

## SEROPREVALENCE OF HEPATITIS A VIRUS INFECTION IN NON-HUMAN PRIMATES IN ASSAM, INDIA

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Hepatitis viruses have long been assumed to be highly host-specific. However, among primates they seem to frequently cross between species. For example infections of nonhuman primates occur due to inoculation with or exposure to human viruses (Robertson 2001). Hepatitis A virus (HAV) is known to be capable of infecting various species of primates, but new molecular biology techniques have necessitated a re-evaluation of the ecology of this agent and the role of non-human primates as natural hosts. Hepatitis A virus infection was reported in wild-caught Chacma baboons (Smith et al. 1980), wild-caught Panamanian owl monkeys (Lemon et al. 1982) and wild Cynomolgus macaques (Burke & Heisey 1984; Slighter et al. 1988). Enterically transmitted hepatitis viruses (hepatitis A virus and hepatitis E virus) can induce hepatitis in a number of old world and new world monkey species. The host range of primates susceptible to hepatitis viruses transmitted by the parenteral route is restricted to a few species of

old world monkeys and apes (Vital et al. 1998). The present study investigated the seroprevalence of hepatitis A virus infection in non-human primates in Assam.

**Materials and Methods:** From December 2007 to November 2009, 37 serum samples of non-human primates were collected from Assam State Zoo and Department of Forest and Environment, Government of Assam. A comprehensive data collection form focused on possible risk factors including age, sex and living status (household or free roaming) was prepared when sampling was carried out. Out of 37 sampled non-human primates, 23 (62.16%) were males and 14 (37.83%) were females. Ten non-human primates were captive and 27 were free living. An additional four serum samples were collected from animal keepers of Assam State Zoo working with non-human primates.

Competitive ELISA was performed using hepatitis A virus ELISA kit (Wanti Hep. AV) to detect hepatitis A virus antibodies. Serum samples from the non-human primates and humans were added in the appropriate wells of the ELISA plate precoated with hepatitis A virus antigen. Monoclonal antibody linked with horse radish peroxidase conjugate was added simultaneously after adding the serum samples. Serum and monoclonal antibody enzyme conjugate were allowed to react for 60 minutes at 37°C. ELISA plate was washed five times with washed buffer supplied along with the kit and after diluting 10 times with distilled water as recommended in the kit. Substrate and chromogen solution A consisting of urea peroxide solution and tetramethyl benzidine (TMB) solution in citric acid was added to each of the wells of the ELISA plate and allowed to react for 15



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minutes at 37°C. The reaction was stopped with 2M H<sub>2</sub>SO<sub>4</sub> and the reading of the plate was taken in ELISA reader (Multiscan Ex) using 450 nm filter. Blank, negative control and positive control were kept in the ELISA plate as recommended in the kit.

The optical density (OD) value for the positive serum control and negative serum control for detectable antibodies were 0.101 and 1.349 respectively. The prevalence was estimated from the ratio of positive results to the total number of animals examined. Assessment of association between seroprevalence of anti-hepatitis A virus antibodies in non-human primates and selected risk factors were made by chi-square test by using SPSS 16 software.

**Results:** In the present investigation, 10 of 37 (27.02%) non-human primates and 3 of 4 (75%) human were found positive for hepatitis A (VAH) infection as indicated by the serum titre (Image 1). The OD value recorded for positive non-human primates samples were 0.130, 0.401, 0.498, 0.151, 0.334, 0.169, 0.156, 0.434, 0.375, 0.168 and for zoo animal keepers were 0.163, 0.122, 0.127. From 10 non-human primates serum samples with detectable antibodies, six (60%) were males and four (40%) were females (Table 1). Statistical analysis using chi-square test showed no correlation between hepatitis A virus infection and gender (P=0.86). To investigate the role of living condition in occurrence of infection, positively reacted serum samples were compared between captive and free living non-human primates. Infection rate had

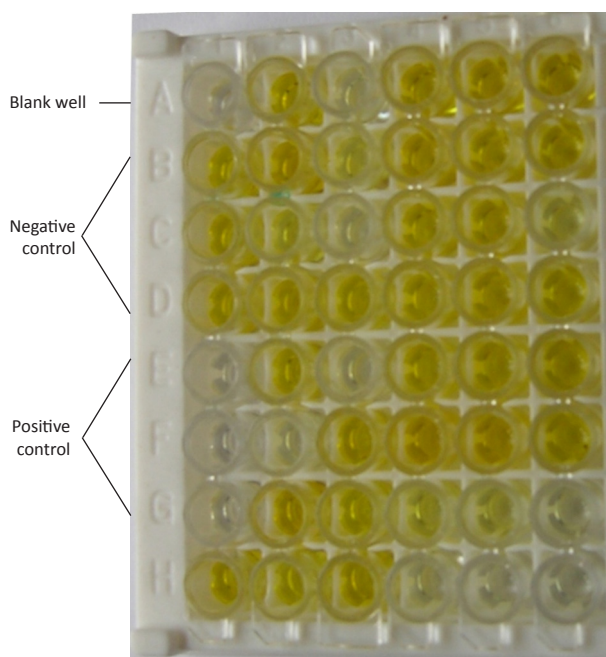


Image 1. ELISA kit showing the result of viral hepatitis A infection

Table 1. Seroprevalence of anti-hepatitis A virus antibodies in different groups

Groups		Total No. of sample	Positive Sample No.	Positive samples (%)
Sex	Male	23	6	60
	Female	14	4	40
Living status	Captivity	10	0	0
	Free living	27	10	100

significant difference between free living non-human primates and captive non-human primates (P<0.05) (Table 1).

**Discussion:** Previously it was widely accepted that the natural infection with HAV is limited to humans, although several nonhuman primate species, including Chimpanzee, tamarins and owl monkeys were susceptible to experimental infection. Serological evidence of infection had been found in various species on non-human primates, including Rhesus and Crab-eating Macaque, African Green Monkeys, baboons and owl monkeys (Dienstag et al. 1976). In the present study viral hepatitis A infection rate in non-human primates was almost similar with the infection rate recorded by Slighter et al. (1988) and Smith et al. (1980).

Comparison of infection rate between two sexes showed no significant differences between males and females, i.e., sex was excluded from possible risk factors of hepatitis A virus infection. A positive association was seen between hepatitis A virus seropositivity and free living non-human primates. No infection in captive non-human primate may be due to the lower chance of exposure, i.e., free living is a possible risk factor for hepatitis A virus infection. Presence of infected non-human primates without clinical signs (even with high titers) indicates the importance of these animals as possible carriers of the virus. The present study could not give any statistical sense for the prevalence of hepatitis A infection in human as less number of samples was examined. The present study indicated the need for screening of a large number of non-human primates and human samples particularly from zoo animal keepers to understand the role of non-human primates in spreading hepatitis A virus infection.

## REFERENCES

- Burke, D.S. & G.B. Heisey (1984). Wild Malaysian Cynomolgus Monkeys are Exposed to Hepatitis A Virus. *The American Journal of Tropical Medicine and Hygiene* 33(5): 940–944.
- Dienstag, J.L., S.M. Feinstone & R.H. Purcell (1976). Non-human

- primate associated viral hepatitis type A: Serological evidence of Hepatitis A viral infection. *The Journal of the American Medical Association (JAMA)*, 236: 462.
- Lemon, S.M., J.W. Leduc, L.N. Binn, A. Escajadillo & K.G. Ishak (1982).** Transmission of hepatitis A virus among recently captured Panamanian owl monkeys. *Journal of Medical Virology* 10(1): 25–36; <http://dx.doi.org/10.1002/jmv.1890100105>
- Robertson, B.H. (2001).** Viral hepatitis and primates: historical and molecular analysis of human and nonhuman primate hepatitis A, B, and the GB-related viruses. *Journal of Viral Hepatitis* 8 (4): 233–242; <http://dx.doi.org/10.1046/j.1365-2893.2001.00295.x>
- Slighter, R.G., J.P. Kimball, T.A. Barbolt, A.D. Sherer & H.P. Drobeck (1988).** Enzootic Hepatitis A infection in *Cynomolgus* monkeys (*Macaca fascicularis*). *American Journal of Primatology* 14(1): 73–81; <http://dx.doi.org/10.1002/ajp.1350140107>
- Smith, M.S., P.J. Swanepoel & M. Bootsma (1980).** Hepatitis A in non-human primates in nature. *Lancet* 2: 1241.
- Vitral, C.L., C.F.T. Yoshida, & A.M.C. Gaspar (1998).** The use of non-human primates as animal models for the study of hepatitis viruses. *Brazilian Journal of Medical and Biological Research* 31(8): 1035–1048; <http://dx.doi.org/10.1590/S0100-879X1998000800003>

