

# Antibodies to human herpes virus type 8 (HHV8) in general population and in individuals at risk for sexually transmitted diseases in Western Sicily

Anna Maria Perna,<sup>a</sup> Filippa Bonura,<sup>a</sup> Francesco Vitale,<sup>a</sup> Enza Viviano,<sup>a</sup> Maria Antonella Di Benedetto,<sup>a</sup> Francesca Ajello,<sup>a</sup> Maria Rosaria Villafrate,<sup>a</sup> Tullio Prestileo,<sup>b</sup> Salvatrice Mancuso,<sup>c</sup> James J Goedert<sup>d</sup> and Nino Romano<sup>a</sup>

<b>Background</b>	Human herpes virus 8 (HHV8) appears to be the primary aetiologic agent of Kaposi sarcoma (KS). To study the distribution of HHV8, a seroepidemiological study was carried out in western Sicily, where a high incidence rate of classical KS is well documented.
<b>Methods</b>	A total of 970 sera of healthy human immunodeficiency virus (HIV) negative individuals of general population (1–70 years old) and 742 sera of individuals in different risk groups for HIV infection were evaluated by means of an indirect immunofluorescence assay able to detect antibodies to lytic and latent HHV8 antigens.
<b>Results</b>	Crude seroprevalence to HHV8 antigens was 11.5% in the general population, and it increased significantly with age from 6% under age 16 to 22% after age 50. Significantly higher HHV8 seroprevalence rates were detected among HIV positive and negative homosexual men (62% and 22%, respectively), men who had sex with prostitutes (40% and 29%, respectively); female prostitutes (42% and 30%, respectively), and clients at a sexually transmitted disease clinic (male: 60% and 33%, respectively, female: 63% and 43%, respectively). In contrast, heterosexual intravenous drug users had seroprevalence rates comparable to those found in the general population.
<b>Conclusions</b>	The results suggest that HHV8 infection is widespread in Western Sicily. The high seroprevalence in individuals with high risk sexual activity point to the role of sexual behaviour in the transmission of the infection in adults, whereas the detection of antibodies in younger population (under 16 years old) is suggestive of a non-sexual route of transmission, probably occurring during childhood by close personal contact.
<b>Keywords</b>	Human herpes virus 8, antibodies, epidemiology, human immunodeficiency virus, risk groups, sexually transmitted diseases
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<sup>a</sup> Servizio di Riferimento Regionale per la diagnostica sierologica e virologica dell'AIDS, Dipartimento di Igiene e Microbiologia, Policlinico, Università di Palermo.

<sup>b</sup> Azienda Unità Sanitaria Locale 6, Presidio Ospedaliero "Casa del Sole", Palermo.

<sup>c</sup> Servizio di Riferimento Regionale per la diagnosi clinica dell'AIDS e Sindromi correlate, Policlinico, Università di Palermo.

<sup>d</sup> Viral Epidemiology Branch, National Cancer Institute, National Institute of Health, Rockville, Bethesda, Maryland, USA.

Reprint requests to: Professor Nino Romano, Dipartimento di Igiene e Microbiologia, Università degli Studi di Palermo, Policlinico, Via del Vespro, 133, 90127, Palermo, Italy.

Kaposi Sarcoma (KS) is the most frequent neoplasm affecting patients with the acquired immunodeficiency syndrome (AIDS) who manifest KS at a rate 106 000 times greater than in the general population.<sup>1</sup> The epidemiology of KS had suggested that it may be caused by a sexually transmitted infectious agent.<sup>2</sup> KS is highly prevalent among HIV-infected homosexual and bisexual males in whom 20–30% develop the neoplasm in contrast to the very low rates among patients with haemophilia or in intravenous drug users.<sup>2,3</sup> The incidence of non-AIDS KS shows marked geographical variations, with endemic

KS occurring predominantly in central Africa and classic KS predominantly in Mediterranean countries.<sup>4</sup>

In 1994, DNA of a previously unknown herpes virus was discovered in KS lesions.<sup>5</sup> DNA sequences of this virus, termed human herpes virus 8 (HHV8) or KS-associated herpes virus (KSHV)<sup>6</sup> were detected by polymerase chain reaction (PCR) in nearly all AIDS-associated KS and in many specimens taken from KS patients who were not infected with HIV.<sup>7</sup> HHV8 also has been identified in the peripheral blood cells of approximately 50% of patients with KS. More significantly, HIV positive patients with HHV8 detected by PCR in their peripheral blood cells are more likely to develop KS than those without detectable HHV8.<sup>8,9</sup> This observation strongly suggests that HHV8 plays a role in the pathogenesis of the disease.

Recently, we detected DNA sequences of HHV8 by PCR in peripheral blood cells and semen of HIV positive and negative individuals in Sicily.<sup>10</sup> The presence of HHV8 in semen of HIV negative individuals implies that HHV8 may be spread in the general population by sexual transmission. However, serological assays for antiviral antibodies may be better suited than PCR methods for addressing the natural history of HHV8 infection. Therefore, we carried out a serological study in Sicily, where a high incidence of classical KS was already present before the appearance of the AIDS epidemic.<sup>11</sup>

## Materials and Methods

### Study populations

#### *Risk groups*

Sera were collected as part of the baseline assessment of HIV prevalence infection in several risk groups in Western Sicily including patients attending HIV clinics, general medical clinics, sexually transmitted disease (STD) clinics, and drug users attending a residential methadone programme or living in the community. In all 782 patients who were referred to our laboratory for HIV testing, during 1995–1997, and gave consent to test their blood for supplemental assays regarding other infectious diseases, were enrolled into the study; 40 individuals (5.1%) refused to participate.

#### *General population*

Sera were also collected from samples of healthy HIV negative people in the general population:

- younger subjects (1–18 years old), were selected from among those attending all levels of schools (nursery, elementary, primary and high schools), using a multi-stage sample methodology. Briefly, a random sample of schools in Palermo was drawn, and then within each selected school a sample of students was drawn. Another group of young people (19–30 years old) was recruited between December 1996 and November 1997, among all university students attending the hygiene course as well as the Department of Hygiene of the Faculty of Medicine.
- middle-aged people (31–50 years old) from among health care workers in the Faculty of Medicine participating in the hepatitis B virus vaccination programme.
- older subjects (51–70 years old) from among adults, excluding those who had infectious diseases and/or tumours, who had been referred to three different test laboratories between June 1997 and December 1997 to be phlebotomized for other assays. All gave their consent.

A total of 1115 people were initially invited to participate in the study; 13% refused and then 970 individuals from all age groups were included in the study. Informed consent was obtained from all the participants or from the parents of the children under 15 years old.

### Cell cultures

BCBL-1 is a B-cell line derived from a body cavity-based B-lymphoma that is Epstein-Barr virus (EBV) negative but is latently infected with HHV8. Molt 4 is a human T-lymphoblastoid cell line and P3HR-1 is a EBV producer cell line, HHV8 negative. All cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 0.02% NaHCO<sub>3</sub>, and 100 U/ml penicillin and 100 mg/ml streptomycin. Cell lines were maintained at 37°C in 5% CO<sub>2</sub>. To induce HHV8 activation, the BCBL-1 were treated with 20 ng/ml tetradecanoyl phorbol-ester acetate (TPA, Sigma Aldrich, Italy) and 200 U/ml human recombinant interleukin 6 (Boehringer Mannheim, Germany).<sup>12</sup>

### Serological techniques

Serum was separated from clotted whole blood by centrifugation and was frozen at –20°C. Serological reactivity to HIV-1 was determined by enzyme immunoassay (Organon Teknika, Boxtel, NL), and positive results were confirmed by Western blot tests using standard techniques.

For detection of HHV8 antibodies by immunofluorescence assay (IFA), uninduced and TPA induced BCBL-1 cells were collected, washed in PBS pH 7.4, spotted on slides, air dried and fixed in cold acetone for 10 minutes. Fixed slides were incubated with human sera diluted 1:120 and then revealed with rabbit anti human IgG-fluorescein isothiocyanate conjugate (Sigma Aldrich, Italy).

The dilution of 1:120 of human sera was determined to be the best cutoff point to avoid homogenous non-specific cytoplasmic background by serial dilution of HH8 negative sera from the general population. All samples that tested HHV8 positive were blinded and retested, along with random samples of those which initially tested negative. Samples that produced disparate results (less than 4%) were retested a third time, and the majority outcome was taken as final. All positive sera were also retested at the same dilution with Molt 4 to control further for non-specific cellular reactivity.

### IFA sensitivity, specificity and cross-reactivity with EBV

Sensitivity of the IFA test was estimated by using sera from KS patients as the gold standard.<sup>7</sup> Sensitivity also may depend upon titre, which was compared among groups as geometric mean titres (GMT). Because a negative gold standard could not be defined to analyse assay specificity, reactive sera were scored positive to lytic antigens only or to both lytic and latent antigens. EBV IgG antibodies were detected by IFA utilizing slides coated with P3HR-1 cells expressing EBV-viral capsid antigen (VCA). To assess possible cross-reactivity, sera from seven subjects (two with classical KS patients and five healthy individuals) who had EBV-VCA titres >1:120 and a range of positive HHV8 titres were absorbed with live P3HR-1 cells, an EBV producer cell line. 1 ml of a serum dilution of 1:60 was mixed and gently shaken with 20 × 10<sup>6</sup> P3HR-1 cells overnight at 4°C, to remove

EBV antibodies. Adsorption with P3HR-1 cells did not alter the reactivity of these sera to HHV8 thus suggesting no interference by cross-reacting EBV antibodies under the conditions of our assay (data not shown).

**Statistical analysis**

The  $\chi^2$  tests or Fisher exact test was applied as appropriate to compare groups.

**Results**

**Detection and measurement of HHV8 antibodies**

All sera were tested with uninduced and TPA induced BCBL-1 cells. When sera were tested with uninduced BCBL-1 cells, a specific granular punctate nuclear fluorescence was observed. This type of reactivity was scored as positive to HHV8 latent antigens. About 2.5 % cells also showed moderate cytoplasmic fluorescence and were presumed to be reacting with HHV8 lytic antigens. When sera were tested with BCBL-1 cells 24 hours after TPA induction, almost all cells showed nuclear fluorescence and 10–12% of cells showed a bright cytoplasmic fluorescence. Five days after TPA induction, 25–30% of the cells showed very bright cytoplasmic fluorescence, and the nuclear, latent-type fluorescence was greatly diminished; therefore these induced cells were used for the detection of the lytic antigens.

**Assay sensitivity and HHV8 antibody titres**

All sera from 14 classical KS patients strongly reacted against both nuclear latent and cytoplasmic lytic antigens in TPA treated BCBL-1 cells. Similarly, all sera from 16 AIDS-associated KS

patients had antibodies to HHV8 antigens; all reacted to lytic antigens and 15 (93.7 %) also had antibodies to latent antigens (Table 1). There was a strong gradient in geometric mean titres (GMT) of serum antibodies to latent and lytic HHV8 antigens, highest among classical KS patients, intermediate among AIDS-associated KS patients, and lowest among seropositive healthy adults in the same age range (50–70 years old) as the classical KS patients (Table 1). Among classical KS patients, GMT to latent and lytic antigens were 14 562 (range 480–61 449) and 9505 (range 240–61 440), respectively. Among AIDS-associated KS patients, GMT to latent antigens was 1559 (range 120–15 360) and to lytic antigens was 5820 (range 120–30 720). Among general population adults, GMT were 822 (range 120–7880) and 607 (range 120–3840), respectively.

**HHV8 seroprevalence**

Of 970 sera 112 had antibodies against HHV8, giving a crude HHV8 seroprevalence in the general population of 11.5%. Seroprevalence increased significantly with age, from 6.3% under age 16 to 22.3% after age 50 ( $P < 0.001$ , Table 2). At every age, seroprevalence was similar in males and females.

Among HIV negative men aged 21–50 years, general population and drug addict subjects had the lowest seroprevalence rates (about 15%, Table 3). Seroprevalence was marginally higher among HIV negative homosexual men (22.1%) and significantly higher than in the general population among male clients of female prostitutes (29.4%,  $P < 0.05$ ) and men at the STD clinic (32.7%,  $P < 0.01$ ). Among the HIV positive men, seroprevalence was very high among homosexuals (62.2%), clients of female prostitutes (40%) and STD patients (60%).

**Table 1** Reactivity of human sera of KS, AIDS-associated KS patients and healthy individuals of general population against latent and lytic HHV8 antigens

Group	No. tested	No. (%) with HHV8 latent antibodies <sup>a</sup>	GMT <sup>b</sup>	(Range)	No. (%) with HHV8 lytic antibodies <sup>d</sup>	GMT <sup>b</sup>	(Range)
Classical KS	14	14 (100)	14 562	(480–61 440)	14 (100)	9505	(240–61 440)
AIDS-associated KS	16	15 (93.7)	1559	(120–15 360)	16 (100%)	5820	(120–30 720)
Healthy adults <sup>c</sup>	70	6 (8.5)	822	(120–7880)	14 (20)	607	(120–3840)

<sup>a</sup> Positive with uninduced BCBL-1 cells (%).

<sup>b</sup> Geometric mean titre.

<sup>c</sup> Healthy adults of similar age (50–70 years) as the classical KS patients.

<sup>d</sup> Pos. with TPA induced BCBL-1 cells (%)

**Table 2** Seroprevalence of HHV8 infection in 970 healthy HIV negative individuals divided by age and by sex

Age group (years)	No. sera tested	No. of males/ No. of females	HHV8 positive sera		
			Total (%)	No. of males (%)	No. of females (%)
1–5	110	63/47	6 (5.4)	3 (4.8)	3 (6.4)
6–10	111	58/53	9 (8.1)	4 (6.9)	5 (9.4)
11–15	98	47/51	5 (5.1)	2 (4.2)	3 (5.9)
Total 1–15 <sup>a</sup>	319	168/151	20 (6.3)	9 (5.4)	11 (7.3)
16–20	113	52/61	9 (8.0)	4 (7.7)	5 (8.2)
21–30	163	84/79	17 (10.4)	10 (11.9)	7 (8.9)
31–40	144	75/69	20 (13.9)	12 (16.0)	8 (11.6)
41–50	137	68/69	25 (18.2)	13 (19.1)	12 (17.4)
51–70 <sup>a</sup>	94	53/41	21 (22.3)	13 (24.5)	8 (19.5)
All ages	970	500/470	112 (11.5)	59 (11.8)	53 (11.2)

<sup>a</sup>  $P < 0.001$  compared with each other (Total 1–15 versus 51–70 age groups).

**Table 3** Seroprevalence against HHV8 lytic antigens in different groups of HIV negative and positive adult (age 21–50) male subjects

Group	HIV negative		HIV positive	
	No. of positive sera/ No. of tested sera	(%)	No. of positive sera/ No. of tested sera	(%)
General population men	35/227	(15.4)	–	–
Heterosexual drug addicts	28/187	(15.0)	28/142	(19.7)
Homosexual men	23/104	(22.1)	23/37 <sup>c</sup>	(62.2)
Clients of prostitutes	10/34	(29.4)	10/25 <sup>b</sup>	(40.0)
Heterosexual STD <sup>a</sup>	17/52 <sup>b</sup>	(32.7)	18/30 <sup>c</sup>	(60.0)

<sup>a</sup> Men attending hospital for sexually transmitted diseases.

<sup>b</sup>  $P < 0.01$ ; <sup>c</sup>  $P < 0.001$  (compared with general population).

**Table 4** Seroprevalence against HHV8 lytic antigens in different groups of HIV negative and positive adult (age 21–50) female subjects

Group	HIV negative		HIV positive	
	No. of positive sera/ No. of tested sera	(%)	No. of positive sera/ No. of tested sera	(%)
General population women	27/217	(12.4)	–	–
Heterosexual drug addicts	2/24	(8.3)	4/21	(19.0)
Prostitutes	9/30 <sup>a</sup>	(30.0)	5/12 <sup>a</sup>	(41.6)
Heterosexual STD <sup>c</sup>	12/28 <sup>b</sup>	(42.8)	10/16 <sup>b</sup>	(62.5)

<sup>a</sup>  $P < 0.01$ .

<sup>b</sup>  $P < 0.001$  (compared with general population).

<sup>c</sup> Women attending hospital for sexually transmitted diseases.

These three rates were statistically different ( $P < 0.01$ ) from the 15.4% in the general population. HHV8 seroprevalence among HIV positive drug addicts (19.7%), was not significantly elevated.

Similar results were observed among HIV negative and positive women. Of 217 women aged 21–50 in the general population, 27 (12.4%) had antibodies to HHV8 (Table 4). Rates were not significantly different in HIV negative (8.3%) and HIV positive (19%) female drug addicts. Significantly higher seroprevalence rates were found among heterosexual female prostitutes (30%,  $P < 0.01$ ) and women attending the STD clinic (42.8%,  $P < 0.001$ ). HHV8 seroprevalence was approximately 1.5 fold higher among HIV positive compared to HIV negative women in the same groups (Table 4).

## Discussion

Our data suggest that infection of HHV8 is widespread in Western Sicily, although corroboration with other serological approaches or, ideally, by detection of HHV8 genome would be helpful. Seroprevalence increased in the general population after age 16, and it was similar among males and females. We also found that HHV8 seroprevalence was much higher in groups postulated *a priori* to have a high risk of acquiring a sexually transmitted disease. HIV positive individuals, either homosexual or heterosexual, appeared to be at higher risk than were HIV negative individuals. Many factors could contribute to these high rates, including many sexual partners, types of sexual practices, and so on. Mode of sexual transmission cannot be defined in our seroprevalence survey. Rather, detailed epidemiological investigations are needed to understand better the key determinants of sexual transmission.

The rate of infection was highest in HIV seropositive homosexual men which fits with the high incidence of KS in this population. However, heterosexual intravenous drug users had seroprevalence rates comparable with those found in the general population, in keeping with the relatively low incidence of KS among drug users with AIDS. These data suggest that parenteral HHV8 transmission is unusual.

The serological response to HHV8 infection consisted in the appearance of antibodies either to lytic antigen(s) alone, or to lytic and latent HHV8 antigen(s). The significance of these types of HHV8 antibody patterns is yet unknown. Recently, Levy<sup>13</sup> suggested that the latent antibodies may be tumour-associated, reflecting cell transformation in the host. We observed that the GMT of anti-latent antibodies was higher in KS and AIDS-associated KS patients, but it is probably too early to conclude these antibodies are a marker of cell transformation. At present we do not know why the incidence of classical KS is higher in males than in females in spite of seeing essentially equal HHV8 seroprevalence between the two sexes. KS development appears to require not only HHV8 infection but also additional environmental or hormonal factors and/or perhaps host genetic susceptibility.

The pattern of seroprevalence of HHV8 in the general population did not quite resemble that of Herpes simplex virus type 2, in which sexual contact appears to be a major mechanism for its transmission. Antibodies to HHV8 were also present in pre-pubertal children suggesting that the virus also has a non-sexual mode of transmission. KS is extremely rare among children in Europe and the US, but it has been noted among African children under age 16.<sup>14</sup> Moreover, the incidence rate of KS among children in Uganda under age 15 has risen dramatically over the past three decades from 0.25 per 100 000 in 1964

through 1968 to 10.1 per 100 000 in 1992 and 1993 among boys. This changing incidence clearly relates to the AIDS epidemic. Since some of these children were 5 years or younger, it has been suggested that the agent can be transmitted perinatally or in infancy.<sup>15</sup> How HHV8 might be transmitted to children is unknown. Recent studies reported the presence of the virus in saliva of infected individuals and indicate a possible HHV8 transmission by this route.<sup>16,17</sup>

Epidemiological data also suggest possible faecal-oral transmission of the Kaposi's sarcoma agent.<sup>18</sup> Possibly, this route could explain the high incidence of KS in heterosexuals and children in Africa, among whom faecal-oral transmission may result from poor sanitation. Although one study found HHV8 DNA in 46% of rectal biopsies of HIV positive patients with gastrointestinal symptoms,<sup>19</sup> the only published report that has looked for HHV8 DNA in faeces did not find it in 18 patients with AIDS-associated KS.<sup>8</sup>

True HHV8 seroprevalence will depend upon the development of the definition of assays that are not only sensitive but also highly specific, particularly for low-titre antibodies. Nonetheless, it seems probable that the spread of HHV8 follows at least two different routes: one which is linked to sexual activity and the other which is not. Intensive study of well characterized populations is needed to clarify these modes of transmission and the factors that influence the viral spread.

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