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## **Age-specific HPV seroprevalence among young females in The Netherlands**

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## **ABSTRACT**

**Objectives:** To obtain insight into the age-specific seroprevalence for human papillomavirus (HPV) 6, 11, 16, and 18 among females before introduction of HPV vaccination in the Netherlands.

**Methods:** In a population-based study in The Netherlands, 637 sera of 11-26-year-old females were tested for HPV6/11/16/18 antibodies. Sera were tested using a competitive Luminex assay with neutralizing monoclonal antibodies specific for each serotype. Associations between HPV seropositivity, demographics, and sexual behaviour were studied with logistic regression. Seroprevalences were standardized for age and urbanization degree to the general female population in the Netherlands.

**Results:** Overall prevalence of antibodies against HPV6/11/16/18 was 7.9%. 4.3% had antibodies against HPV types 6/11, and 4.4% had antibodies against HPV types 16/18. HPV seropositivity significantly increased with age (OR 1.2; 95%CI 1.1-1.4), starting at the age of 16 years (median age of sexual debut in The Netherlands). A former diagnosis with sexually transmitted infections was also significantly associated with HPV seropositivity (OR 6.3; 95%CI 2.2-17.9).

**Conclusions:** In addition to 12-year-old girls who are targeted for routine HPV vaccination, also girls up to 16 years are likely to benefit substantially from HPV16/18 vaccination. Testing for the presence of HPV antibodies in females after introduction of vaccination makes it possible to monitor the impact of immunisation at the population level.

## INTRODUCTION

Persistent infection by certain high risk human papillomavirus (hrHPV) types is known to be responsible for cervical cancer. However, only a small percentage of women who become infected will develop precancerous lesions which can progress to cervical cancer.[1] The majority of genital HPV infections are without symptoms and transient. More than 90% of detected infections are cleared within two years.[2] A quadrivalent (HPV6, 11, 16 and 18) and a bivalent (HPV16 and 18) prophylactic HPV vaccine have been licensed in Europe recently. Both vaccines protect against the hrHPV types 16 and 18, which are responsible for an estimated 73% of cervical cancer cases in Europe.[3] In phase III trials, both vaccines have been shown to prevent more than 90% of precancerous lesions associated with types 16 or 18 among HPV naïve women aged 15 to 26 years.[4,5] The primary target group to consider for routine vaccination is girls at the age just before the onset of sexual activity, because HPV infections are common and frequently acquired soon after initiation of sexual activity.[6] Vaccination does not affect the course of an existing vaccine-type infection and the effectiveness of immunization for subsequent infections (of that type) is uncertain.[4]

In 2008, the Minister of Health in The Netherlands decided to include HPV vaccination in the National Immunization Program (NIP) for girls aged 12-years and a catch-up program for girls aged 13 to 16 years, based on reported age of sexual debut and sexual activity.[7] The catch-up program started in 2009 and HPV vaccination through the NIP will start in 2010, both using the bivalent HPV vaccine.

Pre-vaccination data on the occurrence of HPV infection in the population, in particular in women, are essential to monitor the impact of vaccination. While a lot of data are available from Dutch women 30 years of age onwards as cervical cancer screening starts, data from younger women/girls are scarce.[8] In The Netherlands, an estimated HPV16 and -18 prevalence of respectively 2.8% and 1.4% relies on a HPV DNA study among a convenience sample of unscreened women aged 18-29 years.[9] Other reported HPV16 and -18 DNA

prevalences in the age group 18-24 years, respectively 7.5% and 2.3%, are based on data of opportunistic cervical screening.[10] These data are based on detection of HPV DNA in genital mucosa, where HPV DNA identifies current infections. Although not all infections result in seroconversion, HPV antibodies may persist for many years and can serve as markers of both ongoing and previous infections.[11,12] Several studies have shown that HPV (sero)prevalence increases with age soon after the start of sexual activity.[10,13-18] Maximum seroconversion rate of IgG after incident HPV16 infection was 56.7% by 8 months in a study of Ho et al. among young female students (mean age 20 years).[11] A study of Carter et al. observed seropositivity 18 months after incident HPV6, -16, or -18 infection in about 60% of the women.[12]

Despite incomplete seroconversion and waning of antibodies, population-based sero-epidemiological studies performed before and at regular intervals after introduction of vaccination is one of the tools to monitor the impact of mass vaccination against HPV on the frequency of HPV infection. This paper describes the first age-specific seroprevalence for HPV6, -11, -16, and -18 among girls and women aged 11-26 years in The Netherlands. Sera and questionnaire data were collected in a cross-sectional population-based study in The Netherlands.[19]

## **METHODS**

### **Study population**

A cross-sectional population-based study, the PIENTER 2 project, was performed from 2006 to 2007. Dutch inhabitants aged 0-79 years from 40 municipalities throughout the country were invited to participate. Of all invitees, 33% took part in the study. This sample of the general population in The Netherlands will be used to evaluate the present existing NIP and to determine age-specific seroprevalences against future NIP vaccine candidates, such as HPV. A more detailed description of the study design and objectives of the PIENTER 2 project has been reported previously.[19] The study proposal was approved by the Medical Ethics Testing Committee of the foundation of therapeutic evaluation of medicines (METC-STEG) in Almere, the Netherlands.

The objective of this study was to provide age-specific data on HPV infection in girls/women prior to the national HPV-immunization program. Therefore, all females aged 11-26 years were selected from the nationwide sample of the PIENTER 2 project (n=637).

### **Serological testing**

Serum samples were tested for specific neutralizing antibodies to HPV6, -11, -16 and -18 using a competitive Luminex assay.[20,21] Antigen HPV specific virus-like particles (VLPs) [22] were coupled to distinct fluorescent Luminex microspheres. Sera were incubated with the coupled beads in the presence of HPV type-specific neutralizing monoclonals which compete for the binding sites on the VLPs. The monoclonals are HPV type-specific, bind neutralizing epitopes on the VLPs and show minimal cross-reactivity.[20,23] Sera were called seropositive above the cutoffs as determined in [20]: 20, 16, 20 and 24 milli-Merck Units per milliliter (mMU/ml) for HPV6, -11, -16, and -18 respectively. The serological method used in this study is well validated and bridging to former neutralising assays has been performed.

## **Data analyses**

Information on age, ethnic origin, educational level, and urbanization degree was available for the total study population. Ethnic origin was classified as indigenous Dutch, first generation and second generation of migrants according to the country of birth of the participant and the country of birth of the participant's parents. Educational level of the participant or the mother (for participants <15 years, only the educational level of the mother was available) was classified as low (primary school), medium (lower vocational school, secondary school), or high (higher vocational school, university). Urbanization degree was divided into two classes based on the number of addresses per square meter, namely high or middle/low urbanization degree.[24] Information on marital status and sexual behaviour was only available from participants aged 15-26 years. Information on the sexual behaviour included data on age of sexual debut, steady and casual partnerships, number of partners in the last 6 months, and sexually transmitted infections (STI) including HIV diagnosed ever.

Seroprevalence and geometric mean titres (GMT) were estimated for HPV6, -11, -16, and -18. For GMT, only titres of seropositive samples (i.e. samples with titers above specified cut-off values) were included in the analyses. Associations between HPV6/11/16/18 seropositivity and demographic variables or sexual behaviour were studied with univariate logistic regression. For women aged 15-26 years, variables significantly ( $p < 0.2$ ) related to HPV infection in univariate analyses were entered into multivariate logistic regression analysis. Multivariate analyses were not studied separately by HPV type due to a low number of seropositives. To account for non-response, each person was assigned a sampling weight that incorporated the probability of selection and included adjustment for age and urbanization degree.[19] Moreover, we also adjusted for the two stage cluster sampling by taking into account the strata (regions) and clusters (municipalities). In this way results were representative for the general female population in The Netherlands on January 2007.

## **RESULTS**

### **General characteristics**

The median age of our study population (n=637) was 19 years (Interquartile range 14-23 years). The majority of the total sample population (84%) was of indigenous Dutch origin, 10% was second generation migrant and 6% was first generation migrant. Of those 15 years and older (n=473), 68% had a medium education level (including those who may go on to complete higher vocational school or university) and 73% of the females were not married or living together. Of the 15-year-old females, 6% reported to be sexually active compared to 57% of the 17-year-old females (Figure 1). A quarter of the females older than 15 years reported having had no sexual contact yet.

### **HPV seroprevalence and risk factors**

The overall prevalence of antibodies against at least one of the HPV types 6/11/16/18 amounted to 7.9% among 11-26-year-old females. 4.3% had antibodies against low-risk HPV (lrHPV) types 6 and/or -11 and 4.4% had antibodies against hrHPV types 16 and/or 18 (Table 1). HPV types 6 and 16 were more prevalent (each 3.9%) than HPV11 (1.2%) and -18 (1.1%). Evidence for multiple infections with the different HPV types tested for, was found in the sera of 10 females (1.6%). The majority (8 of those 10 women) had antibodies against HPV16 and any of the other three HPV types. Women with multiple infections were older than 18 years, sexually active for more than two years and reported at least one sex partner in the last 6 months.

None of the girls between 11 and 16 years of age had detectable antibodies against hrHPV16 or -18 (Table 1). After this age the HPV seroprevalence increased with age showing a similar increase with age for HPV6/11 and HPV16/18. Between the ages 21-26 years the overall HPV6/11/16/18 seroprevalence remained at a percentage of about 16% with the highest prevalence of 18% among 23-24-year-old girls (Figure 2).



**Table 1** Weighted age-specific seroprevalences for HPV6, -11, -16, and -18 among 11-26-year-old females, The Netherlands, 2007

Proportion seropositive, % (95%CI)															
Age (yrs)	N	HPV6 <sup>a</sup>		HPV11 <sup>b</sup>		HPV6/11		HPV16		HPV18 <sup>c</sup>		HPV16/18		HPV6/11/16/18	
Overall	637	3.9	(2.1-5.6)	1.2	(0.1-2.4)	4.3	(2.5-6.1)	3.9	(2.0-5.9)	1.1	(0.3-1.9)	4.4	(2.4-6.4)	7.9	(5.0-10.7)
11-12	89	0.0	(0.0-3.5)	0.0	(0.0-4.5)	0.0	(0.0-3.1)	0.0	(0.0-3.1)	0.0	(0.0-3.1)	0.0	(0.0-3.1)	0.0	(0.0-3.1)
13-14	75	0.0	(0.0-4.4)	2.2	(0.0-6.6)	1.7	(0.0-5.2)	0.0	(0.0-3.9)	0.0	(0.0-4.0)	0.0	(0.0-3.9)	1.7	(0.0-5.2)
15-16	64	0.0	(0.0-4.7)	2.7	(0.0-8.0)	2.1	(0.0-6.2)	0.0	(0.0-4.6)	0.0	(0.0-4.6)	0.0	(0.0-4.6)	2.1	(0.0-6.2)
17-18	69	0.0	(0.0-4.4)	0.0	(0.0-5.1)	0.0	(0.0-4.2)	2.8	(0.0-6.4)	1.4	(0.0-4.2)	2.8	(0.0-6.4)	2.8	(0.0-6.4)
19-20	96	4.0	(0.0-8.7)	0.0	(0.0-3.9)	3.8	(0.0-8.2)	3.2	(0.0-7.2)	2.3	(0.0-5.5)	3.9	(0.0-8.0)	7.0	(1.2-12.8)
21-22	78	10.7	(2.7-18.7)	1.1	(0.0-3.7)	10.4	(2.6-18.1)	5.9	(0.2-11.7)	2.2	(0.0-5.4)	7.2	(1.0-13.4)	15.7	(7.5-23.8)
23-24	77	3.7	(0.0-7.7)	2.7	(0.0-6.5)	5.4	(0.0-10.8)	11.7	(1.6-21.8)	3.0	(0.0-6.4)	13.8	(3.2-24.3)	17.9	(7.0-28.7)
25-26	89	12.4	(3.7-21.2)	1.3	(0.0-4.0)	11.3	(3.2-19.4)	7.9	(2.1-13.8)	0.0	(0.0-3.1)	7.9	(2.1-13.8)	16.0	(7.0-25.0)

CI, confidence interval

<sup>a</sup>For 41 females not sufficient material was available for HPV6 test.

<sup>b</sup>For 139 females not sufficient material was available for HPV11 test.

<sup>c</sup>For 4 females not sufficient material was available for HPV18 test.

As described above, increasing age was associated with HPV6, -11, -16, or -18 seropositivity (Table 2). For females in the age of 15-26 years, we also found that those who reported to have ever been diagnosed with an STI were more often HPV seropositive (Table 2). No association was found for ethnicity, educational level, marital status, and number of partners in the last 6 months. Age at sexual debut and the number of years being sexually active were associated univariately with being HPV seropositive for types 6, 11, 16 and/or 18, but were not significant after adjustment in multivariate analysis (Table 2). Table 2 also shows that four women who reported not to be sexually active yet had antibodies against HPV. Three of them had antibodies against HPV6 or -11, while the other one had antibodies against HPV16.

### **Geometric mean titres**

A large proportion of the HPV seronegative samples had titres below the detection limit (i.e. the lower limit of quantitation), respectively 88%, 96%, 95%, and 95%. The GMT was highest for HPV16 (82 mMU/ml (95%CI 57-118)) and lowest for HPV11 (30 mMU/ml (95%CI 14-65)). We found a GMT of 76 mMU/ml (95%CI 45-129) for HPV18 antibodies and of 67 mMU/ml (95%CI 45-102) for HPV6 antibodies.

**Table 2** General characteristics of the total sample population and the sample population of 15 years and older

	Females		HPV seroprevalence		Logistic regression			
	N	(%)	N	(%)*	Univariate		Multivariate	
	N	(%)	N	(%)*	OR (95%CI)	p-value	OR (95%CI)	p-value
<b>Total population</b>	637	(100)	47	(8)				
Age						<0.0001	Not applicable	
11-14	164	(26)	1	(1)	1.0			
15-18	133	(21)	3	(2)	2.8 (0.4-21.2)			
19-22	174	(27)	17	(11)	14.6 (2.4-88.8)			
23-26	166	(26)	26	(17)	23.4 (3.9-139.1)			
Ethnicity						0.1007		
Indigenous Dutch	537	(84)	38	(7)	1.0			
Non-indigenous Dutch	100	(16)	9	(9)	1.8 (0.8-3.6)			
<b>Population ≥15 years</b>	473		46					
Age						<0.0001		0.0007
15-18	133	(28)	3	(2)	1.0		1.0	
19-22	174	(37)	17	(11)	5.2 (1.6-16.5)		5.4 (1.6-17.4)	
23-26	166	(35)	26	(17)	8.3 (2.7-25.6)		9.2 (2.9-29.1)	

Educational level				0.1492
Low	52 (11)	2 (4)	1.0	
Medium	313 (68)	31 (11)	3.2 (0.8-12.6)	
High	98 (21)	13 (14)	4.2 (0.9-17.6)	
Marital status				0.3845
Single	337 (73)	30 (10)	1.0	
Living together/Married	125 (27)	15 (13)	1.4 (0.7-2.7)	
Age first sexual contact				0.0201
No sexual contact	125 (26)	4 (4)	0.3 (0.1-0.7)	
<16 years	68 (14)	11 (27)	1.4 (0.6-2.9)	
≥16 years	229 (48)	26 (13)	1.0	
Missing	51 (11)	5 (8)	0.6 (0.2-1.8)	
Years sexual intercourse				0.0101
No sexual contact ever	125 (26)	4 (4)	1.0	
0-2 years	70 (15)	5 (7)	2.0 (0.5-7.3)	
3-6 years	127 (26)	15 (15)	4.6 (1.6-13.2)	
>6 years	106 (22)	17 (18)	5.5 (1.9-15.9)	
Missing	51 (11)	5 (8)	2.2 (0.6-8.9)	

Partnership last 6 months				0.0258		
No sexual contact ever	125 (26)	4 (4)	1.0			
No partner	28 (6)	1 (4)	1.1 (0.1-8.8)			
1 partner	256 (54)	33 (14)	4.3 (1.6-11.5)			
>1 partner	31 (7)	5 (18)	5.6 (1.5-21.2)			
Missing	33 (7)	3 (9)	2.5 (0.5-11.7)			
STI diagnosed ever	19 (4)	7 (38)	6.6 (2.4-17.9)	0.0008	6.3 (2.2-17.9)	0.0008

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OR, odds ratio; CI, confidence interval; IQR, Interquartile range; STI, sexually transmitted infections

\*seroprevalences weighted by age and urbanization degree

## **DISCUSSION**

Our findings show that the percentage of females aged 11-26 years in the Netherlands with antibodies against HPV6/11 and HPV16/18 amounted both to about 4%. Seroprevalence increased steeply with age from 17 years onwards following the increasing percentage of women who started being sexually active. Below the age of 17, all girls were seronegative for HPV16/18.

In line with our study, large-scale surveys of HPV seroprevalences among children younger than 13 years of age found low seroprevalences.[25] Various studies also have shown increasing HPV seroprevalences with age, starting around the age of sexual debut.[13,17,18] Comparison of HPV serological data should be done with caution due to differences in type of assay used and associated seropositivity cut-off values. Despite they used identical assays and cut-off values as in our study, studies in the United States, England, Norway, Germany, and Australia found higher seroprevalences for HPV in females aged 10-29 years.[13,17,18,26,27] Compared to our study, females in the population-based American study reported more often to have had their sexual debut before their 16<sup>th</sup> birthday. In the Norwegian and German study, regular partners and new partners in the past 6 months were more often reported compared to our study. As both the age of sexual debut and the number of partners were found to be associated with a higher HPV prevalence, this could explain the differences in seroprevalence.[15] However consistent to these studies, we found the highest seroprevalences for HPV6 and -16. Moreover in line with our study, all females younger than 17 years in the German study were seronegative for HPV16 and 18.[27]

For our risk factor analysis we have to acknowledge that the number of seropositives were low and we were unable to perform multivariate analysis separately by HPV type. Our study was population-based and we have no indications that our results are influenced to a considerable extent by demographic characteristics (age, marital status, educational level). The distribution of these characteristics in our study population is similar to that in the general female population in this age group [24], except that our sample population consisting of

more persons of indigenous Dutch ethnic origin compared to the general population (84% vs. 76%). However, no association was found between seropositivity for HPV and ethnicity. The reported median age of sexual debut in our study was similar to those reported by earlier studies on sexual behaviour in The Netherlands (16 vs. 16.7 years).[7,28] Other sexual behavioural characteristics of our study population such as diagnosed with an STI ever, steady partnership and condom use with this partner were comparable to behavioural factors described in earlier studies.[7,28] However, underestimation of the HPV seroprevalence is conceivable as in our study population condom use with casual partners was more frequent and a smaller percentage of females aged 15-17 years reported to be already sexually active (27% vs. 42%).[7]

Sexual behaviour was self-reported which could result in underreporting of sexual activity. This underreporting may potentially have been somewhat larger among young females. Underreporting of sexual behaviour might explain seropositivity among persons who reported no sex partners. Females who reported having had no sexual contact yet were mainly seropositive for HPV6 or -11. LrHPV types 6 and 11 cause the majority of genital warts. Although, in particular lrHPV can be transmitted through skin-to-skin contact as well, without having sexual intercourse. In the present study, HPV6 and/or -11 infections were found from the age of 13 years onwards and increased with age. Genital warts were the most frequently diagnosed viral STI reported in all Dutch STI centres in 2008.[29] In women with genital warts, 59% was younger than 25 years and for 11% a history of gonorrhoea, infectious syphilis or Chlamydia infection was reported.[29] Similar to our study, earlier studies have shown an independent association between diagnosed ever with an STI and HPV infection.[16,30] To be diagnosed with an STI ever could serve as a marker for sexual exposure to HPV among women. Or STIs might increase women's susceptibility to HPV infection.[15] Although the number of years of sexual activity was associated with HPV seroprevalence in univariate analyses, in multivariate analyses age was found to be a stronger predictor for HPV6/11/16/18 seropositivity than age of first sexual contact.

Taking into account that half of the individuals show an antibody response after infection would imply that the percentage of females who have previously been infected with HPV6, -11, -16, or -18 is higher.[12] Although, in the oldest age groups (21-26 years) no further increase in seroprevalence was observed; HPV seroprevalences were about similar, which might suggest waning antibody levels over time.[12] Alternatively this could result from a small sample size or reflect a cohort effect.

This study gives information on the baseline HPV6/11/16/18 seroprevalence in 11-26-year-old girls/women in The Netherlands before introduction of vaccination. No antibodies against HPV16/18 were found among women younger than 17 years. As the median age of sexual debut is 16 years, the majority of the girls aged 13-16 years is not sexually active yet. Taking this and the serological results of our study into consideration, girls aged 13-16 years are likely to benefit substantially from HPV16/18 vaccination in addition to 12-year-old girls. This supports the decision to implement a catch-up HPV vaccination for this age group. Despite the lower sensitivity compared to HPV DNA testing, serological surveillance is an important additional tool to study the impact of HPV vaccination. In contrast to HPV DNA testing where vaginal swabs or cervical smears are needed, serum is easier to collect in younger age groups and less prone to sampling errors. After implementation of routine HPV16/18 vaccination and the catch-up campaign, comparable studies among vaccinated and unvaccinated women will be repeated to study the impact of HPV-vaccination. Testing for the presence of HPV antibodies and DNA in females in a broader age range will make it possible to study the vaccine impact at the population level, i.e. including the effects on those not directly targeted by the HPV-vaccination program.



**Key messages**

- In addition to 12-year-old girls targeted for routine HPV vaccination, girls up to 16 years are likely to benefit substantially from HPV16/18 vaccination.
- Older age and a former diagnosis with sexually transmitted infections were associated with HPV seropositivity.
- Sero-epidemiological studies performed before and after introduction of HPV vaccination is a tool to monitor the impact of vaccination on the frequency of HPV-infection.

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**Contributors**

MK conducted the data analysis and the drafting of the manuscript. LM, GS, HB, HM, FK contributed to the PIENTER study design and contributed to collection of clinical data and samples. LM contributed to the data analysis. GS contributed to laboratory testing. All authors critically revised the manuscript and approved the final version.

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**Figure 1** Reported age of first sexual intercourse (bars) and percentage of ever had sexual intercourse by age (line) for female participants (15-26 years) in the PIENTER 2 project, 2006-2007, The Netherlands

**Figure 2** Weighted seroprevalence for HPV6, -11, -16 and/or -18 among 11-26-year-old girls, 2006-2007, The Netherlands

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