulating among monkeys belonging to the genus *macaca*. The differences between Japanese and rhesus macaques and between Japanese and cynomolgus macaques were statistically significant (*P* < 5.0 × 10⁻⁸ and *P* < 0.05, respectively). The seropositive rate of anti-HEV IgG in Japanese macaques increased with age: 4.2% in 1- and 2-year-old monkeys; 21.1% in 3- and 4-year-old monkeys; 29.6% in 5- and 6-year-old monkeys; and 38.5% in monkeys over 7 years of age (Fig. 2). No significant differences were observed between males and females. We also analyzed the prevalence of anti-HEV IgG in Japanese macaques captured in various geographic regions of Japan.

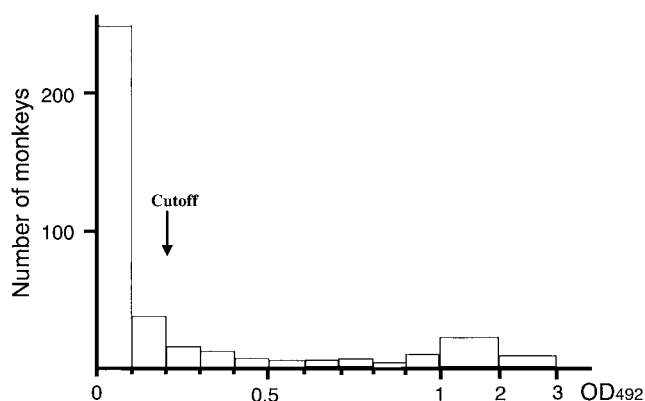


Fig. 1. Distribution of OD₄₉₂ values of monkey sera tested by ELISA for IgG antibody to a recombinant HEV ORF2 protein. Titers of sera of 360 Old World monkeys were determined by ELISA with HRP-conjugated goat anti-monkey IgG as the secondary antibody. The cut-off value was set at 0.2 of OD₄₉₂.

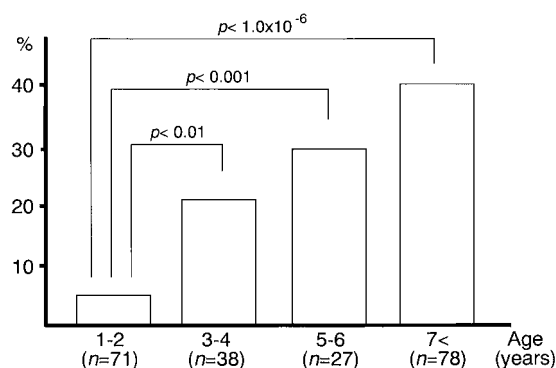


Fig. 2. Relationship between anti-HEV IgG positivity and age of Japanese macaques. Japanese macaques reach sexual maturity at 4-5 years of age.

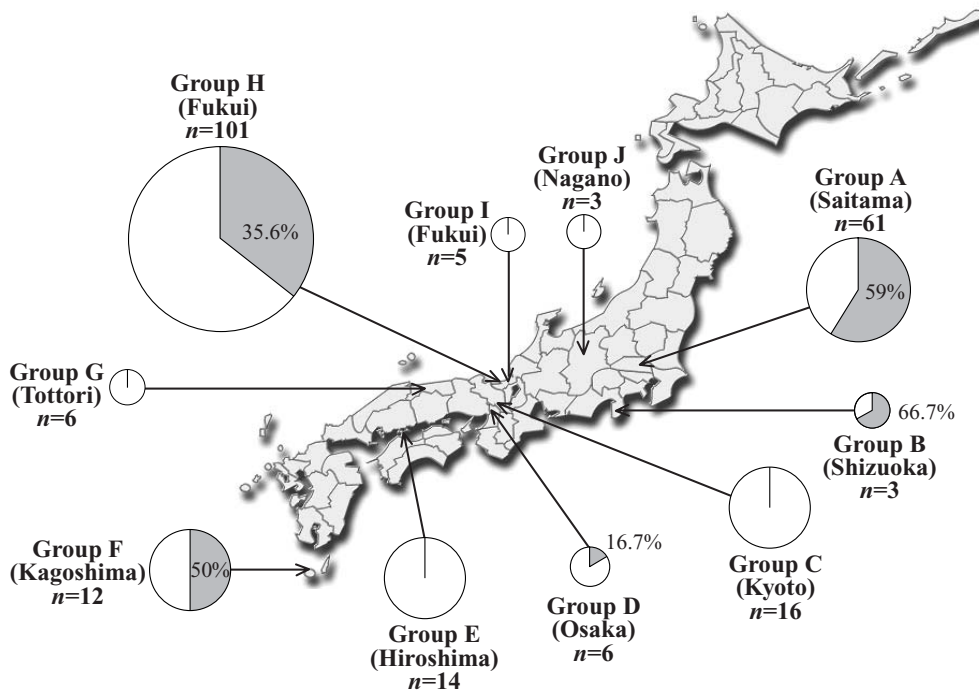


Fig. 3. Prevalence of anti-HEV IgG in Japanese macaques according to their geographic region of capture in Japan. The size of a circle is roughly proportional to the number of animals tested in this study. A blank circle denotes that all macaques in that regional group were negative for anti-HEV IgG.

Seropositive macaques were found throughout Japan, and the positivity of anti-HEV IgG differed remarkably relative to the geographic region of capture (Fig. 3). The prevalence of anti-HEV antibody in group B in Shizuoka (66.7%) was the highest, followed by that in group A in Saitama (59%), in group F in Kagoshima (50%), in group H in Fukui (35.6%), and in group D in Osaka (16.7%). The difference between group A and group H was statistically significant ($P < 0.01$). As shown in Fig. 3, a weak tendency for the rate of seropositivity to be higher in the northern parts of Japan was observed. No positive reaction for anti-HEV IgG was observed in non-human primates other than Japanese, cynomolgus, rhesus, and Taiwanese macaques. All tested samples were negative for anti-HEV IgM.

DISCUSSION

Transmission of HEV is thought to occur primarily via water contaminated with HEV. However, in contrast to the case for other enterically transmitted viruses, person-to-person transmission of HEV appears to occur infrequently. Furthermore, the reservoir of HEV in nature is unknown. In recent studies, HEV infection was demonstrated in swine (6,7) and rodents (8-10). Swine strains of HEV closely related to human strains have been identified in the United States (6) and in Japan (7). A high prevalence of anti-HEV antibody has been reported among persons who work with swine (13). Based on all these findings, hepatitis E is now thought to be a zoonosis.

In this study, we demonstrated the presence of an antibody reactive to a recombinant HEV ORF2 protein in Japanese, rhesus, cynomolgus, and Taiwanese macaques. This finding is consistent with the fact that cynomolgus macaques are susceptible to experimental infection with HEV (14). Surprisingly, however, a high seroprevalence rate (36.2%) was found

in Japanese macaques despite the fact that hepatitis E is not endemic in Japan. This percentage is comparable to the seroprevalence rate of 36.7% in rhesus macaques in India, where hepatitis E is highly endemic (11). In the Japanese macaques used in this study, we also found a high prevalence (63.8%) of antibody to hepatitis A virus (HAV) (15). These serologic findings suggest that HEV and HAV are circulating among macaques, supporting the hypothesis that macaques can serve as reservoirs of enterically transmitted hepatitis viruses. To investigate this hypothesis, a PCR assay is underway to detect HEV RNA, though it has not yet been successful (data not shown), due to the fact that the existence of anti-HEV IgG indicates previous infection, not present infection. In Japanese macaques, the prevalence of anti-HEV IgG is higher in sexually matured adults over 5 years of age than in sexually immature animals under 4 years of age (Fig. 2). Arankalle et al. reported similar results in an epidemiological study of human subjects living in an Indian district, where both HEV and HAV are endemic (16).

In this study, in contrast with the seropositivity in macaques, all hominoids, including chimpanzees, orangutans, and pileated gibbons, showed negativity for anti-HEV IgG. These results were unexpected, because all hominoids were imported from areas where hepatitis E is endemic, and because chimpanzees are susceptible to experimental infection with HEV (17).

In conclusion, our results suggest that HEV is circulating among macaques in the natural environment. It is noteworthy that a high prevalence of anti-HEV IgG was observed in Japanese macaques. Macaques appear to be reservoirs of HEV, and it is possible that HEV can be transmitted from macaques to humans. Further investigation of HEV infection in Japanese macaques may shed light on the enigmatic viral source of sporadic cases of hepatitis E in Japan.

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