

WILD MALAYSIAN CYNOMOLGUS MONKEYS ARE EXPOSED TO HEPATITIS A VIRUS

Principal Investigators : Donald S. Burke¹
Gregory B. Heisey¹

BACKGROUND : Captive non-human primates have been frequently implicated as the source of infection in epidemics of acute hepatitis in humans (1-3). Typically, hepatitis occurs in persons handling animals within the first two months of their importation (2). It has been generally accepted that non-human primates become infected with HAV after contact with humans and thereby become infectious; however, the possibility that the animals are exposed to HAV in nature, before capture, has never been fully explored.

Serum HAV antibodies have been detected in a wide variety of captive non-human primate species in variable proportions, depending on the species (5-9). Recently we have found serologic evidence for epidemic spread of HAV or an HAV-like virus among cynomolgus monkeys (*Macaca fascicularis*) housed in a permanent colony facility in Kuala Lumpur, Malaysia (4), reinforcing the concept that non-human primates develop HAV infections after contact with man. However, we also found that 20% of all freshly captured cynomolgus monkeys had serum HAV antibodies, and that larger (and therefore older) freshly captured monkeys had a higher antibody prevalence rate than did smaller monkeys.

In order to test the hypothesis that cynomolgus monkeys become infected in nature, before intimate contact with man, we captured animals at sites remote from human habitation, obtained serum samples from these animals immediately, and tested the sera for anti-HAV activity.

MATERIALS AND METHODS :

Trapping methods : Cynomolgus monkeys were captured by hired trappers during the months of April, May, and June 1981. The animals were captured in cages measuring approximately 3 x 3 x 2 meters which were constructed on site from wood and galvanized wire. Tunnel entrances constructed of chicken wire allowed ingress but not egress. Traps were baited with bananas, and visited daily. Trapped monkeys were transferred to small metal cages and immediately transported to Kuala Lumpur where they were weighed and a blood sample obtained.

Trapping sites : In order to sample several different monkey troupes, monkeys were trapped in 6 different sites. All sites were at least 10 kilometers from the other trapping sites, and all were at least 10 kilometers from the nearest town which was defined as any aggregation of 10 or

¹ Department of Laboratory Animal Resources, U.S. Army Medical Research Unit, Kuala Lumpur, Malaysia.

more dwellings. Trapping continued at each site until 15-20 animals had been captured. Trapping sites were in two basic locations (Figure 1). Monkeys in Groups A, B, and C were captured at sites in Johor state in southern peninsular Malaysia, in vicinity of Kr. Kahang. This trapping area was virgin forest near a game preserve with practically no human habitation. On several occasions, traps at these sites were disrupted by wild elephants. Monkeys in Groups D, E, and F were captured on the fringes of oil palm and rubber estates in Selangor state approximately 75 kilometers northeast of Kuala Lumpur in the vicinity of Batang Berjuntai. Although these latter areas were removed from human dwellings direct or indirect contact between monkeys and humans was possible because humans did periodically enter these areas to work.

Blood sampling and shipping : In Kuala Lumpur five to ten milliliters of blood was obtained by femoral venipuncture. After clot formation, serum was separated and stored at -20°C until shipped frozen from Kuala Lumpur to Bangkok.

Detection of HAV antibodies : Serum HAV antibodies were detected with a commercial radioimmunoassay (HAVAB (R) Abbot Laboratories) designed for testing human serum specimens. Monkey sera were tested by procedures for human samples. This approach is justified because the distribution of specimens according to the percent blocking was clearly biphasic, with a nadir near 50% blocking as defined by human positive and negative control samples.

Assays for hepatitis B core (HBc) antibodies : Sera from all 37 monkeys weighing 2 kilograms or more were assayed for HBc antibodies with a commercial radioimmunoassay (CORAB (R) Abbot Laboratories).

RESULTS : A total of 106 monkeys, 51 females and 55 males, were trapped and tested: 54 from sites A, B, and C, and 52 from sites D, E, and F. Approximately equal number of males and females were trapped at each site. Larger monkeys were trapped at sites A, B, and C than at D, E, and F: the number of monkeys weighing less than 2 kg, 2-3.9 kg, and 4 kg or more was 31, 15, and 8 respectively at sites A, B, and C and 38, 14, and 0 respectively at sites D, E, and F. Overall 24 monkey sera had greater than 50% blocking activity: 18 of 54 in groups A, B, and C and 6 of 52 in groups D, E, and F. The probability that a given monkey had serum antibody was strongly associated with monkey weight in both groups: only 4 of 69 (6%) monkeys weighing less than two kilograms had antibody, while 20 of 37 (54%) weighing 2 kilograms or more, and six of eight (75%) weighing four kilograms or more had antibody ($\chi^2=39.4$; $p < 10^{-8}$; figures 2A and 2B). There was no association of serum HAV antibody and animal gender: 13 of the 51 females and 11 of the 55 males were positive.

DISCUSSION : HAV antibodies have been found in serum specimens from a wide variety of non-human primates, including both new world (capuchin, marmoset, and owl monkeys) (5-7) and old world (baboon, chimpanzee, gibbon, grivet, and rhesus monkeys) (8-10) species. Despite these reports, there have been no systematic efforts to determine if non-human primates become infected in nature before capture, other than a recent, brief report of the finding of anti-HAV in four freshly captured baboons in South Africa (10).

We found that the majority of cynomolgus monkeys weighing two kilograms or more captured in several different locations in rural Malaysia had serum HAV antibodies. Although exact ages are difficult to determine in captured primates, cynomolgus monkeys weighing two kilograms corresponds roughly to an age of three to four years (11). Thus, the age specific prevalence of HAV among Malaysian cynomolgus monkeys reaches 50% by age 3 to 4 years, both among monkeys captured near plantations and those captured in virgin forest. Although no published data is available regarding the age specific prevalence of HAV antibodies among human populations in Malaysia, data from other human populations in Southeast Asia show an age specific anti-HAV prevalence of 50% by 5 to 10 years (12). These data suggest that cynomolgus monkeys in Malaysian jungles may be infected with HAV at a rate comparable to that of humans in the same region.

The source of infection of cynomolgus monkeys in nature is currently unknown. Although it is possible that monkeys trapped in sites D, E, and F had occasional contact with human urine or feces, it is unlikely that animals trapped in sites A, B, and C did so. Indeed, these sites were selected specifically for their remoteness from man. Also, it is unlikely that the monkeys trapped at these remote sites could have travelled far enough to come into close contact with man, since cynomolgus monkeys have a home range with less than a 5 kilometer radius and rarely venture further (13-14).

If the monkeys do not become infected from human wastes, the next consideration is that they become infected through contact with other infected monkeys. The infectivity of non-human primates for humans is epidemiologically well documented, and is reasonable to assume that HAV infected urine or feces from one cynomolgus monkey could infect another, although this has yet to be demonstrated directly.

If a sylvatic cycle of HAV in monkeys does exist in Malaysia, then it is reasonable to speculate about the nature of the virus. Although it is probably identical or closely related to the human hepatitis A virus, the possibility of a distinct serotype will remain open until virus is isolated from a spontaneously infected monkey and the strain characterized in the laboratory.

Finally, these data suggest that plans for long term control of HAV may have to take into consideration a sylvatic cycle of transmission.

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