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Correlates of Kaposi sarcoma-associated herpesvirus seroprevalence in Sicily

Colleen Pelser^{1,2}, Francesco Vitale³, Denise Whitby⁴, Barry I. Graubard¹, Angelo Messina⁵, Lorenzo Gafà⁶, Elizabeth E. Brown⁷, Lesley A. Anderson^{1,8}, Nino Romano³, Carmela Lauria⁶, and James J. Goedert¹

¹ Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland ² Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, Maryland ³ Dipartimento di Igiene e Microbiologia 'Giuseppe D'Alessandro', Università degli studi di Palermo, Palermo, Italy ⁴ Viral Oncology Section, AIDS and Cancer Virus Program, SAIC-Frederick, National Cancer Institute-Frederick, Frederick, Maryland ⁵ Dipartimento di Scienze Biomediche, Università degli studi di Catania, Catania, Italy ⁶ Lega Italiana per la Lotta Contro i Tumori-Sez Ragusa, Ragusa, Italy ⁷ Departments of Epidemiology, Medicine and Microbiology, University of Alabama at Birmingham, Birmingham AL, USA ⁸ Centre for Public Health, Queen's University Belfast, Northern Ireland

Abstract

Background—Environmental factors, such as plants and soil, may influence Kaposi sarcoma-associated herpesvirus (KSHV) replication or immune responses. However, the relationship of such exposures to KSHV seroprevalence has not been established.

Methods—In 1154 randomly sampled adults (aged 32–92) throughout Sicily, KSHV antibodies were detected with four assays and a conservative algorithm. Seroprevalence was re-weighted to the population. Logistic regression was used to estimate associations of seroprevalence with interview data, including contact with 20 specific plants.

Results—KSHV seroprevalence was 8.5%, including 5.3% among men and 11.5% among women ($P=0.22$). In multivariate models, seroprevalence was consistently higher with residence in a smaller community during childhood ($P_{\text{trend}} \leq 0.03$) and working with plants/soil during adulthood (odds ratio ≥ 2.73). In such models, seroprevalence was higher with exposure to one plant (*Hieracium*, odds ratio ≥ 2.8), but it was lower with three others (*Acanthus mollis*, *Taraxacum officinalis*, and *Trigonella foenum-graecum*) and with cumulative exposure to all 20 plants ($P_{\text{trend}} = 0.03$). Other demographic, household, and water contact variables were unrelated to seroprevalence.

Conclusions—KSHV seroprevalence appears to be increased by contact with soil and to vary with certain plants. Corroboration and investigation of possible effects of soil and plant constituents on KSHV regulation and immune responses are needed.

Keywords

Herpesviridae; Kaposi sarcoma; Italy; ecology; plants; natural products

INTRODUCTION

Kaposi sarcoma-associated herpes virus (KSHV, also known as human herpes virus 8) is considered a necessary but insufficient cause of Kaposi sarcoma (KS). Prevalence of KSHV infection varies worldwide, with seroprevalence being low among general populations in the United States and Northern Europe (0–2%), intermediate in the Mediterranean area (5–25%), and very high ($\geq 50\%$) in areas of sub-Saharan Africa.¹

Modes of KSHV transmission are poorly understood. In endemic areas, primary infection typically occurs during childhood, with transmission through saliva being the most plausible route. Transmission via saliva is supported by a high frequency and load of viral shedding in the saliva of children in Uganda and homosexual men in the U.S.^{2,3} Among children in Uganda, KSHV seropositivity increased with age and with mother's seropositivity.⁴ Molecular analysis of viral DNA sequences revealed that some children acquired KSHV from their mothers, whereas other children apparently acquired it from someone else in the family or community.⁵

Although transmission of KSHV often occurs in childhood, seroprevalence tends to increase during adulthood.^{6–10} Increasing seroprevalence with age among adults has not been clearly explained, but three studies in Italy noted a plateau in seroprevalence after age 70 years.^{11–13} Among younger men, but not women, sexual activity was related to KSHV seropositivity in the U.S.¹⁰ In Uganda, KSHV seroprevalence was not associated with sexual activity, nor with 50 other demographic, environmental, and behavioral variables.⁶

Ecologic and environmental exposures, acting indirectly to facilitate KSHV transmission or antibody seroconversion, should be considered. In central Italy, KSHV seroprevalence was 2-fold higher in rural areas, especially among farmers.⁷ It also was 4.7-fold higher in residents of Sardinia, who are predominantly rural, than in Sardinian emigrants to mainland Italy, who are predominantly urban.⁸ Among the elderly of northern Italy, seroprevalence was 2.2-fold higher in close proximity to the Po River or its tributaries.¹¹ A possible enhancing role for T-helper type 2 (Th2)-predominant immune responses was noted in Egypt, where anti-schistosomal antibodies were associated with a 1.5-fold significantly increased risk of KSHV seroprevalence among women, and with a 2-fold non-significantly increased risk among men.⁹ Among children in Uganda, KSHV seropositivity was associated with low socioeconomic status, and use of surface water or a communal standpipe in lieu of private tap water.⁴

At least three theories have emerged for how ecologic and environmental exposures could affect KSHV seroprevalence. The simplest is the use of KSHV-infected saliva for hygiene, particularly in water-shortage regions⁴ or for soothing insect bites.¹⁴ The second is the alteration of cellular immunity, as induced by insect bites or particularly by parasites,⁹ that may increase susceptible target cells, hamper effective responses, or increase KSHV shedding in close contacts. The third is the induction of KSHV lytic replication by exposure to phorbol esters or other constituents of plants or soil, which would increase shedding by contacts.^{15,16} Exposure to plant or soil constituents could also increase KSHV antigen expression to a critical threshold, resulting in antibody seroconversion among people who are KSHV-infected but seronegative in current assays.

Herein, we sought to identify risk factors for KSHV seropositivity in a population-based study that encompassed the elderly population of Sicily. We focused on socio-demographics, childhood crowding, occupational contact with plants or soil, contact with specific plant species, and various exposures to water.

METHODS

Study Design

Detailed methods for the parent case-control study of classic KS conducted in Sicily during 2002–2006 have been published.¹⁷ Briefly, population-based controls, aged 30–99 years were selected from the National Health System Patients Roster using stratified two-stage cluster sampling. The 4044 primary care physicians were stratified by community size, then randomly selected with the probability proportional to the number of patients on the physician's roster. Up to 12 controls, frequency matched to KS cases by sex and age in 5-year strata, were selected from each of the 450 selected physicians.

Institutional review board approval was obtained from the U.S. National Cancer Institute, local institutions in eastern (Ragusa) and western (Palermo) Sicily, and the coordinating center (RTI International). Following signed informed consent, recruited participants provided a blood sample and responses to a standardized questionnaire. Demographic variables included age, sex, and education level, as well as the community of residence at birth, during childhood to age 12, during the 10 years before enrollment, and at the time of interview. In addition, participants were asked about childhood crowding and family size, source of drinking water, frequency of bathing and showering, occupational contact with open water, contact with plants or soil, and contact with 20 specific plant species. These 20 plants (listed in Table 3, including footnote) were selected on the advice of local botanists based on the prevalence and likelihood of contact in Sicily, known dermal uses or toxicities, or genetic relatedness to plants reported to induce KSHV lytic replication.^{15,16} To optimize the accuracy of recall, the participant was shown color photographs for each of the 20 plants during the interview.

From the Sicilian population of 3.2 million people aged 30–99 years, 4692 controls were selected. Of these, 1232 were enrolled. Major reasons for non-enrollment included termination of recruitment for lack of funds (1268), physician reasons (refused: 510; deceased: 32; closed practice: 48), and patient reasons (refused: 459; not located: 436; too ill: 289; and deceased: 404). Fourteen failed to meet eligibility.

KSHV Serostatus

Plasma aliquots of EDTA anti-coagulated blood were tested for KSHV antibodies using four assays: an immunofluorescence assay (IFA) to the latent nuclear antigen (LANA), an IFA to KSHV lytic antigens, and enzyme-linked immunosorbent assays (ELISAs) against the KSHV gene products K8.1 and open reading frame 73 (orf73).

Laboratory methods for the assays have been described.¹⁸ Briefly, the IFAs were performed with a 1:120 dilution of plasma using the BCBL-1 cell line with (lytic) and without (LANA) induction by phorbol 12-tetradecanoate 13-acetate (TPA). ELISAs with recombinant K8.1 and orf73 proteins were performed with 1:20 and 1:100 dilutions of plasma, respectively.

Participants were considered positive if they had a positive LANA and/or an optical density reading (OD) >1.2 on the K8.1 ELISA. Participants were considered negative if both IFAs were negative, and if they had a K8.1 OD ≤ 0.8 and an orf73 OD ≤ 0.8. This algorithm is a modification of one with an estimated sensitivity of 80%–90% and an estimated specificity of 90%–100%.¹⁸ Participants who did not meet the definition of negative or positive (e.g., reactive by lytic IFA or orf73 ELISA but not by LANA or K8.1 ELISA) were classified as sero-indeterminate and included only in sensitivity analyses.

Statistical methods

Population sampling and weights—To adjust for the multi-stage sampling of the participants, base weights were calculated as the product of the reciprocal of the selection probabilities at each stage of sampling.¹⁷ Non-response adjusted weights were then calculated as the product of these base weights and cross-classified categories of age, region (eastern/western Sicily), and gender. These non-response adjusted weights were further adjusted by using post-stratification to constrain the weights to reflect the population totals by age, gender and six zones (three community sizes $\times$ 2 regions). These non-response/post-stratification-adjusted weights are the final sample weights for each participant's data.

These population-based weights were employed in all analyses using SUDAAN survey procedures. Odds ratios (OR) for each variable were estimated with logistic regression models adjusting for sex and age category (<68, 68–74, 75–80, $\geq$ 81 years). Variables that were statistically significant ($P \leq 0.05$) in initial analyses were considered in multivariate logistic regression modeling, including checking for effect modification.

Variable classification and logistic regression analyses—For all analyses, numerical variables were dichotomized at the median value among all controls. Total hours of work with plants or soil were calculated as: (years of work) $\times$ (hours/week during period of work) $\times$ 52. Participants were then categorized as not exposed, exposed ≤ 900 hours (median value), and exposed > 900 hours.

To evaluate how exposures to plants might be related to KSHV seroprevalence, we conducted a backward-elimination stepwise regression, constructed a cumulative exposure score combining contact with all 20 plants, and employed factor analysis as a dimension-reducing method.

In the stepwise regression, all 20 plants (zero, < 10 , and ≥ 10 exposures), as well as sex, age group, childhood community size, and working with plants/soil, were included. In addition to sex and age group, individual plants and other variables with $P < 0.15$ were retained. For cumulative lifetime exposure, total contacts with all 20 plants, assuming values of 0, 2, 20, 200, and 2000 contacts for the exposure categories (zero, < 10 , 10–100, 100–1000, > 1000 times), were summed (range of values, 0 – 23 224) then divided into quartiles for regression analysis.

Factor analysis uses covariance relationships among multiple observed variables to generate a few underlying, but unobservable, quantities called factors. Four factors were generated with an orthogonal rotation method (VARIMAX and PROC FACTOR, SAS Institute, Cary, NC) based on the proportion of variance explained. These factors were labeled descriptively (Asteraceae, Euphorbia, Iris and Food/Beverage, and Hypericum) and then dichotomized at median values for regression analysis.

All tests of significance tests (except for the stepwise regression) were two-tailed where $P \leq 0.05$ was considered statistically significant.

RESULTS

Socio-demographic correlates

We enrolled an unbiased sample of 1232 adults from the Sicilian population. As they were frequency-matched to the KS cases, there were 907 men and 325 women, with a mean age of 71.3 (range: 32–92). Using four assays, 1031 were KSHV seronegative, 123 were seropositive, and 78 (excluded from analysis, except as noted below) were sero-indeterminate. Using the final sample weights, estimated seroprevalence of KSHV in Sicily was 8.5% (95% CI 5.3 –

13.5%). Sex-specific seroprevalence was 5.3% (95% CI 3.2 – 8.6%) for men and 11.5% (6.0 – 20.8%) for women. Seroprevalence was non-significantly lower (7.2%) in the youngest quartile (age <68 years). With mutual adjustment, KSHV seroprevalence did not differ significantly by sex ($OR_{\text{men}} 0.5$, 95% CI 0.2 – 1.3, $P=0.13$) or age ($P_{\text{trend}} = 0.56$). By province (Figure 1), seroprevalence was relatively high (14–20%) in Ragusa, Caltanissetta, Agrigento, and Messina; lower (3–6%) in Siracusa, Trapani, Catania, and Palermo, and quite low (0.5%) in Enna, the one landlocked province; overall, these did not differ significantly ($P=0.17$).

In logistic regression adjusted for age group and sex, KSHV seroprevalence was unrelated to level of attained education (Table 1). In contrast, seroprevalence was inversely related to community size during childhood ($P_{\text{trend}} = 0.03$). The trend with childhood community size held when the analysis was stratified by whether or not the participant had changed community by adulthood (data not presented). Seroprevalence was not associated with density of residents in the childhood home, including the numbers of older siblings, younger siblings, total residents, and people sharing the participant's bedroom. Seroprevalence was unrelated to currently or ever smoking cigarettes ($P \geq 0.68$).

Environmental correlates

Based on previously reported and postulated associations with KS, we examined whether KSHV seroprevalence was associated with exposures to water, soil, and plants.^{4,15,16} The likelihood of KSHV seropositivity was significantly increased ($OR 2.9$, 95% CI 1.1–7.9) among participants who answered “yes” to the question “During your adult life, have you had a regular activity or paying job working with plants or soil?”. KSHV seroprevalence was 5.5% among those who never worked with plants/soil, 10.5% among those who reported ≤ 900 cumulative hours working with plants/soil, and 19.1% among participants with more cumulative exposure to plants/soil ($P_{\text{trend}}=0.07$). With additional adjustment for community size in childhood, the association with plant/soil work remained elevated but was no longer significant ($OR 2.4$, 95% CI 0.9 – 6.4). Most participants who worked with plants/soil reported seldom or infrequently using masks, gloves, or other protective garments (data not presented).

Bathing less than three times per week was not significantly associated with seroprevalence ($P=0.17$), except for participants who reported working with plants/soil, of whom 24.8% were KSHV seropositive ($OR 5.6$, 95% CI 1.2 – 25.1). Seroprevalence was not associated with ever having worked in water generally, nor specifically in seawater or fresh water (Table 1). Seroprevalence also was not associated with the source of drinking water over the last 10 years.

In sex-stratified analyses (Table 2), KSHV's association with smaller childhood communities was statistically significant and stronger among women ($P_{\text{trend}}=0.02$) than men ($P_{\text{trend}}=0.97$). Similarly, the higher KSHV seroprevalence with plant/soil work was statistically significant and stronger among women ($OR 3.9$, 95% CI 1.2–12.7) than men ($OR 1.4$, 95% CI 0.5–4.4). However, tests of effect modification by sex were not significant ($P=0.23$ and $P=0.22$, respectively).

Botanical correlates

The participants were asked for the frequency and type of contact (direct or as a food or beverage) with 20 species of plants that are prevalent in Sicily and known to have dermal effects or to be genetically related to extracts that induced KSHV lytic replication.^{15,16} With Bonferroni correction for multiple comparisons ($P < 0.0025$), none of the plants was significantly associated with KSHV seroprevalence. Less conservatively, sex- and age-adjusted odds of seropositivity were increased for those reporting contact with *Hieracium* sp. (hawkweed, $OR 2.8$, 95% CI 1.01 – 7.9) or *Chicorium intybus asteraceae* (chicory, $OR 2.9$, 95% CI 1.1 – 7.8); and they were decreased for those reporting contact with *Hypericum*

perforatum guttiferæ (St. John's wort, OR 0.3, 95% CI 0.1 – 1.01), *Inula viscosa* (OR 0.4, 95% CI 0.2 – 0.9), or *Trigonella foenum graecum* (fenugreek, OR 0.4, 95% CI 0.1 – 1.1). These associations were generally consistent by sex (data not presented).

To further explore the relationship of KSHV seropositivity to plant/soil work, each of three logistic regression models included sex, age group, community size during childhood, and exposure to specific plants individually or collectively (Table 3). In model 1, backward stepwise modeling eliminated 16 of the 20 specific plants. Of the retained plants (criterion $P < 0.15$), KSHV seroprevalence was higher with *Hieracium*; and it was lower with *Acanthus mollis*, *Taraxacum officinalis*, and *Trigonella foenum-graecum*. In model 2, none of four independent plant factors – descriptively labeled as Asteraceae, Euphorbia, Iris and Food/Beverage, and Hypericum – were significantly associated with KSHV. In model 3, KSHV seroprevalence was lower with more cumulative exposure to the 20 plants ($P_{\text{trend}}=0.03$). In each of these plant-adjusted models, KSHV seroprevalence was elevated with plant/soil work (OR 2.73 – 3.44, $P = 0.04 – 0.11$).

Sensitivity analysis

KSHV sero-indeterminates represented 4.5% of the population. When these sero-indeterminates were included as seropositive, weighted KSHV seroprevalence was 13.0% (95% CI 9.2 – 18.0%). In sensitivity analyses, correlates of KSHV seroprevalence did not differ substantially with the inclusion of sero-indeterminates. Compared to the primary analysis (Table 1), the inclusion of sero-indeterminates as seropositive tended to strengthen KSHV's association with smaller childhood community ($P_{\text{trend}} < 0.01$) and to attenuate the association with plant/soil work ($P = 0.04$).

DISCUSSION

Consistent with previous reports linking KSHV to rural settings in Italy,^{7,8} we found that KSHV seroprevalence was elevated among elderly Sicilian adults who had grown up in small communities or who worked during adulthood with plants or soil. KSHV seroprevalence was especially high among people who reported high cumulative exposure to plants/soil (19.1%) or infrequent bathing as well as working with plants/soil (24.8%). Higher risk with infrequent bathing suggests an association with dermal contact, as bathing would have no effect on an ingested exposure. Bathing also would have little or no effect on insect bites or arthropod-borne infections that may be intimately related to plants or soil.

The persistent association with plant/soil work, even controlled for exposures to plants in three different ways (Table 3), suggests that KSHV seropositivity is linked to soil exposure. The virus certainly is not acquired from the soil. Rather, we would speculate that soil facilitates KSHV antibody seroconversion by skewing cytokine responses or facilitates the shedding of KSHV in saliva by similarly exposed, close contacts.

KSHV seroprevalence was generally lower with overall exposure to plants categorized in various ways. However, in exploratory analyses, some exceptions were found. Adjusted for exposure to soil and other plants, KSHV seroprevalence was increased more than 3-fold among participants who reported contact with *Hieracium* (hawkweed), a member of the Asteraceae plant family, other members of which induced KSHV reactivation *in vitro*.^{15,16} Another Asteraceae species, *Taraxacum officinalis* (dandelion), and two unrelated plants were associated with low KSHV seroprevalence in our population. Four members of the plant family Euphorbia, another plant family that induced *in vitro* KSHV reactivation, were unrelated to KSHV seroprevalence. Although the 20 plants in our study were selected *a priori*, no firm conclusions can be drawn without independent validation.

Social and behavioral differences might account for the apparently stronger associations in women than men. Compared to men and boys, women and girls generally had closer contact with children who were shedding KSHV, especially in small communities. Against this hypothesis, numbers of siblings and household residents were not associated with seroprevalence (Table 1), even among women (data not presented). Women and girls also were primarily responsible for washing clothes and household items, perhaps sensitizing them to plant/soil contacts. Although social, behavioral and even hormonal hypotheses might be considered, it should be noted that differences by sex were not statistically significant.

Relevant exposures may have been misclassified in our study due to poor recall or insufficient focus on the childhood or young adult years thought to be coincident with most KSHV infections. However, because participants were unaware of their KSHV serostatus, there would be no differential recall bias. Given a KSHV seroprevalence of only 8.5%, our findings are imprecise, but our design assures that they can be generalized to the elderly population of Sicily. Because current KSHV antibody assays are imperfect, some KSHV serostatus misclassification is likely. However, we maximized specificity by using four assays and a conservative algorithm, as well as by excluding the 4.5% of participants who were sero-indeterminate from the primary analyses. Our inclusion of sero-indeterminates in sensitivity analyses suggests that misclassification had no major effect on the associations that we observed.

In summary, in an elderly Mediterranean population with an intermediate prevalence of KSHV, the virus that causes KS, we found that seroprevalence was significantly increased for people who had grown up in small communities or had worked with plants or particularly with soil. As postulated by others,^{4,14,15} such exposures could affect KSHV replication, susceptible target cells, or cellular immune responses resulting in an increased probability of infection or antibody seroconversion. Substantial effort will be needed to corroborate our observations and to identify underlying mechanisms.

KEY MESSAGES

- Approximately 8.5% of elderly adults in Sicily were positive for antibodies against Kaposi sarcoma-associated herpesvirus (KSHV).
- KSHV in this population was more common among those who grew up in small communities or who worked with plants or soil.
- These associations suggest that skin exposures may affect dissemination of or susceptibility to KSHV infection and ultimately to Kaposi sarcoma.

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References

1. Dukers NH, Renwick N, Prins M, Geskus RB, Schulz TF, Weverling GJ, Coutinho RA, Goudsmit J. Risk factors for human herpesvirus 8 seropositivity and seroconversion in a cohort of homosexual men. *Am J Epidemiol* 2000;151(3):213–24. [PubMed: 10670545]
2. Pauk J, Huang ML, Brodie SJ, Wald A, Koelle DM, Schacker T, Celum C, Selke S, Corey L. Mucosal shedding of human herpesvirus 8 in men. *N Engl J Med* 2000;343(19):1369–77. [PubMed: 11070101]
3. Mbulaiteye SM, Walters M, Engels EA, Bakaki PM, Ndugwa CM, Owor AM, Goedert JJ, Whitby D, Biggar RJ. High levels of epstein-barr virus DNA in saliva and peripheral blood from Ugandan mother-child pairs. *J Infect Dis* 2006;193(3):422–6. [PubMed: 16388490]
4. Mbulaiteye SM, Biggar RJ, Pfeiffer RM, Bakaki PM, Gamache C, Owor AM, Katongole-Mbidde E, Ndugwa CM, Goedert JJ, Whitby D, Engels EA. Water, socioeconomic factors, and human herpesvirus 8 infection in Ugandan children and their mothers. *J Acquir Immune Defic Syndr* 2005;38(4):474–9. [PubMed: 15764964]
5. Mbulaiteye S, Marshall V, Bagni RK, Wang CD, Mbisa G, Bakaki PM, Owor AM, Ndugwa CM, Engels EA, Katongole-Mbidde E, Biggar RJ, Whitby D. Molecular evidence for mother-to-child transmission of Kaposi sarcoma-associated herpesvirus in Uganda and K1 gene evolution within the host. *J Infect Dis* 2006;193(9):1250–7. [PubMed: 16586362]
6. Newton R, Ziegler J, Bourboulia D, Casabonne D, Beral V, Mbidde E, Carpenter L, Reeves G, Parkin DM, Wabinga H, Mbulaiteye S, Jaffe H, Weiss R, Boshoff C. Uganda Kaposi's Sarcoma Study Group. The sero-epidemiology of Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in adults with cancer in Uganda. *Int J Cancer* 2003;103(2):226–32. [PubMed: 12455037]
7. Valdarchi C, Serraino D, Cordiali Fei P, Castilletti C, Trento E, Farchi F, Rezza G. General Practitioner Study Group. Demographic indicators and risk of infection with human herpesvirus type 8 in central Italy. *Infection* 2007 Feb;35(1):22–5. [PubMed: 17297585]
8. Angeloni A, Masala MV, Montesu MA, Santarelli R, Satta R, Ceccherini-Nelli L, Valdivieso P, Faggioni A, Cottoni F. Environmental factors influence the rate of human herpesvirus type 8 infection in a population with high incidence of classic Kaposi sarcoma. *Clin Infect Dis* 2006;42(7):e66–8. [PubMed: 16511749]
9. Mbulaiteye SM, Pfeiffer RM, Dolan B, Tsang VC, Noh J, Mikhail NN, Abdel-Hamid M, Hashem M, Whitby D, Thomas Strickland G, Goedert JJ. Seroprevalence and risk factors for human herpesvirus 8 infection, rural egypt. *Emerg Infect Dis* 2008;14(4):586–91. [PubMed: 18394276]
10. Engels EA, Atkinson JO, Graubard BI, McQuillan GM, Gamache C, Mbisa G, Cohn S, Whitby D, Goedert JJ. Risk factors for human herpesvirus 8 infection among adults in the united states and evidence for sexual transmission. *J Infect Dis* 2007;196(2):199–207. [PubMed: 17570106]
11. Tanzi E, Zappa A, Caramaschi F, Amendola A, Lasagna D, Gatti L, Ascoli V, Rezza G, Zanetti AR. Human herpesvirus type 8 infection in an area of northern Italy with high incidence of classical Kaposi's sarcoma. *J Med Virol* 2005;76(4):571–5. [PubMed: 15977229]
12. Vitale F, Briffa DV, Whitby D, Maida I, Grochowska A, Levin A, Romano A, Goedert JJ. Kaposi's sarcoma herpes virus and Kaposi's sarcoma in the elderly populations of 3 Mediterranean islands. *Int J Cancer* 2001;91(4):588–591. [PubMed: 11251987]
13. Serraino D, Cerimele D, Piselli P, Aztori L, Farchi F, Carletti, Navarra A, Masala MV, Rezza G. Infection with human herpesvirus type 8 and Kaposi's sarcoma in Sardinia. *Infection* 2006;34:39–42. [PubMed: 16501902]
14. Ascoli V, Manno D, Coluzzi M. Geographic variation in human herpesvirus 8 seroprevalence and possible association with exposure to bites from bloodsucking arthropods. *J Infect Dis* 2006 Aug 1;194(3):401–2. [PubMed: 16826490]
15. Whitby D, Marshall VA, Bagni RK, Miley WJ, McCloud TG, Hines-Boykin R, Goedert JJ, Conde BA, Nagashima K, Mikovits J, Dittmer DP, Newman DJ. Reactivation of kaposi's sarcoma-associated herpesvirus by natural products from Kaposi's sarcoma endemic regions. *Int J Cancer* 2007;120(2):321–8. [PubMed: 17066452]
16. Cho HJ, Yu F, Sun R, Lee D, Song MJ. Lytic induction of Kaposi's sarcoma-associated herpesvirus in primary effusion lymphoma cells with natural products identified by a cell-based fluorescence moderate-throughput screening. *Arch Virol* 2008;153(8):1517–25. [PubMed: 18607675]

17. Anderson LA, Lauria C, Romano N, Brown EE, Whitby D, Graubard BI, Li Y, Messina A, Gafà L, Vitale F, Goedert JJ. Risk factors for classical Kaposi sarcoma in a population-based case-control study in Sicily. *Cancer Epidemiol Biomarkers Prev* 2008;17(17):1–9. [PubMed: 18187387]
18. Engels EA, Whitby D, Goebel PB, Stossel A, Waters D, Pintus A, Contu L, Biggar RJ, Goedert JJ. Identifying human herpesvirus 8 infection: Performance characteristics of serologic assays. *J Acquir Immune Defic Syndr* 2000 Apr 1;23(4):346–54. [PubMed: 10836758]

Figure 1. Seroprevalence of antibodies against Kaposi sarcoma-associated herpesvirus for the nine provinces of Sicily. Overall, seroprevalence did not differ significantly across the provinces ($P=0.17$).

Table 1

Logistic regression analysis of variables associated with seroprevalence of antibodies against Kaposi sarcoma-associated herpesvirus (KSHV) in Sicily.

Group	Sero -	N*	Sero +	Weighted prevalence	OR	Adjusted for age and sex 95% CI	P-value
Sex							
Males	762		86	5.3%	0.5	0.2 - 1.3	0.13
Females	269		37	11.5%	Reference		
Age Group							
81+	206		29	11.1%	1.4	0.5 - 4.0	0.45 [†]
75 to 80	241		32	10.7%	1.3	0.5 - 3.6	
68 to 74	240		32	15.7%	2.1	0.7 - 6.3	
<68	344		30	7.2%	Reference		
Education							
1st - 5th grade	659		96	10.7%	2.27	0.7 - 6.9	0.29 [†]
6th - 8th grade	172		12	9.8%	2.95	0.5 - 17.4	
9th grade or more	200		15	3.5%	Reference		
Community size during childhood							
less than 1,000	54		11	17.0%	6.9	1.3 - 37.1	0.03 [†]
between 1,000 and 10,000	321		36	12.0%	5.0	1.3 - 19.0	
between 10,000 and 100,000	493		62	8.4%	3.7	1.3 - 10.5	
more than 100,000	157		14	2.6%	Reference		
People living in childhood house							
Six or more	476		58	7.7%	0.6	0.2 - 1.4	0.23
Less than six	555		65	9.0%	Reference		
Number of older siblings							
Two or more	515		57	7.5%	0.7	0.3 - 1.6	0.34
Less than two	516		66	9.2%	Reference		
Number of younger siblings							
Two or more	502		66	9.9%	1.4	0.5 - 3.7	0.50
Less than two	529		57	7.0%	Reference		
People (other than self) sharing childhood bedroom							
Three or more	380		57	8.1%	0.7	0.3 - 1.9	0.49
Less than three	651		66	8.7%	Reference		
Worked with plants or soil during adulthood							
Ever	541		72	13.0%	2.9	1.1 - 7.9	0.03
Never	489		51	5.5%	Reference		
Cumulative plant/soil work							
More than 900 total hours	267		42	19.1%	4.9	1.0 - 23.9	0.03 [†]
Up to 900 total hours	266		30	10.4%	2.3	0.9 - 6.1	
No plant/soil work	489		51	5.5%	Reference		
Frequency of bathing during adulthood							
Three per week or less	512		78	15.0%	2.5	0.7 - 8.8	0.17
More than 3 per week	519		45	5.9%	Reference		
Plants/soil work, bathing frequency							
Worked, <3 baths/week	294		52	24.8%	5.6	1.2 - 25.1	0.03
Worked, ≥3 baths/week	247		20	7.1%	1.7	0.5 - 5.7	0.43
No work, <3 baths/week	218		26	6.7%	1.1	0.4 - 3.2	0.83
No work, ≥3 baths/week	271		25	5.1%	Reference		
Worked in water during adulthood							
Ever	139		24	10.7%	1.7	0.6 - 4.9	0.31
Never	891		99	8.3%	Reference		
Worked in seawater							
Ever	23		4	8.0%	1.4	0.2 - 9.2	0.71
Never	1008		119	8.5%	Reference		
Worked in freshwater							

Group	Sero -	N*	Sero +	Weighted prevalence	OR	Adjusted for age and sex 95% CI	P-value
Ever	131		23	12.0%	1.9	0.7 - 5.6	0.23
Never	900		100	8.2%	Reference		
Drank water from well or cistern during the past 10 years							
ever	303		30	7.8%	1.0	0.3 - 3.1	0.97
never	727		93	8.9%	Reference		
Drank city or community water during the past 10 years							
Always or usually	417		53	6.5%	0.6	0.3 - 1.5	0.28
sometimes or never	614		70	9.7%	Reference		
Drank bottled mineral water during the past 10 years							
always or usually	518		59	9.4%	1.2	0.5 - 3.3	0.69
sometimes or never	513		64	7.5%	Reference		

* Totals do not all sum to 1154 due to missing data.

† P_{trend} with ordered groups.

Table 2
Sex-specific, age-adjusted correlates of Kaposi sarcoma-associated herpesvirus (KSHV) seropositivity in Sicily.

Variable	Men			Women		
	Odds ratio	95% confidence interval	P-value	Odds ratio	95% confidence interval	P-value for sex interaction
Community size during childhood						
<1000	1.0	0.2 – 4.6	0.97	17.51.6 – 198		0.23
1000 – 10,000	1.3	0.3 – 5.9		12.11.5 – 98		
10,000 – 100,000	2.0	0.6 – 6.5		6.51.0 – 43		
>100,000	Reference			Reference		
Worked with plants/soil in adulthood						
Ever	1.4	0.5 – 4.4	0.54	3.91.2 – 13		0.22
Never	Reference			Reference		

Table 3

Age- and sex-adjusted multivariate logistic regression models for risk of KSHV seropositivity by childhood community size and exposures to plants and soil.

Variable	No.	Odds ratio [*]	95% confidence interval	P-value [†]
<i>Model 1[¶]</i>				
Community size during childhood				
<1000	65	36.56	8.36 – 159.93	<0.0001
1000 – 10,000	357	9.87	2.81 – 34.63	
10,000 – 100,000	555	1.89	0.49 – 7.23	
Worked with plants/soil in adulthood	613	3.44	0.81 – 14.60	0.11
Hieracium				
≥10 exposures	185	2.82	1.01 – 7.85	0.01
<10 exposures	57	6.72	1.82 – 24.83	
Acanthus mollis				
≥10 exposures	127	0.15	0.03 – 0.63	0.02
<10 exposures	58	0.96	0.21 – 4.44	
Taraxacum officinalis				
≥10 exposures	300	0.06	0.01 – 0.31	0.001
<10 exposures	118	1.12	0.36 – 3.45	
Trigonella foenum-graecum				
≥10 exposures	120	0.16	0.04 – 0.67	0.01
<10 exposures	35	0.03	0.00 – 0.41	
<i>Model 2</i>				
Community size during childhood				
<1000	65	6.03	0.94 – 38.64	0.06
1000 – 10,000	357	4.47	1.22 – 16.43	
10,000 – 100,000	555	3.36	1.19 – 9.49	
Worked with plants/soil in adulthood	613	2.73	0.90 – 8.30	0.08
Plant factors				
Asteraceae >median	581	0.41	0.14 – 1.20	0.10
Euphorbia >median	592	0.78	0.34 – 1.82	0.57
Ins and plant foods/beverages >median	565	1.15	0.55 – 2.40	0.71
Hypericum >median	574	1.44	0.67 – 3.12	0.35
<i>Model 3</i>				
Community size during childhood				
<1000	65	9.26	1.76 – 48.71	0.02
1000 – 10,000	357	4.18	1.25 – 13.95	
10,000 – 100,000	555	2.92	1.03 – 8.31	
Worked with plants/soil in adulthood	613	2.95	1.02 – 8.55	0.04
Cumulative exposure to 20 selected plants				
More than 864 times	288	0.19	0.04 – 0.91	0.03
245 – 864 times	288	0.69	0.20 – 2.38	
45 – 244 times	288	1.63	0.60 – 4.42	
0 – 44 times	290	Reference		

^{*} Each model includes all variables shown, as well as sex and age group (as in Table 1).

Reference groups are >100,000 population for community size during childhood (N=157), never worked with plants or soil during adulthood (N=489), and no exposure to each of the six specific plants in Model 1.

[†] Heterogeneity tests, except trend test for community size, level of exposure to individual plants (Model 1), and cumulative exposure to 20 plants (Model 3).

The 16 plants that were not retained in the final model ($P > 0.15$) were: Agave (Agavaceae), Ceratonia siliqua (Fabaceae), Chrozophora tinctoria (Euphorbiaceae), Cichorium intybus (Asteraceae), Datura stramonium (Solanaceae), Euphorbia characias (Euphorbiaceae), Euphorbia dendroides (Euphorbiaceae), Euphorbia rigida (Euphorbiaceae), Gladiolus communis o italicus (Iridaceae), Hypericum hircinum (Clusiaceae), Hypericum perforatum guttiferum (Clusiaceae), Inula viscosa (Asteraceae), Iris sisyriuchium (Iridaceae), Lupinus albus (Fabaceae), Matricaria chamomilla (Asteraceae), and Picris echioides (Asteraceae).