ORIGINAL ARTICLE

The seroepidemiology of herpes simplex virus type 1 and 2 in Europe

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Objectives: To describe the seroepidemiology of herpes simplex virus (HSV) types 1 and 2 in the general populations of eight European countries to better understand recent reported changes in disease epidemiology.

Methods: Belgium, Bulgaria, Czech Republic, England and Wales, Finland, Germany, Netherlands, and Slovenia conducted national cross sectional serological surveys for HSV-1 and HSV-2 between 1989 and 2000. Survey sizes ranged from 3000 to 7166 sera. External quality control was ensured through reference panel testing.

Results: Large intercountry and intracountry differences in HSV-1 and HSV-2 seroprevalence were observed. Age standardised HSV-1 seroprevalence ranged from 52% in Finland, to 57% in the Netherlands, 67% in Belgium, 81% in Czech Republic, and 84% in Bulgaria. Age standardised (>12 years) HSV-2 seroprevalence ranged from 24% in Bulgaria, to 14% in Germany, 13% in Finland, 11% in Belgium, 9% in Netherlands, 6% in Czech Republic, and 4% in England and Wales. In all countries, probability of seropositivity for both infections increased with age. A large proportion of teenagers and young adults remain HSV-1 susceptible particularly in northern Europe. Women were significantly more likely to be HSV-2 seropositive in six of seven (p<0.05) countries and HSV-1 seropositive in four of seven (p<0.05) countries, particularly in northern Europe. No significant evidence of a protective role of HSV-1 for HSV-2 infection was found adjusting for age and sex (p<0.05).

Conclusions: There is large variation in the seroepidemiology of HSV-1 and HSV-2 across Europe. The observation that a significant proportion of adolescents are now HSV-1 susceptible may have implications for transmission and clinical presentation of HSV-1 and HSV-2.

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H erpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are among the commonest human viral infections.[1] Transmission is usually through intimate contact, with HSV-2 transmitted predominantly sexually and HSV-1 mainly horizontal in childhood.[2] Exposure to HSV before or during birth through primary infection or reactivation can result in severe systemic neonatal infection.[3][4]

HSV-2 is of public health importance as one of the commonest causes of genital ulceration worldwide and implicated as an important co-factor for HIV infection.[5] HSV-1 was associated predominantly with orolabial ulceration; however, recent changes in HSV-1 and HSV-2 epidemiology have been reported, with an increase in genital[6–10] and neonatal herpes particularly caused by HSV-1.[3][9–12] However, routine surveillance is hindered as many of those infected remain asymptomatic or fail to present to health services.[1] The recent commercial development of type specific enzyme immunoassays (ELISA) that reliably distinguish between antibodies to HSV-1 and HSV-2, enable serological studies which can measure both symptomatic and asymptomatic infection. Subsequently, a small number of population based seroprevalence studies have been reported[1][13–20]; some document recent changes in the seroepidemiology of HSV-1 and HSV-2, with an increase in HSV-2 seroprevalence in some countries[1][13][14][17] and a decline in others.[18] A decline in HSV-1 infection in childhood has been reported,[15] with an increasing proportion of adolescents susceptible. However, some suggest these differences are the result of methodological variations.[19]

With several HSV candidate vaccines in clinical trial and antiviral therapy available,[21] a clear understanding of the epidemiology of HSV in different populations is required to develop the most appropriate prevention and control strategies. The aim of this study was to describe seroepidemiology of HSV-1 and HSV-2 in the general populations of a variety of European countries using comparable methodology.

METHODS

National cross sectional seroprevalence surveys for HSV-1 and HSV-2 were undertaken in eight European countries: Belgium, Bulgaria, Czech Republic, England and Wales, Finland, Germany, Netherlands, and Slovenia. The England and Wales data have previously been reported.[15]

Collection of serum banks

Sample size calculations by age group and sex were based on estimated antibody prevalence. For HSV-1, interest focused primarily on younger age groups, where the majority of transmission occurs. The total number needed was estimated to be 4000 age stratified sera per country (200 sera per 2 year age strata from age 1–24 years and 200 sera per 5 year age strata from age 25–65 years with equal numbers in each stratum by sex). With these sample sizes, differences of 15% to 20% between age and sex subgroups in different populations should be demonstrated.

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Abbreviations: CDSC, Communicable Disease Surveillance Centre; ELISA, enzyme linked immunosorbent assays; HSV, herpes simplex virus

Sampling aimed to provide an estimate of immunity in the general population at a national level and was undertaken either by population based random sampling (four countries) or through unlinked, anonymous, residual sera submitted to laboratories for routine diagnostic purposes (four countries) (table 1). Details of the collection of these serological surveys have been previously reported.[15 22–27] In brief, samples were obtained from a variety of geographical locations stratified by age and sex within each country to provide a reasonably representative estimate of the general population experience. Each specimen had a unique identifier plus sex and age in completed years, the year the specimen was collected. The sole exclusion criterion was sera collected from individuals with known immune deficiencies. Sera were stored at less than −20°C until tested.

In Belgium, the serum bank was residual sera from private and hospital laboratories in Flanders (five of 10 Belgian provinces—that is, 57% of the Belgian population). For the hospital samples, sera were from children and adults admitted to general surgery, traumatology, orthopaedic, and emergency units. For the private laboratories, samples were taken for screening or insurance purposes.[22] In Slovenia, the serum bank was from the national, unlinked, anonymous HIV prevalence survey in pregnant women.[23] In Finland, the serum bank was residual sera submitted from primary and secondary care to the two main microbiology laboratories in the country. In Bulgaria, the serum samples were from a population based survey in healthy children attending daycare centres or kindergartens; schoolchildren and students in colleges and universities, and working adults.[26] In the Czech Republic, in a population based survey, 10 districts were selected at random from all 80 districts with 750 samples per district obtained across all age groups (males and females equally). From each district, 20 paediatricians or general practitioners were selected at random to identify individuals of varying ages of either sex. Individuals with known infection, antibiotic treatment, or immunodeficiency were excluded [27].

The total number of sera collected by each country ranged from 3000 to 7166. Collection took place between 1989 and 2000 (table 1).

Validation and standardisation of laboratory methods

To validate main serum bank testing, the Central Public Health Laboratory (CPHL), London, United Kingdom, created and distributed a panel of reference sera to each laboratory. The panel had been previously tested with an in-house reference HSV type specific blocking assay.[28] The panel consisted of 88 sera including HSV-1 and HSV-2 negative, equivocal, and positive specimens. Panel testing was undertaken before and during main serum bank testing. All testing was undertaken blindly. Qualitative and quantitative results of reference panel testing were returned to CPHL.

All primary testing of reference panels was undertaken using the commercial indirect HSV-1 (gG1) and HSV-2 (gG2) antibody assay, HerpesSelect (Focus Technologies, Cypress, CA, USA). The assays have previously been shown to be sensitive and specific,[29] and approved by the US Food and Drug Administration (FDA). Assay kits from one manufacturing batch were bulk purchased and distributed by CPHL to all laboratories for testing the main serum banks and the reference panel.

All laboratories met kit validation criteria testing the reference panel, achieving >90% specificity and sensitivity for both rounds compared to the consensus results for all laboratories and the reference assay results.[30]

Main serosurvey testing strategy

Primary testing of specimens from national serosurveys was conducted in the seven national laboratories (excluding England and Wales) with the kits as described. England and Wales tested with the in-house type specific binding ELISA assay.[28] Sera from children <12 years were tested only with the Focus HSV-1 antibody assay, and sera from ≥12 year olds were tested with both the Focus HSV-1 and HSV-2 assays. All equivocal sera (for HSV-1 or HSV-2) and a random 5% sample of double (HSV-1 and HSV-2) negative sera from each main serum bank, were retested at CPHL using the in-house type specific binding ELISA assay.[15 28] In total, 1272 (range 12–396 per country) equivocal and 76 (range 0–22 per country) double negative sera were retested.

Statistical analysis

Data entry and analysis were undertaken using Microsoft Excel (version 9.0), Epi-Info 6.04, and Stata 6.0 software. Records of sera with missing variables were excluded. Remaining equivocals were reclassified as negative. For intercountry comparisons across age classes, direct standardisation was undertaken using the European standard population. For further intercountry comparison, the median age of acquisition was calculated for HSV-1 (age group at which 50% of population were HSV-1 seropositive).

Within each country, univariable logistic regression was used to investigate the unadjusted effects of age group (0–4, 5–9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, 40+) and sex on HSV-1 and HSV-2 status. The relation between HSV-1 and

Table 1 Characteristics of national serum surveys for eight participating countries

Country	Year of collection	Total number of sera collected	Age distribution of sera				Sera sex distribution (male:female)	Source of sera	Reference
			(0–9 years)	10–19 years	20–39 years	>39 years)			
Belgium	1999–2000	3892	909 (23%)	1007 (26%)	988 (25%)	988 (25%)	49.1%:50.9%	Residual sera	Mathei et al[22]
Bulgaria	1999	3200	450 (14%)	486 (15%)	1014 (32%)	1250 (39%)	49.7%:50.3%	Population based	Gatcheva et al[26]
Czech Republic	1989	4000	902 (23%)	1060 (27%)	1037 (26%)	1000 (25%)	49.9%:50.1%	Population based	Kriz et al[27]
Finland	1997–8	3346	779 (23%)	797 (24%)	870 (26%)	900 (27%)	53.4%:46.6%	Residual sera	NA
Germany	1997	3792	0 (0%)	244 (6%)	1571 (41%)	1977 (52%)	50.3%:49.7%	Population based†	Thefeld et al[25]
Netherlands	1996	7166	1212 (17%)	979 (14%)	1562 (22%)	3413 (48%)	47.4%:52.6%	Population based	De Melker et al[24]
Slovenia	1993	3000	0 (0%) (<10 years)	664 (22%) (10–19 years)	2336 (78%, 20+years)		0%:100%	Residual sera*	Klavs et al[23]
England and Wales	1994	4948	1000 (20%)	1000 (20%)	1842 (37%)	1106 (23%)	47.5%:52.5%	Residual sera	Vyse et al[15]

*Pregnant women only.
†General population ≥18 years old.

HSV-2 was also investigated in an unadjusted analysis. Country specific multivariable logistic regression was used to estimate the effect of sex adjusted for age group on HSV-1 and HSV-2 status and also to examine the interaction between age and sex. The relation between HSV-1 and HSV-2 was also investigated after adjusting for age and sex in each country. Significance was taken at the 5% level.

RESULTS
HSV-2 seroprevalence
Four key points were noted. Firstly, HSV-2 seropositivity was widely distributed across the general populations ≥12 years of age in the various countries by age group and sex (fig 1).

Secondly, large intercountry differences in the seroepidemiology of HSV-2 were seen (fig 1): with the highest age standardised seroprevalence in Bulgaria (23.9%) and the lowest in England and Wales (4.2%) (in >14 year olds). Germany (13.9%) (in >18 year olds), Finland (13.4%), Belgium (11.1%), Netherlands (8.8%), and the Czech Republic (6.0%) fell between.

Thirdly, women generally had a higher seroprevalence (and thus earlier age of acquisition) than men (fig 1). In six of seven countries, (with the exception of the Czech Republic), women were significantly more likely to be HSV-2 seropositive compared to males after adjusting for age group (table 2).

Finally, from adolescence onwards an increasing proportion in each country was HSV-2 seropositive with increasing age, with a decline in the older age groups in some countries (fig 1). Indeed, in the multivariable analysis for each country, almost all age groups were significantly more likely to be HSV-2 seropositive compared to the youngest (table 2, data not shown) adjusted for sex.

HSV-1 seroprevalence
A further four key issues were highlighted. Firstly, large differences in HSV-1 seroprevalence were observed in the general populations of the participating countries across age group and sex (fig 2). The highest age standardised HSV-1 seroprevalence in each country was observed in Bulgaria (83.9%) and the Czech Republic (80.6%); the lowest in Finland (52.4%). The Netherlands (56.7%) and Belgium (67.4%) fell between.
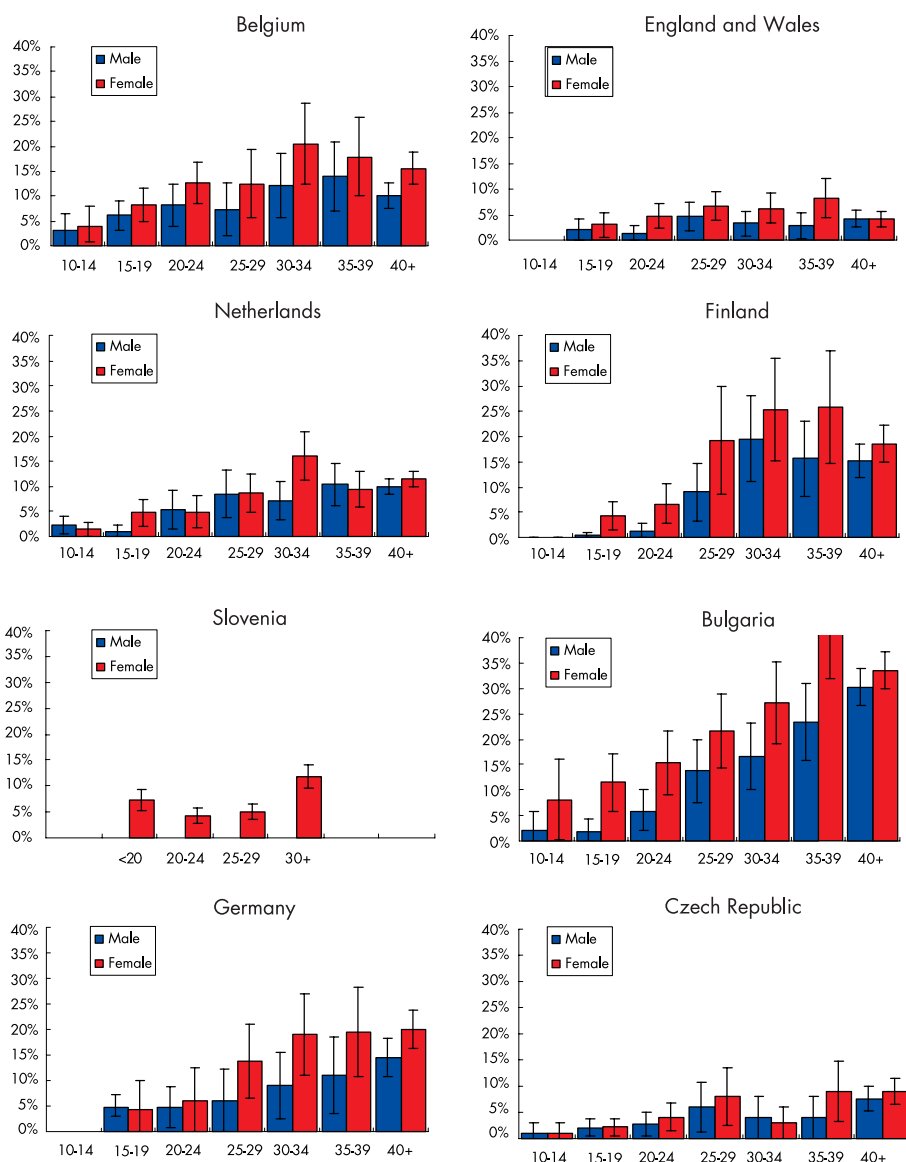

Figure 1 Age and sex specific HSV-2 seroprevalence in eight European countries with 95% CI.

Table 2 Odds ratios for HSV-2 seropositivity (F:M OR; adjusted for age group, 95% CI) in seven European countries

Country	Sex
Belgium	1.59 (1.24 to 2.04) p<0.000
Czech Republic	1.26 (0.90 to 1.77) p=0.175
Netherlands	1.19 (1.00 to 1.42) p=0.05
Finland	1.64 (1.26 to 2.15) p<0.000
Bulgaria	1.51 (1.25 to 1.81) p<0.000
Germany	1.64 (1.35 to 1.98) p<0.000
United Kingdom	1.60 (1.13 to 2.26) p=0.008

Secondly, in all countries, a steady increase in the proportion HSV-1 seropositive occurred with age group (fig 2). In multivariable analysis for each country, almost all age groups were significantly more likely to be HSV-1 seropositive compared to the youngest, adjusting for sex (table 3: data not shown). Furthermore, marked intercountry variation in the median age of HSV-1 acquisition was observed (table 4): ranging from 5–9 years in Bulgaria and the Czech Republic to >25 years in Finland, Netherlands, and England and Wales. Thus, young adults in northern European countries were more likely to be HSV-1 seronegative compared to the remaining countries (table 4).

Thirdly, in four (northern European) countries (Finland, Netherlands, United Kingdom, and Germany), women were significantly more likely to be HSV-1 seropositive than men after adjusting for age group (table 3).

Finally, males were more likely to be seronegative than females as young adults in some countries (table 4). In the multivariable analysis, the sex-age group interaction was
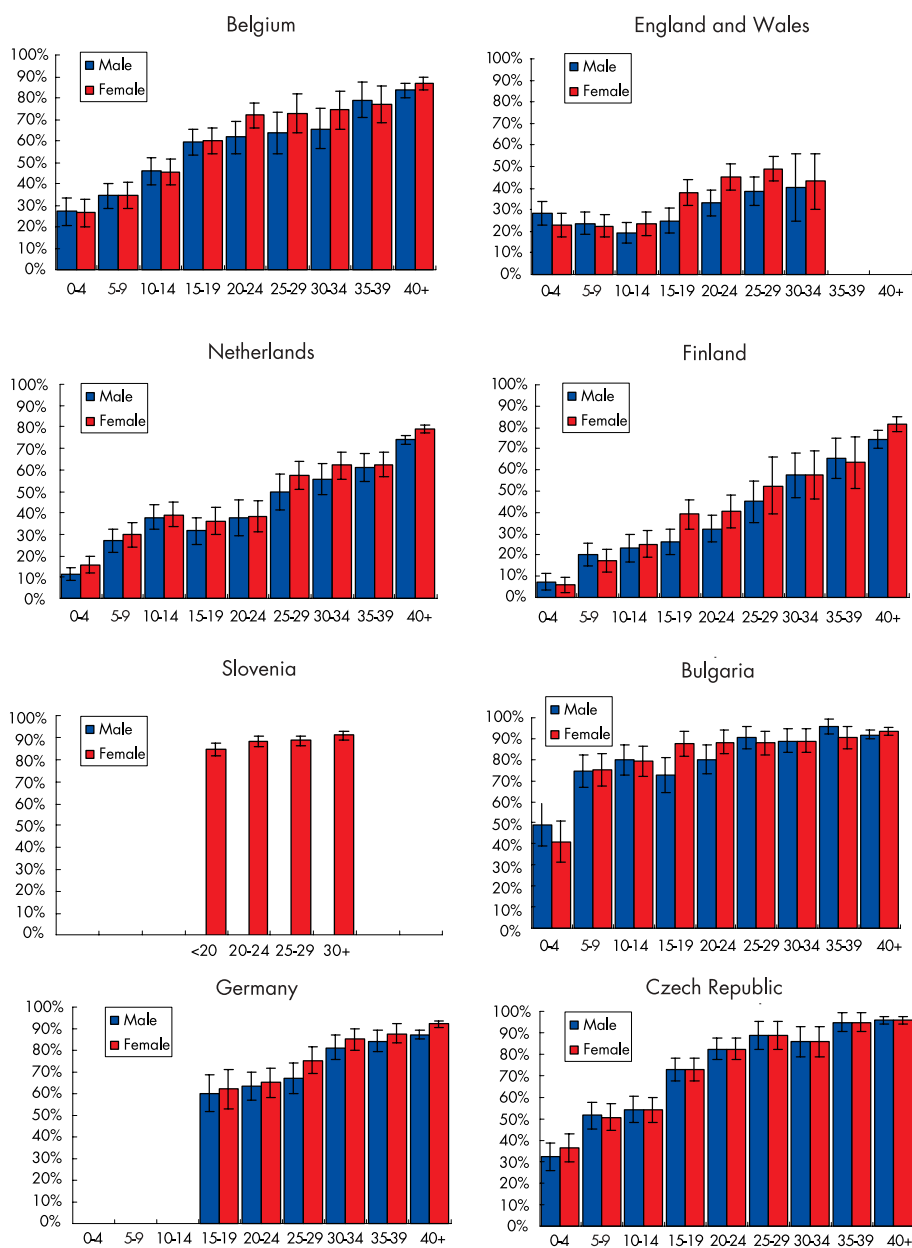

Figure 2 Age and sex specific HSV-1 seroprevalence in eight European countries with 95% CI.

Table 3 Odds ratios for HSV-1 seropositivity (F:M OR; adjusted for age group, 95% CI) in seven European countries

Country	Sex
Belgium	1.14 (0.99 to 1.32) p=0.06
Czech Republic	1.15 (0.98 to 1.35) p=0.085
Netherlands	1.24 (1.12 to 1.38) p<0.000
Finland	1.28 (1.09 to 1.50) p=0.002
Bulgaria	1.12 (0.91 to 1.38) p=0.27
Germany	1.39 (1.16 to 1.65) p<0.000
United Kingdom	1.29 (1.11 to 1.51) p=0.001

significant (LR test p<0.05) in Bulgaria, Czech Republic, and England and Wales (table 3).

Relation between HSV-1 and HSV-2

A crude comparison of the relation between HSV-1 and HSV-2 showed a positive association in four of the seven countries (table 5). After adjusting for age and sex, this association disappeared suggesting that HSV-1 and HSV-2 are both independently related to age and sex, so an unadjusted analysis gives the false impression of a relation between HSV-1 and HSV-2 positivity.

DISCUSSION

This paper is the first to our knowledge to present the comparative seroepidemiology of HSV-1 and HSV-2 in Europe. To directly compare serological surveys, standardised testing and serum sampling are required. Standardised testing in this study was achieved through use of the same batch of kits and distribution of a reference panel to ensure external quality assurance.[30] Sampling was either population based or through collection of residual sera. The latter method of convenience sampling has been shown to provide a good estimate of the population exposure for ubiquitous viral infections, such as measles compared to population based sampling providing sera are representative in terms of geography, age, and sex.[8 31] However, residual sera are obtained from individuals in contact with health services, which creates a potential selection bias for a sexually transmitted infection such as HSV-2. Furthermore, serum banks were collected over a 10 year period, introducing a potential confounding effect of time. Bearing these caveats in mind, a number of conclusions can be drawn.

Firstly large intercountry and intracountry differences in HSV-2 seroprevalence were observed (from 4% to 24% overall, to 40% in some age groups), with no clear pattern according to the method of sampling or time. These figures are consistent with and in some cases exceed previous studies: HSV-2 seroprevalence in the United States has ranged from 2% in teenagers to >25% in adults[14 15]; in the United Kingdom from 2–10%[8 15]; in Germany from 9% in pregnant women[20] to 13%[19]; in the general population and in Sweden from 2% at age 15 years to 25% at 30 years.[16] Acquisition of HSV-2 is frequently cited as a behavioural marker,[32] with HSV-2 antibody status correlated to previous sexual activity.[32 33] The differences by geography and age, we observed presumably reflect historical differences in sexual behaviour. The age effect is consistent with other studies and correlates with cumulative "sexual exposure."[34] The decline in seroprevalence in the oldest age groups could reflect an age-cohort effect, with older age groups having fewer lifetime partners,[32] or waning HSV-2 antibody levels.[35] Whichever, asymptomatic genital shedding of HSV-2 virus plays an important part in transmission. Appropriate and effective prevention and control programmes will need to be designed to take into account these large pools of potentially infectious people.

Secondly, we demonstrated females had a consistently higher risk of HSV-2 infection compared to males in almost all countries, agreeing with previous studies.[7 9 13 15 32] This may reflect the differential role of gender on clinical presentation, with men more likely to have asymptomatic HSV-2 infection,[7] which may impact differentially on subsequent sexual behaviour and could result in higher rates of male to female transmission.

Thirdly, we observed large intercountry differences in the seroepidemiology of HSV-1. Risk of HSV-1 acquisition has been linked to sociodemographic status,[1] which our study partly supports with a north-south/east gradient. Indeed, there is an inverse correlation between the age standardised seroprevalence of HSV-1 and the national gross domestic product (correlation = −0.93). Thus a large proportion of adolescents remain HSV-1 susceptible in northern Europe, suggesting the age dependent force (or risk) of infection for HSV-1 is higher in southern and eastern Europe than the north. These differences in HSV-1 epidemiology probably occurred recently[15] and may reflect changes in socioeconomic status and family size.

Fourthly, in addition to high HSV-1 susceptibility in teenage populations especially in northern Europe, the seroprofiles suggest significant HSV-1 acquisition among young adults. We were not able to estimate what proportion of those infected as young adults acquired infection orally or sexually. However, sexual transmission of HSV-1 is of increasing importance particularly in northern Europe, with an increase in the proportion of genital herpes caused by

Table 4 Proportion HSV-1 susceptible by age group (years) and sex in eight European countries and median age group of HSV-1 seropositivity

Country	0–9		10–19*		20–29		30+		Median age of acquisition
	M	F	M	F	M	F	M	F	
Belgium	69%	69%	47%	47%	38%	28%	20%	16%	15–19
Czech Republic	57%	56%	36%	29%	16%	17%	6%	5%	5–9
Netherlands	78%	73%	66%	63%	47%	40%	33%	28%	25–29
Finland	92%	94%	86%	81%	78%	72%	46%	38%	30–34
Bulgaria	38%	40%	23%	16%	15%	12%	8%	8%	5–9
Slovenia	–	–	–	15%	–	12%	–	9%	–
Germany	–	–	40%	38%	35%	30%	14%	9%	<15
England and Wales	74%	77%	78%	69%	64%	53%	60%	57%	>35

*Only includes sera for 18–19 year olds in Germany.

Table 5 Odds ratio of HSV-1 positivity for HSV-2 positivity (OR; unadjusted and adjusted for age and sex, 95% CI) in seven European countries

Country	OR adj*	OR unadj
Belgium	0.8 (0.6 to 1.1)	1.01 (0.77 to 1.32)
Czech Republic	0.8 (0.5 to 1.4)	1.40 (0.83 to 2.39)
Netherlands	0.9 (0.8 to 1.1)	1.64 (1.36 to 1.97)
Finland	0.8 (0.6 to 1.1)	1.51 (1.16 to 1.98)
Bulgaria	1.3 (0.9 to 1.9)	1.71 (1.22 to 2.39)
Germany	0.9 (0.7 to 1.2)	1.32 (1.02 to 1.71)
England and Wales	0.8 (0.4 to 1.3)	0.86 (0.50 to 1.48)

*Adjusted for age and sex.

HSV-1.[7] [9] [11] [36] [37] Recent changes in adolescent sexual behaviour, particularly the practice of oral-genital sex may partly explain this.[9] Indeed, HSV-1 seropositivity in young adult populations may increasingly be a marker of higher risk sexual behaviour.[6] [9] [11] [36] [38] Similar observations may be seen in the future in central and Eastern Europe.

Fifthly, we demonstrated women had a higher age specific HSV-1 seroprevalence in adolescents and young adults compared to men. The earlier increase in HSV-1 seroprevalence in women seen in this study reflects the higher risk of genitally acquired HSV-1 in young women,[7] [9] [38–41] which may reflect age specific mixing patterns, with women having male partners on average older than themselves.[6] [11] An increase in genitally acquired HSV-1 may have important "knock-on" effects: asymptomatic shedding caused by genital HSV-1 is reportedly less than HSV-2,[2] [42] and the recurrence rate lower for genitally acquired HSV-1.[43] [44] Any impact on vertical transmission is difficult to predict: there may be an increase in neonatal herpes as a result of increased transmission of genital HSV-1 in young women, although vertical transmission is lower for HSV-1 compared to HSV-2[45] and disease is milder.[46] However, an increase in the proportion of neonatal herpes cases due to HSV-1 has been observed in the United Kingdom and Netherlands.[3] [12]

Finally, our cross sectional study found no evidence of a protective role of HSV-1 for acquisition of HSV-2. Previous authors have produced conflicting evidence that previous HSV-1 infection reduces the risk of acquiring HSV-2.[7] However, previous HSV-1 infection does modify HSV-2 clinical presentation[47] and rate of subsequent recurrence.[38] Thus, the observation of increased HSV-1 susceptibility could result in more severe disease manifestations caused by primary HSV-2 infection and potentially increasing vertical and sexual transmission as a result of a higher rate of recurrence. These hypotheses need to be formally addressed through modelling or intervention studies.

CONTRIBUTORS
The European HSV Study Group: The study was designed by RP, DB, RG and NA; RG and DB were responsible for acting as a laboratory reference centre; HM, GF, NG; WH, SJ, IK, MK, TK, BK; KP, KR, PT, WT, MV, PD, and RV were responsible for collection, testing, and interpretation of national serum banks; comparative data analysis was undertaken by RP and NA; preparation of the final manuscript was coordinated by RP on behalf of NA, DB, RG, HM, GF, NG, WH, SJ, IK, MK, TK, BK, KP, KR, PT, WT, MV, PD, and RV.

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