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Mucosal and cutaneous Human Papillomavirus seroprevalence among adults in the prevaccine era in Germany — Results from a nationwide population-based survey

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ABSTRACT

Background: Human Papillomavirus (HPV) vaccination of girls was introduced in Germany in 2007. However, data on the distribution of vaccine-relevant HPV types in the general population in Germany in the prevaccine era are limited.

Methods: Serum samples collected during the German National Health Interview and Examination Survey 1998 (GNHIES98), a nationally representative study including men and women aged 18–79 years, were tested for antibodies to 19 mucosal and cutaneous HPV types. Multivariable regression models were developed to identify associations between demographic and behavioral characteristics and HPV seropositivity.

Results: Of the 6517 serum samples tested, almost a quarter was seropositive for at least one of the nine HPV vaccine types with no clear age-pattern. HPV-6 and HPV-59 were the most common mucosal types, while HPV-1 and HPV-4 were the most common cutaneous HPV types. Factors independently associated with HPV-16 seroprevalence were seropositive to other sexually transmitted infections and lifetime number of sex partners, as well as urbanity (only among females).

Conclusions: Prevalence of naturally acquired antibodies to HPV types which can be prevented by vaccination is high in both sexes and all age groups. These data can serve as baseline estimates to evaluate the population-level impact of the current vaccination strategy.

Background

About 200 different mucosal and cutaneous Human Papillomavirus (HPV) types have been characterized (Van Doorslaer et al., 2017), but only a small fraction is classified as oncogenic by the International Agency for Research on Cancer (IARC) (International Agency on Research on Cancer (IARC), 2012). The so-called high-risk (HR) types are considered to be causative agents of various types of precancerous lesions and cancer in women and men (International Agency on Research on Cancer (IARC), 2012; Schiffman et al., 2007). This group includes HPV types 16 and 18 as well as e.g., 31, 33, 35, 45, 52, and 58. External benign genital warts are mainly caused by low risk (LR) types 6 and 11 (International Agency on Research on Cancer (IARC), 2012). Cutaneous HPV types of different genera are common on healthy

Abbreviations: aPR, adjusted prevalence ratio; CI, confidence interval; DKFZ, German Cancer Research Center; GNHIES98, German National Health Interview and Examination Survey 1998; HPV, human papillomavirus; HPV-LR, low risk HPV types: 6, 11; HPV-HR, high risk HPV types: 16, 18, 31, 33, 35, 39, 45, 52, 58, 59; HPV-cut, cutaneous HPV types: 1, 4, 8, 10, 38, 41, 49; HPV-muc, mucosal HPV types: 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 58, 59; HPV-2val, HPV types in the bivalent vaccine: 16, 18; HPV-4val, HPV types in the quadrivalent vaccine: 6, 11, 16, 18; HPV-9val, HPV types in the nonavalent vaccine: 6, 11, 16, 18, 31, 33, 45, 52, 58; PR, prevalence ratio; Ref, reference; RKI, Robert Koch-Institute; STI, sexually transmitted infections; STI+, seropositivity for at least one of the following sexually transmitted infections: Mycoplasma genitalium (Mg), Herpes simplex 2 (HS2), Chlamydia trachomatis (Ct).

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skin (Antonsson et al., 2000). However, they are also found in several skin lesions such as benign skin warts (de Villiers et al., 2004; International Agency on Research on Cancer (IARC), 2012), and some types have been discussed to be involved in skin carcinogenesis (Hufbauer and Akgül, 2017).

HPV prevalence differs mainly by HPV type but also by anatomical infection site and age (Iftner et al., 2010). Genital mucosal HPV are common sexually transmitted pathogens, but most HPV-infected persons develop no visible signs or symptoms. The majority of genital HPV infections are cleared within 12–24 months (Trottier and Franco, 2006), and persistent HPV infections with particular HR types are associated with an increased risk of anogenital or oropharyngeal cancer (de Villiers et al., 2004; International Agency on Research on Cancer (IARC), 2012; Schiffman et al., 2009). DNA testing has become the reference standard for the detection of current HPV infections (Abreu et al., 2012). However, it is not an appropriate method to assess previous infections (Robbins et al., 2014). Testing for HPV-specific antibodies provides information about past HPV exposure (Robbins et al., 2014; Tiggelaar et al., 2012). Therefore, HPV serology has been established as an important method for population-based studies focusing on type-specific cumulative lifetime exposure to HPV.

In Germany, HPV vaccination of girls has been introduced in 2007. Only limited data are available on the prevalence of HPV HR and LR types in the adult population of Germany (Michael et al., 2008). Previous studies were mainly performed in highly selected populations and focused on DNA prevalence in young women (Delere et al., 2014; Hauck et al., 2015; Iftner et al., 2010; Petry et al., 2013; Remschmidt et al., 2013). Worldwide, only a few population-based studies have focused on type-specific seroprevalence in men and women (Liu et al., 2016; Markowitz et al., 2009; Newall et al., 2008; Scherpenisse et al., 2012). The aim of our study was to determine the seroprevalence and associated risk factors of 19 HPV types in 18–79 year-old women and men living in Germany during the prevaccine era, using blood samples from a large countrywide population-based survey.

Subjects, materials, and methods

Study population and design

HPV seroprevalence was assessed by using archived serum samples from a cross-sectional population-based health survey. The German National Health Interview and Examination Survey 1998 (GNHIES98) was carried out by the Robert Koch Institute from October 1997 to March 1999. It was the first nationwide representative survey on the health status of Germany's adult population after the German reunification (Bellach et al., 1998). Participants were recruited using a two-stage stratified cluster sampling design (for a detailed description of the study design, see (Bellach et al., 1998; Scheidt-Nave et al., 2012)). In brief, the first stage selection comprised 120 cities and municipalities, representative regarding size, location and structure (urbanization, regional population density, and administrative borders) for all German communities. In the second stage a representative sample of the residential population aged 18–79 years was drawn from local population registers of the selected communities. In total 7,124 participants (response rate 61.4%) were interviewed, medically examined, and blood and urine samples were collected. Serum samples were stored at −20 °C. The study was conducted according to the Federal and State Commissioners for Data Protection guidelines and was approved by the Federal Commissioner for Data Protection. Informed consent was obtained from all participants.

Multiplex serology

In 2016/2017, the stored serum samples were tested for antibodies to the major capsid (L1) protein of 19 different HPV genotypes at the German Cancer Research Center (DKFZ) in Heidelberg. HPV types were selected for analysis based on the following criteria: public health relevance, carcinogenic potential, disease outcome, and genus- and species-specific broad distribution (for cutaneous types) (Supplementary Figure 1). The test panel included twelve mucosal (alpha: 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 58, 59) and seven cutaneous (alpha: 10; beta: 8, 38, 49; gamma: 4; nu: 41; mu: 1) HPV genotypes. Serological testing was done by a glutathione S-transferase (GST) capture immunoassay in combination with fluorescent bead technology as previously described (Waterboer et al., 2005). Type-specific HPV seroreactivity was measured in median fluorescence intensity (MFI) units and seropositivity was calculated based on previously established cut-off- values (Clifford et al., 2007). In addition, antibodies against the following other sexually transmitted infections (STI) were determined using the same assay: Mycoplasma genitalium (Mg), Herpes simplex virus 2 (HSV2) and Chlamydia trachomatis (Ct).

Statistical analysis

Seroprevalence was calculated for all 19 types separately and for the following groups of HPV types: HR types (HPV-HR: 16, 18, 31, 33, 35, 39, 45, 52, 58, 59), LR types: (HPV-LR: 6, 11), mucosal types (HPV-muc: 6, 11, 16, 18, 31, 33, 35, 39, 45, 52, 58, 59), cutaneous types (HPV-cut: 1, 4, 8, 10, 38, 41, 49), types included in the bivalent vaccine (HPV-2val: 16, 18), types included in the quadrivalent vaccine (HPV-4val: 6, 11, 16, 18), and types included in the nonavalent vaccine (HPV-9val: 6, 11, 16, 18, 31, 33, 45, 52, 58) (Supplementary Figure 1). Seroprevalence was calculated as the weighted proportion of participants seropositive to at least one of the HPV types included in one group. Differences regarding demographic and behavioral characteristic of participants stratified by sex were evaluated. All estimates were calculated by applying a survey weight, adopting the study sample to the population structure of Germany in 1997 in terms of age, sex, state, size of municipality, education, German/non-German nationality, and the regional distribution between East and West Germany (Thefeld et al., 1999). Age was categorized into 10-year groups (30–39, 40–49, 50–59, 60–69, 70–79), except for younger participants, which were categorized into the age groups 18–24 and 25–29 to be able to identify differences due to sexual behavior. Region of residence was split into two variables. Region of residence I, West and East Germany, takes into account the former boarders of the German Democratic Republic and the Federal Republic of Germany from 1949 to 1990. Region of Residence II describes the different states in Germany and is categorized into northern (Berlin, Brandenburg, Bremen, Hamburg, Lower Saxony, Mecklenburg-West Pomerania, Schleswig-Holstein), central (Hesse, North Rhine-Westphalia, Saxony, Saxony-Anhalt, Thuringia) and southern Germany (Baden-Württemberg, Bavaria, Rhineland-Palatinate, Saarland). Urbanity was categorized as rural (<5000 residents), small city (5000–<20,000 residents), medium-sized city (20,000–<100,000 residents) and large city (≥100,000 residents). Education refers to the highest academic qualification achieved and is categorized into low, middle and high based on the International Standard Classification of Education (ISCED) (1997). Seropositivity for any other sexually transmitted infection (STI+) was categorized as the status of being seropositive for at least one of the three tested STI. We categorized the lifetime number of sexual partners (LNSP) into the following groups: 0, 1, 2–4, 5–9, and ≥10 partners.

Chi2-Tests were used to test for statistical significance in categorical variables ($p < 0.05$) and logit transformation was

applied to calculate 95% confidence intervals (CI). Univariable analysis was applied to identify associations of various demographic and behavioral characteristics with type-specific and HPV group seropositivity. Multivariable weighted binomial regression models were developed estimating adjusted prevalence ratios and their 95% CI for females and males separately. All factors with a p-value <0.05 in univariable analysis were included in the multivariable regression model. Data management and statistical analysis were conducted using Stata, Version 14 (STATA Corp., College Station, TX, US).

Results

Serum samples of 6517 participants were successfully tested for HPV antibodies and included in this study (Figure 1). Included participants were not different to the full GNHIES98 sample regarding all baseline socio-demographic characteristics (Supplementary Table 1). Demographic characteristics of the participants are shown in Table 1.

Mucosal HPV types

Sex- and age-specific seroprevalences of the twelve mucosal HPV types are presented in Table 2. Seroprevalence ranged from 1.3% (HPV-33) to 12.0% (HPV-6). Overall seroprevalence of HPV types 6, 11, 16 and 18 were 12.8%, 4.8%, 8.7% and 6.9% among females, and 11.2%, 3.3%, 4.7% and 5.5% among males, respectively (Table 2). Analyzing all age groups together, we found slightly higher HPV seroprevalences of individual mucosal HPV types in females compared to males in all types except HPV-35.

Seroprevalences of the various HPV types differed by age group, showing heterogeneous age patterns. Among females, the lowest seroprevalences of mucosal HPV types were observed in the youngest age groups, except for HPV-6 and HPV-35, where lowest seroprevalences were found in the oldest age groups (Table 2). Compared to younger age groups, seroprevalences slightly increased in the 30–39 or 40–49-year-olds and remained relatively stable thereafter. Among females, HPV-16 seroprevalence increased from 5.0% in 18–24-year-olds to a first peak in 30–39-year-olds (9.6%) and had a second peak in the age group 60–69 years (10.7%; Table 2). The same pattern was also observed in HPV-6, HPV-11 and HPV-18 but with the first peak in 40–49-year-olds. Among males, the highest seroprevalence was found in the age group 30–59 years. For some HPV types (6, 11, 31, 45, and 52) we found a decrease in seroprevalence between 18–24-year-old and 25–29-year-old males. HPV-16 slightly increased from 2.7% in 18–24-year-olds to 4.5% in 25–29-year-olds and thereafter remained relatively stable with the highest prevalence of 5.7% among men aged 50–59 years.

There were only slight regional differences in type-specific HPV seroprevalence. HPV-16 seroprevalence was lowest among males in South Germany (3.4%, 95% CI 2.4%–4.7%) compared to North (5.2%, 95% CI 4.1%–6.6%) and central Germany (5.3%, 95% CI 4.2%–6.8%) (p = 0.042). Compared to males living in East Germany (5.9%, 95% CI 4.8%–7.4%), males in West Germany had lower HPV-16 seroprevalence (4.3%, 95% CI 3.6%–5.3%, p = 0.039). We observed no regional differences in HPV-16 seroprevalence among females (data not shown).

Cutaneous HPV types

Type-specific cutaneous HPV seroprevalence ranged from 8.7% (HPV-41) to 34.7% (HPV-4). The most prevalent cutaneous HPV types were 1, 4, and 8 with 34.5%, 33.9%, 18.3% among females, and

Table 1
Demographic Characteristics of the Study Participants by Sex, HPV Seroprevalence Study (n = 6517), 2016/2017 (sera collected 1997–1999).

	Females		Males	
	Subjects, No.	%[a]	Subjects, No.	%[a]
Overall	3,356	50.6	3,161	49.4
Age group (y)				
18–24	305	9.4	342	10.0
25–29	274	9.2	237	9.2
30–39	729	21.4	709	24.2
40–49	635	17.8	588	19.1
50–59	636	16.5	624	17.1
60–69	492	14.7	448	13.5
70–79	285	11.0	213	6.8
Region of Residence I				
East	1,165	20.9	1,060	20.9
West	2,191	79.1	2,101	79.1
Region of Residence II				
Northern Germany	872	25.8	794	26.4
Central Germany	1,535	41.1	1,454	40.7
Southern Germany	949	33.1	913	33.0
Country of Birth				
Germany	2,943	83.8	2,797	84.1
Other	315	13.5	286	13.2
NA	98	2.7	78	2.7
Urbanity				
Rural	787	20.1	758	20.3
Small City	694	18.8	694	20.6
Medium-sized City	863	28.5	784	27.0
Large City	1,012	32.6	925	32.1
History of Smoking				
No	1,823	54.6	1,070	33.4
Yes	1,443	42.8	2,029	64.4
NA	90	2.6	62	2.3
Education				
Low	736	29.6	378	16.7
Middle	1,960	53.4	1,695	54.0
High	562	14.2	1,011	26.5
NA	98	2.7	77	2.8
Seropositive for any other STI				
No	1,462	44.2	1,344	42.3
Yes	1,894	55.8	1,817	57.7
Lifetime sex partners				
0	29	1.1	30	0.9
1	702	20.9	442	13.6
2–4	838	24.0	604	18.4
5–9	311	9.3	402	12.4
≥10	136	3.9	387	12.8
NA	1,340	40.9	1,296	41.8
History of Use of Contraceptives				
No	2,637	79.8	–	–
Yes	704	19.8	–	–
NA	15	0.5	–	–

NA, not available.
[a] Weighted percentage STI, sexually transmitted infection (i.e. Mycoplasma genitalium, Herpes simplex virus 2, or Chlamydia trachomatis).

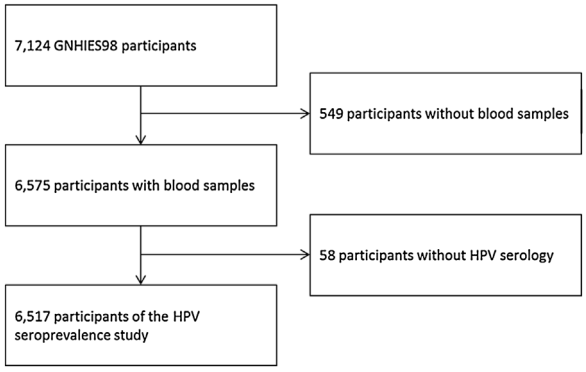

Figure 1. Flow chart of study participants of the HPV seroprevalence study, 2016/2017. Sera were collected throughout the German National Health Interview and Examination Survey (GNHIES98).1997–1999.

Table 2
Seroprevalence of Individual Mucosal Human Papillomavirus Types by Sex and Age, HPV Seroprevalence Study (n = 6517), 2016/2017 (sera collected 1997–1999).

Group	Subjects, no.	Seroprevalence by HPV type, % (95% CI)											
		6[#]	11[#]	16[#]	18	31	33	45	52	58	35	39	59
Overall	**6517**	**12.0 (11.1–13.0)**	**4.1 (3.4–4.8)**	**6.7 (6.0–7.4)**	**6.2 (5.5–7.0)**	**3.2 (2.7–3.7)**	**1.2 (0.9–1.6)**	**2.6 (2.2–3.1)**	**2.0 (1.6–2.5)**	**4.0 (3.4–4.7)**	**5.8 (5.1–6.6)**	**8.3 (7.5–9.1)**	**9.6 (8.8–10.5)**
		782	**253**	**455**	**395**	**204**	**82**	**165**	**121**	**262**	**376**	**543**	**629**
Female, overall	**3356**	**12.8 (11.7–14.1)**	**4.8 (4.0–5.8)**	**8.7 (7.6–9.8)**	**6.9 (5.9–7.9)**	**3.5 (2.9–4.3)**	**1.3 (0.9–1.8)**	**3.0 (2.4–3.7)**	**2.2 (1.7–3.0)**	**4.2 (3.5–5.1)**	**5.3 (4.4–6.3)**	**8.7 (7.7–9.9)**	**10.2 (9.1–11.5)**
		439	**161**	**299**	**228**	**119**	**47**	**101**	**67**	**143**	**176**	**306**	**355**
Age groups													
18–24	305	8.5 (5.4–13.3)	2.2 (1.1–4.7)	5.0 (3.0–8.2)	4.8 (2.7–8.4)	3.3 (1.8–6.1)	0.5 (0.1–2.2)	1.0 (0.3–2.9)	0.7 (0.1–3.2)	0.9 (0.2–3.3)	4.0 (2.0–7.9)	6.1 (3.5–10.5)	6.5 (4.0–10.3)
		28	8	15	13	10	2	4	2	3	13	17	19
25–29	274	14.3 (10.0–20.0)	4.5 (2.1–9.7)	7.4 (4.4–12.4)	6.2 (3.7–10.2)	2.3 (1.1–4.8)	0.2 (0.0–1.7)	1.0 (0.4–2.8)	0.8 (0.2–3.3)	3.6 (1.7–7.4)	5.3 (3.0–9.0)	5.5 (2.9–10.3)	8.0 (5.2–12.0)
		37	11	19	17	7	1	4	2	10	15	11	22
30–39	729	14.6 (11.9–17.9)	5.0 (3.5–7.0)	9.6 (7.5–12.2)	6.9 (5.0–9.5)	4.7 (3.2–6.9)	0.6 (0.2–1.4)	3.2 (2.2–4.8)	1.7 (0.9–3.2)	6.8 (4.9–9.2)	6.9 (5.0–9.5)	7.7 (5.7–10.2)	9.8 (7.7–12.5)
		111	37	75	51	32	5	25	10	47	46	66	82
40–49	635	16.1 (13.3–19.3)	5.5 (3.8–7.9)	8.2 (6.2–10.7)	8.1 (6.0–10.7)	3.6 (2.2–5.7)	1.8 (0.9–3.4)	3.6 (2.2–5.8)	2.0 (1.1–3.4)	5.6 (4.1–7.6)	5.2 (3.6–7.4)	10.5 (8.1–13.5)	10.8 (8.1–14.2)
		102	34	55	51	22	12	22	13	40	36	68	70
50–59	636	10.5 (8.0–13.7)	4.2 (2.8–6.5)	9.3 (7.1–12.0)	5.9 (4.0–8.5)	2.9 (1.7–4.9)	2.4 (1.3–4.6)	2.0 (1.0–4.1)	2.5 (1.3–5.0)	2.7 (1.5–4.9)	5.0 (3.2–7.8)	10.4 (8.0–13.4)	10.0 (7.7–13.1)
		67	28	57	36	20	13	12	12	15	28	64	66
60–69	492	14.5 (11.2–18.7)	5.7 (3.6–8.7)	10.7 (7.4–15.2)	8.2 (5.8–11.5)	3.6 (2.2–5.8)	1.6 (0.8–3.3)	4.4 (2.6–7.2)	3.6 (2.0–6.4)	4.3 (2.5–7.5)	3.8 (2.4–6.1)	10.8 (8.1–14.3)	14.2 (11.1–17.9)
		67	27	54	40	19	9	20	15	19	20	57	66
70–79	285	7.6 (5.1–11.3)	5.6 (2.9–10.6)	8.1 (5.1–12.7)	6.9 (4.3–11.0)	3.3 (1.3–8.0)	1.5 (0.6–4.0)	4.5 (2.6–7.8)	4.1 (2.0–8.4)	2.3 (0.8–6.6)	5.6 (3.1–10.0)	7.7 (4.5–12.6)	10.4 (7.0–15.1)
		27	16	24	20	9	5	14	13	9	18	23	30
Males, overall	**3161**	**11.2 (10.0–12.5)**	**3.3 (2.5–4.2)**	**4.7 (4.0–5.5)**	**5.5 (4.5–6.7)**	**2.8 (2.2–3.6)**	**1.1 (0.7–1.7)**	**2.2 (1.7–2.9)**	**1.7 (1.2–2.3)**	**3.8 (3.1–4.7)**	**6.4 (5.4–7.4)**	**7.8 (6.8–9.0)**	**9.0 (7.9–10.3)**
		343	**92**	**156**	**167**	**85**	**35**	**64**	**54**	**119**	**200**	**237**	**274**
Age groups													
18–24	342	12.5 (8.8–17.4)	3.5 (1.8–6.7)	2.7 (1.4–5.2)	4.9 (3.0–7.9)	2.6 (1.4–4.9)	0.5 (0.1–2.1)	2.2 (1.1–4.4)	1.7 (0.8–3.7)	3.3 (1.7–6.4)	5.6 (3.6–8.7)	5.2 (3.2–8.5)	6.1 (3.9–9.3)
		42	11	9	18	9	2	8	7	11	19	20	25
25–29	237	9.0 (5.6–14.1)	0.8 (0.2–3.6)	4.5 (2.3–8.5)	5.5 (3.3–9.2)	1.5 (0.6–3.8)	0.5 (0.1–3.6)	1.7 (0.7–4.5)	1.0 (0.3–3.3)	3.3 (1.5–7.0)	7.7 (4.6–12.6)	6.1 (3.5–10.4)	10.9 (6.6–17.4)
		22	2	13	14	5	1	5	3	8	18	14	26
30–39	709	14.3 (11.5–17.6)	5.0 (3.4–7.2)	4.7 (3.3–6.7)	5.5 (3.6–8.5)	3.7 (2.2–6.3)	1.9 (0.9–3.9)	2.9 (1.6–5.3)	2.3 (1.2–4.3)	6.2 (4.4–8.7)	7.2 (5.2–9.9)	7.9 (5.9–10.6)	7.2 (5.3–9.6)
		96	30	35	33	20	11	17	12	43	50	53	48
40–49	588	15.5 (12.3–19.2)	3.8 (2.2–6.4)	5.4 (3.5–8.1)	5.0 (3.0–8.3)	3.1 (1.9–4.8)	1.5 (0.8–2.8)	1.8 (0.8–4.0)	2.1 (1.0–4.2)	5.7 (3.6–8.7)	7.2 (5.1–9.9)	7.2 (5.1–10.0)	7.4 (5.3–10.2)
		83	18	30	24	18	10	8	10	34	40	38	42
50–59	624	7.4 (5.6–9.8)	2.3 (1.3–4.0)	5.7 (4.2–7.7)	6.4 (4.6–8.8)	2.6 (1.6–4.2)	0.8 (0.4–2.0)	1.8 (1.0–3.2)	1.5 (0.8–2.7)	2.6 (1.5–4.5)	5.2 (3.6–7.4)	9.3 (6.9–12.5)	11.1 (8.4–14.4)
		49	12	37	39	18	6	12	11	15	33	52	63
60–69	448	7.4 (5.2–10.5)	2.8 (1.6–4.8)	4.0 (2.6–6.2)	5.4 (3.4–8.3)	2.1 (1.1–3.8)	0.5 (0.1–2.0)	2.8 (1.4–5.5)	1.1 (0.5–2.4)	0.5 (0.2–1.4)	4.6 (2.9–7.2)	9.0 (6.6–12.1)	11.6 (8.7–15.2)
		40	14	22	25	10	2	10	7	4	25	40	48
70–79	213	5.7 (3.1–10.3)	2.2 (0.9–5.6)	4.3 (2.2–8.2)	5.7 (3.1–10.3)	2.6 (1.1–6.5)	1.1 (0.3–3.6)	1.3 (0.5–3.6)	1.1 (0.4–3.2)	1.3 (0.5–3.7)	6.7 (3.8–11.4)	9.0 (5.6–14.2)	11.3 (7.6–16.4)
		11	5	10	14	5	3	4	4	4	15	20	22

Note. CI, confidence interval.
[#] Female overall vs male overall, $p < 0.05$.

33.6%, 35.6%, 19.6% among males, respectively. Additional data on cutaneous HPV seroprevalence are provided in Supplementary Table 2. Compared to mucosal types, we did not find any significant type-specific differences between both sexes. For some cutaneous HPV types, i.e., 8, 10, 38 and 49, we observed a general increase in HPV seroprevalence with age. The age pattern of HPV-1 clearly differed with a steady decrease from 46.4% to 21.3% among females and 39.8% to 26.6% among males.

Grouped HPV types

Seropositivity against at least one mucosal HPV type (HPV-muc, 35.3%) or any HPV-HR type (HPV-HR, 27.9%) showed slight but statistically significant higher seroprevalences in females as compared to male participants (Table 3). This constellation was also observed in other subgroups (HPV-2val, HPV-4val, HPV-9val, HPV-LR), reflecting mainly the sex-specific seroprevalence differences in HPV-6, HPV-11 and HPV-16. Age-specific seroprevalences for HPV types included in the vaccines and for those assigned to the HR and LR group are shown in Figure 2 and Figure 3.

Multiple seropositivity

While 18.4% were positive for only one HPV-HR type, 4.9% were positive for two and 4.6% for ≥3 different HPV-HR types (Supplementary Table 3). There was no significant difference in this distribution between females and males.

Risk factors

The risk factor analysis was restricted to HPV types included in the 4-valent vaccine and the HR and LR type groups. The results related to HPV-16 are shown in Table 4; the remaining results are shown in the supplement (Supplementary Table 4–Supplementary Table 8). In univariable analysis, STI+, LNSP, age and urbanity were significantly associated with HPV-16 seropositivity among females (Table 4), and were therefore included in our final multivariable model. In the model, all variables remained significantly associated with HPV-16 seropositivity except age. Females living in medium-sized cities were more likely to be seropositive than females living in rural areas, small or large cities. STI+ females as well as females with higher LNSP were more likely to be HPV-16 seropositive. Among males, STI+, LNSP, age and region of residence were significantly associated with HPV-16 seropositivity in the univariable analysis. In the multivariable analysis, STI+ was associated with higher HPV-16 seropositivity, while living in the southern part of Germany was associated with lower HPV-16 seropositivity.

Discussion

We assessed the prevalence of antibodies against 19 different mucosal and cutaneous HPV types in the adult population in Germany before the introduction of routine female HPV vaccination. Therefore, these data provide an estimate of naturally acquired and cumulative type-specific HPV exposure (Robbins et al., 2014). Being positive for any HPV type included in the recently licensed 9-valent HPV vaccine was common with 26.9% among females and 22.8% among males. Being seropositive for any other tested STI and having a higher lifetime number of sexual partners were the strongest factors associated with HPV-16 seropositivity.

Most previous studies on HPV infections in Germany were based on HPV DNA in genital samples collected from young women (Delere et al., 2014; Iftner et al., 2010; Petry et al., 2013). Using this approach, one study showed that HPV-16 was the predominant vaccine-relevant HPV-HR genotype in Germany with 19.5% among 20–22-year-old females (Delere et al., 2014). This was also observed in other HPV DNA studies in Germany and is in accordance with data collected worldwide (Bruni et al., 2010; de Sanjose et al., 2007; Iftner et al., 2010; Petry et al., 2013). Compared

Table 3
Seroprevalence of grouped Human Papillomavirus (HPV) Types by Sex, HPV Seroprevalence Study (n = 6517), 2016/2017 (sera collected 1997–1999).

		Seroprevalence, % (95% CI)		
HPV types, grouped	Overall	Female	Male	p-Value*
≥1 of 2-valent vaccine (HPV-2val)	10.7 (9.9–11.6)	12.7 (11.4–14.1)	8.7 (7.5–9.9)	<0.01
≥1 of 4-valent vaccine (HPV-4val)	20.6 (19.5–21.8)	22.9 (21.3–24.6)	18.3 (16.9–19.8)	<0.01
≥1 of 9-valent vaccine (HPV-9val)	24.9 (23.7–26.1)	26.9 (25.1–28.8)	22.8 (21.4–24.3)	<0.01
≥1 of mucosal (HPV-muc)	35.3 (33.7–36.8)	36.9 (34.8–39.0)	33.6 (31.7–35.6)	<0.05
≥1 of cutaneous (HPV-cut)	63.6 (61.9–65.2)	62.5 (60.6–64.4)	64.7 (62.2–67.0)	0.14
≥1 of High Risk (HPV-HR)	27.9 (26.4–29.4)	29.3 (27.3–31.4)	26.4 (24.5–28.3)	<0.05
≥1 of Low Risk (HPV-LR)	13.3 (12.3–14.4)	14.6 (13.2–16.0)	12.0 (10.8–13.4)	<0.01

* Females vs males.

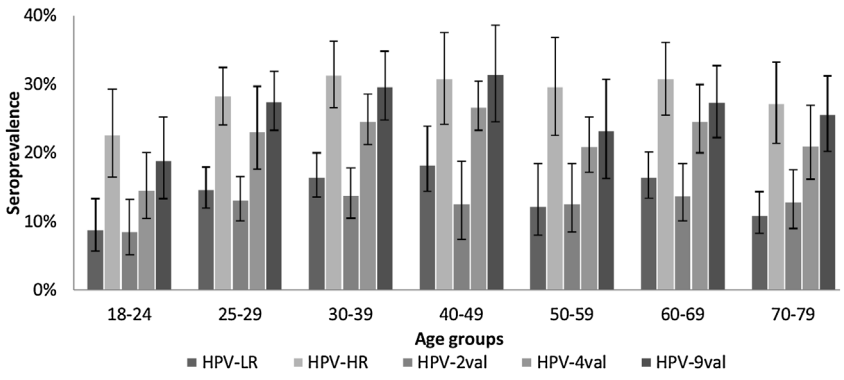

Figure 2. Seropositivity of grouped Human Papillomavirus (HPV) types in 3356 females living in Germany by age (HPV-LR: ≥1 of 6 or 11; HPV-HR: ≥1 of 16 or 18; HPV-2val: ≥1 of 6 or 11; HPV-4val: ≥1 of 6, 11, 16 or 18; HPV-9val: ≥1 of 6, 11, 16, 18, 31, 33, 45, 52 or 58). HPV seroprevalence study 2016/2017 (sera collected 1997–1999).

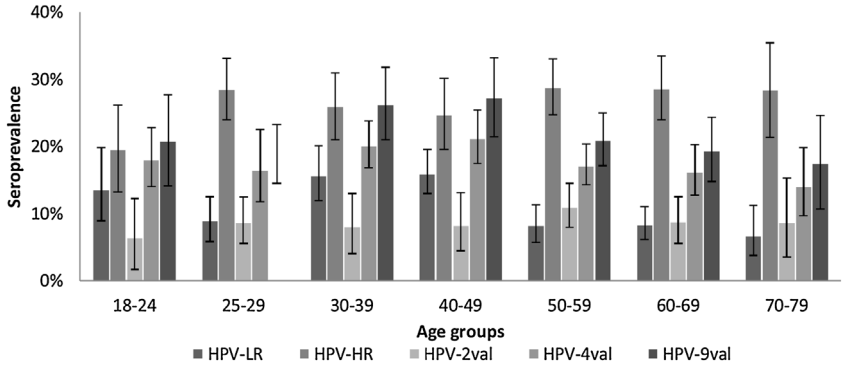

Figure 3. Seropositivity of grouped Human Papillomavirus (HPV) types in 3161 males by age (HPV-LR: ≥1 of 6 or 11; HPV-HR: ≥1 of 16 or 18; HPV-2val: ≥1 of 6 or 11; HPV-4val: ≥1 of 6, 11, 16 or 18; HPV-9val: ≥1 of 6, 11, 16, 18, 31, 33, 45, 52 or 58). HPV seroprevalence study 2016/2017 (sera collected 1997–1999).

Table 4
Factors Associated with Seropositivity to Human Papillomavirus Type 16 Stratified by Sex, HPV Seroprevalence Study, 2016/2017 (sera collected 1997–1999).

Variable		Females		Males	
		Crude PR (95% CI)	Fully aPR (95% CI) [$]	Crude PR (95% CI)	Fully aPR (95% CI) [$]
Age groups (y)					
	18–24	Reference	Reference	Reference	Reference
	25–29	1.49 (0.69–3.18)	1.17 (0.54–2.53)	1.68 (0.64–4.40)	1.61 (0.61–4.22)
	30–39	**1.91 (1.09–3.35)**	1.45 (0.83–2.55)	1.76 (0.81–3.84)	1.51 (0.67–3.42)
	40–49	1.63 (0.93–2.87)	1.21 (0.69–2.10)	2.00 (0.86–4.62)	1.67 (0.71–3.93)
	50–59	**1.85 (1.06–3.23)**	1.45 (0.84–2.50)	**2.12 (1.03–4.38)**	1.90 (0.90–4.00)
	60–69	**2.13 (1.16–3.93)**	1.61 (0.90–2.89)	1.51 (0.69–3.29)	1.32 (0.59–2.94)
	70–79	1.62 (0.85–3.08)	1.16 (0.61–2.20)	1.61 (0.61–4.27)	1.43 (0.53–3.88)
Region of residence I					
	East Germany	Reference	na*	Reference	Reference
	West Germany	0.81 (0.65–1.02)	na*	**0.73 (0.55–0.98)**	0.89 (0.65–1.22)
Region of residence II					
	Northern Germany	Reference	na*	Reference	Reference
	Central Germany	1.17 (0.86–1.60)	na*	1.02 (0.73–1.43)	1.04 (0.75–1.44)
	Southern Germany	1.03 (0.71–1.48)	na*	**0.64 (0.43–0.97)**	0.69 (0.45–1.07)
Country of birth					
	Germany	Reference	na*	Reference	na*
	Other	1.01 (0.63–1.62)	na*	0.88 (0.43–1.80)	na*
Urbanity					
	Rural	Reference	Reference	Reference	na*
	Small city	0.94 (0.63–1.40)	0.95 (0.64–1.39)	1.25 (0.75–2.06)	na*
	Medium-sized city	**1.44 (1.02–2.02)**	**1.48 (1.07–2.04)**	1.47 (0.94–2.30)	na*
	Large city	1.06 (0.74–1.51)	1.01 (0.72–1.43)	1.26 (0.83–1.91)	na*
History of smoking					
	No	Reference	na*	Reference	na*
	Yes	1.12 (0.86–1.46)	na*	0.73 (0.51–1.06)	na*
Education					
	Low	1.24 (0.90–1.72)	na*	0.81 (0.41–1.59)	na*
	Middle	Reference	na*	Reference	na*
	High	0.95 (0.68–1.32)	na*	1.12 (0.81–1.55)	na*
Seropositive for any other STI					
	No	Reference	Reference	Reference	Reference
	Yes	**2.48 (1.84–3.34)**	**2.29 (1.67–3.12)**	**2.73 (1.72–4.35)**	**2.51 (1.56–4.04)**
Lifetime no. of sex partners					
	0	no+	no+	2.65 (0.53–13.16)	3.08 (0.63–15.09)
	1	Reference	Reference	Reference	Reference
	2–4	1.31 (0.91–1.88)	1.23 (0.85–1.79)	1.46 (0.66–3.20)	1.36 (0.63–2.93)
	5–9	**2.17 (1.33–3.52)**	**1.83 (1.10–3.04)**	1.70 (0.76–3.81)	1.55 (0.70–3.41)
	> = 10	**2.40 (1.39–4.13)**	**1.97 (1.07–3.63)**	**2.46 (1.24–4.87)**	**2.00 (1.02–3.93)**
	NA	**1.85 (1.25–2.74)**	**1.63 (1.13–2.36)**	**2.09 (1.18–3.71)**	**1.88 (1.07–3.29)**
History of use of oral contraceptives					
	No	Reference	na*	–	–
	Yes	1.02 (0.77–1.35)	na*	–	–

Abbreviations: aPR, adjusted prevalence ratio; CI, confidence interval; na, not available; STI, sexually transmitted infection.
na* variables were not significantly associated with HPV seroprevalence in the univariable model and therefore not included in the fully adjusted model.
no+ no observations in categories.
$ the aPR are adjusted for all variables which are listed in the table and remained in the final model.
Statistically significant PR are shown in **bold font**.

to other vaccine-relevant HPV-HR types, HPV-16 was also the most prevalent in our study, which is in agreement with previous HPV seroprevalence studies (Liu et al., 2016; Scherpenisse et al., 2012), even though comparing serological results based on different assays remains difficult. Apart from methodological differences, heterogeneity in HPV-16 seroprevalence among populations in different countries and regions has to be taken into account (Vaccarella et al., 2010). In general, HPV-16 seroprevalence was lower in our study compared to other population-based seroprevalence studies (Liu et al., 2016; Markowitz et al., 2009; Newall et al., 2008; Wang et al., 2003). However, in a seroprevalence study with sera from 34–82-year-old adults in Germany collected in the late 1980s, HPV-16 seroprevalence among females was only slightly higher than in our study (10.4%), and nearly the same for males (4.3%) (Michael et al., 2008). Regarding the four vaccine-relevant HPV types 6, 11, 16 and 18, we observed similar results with the highest seroprevalence in HPV-6 as reported in other population-based HPV seroprevalence studies (Introcaso et al., 2014; Liu et al., 2016; Markowitz et al., 2009; Newall et al., 2008).

The overall lower seroprevalence in mucosal HPV types among males compared to females is consistent with other studies (Markowitz et al., 2009; Michael et al., 2008; Newall et al., 2008; Scherpenisse et al., 2012), even though the quantitative difference of HPV seroprevalence between men and women varies. While population studies in the US (Markowitz et al., 2009) and England (Desai et al., 2011) showed a greater difference (depending on the HPV types) in seroprevalence between women and men, the observed differences were less strong in our results as also observed in other population surveys in the Netherlands (Scherpenisse et al., 2012) or Australia (Newall et al., 2008). The overall sex difference has not been observed in studies using HPV DNA assays, where men had similar HPV infection rates and a relative stable risk for acquiring new HPV infections over age (Dunne et al., 2006; Giuliano et al., 2011). The difference in mucosal HPV seroprevalence might be a result of a different (keratinized) epithelium at the infection site, but has also been attributed to a shorter average duration of infection in men, leading to a reduced chance of developing HPV antibodies (Carter et al., 2000; Markowitz et al., 2009). Our present findings showed that HPV-4val seropositivity peaked later among men (40–49-year-olds) than among women, which was comparable to seroprevalence data from Australia (32% at 40–49-year-olds) and the US (18% at 50–59-year-olds) (Markowitz et al., 2009; Newall et al., 2008).

Population-wide surveys offer the opportunity to draw conclusions on trends over age groups. We observed a slight increase in HPV-HR seroprevalence mainly among females in the youngest age groups, from 18 to 24 years to 30–39 years, which is consistent with other seroprevalence studies (Liu et al., 2016), probably reflecting the onset of sexual activity and the increasing number of sex partners in those age groups. HPV-4val among females was generally lower and reached a later age peak with 26.7% in 40–49-year-olds compared to peak seropositivity at 30–39-year-olds in Australia (39%) and the US (42%) (Markowitz et al., 2009; Newall et al., 2008). We also observed a lower HPV-9val seropositivity in females in our study as compared to a study from the US (40.5%), while HPV-9val prevalence in males was comparable between these two studies (Markowitz et al., 2009).

In contrast to other studies, we did not observe in older age groups a lower seroprevalence in vaccine-relevant HPV types, except for HPV6 and HPV58 in males. This lower seroprevalence in older age groups was explained by other authors as a result of cohort effects or waning antibodies, even though the humoral response is considered to be relatively stable over time (Newall et al., 2008; Ryser et al., 2017; Tiggelaar et al., 2012; Wang et al., 2003). In general, HPV prevalences in men are described to be less influenced by age compared to women (Giuliano et al., 2011). However, in our study a relatively stable age-related seroprevalence trend was observed in both sexes.

Previous studies described a second peak in HPV DNA prevalence among older women in some but not all geographic regions (Castle et al., 2005; de Sanjose et al., 2007; Gravitt et al., 2013; Trottier et al., 2010). However, this second peak was not observed in most population-based seroprevalence studies (Liu et al., 2016; Markowitz et al., 2009; Newall et al., 2008). Despite a relatively stable seroprevalence over age in our present findings, we observed a second age peak in HPV seroprevalence for some HPV types (6, 11, 16, 39, 58, and 59) among 60–69-year-old women. The second age peak in older women is discussed as a menopause-related hormonal change which possibly reactivates latent HPV infections (Althoff et al., 2009; Castle et al., 2005; de Sanjose et al., 2007; Gravitt et al., 2013; Gravitt and Winer, 2017). Another hypothesis is that the peak might reflect new HPV infections because of sexual behavior change in women of older ages and their partners (Trottier et al., 2010). However, it is also discussed that seroprevalence differences in older women are a cohort effect (de Sanjose et al., 2007; Ryser et al., 2017; Trottier and Franco, 2006).

As compared to individual mucosal HPV seroprevalence among older age groups, our data showed that HPV seroprevalence in the youngest age groups was relatively high. The HPV-HR seroprevalence of nearly 10% in 18–24-year-old participants underlines the importance of vaccination at a young age before sexual debut. However, the observed second peak of individual mucosal HPV seroprevalence indicates that in older women reactivation of prior infections as well as incident HPV infections should be considered.

Type- and age-specific seroprevalence of cutaneous HPV types was comparable with other studies with an age-related increase in cutaneous HPV types except for HPV-1 (Antonsson, 2012; Rahman et al., 2016b). Since HPV-1- and HPV-4-related warts are common among children and adolescents the high prevalence of HPV-1 and HPV-4 among the youngest participants of our study is not an unexpected finding (Antonsson, 2012).

Behavioral factors associated with seropositivity in our study were similar to those reported elsewhere (Liu et al., 2016; Markowitz et al., 2009). Age was associated with HPV-16 seropositivity in both sexes only in univariable analysis. In the final model, independent predictors of HPV-16 seropositivity were the presence of antibodies to other STI and LNSP, which was similar to those reported elsewhere (Liu et al., 2016; Scherpenisse et al., 2012; Vaccarella et al., 2010; Wang et al., 2003). Given that being STI+ was the strongest associated factor in nearly all HPV types, STI + could be a useful marker of sexual behavior, in addition to the potentially biased self-reported LNSP. In contrast to previous studies (Liu et al., 2016; Rahman et al., 2016a; Vaccarella et al., 2010), we did not observe any association between age and HPV-16 and HPV-18 seropositivity, which could be explained by different age categorizations.

Several studies have supported HPV antibody seropositivity as a biomarker of past HPV exposure (Hariri et al., 2008; Liu et al., 2016; Markowitz et al., 2009; Newall et al., 2008; Wang et al., 2003). In our study, seropositivity was measured by a GST fusion protein-based multiplex serological assay that has been used in several other HPV seroprevalence studies (Clifford et al., 2007; Michael et al., 2008; Rahman et al., 2016a; Rahman et al., 2016b; Wilson et al., 2014). However, our prevalence data may not be directly comparable with studies using different antigen preparations (e.g. virus-like particles) and other cut-off definitions in their assays. Besides these technique-based differences, seroprevalence differences between countries may also be explained by underlying populations, sampling time, and differences in exposures related to HPV transmission like sexual behaviors. Seropositivity is not a perfect HPV exposure measure as it tends to underestimate the

cumulative exposure (Introcaso et al., 2014). This is due to various factors, i.e., the impact of a broad range of sex-, type- and infection site-specific seroconversion rates, infection clearance and the still unknown seropositivity duration (Brouwer et al., 2015; Carter et al., 2000; Edelstein et al., 2011; Giuliano et al., 2015; Lu et al., 2012). Furthermore, natural HPV infections result in much lower immune response and seroconversion rates, compared to HPV vaccination (Harper et al., 2006). Therefore, the presented estimates of seroprevalence also might be an underestimation of cumulative HPV exposure.

Our study has several strengths. It was based on a large, representative sample of the population of Germany, and a large demographic and behavioral data set; blood samples were collected using highly standardized sampling methods. Using these blood samples from the prevaccine era, we were able to measure naturally acquired antibodies to 19 mucosal and cutaneous HPV types in a single laboratory test. In addition, the antibody prevalence against other common STI was used as a proxy for sexual activity. Finally, using nationally representative data of GNIEHS98 it was possible to estimate the type-specific HPV seroprevalence in demographic groups in Germany and to identify factors associated with type-specific seropositivity in men and women.

However, we are also aware of some limitations of our study which should be considered when interpreting the results. Serum samples were properly stored at −20 °C since the collection in 1997–1999, but storage time could still have an effect on sera quality and antibody concentration. However, this would affect all samples and could not explain group-specific differences, like age-, sex- or type-specific prevalence.

In conclusion, we generated data on type-specific HPV seroprevalence in the general population of adults living in Germany, for all age groups and both sexes. These data were collected before HPV vaccine introduction and will serve as baseline estimates for naturally derived cumulative type-specific HPV exposure in Germany in non-vaccinated individuals. HPV seroprevalence studies in the adult population after vaccine introduction as well as in children and adolescents are currently underway in Germany and can be used to assess the population-level (indirect) effects of HPV vaccination, which has been initially carried out in Germany mainly with 4-valent and since 2016 mainly with 9-valent HPV vaccines.

Conflict of interest

A.L., C.P.-M., M.P., M.T., T.H., T.W., J.S., Y.D., O.W., M.W.-P. declared that they have no conflict of interest.

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Ethical approval

Although there was no law or regulation on Ethic Committees in Germany at the time of the conduct of the study, the study was approved by the Board of the Federal Commissioner for Data Protection Berlin, Germany. The study was conducted according to the Federal and State Commissioners for Data Protection guidelines. Informed written consent and assent were obtained from all participants.

Meetings information

Part of the data was presented at the EUROGIN 2016 Congress, Salzburg/Austria, June 15–18, 2016 and the 31st International Papillomavirus Conference (HPV 2017), Cape Town/South Africa, 28 February–5 March 2017.

Author contribution

Analyzed the data: AL; Developed the IgG Multiplex assay and did the experiments for the study: TW, MP; Collected data for the study: CPM, MT, TW; Wrote the first draft of the paper: AL; Contributed to the writing of the paper: AL, MWP, TH, CPM, MT, TW, MP, OW, JS; Conception and design of the study: YD, MP, MWP, OW.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.03.022.

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