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Brief report

Pre-vaccination seroprevalence of 15 human papillomavirus (HPV) types among women in the population-based Slovenian cervical screening program☆

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ABSTRACT

Objectives: To estimate seroprevalence of 11 high-risk (hr) HPV types and four low-risk (lr) HPV types among 20–64 years old Slovenian women participating in the population-based cervical cancer screening program.
Methods: Serum samples from 3259 women were tested for HPV type-specific antibodies with a multiplexed pseudovirion-based serological assay (PsV-Luminex).
Results: Seropositivity for any of the 15 HPV types was 65.7%, any of the 11 hr-HPV types 59.2%, and any of the four lr-HPV types 33.1%. Antibodies against at least one of the four vaccine HPV types (HPV 6, 11, 16, 18) were detected in 40.8% women. Among hr-HPV types, seropositivity was highest for HPV 16 (25.2%) and among lr-HPV types for HPV 6 (19.1%). Age-specific HPV16 seropositivity was highest among 30–39 years old (29.6%) and decreased with increasing age to 14.0% among 60–64 years old.
Conclusion: The lifetime sexual exposure to genital HPV types is substantial, emphasising the need for HPV vaccination.

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1. Introduction

Genital human papillomavirus (HPV) types cause the most common sexually transmitted infection [1]. High-risk (hr) HPV types cause cervical cancer in women and other cancers in both genders [1,2] and low-risk (lr) HPV types genital warts and recurrent respiratory papillomatosis [3]. HPV DNA detection is the standard method for diagnosing current cervical infection of which most clear or became undetectable within two years [4]. Approximately 60% of women with detectable HPV DNA in cervical smears do not have a detectable type-specific HPV antibodies [5–8]. When a detectable serum antibody response to the viral major capsid protein L1 occurs, it remains relatively stable [5]. Thus, measuring HPV type specific antibodies can be a useful, although imperfect, tool for estimating cumulative HPV type specific lifetime exposure. To date, there were relatively few HPV seroepidemiological studies conducted among general female populations and they were mostly restricted to estimating seropositivity for HPV 6, 11, 16, and 18 [9–14].

Two vaccines against HPV infection have been licensed, the quadrivalent containing L1 virus-like particles for types 6, 11, 16 and 18 and bivalent containing L1 virus-like particles for types 16 and 18. Vaccination of 11–12 years old girls with quadrivalent vaccine was introduced in Slovenia in 2009 in the absence of data on the current prevalence and lifetime risk for HPV infections in the population.

In 2010, we conducted the Slovenian HPV prevalence survey (SHPVS) among women screened for cervical cancer to estimate the pre-vaccination prevalence of cervical infections (HPV DNA

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Table 1
Seropositivity (%) for 15 HPV types overall and according to age groups among 3259 women screened for cervical cancer, Slovenia, 2010.

HPV types	All women (*N* = 3259)			Age groups							
				20–29 (*N* = 945)		30–39 (*N* = 1171)		40–49 (*N* = 707)		50–64 (*N* = 436)	
	N	S (%)	95% CI	*N*	S (%)	*N*	S (%)	*N*	S (%)	*N*	S (%)
HPV	2141	65.7	64.0–67.3	592	62.6	812	69.8	462	65.3	269	61.7
Single HPV	664	20.4	19.0–21.7	180	19.0	256	21.9	139	19.7	89	20.4
Multiple HPV	1477	45.3	43.6–47.0	412	43.6	562	48.0	323	45.7	180	41.3
hr-HPV	1930	59.2	57.5–60.9	535	56.6	750	64.0	407	57.6	238	54.6
Single hr-HPV	497	15.2	14.0–16.4	134	14.2	204	17.4	96	13.5	63	14.4
Multiple hr-HPV	1433	44.0	42.3–45.7	401	42.4	546	46.6	311	44.0	175	40.1
Only hr-HPV	1062	32.6	31.0–34.2	291	30.8	405	34.6	235	33.2	131	30.0
HPV16 or 18	976	29.9	28.3–31.5	258	27.3	412	35.2	200	28.3	106	24.3
HPV16 and 18	151	4.6	3.9–5.3	46	4.9	64	5.5	21	3.0	20	4.6
HPV16	820	25.2	23.7–26.6	217	23.0	347	29.6	164	23.0	92	21.1
HPV18	307	9.4	8.4–10.4	87	9.2	129	11.0	57	8.1	34	7.8
HPV31	564	17.3	16.0–18.6	153	16.2	214	18.3	125	17.7	72	16.5
HPV33	365	11.2	10.1–12.3	113	12.0	134	11.4	70	9.9	48	11.0
HPV35	406	12.5	11.3–13.6	127	13.4	154	13.1	86	12.2	39	8.9
HPV39	570	17.5	16.2–18.8	162	17.1	212	18.1	123	17.4	73	16.7
HPV45	185	5.7	4.9–6.5	39	4.1	75	6.4	50	7.1	21	4.8
HPV52	303	9.3	8.3–10.3	92	9.7	123	10.5	61	8.6	27	6.2
HPV56	340	10.4	9.4–11.5	96	10.2	130	11.1	72	10.2	42	9.6
HPV58	640	19.6	18.3–21.0	177	18.7	254	21.7	127	18.0	82	18.8
HPV59	409	12.5	11.4–13.7	127	13.4	154	13.1	77	11.0	51	11.7
lr-HPV	1079	33.1	31.5–34.7	301	31.8	413	35.3	227	32.1	138	31.6
Single lr-HPV	167	5.1	4.4–5.9	46	4.9	52	4.4	43	6.1	26	6.0
Multiple lr-HPV	912	28.0	26.4–29.5	255	27.0	361	30.8	184	26.0	112	25.7
Only lr-HPV	211	6.5	5.6–7.3	57	6.0	68	5.8	55	7.8	31	7.1
HPV6 or 11	681	20.9	19.5–22.3	194	20.5	248	21.2	155	21.9	84	19.3
HPV6 and 11	130	4.0	3.3–4.7	32	3.4	41	3.5	35	4.9	22	5.0
HPV6	622	19.1	17.7–20.4	183	19.4	230	19.6	139	19.7	70	16.1
HPV11	189	5.8	5.0–6.6	43	4.5	59	5.0	51	7.2	36	8.3
HPV68	397	12.2	11.1–13.3	108	11.4	153	13.1	78	11.0	58	13.3
HPV73	404	12.4	11.3–13.5	108	11.4	174	14.9	78	11.0	44	10.1
HPV 6 or 11 or 16 or 18	1331	40.8	39.1–42.5	366	38.7	531	45.3	282	39.9	152	34.9
HPV 6 and 11 and 16 and 18	30	0.9	0.6–1.2	11	1.2	9	0.8	6	0.8	4	0.9
HPV not in bivalent vaccine	2578	79.1	77.7–80.5	751	79.5	923	78.8	552	78.1	352	80.7
HPV not in quadrivalent vaccine	1928	59.2	57.5–60.8	579	61.3	640	54.6	425	60.1	284	65.1

Due to antibodies to multiple HPV types present in the serum of one woman the sums may not add up.
HPV: human papillomavirus; N: number of seropositive women; S: seropositivity; CI: confidence interval.
hr-HPV types: high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59 (Group 1 by the IARC Monograph Working Group with the exception of HPV 51)).
lr-HPV types: low-risk HPV types (6, 11, 68, 73).

positivity) with hr-HPV and lr-HPV types [15] and to obtain estimates of seroprevalences for 11 hr-HPV types and four lr-HPV types.

2. Methods

We conducted a cross-sectional study among 20–64 years old women screened for cervical cancer within a convenience sample of 22 outpatient gynaecology services with nationally wide geographical coverage as described previously [15,16]. Briefly, they were enrolled consecutively during the study period. Cervical smear specimens obtained from each participant were sent to certified cytological laboratories. In addition, cervical smear specimens were obtained for HPV DNA testing and blood specimens for HPV serology testing. Information about vaccination against HPV and the number of lifetime male sexual partners was collected anonymously.

Whole blood samples (5 mL) were stored at +4 °C for less than a week before centrifugation for 5 min at 3000 rpm. The serum was aliquoted and stored at -30 °C until further analysis. Serology testing was performed with a multiplexed pseudovirion-based serological assay, as described previously [8]. Briefly, pseudovirions for 15 HPV types: 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 68, 73 were used to detect seropositivity for indicated HPV types. Pseudovirions were generated by transfection of 293TT cells [17]. To define seropositivity, cut-off values were calculated independently for each HPV type by analysing mean fluorescence intensity unit (MFI) values for 133 sera from children aged less than 12 years. The global HPV LabNet recommended cut-off algorithm [18] was applied: mean MFI value of a negative control serum panel plus 3 standard deviations. If the calculated cut-off value was less than 400 MFI, the 400 MFI was used as cut-off.

Statistical analyses were performed using the STATA package version 10.0 (Stata Statistical Software: release 10.0 College Station. TX: Stata Corporation). We estimated the HPV seropositivity with 95% confidence intervals (CI) for selected HPV types (overall, according to age and according to cytology result) and odds of seropositivity with 95% CI in women with pathological cytology result against odds among those with normal cytology result. Chi-square test and/or test for linear trend was used to assess the association between seropositivity and age groups and seropositivity and cytology results.

The study has been carried out in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) and has been approved by the Medical Ethics Committee of the Republic of Slovenia (Consent number: 83/11/09).

3. Results

Among 4514 participants (survey response: 98.1%), 3321 (73.6%) contributed a blood specimen. We excluded 62 women because they were not 20–64 years old or information on age was

Table 2
Seropositivity (%) for different hr-HPV types by cytology result among 3230 women screened for cervical cancer, Slovenia, 2010.

HPV types	Cytology result																				P trend
	NILM (*N* = 3088)				ASC-US (*N* = 69)					LSIL (*N* = 51)					HSIL (*N* = 22)					
	N	S (%)	95% CI	OR[a]	*N*	S (%)	95% CI	OR	95% CI	*N*	S (%)	95% CI	OR	95% CI	*N*	S (%)	95% CI	OR	95% CI	
hr-HPV	1802	58.3	56.6–60.1	1	52	75.4	64.9–85.8	2.2	1.2–3.8	41	80.4	69.1–91.7	2.9	1.4–5.9	17	77.3	58.2–96.3	2.4	0.9–6.6	<0.001
Single hr-HPV	466	15.1	13.8–16.3	1	16	23.2	13.0–33.4	1.7	1.0–3.0	8	15.7	5.3–26.0	1.0	0.5–2.3	4	18.2	0.7–35.7	1.2	0.4–3.7	0.312
Multiple hr-HPV	1336	43.3	41.5–45.0	1	36	52.2	40.1–64.3	1.4	0.9–2.3	33	64.7	51.1–78.3	2.4	1.3–4.3	13	59.1	36.8–81.4	1.2	0.8–4.4	<0.001
Only hr-HPV	989	32.0	30.4–33.7	1	30	43.5	31.5–55.5	1.6	1.0–2.6	23	45.1	31.0–59.2	1.7	1.0–3.0	10	45.4	22.8–68.0	1.8	0.8–4.1	0.004
HPV16 or 18	908	29.4	27.8–31.0	1	26	37.7	25.9–49.4	1.4	0.9–2.4	24	47.1	32.9–61.2	2.1	1.2–3.7	11	50.0	28.3–72.7	2.4	1.0–5.6	<0.001
HPV16 and 18	138	4.5	3.7–5.2	1	3	4.3	0.0–9.3	1.0	0.3–3.1	6	11.8	2.6–20.9	2.8	1.2–6.8	3	13.6	0.0–29.2	3.4	1.0–11.5	0.004
HPV16	764	24.7	23.2–26.3	1	23	33.3	21.9–44.7	1.5	0.9–2.5	20	39.2	25.3–53.1	2.0	1.1–3.5	9	40.9	18.6–63.2	2.1	0.9–4.9	0.001
HPV18	282	9.1	8.1–10.1	1	6	8.7	1.9–15.5	0.9	0.4–2.2	10	19.6	8.3–30.9	2.4	1.2–4.9	5	22.7	3.7–41.7	2.9	1.1–8.0	0.002
HPV31	516	16.7	15.4–18.0	1	23	33.3	21.9–44.7	2.5	1.5–4.1	11	21.6	9.9–33.2	1.4	0.7–2.7	7	31.8	10.7–52.9	2.3	0.9–5.7	0.002
HPV33	337	10.9	9.8–12.0	1	8	11.6	3.8–19.3	1.1	0.5–2.2	11	21.6	9.9–33.2	2.2	1.1–4.4	5	22.7	3.7–41.7	2.4	0.9–6.5	0.006
HPV35	380	12.3	11.1–13.5	1	11	15.9	7.0–24.8	1.3	0.7–2.6	10	19.6	8.3–30.9	1.7	0.9–3.5	1	4.5	0.0–14.0	0.3	0.1–2.5	0.522
HPV39	526	17.0	15.7–18.4	1	14	20.3	10.5–30.0	1.2	0.7–2.2	13	25.5	13.1–37.9	1.7	0.9–3.1	8	36.4	14.5–58.2	2.8	1.2–6.7	0.005
HPV45	170	5.5	4.7–6.3	1	7	10.1	2.8–17.4	1.9	0.9–4.3	3	5.9	0.0–12.6	1.1	0.3–3.4	3	13.6	0.0–29.2	2.7	0.8–9.2	0.082
HPV52	280	9.1	8.0–10.1	1	9	13.0	4.9–21.2	1.5	0.7–3.1	10	19.6	8.3–30.9	2.4	1.2–4.9	2	9.1	0.0–22.1	1.0	0.2–4.3	0.037
HPV56	308	10.1	8.9–11.0	1	13	18.8	9.4–28.3	2.1	1.1–3.9	12	23.5	11.5–35.6	2.8	1.4–5.4	4	18.2	0.7–35.7	2.0	0.7–6.0	<0.001
HPV58	599	19.4	18.0–20.8	1	14	20.3	10.5–30.0	1.1	0.6–1.9	14	27.4	14.8–40.1	1.6	0.8–2.9	6	27.3	7.1–47.5	1.5	0.6–4.0	0.109
HPV59	379	12.3	11.1–13.4	1	12	17.4	8.2–26.6	1.5	0.8–2.8	11	21.6	9.9–33.2	2.0	1.0–3.9	5	22.7	3.7–41.7	2.1	0.8–5.7	0.007
lr-HPV	1016	32.9	31.2–34.5	1	24	34.8	23.2–46.3	1.1	0.6–1.8	21	41.2	27.2–55.1	1.4	0.8–2.5	8	36.4	14.5–58.2	1.2	0.5–2.8	0.249
Single lr-HPV	156	5.1	4.4–5.9	1	2	2.9	0.0–6.9	0.5	0.1–2.3	3	5.9	0.0–12.6	1.1	0.3–3.7	1	4.5	0.0–14.0	0.9	0.1–6.6	0.813
Multiple lr-HPV	857	27.7	26.2–29.3	1	22	31.9	20.6–43.2	1.2	0.7–2.0	18	35.3	21.7–48.9	1.4	0.8–2.5	7	31.8	10.7–52.9	1.2	0.5–3.0	0.185
Only lr-HPV	203	6.6	5.7–7.4	1	2	2.9	0.0–6.9	0.4	0.1–1.7	3	5.9	0.0–12.6	0.9	0.3–2.9	1	4.5	0.0–14.0	0.7	0.1–5.0	0.408
HPV6 or 11	639	20.7	19.3–22.1	1	16	23.2	13.0–33.4	1.1	0.6–2.0	15	29.4	16.5–42.3	1.6	0.9–2.9	6	27.3	7.1–47.5	1.4	0.5–3.7	0.097
HPV6 and 11	120	3.9	3.2–4.6	1	4	5.8	0.1–11.4	1.5	0.5–4.2	4	7.8	0.2–15.5	2.1	0.7–5.9	2	9.1	0.0–22.1	2.5	0.6–10.7	0.043
HPV6	583	18.9	17.4–20.3	1	15	21.7	11.7–31.7	1.2	0.7–2.1	15	29.4	16.4–42.3	1.8	1.0–3.3	6	27.3	7.1–47.5	1.6	0.6–4.1	0.036
HPV11	176	5.7	4.9–6.5	1	5	7.2	1.0–13.5	1.3	0.5–3.2	4	7.8	0.2–15.5	1.4	0.5–3.9	2	9.1	0.0–22.1	1.6	0.4–7.1	0.289
HPV68	371	12.0	10.9–13.2	1	12	17.4	8.2–26.6	1.5	0.8–2.9	7	13.7	3.9–23.5	1.2	0.5–2.6	2	9.1	0.0–22.1	0.7	0.2–3.1	0.642
HPV73	376	12.2	11.0–13.3	1	9	13.0	4.9–21.2	1.1	0.5–2.2	12	23.5	11.5–35.6	2.2	1.1–4.3	3	13.6	0.0–29.2	1.1	0.3–3.9	0.071

9 women with atypical glandular cells or atypical squamous cells – cannot exclude HSIL (ASC-H) were excluded from analyses.
Due to antibodies to multiple HPV types present in the serum of one woman the sums may not add up.
HPV: human papillomavirus; NILM: negative for intraepithelial lesion or malignancy; ACS-US: atypical squamous cells of undetermined significance; LSIL: low-grade squamous intraepithelial lesions.
HSIL: high grade squamous intraepithelial lesion; N: number of seropositive women; S: seropositivity; CI: confidence interval for seropositivity; OR: odds ratio (odds of seropositivity in women with particular pathological cytology result against odds of seropositivity among women with NILM).
hr-HPV types: high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59 (Group 1 by the IARC Monograph Working Group with the exception of HPV 51)).
lr-HPV types: low-risk HPV types (6, 11, 68, 73).
[a] Since seroprevalence of HPV infection is common, ORs are not numerically similar to prevalence ratios.

missing or they have been vaccinated against HPV. The mean age of the remaining 3259 women was 36.5 years (median: 35). Among 3239 women with available cytology result, 95.3% were negative for intraepithelial lesion or malignancy (NILM), 2.1% had atypical squamous cells of undetermined significance (ACS-US), 0.1% atypical glandular cells, 1.6% low-grade squamous intraepithelial lesions (LSIL), 0.7% high grade squamous intraepithelial lesion (HSIL), and 0.2% atypical squamous cells-cannot exclude HSIL (ASC-H).

Table 1 shows the overall seropositivity and age specific seropositivity for individual HPV types and for different combinations of HPV types. The overall seropositivity for any of the 15 HPV types was 65.7% (CI:64.0–67.3%), for any of the 11 hr-HPV 59.2% (CI:57.5–60.9%), and any of the four lr-HPV 33.1% (CI:31.5–34.7%). Antibodies against at least one of the four vaccine HPV types (HPV 6, 11, 16, 18) were detected among 40.8% women (CI:39.1–42.5%). Overall seropositivity for multiple HPV types was higher (45.3%; CI:43.6–47.0%) than seropositivity for single HPV types (20.4%; CI:19.0–21.7%). The HPV type specific seropositivity was the highest for HPV 16 (25.2%; CI:23.7–26.6%), followed by HPV 58 (19.6%; CI:18.3–21.0%), and HPV 6 (19.1%; CI:17.7–20.4%). Seropositivity for HPV 18 was 9.4% (CI:8.4–10.5%), and for HPV 11 5.8% (CI:5.0–6.6%).

Women with at least five lifetime heterosexual partners had 5.1 (95% CI:4.0–6.4) times higher odds for seropositivity with any of the hr-HPV types in comparison to those with only one heterosexual partner ever ($p < 0.001$) and those with seropositivity for HPV 16 and HPV 18, had 5.3 (CI:4.0–7.0) and 7.6 (CI:4.7–12.3) times higher odds in comparison to those with only one heterosexual partner ever (both $p < 0.001$). Corresponding odds ratios for lr-HPV, HPV 6, and HPV 11 were 4.1 (CI:3.3–5.2), 4.1 (CI:3.1–5.5), and 2.7 (CI:1.7–4.4) (all $p < 0.001$).

The age-specific seropositivity for HPV 16 increased from 17.8% among women 20–24 years old to 32.4% among those 30–34 years old ($p_{trend} < 0.001$), and then decreased with increasing age to 14.0% among 60–64 years old women ($p_{trend} = 0.014$), while the age-specific seropositivity for HPV 18 increased from 5.8% among women 20–24 years old to 11.7% among those 25–34 years old ($p < 0.001$), which was not followed by a decrease with increasing age ($p_{trend} = 0.159$) (Fig. 1).

Seropositivity for hr-HPV and lr-HPV types according to cytology result is shown in Table 2. Seropositivity for any of the 11 hr-HPV was lower among women with NILM (58.3%; CI:56.6–60.1%) than among women with pathological cytology results (76.8%; CI:70.0–83.6%; $p < 0.001$). In comparison to women with NILM, women with ASC-US had 2.2 (CI:1.2.-3.8) times higher odds for seropositivity for any of the hr-HPV types, women with LSIL 2.9 (CI:1.4–5.9) times, and women with HSIL 2.4 (CI:0.9–6.6) times higher odds ($p_{trend} < 0.001$). Increasing seropositivity with increasing pathology of the cytology result was observed for the majority of individual hr-HPV types.

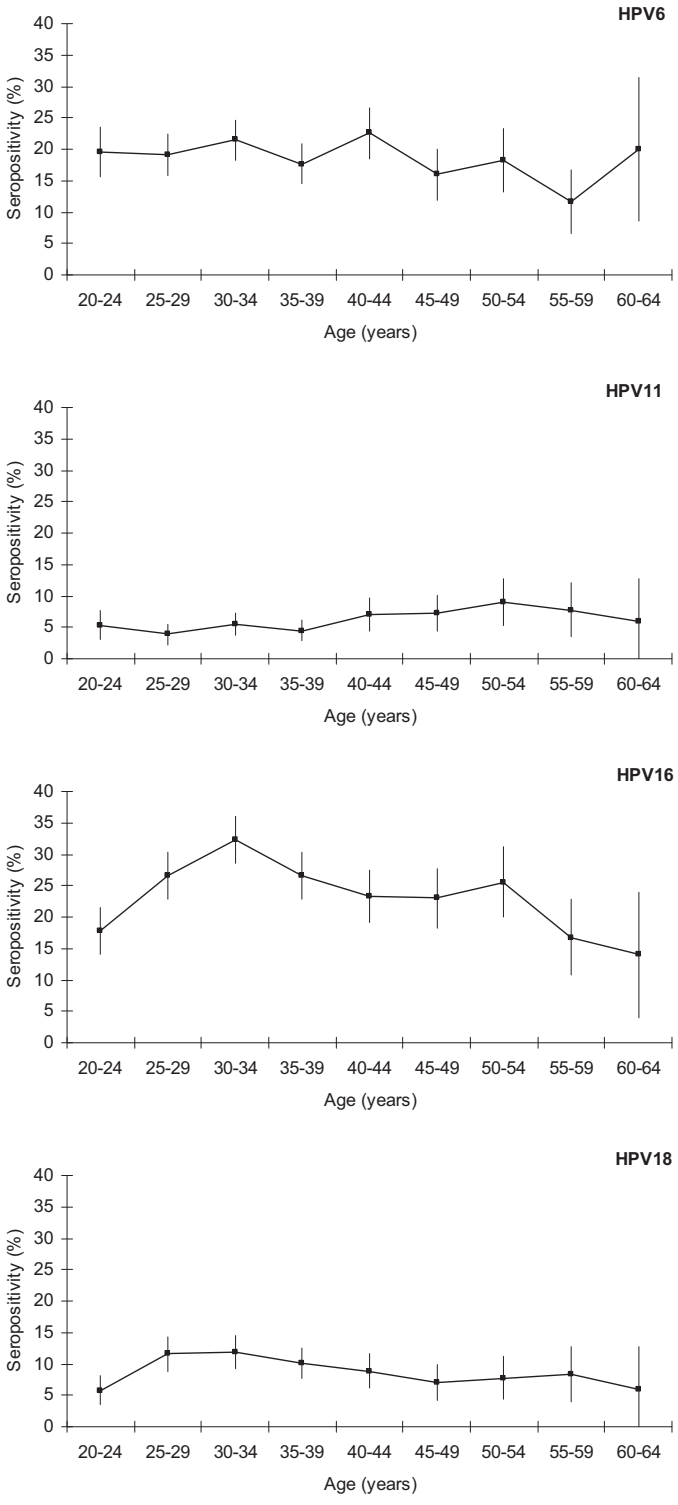

Fig. 1. Seropositivity (%) for HPV 6, HPV 11, HPV 16 and HPV 18 with 95% confidence intervals according to age among 3259 women screened for cervical cancer, Slovenia, 2010.

4. Discussion

We obtained the first seroprevalence estimate for as many as 15 selected genital HPV types in a survey of 3259 Slovenian women 20–64 years old screened for cervical cancer, the largest such population-based cross-sectional study to date.

Comparison of reported results of different HPV seroepidemiological studies should be done with caution, because of differences in study periods, age range of the studied populations, and the methods used for detecting HPV type specific antibodies. Our overall seroprevalence estimate for any of the 11 hr-HPV types examined was high in comparison to other similar studies [9–14]. The HPV 16 seroprevalence was higher than in all similar studies. Also our HPV 18 seroprevalence estimate was the highest, except for the Czech study [14]. We observed a higher proportion of women with antibodies against multiple HPV types than against single HPV types. Some overestimation of seropositivity to multiple HPV types could be attributed to a possible cross-reactivity of HPV specific antibodies induced by infection with phylogenetically related HPV types [11,12]. However, our method had a high specificity and low cross-reactivity [8]. The association of seropositivity with higher numbers of lifetime sexual partners suggests that

we have used a specific assay [9,10,12]. As others, we also showed lower seroprevalence for any of the 11 hr-HPV types among women with NILM in comparison to women with pathological cytology results [19,20].

The decreasing seropositivity for any HPV and many individual HPV types with increasing age in women after their thirties seems to be mainly due to a cohort effect and not to waining antibody response. Women born during 1950-ties and 1960-ties reported lower numbers of lifetime male partners and were thus at a lower cumulative lifetime risk for HPV infection in comparison to women born during 1970-ties that were 30–39 years old at the time of the survey (results not shown).

Possible constrain in extrapolating our results to all Slovenian women 20–64 years old is the possibility that women who are not screened for cervical cancer are at a different risk of natural exposure to HPV infection. However, we were able to ensure that with respect to age and cervical pathology, our study population had fairly similar characteristics to the total population of women screened for cervical cancer during 2010 and with respect to age, to Slovenian women 20–64 years old (results not shown). Also, measuring HPV type specific antibodies underestimates the cumulative lifetime risk of infections, as not all infected individuals develop detectable levels, antibodies can be detected only several months after infection, and because of waning of seropositivity [6,7]. However, we believe that our estimates fairly well reflect the substantial burden of lifetime sexually transmitted infections with the examined 15 HPV types in the reference population.

We have shown a substantial cumulative lifetime sexual exposure to at least one of the 15 HPV types examined as well as a relatively high cumulative lifetime sexual exposure to at least one of the four vaccine HPV types among Slovenian women. Thus, vaccination of females before sexual debut with a quadrivalent HPV vaccine has a potential to contribute to a substantial reduction of the burden of cervical infections and cervical cancer as well as some other HPV related morbidity, including genital warts among women and their male sexual partners.

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