Seroprevalence of antibodies against human herpesvirus 6 in the Quebec City area

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Seroprevalence of antibodies against human herpesvirus 6 was determined in a sample of 303 randomly selected individuals from the Quebec City area. The influence of different variables on antibody titres was also evaluated. Human herpesvirus 6 was grown in the HSB-2 cell line, and antibody titres were measured by indirect immunofluorescence. Serum samples were collected from 177 females and 126 males ranging in age from two months to 88 years. Ninety-nine per cent (300 of 303) of this population had an antibody titre of at least 1:10, whereas 75% had a titre of at least 1:80. Women had a higher geometric mean titre than men (P=0.06). This difference between sexes varied according to age and became statistically significant in subjects older than 20 years of age (P=0.04). It was found that this difference was attributable to higher antibody titres in women in the 15 to 40 year age group who had previously had children.

Key Words: Exanthem subitum, Human herpesvirus 6 (HHV6), Indirect immunofluorescence, Roseola, Seroprevalence

Séroprévalence des anticorps anti-virus herpes hominis 6 dans la région de Québec

RÉSUMÉ: On a déterminé la séroprévalence des anticorps anti-virus herpes hominis 6 dans un échantillon aléatoire de 303 personnes issues de la région de Québec. L'influence de diverses variables sur le titre des anticorps a également été évaluée. Le virus a été cultivé sur une lignée cellulaire de type HSB-2 et les titres d'anticorps ont été mesurés par immunofluorescence indirecte. Les échantillons de sérum ont été prélevés chez 177 femmes et 126 hommes dont l'âge variait entre 2 mois et 88 ans. Quatre-vingt-dix-neuf pour cent (300 sur 303) de cette population présentaient un titre d'anticorps supérieur ou égal à 1:10, alors que le titre était supérieur ou égal à 1:80 dans 75% des cas. La moyenne géométrique des titres était plus élevée chez les femmes que chez les hommes (P=0.06). Cette différence entre les sexes variait selon l'âge et devenait significative chez les sujets âgés de plus de 20 ans (P=0.04); on a établi qu'elle était imputable à des titres d'anticorps supérieurs chez les femmes appartenant au groupe d'âge des 15 à 40 ans ayant eu des enfants.
In 1986, SalahuDDin et al. (1) described a new human herpesvirus isolated from patients with various lymphoproliferative disorders and the acquired immune deficiency syndrome (AIDS). Ultrastructural studies have shown that this 160 to 200 nm DNA enveloped virus, with an icosahedral nucleocapsid made up of 162 capsomeres, belongs to the Herpesviridae family (2). According to DNA hybridization studies, the double-stranded DNA genome (110 kilobase pairs) is distinct from the DNA of other herpesviruses (3,4). It is also biologically and immunologically distinct from herpes simplex virus, cytomegalovirus, Epstein-Barr virus and varicella-zoster virus.

Initially called 'human B cell lymphotropic virus', this agent was redesignated 'human herpesvirus 6' because of an expanded cell tropism (5,6). In fact, human herpesvirus 6 can infect fresh mononuclear cells, T and B lymphocytes, and other human cells (megakaryocytes and glioblastoma cells) (7,8). In addition, human herpesvirus 6 predominantly infects mature CD4+ T lymphocytes (9) and exerts a strong cytopathic effect on them (5), although CD4 is not the membrane receptor for human herpesvirus 6 (10).

This virus has been causally linked, by viral isolation and seroconversions, to exanthem subitum (roseola) by Yamanishi et al (11). Another research team reported possible serological association with sarcoidosis, malignant lymphoma and, to a lesser extent, Sjögren's syndrome (12). Furthermore, the fact that human herpesvirus 6 and human immunodeficiency virus type 1 (HIV-1) can co-infect human CD4+ T lymphocytes in vitro suggests a role for human herpesvirus 6 as a cofactor in AIDS (13,14), although this issue is still debated (15,16). Recently, Dubedat and Kappagoda (17) reported human herpesvirus 6 as a possible cause for hepatitis. Except for roseola, all other disease associations are purely speculative.

Knowledge of the epidemiology of human herpesvirus 6 is essential if one is to understand its natural history and evaluate the role of this virus in different clinical settings. Highly discrepant results have been reported in seroprevalence studies (1,18-26). In these reports, prevalence of antibodies against human herpesvirus 6 has ranged from 2 to 95%.

The authors determined the presence of antibodies against human herpesvirus 6 in 303 randomly selected individuals from the Quebec City area. The importance of different variables (age, sex, parity) on antibody titres was also evaluated.

**MATERIALS AND METHODS**

**Viral culture:** Human herpesvirus 6 (Dv strain) (19) was propagated in the HSB-2 cell line (ATCC CCL 120.1), a T lymphoblastoid cell line established from the bone marrow of a patient with acute lymphoblastic leukemia. The technique used in this study is a modification of that established by Ablashi and collaborators (7) and modified by one of the authors (27). Briefly, actively growing HSB-2 cells maintained at a concentration of 1 to 2 x 10^5 in RPMI 1640 medium (Flow Laboratories Inc) were pretreated with hydrocortisone phosphate (5 μg/mL) (Merck, Sharp & Dohme, New Jersey) and mixed with previously infected (for seven days) HSB-2 cells. The ratio of infected to uninfected cells was fixed at 1:10. Culture were maintained at 37°C in a carbon dioxide incubator, and two-thirds of the medium was changed after three days.

**Immunofluorescence method:** Human herpesvirus 6 antibody titres were measured in infected HSB-2 cells using an indirect fluorescent antibody technique (27). Decomplemented serum samples (25 μL per well) were incubated with antigen preparation for 30 mins at 37°C. Washing was carried out in three changes of phosphate-buffered saline (2 mins each) followed by air-drying. Afterwards, fluorescein isothiocyanate (FITC) goat anti-human immunoglobulins (Pasteur Institute, France) diluted 1:400 in phosphate-buffered saline and mixed with eriochrome black T (2.83x10^-3 M, diluted 1:200) (Sigma, Missouri), was added. Incubation and washings were as above. Titres of antibodies were expressed as the highest serum dilution yielding detectable and specific immunofluorescence. Positive and negative serum controls were included in each series of tests. Mock-infected HSB-2 cells were also used as negative cell controls.

**Serology for cytomegalovirus and Epstein-Barr virus:** The same sera were also tested for cytomegalovirus and Epstein-Barr virus viral capsid antigen antibodies. Antibody determinations for cytomegalovirus and viral capsid antigen were also performed by indirect fluorescent antibody test according to standard procedures (28,29).

**Serum sampling:** Random digit dialing was used to obtain a sample of the Quebec City area population. A research nurse initially contacted selected individuals and explained the purpose of the study. A particular telephone number would be abandoned after three failed attempts made at different times. Individuals that agreed to participate in the study were visited at home by the same nurse and serum samples were collected after informed consent. All individuals living in a given household were eligible for the study. All participants responded to a short questionnaire focusing on various demographic parameters such as age, sex, race, civil status, type of household, occupation and day care centre attendance. Serum samples were preserved at -20°C until use.

**Statistical analysis:** Data were analyzed using Statistical Analysis System (SAS) software (SAS Institute, North Carolina). Student's t test was used to evaluate differences between geometric mean titres, and linear regression modelling was used to analyze geometric mean titres with respect to sex and age groups. Values were expressed as mean ± standard error. Possible
TABLE 1
Distribution of subjects in study according to age and sex

<table>
<thead>
<tr>
<th>Age (years)</th>
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<th>Males</th>
<th>Total</th>
<th>Percentage of total</th>
</tr>
</thead>
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<td>21</td>
<td>6.9</td>
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<tr>
<td>≥60</td>
<td>22</td>
<td>13</td>
<td>35</td>
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</tr>
</tbody>
</table>

Results

Serological cross-reactions between human herpesvirus 6 and cytomegalovirus or Epstein-Barr virus were assessed by correlation analysis of antibody titres against these viruses.

**RESULTS**

**Selected population:** A total of 1200 numbers were called: 760 resulted in household contacts. The 440 remaining numbers were connected either to industrial or commercial outlets or were out of service. Of the 760 household contacts, 161 (21%) agreed to participate in the study; all subjects were living within 30 km of the Quebec City area.

From these households, 303 serum samples were collected between February and May 1988 from 177 females and 126 males. Subjects ranged in age from two months to 88 years with a median age of 37 years (mean 36.9 years). The 20 to 49 years group accounted for 65.3% of the population sample; the remainder were evenly distributed in the other age groups (Table 1). All but three subjects were Caucasian.

**Seroprevalence:** Seropositivity rates varied according to the cut-off dilutions selected. When a titre greater or equal to 1:20 was considered positive for human herpesvirus 6, 99% (300 of 303) of the population was seropositive. When the cut-off was considered to be greater or equal to 1:40 or 1:80, seropositivity rates were 96%, 88% and 75%, respectively.

Serological results were analyzed using geometric mean titres. The crude geometric mean titre was 112.5. Women had a higher geometric mean titre than men (124 versus 98; P=0.06, Student’s t test).

Differences in geometric mean titres between males and females varied according to age. In individuals younger than 20 years, nearly identical geometric mean titres were observed in men and women (133.7 versus 131.7; P=0.97, Student’s t test). In subjects older than 20 years of age, geometric mean titres were highly variable between males and females (92.8 versus 122.8; P=0.04, Student’s t test). In those older than 30 years a similar difference was observed (85.4 versus 122.2; P=0.02, Student’s t test). Interestingly, although geometric mean titres in women tended to remain constant over time, a steady and statistically significant decline in titres was observed in men with advancing age (linear regression P=0.05) (Figure 1). To explain the differences in geometric mean titres between men and women, the possible role of several variables was investigated. Previous pregnancy was the only variable that was somewhat related to this phenomenon. Indeed, women who had been pregnant previously tended to have a higher geometric mean titre than women who had not experienced full term pregnancy (Figure 2). In the 15 to 40 years age group, women who had never been pregnant had a geometric mean titre similar to that of men (102 for nulliparous women versus 98 for men; P=0.9, Student’s t test), whereas previously pregnant women had a geometric mean titre significantly higher than that of men (146; P=0.05, Student’s t test).

The geometric mean titre for day care centre attendees was much higher than that found for children staying home (403 versus 103, respectively P=0.08, Student’s t test). The lack of statistical significance is probably due to the small number of children young enough to attend day care centres (11 subjects).

Analysis of other factors did not disclose any particular trend. Geometric mean titres were similar in health care and other occupations (P=0.96). Marital status and area of residence (rural versus urban) did not influence geometric mean titres.

**Epstein-Barr virus and cytomegalovirus seroprevalences:** Seroprevalences of antibodies against Epstein-Barr virus and cytomegalovirus were 88% and 67%, respectively, similar to titres reported in equivalent populations. Antibody titres against these two viruses were similar in males and females. The presence of antibodies against human herpesvirus 6 did not correlate with the presence or the quantity of antibodies against Epstein-Barr virus (r=0.03; P=0.4877) or cyto-
megalovirus (r=0.04; P=0.5935), suggesting that a positive antihuman herpesvirus 6 indirect fluorescent antibody was quite specific and unlikely to be related to exposure to Epstein-Barr virus or cytomegalovirus.

**DISCUSSION**

Knowledge of the prevalence of antibodies against human herpesvirus 6 in a randomly selected population is essential to the understanding of the natural history of the infection caused by this virus. In the present study, subjects were selected by random digit dialing, a procedure that is widely used in epidemiological research. This selection procedure allows a similar probability of participation to nearly all citizens, since over 98% of Canadian households do have a telephone (30).

Using this method, the authors were able to recruit 303 participants to the present study. The age and sex distribution of the selected individuals was slightly different than that of the general population of the Quebec City area. Indeed, women were over-represented in the present sample (female to male ratio of 1.4 in the sample versus 1.1 in the general population), whereas subjects younger than 20 years of age were under-represented (14.5% in the sample versus 25.5% in the general population) (31). Individuals between 20 and 50 years of age were slightly over-represented, and the proportion of subjects older than 50 years of age was similar to that of the reference population. The voluntary nature of this study probably explains the small number of infants and children. The under-representation of male subjects is more difficult to assess, but a similar phenomenon has previously been reported in another telephone survey where male subjects more readily declined participation (32).

Despite these differences, the subjects that did agree to participate in the study came from diverse backgrounds and socio-economic statuses. In addition, seropositivity rates against cytomegalovirus and Epstein-Barr virus (67% and 88%, respectively) were similar to those reported in other North American populations (33-35).

The present results suggest that a large segment of the population has previously been exposed to human herpesvirus 6. However, because clinical correlations were impossible in this study and because viral neutralization assays were not performed, it is difficult to set a relevant cut-off dilution for positive and negative serum samples. Because of this, seropositivity rates are presented at different dilutions (1:10, 1:20, 1:40 and 1:80). Nevertheless, the present results are generally higher than those previously reported, even at the 1:40 dilution.

Differences in antibody titres between men and women have been suggested previously (18,36). In the present study, analysis of geometric mean titres clearly demonstrates that, for subjects older than 20 years of age, antibody titres are higher in women than in men. In addition, trends in antibody titres were different in men versus women. A significant drop in antibody titres was seen below the age of 60 years in men (linear regression: P=0.05), whereas a similar drop could not be found in women (Figure 1). For both sexes, however, an increase in geometric mean titres was noted in subjects older than 60 years of age. Such a phenomenon could be due to reactivation or reinfection with the virus. Indeed, immunity is known to decrease with age.

The importance of various factors to explain the difference between the sexes was examined. The only factor that could be identified as significant was previous pregnancy. When previously pregnant women were compared with those that had never been pregnant, geometric mean titres were found to be different (Figure 2). However, when nulliparous women were compared with men, no differences could be found in geometric mean titres. These results strongly suggest that previous pregnancy has a major impact on antibody titres against human herpesvirus 6.

The exact reason for differences in geometric mean titres between previously pregnant and nonpregnant subjects is currently unknown. It is possible that human herpesvirus 6, like other herpesviruses, is reactivated during pregnancy with a subsequent increase in antibody titres. Balachandra et al (25) have reported that the prevalence of antibodies against human herpesvirus 6 in pregnant and age-matched nonpregnant women is similar in Thailand. Information on previous pregnancy was, however, not provided for controls in their study. A second explanation for these differences may relate to a greater contact between mother and child especially during early infancy, the time at which most individuals appear to be initially infected by human herpesvirus 6. Unfortunately, the present ques-

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**Figure 2** Geometric mean titres of antibodies to human herpesvirus 6 in previously pregnant and nulliparous women. ■ Nulliparous; □ Females
tionnaire did not provide information regarding time elapsed between the last pregnancy and the date of serum sampling. Transmission of cytomegalovirus from infant to mother has previously been reported (37), and a similar situation might be true for human herpesvirus 6.

Correlation analysis did not show any relationship between antibody titres against cytomegalovirus or Epstein-Barr virus and human herpesvirus 6. Furthermore, despite findings of similarities between genomes of human herpesvirus 6 and cytomegalovirus (38), serological cross-reactions between these two viruses have not been demonstrated conclusively (39,40). The few such cases previously reported could represent dual infections, concomitant reactivation of human herpesvirus 6 or stimulation of human herpesvirus 6-specific memory cells with subsequent augmentation of antibody titres (41).

Although the prevalence of antibody against human herpesvirus 6 was similar in day care and non-day care centre attendees, the antibody titres were much higher in the former group. Numerous infections are known to be transmitted in these centres. Geometric mean titre in these children may suggest prolonged contact with the virus and subsequently higher antibody titres. Additional studies in day care centres are warranted.

In conclusion, it appears that human herpesvirus 6 infection, like other herpesvirus infections, is frequent in the Quebec City area, are acquired relatively early in life. Antibody titres are significantly higher in women who have previously had children. Antibody titres declined with age in men.

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