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Serological evidence of herpesvirus infection in gibbons

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Abstract

Background: Herpesviruses are not only infectious agents of worldwide distribution in humans, but have also been demonstrated in various non-human primates as well. Seventy-eight gibbons were subjected to serological tests by ELISA for herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), Epstein-Barr virus (EBV) and cytomegalovirus (CMV).

Results: The prevalence of IgG antibodies against HSV-1, HSV-2, EBV and CMV was 28.2%, 28.2%, 14.1% and 17.9%, respectively.

Conclusions: Antigenic cross-reactivity is expected to exist between the human herpesviruses and gibbon herpesviruses. Gibbons have antibodies to human herpesviruses that may reflect zoonotic infection with human herpesviruses or infection with indigenous gibbon herpesviruses. Therefore, it is difficult to draw concrete conclusions from serological studies alone. Identification should be based on further isolation and molecular characterization of viruses from seropositive animals.

Background

Gibbons (*Hylobates spp.*) have become valuable animals for zoological, medical and psychological research. Their small size (the smallest of the anthropoids), ease of handling and maintenance in captivity and their close phylogenetic relationship to humans represent only a few of their desirable characteristics as laboratory animals. Gibbons are found throughout the tropical rainforest of South and Southeast Asia, including Thailand, Malaysia and Indonesia. Illegal pet trade is the main cause of the decreasing gibbon population in Thailand. Since gibbons were categorized as a conserved species in Thailand, hundreds of appropriated and abandoned animals have been handed over to the authorities of the Royal Forest Department (RFD). An infectious disease screening process is necessary to interrupt the spread of diseases, including herpesvirus infection. Little is known regarding natural or experimental herpesvirus infections in this interesting arboreal primate and reports of outbreaks or natural disease are still limited. Hence, screening is required to prevent the spread of infectious diseases, including herpesvirus.

Herpesviruses have been isolated from a wide variety of mammalian and non-mammalian species. The eight human herpesviruses, herpes simplex virus type 1 and 2 (HSV-1 and -2), varicella-zoster virus (VZV), Epstein-Barr

virus (EBV), human cytomegalovirus (CMV), and human herpesviruses-6, -7 and -8 (HHV-6, -7 and -8), represent a significant public health problem worldwide. These viruses have been further classified as members of the alpha-herpesvirus (HSV-1, HSV-2 and VZV), the beta-herpesvirus (CMV, HHV-6 and HHV-7) and the gamma-herpesvirus subgroups (EBV and HHV-8). The virus-host relationship is characterized by the benign nature of HSV infection in its usual host, man, and by the fatal disease it causes in accidental hosts, such as the owl monkey or gibbon. Fatal infections caused by HSV have been documented in gibbons, patas and colobus monkeys [1,2]. A natural epizootic model of *Herpesvirus hominis* is splenectomized gibbons, which clinically mimic the disease as it occurs in man [3]. EBV causes 85% of infectious mononucleosis cases, whereas CMV is responsible for most of the remaining cases. Both are mainly transmitted via exchange of saliva. Cells infected with human herpesviruses such as CMV, HSV-1, HSV-2 and EBV express specific virus-encoded receptors capable of binding the F_C domain of IgG [4]. Three major approaches can be employed for herpesvirus diagnosis. The first is isolation of virus from clinical specimens using mammalian cell culture systems and subsequent identification by biological, biochemical and immunological procedures. The second is by identification of anti-herpesvirus antibodies in sera of infected individuals. A third method, PCR, can also be used to amplify and sequence herpesvirus DNA. Although the PCR technique is widely used to detect herpesviruses, we did not utilize it for this study due to the high costs. Since clinical specimens are often unavailable, the first approach is not always possible; the second approach is therefore more routinely used.

In this study, our objective was to determine whether there is evidence of herpesvirus infection in gibbons. Since test kits for gibbon herpesviruses are not currently available, the serological tests were performed using human HSV-1, HSV-2, EBV and CMV strains by detecting IgG antibodies to these viruses. However, due to possible cross-reaction, further studies would need to be performed to differentiate between actual human herpesviruses and indigenous gibbon herpesviruses.

Results

The results of the serological tests on gibbon sera for anti-HSV-1, HSV-2, EBV and CMV show evidence of mixed herpesvirus infections in healthy gibbons as shown in Table 2. There were 22 gibbons positive for HSV-1. These same gibbons were also positive for HSV-2. Anti-herpesvirus antibody was detected in 39 of 78 (50.0%) gibbons tested. Among these, 16 of 39 (41.0%) had antibody to HSV-1 and HSV-2, 8 of 39 (20.5%) had antibody to EBV, 8 of 39 (20.5%) had antibody to CMV, 1 of 39 (2.5%) had antibody to EBV and CMV, 4 of 39 (10.2%) had antibody to HSV-1, HSV-2, and EBV, 1 of 39 (2.5%) had antibody to HSV-1, HSV-2, and CMV and 1 of 39 (2.5%) had antibody to HSV-1, HSV-2, EBV and CMV. The cut-off value (COV), mean and range of positive optical density (OD) were shown in Table 2.

Discussion

Despite the rather limited data on herpesvirus infection in wild animals, our results showed a high prevalence of HSV-1 and HSV-2 infection in gibbons, comparable with previous serological studies on the incidence variation of herpesvirus infections in different species of apes. Of 24 gibbon serum samples tested, 8 (33.3%) were positive and reacted more strongly with the HSV-1 antigen than with any of the other herpesvirus antigens [5].

Both western blot assay and virus neutralization tests were done with sera from 15 gibbons. Antibodies against HSV-1 and HSV-2 were detected in four (26.6%) healthy gibbons [6]. In addition, neutralizing antibodies against HSV were found in 16 of the 84 (19.0%) animals in the colony [3]. Cerebral infarction and myocardial fibrosis were reported in a white-handed gibbon (*Hylobates lar*), which was serologically positive for HSV-1 and EBV [7]. However, the serological tests can not positively determine if this was the cause of illness. Most of the gibbons are healthy, with herpesvirus infection in those animals apparently being in the latent phase.

Isolation of viruses related to HSV from primates is scarce. However, a number of primate species, including apes, have been surveyed for antibodies to many different simian and related human viruses [5]. These studies have reported that the incidence of herpesvirus antibody in gorillas, orangutans, and gibbons is very low, whereas chimpanzees showed a much higher prevalence. In our study, we found that the prevalence for HSV-1 and HSV-2 was equal (28.2%). It has been reported that both viruses share a common antigen, which can cause serologic cross-reaction [8].

Sera obtained from 50 non-human primates and 45 lower mammals were tested for complement fixation (CF) antibodies to partially purified antigens prepared from virus-containing cells of Burkitt's lymphoma cultures. All five non-human primate species tested (chimpanzee, baboon, cynomolgous, rhesus macaque and African green monkey) displayed a high incidence of CF antibodies to EBV, whereas none of the sera from nine species of laboratory or domestic animals reacted with this antigen [9]. The prevalence of herpesvirus antibody among non-human primate is shown in Table 3.

Table 2: Evidence of serological of herpesviruses (HSV-1, HSV-2, EBV and CMV) infection in healthy gibbons (n = 78 cases).

Herpesvirus Serological markers	Positive serological marker No.	(%)	Cut-off value (COV)	Mean positive OD. Range
HSV-1	22	(28.2)	0.118	0.591 0.286–0.692
HSV-2	22	(28.2)	0.093	0.361 0.137–0.607
EBV	14	(17.9)	0.143	0.395 0.203–0.653
CMV	11	(14.1)	0.085	0.213 0.106–0.375
Herpesvirus mixed infection				
HSV-1 and HSV-2	16	(20.5)	-	
HSV-1, HSV-2 and EBV	4	(5.1)	-	
HSV-1, HSV-2 and CMV	1	(1.3)	-	
HSV-1, HSV-2, EBV and CMV	1	(1.3)	-	
EBV and CMV	1	(1.3)	-	

Table 3: Number of animals serological positive/total to herpesviruses in non-human primate sera.

Species	Tested method	HSV-1	HSV-2	EBV	CMV
Gorilla [5]	ELISA, Immunoblot	49/53(92.5%)	4/53 (7.5%)	-	-
Chimpanzee [5]	ELISA, Immunoblot	2/38 (5.3%)	5/38(13.2%)	-	-
Orangutan [5]	ELISA, Immunoblot	5/70 (7.1%)	-	-	-
Gibbon [5]	ELISA, Immunoblot	8/24 (33.3%)	-	-	-
Chimpanzee [5]	Complement-Fixation	-	-	10/10 (100%)	-
Baboon [9]	Complement-Fixation	-	-	2/10 (20%)	-
Cynomolgous [9]	Complement-Fixation	-	-	10/10 (100%)	-
Rhesus [9]	Complement-Fixation	-	-	6/9 (66.7%)	-
African Green [9]	Complement-Fixation	-	-	9/10 (90%)	-
Rhesus [14]	Serum neutralization	67/164 (40.9%)	-	-	-
	Dot-immunobinding	77/164 (47.0%)	-	-	-
Chimpanzee [14]	Fluorescent antibody		-	-	2/8 (25%)
	Dot-immunobinding	-	-	-	5/8 (62.5%)
Gibbon (our study)	ELISA	22/78 (28.2%)	22/78 (28.2%)	11/78 (14.1%)	14/78 (17.9%)

Since it was not possible to use species-specific antibodies in the ELISA test, the negative results obtained for gibbons might be attributable to non-reactivity of their sera with anti-human IgG. There has been evidence of cross-species reactivity as both human and non-human (Old World) primates exhibit close antigenic relationships between their immunoglobulins. In previous studies, both fluorescent-labeled goat anti-human globulin and anti-monkey globulin reacted well with chimpanzee [10] and non-human primate globulins [11]. For this reason, goat anti-human immunoglobulin G (IgG) conjugated with alkaline phosphatase may be used for both human and non-human (Old World) primates based on the close antigenic relationship between their immunoglobulins [12]. Cross-reactivity has also been reported between human and monkey herpesviruses [13]. Hence, it is important to determine whether the antibodies detected in the gibbons in this study are actually directed against human herpesviruses (HSV-1, HSV-2, EBV and CMV) or other related viruses. To resolve this problem, ELISA assays using human herpesvirus and monkey herpesvirus infected cell antigens could be used. The non-human primate herpesviruses are much more similar to each other than to human herpesviruses. Therefore, the sera will react more strongly with

the human than with the non-human primate herpesvirus, suggesting that the gibbon is infected with a human herpesvirus or vice versa.

The animals we screened were at high risk of contracting herpesviruses from humans. For example, hand rearing of gibbon infants by zoo personnel would provide ample opportunity for human herpesvirus transmission to gibbons. Moreover, the exclusive HSV serotypes within species suggests that these viral infections may have originated from a common source within a species, with subsequent spread from animal to animal, rather than from isolated human-to-gibbon transmissions. Accordingly, HSV-2 infections have been observed to spread from chimpanzee to chimpanzee [5].

Based on the high level of cross-reactivity expected to exist between the human herpesviruses likely to be contracted by animals in captivity and any indigenous gibbon viruses, it is difficult to draw any concrete conclusions from serological studies alone. The most convincing approach toward identification of an indigenous gibbon herpesvirus would be virus isolation from seropositive animals and subsequent molecular characterization.

Conclusions

It can be stated that infections with herpesvirus-like viruses are relatively common in gibbons. The viruses found in these gibbons are either human herpesviruses or indigenous gibbon herpesviruses that are antigenically similar to human herpesviruses. Further studies on molecular characterization of gibbon herpesviruses should be performed in order to arrive at concrete conclusions.

Materials and methods

Seventy-eight healthy gibbons kept at the Krabok Koo Wildlife Breeding Center, Royal Forest Department, Cha-Cheng-Sao province, Thailand were included in the study. All of the animals examined, although born in the wild, were raised by humans and have since been kept in captivity at the National Wildlife Research Center. Demographic data for the gibbons are shown in Table 1. Blood samples were obtained by femoral venipuncture during a brief period of anesthesia by Ketamine hydrochloride, according to routine health care procedures. The sera were separated by centrifugation and stored at -70°C until further analysis.

As no screening test is available for gibbon herpesviruses, screening for serum antibodies against HSV-1, HSV-2, EBV and CMV was performed by ELISA using the HUMAN-ELISA-Antibodies-Test (Human, Wiesbaden, Germany). The procedure applied followed the manufactured recommendations. Calculation of control values and cut-off, mean absorbance values of the negative control in wells B1 and C1 (MNC) and the positive control in wells D1 and E1 (MPC) are calculated according to: MNC = $[A_{450}(B1)+A_{450}(C1)]/2$; MPC = $[A_{450}(D1)+A_{450}(E1)]/2$. The cut-off value (COV) is calculated using the formula: COV = MNC+0.1× MPC. The OD more than COV is considered positive.

Table 1: Demographic data of the healthy gibbons at the Krabok Koo Wildlife Breeding Center, Cha-Cheng-Sao province, Thailand.

Species	No.	Sex (Male/Female)	Age [a)] (n) (X +SD) (range)
Hylobates pileatus	19	9/10	15 12.4 ± 3.52
Hylobates lar	58	32/26	30 11.53 ± 4.01 5–21
Hylobates agilis	1	1/0	5

a) only known age

Authors' contributions

Author 1 (KS) carried out the conception and design of the study, collection and assembly of data, analysis and interpretation of the data and drafting of the manuscript. Author 2 (AT) carried out the serological testing. Author 3 (PC) performed assistance in collection of data. Author 4 (PR) participated in the critical revision of the article for important intellectual content. Author 5 (YP) participated in the study design, coordination of the study and request for funding.

All authors read and approved the final manuscript.

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References

1. MR Loomis, O'Neill T, Bush M, Montail J: **Fatal herpesvirus infection in patas monkeys and a black and white colobus monkey.** *J Am Vet Med* 1981, **179:**1236-1239
2. Ramsay E, stair EL, Castro AE, Marks MI: **Fatal *Herpesvirus* hominis encephalitis in a white-handed gibbon.** *J Am Vet Med* 1982, **181:**1429-1430
3. Smith PC, Yuill TM, Buchanan RD, Stanton JS, Chaicumpa V: **The gibbon *(Hylobates lar)*; a new primate host for Herpesvirus hominis I. A natural epizootic in a laboratory colony.** *J Infect Dis* 1969, **120:**292-297

4. Antonsson A, Johansson H: **Binding of human and animal immunoglobulins to the IgG Fc receptor induced by human cytomegalovirus.** *J Gen Virol* 2001, **82:**1137-1145
5. Eberle R, Hilliard JK: **Serological evidence for variation in the incidence of herpesvirus infections in different species of apes.** *J Clin Microbiol* 1989, **27:**1357-1366
6. Mootnick AR, Reingold M, Holshuh HJ, Mirkovic RR: **Isolation of a herpes simplex virus type 1-like agent from the brain of a mountain agile gibbon (*Hylobates agilis*) with encephalitis.** *J Zoo Wild Med* 1998, **29:**61-64
7. Borkowski R, Taylor TG, Rush J: **Cerebral infarction and myocardial fibrosis in a white-handed gibbon (*Hylobateslar.*** *J Zoo Wild Med* 2000, **31:**65-70
8. Pedro NA, Boris S: *Zoonoses and Communicable Diseases Common to Man and Animals (2nd ed.). Pan American Health Organization, Washington, D.C.* 1994
9. Gerber P, Birch SM: **Complement-fixing antibodies in sera of human and non-human primates to viral antigens derived from Burkitt's lymphoma cells.** *Proc Natl Acad Sci USA* 1967, **58:**478-484
10. Levy JA, Levy SB, Hirshaut Y, Kafuko G, Prince A: **Presence of EBV antibodies in sera from wild chimpanzees.** *Nature* 1971, **233:**559-560
11. Kalter SS, Heberling RL, Ratner JJ: **EBV antibody in sera of non-human primates.** *Nature* 1972, **238:**353-354
12. Shuster JJ, Warner NL, Fudenburg HH: **Discussion paper: cross-reactivity of primate immunoglobulins.** *Ann NY Acad Sci* 1969, **162:**195-201
13. Hull RN, Dwyer AC, Holnes AW, Nowakowski E, Deinhardt F, Lennette EH, Emmons RW: **Recovery and characterization of a new simian herpesvirus from a fatally infected spider monkey.** *J Natl Cancer Inst* 1972, **49:**225-231
14. Heberling RL, Kalter SS: **Rapid dot-immunobinding assay on nitrocellulose for viral antibodies.** *J Clin Microbiol* 1986, **23:**109-113