Human Herpesvirus 8 seroprevalence among internationally adopted children coming from Eastern Europe

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SUMMARY

The seroprevalence of Human Herpes Virus 8 (HHV8) and its transmission pattern were assessed testing serum samples of 120 internationally adopted children (aged 1-15 years) coming from Eastern Europe. Determinations of IgG antibodies against both latent and lytic HHV-8 antigens were performed by indirect immunofluorescence assay. Antibodies were detected only for lytic antigen of the virus in 12.5% of children with a seroprevalence significatively higher (19.6%) in young children (age 1-6). No correlation was observed between HHV8 seropositivity and serological markers for hepatitis A, B and C viruses and Human Immunodeficiency virus.
In conclusion, our findings suggest that HHV8 infection is widespread in some populations from the East Europe, and that person to person contacts among children could be considered the predominant mode of HHV8 transmission in younger age.

KEY WORDS: Adopted children, Eastern Europe, HHV8, Seroepidemiology

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INTRODUCTION

Seroepidemiological studies have shown that the spread of HHV8 infection varied in different geographic areas and populations. Among general population the seroprevalence of HHV8 ranges between 40-50% in sub-Saharan Africa and 2-5% in the United States and in North Europe (Martin, 2003). In the Mediterranean countries, including Italy, the HHV8 infection is relatively common, although wide geographic variations were reported within this area (Stratigos *et al.*, 1997, Perna *et al.*, 2000, Gambus *et al.*, 2001, Santarelli *et al.*, 2001).

Data about HHV8 prevalence and/or KS incidence are still extremely limited for Eastern European populations, although high seroprevalence rates in immigrant and refugee populations from Albania and Kosovo were reported recently (Graffeo *et al.*, 2003, Schinaia *et al.*, 2004, Chironna *et al.*, 2006).

A main point of debate is how HHV8 is transmitted from infected to uninfected persons. Originally HHV8 infection was associated with high-risk sexual practices, particularly among homosexual males either HIV positive or not, and there was some evidence pointing to sexual transmission of HHV8 in general adult population (Perna *et al.*, 2000, Melbye *et al.*, 1998, Martin *et al.*, 1998). However, reports of elevated rates of HHV8 infection among children show that non-

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sexual routes of viral transmission could play a major role particularly in developing regions where the virus appears to be widespread and social and hygienic condition are scarce (Perna *et al.*, 2000, Mayama *et al.*, 1998, Andreoni *et al.*, 1999, Mbulaiteye *et al.*, 2005).
The exact route of transmission to children remains to be determined, but infectious HHV8 has been detected in saliva suggesting this as a possible source of horizontal transmission (Vieira *et al.*, 1997, Vitale *et al.*, 2000, Corey *et al.*, 2002).
To clarify the epidemiology of this virus, the aim of the present study was to assess seroprevalence of HHV8 among internationally adopted children coming from different countries of eastern Europe, and its association with other viral infections (Hepatitis virus A, B, and C and Human Immunodeficiency Virus).

STUDY POPULATION AND METHODS

The study population consisted of 120 internationally adopted children evaluated between April 2002 and May 2006 at the International Adoption Centre of the Pediatrics Department of Palermo University.
All of the children came from Eastern Europe: Ukraine (n= 72), Russia (n=21), Poland (n=8), Hungary (n=6), Latvia (n=5), Romania (n=4), Bulgaria (n=2), and Macedonia (n=2). The median age of the children was 6 years (range: 18 months -15 years), 55 children were girls and 65 were boys.
All of them resided in an orphanage in their original country before adoptions and they were evaluated within 4-6 weeks of their arrival in Italy. The health and immunization status of these children was previously evaluated (Viviano *et al.*, 2006, Cataldo *et al.*, 2007).
Antibodies to latent and lytic antigens of HHV8 were detected using an immunofluorescence assay (IFA) based on BCBL-1 cell line as previously described (Perna *et al.*, 2000). Samples were considered positive if reactive at diluition ≥1:120.
Serum samples were also tested for hepatitis A virus (HAV) total antibodies, hepatitis B virus (HBV) core antibody (HBcAb), hepatitis C virus (HCV) antibodies and Human Immunodeficiency Virus (HIV) antibodies, using respectively specific and standardized enzyme-linked immunosorbent assay (*Vitros*®-Ortho Clinical Diagnostics-Amersham UK).
Informed consent to partecipate in the study was requested to adoptive parents of the children.
Statistical analysis were performed with Epi-Info software. Fisher's exact test was used to compare percentages. If a *P*-value was <0.05, the difference between percentages was considered statistically significant.

RESULTS

The distribution of HHV8, seroprevalence in adopted children, classified by age and gender, is shown in Table 1.
Fifteen subjects out 120 tested positive for HHV8 (12.5%) and seroprevalence observed in females was lower than in males (7.2% vs 16.9%) although this difference was not statistically significant (p= 0.093). Of the 15 children HHV8 positive, 8 were from Ukraine, 3 from Russia, 2 from Bulgaria, 1 from Macedonia and 1 from Latvia.

TABLE 1 - *Seroprevalence of HHV8 infection in 120 adopted children divided by age and by sex.*

Age group (years)	*N° of sera tested*	*N° of males/ N° of females*	*HHV8 positive sera Total*		*HHV8 positive sera Males*		*HHV8 positive sera Females*	
			N°	*(%)*	*N°*	*(%)*	*N°*	*(%)*
1-6	61	37/24	12	(19.6)*	9	(24.3)	3	(12.5)
7-15	59	28/31	3	(5.0)*	2	(7.1)	1	(3.2)
Total 1-15	120	65/55	15	(12.5)	11	(16.9)°	4	(7.2)°

*p= 0.015 compared with each other (1-6 versus 7-15 age groups); °p= 0.093 (males versus females)

TABLE 2 - *Prevalence of HAV, HBV, HCV and HIV antibodies (A) and prevalence of coinfection with HHV8 (B) in 120 adopted children.*

Viral infection	*A - Pos/tested*		*B - HHV8 positive/positive for other infections*		
	N°	*(%)*	*N°*	*(%)*	*P-value*
HAV	32/120	(26.6)	2/32	(6.3)	0,126
HBV*	12/120	(10)	1/12	(8.3)	0,35
HCV	1/120	(0.8)	0/1	(0)	-
HIV	0/120	(0)	0/0	(0)	-

*Presence of antibodies to HBV core antigen

All the 15 children HHV8 positives were seroreactive only for lytic antigen of the virus.
HHV8 positivity showed a decreasing trend with age, ranging from 19.6% for age 1-6 years to 5.0% among children aged 7-15 (p value = 0.015).
Table 2 shows the prevalence of HAV, HBV, HCV and HIV antibodies among 120 children studied. Thirty-two children out 120 were positive for HAV antibodies (26.6%). Antibodies to hepatitis B core antigen (anti HBc) were found in 12 children (10%). Only one child had HCV antibodies and none tested positive for HIV antibodies.
Two of the HHV8 positive children were also HAV positive (p value = 0.126), whereas just one tested anti-HBc positive (p value = 0.35) and none showed a concomitant HCV and/or HIV infection.

DISCUSSION

This seroprevalence study carried out among 120 adopted children, 1-15 years old, coming from Eastern Europe, demonstrated the presence of HHV8 antibodies in 12.5% of adopted children. This result agrees with those found among immigrants and refugees adult and children coming from Albania and Kosovo (Graffeo *et al.*, 2003, Schinaia *et al.*, 2004, Chironna *et al.*, 2006).
Although our study population is not representative of all children of the Eastern Europe, our results do help to elucidate the rate of HHV8 infection in these countries. All together, these findings indicated that children in Eastern Europe could have an intermediate rate of HHV8 infection, higher of that reported in United States and Northern Europe (Simpson *et al.*, 1996, Anderson *et al.*, 2008) but lower than that observed among African children (Mayama *et al.*, 1998, Andreoni *et al.*, 1999, Kasolo *et al.*, 1997, Gessain *et al.*, 1999).
All of these adopted children in our study resided in orphanages before adoption; however, because of the lack of the data of entrance in the orphanage, it is difficult to determine whether these children acquired the infection from family member or the community. Moreover all of these infection are recent ones, as assessed by the detection of only anti-lytic antibodies, which are believed to be a marker of a recent infection (Andreoni *et al.*, 1999, Mbulaiteye *et al*., 2004). An interesting point to consider is that HHV8 seroprevalence was highest in our youngest children (age 1-6), presumably due to more person-to person exposure in this age bracket.
The presumed shedding of virus in saliva, especially during acute infection, may be the main determinant for dissemination of HHV8 infection. This point has recently stressed by other authors that reporting a frequent shedding of HHV8 in saliva of children (Mbulaiteye *et al*., 2004) indicated this group as a significative source of infection, particularly to their younger siblings or playmates (Mbulaiteye *et al*., 2006).
However, overall our finding suggests that HHV8 infection in this group was acquired early in childhood, perhaps as a consequence of transmission in an overcrowding, poor hygienic orphanage community.
In the current study, the seroprevalence for HAV, HBV, HCV and HIV infections were evaluated in our study population as potential indicators of different transmission patterns.

The high rates of HAV seroprevalence that we found, demonstrate that person to person transmission by the fecal oral route was operating in this group of children. This confirms the poor sanitation level and low hygiene conditions. However, the lack of correlation between HHV8 infection with concomitant hepatitis A infection, as demonstrated by little overlap between HAV and HHV8 infections indicates that a fecal- oral route of transmission of HHV8 is unlikely, as also demonstrated in others studies (Chironna *et al.*, 2006, Mayama *et al.*, 1998). Moreover, inadequate sanitation and use of bath items in common as well as low access to the health care system could offer a plausible hypothesis for high HBV prevalence seen in this setting. However, only few children had a HHV8 and HBV coinfection. This fact, together with the low prevalence of HCV antibodies and the absence of HIV infection in these children, argue against the possibility of parenteral or sexual transmission of HHV8 infection in institutionalised children.

In conclusion, our findings indicate that the seroprevalence rate of HHV8 infection is relatively high among children coming from Eastern European countries, exclude a common mode of diffusion with HAV, HBV, HCV and HIV infections and, finally, reinforce the hypothesis that close person to person contacts among children could be considered a major mode of HHV8 transmission.

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