Short Communication

Increasing seroprevalence of *Human herpesvirus 8* (HHV-8) with age confirms HHV-8 endemicity in Amazon Amerindians from Brazil

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Human herpesvirus 8 (HHV-8) seroprevalences were determined in two isolated Amazon Amerindian tribes, according to age, gender and familial aggregation. Plasma and serum samples obtained from 982 Amazon Amerindians (664 Tiriyó and 318 Waiampi) were tested for antibodies against lytic and latent HHV-8 antigens by using 'in-house' immunofluorescence assays. Overall, HHV-8 seroprevalence was 56·8 % (57·4 % in the Tiriyó tribe and 55·7 % in the Waiampi tribe). Seroprevalence was independent of gender and increased linearly with age: it was 35·0 % among children aged 2–9 years, 51·4 % in adolescents (10–19 years), 72·9 % in adults and 82·3 % in adults aged >50 years. Interestingly, 44·4 % of children under 2 years of age were HHV-8-seropositive. No significant differences in seroprevalence between tribes and age groups were detected. It is concluded that HHV-8 is hyperendemic in Brazilian Amazon Amerindians, with vertical and horizontal transmission during childhood, familial transmission and sexual contact in adulthood contributing to this high prevalence in these isolated populations.

Human herpesvirus 8 (HHV-8) is associated with the aetiopathogenesis of Kaposi's sarcoma (KS), body-cavity B-cell lymphoma (BCBL) and multicentric Castleman's disease (Chang *et al.*, 1994; Schulz, 1998; Hengge *et al.*, 2002b). Current serological and molecular assays indicate that HHV-8 infection is not widespread (Schulz & Moore, 1999), presenting variable routes of transmission throughout the world. In areas with low seroprevalence, HHV-8 appears to be acquired predominantly through sexual contact, and sex between men may be an important route of transmission (Schulz & Moore, 1999; Hengge *et al.*, 2002a). In regions with high endemicity, HHV-8 seems to be transmitted by several routes, both sexual and non-sexual (Vieira *et al.*, 1997; Blackbourn *et al.*, 1998; Olsen *et al.*, 1998; Plancoulaine *et al.*, 2000; Whitby *et al.*, 2000; Brayfield *et al.*, 2004; Dedicoat *et al.*, 2004). Familial clustering of HHV-8 is frequently observed in endemic regions (Plancoulaine *et al.*, 2000; Davidovici *et al.*, 2001; Mbulaiteye *et al.*, 2003; Guttman-Yassky *et al.*, 2004).

HHV-8 seroprevalence in Brazil varies according to the region and the population analysed. In the south-east, low HHV-8 seroprevalences (2·4–7·4 %) were detected among blood donors, healthy children, adolescents and adults from some cities in São Paulo state (Caterino-de-Araujo *et al.*, 1999; Pérez *et al.*, 2004; Souza *et al.*, 2004; Cunha, 2005). Intermediate to high HHV-8 seroprevalences (12–33 %) were detected among healthy individuals from an urban area near the Amazon rainforest in the northern region of Brazil (Freitas *et al.*, 2002) and high HHV-8 prevalences were detected in diverse, isolated Brazilian Amerindian tribes (53 % prevalence overall), implying that they are hyperendemic populations (Biggar *et al.*, 2000).

In order to investigate HHV-8 infection in two of these isolated tribes, we used 'in-house' immunofluorescence assays (IFA) to identify antibodies against HHV-8 in plasma samples of 982 Amerindians: 664 Tiriyó Indians from 148 families and 318 Waiampi Indians. Results were analysed according to gender, age and familial aggregation.

We conducted a retrospective, cross-sectional prevalence study of plasma and serum samples from Brazilian Amerindians living in the Amazon rainforest. The Tiriyó tribe

speaks the Caribe language and numbers approximately 1700 individuals, 750 of whom live in the state of Pará, near the border with Surinam. The Waiampi tribe speaks the Tupi-Guarani language and lives along the border with French Guyana: 450 of the tribe's 1200 individuals live in the state of Amapá, Brazil (Brazilian Ministry of Health, 1997; Shindo et al., 2002).

The samples analysed in this study were originally collected to determine the seroprevalence of human immunodeficiency virus (HIV) in these tribes following a report of AIDS in an Amerindian female, probably due to contact with a non-Amerindian male (Shindo et al., 2002). Blood samples were collected in 1997 and were sent to the Advanced Public Health Laboratory in Salvador, Brazil, where they were stored at $-20\,^{\circ}$C until use.

Serum samples from 664 Tiriyó Amerindians (332 of each gender, aged 0–81 years) and 318 Waiampi Amerindians (164 men and 154 women, aged 0–67 years) were tested for the presence of HHV-8-specific antibodies. Sociodemographic information was only available for 148 families from the Tiriyó tribe. This study was approved by the regional Ethnic Committee of the Brazilian Indian Service and the Ethnic Committee of the Brazilian Ministry of Health (CONEP).

Antibodies against lytic and latent antigens of HHV-8 were detected by 'in-house' indirect IFA as described elsewhere (Caterino-de-Araujo & Cibella, 2003; Caterino-de-Araujo et al., 2003). Briefly, cells of the BCBL-1 line, latently infected with HHV-8 or stimulated to enter lytic replication with tetradecanoyl phorbol ester acetate (TPA), were used to prepare slides for IFA-LANA and IFA-Lytic, respectively. Serum samples were diluted to 1 : 50 in PBS/non-fat milk and slides were read immediately by three independent observers using a fluorescent microscope. A sample was scored as positive by IFA-LANA if it showed clear, punctate, nuclear staining in $>90\,\%$ of cells, and as positive by IFA-Lytic if it showed strong membrane staining and diffuse cytoplasmic staining in 5–10 % of cells (Fig. 1). Positive samples were retested at dilutions of 1 : 50 and 1 : 100. Samples were considered seropositive for HHV-8-specific antibodies when at least one of the IFA-LANA or IFA-Lytic assays was positive (Carbone, 2003; Caterino-de-Araujo & Cibella, 2003; Caterino-de-Araujo et al., 2003). IFA-LANA or IFA-Lytic assays present sensitivities of 75·1 and 90·9 % and specificities of 97·8 and 99·7 % in detecting anti-latent and anti-lytic antibodies in Brazilian samples, respectively (Carbone, 2003).

Results obtained are expressed as proportions or percentages (i.e. no. positive samples/no. tested samples) and as mean $\pm$ SD for quantitative variables. Because ages were not distributed normally, the mean age of each tribe was compared by using the Mann–Whitney test. The χ^2 test was used to examine the association of HHV-8 seroprevalence with gender and age groups in both tribes. Prevalence and 95 % confidence intervals (CI) were calculated by using Microsoft Excel for Windows. We used SPSS for Windows software version 10.0 for other statistical analyses. Statistical significance was defined to be when $P < 0.05$.

The present study analysed serum samples from 88·5 % (664/750) of the Tiriyó and 70·6 % (318/450) of the Waiampi residing in Brazil in 1997 (Brazilian Ministry of Health, 1997; Shindo et al., 2002). Mean age was different for each tribe (20·50 $\pm$ 17·45 years for Tiriyó and 21·12 $\pm$ 14·02 years for Waiampi; $U = 93635·5$; $P = 0·014$), with a higher frequency of younger individuals in both tribes.

Overall prevalence of HHV-8-specific antibodies in these populations is presented according to gender in Table 1. No significant differences were observed in tribal seroprevalences [57·4 % (95 % CI, 53·6–61·1 %) vs 55·7 % (95 % CI, 50·2–61·1 %), respectively], or in gender-specific
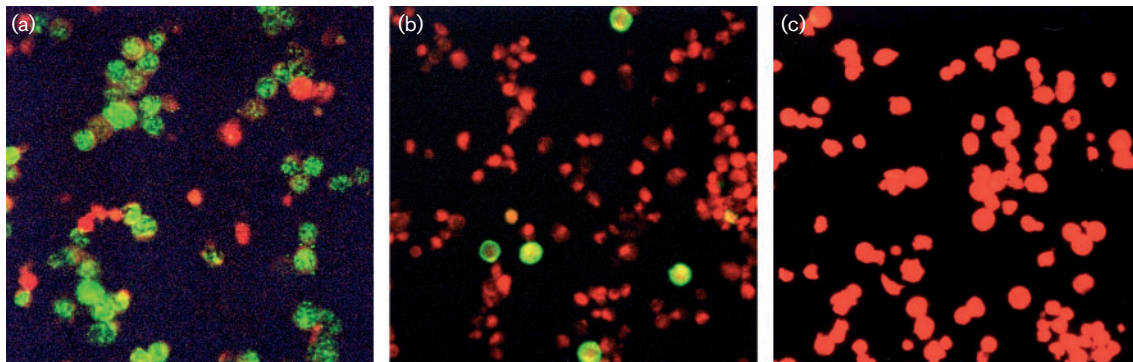
Fig. 1. Pattern obtained with HHV-8-specific antibody-positive and -negative sera in IFA-LANA and IFA-Lytic assays (400 ×). (a) IFA-LANA-positive slide with a typical speckled, nuclear green fluorescence, indicating reactivity of antibodies to latent antigens. (b) IFA-Lytic-positive slide with a cytoplasmic green fluorescence pattern observed during the lytic phase of virus replication. (c) Negative-control slide.

Table 1. Seroprevalence of HHV-8 antibodies (IFA-LANA and/or IFA-Lytic) in Amerindians from the Tiriyó and Waiampi tribes, according to gender

IFA-LANA and IFA-Lytic were conducted as described in Methods. No significant difference in seroprevalence was detected between tribes or according to gender ($P=0.695$ for Tiriyó; $P=0.606$ for Waiampi).

Tribe	No. seropositive/no. tested (%)			
	Male	**Female**	**Total**	**95 % CI***
Tiriyó	193/332 (58·1 %)	188/332 (56·6 %)	381/664 (57·4 %)	53·6–61·1 %
Waiampi	89/164 (54·3 %)	88/154 (57·1 %)	177/318 (55·7 %)	50·2–61·1 %
Total	282/496 (56·8 %)	276/486 (56·8 %)	558/982 (56·8 %)	53·7–59·9 %

*95 % confidence intervals for overall HHV-8 seroprevalence.

seroprevalences. When serum results were stratified by age, an increase in the number of seropositive cases was detected with age, varying from 35·0 % in children (2–9 years) to 82·3 % in adults of over 50 years of age (Table 2). However, children younger than 2 years of age showed an overall prevalence of 44·4 %. No significant differences in age-specific seroprevalence were detected between tribes (Table 2). The prevalence ratio increased significantly with age, and mean age was different in HHV-8-seropositive and -seronegative individuals ($23·95 \pm 14·44$ and $17·57 \pm 12·65$ years, respectively; $U=9008·5$; $P<0·001$).

Of the 148 Tiriyó families analysed, 94 % had at least one infected member, and in 70 % of families, more than half of the members were HHV-8-seropositive. In this tribe, the majority of individuals had positive results for both IFA-LANA and IFA-Lytic (39·1 % of samples), whilst 9·9 % were seropositive only by IFA-LANA and 8·4 % only by IFA-Lytic. Taken together, the overall antibody prevalence was 57·4 %. Titration of 50 positive sera, using two-fold serial dilution, showed high titres of anti-latent and anti-lytic antibodies that ranged from 1 : 100 to 1 : 3200.

The results showed that 24·9 % of Waiampi samples were positive by both IFA-LANA and IFA-Lytic, whilst 22·9 % of samples were positive only by IFA-LANA and 7·9 % only by IFA-Lytic; the overall seroprevalence was 55·7 %.

The present study provides additional insight into the epidemiological profile of hyperendemic HHV-8 infection in two isolated Amerindian tribes from northern Brazil. An overall HHV-8 seroprevalence of 53 % was identified previously in Brazilian Amerindians from the Amazon region (Biggar *et al.*, 2000). Those authors analysed 721 serum samples from several tribes and detected an increase in HHV-8 seroprevalence with age. We were able to confirm and extend these data by searching for HHV-8-specific antibodies in 982 plasma samples from almost all individuals of two tribes, the Tiriyó and the Waiampi.

A traditional method used to evaluate whether an infectious agent is endemic or epidemic is to examine the age-specific seroprevalence, which increases with age in endemic populations because of continuous or steady-state transmission (Olsen *et al.*, 1998). We analysed our data according to age group and found that HHV-8 seroprevalence was higher in children aged 0–2 years (44·4 %) than in those aged 2–9 years (35·0 %), increasing constantly, after that, with every age group, from adolescents (51·4 %) to adults (72·9 %) and adults older than 50 years (82·3 %). Familial clustering of HHV-8 is frequently observed in endemic regions (Plancoulaine *et al.*, 2000; Davidovici *et al.*, 2001; Mbulaiteye *et al.*, 2003; Guttman-Yassky *et al.*, 2004). Thus, the results obtained in these tribes are consistent with endemic transmission of HHV-8.

Table 2. Prevalence of HHV-8 antibodies (IFA-LANA and/or IFA-Lytic) in Amerindians from Tiriyó and Waiampi tribes, according to age group

Tribe	No. seropositive/no. tested (%) in age group:				
	<2 years	**2–9 years**	**10–19 years**	**20–49 years**	**>50 years**
Tiriyó	11/25 (44·0 %)	65/191 (34·0 %)	91/168 (54·2 %)	154/205 (75·1 %)	54/63 (85·7 %)
Waiampi	5/11 (45·4 %)	18/46 (39·1 %)	58/122 (47·5 %)	85/123 (69·1 %)	11/16 (68·7 %)
Total*	16/36 (44·4 %)	83/237 (35·0 %)	149/290 (51·4 %)	239/328 (72·9 %)	65/79 (82·3 %)
P value†	0·936	0·515	0·265	0·235	0·113

*Significant differences were detected among age groups ($P<0·001$, $\chi^2=106·9843$).
†No significant difference in age-specific seroprevalence was detected between the tribes.

Overall HHV-8 seroprevalence of 44·4 % detected in children under 2 years of age initially suggests non-sexual transmission, most probably during pregnancy, delivery or breastfeeding. The possibility of vertical transmission was reported in a preliminary study of HHV-8 prevalence in Brazilian Ameridians (Nicastri *et al.*, 2000). HHV-8 DNA was detected in breast milk by using real-time PCR in South Africa (Dedicoat *et al.*, 2004) and Brazilian Amerindians are described as having long-term maternal breastfeeding (Mattos *et al.*, 1999), but this potential route of transmission needs to be better confirmed, as another study failed to detect HHV-8 DNA in breast milk in Zambia (Brayfield *et al.*, 2004).

Meanwhile, a study conducted in children from Italy (Whitby *et al.*, 2000) showed an HHV-8-seroprevalence profile similar to that detected in the present study (i.e. HHV-8-seropositive children under 2 years of age and declining seroprevalence in children aged 3–5 years) and suggested that maternal antibody in younger children can give false-positive signals in diagnoses. However, persistently high prevalence in children aged 2–9 years in our study also suggests non-sexual transmission, either vertically or by other body fluids.

Saliva has been indicated as a potentially important route of transmission in endemic populations, with HHV-8 DNA detected in saliva by several studies (Vieira *et al.*, 1997; Blackbourn *et al.*, 1998; Dedicoat *et al.*, 2004). In fact, exposure to saliva may occur in sub-Saharan Africa and in Brazil, when mothers premasticate food for infants or clean children's faces with saliva. In addition, children may also be exposed during play and by sharing utensils with siblings (Biggar *et al.*, 2000; Mbulaiteye *et al.*, 2003).

The highest seroprevalence (82·3 %) was detected in adults older than 50 years. The differences in the HHV-8 seroprevalences obtained in older Tiriyó and Waiampi (85·7 vs 68·7 %) did not quite reach statistical significance, but we suspect that this difference is due to the different age profiles of the two tribes: the Tiriyó subjects had a mean age of 20·50 years (range, 0–81 years), whereas the Waiampi individuals had a mean age of 21·12 years (range, 0–67 years). The strong increase in HHV-8 seroprevalence according to age may reflect either a cohort effect or suppressed immunity in elderly people, which could either reactivate silent HHV-8 infection or facilitate a new HHV-8 infection. An increase in anti-HHV-8 antibody titres was detected in subjects older than 40 years (Plancoulaine *et al.*, 2002) and HHV-8 DNA was also detected in saliva of women presenting high titres of anti-lytic antibodies (Dedicoat *et al.*, 2004). Of note, we detected more serum samples presenting anti-lytic antibodies in Tiriyó Indians, although these antibodies were detected in all age groups analysed from both tribes.

We do not know the exact impact of HHV-8 infection in Amerindians, as no cases of KS or other HHV-8-associated diseases have yet been identified (Biggar *et al.*, 2000). This fact may suggest that there is a gene that provides resistance against HHV-8 infection, as proposed by Plancoulaine *et al.* (2003) in French Guyana, or there may be a less virulent strain of HHV-8 in this population, as only one subtype was described by Biggar *et al.* (2000). The implications of HHV-8 biology and pathogenesis in these isolated tribes should be investigated to answer these questions as, recently, one Tiriyó Indian was found to be infected with HIV-1 (Shindo *et al.*, 2002).

Finally, the results obtained confirmed that both tribes are hyperendemic for HHV-8 infection and suggest early HHV-8 infection during childhood, as well as multiple routes of virus transmission.

Aside from protecting these populations, we are now stimulated to continue studying the genetic background of Amerindians, as well as the HHV-8 subtypes that circulate in these tribes.

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