

# Seroprevalence of Human Herpesvirus 6 and 7 Infections in the Thai Population

Uraivan Kositanont,<sup>1</sup> Chantapong Wasi,<sup>1</sup> Namtip Ekpatcha,<sup>1</sup> Anong Poomchart,<sup>2</sup> Sirirut Likanonsakul,<sup>3</sup> Isaaracha Suphanip,<sup>4</sup> Kruavon Balachandra,<sup>5</sup> and Koichi Yamanishi,<sup>6</sup>

Human herpesviruses 6 and 7 (HHV-6 and HHV-7), are T-lymphotropic viruses. HHV-6 was isolated from patients with lymphoproliferative disorders<sup>1</sup> and acquired immune deficiency syndrome.<sup>2</sup> HHV-6 is the causative agent of exanthem subitum (ES).<sup>3</sup> In addition, HHV-6 has been associated with other diseases such as infectious mononucleosis,<sup>4,5</sup> neurological disorders<sup>6,7</sup> and liver dysfunction.<sup>4,8</sup> The possible co-tumorigenic role of HHV-6 has been studied in an NIH-3T3 mouse fibroblast system<sup>9</sup> and in tissue biopsies of Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma.<sup>10</sup>

HHV-7 was first isolated from peripheral blood of a healthy individual.<sup>11</sup> Recently, HHV-7 has been isolated from saliva of healthy children and adults with high frequency.<sup>12-14</sup> HHV-7 infection is not known to be associated with any diseases, although HHV-7 was recently isolated from a patient with chronic fatigue syndrome<sup>15</sup> and from a child with chronic EBV infection.<sup>16</sup> Recently, it was reported that HHV-7 is another causative agent of ES.<sup>17</sup>

The major target of the two viruses is the CD4<sup>+</sup> T cell. HHV-6 and HHV-7 have the ability to establish life-long latency and can be

**SUMMARY** Seroprevalence of human herpesvirus 6 (HHV-6) and 7 (HHV-7) was estimated in the Thai population using indirect immunofluorescence assay to determine serum antibodies to HHV-6 and HHV-7. A total of 333 serum samples obtained from umbilical cord blood and venous blood of healthy persons at Siriraj Hospital and Krabi Hospital during 1990-1993 were investigated. Of 73 infants aged 0-1 month, 73% and 78% were found to be positive for HHV-6 and HHV-7 antibodies, respectively. Antibody to HHV-6 was detected in age groups 2-3 months (38%), 4-5 months (14%), 6 months (44%), 7-11 months (66%), 1-2 year (84%), 3-4 years (82%), 5-9 years (83%), 10-19 years (83%), 20-29 years (80%), 30-39 years (67%), and over 40 years (58%), respectively. The positive rates of HHV-7 antibody in age groups 2-3 months, 4-5 months, 6 months, 7-11 months, 1-2 years, 3-4 years, 5-9 years, 10-19 years, 21-29 years, 30-39 years, and over 40 years were 50%, 21%, 10%, 37%, 47%, 82%, 75%, 72%, 72%, 67%, and 67%, respectively. At 6 months of age as the starting time of infections, 34% (14/41) and 9% (3/41) of infants had presumed primary infections of HHV-6 and HHV-7, respectively. In the follow-up study, 53% (20/38) of children were infected with HHV-6 prior to HHV-7 and only 5% *vice versa*. Eighty-four percent of children had acquired antibody to HHV-6 by 1-2 years old while 82% of children had acquired antibody to HHV-7 by 3-4 years old. These results suggest that HHV-6 and HHV-7 are prevalent viruses in the Thai population. The infections of both viruses begin at 6 months of age. However, infection of HHV-7 in most children begins later. The data also provided evidence that antigenic distinction between HHV-6 and HHV-7 existed with a limited cross-reactivity in an antibody test. The antibody responses to HHV-6 and HHV-7 occurred independently.

reactivated in the host. A study has suggested that HHV-7 resides latently in T cells and that it could be induced from latency by T cell activation. It can also act as a helper virus for the reactivation of HHV-6 from latency.<sup>18</sup> HHV-6 isolates are divided into 2 groups, A and B.<sup>19</sup> HHV-6 group A includes the strains GS, Davilla and U1102. HHV-6 group B includes the strains Z29, HST and SF. In fact, the strains of group A and B show high cross-reactions in antibody testing; either strain can be

From the <sup>1</sup>Department of Microbiology, <sup>4</sup>Department of Obstetrics and Gynaecology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, <sup>2</sup> Krabi Hospital, Krabi Province, <sup>3</sup>Bamrasnaradura Hospital, <sup>5</sup>National Institute of Health, Department of Medicine Sciences, Ministry of Public Health, Thailand, <sup>6</sup>Research Institute for Microbial Diseases and Osaka University Medical School, Osaka University, Osaka, Japan.

Correspondence : Uraivan Kositanont, Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

selected for seroprevalence study. HHV-6 and HHV-7 are prevalent viruses which infect children in very early life. As previously reported by others,<sup>20-27</sup> infection with HHV-6 occurs prior to 2 years of age and HHV-7 infects children later in childhood. On the basis of a limited survey of age-related HHV-7 infection, the aim of this study is to determine the seroprevalence of HHV-7 infection in Thai children and adults. The data provide basic information on HHV-7 infection and are compared to those of HHV-6 infection in Thailand.

## MATERIALS AND METHODS

### Study population

Three hundred and thirty-three sera were obtained from umbilical cord blood, designated as 0 month, and venous blood of healthy Thai people ranging in age from 1 month to 70 years. Serum samples were collected at Siriraj Hospital and Krabi Hospital during 1990 to 1993. Five paired sera for cross-reactivity testing were obtained from ES patients.

### Preparation of viral antigens

HHV-6 (HST strain) was isolated in the laboratory from a patient with ES as described previously<sup>3</sup> and HHV-7 (RK strain) was kindly provided by Dr N Frenkel.<sup>11</sup> HHV-6 was grown in MT-4 cells<sup>28</sup> and HHV-7 was grown in SUP-T1 cells.<sup>29</sup> The culture medium was RPMI 1640 medium with fetal calf serum [10%], glutamine [2mM], and kanamycin [100 µg/ml]. Cytopathic effect (CPE) of both viruses showing balloon-like syncytial cells was observed. Approximately 30-50% of cell population were infected as measured by CPE and indirect immunofluorescence assay (IFA) at 5-9 days postinfection. The cells were harvested, washed with phosphate buffer saline, and smeared on glass slides.

### Antibody test by indirect immunofluorescence assay

The cells on glass slides were fixed in cold acetone at -20°C for 10 minutes. The fixed cells were incubated with human sera for 1 hour at 37°C and then with fluorescein isothiocyanate-conjugated goat anti-human IgG (Dako, Denmark) for 1 hour. The sera were serially diluted from 1:10 to 1:320. The fluorescence was visualized on slides under fluorescence microscope (Nikon, Japan) and the titer was defined as reciprocal of the last dilution which gave a positive reaction. A titer of  $\geq 10$  was considered as a positive result.

## RESULTS

### Reactivity of antibodies to HHV-6 and HHV-7 in paired sera from ES patients

Antibody titers to HHV-6 and HHV-7 in five paired sera from ES patients are compared in Table 1. All 5 paired sera showed a four-fold rise in titers of anti-HHV-6. Antibody to HHV-7 was detected in these convalescent sera with a low titer of 10. This observation revealed that

there was an antigenic distinction between HHV-6 and HHV-7 with limited cross-reactivity.

### Prevalence of HHV-6 and HHV-7 antibodies in children and adults

The results of sera from 333 individuals assayed by IFA for antibodies to HHV-6 and HHV-7 are shown in Table 2. The seropositive rate of HHV-6 infection was lowest in the age-group 4-5 months (2/14, 14%). The seropositive rate started to increase to 44% at the age of 6 months. After 6 months of age, the prevalence of antibody increased to 66% (25/38) in the age-group 7-11 months. It rose rapidly to 84% (41/49) at 1-2 years of age. The prevalence of antibody was maintained at 82% (9/11), 83% (20/24), 83% (20/24), 83% (15/18) and 80% (20/15) at 5-9, 10-19 and 20-29 years of age, respectively. The seropositivity rate declined gradually to 67% (8/12) and 58% (7/12) in age-groups of 30-39 and over 40 years, respectively.

The prevalence of anti-HHV-7 was also analyzed. The data were similar to those of HHV-6 antibody. There was a decline in seropositive

Table 1. Antibody of HHV-6 and HHV-7 in paired sera from ES patients.

Case No.	Serum phase	Titers of antibody to	
		HHV-6	HHV-7
1	acute	< 10	< 10
	convalescent	80	10
2	acute	< 10	< 10
	convalescent	40	10
3	acute	< 10	< 10
	convalescent	80	10
4	acute	< 10	< 10
	convalescent	160	10
5	acute	< 10	< 10
	convalescent	80	10

**Table 2.** Prevalence of HHV-6 and HHV-7 antibodies in healthy individuals.

Age	No. of sera	HHV-6		HHV-7	
		No. (%) seropositive	GM titer	No. (%) seropositive	GM titer
0-1 month	73	53 (73)	21.2	57 (78)	17.3
2-3 months	16	6 (38)	10.4	8 (50)	11.4
4-5 months	14	2 (14)	6.4	3 (21)	6.4
6 months	41	18 (44)	19.3	4 (10)	6.3
7-11 months	38	25 (66)	37.9	14 (37)	11.4
1-2 years	49	41 (84)	100.3	23 (47)	29.3
3-4 years	11	9 (82)	48.3	9 (82)	51.5
5-9 years	24	20 (83)	49.0	18 (75)	16.8
10-19 years	18	15 (83)	14.7	13 (72)	15.9
20-29 years	25	20 (80)	18.4	18 (72)	17.9
30-39 years	12	8 (67)	12.6	8 (67)	14.1
≥40 years	12	7 (58)	10.0	8 (67)	10.6
Total	333	226 (68)		186 (56)	

rate of HHV-7 antibody from 78% at 0-1 month of age to 10% (4/41) at the age of 6 months. The prevalence of antibody increased to 37% (14/38) and 47% (23/49) in age-groups 7-11 months and 1-2 years, respectively. The highest seropositivity rate was 82% (9/11) in age-group 3-4 years and the value maintained through age-group 10-29 years. The seropositive rate declined to 67% (8/12) in age-group over 30 years. The seroprevalence of HHV-6 and HHV-7 infections in children and adults was 68% (226/333) and 56% (186/333), respectively.

The geometric mean (GM) titers of anti-HHV-6 and HHV-7 are shown in Table 2. The GM titers of HHV-6 antibody increased rapidly at 6 months of age and reached the highest GM titer of 100.3 in age-group 1-2 years. Although the GM titer decreased to 48.3 and 49.0 between age-groups 3-4 years and 5-9 years, they were considerably

higher than that of 14.7 in the age-group 10-19 years. In contrast to HHV-6, GM titers of HHV-7 antibody rose gradually to 11.4 at 7 months, rapidly reached a peak of 51.5 at 3-4 years, and then declined to 16.8 in the age group 5-9 years.

#### Age of acquired antibodies to HHV-6 and HHV-7

Distribution of HHV-6 and HHV-7 antibody titers is shown in Fig. 1a and 1b. Seventy-four percent and 79% of infants aged 0 month had antibodies to HHV-6 and HHV-7, respectively, which reflected to be transferred antibodies from mothers. Transplacental antibody to HHV-6 decreased to the lowest levels at 4 and 5 months of age and HHV-6 infection began at 6 months of age. In Fig. 1b, the maternal antibody to HHV-7 declined to the lowest level at 5 months of age. At 6 months of age, 34% (14/41) and 9% (3/41) of infants whose antibody titers were  $\geq 40$

showed presumable primary infections of HHV-6 and HHV-7, respectively. Almost all children (84%) had acquired antibody to HHV-6 by 1-2 years old while 47% of children had acquired antibody to HHV-7. Most children (82%) had acquired antibody to HHV-7 at 3-4 years of age.

#### Follow-up study of HHV-6 and HHV-7 infections

To clarify the infections of HHV-6 and HHV-7 in early age of life, a follow-up study of 38 children aged 1-18 months was carried out. Data in Fig. 2 show seroconversion patterns of HHV-6 and HHV-7 infections. It is clear that most children 53% (20/38) were infected with HHV-6 prior to HHV-7 (pattern P1). Only 5% (2/38) of children had experienced HHV-7 prior to HHV-6 (pattern P2). In 34% (13/38) of children, the data could not show the infection time of both viruses (pattern P3). Pattern P4

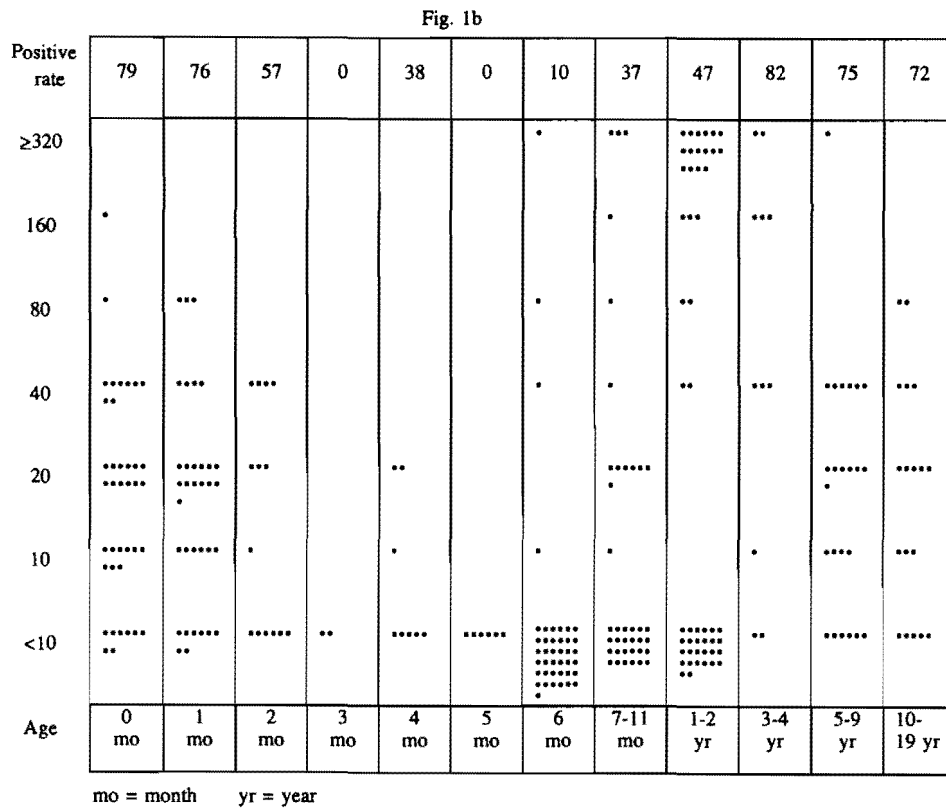
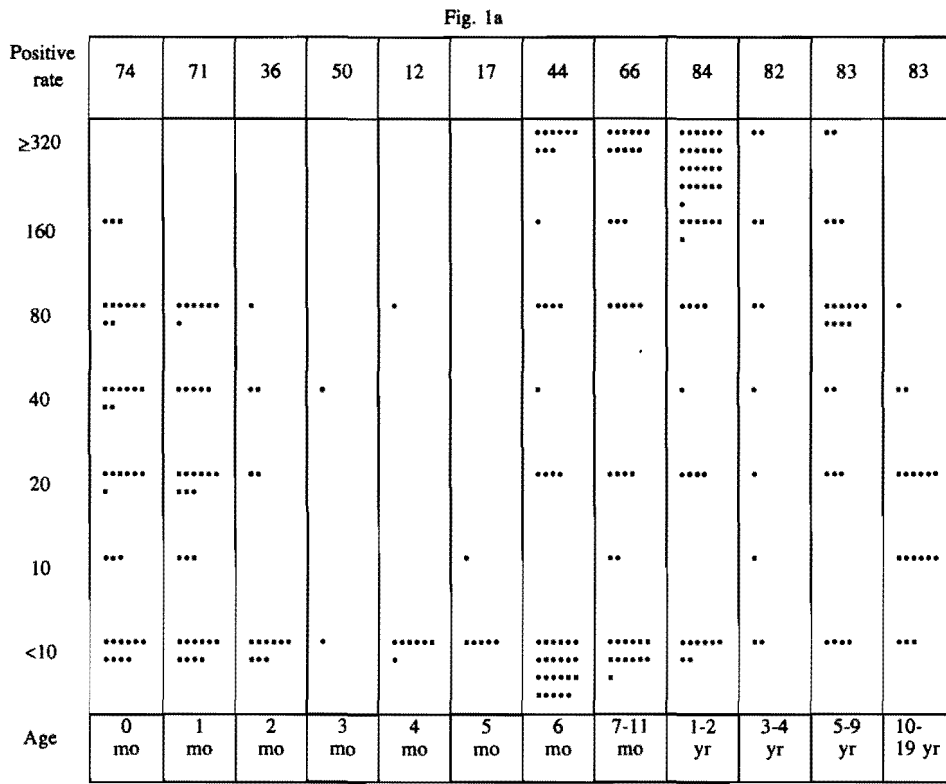
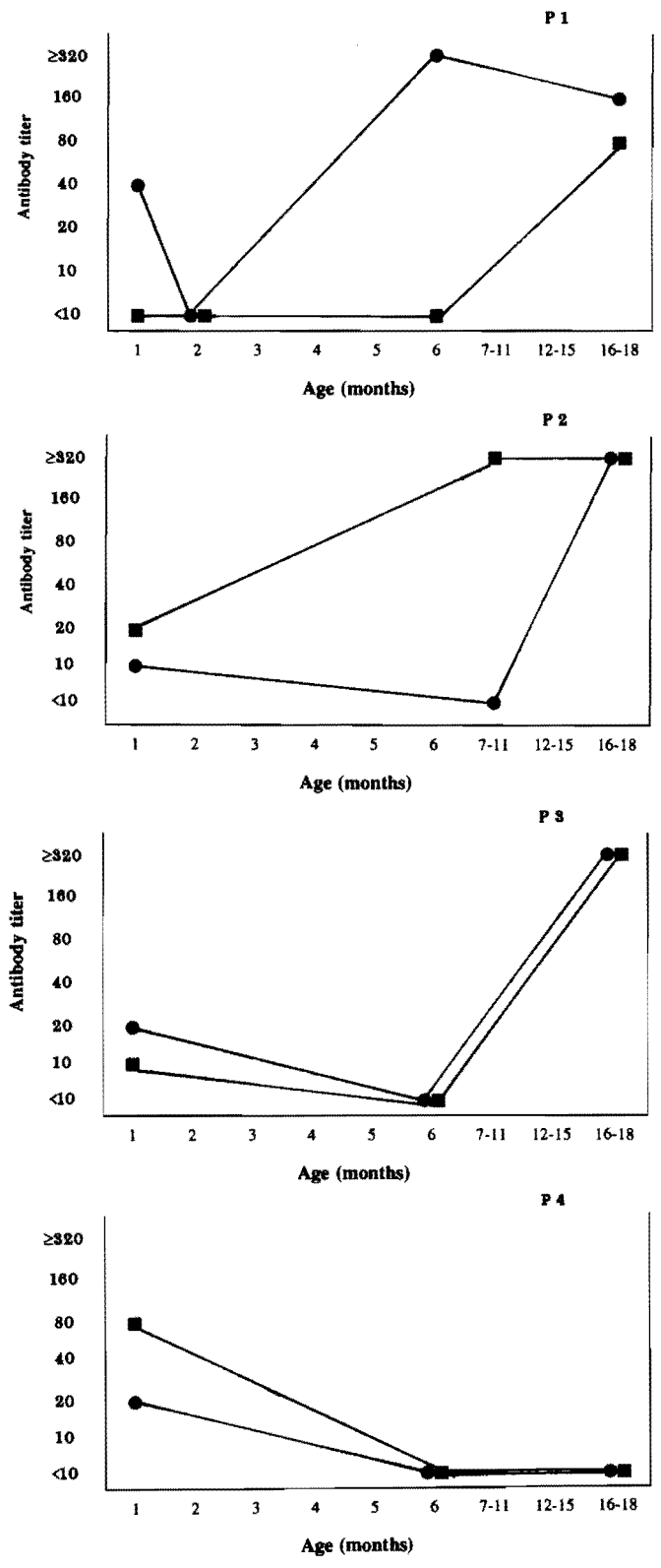


Fig. 1 Distribution of HHV-6 antibody titers (Fig. 1a) and HHV-7 antibody titers (Fig. 1b) in various age groups.



**Fig. 2** Seroconversions to HHV-6 and HHV-7 in children in the follow-up study. Filled circle: (●) HHV-6 antibody and filled square: (■) HHV-7 antibody.

demonstrated that 13% (5/38) of children were not infected with both viruses throughout 18 months of age. The data in Fig. 2 also shows the discordant presence of HHV-6 and HHV-7 antibodies in same sera. Some sera from children whose maternal antibodies declined showing high HHV-6 antibody titers of  $\geq 320$  were negative for HHV-7 antibody. Some children without detectable anti-HHV-6 had acquired antibody to HHV-7. It was confirmed that antibody responses to HHV-6 and HHV-7 occurred independently.

**DISCUSSION**

HHV-6 and HHV-7, two recently discovered viruses, are T-lymphotropic human herpesviruses. The molecular, immunological, and biological properties of HHV-7 are related to HHV-6, but the two viruses differ. Although Southern blot analysis with DNA probes of HHV-6 revealed homology to HHV-7 for 37.4% of the total probe length,<sup>29</sup> immunologic properties were different.<sup>25</sup> The finding in this study also supports the evidence of immunological distinction between HHV-6 and HHV-7 with a limited cross-reactivity (Table 1). The antibody responses to HHV-6 occurred independently since sera from some children had high anti-HHV-6 titers without detectable anti-HHV-7 and *vice versa* (Fig. 2). Furthermore, the results from this study show definitely that some children had experienced HHV-7 infection prior to HHV-6 infection (Fig. 2).

Several studies regarding HHV-6 and HHV-7 seroepidemiology in different countries demonstrate that HHV-6 and HHV-7 are prevalent viruses in the population. The seroprevalence of HHV-6 infection in this study was 68%. These data were similar to those reported in previous studies in Thai population<sup>27</sup> and in other countries.<sup>20,22-24</sup> However, both studies in Thailand

showed higher seropositivity than that in another study<sup>21</sup> due to different serum dilution screening. The seroprevalence of HHV-7 infection of healthy adults in this first study in Thailand was 72% (18/25). These data were not different from previous studies in other countries.<sup>25,26</sup>

At birth, 74% and 79% of cord blood samples were positive for HHV-6 and HHV-7 antibodies, respectively, due to the presence of maternal antibody. The positive rates of HHV-6 and HHV-7 infections decreased from birth to their lowest values at 4-5 months and at 6 months of age, respectively, because of the half-life of IgG class in transplacental antibody. The data here indicated that HHV-6 infection began at 6 months of age while HHV-7 infection in most children occurred later. The data of HHV-6 infection related to the results reported previously<sup>30</sup> showed that ES occurred in infants aging from 3 months to 1 year and most frequently at age 4-6 months. The acquired antibody to HHV-6 which reached 84% at 1-2 years of age agreed with those reported previously.<sup>20-24,27</sup>

The finding in this study that HHV-7 infection which began at 6 months agreed with findings from previous reports.<sup>14,26</sup> However, these data were in contrast to the children at 15-25 months without seropositivity for HHV-7.<sup>25</sup> Interestingly, the GM titers of anti-HHV-6 and seropositive rates were maintained at high values in age group 3-4 years and 5-9 years (Fig. 1). The explanation of these results may be that some viruses such as HHV-7<sup>18</sup> and dengue virus<sup>31</sup> can also act as helper viruses for the reactivation of HHV-6 from latency.

In conclusion, the results confirm the evidence that HHV-6 and HHV-7 are prevalent viruses with widespread infections in children and adults. Primary infection of the two viruses occurs in early life.

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