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Original Contribution

Population- and Type-Specific Clustering of Multiple HPV Types Across Diverse Risk Populations in the Netherlands

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In view of possible type replacement upon introduction of human papillomavirus (HPV) vaccination, we aimed to explore patterns of type-specific clustering across populations with various background infection risks. A total of 3,874 women from 3 cross-sectional studies in the Netherlands (in 2007–2009) provided vaginal self-samples, which were tested for 25 HPV genotypes by a sensitive molecular assay (SPF_{10} line probe assay, DDL Diagnostic Laboratory, Voorburg, the Netherlands). The number of concurrent HPV infections per woman was studied by Poisson regression. Associations between HPV types were investigated by generalized estimating equation analyses. The prevalence of any HPV type was 14% in a population-based study, 54% in a chlamydia screening intervention study, and 73% in a study among attendees of sexually transmitted infection clinics. Overall, multiple HPV infections were detected in 26% of the women. The number of concurrent HPV infections conformed to an overdispersed Poisson distribution, even after correction for known risk factors. Types differed significantly in their tendencies to be involved in coinfections, but no evidence for particular type-type interactions was found. Moreover, the strongest associations were observed in the lowest-risk population and vice versa.We found no indications of pairwise interactions, but our findings do suggest that clustering differs among HPV types and varies across risk groups.

antagonism; coinfection; human papillomavirus; monitoring; multilevel analysis; synergism; type replacement; vaccination

Abbreviations: CI, confidence interval; CSI, chlamydia screening intervention; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; lrHPV, low-risk human papillomavirus; OR, odds ratio; STI, sexually transmitted infection.

More than 40 human papillomavirus (HPV) types can infect the genital epithelia. Of these, at least 12 can cause cancer and are referred to as high-risk HPV (hrHPV) types (1). Persistent infection with a hrHPV type can lead to various forms of anogenital and oropharyngeal carcinoma (2–4). This knowledge has led to the development of prophylactic vaccines targeting HPV types 16 and 18, which are most frequently associated with cervical cancer.

In 2009, HPV vaccination was introduced in the Netherlands with the specific aim to protect women from cervical cancer (5). The HPV16/18 vaccine has the potential to reduce the number of cervical cancer cases by at least 70% if other HPV types do not take the resulting ecological niche. An apparent increase in disease might occur when vaccine types are removed and stop masking the types not included in the vaccine (referred to as "unmasking"). Model-based estimates for the size of this effect are on the order of a 3%–10% diminished reduction in long-term cervical cancer incidence, depending on the assumptions made about the existence of natural immunity (6, 7). The ecological niche could also be taken through type replacement, which refers to the possibility that elimination of HPV16 and HPV18 could lead to an increased transmission of nonvaccine types. For this to occur, antagonistic interactions are required between vaccine types and those not included in the vaccine (8, 9). Type replacement has been observed following vaccination against other

pathogens (e.g., *Streptococcus pneumoniae*) (10) and is plausible whenever genotypically diverse pathogen strains compete for the same hosts.

Because an estimated 20%–50% of HPV-infected women harbor multiple HPV types (11, 12), understanding the possible interactions among HPV types is vital for predictions regarding the effects of HPV vaccination (13, 14). So far, several longitudinal studies have shown that a person at high risk of infection with 1 HPV type is also at high risk of infection with another type (15–18). The elevated risk of coinfection has generally been supported by the results of cross-sectional studies, which typically report odds ratios above 1 for the occurrence of any 2 HPV types, meaning that HPV infections occur more often together than would be expected by chance (19–21). In addition, these studies have investigated whether particular type-type combinations occur more often than other combinations but have concluded that pairwise interactions are not likely, even among closely related HPV genotypes.

The uniformly elevated risk of coinfection across HPV genotypes is usually attributed to common risk factors for HPV acquisition (22). However, adjustment for known risk factors in multilevel analysis is rarely sufficient to eliminate associations in the occurrence of multiple HPV types. Although such elimination may be achieved in random effect models, allowing adjustments to be made for all sources of residual variation other than those already represented by the covariates, these models offer no explanation for the elevated risk of coinfection. Identification of the factors that account for clustering of multiple HPV types is paramount to assessing the potential consequences of vaccination. Previously, elevated odds ratios were usually interpreted as indicating the absence of antagonistic interactions between types, suggesting a low probability of type replacement. However, recently it was shown that elevated odds ratios could also be consistent with cross-immunity between HPV types, a condition that would facilitate type replacement (13).

The net effects on the occurrence of multiple infections of variation on the part of the host (sexual risk behavior, susceptibility to infection, immune response), as well as HPV (transmissibility, persistence, immunogenicity) and possible interactions between types, are difficult to disentangle. Nonetheless, comparisons across risk groups may help to elucidate the relative role of either component.

In this study, we aimed to explore the clustering patterns of multiple HPV types in diverse populations at various risks of infection by pooling data from 3 prevaccine studies of HPV infection in young women. The use of a novel approach to model pairwise odds ratios allowed us to study clustering patterns more carefully than has been done before.

METHODS

Study population and design

In 2007–2009, several HPV monitoring studies were carried out in the Netherlands, prior to HPV vaccination. Data from 3 of these studies were combined for the current analysis and included women aged 18–24 years. Study protocols have been described elsewhere (23–26). The 3 studies were 1) a population-based study, herein referred to as Nijmegen, which included 1,145 women aged 18–29 years who were selected through internet advertisements and posters at general health care practices in the regions of Arnhem, Nijmegen, and Den Bosch; 2) a chlamydia screening intervention (CSI) study, which included 3,282 women aged 16–29 years who were selected from South Limburg, Rotterdam, and Amsterdam; and 3) the Papillomavirus Surveillance Among STI [sexually transmitted infection] Clinic Youngsters in the Netherlands Study of attendees of 12 sexually transmitted infection (STI) clinics (herein referred to as STI clinics) throughout the Netherlands, which included 1,072 women aged 16–24 years.

HPV DNA detection and genotyping

Each participant provided a vaginal self-sample, which was tested for the presence of HPV. All studies used the same HPV genotyping method (27–29). Briefly, HPV DNA was extracted from the vaginal self-samples using the MagNA Pure platform (Roche Deutschland Holding GmbH, Penzberg, Germany) and amplified using the SPF_{10} primer set according to the manufacturer's instructions (DDL Diagnostic Laboratory, Voorburg, the Netherlands). The presence of HPV amplicons was assessed by an HPV DNA enzyme immunoassay. Genotyping of the HPV-positive DNA samples was done by reverse hybridization in a line probe assay. The polymerase chain reaction fragment SPF_{10} primer set amplifies 12 hrHPV genotypes (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and 12 low-risk human papillomavirus (lrHPV) genotypes that have limited evidence for a causal link with cancer (HPV types 6, 11, 34, 40, 42, 43, 44, 53, 54, 66, 70, and 74) (classification based on the last International Agency for Research on Cancer report (1)). Because no distinction can be made between HPV types 68, 73, and 97, they were classified as HPV68 (a lrHPV genotype). Samples that were HPV-positive in the DNA enzyme immunoassay analysis but did not reveal any of the 25 HPV genotypes in the line probe assay were classified as negative.

Statistical analysis

We present descriptive data on overall and type-specific HPV prevalence (percentages of women testing positive) and the number of concurrent HPV types by study population. For every hrHPV type, we present the (relative) proportion of single versus multiple infections, stratified by study population.

We used Poisson regression models for the number of HPV types per woman, and we calculated observed-to-expected ratios for the counts of multiple infections. The following categorical variables, available in all 3 studies, were included in the multivariable models: age, ethnicity (Dutch vs. non-Dutch), education (low vs. high), living situation (being single vs. in a relationship), age of sexual debut (≤13 years, 14–16 years, 17–19 years, >19 years), number of partners in the last 6 months (0–1 partners, >1 partner), and ever had an STI (no, yes, never been tested). No information was available on human immunodeficiency virus status, smoking behavior, and cervical disease status. In case of

Table 1. Demographic Characteristics, Sexual Behavior, and HPV Prevalence Among 3,874 Women, Stratified by Study Population, the Netherlands, 2007–2009

Characteristic	Nijmegen ($n = 1,145$)			CSI Study ($n = 1,657$)			STI Clinics ($n = 1,072$)		
	No.	% of Total	% hrHPV	No.	% of Total	% hrHPV	No.	% of Total	% hrHPV
Age, years[a]									
18	118	10	7	192	12	33	81	8	63
19	147	13	9	213	13	40	157	15	55
20	174	15	9	274	17	41	193	18	57
21	172	15	13	261	16	43	187	17	55
22	176	15	11	253	15	45	187	17	66
23	176	15	10	243	15	47	155	15	60
24	182	16	14	221	13	49	112	11	61
Ethnicity									
Dutch	1,117	98	11	1,383	83	42	932	87	59
Non-Dutch	20	2	10	274	17	47	140	13	59
Missing	8								
Education									
Low	33	3	6	105	7	40	59	6	75
High	1,104	97	11	1,448	93	43	970	94	58
Missing	8			104			43		
Living situation									
Partner	811	71	9	815	53	36	549	53	60
Single	328	29	14	735	47	50	494	47	59
Missing	6			107			29		
Age at sexual debut, years									
<13	25	2	24	50	3	42	17	2	71
14–16	581	51	12	838	54	48	576	55	61
17–19	465	41	8	559	36	38	403	39	57
>19	71	6	10	98	6,3	34	44	4	45
Missing	3			112			32		
No. of sexual partners in last 6 months									
0–1	974	86	9	1,037	67	36	370	35	50
>1	165	14	20	512	33	57	700	65	64
Missing	6			108			2		
Ever had STI									
No	1,074	94	10	182	68	53	673	65	56
Yes	66	6	20	87	32	68	228	22	73
Never tested							137	13	53
Missing	5			1,388			34		

Abbreviations: CSI, chlamydia screening intervention; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; STI, sexually transmitted infection.

[a] Median ages were as follows: in Nijmegen, 21.2 (standard deviation, 1.9) years; in the CSI study, 21.1 (standard deviation, 1.9) years; and in STI clinics, 21.1 (standard deviation, 1.8) years.

more than 5% of missing values per variable in the overall analysis, an extra category for missing values was introduced.

Observed-to-expected ratios were calculated before and after adjustment for covariates, stratified by study population. Overdispersion was tested by comparing the adjusted model with an alternative, which specifies a negative binomial distribution for the counts of multiple infections.

To look at type-type interactions, pairwise odds ratios were calculated for each HPV type with all other HPV types that had a prevalence of more than 1% in the combined study

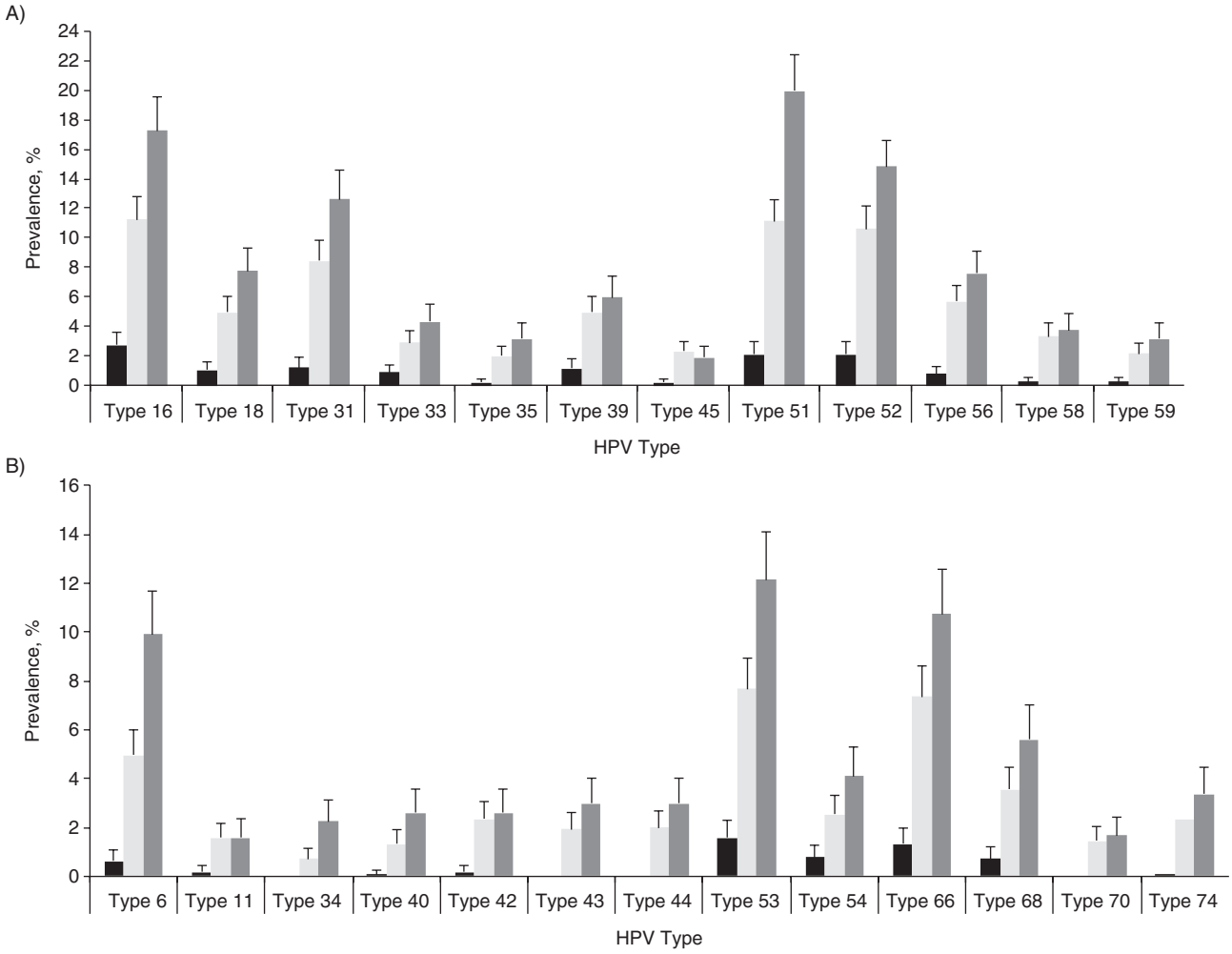

Figure 1. Human papillomavirus (HPV) type–specific prevalence in the Netherlands, 2007–2009, for A) low-risk HPV and B) high-risk HPV among 3,874 women aged 16–24 years, stratified by study population (Nijmegen (in black), a chlamydia screening intervention study (in light gray), and sexually transmitted infection clinics (in dark gray)).

populations (all types except HPV34). For each type, we calculated a Mantel-Haenszel estimate of the pooled odds ratio after stratification by all other HPV types. This pooled odds ratio represents the affinity of the index type to be involved in a coinfection with another HPV type (21). Pairwise odds ratios were subsequently compared with the bootstrapped pooled odds ratio by HPV type to assess whether the occurrence of particular combinations differed from the underlying affinity of either type (see Web Appendix 1 available at http://aje.oxfordjournals.org/ for a detailed description of statistical methods). We also compared the affinity of HPV types to be involved in a coinfection by means of generalized estimating equations (30). In modeling the association between pairs of responses, we used the alternating logistic regression algorithm of the GENMOD procedure in SAS statistical software (31, 32). This algorithm models pairwise odds ratios in a regression framework. By correct model specification, one obtains estimates that should be comparable to the Mantel-Haenszel estimates of the pooled odds ratio for each type separately. In addition, the regression framework allows for alternative model specifications (e.g., a common log odds ratio between any pair of HPV genotypes or differences in affinity for clustering between lrHPV vs. hrHPV types).

Finally, to look at population-specific clustering of HPV, we specified a model with distinct affinities for clustering in each study population separately. For this analysis, we looked only at HPV types with a prevalence of more than 1% in all study populations (i.e., HPV types 16, 18, 31, 39, 51, 52, 53, and 66).

RESULTS

Subject characteristics and HPV prevalence stratified by study population are listed in Table 1. The ages of the 3,874 women in the 3 studies ranged from 18 to 24 (median, 21) years. Overall HPV prevalence was 47% (14% in Nijmegen, 54% in the CSI study, and 73% in the STI clinics). In addition, the largest proportion of hrHPV was found in Nijmegen (69%)

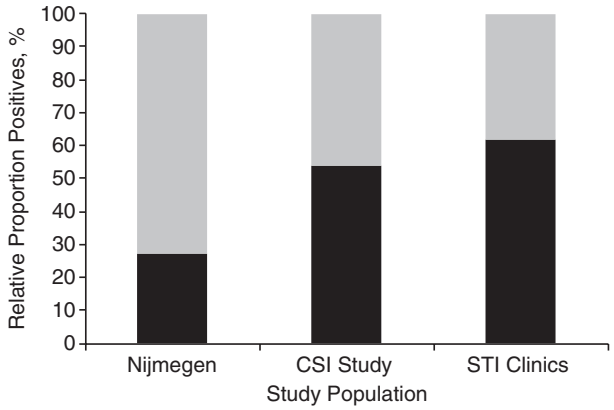

Figure 2. Relative distribution of single (black) versus multiple (gray) human papillomavirus (HPV) infections in the Netherlands, 2007–2009, among 1,839 HPV–positive women aged 16–24 years, stratified by study population (Nijmegen, a chlamydia screening intervention (CSI) study, and sexually transmitted infection (STI) clinics). Single human papillomavirus distributions were 27% in Nijmegen, 54% in the CSI study, and 62% in STI clinics.

compared with the CSI study (59%) and the STI clinics (57%). The most common HPV types in all studies were types 16, 51, and 52 (Figures 1A and 1B). HPV types 54, 42, 16, and 70 were the types most often found alone, whereas HPV types 43, 44, 45, 35, and 11 were most often found as part of a multiple infection (Web Figure 1). In general, multiple infections were detected in 26% of the women. Both in absolute and relative terms, most multiple infections were found in subjects from the STI clinics, followed by those from the CSI study, and then those from Nijmegen (Figure 2).

The number of HPV infections within a woman ranged from 0 to 4 (median, 0) in the Nijmegen study, 0–8 (median, 1) in the CSI study, and 0–9 (median, 1) in the STI clinics. The observed number of infections per woman differed from expectation under a Poisson distribution (variance equal to the mean) (Table 2). In Nijmegen, the number of women with 1 infection was significantly lower than expected, whereas the numbers of women without infection and with more than 1 infection were significantly higher than expected. A similar pattern was observed in the CSI study and in the STI clinics, except that the number of women with 2 infections was also less than expected in these populations (Table 2). After adjustment for potential risk factors for HPV, the observed-to-expected ratios moved slightly toward 1, but the variance in the counts of HPV infections remained greater than the mean, indicating an overdispersed Poisson model. Overdispersion was also supported by the fact that a negative binomial distribution

Table 2. Observed/Expected Ratios of Multiple Infections Among 3,723 Women, Stratified by Study Population, the Netherlands, 2007–2009

No. of HPV Types, by Population	Observed No. of Coinfections	Expected[a] No. of Coinfections	Ratio of Observed to Expected[a] No. of Coinfections	95% CI	Adjusted Expected[a] No. of Coinfections	Adjusted Ratio of Observed to Expected[a] No. of Coinfections	95% CI
Nijmegen							
0	964	929.71	1.01	1.01, 1.07	933.15	1.03	0.97, 1.13
1	113	170.63	0.74	0.59, 0.74	164.55	0.69	0.51, 0.96
2	32	15.66	2.04	1.60, 2.63	17.60	1.82	0.92, 3.70
3	4	0.96	4.18	2.85, 6.15	1.56	2.53	0.89, 7.60
≥4	4	0.04	91.02	54.08, 153.72	0.14	28.67	6.50, 128.76
CSI study							
0	700	537.58	1.30	1.24, 1.37	550.12	1.27	1.12, 1.48
1	385	567.18	0.68	0.68, 0.68	536.24	0.72	0.70, 0.76
2	232	299.20	0.78	0.74, 0.81	288.43	0.80	0.72, 0.91
3	128	105.22	1.22	1.11, 1.34	115.36	1.11	0.89, 1.42
≥4	99	27.75	3.57	3.10, 4.12	53.85	1.84	1.24, 2.76
STI clinics							
0	275	221.96	1.30	1.21, 1.41	201.97	1.36	1.10, 1.74
1	282	349.13	0.85	0.83, 0.88	319.52	0.82	0.82, 0.98
2	188	274.57	0.72	0.71, 0.74	259.98	0.70	0.70, 0.77
3	147	143.96	1.07	1.01, 1.15	144.72	1.02	0.86, 1.24
≥4	126	56.61	2.33	2.08, 2.63	91.81	1.37	0.95, 2.02

Abbreviations: CI, confidence interval; CSI, chlamydia screening intervention; STI, sexually transmitted infection.
[a] Based on Poisson regression.

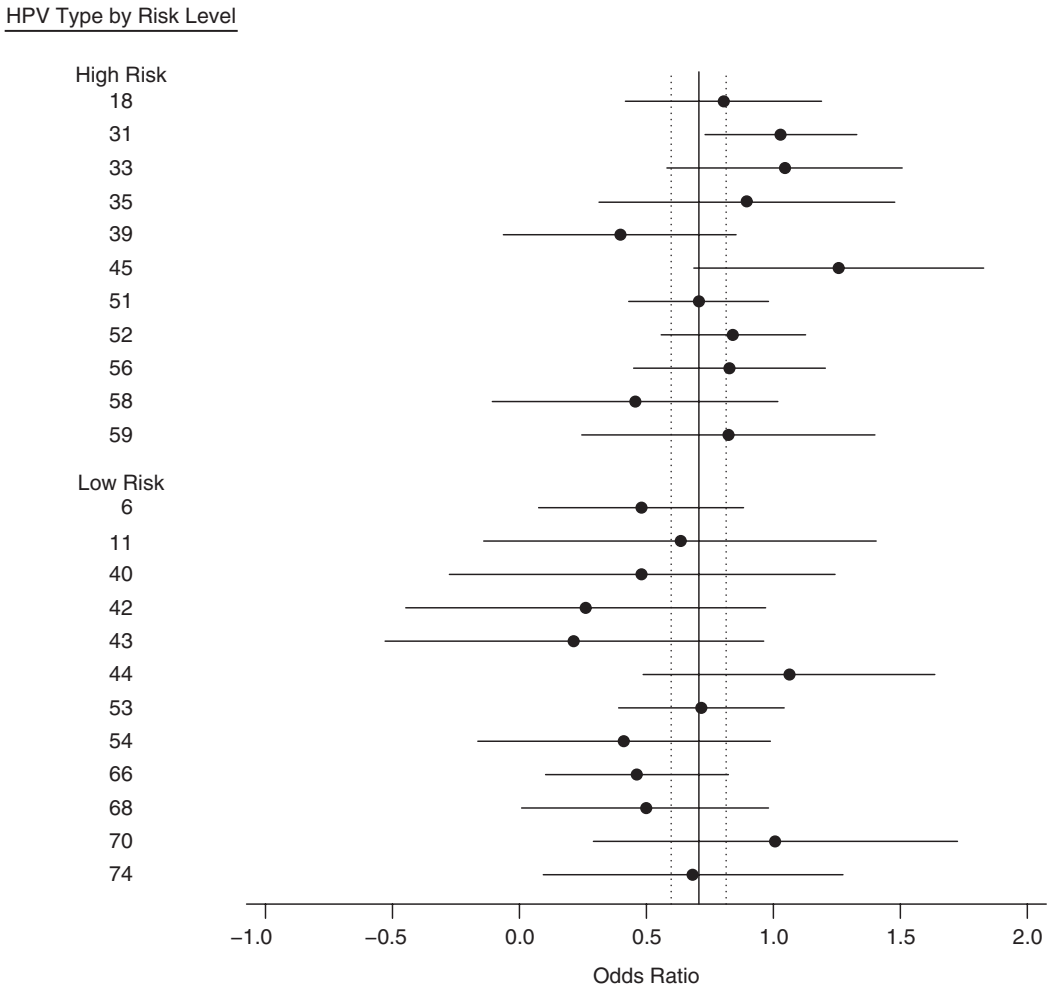

Figure 3. Bootstrapped pooled odds ratios (solid lines) and bootstrapped 95% confidence intervals (dotted lines) for human papillomavirus (HPV) type 16 and coinfection with 23 other HPV types in the Netherlands, 2007–2009.

(variance greater than the mean) provided significantly better fit to the data ($P < 0.001$ based on the likelihood ratio test).

The assumption of homogeneous odds ratios for pairwise interactions was violated only sporadically; 8 of 276 pair-specific odds ratios deviated from the pooled odds ratio of the reference type ($P < 0.05$). Although this is still less than expected at a 5% false positivity rate (which would be 14 deviations), it appeared that 5 of those 8 deviations involved either HPV31 or HPV58. HPV31 was found to cluster significantly more often with HPV types 33, 44, and 58 than with other types, whereas HPV58 clustered significantly more often with HPV types 31, 43, and 59. The other interactions were HPV type 42 with 70, HPV type 54 with 53, and HPV type 74 with 68. The (bootstrapped) pooled odds ratios for coinfection with other types were 2.0 (95% confidence interval (CI): 1.8, 2.3) for HPV16 (Figure 3) and 2.4 (95% CI: 2.1, 2.8) for HPV18 (Figure 4).

Although we found no evidence for particular pairwise interactions (except perhaps those involving HPV31 or HPV58), we did find significant differences among the 24 types regarding their tendencies to be involved in a coinfection (Figure 5). After adjustment for potential risk factors common to all types, HPV types 31, 33, 45, 52, 53, and 58 showed a greater affinity to be involved in a coinfection relative to HPV16 (the most prevalent type in all study populations), whereas HPV54 was found less often in a multiple infection (Web Table 1).

When studying the association between lrHPV versus hrHPV types, we found that, when taking the association between 1 lrHPV and 1 hrHPV as a reference (odds ratio (OR) = 1.62), 2 hrHPV types clustered significantly more often (OR = 1.81, $P = 0.03$) compared with 2 lrHPV types (OR = 1.61, $P = 0.87$). However, no correlation was found between the pooled odds ratio of a particular HPV type and its prevalence (Pearson's correlation coefficient = −0.09).

Lastly, we investigated whether odds ratios for coinfection were different among the 3 study populations. The stratified model showed that the association between any pair of HPV types was highest in Nijmegen (pooled OR = 4.5, 95% CI: 3.0, 6.7) and lowest in the STI clinics (pooled OR = 1.5,

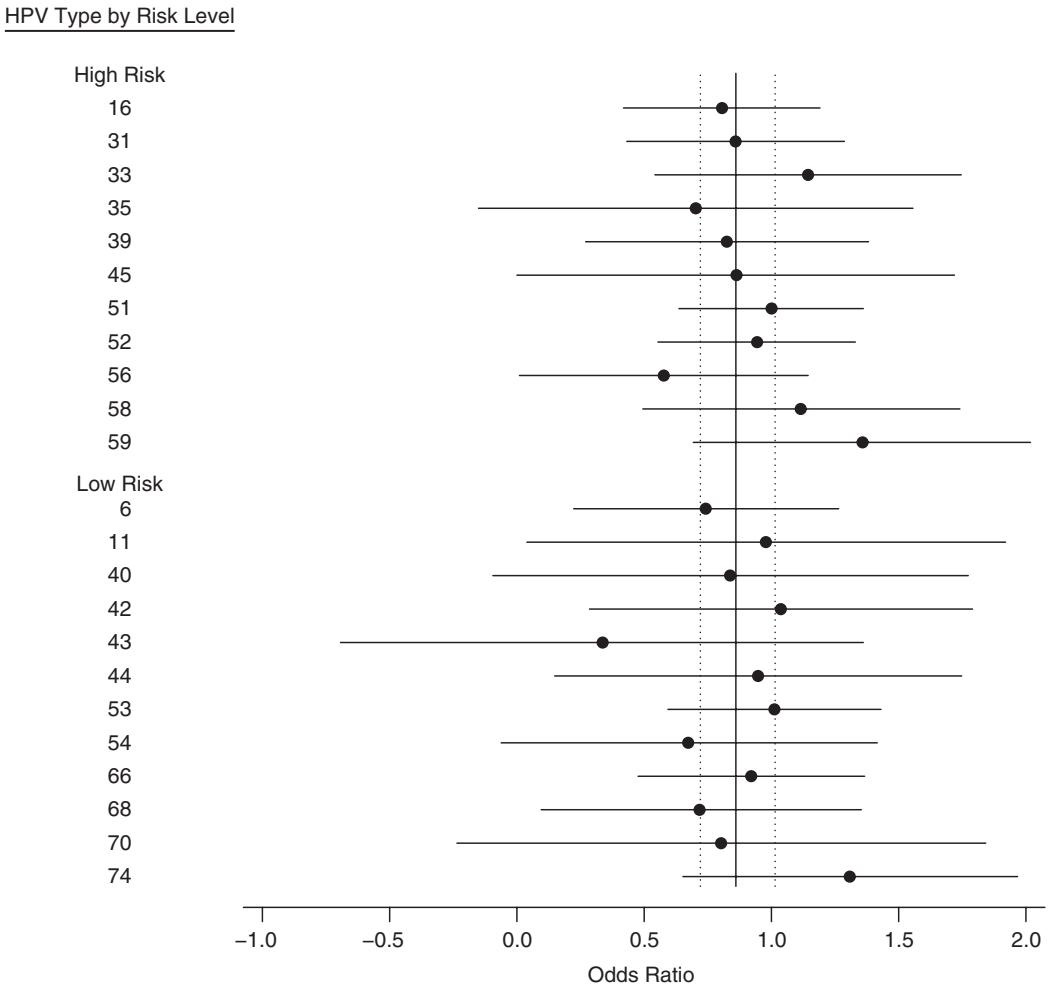

Figure 4. Bootstrapped pooled odds ratios (solid lines) and bootstrapped 95% confidence intervals (dotted lines) for human papillomavirus (HPV) type 18 and coinfection with 23 other HPV types in the Netherlands, 2007–2009.

95% CI: 1.3, 1.6), with the CSI study population in between (pooled OR = 1.8, 95% CI: 1.6, 2.2). After adjustment for potential confounders, differences between the study populations became somewhat smaller, but the gradient with background infection risk remained (Figure 6).

DISCUSSION

In part, the current study confirms previous findings by showing that associations between HPV types were unanimously positive, and that pairwise interactions were apparently nonexistent (19–21). However, the use of a novel approach to model pairwise odds ratios allowed us to study clustering patterns more carefully than has been done before, and as a result, we did find differences in the tendency per HPV type to cluster together with other HPV types. For instance, HPV54 had a significantly lower affinity to be involved in a coinfection than HPV45. In addition, we showed that associations between HPV genotypes differed among study populations, with the strongest clustering found in the population at lowest risk of infection and vice versa.

The association in the occurrence of multiple HPV types likely depends on many factors, such as the risk heterogeneity of a population, the per-partnership transmission probability, differences in the persistence of lrHPV and hrHPV, and possibly immunological factors, such as (partial) immunity against reinfection with the same HPV type or cross-immunity to other types. For example, an increased heterogeneity in the risk of infection would result in an increased clustering of multiple HPV types, analogous to the observed coepidemic of hepatitis C virus and human immunodeficiency virus in populations of injection drug users (33, 34). The same holds for the per-partnership transmission probability; 2 types that transmit easily but must do so at few occasions (e.g., in the case of serial monogamy) will end up together more frequently than 2 types that transmit less easily but can do so at inversely proportionally more occasions (e.g., in the case of partnership concurrency). This can be illustrated by a simple probability example. If a susceptible person

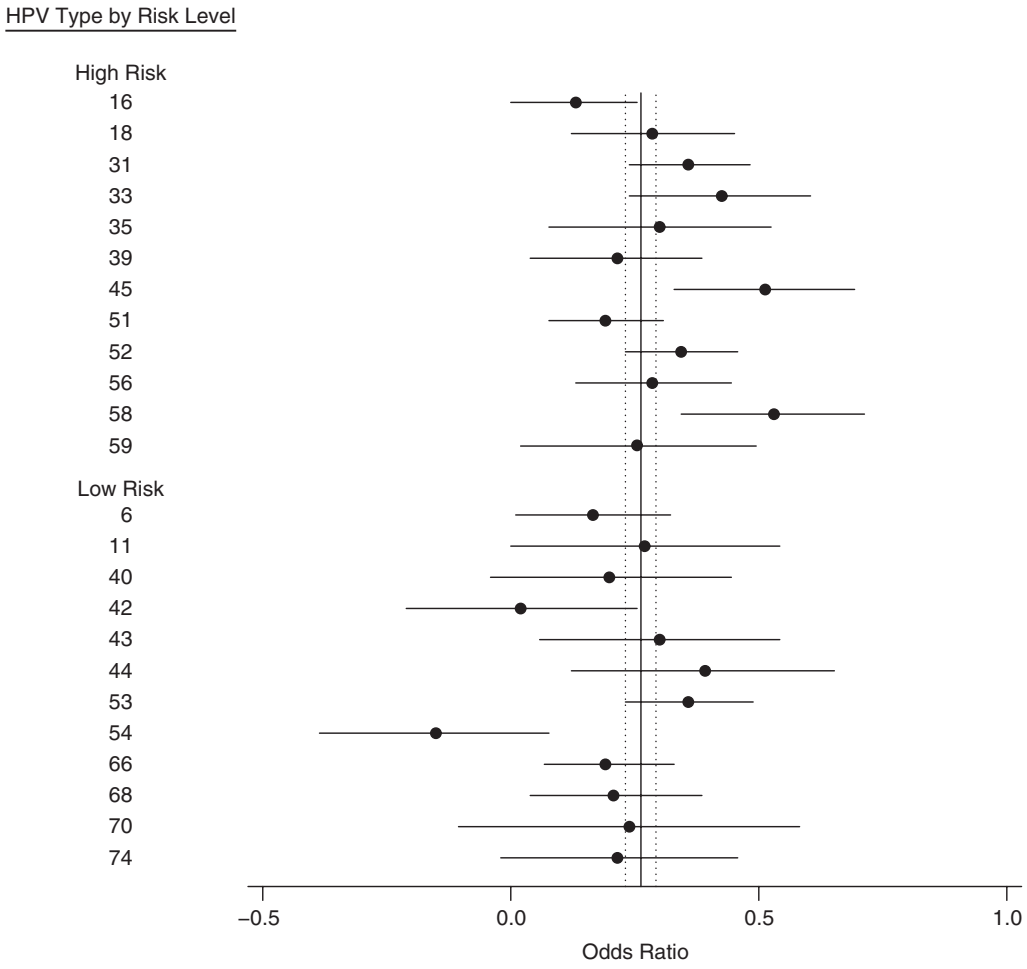

Figure 5. Pooled odds ratio (solid lines) and 95% confidence intervals (dotted lines) of 23 human papillomavirus (HPV) types by referent type among 3,679 women aged 16–24 years in the Netherlands, 2007–2009. Results obtained using generalized estimating equations, adjusted for age, ethnicity, education, having a partner, age at sexual debut, number of sexual partners in the last 6 months, and ever having had a sexually transmitted infection.

forms a sexual partnership with someone who is doubly infected, with a transmission probability (β) of 0.8 for both types, then the probability that this person will become doubly infected is 0.64. If β is 0.4 for both types, then the probability of becoming doubly infected in the first partnership is 0.16. After another partnership with a doubly infected person, the probability of being doubly infected is $\beta^2 + 2(1-\beta)\ \beta^2 + (1-\beta)\ (1-\beta)\ \beta^2 = \beta^2(2-\beta)^2$, which is still smaller than $(2\beta^2)$. The negative correlation between the association in the occurrence of multiple HPV types and background infection risk might be attributed to either of those factors if it is assumed that populations who have STIs have reduced risk heterogeneity relative to the general population, and that HPV transmission probability is lower in short-term sexual encounters than in longer-lasting partnerships.

Our results seem to counter a predominant role for cross-immunity in determining clustering patterns of multiple HPV types if one supposes that clustering due to preexisting immunity would be more likely to show up in populations with a high degree of prior exposure to HPV. The lack of clustering between closely related genotypes also seems to argue against cross-immunity. However, one might as well reason that cross-immunity leads to a relatively stronger clustering in populations with less exposure to HPV. A formal investigation (e.g. based on a transmission model) could be used to sharpen our intuition on this particular topic. Alternative factors, such as increased host susceptibility due to coinfections with other STIs, can also be of interest (35). However, in a scenario where concurrent STIs would render an individual more susceptible to HPV infection (36–38), an increased likelihood of coinfection would be expected in the STI clinics, whereas we found the opposite.

Given the various factors involved in determining coinfection patterns, the assessment of interactions among HPV types is methodologically challenging (22). A strength of our study is the use of a generalized estimating equation regression framework. Generalized estimating equation models permit separate modeling of the relationship of the multivariate

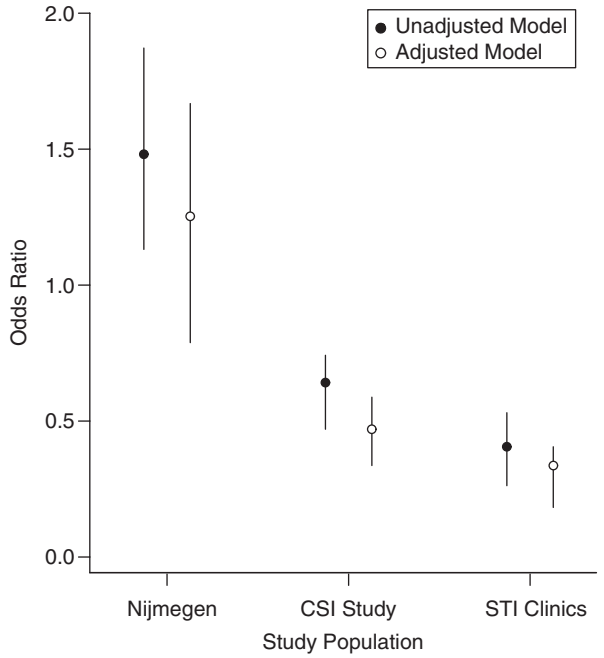

Figure 6. Pooled odds ratios and 95% confidence intervals for the occurrence of multiple human papillomavirus (HPV) infections among 3,679 women aged 16–24 years, stratified by study population (Nijmegen, a chlamydia screening intervention (CSI) study, and sexually transmitted infection (STI) clinics) in the Netherlands, 2007–2009. Results obtained by generalized estimating equations for HPV types with a prevalence of 1% or more in all study populations (HPV types 16, 18, 31, 39, 51, 53, and 66). Black dots, unadjusted odds ratios; white dots, odds ratios adjusted for age, ethnicity, education, partner, number of partners in the last 6 months, age at sexual debut, and ever having had a sexually transmitted infection.

binary response with explanatory variables and of the association between pairs of responses. In this sense, they offer a natural way of separating individual risk factors common to all HPV types from the residual tendency of types to cluster together (22). We made particular use of the alternating logistic regression algorithm, which gives robust and efficient estimates when the association model is a scientific focus in itself (31). It should be noted that this algorithm considers only pairwise associations and leaves higher-order interactions unspecified. This is a critique of marginal models (39), but the same applies to alternative approaches that are used to evaluate the potential for type replacement following HPV vaccination, both in mathematical modeling (8, 13, 14) and in statistical analysis (21, 40). We show that generalized estimating equation models yield results that are comparable to those obtained with an approach that is more familiar to HPV researchers, but the ability to use a regression framework for the association model has substantial benefits. It allows the formulation of testable hypotheses to study pairwise interactions and specification of type- or population-specific differences in the tendency to cluster.

Another strength of our study is that we pooled data from 3 HPV monitoring studies that used the same HPV genotyping method, which provided enough data to identify significant differences between HPV types. Our analyses regarding pairwise interactions showed 8 associations that were significantly different from the pooled average of either reference type, 3 of which involved HPV31 (HPV type 31 with 33, HPV type 31 with 58, and HPV type 31 with 44) and 3 of which involved HPV58 (HPV type 58 with 59, HPV type 58 with 43, and HPV type 58 with 31). Although false positive findings should be expected in multiple testing, the deviations relating to HPV31 or HPV58 cannot simply be ascribed to chance and merit more scrutiny. Besides a possible biological interpretation, a technical explanation for these findings is available. The broad-spectrum DNA assay that was used for genotyping does not have the same sensitivity and specificity for each HPV type (28). It has been shown before that HPV31 has a higher positivity rate in our test (SPF_{10} line probe assay) compared with other tests (28). Except for the association between HPV types 31 and 33 (21), the other associations with HPV31 that we detected were not found in previous studies using a similar testing method (21, 41). The HPV genotyping algorithm could also explain some of the type-specific differences in the affinity to cluster that we found. For example, HPV54, which is found least often with other HPV types, is on the same probe line as HPV31 and HPV33. Therefore, no distinction can be made between a coinfection including HPV31 or HPV33 with HPV54 versus a monoinfection of HPV31 or HPV33. Because HPV54 is a lrHPV type, the chosen algorithm does not "score" HPV54 if either HPV31 or HPV33 is present. The faculty to pick up such technical limitations underscores the strength and sensitivity of our analysis method.

Type-specific differences in the tendency to cluster did not show a clear correlation with viral characteristics, such as immunogenicity or prevalence of the HPV type. However, we did find significant differences according to oncogenicity, in that pairwise odds ratios were higher if the types involved were both hrHPV compared with 1 lrHPV and 1 hrHPV. These differences might be attributed to the fact that hrHPV infections generally have lower clearance rates than lrHPV infections (16, 17, 24); 2 high-risk types thus have greater opportunity to be detected together, even if they are acquired and cleared independently. The significantly higher clustering among 2 hrHPV types compared with 2 lrHPV types offers an additional explanation for the observed gradient with background infection risk, because we found the highest proportion of hrHPV among HPV-positive cases in the Nijmegen study. These findings are in line with a modeling study by Orlando et al. (42), who hypothesized that HPV dynamics depend on the turnover rate of sexual relationships; a slow turnover of sexual partners favors hrHPV, whereas a high turnover of sexual partners selects for lrHPV. Again, the numerous factors involved in determining coinfection patterns strongly suggest that different tendencies to cluster according to HPV type and population are to be expected.

Currently, we are performing several ongoing studies among different risk groups in the Netherlands, which allows monitoring of possible type replacement in young and sexually active adults. The HPV Amongst Vaccinated and Nonvaccinated Adolescents (HAVANA) Study follows a cohort of young and partly vaccinated girls who provide a vaginal self-swab and serum for detection of HPV DNA and antibodies on an annual

basis (43). A biannual sentinel surveillance study at STI clinics (baseline data from which we used in the current analysis) provides information on the opposite side of the risk spectrum (44). It would be worthwhile to analyze forthcoming data with the same analysis method, because it has been shown to provide sensitive and robust estimates of coinfection patterns and is fairly simple to use with standard statistical software.

In conclusion, this study provides information about HPV clustering patterns in the prevaccination era and can further our understanding of changes in HPV dynamics over time after the introduction of the HPV vaccine. The current study shows that, prior to vaccination, the affinity of HPV types to cluster with other types is not solely determined by heterogeneities on the host level, but may also be dependent on HPV type. However, we found no indication of specific pairwise interactions, nor that cross-immunity is a dominant factor in determining coinfection patterns. Our findings are compatible with the working hypothesis that HPV transmission dynamics from 1 type are largely independent of other types, supporting the view that, at present, there is no reason to suspect detrimental consequences of vaccination against a limited set of HPV types.

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