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Type-specific herpes simplex virus-1 and herpes simplex virus-2 seroprevalence in Romania: comparison of prevalence and risk factors in women and men

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ABSTRACT

Objective: To determine herpes simplex virus (HSV)-2 and HSV-1 seroprevalence in women and men in Romania.
Methods: A cross-sectional seroprevalence survey was conducted between 2004 and 2005 on a total of 1058 women and men representative of the population of Bucharest. All participants were aged 15–44 years and completed a structured questionnaire. A blood sample was collected to detect IgG anti-HSV-1 and HSV-2 serum antibodies using the HerpeSelect ELISA (Focus Diagnostics).
Results: A total of 761 women (median age 29 years) and 297 men (median age 29 years) were included. Overall, HSV-2 seroprevalence (15.2%) increased with age. Among women, HSV-2 seroprevalence increased from 11.0% in 15–19-year-olds to 38.3% in 40–44-year-olds. Among men, seroprevalence increased from 4.0% in 20–24-year-olds to 27.1% in 40–44-year-olds. HSV-2 seroprevalence was significantly higher among women than men (17.0% vs. 10.8%). HSV-1 seropositivity was high (87.2%) in all age groups, with no clear trend by age or by sex. In addition to older age and female sex, risk factors for HSV-2 included greater number of lifetime sexual partners, lower educational attainment, and history of genital vesicles. Lower educational level and rural residence were associated with a higher risk of HSV-1 seropositivity.
Conclusions: In Romania, HSV-2 seroprevalence was higher in women than men, and was within European limits and lower than that in Africa and the USA. In contrast, HSV-1 seroprevalence was generally higher than that previously recorded in similarly aged populations in Western Europe.

1. Introduction

Herpes simplex virus type-2 (HSV-2) infection is endemic in many geographical areas[1] and associated with a higher risk of HIV infection.[2,3] As one of the most prevalent sexually transmitted infections (STIs) worldwide,[4] HSV-2 is the primary cause of genital ulcerations in both developed and developing countries.[5] HSV-2 and HSV-1 are alpha-herpes viruses which share approximately 50% nucleotide sequence homology[6,7] and can infect both orolabial and anogenital sites. Genital lesions induced by these two herpetic viruses are clinically indistinguishable. Classically, HSV-2 is characterized by anogenital infection, whereas HSV-1 is associated with orolabial infections. Over the past decade, a higher proportion of genital herpes has been attributed to HSV-1 in several world regions,[8,9] likely due to changes in sexual behavior and in the incidence of orolabial HSV-1 infection acquired during childhood.

As both HSV-2 and HSV-1 infections are generally asymptomatic,[10] estimations of their population-based seroprevalence are most reliably made by determining type-specific IgG HSV-2 and HSV-1 seropositivity.[8] The seroprevalence of HSV-2 and HSV-1 vary considerably by continent, with seropositivity clearly being dependent upon age.[1] There are certain geographical regions, however, with limited or no data on type-specific HSV seroprevalence, notably in Central and Eastern Europe. Further, only a few studies have reported differences in the seroprevalence and risk factors for HSV-2 seropositivity for women vs. men.

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To date, there are no published data on HSV-2 and HSV-1 seroprevalence using validated type-specific serological assays in Bucharest, Romania, an ex-communist country with a population of 21 million.[11] To determine the burden of HSV-2 and HSV-1 infection in Bucharest, we conducted a study to estimate type-specific HSV-1 and HSV-2 seroprevalence and to identify associated HSV-2 risk factors, stratified by sex.

2. Materials and methods

2.1. Population surveyed

Between January 2004 and June 2005, an HSV seroprevalence survey was conducted among 1066 hospital attendees in Bucharest, Romania. Study participants were eligible if they were over 15 years of age and permanent residents of Bucharest. We excluded those patients who, at the time of enrollment in the study, had labial and genital herpes (recurrent or not), varicella, herpes zoster, viral encephalitis, a symptomatic sexually transmitted disease (STD; genital ulcerative disease, dysuria, genital discharge, genital warts), AIDS, or a severe mental disorder. Symptomatic HIV infection was defined by known HIV infection at stage B or C of the Centers for Disease Control and Prevention (CDC) classification. The study was approved by the ethical review committees of the two participating hospitals.

Study participants were selected from two different populations. The first population, consisting of 452 pregnant women aged 15–39 years, was recruited during prenatal visits to the Elias University Emergency Hospital. The second population group included 606 patients (297 men and 309 women) aged between 15 and 44 years attending the Professor Dr. Matei Bals National Infectious Diseases Institute. The present analysis includes HSV-2 type-specific data on a subset of 452 pregnant women. In both populations, sampling was conducted within stratified age groups of five-years to include approximately equal sample sizes per five-year age group (15–19, 20–24 years, etc.), stratified by sex. Both Bucharest hospitals have university staff and catchment areas including all parts of Bucharest. Of the total eligible subjects, 90% of the women and 84% of the men participated.

2.2. Questionnaire and laboratory testing

After obtaining informed consent, each participant completed an anonymous standardized questionnaire to collect information on sociodemographics, sexual behavior, reproductive and gynecological history, family planning, and smoking habits. During clinical examination, a 5-ml blood sample was collected for the detection of antibodies to HSV-1 and HSV-2. Collected samples were centrifuged at 1000 *g* for 10 min. Serum samples were transferred to sterile cryotubes of 0.5–1 ml, stored at −70 °C, and processed in the laboratory at the Professor Dr. Matei Bals National Infectious Diseases Institute.

Type-specific serum antibodies to HSV-1 and HSV-2 were detected using HerpeSelect 1 and HerpeSelect 2 IgG ELISAs (Focus Diagnostics, Cypress, CA, USA), as per the manufacturer's instructions. The HerpeSelect HSV serological tests are based on glycoprotein G antigens to elicit type-specific antibody responses: gG1 from HSV-1 and gG2 from HSV-2. In accordance with the manufacturer's instructions, an optical density cut-off value of 1.1 was used to define HSV-1 and HSV-2 seropositivity. In terms of the specified cut-off point for the HerpeSelect HSV-2 ELISA to define HSV-2 seropositivity, we chose not to use the modified higher cut-off value of >3.5, as we have found that this cut-off does not necessarily result in optimal performance (largely due to inadequate sensitivity) based on a validation study in Kenya.[12] Equivocal samples were retested using the same ELISA test. In total, four samples were equivocal for HSV-1 and four samples for HSV-2. All eight samples remained equivocal after retesting and were excluded from statistical analyses. All samples were tested blinded of the original questionnaire results.

For quality control purposes, a total of 74 samples (7%) were retested in a blinded fashion with the HerpeSelect HSV-1 and HSV-2 ELISAs (Focus Diagnostics). For all samples, the retest results agreed with the original test for both HSV viral types. Type-specific HSV test results were not systematically reported back to the study participants, given that treatment based on HSV serostatus is not currently the recommended standard of care.

2.3. Statistical analyses

A Mantel–Haenszel Chi-square test was used to determine differences in HSV-1 and HSV-2 seroprevalence. To evaluate risk factors for type-specific HSV-2 seropositivity, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by multiple logistic regression, controlling for age, study site and, if appropriate, sex, using SPSS v. 12.0 (SPSS Inc., Chicago, IL, USA). A similar analysis was performed for HSV-1 infection. Data were combined for pregnant and non-pregnant women because demographic characteristics including age were comparable between the two groups. Tests for trend for odds ratios were assessed by considering the categorical variable as a continuous variable in the logistic model. A multiple logistic regression model was fitted by starting with a model containing all variables considered to be predictive for HSV-2 infection and proceeding by backward selection.

3. Results

Type-specific HSV serological results were available for 1058 patients (761 females, 297 males), with a median age for both sexes of 29 (range 15–44) years (Table 1). Overall, HSV-2 seroprevalence was 15.2%, with seroprevalence among women

Table 1
HSV-2 and HSV-1 seroprevalence in Bucharest, Romania, in the total population and by sex

	Total sample	Females	Males	*p*-Value
Sample size	1058	761	297	
Median age in years (range)	29 (15–44)	29 (15–44)	29 (15–44)	
HSV-2 (%)	15.2	17.0	10.8	0.016[a]
HSV-1 (%)	87.2	88.0	85.2	0.251[a]
Both HSV-1 and HSV-2 positive (%)	12.9	14.7	8.4	0.015[b]
Both HSV-1 and HSV-2 negative (%)	10.5	9.7	12.5	0.015[b]
Genital herpes history in HSV-2 positives (%)	23.0	21.7	28.1	0.129[a]
Oral herpes history in HSV-1 positives (%)	63.1	64.8	58.5	0.768[a]

HSV, herpes simplex virus.
[a] By Mantel–Haenszel test between males and females.
[b] By Pearson Chi-square test between males and females.

(17.0%) higher than men (10.8%). HSV-1 seroprevalence was 87.2% overall, and similar among women (88.0%) and men (85.2%). Dual seropositivity was present in 12.9% of samples (females 14.7%, males 8.4%), while 10.5% of the overall study samples had no antibodies for either subtype (females 9.7%, males 12.5%). A history of genital herpes in HSV-2 positive participants was less common (23.0% symptomatic genital herpes in the HSV-2 seropositive group) than a history of oral herpes in the HSV-1 positive participants (63.1%).

HSV-2 seroprevalence gradually increased with age to peak at the oldest age surveyed of 40–44 years of age for both sexes (Figure 1A). Among women, HSV-2 seroprevalence increased from 11.0% in those aged 15–19 years to 38.3% in those aged 40–44 years; among men it increased from 4.0% in 20–24-year-olds to 27.1% in 40–44-year-olds. There was little variation across age groups for HSV-1, reflective of the high seroprevalence (≥80%) for all age groups (Figure 1B). For both serotypes, the seroprevalence in males was consistently lower than that in females, with the one exception of HSV-1 in the 35–39 years age group.

In univariate analyses, the strongest risk factors for HSV-2 seropositivity among all participants in the age-, center-, and sex-adjusted model (Table 2) included age (OR 6.6, 95% CI 3.3–13.0 for 40–44 vs. 20–24 years) and history of genital herpes (OR 7.1, 95% CI 4.2–12.1 vs. without history of genital herpes). Additional risk factors included the total number of sex partners (OR 3.5, 95% CI 2.1–5.9 for 3+ vs. 0–1), number of occasional sex partners, defined by persons with whom the subject had a rare, irregular sexual relationship, for less than 6 months (OR 1.9, 95% CI 1.1–3.3 for 3+ vs. none), reporting lower educational attainment (OR 2.3, 95% CI 1.2–4.6 for primary education vs. higher education), age at first intercourse (OR 2.6, 95% CI 1.3–5.1 for age ≤16 vs. ≥21 years), and any STI history (OR 2.1, 95% CI 1.3–3.1). Other potential risk factors that were not associated with overall HSV-2 seropositivity in the combined study population included smoking status and ever use condoms. HSV-2 risk factors were generally similar between women and men, although specific associations for women appeared stronger than those for men: lower educational attainment (OR 2.9, 95% CI 1.3–6.5 for primary vs. higher), reporting two lifetime sexual partners (OR 2.2, 95% CI 1.4–3.5 vs. 0–1), having had three or more occasional sexual partners (OR 2.5, 95% CI 1.2–5.2), age at first intercourse ≤16 years (OR 2.6, 95% CI 1.1–6.0), and any STI history (OR 1.88, 95% CI 1.2–3.0).

Overall HSV-1 seropositivity among all study participants in the age-, center-, and sex-adjusted model was associated with lower educational attainment (OR 2.3, 95% CI 1.1–4.9 for primary education vs. higher education), being born in rural areas (OR 2.4, 95% CI 1.3–4.5 vs. urban), younger age at first intercourse (OR 3.0, 95% CI 1.4–6.5 for ≤16 vs. ≥21 years), and a greater number of sexual partners (OR 2.1, 95% CI 1.2–3.7 for 3+ vs. 1 sex partner) (Table 3). HSV-1 risk factors in women included lower education (OR 2.8, 95% CI 1.6–4.6 for secondary vs. higher) and rural place of birth (OR 2.4, 95% CI 1.1–5.2 vs. urban). Property ownership was also a protective factor for HSV-1 in women (OR 0.5, 95% CI 0.3–0.9 vs. non-owners). For analyses limited to men, risk factors included current smoking (OR 3.7, 95% CI 1.5–9.2 vs. never smokers), age at first intercourse (OR 6.5, 95% CI 1.9–21.7 for ≤16 vs. ≥21 years), total number of sex partners (OR 4.6, 95% CI 1.9–11.0 for 3+ vs. 1), and occasional sex partners (OR 3.6, 95% CI 1.3–10.3 for 3+ vs. 0).

In a multivariate analysis, risk factors for HSV-2 seropositivity (Table 4) were the same as those previously found in the age-, recruitment site- and sex-adjusted analyses: age, female sex, lower educational attainment, reporting a greater number of sexual partners, and a reported history of genital herpes. Among males, current smoking appeared to be associated with a lower risk of HSV-2 seropositivity, and condom use did not appear to be protective in either women or men. HSV-1 was also not associated with HSV-2 in the multivariate model (OR 0.7, 95% CI 0.4–1.2) (data not shown).

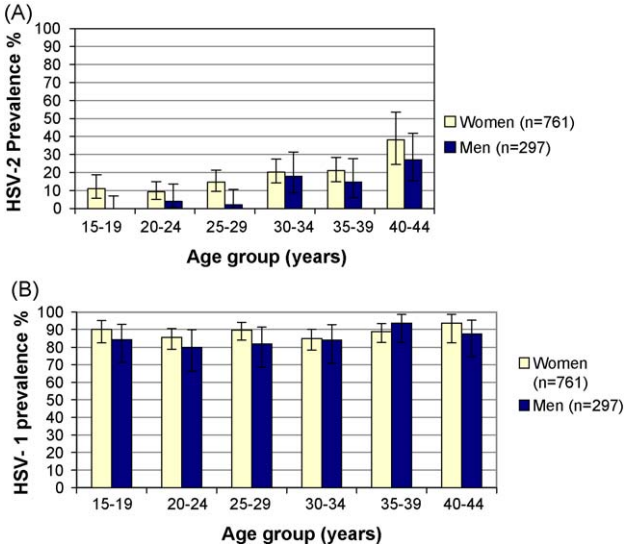

Figure 1. Seroprevalence of (A) HSV-2 and (B) HSV-1, by sex and age, Romania.

4. Discussion

This study is the first, to our knowledge, to document the type-specific HSV seroprevalence in Bucharest among both females and males. HSV-1 seropositivity was consistently high across age and sex, whereas HSV-2 seropositivity appeared to increase with age and was generally higher in women than in men. A higher HSV-2 seropositivity overall was found among participants who reported a previous history of genital herpes, a greater number of lifetime or occasional sexual partners, and lower educational attainment. In contrast, HSV-1 seropositivity was more strongly associated with factors related to socio-economic status than HSV-2, including rural residence, educational attainment, and home ownership.

Few data are currently available on HSV seroprevalence in Eastern Europe. A multi-country study was conducted across Europe and included data from the Czech Republic, Bulgaria, and Slovenia using type-specific Focus Diagnostics HSV-1 and HSV-2 tests. HSV-2 seropositivity ranged from a low 6% among 3999 women aged >12 years in the Czech Republic to a high of 23.9% in 3200 women in Bulgaria,[13] likely reflecting differences in sexual behavior across geographical sites. An overall seroprevalence of 9.3% was found in a study of 2257 blood donors from four areas of Poland.[14] Thus, the HSV-2 seroprevalence of 15.2% in Bucharest found in the present study falls within the Eastern European range, and is also consistent with HSV-2 seroprevalence data from Europe.[13]

In the same European multi-country study, HSV-1 seroprevalence appears to be higher in Eastern and Central Europe than Western and Southern Europe.[13] The 87% HSV-1 seropositivity found in Romania is similar to the seroprevalence in equivalent age groups of women surveyed in the Czech Republic, Bulgaria, Slovenia, and Germany. The study across four regions of Poland also found similar HSV-1 rates among those participants aged 15–44 years, comparable to results from this Romanian study.[14] The HSV-1 seroprevalence found, although high, is similar to that found in other developed countries, including Australia.[15]

Consistent with previous studies in Europe[13,14] and the USA,[1] women appeared to have a higher HSV-2 seropositivity than men. Women may be more biologically susceptible to HSV infection, as is the case for HIV infection.[16] We found that HSV-1 seropositivity was not associated with lower HSV-2 seropositivity in Bucharest,

Table 2
Odds ratios and 95% confidence intervals for the association between HSV-2 seropositivity and sociodemographic and behavioral factors in adults aged 15–44 years in Romania[a]

Factor	All participants OR (95% CI)	*n*	Females OR (95% CI)	*n*	Males OR (95% CI)
Age (years)					
15–19	1.0 (0.4–2.1)	100	1.0 (0.5–2.7)	50	-
20–24	1	152	1	57	1
25–29	1.5 (0.8–3.0)	157	1.7 (0.8–3.4)	54	0.5 (0.1–5.6)
30–34	2.9 (1.6–5.4)	153	2.5 (1.3–5.0)	49	5.3 (1.1–26)
35–39	2.8 (1.5–5.3)	152	2.6 (1.3–5.2)	52	4.1 (0.8–21.0)
40–44	6.6 (3.3–13.0)	47	5.7 (2.5–13.0)	47	8.9 (1.9–42.0)
	p for trend <0.001		*p* for trend <0.001		*p* for trend <0.001
Education					
Primary	2.3 (1.2–4.6)	82	2.9 (1.3–6.5)	49	1.1 (0.3–4.6)
Secondary	1.3 (0.9–2.0)	358	1.5 (1.0–2.4)	161	0.68 (0.3–1.6)
Higher	1	316	1	86	1
	p for trend 0.020		*p* for trend 0.006		*p* for trend 0.74
Place of birth					
Urban	1	246	1	618	1
Rural	1.2 (0.8–1.8)	51	1.3 (0.8–2.0)	143	0.63 (0.2–1.7)
Property owner					
No	1	481	1	168	1
Yes	0.8 (0.6–1.2)	264	0.8 (0.5–1.3)	113	1.1 (0.4–2.9)
Marital status					
Married	1	489	1	149	1
Cohabitating	1.7 (1.1–2.6)	138	1.9 (1.1–3.2)	32	1.1 (0.3–4.3)
Single/divorced	1.2 (0.6–2.0)	132	1.4 (0.8–2.7)	110	0.8 (0.2–3.9)
Smoking status					
Never	1	352	1	97	1
Former smoker	1.2 (0.8–1.7)	227	1.2 (0.8–1.9)	91	0.74 (0.3–1.8)
Present smoker	1.3 (0.8–2.0)	180	1.6 (1.0–2.5)	107	0.5 (0.2–1.4)
Age at first intercourse (years)					
≤16	2.6 (1.3–5.1)	77	2.6 (1.1–6.0)	103	3.5 (0.7–18)
17–18	2.3 (1.4–3.9)	202	2.0 (1.2–3.5)	90	4.1 (0.8–20)
19–20	1.6 (1.0–2.7)	226	1.6 (0.9–2.9)	48	2.2 (0.4–12)
21+	1	212	1	28	1
	p for trend 0.001		*p* for trend 0.005		*p* for trend 0.107
Total number of sexual partners					
0–1	1	400	1	53	1
2	2.1 (1.3–3.2)	264	2.2 (1.4–3.5)	76	1.0 (0.2–6.2)
3+	3.5 (2.1–5.9)	94	3.1 (1.7–5.4)	164	3.7 (0.8–17)
	p for trend 0.000		*p* for trend 0.000		*p* for trend 0.018
Partner had other sexual partners (ever)					
No	1	276	1	148	1
Don't know	1.6 (0.8–1.7)	292	1.8 (1.0–3.1)	106	0.5 (0.1–4.0)
Yes	1.9 (1.3–2.8)	142	2.1 (1.3–3.4)	19	1.2 (0.5–2.7)
Occasional sex partner					
0	1	366	1	35	1
1–2	1.3 (0.7–2.1)	135	1.5 (0.9–2.5)	63	0.9 (0.1–6.7)
3+	1.9 (1.1–3.3)	47	2.5 (1.2–5.2)	139	3.9 (0.8–19)
	p for trend 0.023		*p* for trend 0.012		*p* for trend 0.026
Condom use with occasional partner					
No	1	48	1	26	1
Yes	1.4 (0.6–3.4)	67	1.4 (0.5–4.2)	127	1.2 (0.2–5.7)
Ever use condoms					
No	1	479	1	136	1
Yes	0.8 (0.5–1.1)	282	0.8 (0.5–1.2)	161	0.7 (0.3–1.5)
Oral contraceptive use					
No	1	498	1	259	1
Yes	0.8 (0.6–1.3)	263	0.9 (0.6–1.4)	0	-
Any STI history					
No	1	528	1	203	1
Yes	2.1 (1.3–3.1)	127	1.9 (1.2–3.0)	49	2.2 (0.9–5.5)
Genital herpes history					
No	1	605	1	231	1
Don't know	2.2 (1.4–3.4)	104	2.6 (1.6–4.3)	44	1.0 (0.3–3.3)
Yes	7.1 (4.2–12.1)	52	7.2 (3.8–13)	22	5.2 (1.8–15)

HSV, herpes simplex virus; OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection.
[a] OR = odds ratios adjusted for age, recruitment site and, where appropriate, sex.

despite conflicting evidence of a potential association elsewhere.[17] The cross-sectional design of the current analyses limited our ability to investigate potential temporal relationships between HSV-2 and HSV-1 infections.

We found a notably higher HSV-2 seroprevalence among participants from Bucharest reporting lower rather than higher educational attainment. Study participants reporting a primary school level of education were more likely to report younger age at first intercourse (median 16 years for primary vs. 18.5 years for secondary) and less regular use of condoms with regular or occasional sexual partners (28% and 42% for primary, respectively vs. 53% and 82% for higher education, respectively). In contrast,

Table 3
Odds ratios and 95% confidence intervals for the association between HSV-1 seropositivity and sociodemographic and behavioral factors in adults aged 15–44 years in Romania[a]

Factor	All participants OR (95% CI)	*n*	Females OR (95% CI)	*n*	Males OR (95% CI)
Age (years)					
15–19	1	100	1	50	1
20–24	0.7 (0.4–1.3)	152	0.7 (0.3–1.5)	57	0.7 (0.3–2.1)
25–29	1.0 (0.5–2.9)	157	1.0 (0.4–2.3)	54	0.9 (0.3–2.4)
30–34	0.7 (0.4–1.4)	153	0.6 (0.3–1.4)	49	1.0 (0.3–2.8)
35–39	1.2 (0.6–2.4)	152	0.9 (0.4–2.1)	52	2.8 (0.7–11)
40–44	1.3 (0.6–3.1)	47	1.5 (0.4–6.0)	47	1.3 (0.4–4.1)
	p for trend 0.26		*p* for trend 0.38		*p* for trend 0.17
Education					
Primary	2.3 (1.1–4.9)	82	1.9 (0.8–4.8)	49	2.1 (0.5–8.0)
Secondary	1.8 (1.2–2.8)	358	2.8 (1.6–4.6)	161	0.7 (0.3–1.6)
Higher	1	316	1	86	1
	p for trend 0.003		*p* for trend 0.002		*p* for trend 0.50
Place of birth					
Urban	1	246	1	618	1
Rural	2.4 (1.3–4.5)	51	2.4 (1.1–5.2)	143	2.1 (0.7–6.7)
	p for trend 0.008		*p* for trend 0.024		*p* for trend 0.19
Property owner					
No	1	264	1	113	1
Yes	0.7 (0.4–1.0)	481	0.5 (0.3–0.9)	168	1.0 (0.5–2.1)
	p for trend 0.050		*p* for trend 0.019		*p* for trend 0.99
Marital status					
Married	1	489	1	149	1
Cohabitating	1.5 (0.8–2.6)	138	1.3 (0.7–2.4)	32	4.8 (0.6–40)
Single/divorced	0.8 (0.5–1.5)	132	1.4 (0.6–3.0)	110	0.51 (0.2–1.5)
	p for trend 0.67		*p* for trend 0.38		*p* for trend 0.17
Smoking status					
Never	1	352	1	97	1
Former smoker	1.0 (0.7–1.5)	227	0.8 (0.5–1.3)	91	1.6 (0.7–3.4)
Present smoker	1.6 (1.0–2.6)	180	1.0 (0.6–1.9)	107	3.7 (1.5–9.2)
	p for trend 0.085		*p* for trend 0.91		*p* for trend 0.004
Age at first intercourse(years)					
≤16	3.0 (1.4–6.5)	77	2.2 (0.7–6.7)	103	6.5 (1.9–21.7)
17–18	1.3 (0.8–2.2)	202	1.0 (0.5–1.7)	90	3.7 (1.2–11)
19–20	1.2 (0.7–1.9)	226	1.1 (0.6–1.9)	48	1.9 (0.6–5.5)
21+	1	212	1	28	1
	p for trend 0.016		*p* for trend 0.50		*p* for trend 0.001
Total number of sex partners					
1	1	400	1	53	1
2	0.9 (0.6–1.4)	264	0.8 (0.5–1.3)	76	1.4 (0.6–3.3)
3+	2.1 (1.2–3.7)	94	1.1 (0.5–2.3)	164	4.6 (1.9–11.0)
	p for trend 0.043		*p* for trend 0.85		*p* for trend 0.000
Partner had other sexual partners (ever)					
No	1	276	1	148	1
Don't know	1.5 (1.0–2.3)	292	1.3 (0.8–2.2)	106	2.5 (1.1–5.6)
Yes	0.8 (0.5–1.4)	142	0.8 (0.5–1.5)	19	0.6 (0.2–1.8)
Occasional sex partner					
0	1	366	1	35	1
1–2	1.0 (0.6–1.6)	135	1.1 (0.6–2.1)	63	0.9 (0.3–2.4)
3+	2.4 (1.2–4.8)	47	1.0 (0.4–2.6)	139	3.6 (1.3–10.3)
	p for trend 0.033		*p* for trend 0.81		*p* for trend 0.003
Condom use with occasional partner					
No	1	48	1	26	1
Yes	0.4 (0.1–1.2)	67	0.4 (0.1–2.0)	127	0.4 (0. 1–1.9)
Any STI history					
No	1	528	1	203	1
Yes	1.3 (0.8–2.2)	127	1.1 (0.6–2.0)	49	1.9 (0.7–5.4)
	p for trend 0.35		*p* for trend 0.79		*p* for trend 0.20
Genital herpes history					
No	1	605	1	231	1
Don't know	1.3 (0.7–2.2)	104	1.0 (0.5–2.0)	44	1.8 (0.6–5.5)
Yes	0.6 (0.3–1.2)	52	0.5 (0.2–1.0)	22	1.1 (0.3–1.2)

HSV, herpes simplex virus; OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection.
[a] OR = odds ratios adjusted for age, recruitment site and, where appropriate, sex.

participants with a primary school level education also reported fewer lifetime sexual partners than those with higher educational attainment (1 for primary vs. 2 for university). We do not have information concerning potential differences in the sexual risk profiles of participant's chosen sexual partners with lower vs. higher educational attainment, although network analyses would have proven useful.[18]

Among study strengths, a well validated serological assay (HerpeSelect)[19] was used for the determination of type-specific serological antibodies in a central laboratory, with high quality control standards. The inclusion of men in addition to women allowed for the comparison of a wide range of risk factors by sex. Limitations include the relatively small sample size of 297 men, which limited our ability to clearly determine HSV-2 risk factors,

Table 4
Results of the multivariate analysis by risk factors for HSV-2 seropositivity in male and female adults aged 15–44 years in Bucharest, Romania[a,b]

Factor	All subjects OR (95% CI)	*n*	Females OR (95% CI)	*n*	Males OR (95% CI)
Sex					
Female	1		-		-
Male	0.3 (0.2–0.7)		-		-
Age (years)					
15–19	0.7 (0.3–2.0)	100	0.8 (0.3–2.4)	50	-
20–24	1	152	1	57	1
25–29	1.2 (0.5–2.7)	157	1.2 (0.5–2.9)	54	0.5 (0.1–8.9)
30–34	2.6 (1.3–5.5)	153	2.4 (1.1–5.4)	49	6.1 (0.9–40)
35–39	2.2 (1.0–4.7)	152	1.8 (0.8–4.1)	52	7.6 (1.0–57)
40–44	4.3 (1.7–11)	47	2.8 (0.8–10)	47	11 (1.7–78)
	p for trend < 0.001		p for trend 0.019		p for trend < 0.001
Education					
Primary	5.2 (2.0–13)	82	4.7 (1.5–14)	49	10 (1.2–91)
Secondary	1.8 (1.1–3.0)	358	1.9 (1.0–3.5)	161	0.37 (0.1–1.1)
Higher	1	316	1	86	1
	p for trend 0.001		p for trend 0.004		p for trend 0.052
Smoking status					
Never	1	352	1	97	1
Former smoker	0.8 (0.5–1.5)	227	1.2 (0.6–2.4)	91	0.2 (0.1–0.9)
Present smoker	0.9 (0.5–1.5)	180	1.1 (0.6–2.0)	107	0.2 (0.1–0.7)
Age at first intercourse (years)					
≤16	1.0 (0.4–2.5)	77	1.1 (0.3–3.3)	103	0.7 (0.1–5.4)
17–18	1.7 (0.9–3.4)	202	1.5 (0.7–3.2)	90	2.3 (0.3–15)
19–20	1.4 (0.7–2.6)	226	1.4 (0.7–2.7)	48	1.2 (0.2–8.9)
21+	1	212	1	28	1
	p for trend 0.521		p for trend 0.508		p for trend 0.642
Total number of sex partners					
0–1	1	400	1	53	1
2	2.0 (1.0–3.8)	264	2.1 (1.0–4.2)	76	0.2 (0.1–8.3)
3+	2.8 (1.1–7.2)	94	2.7 (0.9–7.8)	164	0.5 (0.1–23)
	p for trend 0.023		p for trend 0.038		p for trend 0.771
Occasional sex partners					
0	1	366	1	35	1
1–2	0.8 (0.4–1.5)	135	0.8 (0.4–1.6)	63	3.2 (0.2–67)
3+	1.8 (0.7–4.6)	47	1.6 (0.5–4.8)	139	10 (0.5–221)
	p for trend 0.443		p for trend 0.834		p for trend 0.096
Ever use condoms					
No	1	479	1	136	1
Yes	0.7 (0.43–1.1)	282	0.8 (0.4–1.3)	161	0.4 (0.1–1.1)
Genital herpes history					
No	1	605	1	231	1
Don't know	2.3 (1.3–4.0)	104	3.0 (1.6–5.7)	44	0.7 (0.2–3.8)
Yes	6.1 (3.1–12)	52	5.5 (2.5–12)	22	7.5 (1.9–30)

HSV, herpes simplex virus; OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection.
[a] OR = odds ratios adjusted for all other variables in the table.
[b] Results are adjusted for recruitment site.

given the relatively low HSV-2 seroprevalence in men (10.8%). Another limitation is that the HerpeSelect HSV-1 ELISA may have been less specific for the detection of type-specific HSV-1 antibodies as compared to the specificity of the HerpeSelect HSV-2 ELISA test for the detection of type-specific HSV-2 antibodies.[19]

Furthermore, sample representativeness may have been reduced by the exclusion of those with recurrent HSV-1/2 infections or other symptomatic STIs. We should emphasize that this is a convenience sample; although this study was conducted at two hospitals in Bucharest with wide catchment areas, HSV-2 and HSV-1 seroprevalence figures may not be representative of the general Romanian population of reproductive age. It is also possible that the participants enrolled at the infectious diseases institute were more likely to have been exposed to an infectious disease such as HSV, thus may have been expected to have a higher seroprevalence than at the university hospital. We believe, however, that this did not represent an important bias, given that HSV-2 seroprevalence was similar among participating women from these two clinic sites. The selection criteria used in this study excluded individuals who reported recurrent labial and genital herpes as well as a symptomatic STD or AIDS. These enrollment criteria potentially excluded a proportion of the population who may have been more likely to be HSV-2-seropositive, resulting in a potential underestimation of the overall HSV seroprevalence in our survey sample. Representativeness could be increased by examining HSV seroprevalence in other geographical areas of Romania, and by including populations that may not seek clinical care at hospitals, such as potentially vulnerable subgroups within Romania.[20] A sub-analysis among ethnic subgroups found higher HSV-2 seroprevalence amongst 21 Roma (19%) vs. 1032 ethnic Romanians (15.2%), although associations were not significant due to the limited sample size.

Longitudinal studies in Romania could potentially provide valuable information on HSV-2 epidemiology over time, particularly as HSV-2 seropositivity is a relatively reliable marker of higher risk sexual behavior within a population.[21] Once high-risk HSV-2-seropositive population groups are detected, long-term sexual risk prevention interventions could be established to reduce the spread of HSV-2, as well as other STIs such as HIV.[22]

Our results of HSV seroprevalence in Bucharest, Romania should be viewed in the context of rates of other STIs at the country-level. Although rates of HIV (0.93/100 000 population in 2006)[23] and *Chlamydia trachomatis* (1.1/100 000 in 2006)[23] remain relatively low, the incidence of invasive cervical cancer is amongst the highest reported in Europe (23.4/100 000 women in 2002),[24] likely attributable to a relatively lower coverage of regular

cytological screening services. In 2006, rates of syphilis (6.2/100 000) and gonorrhea (26/100 000) were similar to those in other Eastern and Western European countries.[23] With the recent entry of Romania into the European Union,[25] socio-economic changes[25] could lead to higher levels of risky sexual behavior and corresponding higher rates of HSV-2 and other STIs. Monitoring HSV-2 seroprevalence could assist in mapping changes in sexual behavior in these times of social and economic change.

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