

# Epidemiology of herpes simplex virus type 1 and 2 in Italy: a seroprevalence study from 2000 to 2014

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## Keywords

Herpes simplex virus • HSV-1 • HSV-2 • Seroprevalence • Italy

## Summary

*Herpes simplex viruses (HSV) are among the most widespread causative agents of human viral infections. HSV-2 is one of the commonest causes of genital disease, while HSV-1 is associated primarily with orolabial ulceration; however, recent changes in HSV epidemiology showed an increase in genital and neonatal herpes particularly caused by HSV-1. The main purpose of this study was to assess the seroprevalence of HSV-1 and HSV-2 in a random population in Siena (central Italy) in 2000, 2005 and 2013-2014 and in Bari (southern Italy) in 2005. Moreover, a preliminary study was conducted to investigate the spread of HSV infection in a population of pregnant women and infants in Bari in 2003, 2004 and 2005. Human serum samples were tested for the presence of specific anti-HSV-1 and anti-HSV-2 IgG antibodies*

*using a commercially available ELISA test. For the primary purpose, seroprevalence rates observed in Siena were compared over the years sampled and with the seroprevalence rate found in Bari. Results of seroprevalence in Siena show a decreased trend for both viruses, especially in adolescents and young adults; moreover, HSV-2 seroprevalence rates found in the two cities suggest geographical differences. For the secondary purpose, prevalence rates among pregnant women were compared with the seroprevalence found in women of the general population. No significant difference in prevalence rates were found among pregnant women, while results indicate both viruses are a source of infection in infants.*

## Introduction

Herpes simplex virus (HSV) is one of the most widespread infections in humans, affecting 60-95% of the adult population worldwide. After entering the host, the virus establishes persistent and latent infection in neuronal ganglia, from which it can reactivate periodically causing recurrent infections. Most infections are sub-clinical; symptoms consist primarily of ulcerative lesions at the site of infection and, although rare, complications such as blindness, encephalitis and aseptic meningitis can occur, especially in immunocompromised hosts [1, 2]. Moreover, the acquisition of HSV during pregnancy is associated with miscarriage, prematurity, and congenital and neonatal herpes. HSV can cause severe infections in newborn by vertical transmission in utero or, in most cases, during vaginal delivery through contact with HSV-infected genital tract secretions, with a high mortality and neurodevelopmental disability [3-5].

There are two types of HSV, HSV-1 and HSV-2, both transmitted by direct contact with infected secretions. HSV-2 is transmitted sexually, resulting in one of the most common genital diseases, affecting adolescents and adults and facilitating HIV transmission [6, 7]. HSV-1 infection is associated predominantly with orolabial ulceration and occurs mostly by nonsexual contacts during

childhood after the disappearance of maternal antibodies in the first year of life [7, 8]. However, recent changes in herpes infection epidemiology reported an increase in genital and neonatal herpes due to HSV-1 [9].

Nevertheless, many infections remain asymptomatic or do not require health care intervention, resulting in an underestimation of the spread of HSV. Serological tests based on the use of type-specific immunoassays that distinguish antibodies to HSV-1 and HSV-2, can recognize both symptomatic and asymptomatic infections, facilitating a better understanding of HSV epidemiology [6]. In Italy, some studies have investigated the seroprevalence of these viruses in different populations considered to be at low or high risk of acquiring the infection. According to these studies, HSV-1 infection is widespread, with seroprevalence rates of 93% in the adult population [10]. Conversely, the circulation of HSV-2 is more limited (5.5% in adults, according to Suligoj et al. [11]) and frequently associated with other sexually transmitted diseases (STD) [12-14]. Moreover, a HSV-2 seroprevalence study conducted on samples collected in 1998 showed 7.6% positivity to HSV-2 antibodies among pregnant women [11], comparable to 8.4% seroprevalence in a similar survey conducted in Northern Italy [15].

HSV infection control is of relevance for public health especially for mitigating the risk of neonatal herpes and as-

sociated diseases; thus, monitoring the epidemiology of HSV infections is an important tool for prevention and control strategies, as suggested by Woestenberget al. [16]. In Italy, a characterization of HSV infections in the general population over time is lacking. To revise the epidemiology of HSV in Italy, a seroepidemiological study was carried out to determine the HSV-1 and HSV-2 antibody prevalence in the general population of two cities from different geographical areas (Siena, central Italy and Bari, southern Italy), making a comparison between seroprevalence rates obtained over a period of nearly 15 years. Moreover, a preliminary study was carried out in a small population of samples of pregnant women and infants, to evaluate HSV as a source of infection in infants.

## Materials and methods

HSV antibody tests were performed on human serum samples from the internal serum bank of the Laboratory of Molecular Epidemiology, Department of Molecular and Developmental Medicine, University of Siena. The samples had been anonymously collected in compliance with Italian law on Ethics; the only information available for these subjects were age, gender and state of pregnancy.

1,776 samples (640 in 2000, 636 in 2005 and 500 in 2013-2014) collected in the Siena area and 168 samples collected in 2005 in the province of Bari were tested for the presence of specific anti-HSV-1 and anti-HSV-2 IgG antibodies. In addition, 91 samples from pregnant women and 70 samples from infants collected in the province of Bari in 2003, 2004 and 2005, were tested. In particular, infants were subdivided in two age-groups, 0-11 months of age and 1 year of age, considering the waning of maternal antibodies over a period of 6-12 months [17]. A population summary is shown in Table Ia, Ib and Ic.

Type specific serum antibodies to HSV-1 and HSV-2 were detected by commercial ELISA kits BEIA HSV 1 IgG and BEIA HSV 2 Rec IgG (TechnoGenetics, Milano, Italy). ELISA assays were performed in accordance with the manufacturer's instructions. According to the manufacturer, the tests have sensitivity and specificity of 98.7% and 100% for HSV-1 and 94.1% and 98.5% for HSV-2 respectively. Cut-off levels were  $> 1.1$  for seropositivity and  $< 0.9$  for seronegativity; samples with borderline results (between 0.9 and 1.1) were excluded from the study.

Sex and age-specific seroprevalence rates were calculated, along with the corresponding 95% CI. Statistical analysis was performed using the Yates corrected chi-squared test to compare prevalence rates among different groups and the chi-squared test for the trend to evaluate possible tendencies in seroprevalence rates over time. Statistical significance was set at  $p < 0.05$ , two tailed.

Tab. I. General population of Siena in 2000, 2005 and 2013-2014 (a), general population of Bari 2005 and pregnant women of Bari 2003, 2004 and 2005 (b) and infants of Bari 2003, 2004, 2005 (c).

a)									
Age groups	Siena 2000			Siena 2005			Siena 2013-2014		
	M	F	TOT	M	F	TOT	M	F	TOT
0-14	60	40	100	39	19	58	10	5	15
15-19	8	41	49	10	10	20	9	13	22
20-24	12	39	51	10	11	21	23	22	45
25-29	12	42	54	7	29	36	26	23	49
30-34	16	50	66	11	28	39	19	31	50
35-39	20	31	51	16	37	53	25	36	61
40-49	43	63	106	39	59	98	51	59	110
50+	54	90	144	149	141	290	74	71	145
Total	225	396	621	281	334	615	237	260	497

b)				
Age groups	Bari 2005			Bari 2003-2004-2005
	M	F	TOT	Pregnant
0-14	28	28	56	-
15-19	10	6	16	-
20-24	5	7	12	9
25-29	6	15	21	30
30-34	7	25	32	34
35-39	13	18	31	18
Total	69	99	168	91

c)	
Age groups	Bari 2003-2004-2005
0-11 months	34
1 year old	34
Total	68

## Results

A total of 2105 serum samples were tested by ELISA assay, 45 of which yielded borderline results and were excluded from the statistical analysis. Samples collected in the general population of Siena and Bari were divided by sex and classified into eight age groups: 0-14 (children), 15-19 (adolescents), 20-24 (young adults), 25-29, 30-34, 35-39, 40-49 and  $> 50$  years old. Samples collected from pregnant women were divided in four age groups: 20-24, 25-29, 30-34 and 35-39 years old, while samples from infants were divided into 0-11 months and 1-year-old.

### HSV-1 SEROPREVALENCE IN THE GENERAL POPULATION

The results for specific IgG antibodies against HSV-1 in samples collected in Siena are reported in Table II.

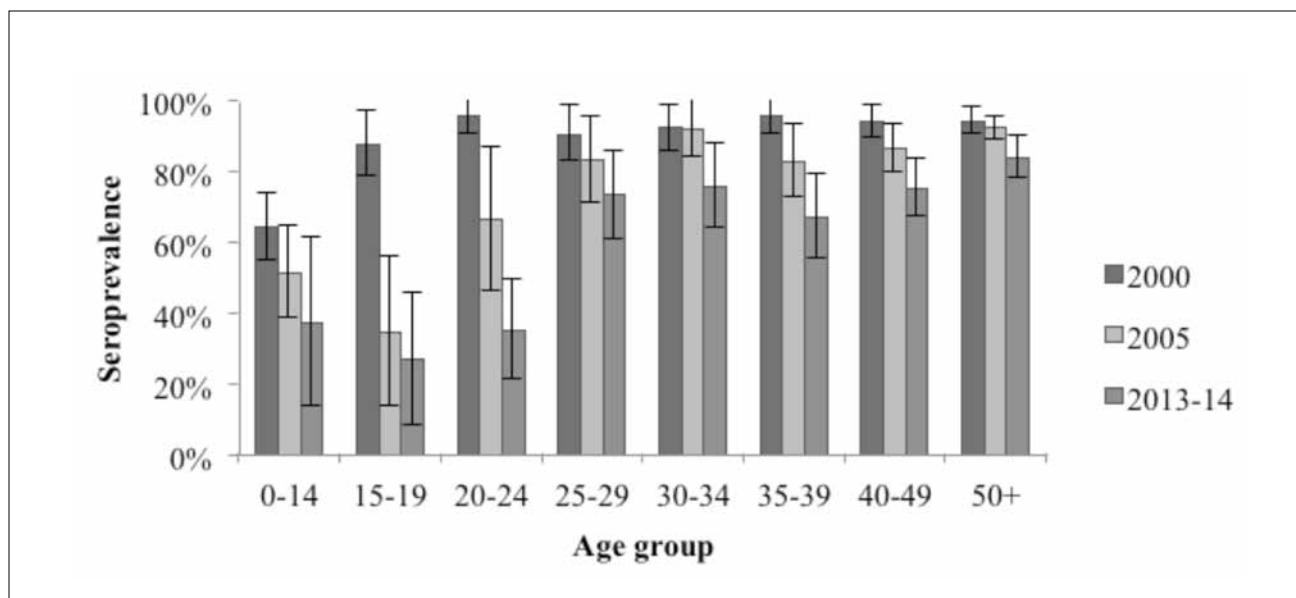
Overall, HSV-1 seroprevalence declined significantly ( $p < 0.001$ ) from 2000 to 2013-2014 in the study population, in particular rates by sex showed a decrease of 14.5% in women and 22% in men; however, the prevalence remained significantly higher among women ( $p = 0.003$ ).

In all the years considered in this study, a steady increase in the proportion of HSV-1 seropositive occurred

Tab. II. HSV-1 seroprevalence in population of Siena in 2000, 2005 and 2013-2014, divided by sex (male, M and female, F) and age groups.

Age groups	Siena 2000 (%)			Siena 2005 (%)			Siena 2013-2014 (%)		
	M	F	TOT	M	F	TOT	M	F	TOT
0-14	42/60 (70)	23/40 (57.5)	65/100 (65)	21/39 (53.8)	9/19 (47.4)	30/58 (51.7)	3/10 (30)	3/5 (60)	6/15 (40)
15-19	8/8 (100)	35/41 (85.4)	43/49 (87.8)	6/10 (60)	1/10 (10)	7/20 (35)	2/9 (22.2)	4/13 (30.8)	6/22 (27.3)
20-24	10/12 (83.3)	39/39 (100)	49/51 (96.1)	6/10 (60)	8/11 (72.7)	14/21 (66.7)	7/23 (30.4)	9/22 (40.9)	16/45 (35.6)
25-29	11/12 (91.7)	38/42 (90.5)	49/54 (90.7)	5/7 (71.4)	25/29 (86.2)	30/36 (83.3)	18/26 (69.2)	18/23 (78.3)	36/49 (73.5)
30-34	14/16 (87.5)	47/50 (94)	61/66 (92.4)	9/11 (81.8)	27/28 (96.4)	36/39 (92.3)	10/19 (52.6)	28/31 (90.3)	38/50 (76)
35-39	20/20 (100)	29/31 (93.5)	49/51 (96.1)	15/16 (93.7)	29/37 (78.4)	44/53 (83)	14/25 (56)	27/36 (75)	41/61 (67.2)
40-49	38/43 (88.4)	62/63 (98.4)	100/106 (94.3)	31/39 (79.5)	54/59 (91.5)	85/98 (86.7)	36/51 (70.6)	47/59 (79.7)	83/110 (75.4)
50+	47/54 (87)	89/90 (98.9)	136/144 (94.4)	138/149 (92.6)	131/141 (92.9)	269/290 (92.8)	58/74 (78.4)	64/71 (90.1)	122/145 (84.1)
Total	190/225 (84.4)	362/396 (91.4)	552/621 (88.9)	231/281 (82.2)	284/334 (85)	515/615 (83.7)	148/237 (62.4)	200/260 (76.9)	348/497 (70)

Fig. 1. HSV-1 seroprevalence in population of Siena in 2000, 2005 and 2013-2014, divided by age groups with 95%CI.



with age ( $p < 0.001$ ). As shown in Figure 1, from 2000 to 2013-2014 seroprevalence declined significantly ( $p < 0.001$ ) among adolescents and young adults (from 87.8% and 96.1% in 2000 to 27.3% and 35.6% in 2013-2014 respectively) as well as for 35-39- and 40-49-years-old groups; moreover, there is an important decrease among 30-34 years old ( $p = 0.018$ ) and in subjects over 50 ( $p = 0.003$ ). Among children and 25-29 years old subjects, there were no significant changes.

The greatest increase in infections in 2000 was amongst adolescents ( $p = 0.006$  with children), whilst in 2013-2014 it was in the 25-29 years group (73.5%;  $p < 0.001$  with 20-24 age group of the same year). In particular,

a significant difference was found between 20-24 and 25-29 year old men ( $p = 0.015$ ), but also in women ( $p = 0.024$ ).

The seroprevalence in samples collected in Siena in 2005 was compared with the results of the samples collected in Bari in the same year (Tab. III). No significant differences were found between the two cities.

#### HSV-2 SEROPREVALENCE IN THE GENERAL POPULATION

The results for specific IgG antibodies against HSV-2 in the samples collected in Siena are reported in Table IV.

HSV-2 seroprevalence was halved between 2000 and 2013-2014 (from 22.4% to 11.5%,  $p < 0.001$ ). In 2000 the prevalence among males and females exhibited no difference; in almost 15 years, both decreased ( $p < 0.001$ ), but in 2013-2014 the prevalence was higher among males than females, even if this difference is not considered to be statistically significant.

In almost all age groups, seroprevalence decreased in the time span considered. The greatest decreases were observed particularly in adolescents ( $p = 0.005$ ) and young adults ( $p < 0.001$ ); a decrease was observed also in children from a value of 8% in 2000 to 0% in 2013-2014, but the difference was considered to be not statistically significant. In contrast, in 35-39 and 40-49 year old subjects no significant differences over the years were observed. Considering the period 2013-2014, HSV-2 seropositivity is distributed only among the general population

**Tab. III.** HSV-1 seroprevalence in Siena and in Bari in 2005, divided by sex and age groups.

Age groups	Siena 2005 (%)			Bari 2005 (%)		
	M	F	TOT	M	F	TOT
0-14	21/39 (53.8)	9/19 (47.4)	30/58 (51.7)	18/28 (64.3)	13/28 (46.4)	31/56 (55.4)
15-19	6/10 (60)	1/10 (10)	7/20 (35)	5/10 (50)	4/6 (66.7)	9/16 (56.2)
20-24	6/10 (60)	8/11 (72.7)	14/21 (66.7)	4/5 (80)	5/7 (71.4)	9/12 (75)
25-29	5/7 (71.4)	25/29 (86.2)	30/36 (83.3)	3/6 (50)	13/15 (86.7)	16/21 (76.2)
30-34	9/11 (81.8)	27/28 (96.4)	36/39 (92.3)	6/7 (85.7)	22/25 (88)	28/32 (87.5)
35-39	15/16 (93.7)	29/37 (78.4)	44/53 (83)	12/13 (92.3)	16/18 (88.9)	28/31 (90.3)
Total	62/93 (66.7)	99/134 (73.9)	161/227 (70.9)	48/69 (69.6)	73/99 (73.7)	121/168 (72)

over 20 years of age, but over the years it was possible to observe some differences in seroprevalence patterns (Fig. 2). While in 2000 30.6% of adolescents showed HSV-2 positivity and the peak of infection was achieved in 20-24 year old subjects (47.1%) with a decrease up to 13.7% in 35-39-year old group, in 2013-2014 none of the adolescents in the study showed positivity to the virus. In the same years, the 20-24 year old age group remains the most affected in conjunction with that of 25-29 year olds; also there is a second increase in seroprevalence in people over 35 years.

The seroprevalence in samples collected in Siena in 2005 was compared with the results of samples collected in Bari in the same year (Tab. V).

The seroprevalence observed in the two cities showed a significant difference in the general population (13.2%

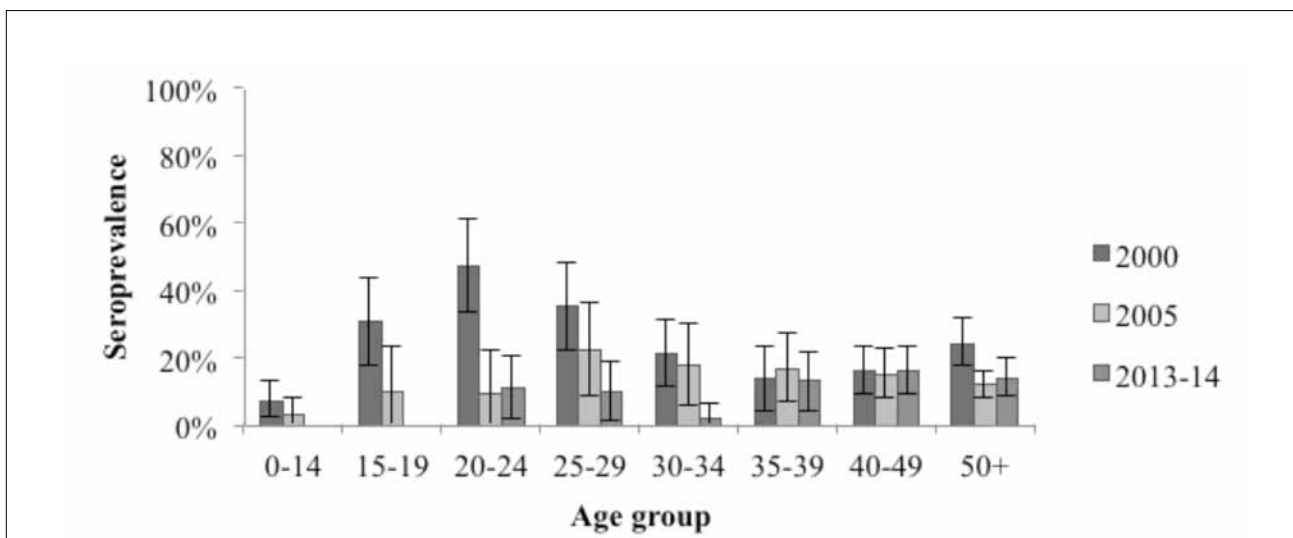
**Tab. V.** HSV-2 seroprevalence in Siena and in Bari in 2005, divided by sex and age groups.

Age groups	Siena 2005 (%)			Bari 2005 (%)		
	M	F	TOT	M	F	TOT
0-14	1/39 (2.6)	1/19 (5.3)	2/58 (3.4)	1/28 (3.6)	1/28 (3.6)	2/56 (3.6)
15-19	1/10 (10)	1/10 (10)	2/20 (10)	0/10 (-)	0/6 (-)	0/16 (0)
20-24	1/10 (10)	1/11 (9.1)	2/21 (9.5)	1/5 (20)	0/7 (-)	1/12 (8.3)
25-29	1/7 (14.3)	7/29 (24.1)	8/36 (22.2)	0/6 (-)	2/15 (13.3)	2/21 (9.5)
30-34	3/11 (27.3)	4/28 (14.3)	7/39 (17.9)	0/7 (-)	2/25 (8)	2/32 (6.2)
35-39	2/16 (12.5)	7/37 (18.9)	9/53 (17)	1/13 (7.7)	0/18 (-)	1/31 (3.2)
Total	9/93 (9.7)	21/134 (15.7)	30/227 (13.2)	3/69 (4.3)	5/99 (5)	8/168 (4.8)

**Tab. IV.** HSV-2 seroprevalence in population of Siena in 2000, 2005 and 2013-2014, divided by sex and age groups.

Age groups	Siena 2000 (%)			Siena 2005 (%)			Siena 2013-2014 (%)		
	M	F	TOT	M	F	TOT	M	F	TOT
0-14	5/60 (8.3)	3/40 (7.5)	8/100 (8)	1/39 (2.6)	1/19 (5.3)	2/58 (3.4)	0/10 (-)	0/5 (-)	0/15 (-)
15-19	1/8 (12.5)	14/41 (34.1)	15/49 (30.6)	1/10 (10)	1/10 (10)	2/20 (10)	0/9 (-)	0/13 (-)	0/22 (-)
20-24	7/12 (58.3)	17/39 (43.6)	24/51 (47.1)	1/10 (10)	1/11 (9.1)	2/21 (9.5)	3/23 (13)	2/22 (9.1)	5/45 (11.1)
25-29	3/12 (25)	16/42 (38.1)	19/54 (35.2)	1/7 (14.3)	7/29 (24.1)	8/36 (22.2)	4/26 (15.4)	1/23 (4.3)	5/49 (10.2)
30-34	6/16 (37.5)	8/50 (16)	14/66 (21.2)	3/11 (27.3)	4/28 (14.3)	7/39 (17.9)	1/19 (5.3)	0/31 (-)	1/50 (2)
35-39	7/20 (35)	0/31 (-)	7/51 (13.7)	2/16 (12.5)	7/37 (18.9)	9/53 (17)	5/25 (20)	3/36 (8.3)	8/61 (13.1)
40-49	4/43 (9.3)	13/63 (20.6)	17/106 (16)	1/39 (2.6)	14/59 (23.7)	15/98 (15.3)	9/51 (17.6)	9/59 (15.2)	18/110 (16.4)
50+	16/54 (29.6)	19/90 (21.1)	35/144 (24.3)	15/149 (10.1)	20/141 (14.2)	35/290 (12.1)	11/74 (14.9)	9/71 (12.7)	20/145 (13.8)
Total	49/225 (21.8)	90/396 (22.7)	139/621 (22.4)	25/281 (8.9)	55/334 (16.5)	80/615 (13)	33/237 (13.9)	24/260 (9.2)	57/497 (11.5)

Fig. 2. HSV-2 seroprevalence in population of Siena in 2000, 2005 and 2013-2014, divided by age groups with 95% CI.



in Siena vs 4.8% in Bari,  $p = 0.008$ ), in particular in women ( $p = 0.02$ ).

**HSV-1 AND HSV-2 SEROPREVALENCE AMONG PREGNANT AND INFANTS**

The prevalence of IgG antibodies against HSV-1 and HSV-2 in samples collected from pregnant women in Bari in 2003, 2004 and 2005 is shown in Table VI.

Among pregnant women, 91.2% and 9.9% showed antibodies against HSV-1 and HSV-2 respectively, while 8.8% were negative for both viruses. As shown in Table V, no significant differences were observed comparing pregnant and women of the same age of the general population collected in Bari 2005. In both populations, all

Tab. VI. HSV-1 and HSV-2 seroprevalence in Bari in 2003, 2004 and 2005 in pregnant women and not pregnant women, divided by age groups..

Age groups	Pregnant		No pregnant	
	HSV-1 (%)	HSV-2 (%)	HSV-1 (%)	HSV-2 (%)
20-24	8/9 (88.9)	2/9 (22.2)	5/7 (71.4)	0/7 (-)
25-29	27/30 (90)	5/30 (16.7)	13/15 (86.7)	2/15 (13.3)
30-34	32/34 (94.1)	1/34 (2.9)	22/25 (88)	2/25 (8)
35-39	16/18 (88.9)	1/18 (5.6)	16/18 (88.9)	0/18 (-)
Total	83/91 (91.2)	9/91 (9.9)	56/65 (86.1)	4/65 (6.1)

Tab. VII. HSV-1 and HSV-2 seroprevalence in Bari in 2003, 2004 and 2005 among infants 0-11 months and 1-year-old.

Age groups	HSV-1 (%)	HSV-2 (%)
0-11 months	18/34 (52.9)	11/34 (32.3)
1 year old	8/34 (23.5)	10/34 (29.4)
Total	26/68 (38.2)	21/68 (30.9)

subjects positive for anti HSV-2 IgG were also positive for anti HSV-1 IgG.++

Among infants, 38.2% and 30.9% showed anti-HSV-1 and anti-HSV-2 antibodies respectively (Tab. VII). 16.2% of them had antibodies directed against both viruses, in particular 23.5% of the infants 0-11 months of age and 8.8% of infants 1-year-old. HSV-1 seroprevalence showed a significant difference ( $p = 0.024$ ) between infants 0-11 months of age and infants 1-year-old, while no difference was observed for HSV-2, or for co-detection of both viruses.

**Discussion**

HSV-1 and HSV-2 are among the most widespread human viral infections. HSV-2 is one of the most common causes of genital disease, while HSV-1 is associated primarily with orolabial ulceration; however, recently an increase in genital and neonatal herpes caused by HSV-1 has been reported [7-9]. In Italy, the seroepidemiology of HSV infection has been investigated in populations associated with some risk factors, such as HIV infection [13] or other STDs [12, 14, 18], or considering a single serotype [10, 11].

The serological study presented here was conducted in order to estimate the seroprevalence of HSV-1 and HSV-2 in a general population from Siena, analyzing potential differences in the spread of infection from 2000 to 2014 and making a comparison with the seroprevalence in a general population from Bari. Moreover, a preliminary study was carried out in a small population of samples of pregnant women and infants, to investigate the spread of HSV as infection source of infection in infants.

As expected, analysis of data obtained from this study confirms the widespread prevalence of HSV-1 in the general population with seroprevalence rates higher than those to HSV-2.

The seroprevalence of HSV-1 declined significantly in the general population of Siena from 88.9% in 2000 to

70% in 2013-2014. This decrease has also been observed in other industrialized countries [6, 7, 16, 19] and associated with changes in socioeconomic status and family size, with improvements in living and hygiene conditions, as suggested by the same studies. The strongest decrease is observed in adolescents and young adults; in 2000 the peak of infection was achieved among adolescents, while in 2013-2014 HSV-1 infection was mainly acquired in 25-29 year-old subjects. Lack of HSV-1 immunity at the start of sexual activity makes young people more susceptible to genitally acquired HSV-1 infection [6, 19, 20]; women in particular are more susceptible to acquiring HSV-1, as well as HSV-2, as a genital infection because of a more vulnerable mucosal lining of the external genitalia [21]. In this study it was not possible to distinguish orolabial infections from genital infections. However, considering the 2013-2014 population, in every age group women show a higher rate of positivity than males of the same age, especially in the 30-34-year-old group.

HSV-2 prevalence declined significantly from 22.4% in 2000 to 11.5% in 2013-2014 among the general population and, as with HSV-1, the strongest decrease is observed in adolescents and young adults. In samples collected in 2000, 30.6% of adolescents exhibited positivity to HSV-2 antibodies, and the peak of infection was reached among young adults, while in 2013-2014 subjects who showed positivity to HSV-2 antibodies were over 20 years old. These results may reflect changes in sexual behavior, as acquisition of HSV-2 is considered a marker of previous sexual activity [6]. A second increase in HSV-2 seroprevalence was observed in people over 35 years of age without significant differences over the years, potentially correlated to a cumulative "sexual exposure" in older age groups. Considering the 2013-2014 population, women have a lower seroprevalence value than men, even if the difference is not statistically significant. This figure is in contrast with what has been observed by Pebody et al. [6], which states that women generally have a higher seroprevalence than men. A possible explanation could be that prior HSV-1 infection may protect against HSV-2 by conferring cross-immunity [7, 8, 22], even if it is still not known if a previous HSV-1 oral infection could confer the same protection as a HSV-1 genital infection.

While no differences were found among the general population of Siena and Bari for HSV-1 seroprevalence, HSV-2 antibody prevalence in Siena was higher than in Bari, especially in women, and additionally higher than those reported in Rome by Suligoj et al. [11]. These geographic differences could reflect historical differences in sexual behavior, as suggested by Pebody et al. [6], or differences in prevention and control programmes designed into these population.

Among pregnant women 91.2% and 9.9% showed antibodies against HSV-1 and HSV-2 respectively. HSV-2 seroprevalence in this study is consistent with those observed in Rome (7.6%) and Northern Italy (8.4%) [11, 15], but nevertheless it is lower than that reported among pregnant women in other countries, such

as Germany (18%) and Finland (15.7%). Conversely, HSV-1 seroprevalence was higher (82% in Germany and 70% in Finland) [7, 23]. 8.8% of the pregnant women in this study are negative to both viruses and therefore are at greater risk to acquire the infection during pregnancy and transmit it to a newborn [5, 24], considering that approximately 3% of women in Italy acquires HSV infection during pregnancy [25].

These data are confirmed by seroprevalence rates found in infants: 52.9% and 32.3% of children of 0-11 months of age show antibodies against HSV-1 and HSV-2 respectively, resulting from an infection or inherited passively from the mother. After the first year of life HSV-1 seroprevalence declines to 23.5%, while 29.4% of infants show antibodies against HSV-2. Because maternal antibodies wane within the first year of life [17], the presence of anti HSV-1 or HSV-2 antibodies in children 1-year-old is indicative of infection. Comparing seroprevalence rates among infants 0-11 months and infants 1-year-old it could be postulated that the presence of anti-HSV-1 antibodies in the first months of life could be associated with infection or passively acquired maternal immunity, whilst antibodies directed against HSV-2 are almost always associated with HSV-2 infection, which is vertically transmitted more frequently than HSV-1 as suggested by other studies [5, 6].

The main importance of this study is that the detection of specific antibodies against HSV-1 and HSV-2 was conducted on samples that had been randomly collected in a general population, allowing characterization of the spread of the infections not only in high-risk subgroups (e.g. STD clinic attendees). On the other hand, some age groups are numerically limited because of the method of the sample collection and the lack of information (e.g. marital status, living area, education level, employment, sexual activity and number of partners, symptoms or previous diagnosis of genital herpes) on the subjects involved did not allow an evaluation of the influence of factors that may be related to an increased risk of HSV infection. Unfortunately, no data about the circulation of HSV-1 and HSV-2 in the years between those studied were available, therefore it was not possible to assess any changes in trends or possible epidemic outbreaks in the population studied.

## Conclusions

In conclusion, this study highlights a decreased trend in seropositivity for both HSV viruses, especially in adolescents and young adults, updating data from previous studies and providing important information about the changing epidemiology of these infections in Italy as already observed in other countries. Moreover, prevalence rates found among pregnant women and infants indicate both viruses as a source of infection in infants, emphasizing the importance of prevention with vaccine development and adequate communication to healthcare workers and public.

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## Authors' contributions

SM and CMT performed laboratory work. SM wrote the first draft of the manuscript. NT performed the linguistic revision of the manuscript. All authors critically read and revised the manuscript and approved the final version.

## References

- [1] Brady RC, Bernstein DI. *Treatment of herpes simplex virus infections*. *Antiviral Res* 2004;61:73-81. doi: 10.1016/j.antiviral.2003.09.006.
- [2] Whitley RJ, Roizman B. *Herpes simplex virus infections*. *Lancet* 2001;357(9267):p 1513-8. doi: 10.1016/S0140-673604638-9.
- [3] Whitley RJ. *Neonatal Herpes simplex virus infections*. *J Med Virol* 1993;(Suppl 1):13-21. doi: 10.1002/jmv.1890410505.
- [4] Cherpès TL, Matthews DB, Maryak SA. *Neonatal Herpes simplex virus infection*. *Clin Obstet Gynecol* 2012;55:938-44. doi: 10.1097/GRF.0b013e31827146a7.
- [5] Brown ZA, Selke S, Zeh J, Kopelman J, Maslow A, Ashley RL, Watts DH, Berry S, Herd M, Corey L. *The acquisition of herpes simplex virus during pregnancy*. *N Engl J Med* 1997;337::509-15. doi: 10.1056/NEJM199708213370801.
- [6] Pebody RG, Andrews N, Brown D, Gopal R, De Melker H, Francois G, Gatcheva N, Hellenbrand W, Jokinen S, Klavs I, Kojouharova M, Kortbeek T, Kriz B, Prosenc K, Roubalova K, Teocharov P, Thierfelder W, Valle M, Van Damme P, Vranckx R. *The seroepidemiology of herpes simplex virus type 1 and 2 in Europe*. *Sex Transm Infect* 2004;80:185-91. doi: 10.1136/sti.2003.005850.
- [7] Sauerbrei A, Schmitt S, Scheper T, Brandstadt A, Saschenbrecker S, Motz M, Soutschek E, Wutzler P. *Seroprevalence of herpes simplex virus type 1 and type 2 in Thuringia, Germany, 1999 to 2006*. *Euro Surveill* 2011;16 pii: 20005.
- [8] Brugha R, Keersmaekers K, Renton A, Meheus A. *Genital herpes infection: a review*. *Int J Epidemiol* 1997;26:698-709. doi: 10.1093/ije/26.4.698.
- [9] Lafferty WE, Downey L, Celum C, Wald A. *Herpes simplex virus type 1 as a cause of genital herpes: impact on surveillance and prevention*. *J Infect Dis* 2000;181:1454-7. doi: 10.1086/315395.
- [10] Franco E, Caprilli F, Zaratti L, Pasquini P. *Prevalence of antibodies to Herpes simplex virus type 1 in different population groups in Italy*. *Eur J Clin Microbiol* 1987;6:322. doi: 10.1007/BF02017628.
- [11] Suligoï B, Cusani M, Santopadre P, Palu G, Catania S, Girelli G, Pala S, Vullo V. *HSV-2 specific seroprevalence among various populations in Rome, Italy. The Italian Herpes Management Forum*. *Sex Transm Infect* 2000;76:213-4. doi: 10.1136/sti.76.3.213.
- [12] Suligoï B, Calistri A, Cusani M, Palu G, Italian Herpes Management Forum. *Seroprevalence and determinants of herpes simplex type 2 infection in an STD clinic in Milan, Italy*. *J Med Virol* 2002;67:345-8. doi: 10.1002/jmv.10072.
- [13] Suligoï B, Dorrucchi M, Volpi A, Andreoni M, Zerboni R, Rezza G; Italian Seroconversion Study Group. *Prevalence and determinants of Herpes simplex virus type 2 infection in a cohort of HIV-positive individuals in Italy*. *Sex Transm Dis* 2002;29:665-7.
- [14] Cusani M, Cusan M, Parolin C, Scioccati L, Declèva I, Mengoli C, Suligoï B, Palu G. *Seroprevalence of Herpes simplex virus type 2 infection among attendees of a sexually transmitted disease clinic in Italy*. *Italian Herpes Forum*. *Sex Transm Dis* 2000;27:292-5.
- [15] Nahmias AJ, Lee FK, Beckman-Nahmias S. *Sero-epidemiological and -sociological patterns of Herpes simplex virus infection in the world*. *Scand J Infect Dis Suppl* 1990;69:19-36.
- [16] Woestenbergh PJ, Tjhe JH, de Melker HE, van der Klis FR, van Bergen JE, van der Sande MA, van Benthem BH. *Herpes simplex virus type 1 and type 2 in the Netherlands: seroprevalence, risk factors and changes during a 12-year period*. *BMC Infect Dis* 2016;16:364. doi: 10.1186/s12879-016-1707-8.
- [17] Niewiesk S. *Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies*. *Front Immunol* 2014;5:446. doi: 10.3389/fimmu.2014.00446.
- [18] Mele A, Franco E, Caprilli F, Gentili G, Capitano B, Crescimbeni E, Di Napoli A, Zaratti L, Conti S, Corona R, Rezza G, Pana A, Pasquini P. *Genital herpes infection in outpatients attending a sexually transmitted disease clinic in Italy*. *Eur J Epidemiol* 1988;4:386-8. doi: 10.1007/BF00148930.
- [19] Bradley H, Markowitz LE, Gibson T, McQuillan GM. *Seroprevalence of herpes simplex virus types 1 and 2 - United States, 1999-2010*. *J Infect Dis* 2014;209:325-33. doi: 10.1093/infdis/jit458.
- [20] Xu F, Lee FK, Morrow RA, Sternberg MR, Luther KE, Dubin G, Markowitz LE. *Seroprevalence of herpes simplex virus type 1 in children in the United States*. *J Pediatr* 2007;151:374-7. doi: 10.1016/j.jpeds.2007.04.065.
- [21] Wald A. *Genital HSV-1 infections*. *Sex Transm Infect* 2006;82:189-90. doi: 10.1136/sti.2006.019935.
- [22] Pereira VS, Moizeis RN, Fernandes TA, Araujo JM, Meissner RV, Fernandes JV. *Herpes simplex virus type 1 is the main cause of genital herpes in women of Natal, Brazil*. *Eur J Obstet Gynecol Reprod Biol* 2012;161:190-3. doi: 10.1016/j.ejogrb.2011.12.006.
- [23] Arvaja M, Lehtinen M, Koskela P, Lappalainen M, Paavonen J, Vesikari T. *Serological evaluation of herpes simplex virus type 1 and type 2 infections in pregnancy*. *Sex Transm Infect* 1999;75:168-71. doi: 10.1136/sti.75.3.168.
- [24] Straface G, Selmin A, Zanardo V, De Santis M, Ercoli A, Scambia G. *Herpes simplex virus infection in pregnancy*. *Infect Dis Obstet Gynecol* 2012;385697. doi: 10.1155/2012/385697.
- [25] Ciavattini A, Vichi M, Rinci A, Tsioglou D. *Infezioni virali in gravidanza: gestione e raccomandazioni*. *La Colposcopia in Italia Anno 2007;XXI:11-16*.

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