

Human Herpesvirus 8 in Brazilian Amerindians: A Hyperendemic Population with a New Subtype

Robert J. Biggar,¹ Denise Whitby,² Vickie Marshall,²
Alexandre C. Linhares,³ and Francis Black⁴

¹Viral Epidemiology Branch, National Cancer Institute, National Institutes of Health, Bethesda, and ²Viral Epidemiology Section, AIDS Vaccine Program, National Cancer Institute–Frederick Cancer Research and Development Center, Scientific Application International Corporation–Frederick, Frederick, Maryland; ³Instituto Evandro Chagas, Fundacao Nacional de Saude, Belém, Brazil; ⁴Department of Epidemiology, School of Public Health, Yale University, New Haven, Connecticut

Human herpesvirus 8 (HHV-8) epidemiology in Brazilian Amerindians was studied. Use of an immunofluorescence (IFA) test for latent antibody demonstrated that the prevalence of HHV-8 in 781 Amerindians of diverse tribes (overall, 53% prevalence) was not related to language group or sex but rather increased gradually from 41% in children <10 years of age to 65% in adults ≥30 years of age. In IFA-positive subjects, HHV-8 DNA was detected in 3 (16%) of 19 mononuclear cell samples from peripheral blood and in 1 of 16 saliva samples. The sequences of conserved *ORF22* and *K6* genes were typical of HHV-8, but the variable *K1* gene sequences were only 70%–75% identical to other known HHV-8 strains. Thus, a new HHV-8 subtype, E, is hyperendemic in Brazilian Amerindians, although Kaposi's sarcoma has not been reported. Transmission is probably oral rather than sexual. The limited genetic pool in isolated groups may permit more frequent transmission of a virus with a low prevalence in heterogeneous populations.

Human herpesvirus 8 (HHV-8, also called Kaposi's sarcoma-associated herpesvirus) is a newly described gamma herpesvirus that is etiologically important in causing Kaposi's sarcoma (KS) [1], primary effusion lymphoma [2], and multicentric Castlemann's disease [3]. This virus has a worldwide distribution. The prevalence among healthy heterosexual adults in North American and Western European populations is reported to be 1%–2% in most studies [4–6], but in southern European and Middle Eastern Mediterranean populations prevalence is higher, 5%–35% [7]. In Greece, Sicily, and Sardinia, areas in which classic KS incidence is especially high [8–10], the prevalence in adults is reported to be 20%–35% [6, 11, 12]. HHV-8 prevalence varies regionally within Italy, and regional variation corresponds with variation in the incidence of classic KS [11, 12]. Antibody titer in HHV-8-infected healthy adults is higher in regions with high HHV-8 prevalence and KS incidence [11]. In eastern and southern African countries, which are known to have a high rate of endemic KS [13], the reported

prevalence in adults has ranged from 30% to 60% [4, 6, 14, 15]. However, high HHV-8 prevalence has also been reported in West Africa, where the incidence of KS is low [16, 17]. Variation in testing approaches could have influenced these results; whereas most tests give a similar frequency of positive results in panels of sera, they often identify different people as positive [18]. Nevertheless, the prevalence of HHV-8 has been reported to vary by area and is highest in areas with a high incidence of KS, with the important exception being West Africa.

Studies of isolated populations may lead to insights into the antiquity and epidemiology of infections. In previous studies, we have shown human T-lymphotropic virus type II (HTLV-II) to be highly endemic in Amerindians [19–21], and others [22] have found HTLV-II to be endemic in pygmies. These studies demonstrated that HTLV-II is a very ancient virus of humans, one that was brought over the Bering Strait with migrations that occurred many millennia ago [23]. We now report results of testing Amerindians for HHV-8. These samples were collected mostly in the 1970s and 1980s, soon after the first friendly contact with some of these groups. Thus, these findings represent the HHV-8 status in Amerindians at a time when some Amerindians, such as the Parakana, were still very isolated from external contact and before their ways of life had changed greatly.

Materials and Methods

In a follow-up to early work on HTLV-II, we obtained fresh blood samples from Amerindians in Belém and Altamira, Para State, Brazil, in 1997. A convenience sample of 35 was obtained

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Reprints or correspondence: Dr. Robert J. Biggar, EPS 8014, 6120 Executive Blvd., Bethesda, MD 20852 (biggarb@epndce.nci.nih.gov).

from Amerindians from 7 villages that represented 3 language groups: Tupi, Ge, and Carib; these groups were staying at the Casas dos Indios in Belém and Altamira (figure 1). Some residents were visiting for medical reasons: suspected tuberculosis (5 cases), gonorrhea (3 cases), and 1 case each of fever, malaria, chronic diarrhea, swollen joints, cervical cancer, mental derangement, broken arm, and a vision problem. The other individuals were healthy family members who were accompanying the person requiring the medical care. Samples of plasma were saved and tested for HHV-8 antibodies by latent immunofluorescence (IFA) at a minimum dilution of 1 : 100, using BCP-1 cells as the target, as described elsewhere [6, 18]. These samples were also examined by a whole-virus EIA (Advanced Biotechnologies, Rockville, MD).

For DNA, we allowed the samples to settle at room temperature for several hours. The leukocyte-rich supernatants were then removed and immediately mixed thoroughly with a nucleic acid-preserving solution (Gentra Systems, Minneapolis). Saliva samples were frozen and DNA was extracted at the time of polymerase chain reaction (PCR) testing using the PureGene DNA Isolation Kit (Gentra Systems). The quality and quantity of DNA were determined with a quantitative PCR assay for human endogenous retrovirus-3 [1]. Extracted DNA was examined by quantitative Taqman PCR, with primer-probe sets used for sequences in HHV-8 *ORF22* (primers: 5'-CACCTTGGCGGATTTGGGATC-3' and 5'-ACGGCCATGACAATCATTGGG-3'; probe: 5'-CGCGTCTGTACTGGGTGATATCTTCGC-3') and in *K6* (primers: 5'-CGCCTAATAGCTGCTGCTACGG-3' and 5'-TGCATCAGCTGCTAACCAG-3'; probe: 5'-CACCCACCGCCCGTCCAAATTC-3'), with an ABI Prism 7700 (P.E. Biosystems, Foster City, CA). Nested PCR for the hypervariable HHV-8 *K1* gene was carried out on HHV-8 genome-positive samples for HHV-8 genotyping, as described elsewhere [24]. Sequence analysis was performed by use

of the Seqed, Pileup, and Pretty programs (Genetics Computer Group, Madison, WI). Phylogenetic analysis was performed with the MEGA program (Molecular Evolutionary Genetic Analysis, version 1.0; The Pennsylvania State University, University Park) [25].

The researchers conducting the earlier studies had initially collected samples for genetic studies. Because of the antiquity of these samples, we refer to them as historic samples. Complete descriptions of recent studies that employed these samples have been published [19–21]. Samples were collected from residents of 16 Amerindian villages throughout central and northern Brazil. By design, the investigators sought a wide representation of villages comprising residents from different language groups. Serum samples were stored at –20°C until use. We had previously used these samples to study HTLV-II epidemiology by antibody techniques and had found them to be of good quality [19–21]. No DNA-containing samples were available in the historic studies. These studies had all been approved both in Brazil (Brazilian Indian Service) and in the United States (National Cancer Institute).

Results

Recent samples. All 11 residents (ages 3–55 years) of 1 Carib-speaking village, Larangal, were latent IFA negative. However, 19 (79%) of 24 subjects (2 children and 22 adults, ages 6–68 years) from 6 other villages were positive in a latent IFA test. Further testing with a whole-virus EIA showed agreement, with 15 positives and 15 negatives (including the 11 persons from Larangal). One sample was positive on EIA only. Four latent IFA-positive samples were EIA negative; all 4 sam-

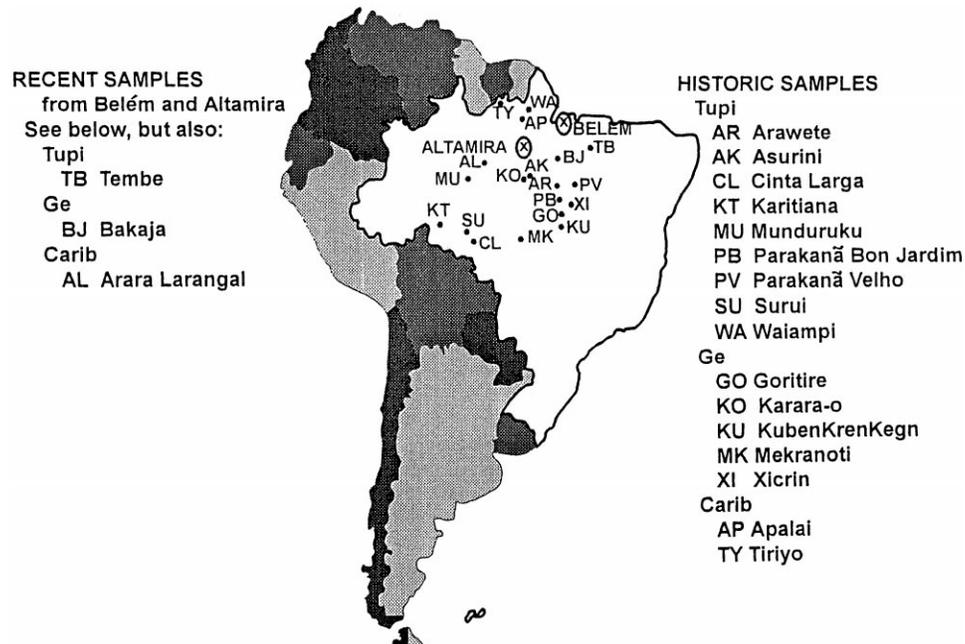


Figure 1. The locations of Brazilian Amerindian villages studied in this survey. Recent samples refer to 1997 studies, which included molecular characterization of human herpesvirus 8. Historic samples comprised sera obtained in the 1970s and 1980s.

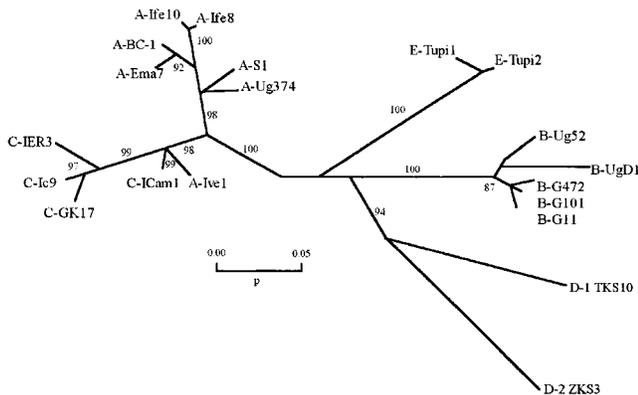


Figure 2. Neighbor-joining tree [28] based on the proportion of amino acid difference (p) for 20 full-length *K1* protein sequences aligned by use of the ClustalW program (Genetics Computer Group, Madison, WI). Previously published sequences representative of human herpesvirus 8 subtypes [24, 26] (obtained from GenBank) were used for a comparison with 2 new samples from Amerindians. Branch lengths correspond to the proportion of amino acid differences. Internal branches were tested by the bootstrap test. A total of 1000 bootstrap samplings were used, and bootstrap confidence levels >75% are shown. The Amerindian samples represent a distinct new subtype, E. Although the E subtype clusters with subtypes B and D, the relative positions of these 3 relationships and the cluster of A and C subtypes cannot be inferred by the placement of the branches of the tree.

ples had low latent IFA titers (3 samples were 1 : 100 and 1 sample was 1 : 400). Titers in concordant positives ranged from 1 : 100 to 1 : 52,600.

Peripheral blood DNA samples from the 19 subjects who were positive in latent IFA were examined by quantitative Taqman PCR. Three (16%) persons were concordantly positive, with a range of copy numbers of 11–156 copies/10⁶ cells in assays using both the highly conserved *ORF22* and *K6* regions. Direct sequencing of *ORF22* and *K6* amplimers showed all 3 positives to be 98%–100% identical to published HHV-8 sequences. These subjects, all of whom had high HHV-8 antibody titers, were among the oldest persons we tested: a 66-year-old Asurini woman (IFA titer 1 : 6400), a 64-year-old Arawete woman (1 : 12,800), and a 50-year-old Arawete man (1 : 25,600). While all 3 subjects were Tupi-speaking, 7 of 8 persons ≥50 years old in the recent study were Tupi-speaking. Saliva samples were available from 16 IFA-positive subjects. One sample from a 22-year-old Ge-speaking man was PCR positive, with 125,000 copies/10⁶ cells. His peripheral blood sample was PCR negative.

K1 was amplified from blood samples from 2 of the 3 PCR-positive subjects (1 Arawete and 1 Asurini), using nested PCR, and samples were sequenced. The Amerindian *K1* sequences were 95% identical to each other, but they differed from previously reported A, B, C, and D subtype HHV-8 *K1* sequences [24, 26, 27] by 25%–30%. Phylogenetic analysis with reported sequences confirmed that the Amerindian sequences clustered

together as a separate subtype. The relative positions of the B, D, and E subtypes were unstable among different trees, but all trees placed the A and C subtypes close to each other and away from the B, D, and E subtypes (figure 2). An alignment of consensus amino acid sequences from known subtypes and from the 2 Amerindian samples is provided in figure 3.

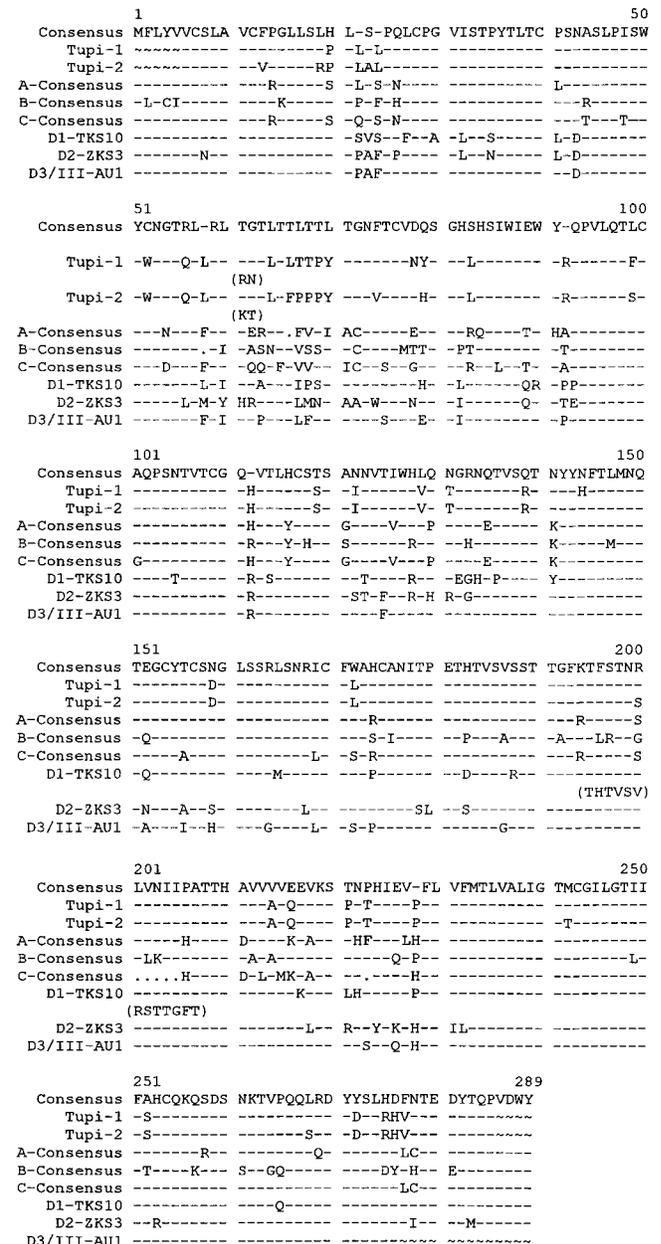


Figure 3. Alignment of New Amerindian sequences with consensus A, B, and C subtypes and 3 D subtype sequences, constructed by use of the Pretty program (Genetics Computer Group, Madison, WI). The A, B, and C consensus sequences were derived by use of Pretty from 13, 15, and 18 previously reported sequences, respectively [24, 26]. GenBank accession numbers for Tupi 1 and 2 sequences are AF220292 and AF220293, respectively.

Table 1. HHV-8 antibody prevalence (latent IFA) by language group and tribe and village in 344 adults who were ≥ 30 years old at the time of first contact.

Language, survey	Tribe, village ^a	First contact ^b	Blood sample obtained ^c	No. positive/ no. tested	Prevalence (%)
Tupi					
Recent	Arawete	1975	1997	5/5	100
	Assurini Koatinema	1971	1997	2/2	100
	Mundurucu Cabrua	Early	1997	1/2	50
	Tembe	Early	1997	1/3	33
Historic	Arawete	1975	1986	7/12	58
	Assurini Koatinema	1971	1984–1985	5/8	62
	Cinta Larga	Early	1987	1/14	7
	Karitiana	1970	1986–1987	12/25	48
	Mundurucu Sae Cinza	Early	1985	35/38	92
	Parakana Bon Jardim	1984	1984	13/13	100
	Parakana Velho	1974	1974, 1977, 1981	14/17	82
	Surui Rondonia	1969	1987	15/17	88
	Waiampi	1970	1980, 1981, 1987	28/33	85
Ge					
Recent	Kayapo Bakaja	1964	1997	5/6	81
	Kayapo Goritire	1939	1997	4/4	100
Historic	Kapayo Goritire	1939	1980, 1985	2/2	100
	Kayapo Karara-o	1969	1986	3/3	100
	Kayapo Kubenrankegn	1945	1974	4/5	80
	Kayapo Mekranoti	1970	1972, 1978	34/52	65
	Kayapo Xicrin	1961	1970, 1981, 1985	15/28	54
Carib					
Recent	Arara Larangal	1982	1997	0/11	0
	Tiriyo	1961	1997	2/2	100
Historic	Apalai	Early	1977, 1983, 1984	8/44	18
	Tiriyo	1961	1966, 1984	28/33	85

NOTE. HHV-8, human herpesvirus 8; IFA, immunofluorescence assay.

^a Language groups are established, but tribes self-identify in different ways. Some researchers group tribes by their self-identification with other groups, and others group them by their village name. Both groupings are provided when they differ. For example, the Ge-speaking Kayapos all identified themselves as Kayapos, but they have different villages, whereas the Tupi-speaking Arawetes identified themselves only with their own village.

^b Dates of first peaceful contact are approximate. In some cases, outside persons might have joined a village much earlier.

^c Some villages were visited more than once, and therefore several blood samples were obtained. Only the earliest blood sample (1 sample per subject) was used in this study.

Historic samples. The earliest serum samples of 746 subjects from 16 villages, representing Tupi-, Ge-, and Carib-speaking peoples, were tested. The prevalence by latent IFA was similar in men (53%) and women (54%). The prevalence in children <10 years of age was 41%, and it increased slowly to 65% in persons ≥ 30 years of age. This increase was steady and was similar in both sexes (figure 4). To ensure comparability in age groups, we then examined prevalence only in 344 adults who were ≥ 30 years of age. Prevalences were 64% (58 of 90) in 5 Ge villages, 73% (130 of 177) in 9 Tupi villages, and 47% (36 of 77) in 2 Carib villages. Prevalences differed widely by village, even among those sharing the same language group (table 1). For example, in the 2 Carib villages, 85% (28 of 33) and 18% (8 of 44) of older adults were positive.

Discussion

HHV-8 infection is endemic in Brazilian Amerindians. HHV-8 cannot have been newly introduced in Amerindians, since many of our samples were obtained within a few years of the first friendly contacts with these peoples. The distribution of

HHV-8 was widespread, including diverse language groups and geographic areas. However, not all villages had high infection rates. We previously found HTLV-II to be hyperendemic only in Ge-speakers [19–21, 29], but HHV-8 was hyperendemic in all language groups. Although this is the first full report of HHV-8 in Amerindians, we note that a recently published meeting abstract also describes detection of HHV-8 by PCR in the peripheral blood of Yanomama Indians in Venezuela [30].

We detected the presence of HHV-8 by antibody testing but confirmed it by PCR of peripheral blood mononuclear cells. Although only a small proportion of antibody-positive subjects was positive by PCR, this pattern is typical of findings in other areas of the world [31, 32]. The HHV-8 genome shows little overall variation except in the *K1* gene, which can vary by up to 30% at the amino acid level. Phylogenetic analysis of previously described *K1* sequences verified the existence of 4 distinctive subtypes—A, B, C, and D—which were estimated to have diverged at least 10,000 years ago [24, 26, 27, 33]. The B subtype predominates in Africa and is more distant from subtypes A and C than A and C are distant from each other. Subtypes A and C predominate in Europe [24], whereas the

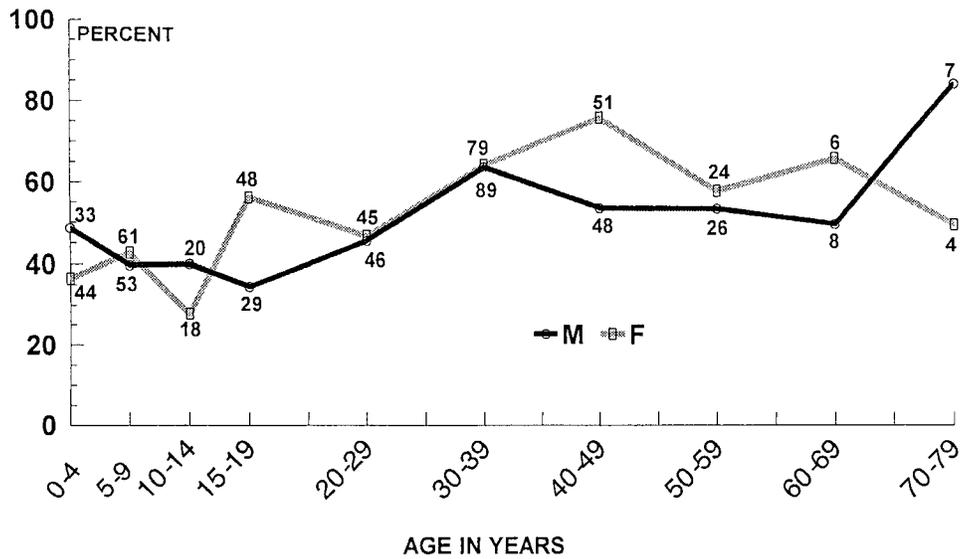


Figure 4. The prevalence of antibodies to human herpesvirus 8, measured by latent immunofluorescence assay, by age and sex, in all 746 subjects in the historic samples. Prevalence patterns within individual larger villages showed similar profiles. For subjects who had serum samples collected at multiple time points, only the earliest test result was included in this analysis. The number at each time point provides the number of subjects tested at that point.

rare D subtype has been found only in 4 classic KS patients of Polynesian and Australian aboriginal origin [26, 27]. Several clades can be identified within each subtype [24, 26, 27]. The sequences derived from Amerindian DNA differ from all other subtypes by 25%–30%; the Amerindian sequences are closest to subtype D. These sequences therefore appear to represent a new lineage, subtype E.

Several striking epidemiological observations can be made from these data. These Amerindians have the highest HHV-8 prevalence yet reported. We postulate that genetic homogeneity may underlie the high prevalence. What characterizes these villages is their isolation from outside contact. Therefore, the gene pool within each village is relatively limited. HHV-8 may be more readily transmitted because the virus, which is normally difficult to transmit, is already preadapted to a new host, since that host shares a similar genetic background with the donor. This pattern could be important in preserving the existence of this and other viruses that are otherwise hard to transmit. If so, prevalence may be high in isolated populations, assuming that the virus is present at all.

The routes of transmission of HHV-8 between Amerindians are unclear. Sexual transmission of HHV-8 has been suggested [5, 6], in particular as the explanation for the high prevalence among homosexual men [34, 35]. Homosexuality in these Amerindian groups is uncommon, if it occurs at all. However, mixing of heterosexual partners is common in these Amerindian groups, particularly during adolescence and young adulthood. We found no sharp increase in prevalence among adolescents and young adults in this study. Evidence for heterosexual transmission of HHV-8 is inconsistent in other studies [15, 36], and

those with large numbers of sexual partners are also likely to have other body contact, such as kissing.

In contrast, we found a high prevalence of HHV-8 in young children. Children younger than the age of sexual activity have also been found to be HHV-8 positive in other studies [37–39], although positive status appears to be uncommon in areas of low HHV-8 prevalence [40]. Mother-to-child transmission could play a role [38], but the prevalence increases in older children, indicating that child-to-child transmission also occurs [7, 37, 39]. Prior to the early studies, these Indians had almost no exposure to medical practices that might have resulted in transfusions. Tattoos were not used in these populations. Poor sanitary conditions, such as those in Amerindian villages, are found in many areas of the world and are not an adequate explanation for the exceptionally high prevalence of HHV-8.

The finding of HHV-8 in the saliva of a young man raises the possibility that this herpesvirus is being spread orally. Interestingly, the relatively high copy number of HHV-8 DNA detected suggests a cell-free virus, since this individual had little human DNA in his saliva sample. HHV-8 has been cultured from the cell-free saliva of KS patients [41]. This finding, in conjunction with the high infection rates in children, the lack of prevalence differences between sexes, and the continuing increase in prevalence among older subjects who were unlikely to have been sexually active, suggests that saliva exchange may be an important route of transmission in these people.

The health impact of having HHV-8 is unclear. We are not aware of any cases of KS or lymphoma resembling the primary effusion type among these Amerindians. However, the quality of medical service available to these villagers raises doubts

about the diagnosis and recording of obscure diseases of the elderly. Therefore, we cannot be sure that the incidence of KS is low. We note that a pattern of high HHV-8 prevalence but little KS has been reported from Gambia [16]. The risk of KS among HHV-8-infected persons is commonly acknowledged to increase greatly as a result of human immunodeficiency virus (HIV) infection and its associated immunosuppression [15, 35], but, so far, HIV has not been described in these Brazilian Amerindian groups. If HIV penetrates the hyperendemic villages, an increase in KS diagnoses could be an early indication. Long-term natural history studies of HHV-8 infection and its relationship to diseases in these populations are greatly needed.

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