Detection of human herpesvirus 7 infection in young children presenting with exanthema subitum

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In this study, we assessed the prevalence of human herpesvirus-7 (HHV-7) in 141 serum samples from children less than four years of age with exanthematic disease. All samples were negative for measles, rubella, dengue fever and parvovirus B19 infection. Testing for the presence of human herpesvirus-6 (HHV-6)-specific high avidity IgG antibodies by indirect immunofluorescence assay (IFA) revealed two main groups: one composed of 57 patients with recent primary HHV-6 infection and another group of 68 patients showing signs of past HHV-6 infection. Another 16 samples had indeterminate primary HHV-6 infection, by both IgG IFA and IgM IFA. Serum samples were subjected to a nested polymerase chain reaction to detect the presence of HHV-7 DNA. Among patients with a recent primary HHV-6 infection, HHV-7 DNA was present in 1.7% of individuals; however, 5.8% of individuals tested positive for HHV-7 DNA in the group with past primary HHV-6 infection. Among the 16 samples with indeterminate diagnosis, 25% (4/16) had HHV-7 DNA (p < 0.002). We hypothesise that HHV-7 might be the agent that causes exanthema. However, a relationship between clinical manifestations and the detection of virus DNA does not always exist. Therefore, a careful interpretation is necessary to diagnose a primary infection or a virus-associated disease. In conclusion, we detected HHV-7 DNA in young children from the state of Rio de Janeiro, Brazil.

Key words: HHV-7 - children - exanthema subitum - PCR

Human herpesvirus-7 (HHV-7) is a member of the Roseolovirus genus within the Betaherpesvirinae subfamily, which was first isolated from purified, activated CD4⁺ T lymphocytes from the peripheral blood of a healthy individual by Frenkel et al. (1990). HHV-7 is closely related to human herpesviruses-6 (HHV-6). Both cause infections that occur in early childhood and cause short febrile diseases and are sometimes associated with cutaneous rash [exanthema subitum (ES)]. Furthermore, HHV-7 and HHV-6 are highly prevalent in the healthy population and are known to establish latency in macrophages and T-lymphocytes. Healthy carriers also frequently shed virus in their saliva. Genetically, their nucleic acid sequence identity ranges from 20-75% in various genes and their virions share several common antigenic epitopes. These similarities have made the development of diagnostic assays challenging (Ward 2005, Caselli & Di Luca 2007). Along with ES, HHV-7 infections have been associated with sporadic cases of pityriasis rosea, hepatitis, neurological manifestations and transplant complications (Black & Pellett 1999).

ES is a classical rash disease of early childhood that is accompanied by the abrupt onset of a high fever that lasts three-four days; a maculopapular rash appears as the child’s temperature falls by crisis. As hypothesised by Yamanishi et al. (1988), HHV-6B represents the primary etiological agent of ES and second exanthema episodes are caused by HHV-6 reactivation. However, HHV-7 was isolated from the peripheral blood lymphocytes (PBL) of children with ES that had a previously documented episode of HHV-6-associated ES; furthermore, examination of sera samples revealed IgG seroconversion for HHV-7 (Ueda et al. 1994). In conjunction with HHV-7 seroconversion, many primary cases also showed simultaneous increases in HHV-6 antibody titres. As multiple serological studies indicate that most children acquire HHV-6 prior to HHV-7 (Caserta et al. 1998, Black & Pellett 1999) and Frenkel et al. (1990) showed that HHV-7 infection can reactivate HHV-6 infection, it was hypothesised that reactivated HHV-6 may be the true cause of the clinical symptoms. However, more recent studies have found seroconversion and isolated HHV-7 DNA from the PBL of children with ES symptoms that do not exhibit evidence of prior HHV-6 infection (Torigoe et al. 1995, Caserta et al. 1998). Other explanations for increased HHV-6 antibody titres during primary HHV-7 infection include immune cell stimulation by common antigenic epitopes.

Although exanthematic syndrome is well characterised, it was noted many years ago that the rash is frequently misdiagnosed as measles, rubella or dengue infection (Tait et al. 1996, Oliveira et al. 2003). Thus, primary HHV-6 and HHV-7 infection should be included in the differential diagnosis of rash illnesses in young children. As described by Black et al. (1996), nearly 10% of rash illness patients who were presumptively diagnosed with measles or rubella were seronegative for these viruses.

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in laboratory assays; these patients had seroconverted for HHV-7 and were most likely experiencing primary HHV-7 infection (Black et al. 1996). HHV-6 and HHV-7 should also be ruled out during rash presentations attributed to antimicrobial sensitivity to prevent children from incorrectly being diagnosed with allergies to antibiotics. In addition, the accurate diagnosis of HHV-7 and HHV-6 infections would decrease unnecessary antimicrobial prescriptions, reducing the potential for generating antibiotic-resistant microbes (Black & Pellet 1999).

Serological methods can be used for the detection of primary HHV-7 infection; however, immunological cross-reactivity between HHV-6 and HHV-7 is well documented (Ward 2005) and HHV-7 viremia can represent either a primary or a reactivated infection (Hall et al. 2006). Currently, although a specific immunofluorescence technique is accepted as the gold standard for HHV-6 diagnosis, no commercial test is yet available to screen for HHV-7 in the general population.

HHV-7-specific nested and conventional polymerase chain reaction (PCR) primer sets for qualitative, quantitative and multiplex assays have been developed and are useful for detection of viral DNA in human tissue samples and body fluids (Ward 2005).

In this study, we used a nested technique PCR to assess the prevalence of HHV-7 in serum samples from children less than four years of age with exanthemic disease.

The study was conducted between January 1998-December 2006 at the Antônio Pedro University Hospital and a large primary health care unit in Niterói, state of Rio de Janeiro (RJ), Brazil. A total of 141 serum samples were obtained from children younger than four years old who presented with rashes. All samples tested were previously screened and were negative for measles, rubella, dengue fever and parvovirus B19 infections. All samples were also PCR negative for the presence of HHV-6 DNA, as described by Magalhães et al. (2005a). Additionally, samples were tested for the avidity of HHV-6-specific IgG antibodies by indirect immunofluorescence assay (IFA), as described by de Oliveira Vianna et al. (2008). Overall, 57 patients exhibited recent HHV-6 primary infection, defined by low antibody avidity detected by IFA. In contrast, 68 had past HHV-6 primary infection, determined by the presence of high avidity antibodies detected by IFA; thus, the patients in this group did not exhibit any agent associated with rash. The other 16 samples had indeterminate primary HHV-6 infection; in these patients, the only serum available was obtained during the acute phase and was negative HHV-6-specific antibody. In general, these samples were taken less than nine days after rash onset, at a medium of five days after fever onset. In these cases, primary infection could not be excluded by IFA (Oliveira et al. 2003). De Oliveira Vianna et al. (2008) also investigated IgM antibodies by using IFA, but no IgM positivity was detected (Biotrin Human Herpesvirus 6 IgM IFA, Biotrin, Ireland).

Informed consent was obtained from the parents or guardians of the patients. The study protocol was approved by the Hospital’s Research Ethics Committee (CEP-CMM/HUAP 85/02).

To detect HHV-7 infection, DNA was extracted from 500 μL of serum by using QIAmp Kit (QIAGen, Germany) and tested for HHV-7 DNA with the use of a nested PCR assay; reaction conditions and primer sets were as previously described in Magalhães et al. (2010a). Data were analysed using the EPIinfo 2004 Statistical Software Package (CDC, Atlanta, EUA, 2004). Prevalence rates were compared through Chi-square tests with a Yates correction. The significance level (p) was set at 0.05.

PCR testing revealed that of the 141 samples, nine were positive for HHV-7 DNA (6.4%). Among the 57 samples that exhibited a recent primary HHV-6 infection, HHV-7 DNA was present in 1.7% (1/57). When we analysed the 68 samples with past primary HHV-6 infection, we observed a frequency of 5.8% (4/68) for the detection of HHV-7 DNA. It is interesting to note that among the 16 acute serum samples with indeterminate diagnosis, 25% (4/16) had HHV-7 DNA (p < 0.002). Although no statistical correlation was observed between female and male patients for cases (1 male) or controls (2 males, 2 females), all four inconclusive samples were from male patients. However, our casuistic was too small to suggest any correlation, as has been described in the literature, associating young girls with a higher sensitivity (Ward 2005).

The frequency rates described here are lower than those previously published (Kidd et al. 1998). Our findings may have resulted from the fact that our study group was very young, mainly comprised of children less than three years of age, making it possible that these children had not been infected yet; this hypothesis was also proposed by Ward (2005). However, these results could also be attributed to the lack of standardisation of Roseolovirus diagnosis. As suggested by Black and Pellet (1999), detection of viral DNA in the serum is indicative of an active infection, but less sensitive primer pairs, low viral loads or inhibitors present in the serum could cause false negatives.

Notably, the laboratory diagnosis of HHV-6 and 7 infections is confounded by the limited availability of antibody and DNA tests, problems with antigenic cross-reaction and a lack of understanding of the clinical relevance and epidemiology of these two viruses (Ward 2005).

Regarding Roseolovirus primary infection epidemiological data, several authors have proposed that HHV-7 infection usually occurs later than HHV-6 infection (Wyatt et al. 1991, Torigoe et al. 1995, Tanaka-Taya et al. 1996, Ward et al. 2001). However, HHV-7 infection may sometimes occur earlier or as soon as infection by HHV-6 (Oliveira et al. 2003). Consistent with these findings, we examined a one-year old child with a recent HHV-6 infection and HHV-7 DNA, suggesting the occurrence of a coinfection. Such events have been previously described by Hall et al. (2006). Notably, our study population exhibited a low socio-economic status, which has been associated with early disease exposure (Ongrádi et al. 1999).

In this study, we found that 5.8% of individuals had a past primary HHV-6 infection and HHV-7 DNA. In these patients, the observed exanthema could have been caused by either HHV-7 or by HHV-6 reactivation, as previously observed by Tanaka-Taya et al. (2000) in vitro and by Frenkel and Roffman in 1996 in vivo. Although
antibody detection tests to confirm HHV-7 seroconversion were not performed in these studies, these children were three years old or younger, suggesting that HHV-7 could be the agent causing the clinical signs (Oliveira et al. 2003). It is important to emphasise that HHV-6-specific PCR was performed and that no positive cases were found (Magalhães et al. 2010b). However, although herpesvirus DNA was detected in the serum of these immunocompetent children, there is not always a relationship between clinical manifestations and the detection of virus DNA. Therefore, a careful interpretation is necessary to diagnose a primary infection or a virus-associated disease (Hara et al. 2002). Prevalence rates described here were similar between cases and controls and no statistical differences were found (p = 0.52).

Among our samples, we detected HHV-7 DNA in 25% of the samples with previously undetermined diagnosis. We speculate that these patients, who were negative for prior exposure to other exanthem agents, such as rubella, measles, parvovirus B19 and dengue fever, could be presenting an exanthematic episode due to primary HHV-7 infection. This group was previously tested for HHV-6 exposure by IFA detection of IgM antibodies, but no positivity was found and they were still undetermined. In fact, IgM tests usually have low accuracy, related to serum inhibitors and cross-reactivity among herpesviruses; these issues can give rise to both false positive and false negative results (de Oliveira Vianna et al. 2008).

Although PCR detection has not been validated for the clinical diagnosis of HHV-7 infection, given the lack of proper antibody detection assays, it might be further studied as a possible tool for the differential diagnosis of exanthematic disease in young children. In conclusion, we describe HHV-7 DNA detection in young children, corroborating previous findings of HHV-7 infection in RJ.

REFERENCES
