

# Seroprevalence of herpes simplex virus type 1 and type 2 - data from a hospital-based study in Varna, northeastern Bulgaria, 2019-2021

Gabriela Tsankova<sup>1</sup>, Tatina Todorova<sup>1,2</sup>, Neli Ermenlieva<sup>1</sup>, Gergana Nedelcheva<sup>1</sup>, Zhivka Stoykova<sup>1,2</sup>, Tsvetelina Kostadinova<sup>2,3</sup>

<sup>1</sup>Department of Microbiology and Virology, Varna Medical University, Bulgaria;

<sup>2</sup>Laboratory of Virology, "St. Marina" University Hospital, Varna, Bulgaria;

<sup>3</sup>Medical Laboratory Technicians Section, Medical College, Varna Medical University, Bulgaria

## SUMMARY

The purpose of the current study was to describe the prevalence of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in northeastern Bulgaria. From January 2019 to December 2021, we tested 1493 samples for anti-HSV-1 IgG and 817 samples for anti-HSV-2 IgG antibodies in the Virology Laboratory, "St. Marina" University Hospital, Varna, Bulgaria. HSV-1 was considerably more widespread, with an overall seroprevalence of 73.3% (95% CI: 71.0-75.5%), than HSV-2 infection, which showed a seropositive rate of 10.0% (95% CI: 8.1-12.4%). Age was the most significant risk factor for both infections, while gender had no role in herpes simplex seropositivity.

Received March 21, 2023

Accepted June 20, 2023

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are common human pathogens (Zhu and Viejo-Borbolla, 2021). HSV-1 infects mainly by closed oral-to-oral contact and affects the skin or mucous membranes in the area of the mouth (oral herpes). Sometimes it can also spread to the genitalia, where it causes genital herpes. HSV-2 is usually sexually acquired and provokes genital herpes. Similarly, it can also lead to orolabial infection.

Primary infections with HSV-1 or HSV-2 vary from asymptomatic and self-limited to severe cases characterized by extensive, painful oral or genital ulceration and systemic symptoms (Ostler, 1976). Both can progress to fulminant diseases in neonates, young children, and immunocompromised hosts. Once acquired, HSV infection becomes latent, and symptoms can recur at different triggering events over many years.

After the first HSV infection, specific immunoglobulin M (IgM) antibodies appear approximately one week later and persist for 4-6 weeks, while anti-HSV immunoglobulin G (IgG) antibodies are produced from 21-28 to over 42 days after exposure and prob-

ably last for life (Page *et al.*, 2003). Both antibodies can be identified with laboratory tests and can serve as a reliable indicator for active infection and seroprevalence level, respectively (Wald and Ashley-Morrow, 2002).

A study from 2016 estimated a global HSV-1 seroprevalence of 67% and an HSV-2 seroprevalence of 13% (James *et al.*, 2020). Similar to the worldwide data, knowledge about HSV seroprevalence in Bulgaria has not been recently updated: the only nationwide study (which found a seroprevalence of 84% and 24% for HSV-1 and HSV-2, respectively) was conducted more than 20 years ago (Pebody *et al.*, 2004). Besides some new data for neurological patients (Shikova *et al.*, 2022), there is no information about the general population. In this context, the purpose of the present study was to add new epidemiological data about the distribution of HSV-1 and 2 in northeastern Bulgaria.

We retrospectively analyzed the HSV-1 and HSV-2 serological testing results obtained in the Virology Laboratory, "St. Marina" University Hospital, Varna, Bulgaria for three years. From January 2019 to December 2021, we tested 1493 serum samples for anti-HSV-1 IgG and 817 serum samples for anti-HSV-2 IgG. Commercially available indirect ELISA kits were used - Anti-HSV-1 (gC1) ELISA (IgG) and anti-HSV-2 (gG2) ELISA (IgG) (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). The corresponding antigens were glycoproteins C1 and G2 - a membrane and an envelope protein

### Key words:

HSV-1, HSV-2, HHV-1, HHV-2, prevalence, epidemiology.

### Corresponding author:

Tatina T. Todorova

E-mail: Tatina.Todorova@mu-varna.bg

of HSV-1 and HSV-2, respectively. No exclusion criteria were applied for sampling – all ordered tests were included in the retrospective analysis. Samples were obtained mainly from hospitalized patients in the neurological, dermatological and pediatric clinics, but also ambulatory and other hospitalized patients suspected of herpes simplex-associated diseases were included. The age of the tested individuals ranged from 2 days to 93 years.

We performed Wilson score for the confidence interval calculation and Chi-square test with a significance level of 0.05 with the R project for statistical computing (version 4.0.4/2021-02-15).

The overall rate of HSV-1 IgG seropositivity was 73.3% (95% CI: 71.0-75.5%) (Table 1). The proportions of positive HSV-1 IgG samples in females and males did not differ significantly - 71,7% and 74.6%, respectively (Chi-square =1.55, p=0.21).

Children less than one year of age had 57% of HSV-1 IgG-positive samples. The proportion of the HSV-1 IgG-positive samples fell to 28.1% among those aged 1-7 years. There was then a gradual increase in the proportion of seropositive individuals - individuals over 70 showed the highest seropositive rate of HSV-1 IgG (Table 1).

The HSV-2 IgG seropositive rate was 10.0% (95% CI: 8.1-12.4%) (Table 2). The proportion of positive HSV-2 IgG samples was slightly higher among females 10.8% (95% CI: 8.0-14.4%) than among males 9.4% (95% CI: 7.0-12.5%), but was not statistically significant (Chi-square =0.3, p=0.6).

Similar to the HSV-1 IgG prevalence, the HSV-2 IgG seropositive rate in infants aged less than one year was a relatively high - 3.3%, then seropositivity decreased to 1.0% with the increase of age and gradually increased again to reach the highest value of 32.3% among 60-69-year-olds.

The proportion of the HSV-1 and HSV-2 IgG positive samples was similar to that reported in other European and Asian countries (Bünzli *et al.*,

2004; Pebody *et al.*, 2004; Shen *et al.*, 2015; Olsson *et al.*, 2017). Compared to data from a study in the same hospital but conducted 20 years ago (Ivanova, 2007), HSV-1 seropositivity increased by 3-7%, which was not a statistically significant difference. Interestingly, the HSV-2 seropositivity found in the current study was several times lower than the prevalence detected 20 years ago (10% versus 56%). Although this difference can derive from the different methodology used, a similar significant decrease in HSV-2 seropositivity was detected when compared to another past study in Bulgaria (also performed more than 20 years ago) - that of Pebody *et al.* (Pebody *et al.*, 2004), which found an HSV-2 IgG seroprevalence of 24%. Therefore, we could speculate that HSV-2 has become less prevalent among the Bulgarian population.

Children under one year of age had a relatively very high seroprevalence of 57% for HSV-1 IgG and 3.3% for HSV-2 IgG. This might be attributed to the passive transition of maternal antibodies. In older ages, children (between one and seven years for HSV-1 and between one and 13 years for HSV-2) had lower seropositivity rates. With the beginning of puberty and sexual life, it increased again. Compared to HSV-1 IgG seropositivity, which gradually increased with age, HSV-2 IgG seropositivity showed a fluctuant dynamic among the age groups - a possible explanation could be the effect of the small sample size and the isolated HSV-2 cases detected in some of the age groups. It is also possible that more complicated behavioral and social factors play a role in HSV-2 dynamics. In general, we can conclude that age is the most significant risk factor for HSV infection.

We found no significant association between gender and HSV-1 seropositivity, which was also described in previous studies (Pebody *et al.*, 2004; Ivanova, 2007). Our analysis showed the same lack of statistically significant difference between men and women for HSV-2 (the rates of HSV-2 IgG were

**Table 1** - Prevalence of IgG antibodies against HSV-1, 2019-2021.

Age group	Males		Females		Total	
	n/N	% (95% CI)	n/N	% (95% CI)	n/N	% (95% CI)
<1	46/76	60.5 (49.3-71.5)	40/75	53.3 (42.2-64.2)	86/151	57.0 (49.0-64.6)
1-7	22/73	30.1 (20.8-41.4)	14/55	25.5 (15.8-38.3)	36/128	28.1 (21.1-36.5)
8-13	21/63	33.3 (23.0-45.6)	13/42	31.0 (19.1-46.0)	34/105	32.4 (24.2-41.8)
14-19	43/63	68.3 (56.0-78.4)	16/47	34.0 (22.2-48.3)	59/110	53.6 (44.4-62.7)
20-29	25/37	67.6 (51.5-80.4)	29/46	63.0 (48.6-75.5)	54/83	65.1 (54.3-74.4)
30-39	95/107	88.8 (81.4-93.5)	54/63	85.7 (75.0-92.3)	149/170	87.7 (81.9-91.8)
40-49	92/107	86.0 (78.2-91.3)	54/57	94.7 (85.6-98.2)	146/164	89.0 (83.3-92.9)
50-59	98/105	93.3 (86.9-96.7)	72/81	88.9 (80.2-94.0)	170/186	91.4 (86.5-94.6)
60-69	95/110	86.4 (78.7-91.6)	77/84	91.7 (83.8-96.0)	172/194	88.7 (83.4-92.4)
>70	94/105	89.5 (82.2-94.1)	95/97	97.9 (92.8-99.4)	189/202	93.6 (89.3-96.2)
Total	631/846	74.6 (71.6-77.4)	464/647	71.7 (68.1-75.1)	1095/1493	73.3 (71.0-75.5)

n - number of positive samples; N - total number of samples

**Table 2** - Prevalence of IgG antibodies against HSV-2, 2019-2021.

Age group	Males		Females		Total	
	n/N	% (95% CI)	n/N	% (95% CI)	n/N	% (95% CI)
<1	3/72	4.2 (1.4-11.6)	2/78	2.6 (0.7-8.9)	5/150	3.3 (1.4-7.6)
1-7	1/63	1.6 (0.3-8.5)	1/46	2.2 (0.4-11.3)	2/109	1.8 (0.5-6.4)
8-13	0/50	–	1/47	2.1 (0.4-11.1)	1/97	1.0 (0.2-5.6)
14-19	2/52	3.8 (1.1-13.0)	0/41	–	2/93	2.2 (0.6-7.5)
20-29	2/24	8.3 (2.3-25.9)	3/21	14.3 (5.0-34.6)	5/45	11.1 (4.8-23.5)
30-39	3/33	9.1 (3.1-23.6)	2/25	8.0 (2.2-25.0)	5/58	8.6 (3.7-18.6)
40-49	11/52	21.2 (12.2-34.0)	6/22	27.3 (13.2-48.2)	17/74	23.0 (14.9-33.8)
50-59	9/54	16.7 (9.0-28.7)	13/45	28.9 (17.7-43.4)	22/99	22.2 (15.2-31.4)
60-69	10/34	29.4 (16.8-46.2)	11/31	35.5 (21.1-53.1)	21/65	32.3 (22.2-44.4)
>70	1/12	8.3 (1.5-35.4)	1/15	6.7 (1.2-29.8)	2/27	7.4 (2.1-23.4)
Total	42/446	9.4 (7.0-12.5)	40/371	10.8 (8.0-14.4)	82/817	10.0 (8.1-12.4)

n - number of positive samples; N - total number of samples.

slightly but not significantly higher among females). This result differs from previous studies that found females to have a consistently higher risk of HSV-2 infection than males (Smith and Robinson, 2002; Pebody *et al.*, 2004; Sauerbrei *et al.*, 2011; Shen *et al.*, 2015).

Among the strengths of the study are the high number of tested samples and the large area of territory covered by the hospital, which ensures representativity for the entire territory of northeastern Bulgaria. However, the present work has some limitations that are worth discussing. First, this was a retrospective, observational and single-center study. Second and more important was the sample used for the analysis. Unfortunately, we could not perform random sampling to ensure the results were valid for the general population. Our sample consisted mainly of hospitalized patients with ordered HSV tests for any reason - most of them with severe conditions that might be related to HSV infection. Thus, a bias that may have led to overestimating the actual prevalence could exist.

To conclude, the population in northeastern Bulgaria is widely infected with HSV-1 - 73.3% of the samples tested in our study were HSV-1 IgG seropositive. Our study shows a significant drop in the frequency of HSV-2 compared to previous studies: currently only 10% of the tested samples are positive for HSV-2 IgG. Both herpes infections are acquired mainly during childhood and adolescence, and seropositivity increases with age. Also, the widely accepted higher risk of HSV-2 for women has not been confirmed in the current Bulgarian population.

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