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Occurrence of vaccine and non-vaccine human papillomavirus types in adolescent Finnish females 4 years post-vaccination

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Control of human papillomavirus (HPV)-related cancers by inclusion of HPV vaccination into national vaccination programmes is likely. One open question is replacement of the vaccine types with other high-risk (hr) HPV types in the vaccination era. We studied occurrence of HPV types in adolescent females participating in a population-based vaccination trial. A total of 4,808 16- to 17-year-old females from Finland were enrolled in the 1:1 randomized phase III (PATRICIA) trial of the efficacy of vaccination with the AS04-adjuvanted HPV-16/18 virus-like particle vaccine as compared to hepatitis A virus (HAV) vaccine. HPV infection was assessed from cervical samples taken every 6 months for 4 years post-vaccination by polymerase chain reaction (PCR) for genital oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 68, and 73 as well as low-risk types HPV-6 and HPV-11. The HPV-16/18 vaccine coverage ranged between 1 and 22% by age-cohort and study community. Odds ratios (ORs) for infections with different HPV types in baseline PCR negative HPV-16/18 *vs.* HAV vaccinated women, and Poisson regression derived HPV incidence rate ratios (IRRs) in baseline positive *vs.* negative women were calculated. The OR and IRR estimates for acquisition of any genital HPV types showed no excess risk neither in baseline HPV DNA-negative HPV-16/18-vaccinated women compared to baseline HPV DNA-negative HAV vaccinated women nor in HPV-16/18-vaccinated baseline HPV-16/18-positive women compared to baseline HPV-16/18-negative women. In the HAV-vaccinated, baseline HPV-18-positive women showed an increased risk of acquiring other clade A7 HPV types (39, 45, 59, 68) (IRR 1.8, 95% confidence interval = 1.01.-3.1). We found no increased occurrence of non-vaccine HPV types suggestive of type-replacement 1–4 years post-vaccination among HPV-16/18-vaccinated Finnish adolescents.

As predicted, 15 years ago,[1] replacement of multivalent vaccine covered *Streptococcus pneumoniae* serotypes by non-vaccine serotypes may already with as low-vaccine coverage as <20% endanger the effectiveness of pneumococcal mass vaccination.[1,2] A recent meta-analysis indicates that this phenomenon usually takes place within <5 years from the introduction of mass vaccination.[2] There are fundamental differences between pneumococci and human papillomavirus (HPV) infections and infection immunology. Depending on the extent population biology differences of the various pneumococci and HPV types, vaccine cross-protection and vaccination coverage type-replacement may or may not be an issue for the HPV vaccines licensed worldwide.[3–6] National HPV vaccination programs have been implemented in many countries with coverage ranging from 20 to 70%,[7–10] and good vaccine efficacy against HPV16/18-related types 31/33/45, and against HPV-51 has been reported.[11–13]

We cannot predict whether mass vaccination will change the distribution of HPV types because the population biology and the potential risk for competition between HPV types are not well understood aside from short/narrow protection gained from natural infections.[14–19] Due to risk-taking behaviour 20–50% of women with cervical HPV infections have or have had more than one HPV types regardless of analytical methods.[14–19] These, often concomitant, infections with multiple HPV types favor a coinfection with equal types rather than a superinfection model, with a competitively more advantaged type for the HPV population biology.[20,21] A competitive advantage for HPV33 over other HPV types in

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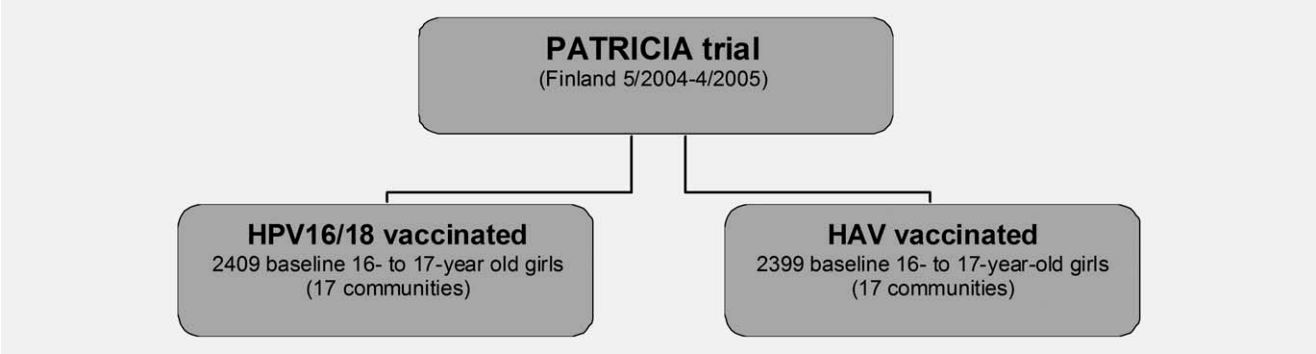

Figure 1. Enrolment of HPV-008 (PATRICIA) trial participants in Finland.

unvaccinated fertile-aged female population was recently reported, suggesting that superinfection might also be a possibility.[19]

Less common HPVs, with competitive edge, might fill in an ecological niche following HPV-16/18 vaccination. Therefore, we studied the incidence of non-vaccine HPV types in a large cohort of young females followed for up to 4 years after the start of HPV vaccination.[12,22–24]

Material and Methods

Participants

All (24,046) healthy Finnish women aged 16–17 years from 17 Finnish study site communities were eligible and invited into an international phase III vaccination trial: PApilloma TRIal against Cancer In young Adults (PATRICIA) by two personal invitation letters between May 2004 and April 2005. They were eligible regardless of their baseline HPV DNA status, HPV serostatus or cytology. No exclusion criteria based on the lifetime number of sexual partners were used. The study design and setting at the Finnish study site communities where the recruitment was reinforced by sexual health education at secondary high schools and technical schools as described,[12,22–24] and the PATRICIA study protocol was approved by the Finnish national ethical review board. Written informed consent was obtained from the participants, who consented to adequate contraception, for example hormonal contraception or barrier methods (condom use) up to 2 months after the vaccination period (a total of 8 months).

Procedures

A total of 4,808 young females were randomised in a 1:1 ratio to receive either the HPV-16/18 AS04-adjuvanted vaccine (Cervarix™, GlaxoSmithKline Biologicals) or a control hepatitis A (HAV) vaccine (investigational formulation based on licensed Havrix vaccine; GlaxoSmithKline) using an internet-based centralised randomisation system, and a double-blind, three-dose vaccination schedule at 0, 1 and 6 months. Cervical samples were collected every 6 months for cytology and HPV DNA detection and typing for 4 years after vaccination. The clinical management algorithm for abnormal cytology results, colposcopy referral and collection of histopathology specimens have been described.[12,23,24]

A broad spectrum polymerase chain reaction (PCR) SPF_{10}-$LiPA_{25}$ (version 1) manufactured by Labo Biomedical products, Rijswijk, The Netherlands, based on licensed Innogenetics SPF10 technology, and type-specific (TS) PCR TS16/TS18 DEIA assays were used to test cervical samples and biopsy material for HPV DNA from 11 non-oncogenic HPV types (6, 11, 34, 40, 42, 43, 44, 53, 54, 70 and 74) and 14 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, 73).[13,24,25] These types were selected based on the IARC list of oncogenic HPV types even if the evidence for the carcinogenicity of HPV-66 is currently thought to be limited.[26]

Identification of an incident infection with a previously undetected HPV type by PCR from a cytological sample taken within a minimum of 5 months from the baseline sample during the follow-up time was a PATRICIA study endpoint. In the current study, stratification of the subjects was performed as follows: those who were at least once HPV DNA positive for one of the above-mentioned 14 oncogenic HPV types or HPV-6 or 11, and those always HPV DNA negative. Statistical analyses were performed in the former.

Incident infection was defined as identification of one of the 12 oncogenic HPV types or HPV-6/11 by PCR in a sample subsequent to a preceding sample with a defined, positive or negative among HPV16/18, 31, 33 or 45 negatives, HPV-16 or HPV-18 PCR finding. This resulted in 242 and 250 cases eligible for statistical analysis in the HPV16/18 and HAV vaccine arms, respectively.

Statistical analysis

We calculated odds ratios [OR with for 95% confidence intervals (95% CI)] for occurrence of hrHPV infections in baseline HPV DNA-negative women.

Time elapsed between withdrawals of two consecutive and two consecutive positive samples were 6 months and 6–48 months, respectively. Hence, we used person–time-based statistical approach to evaluate if a cervical infection as indicated by baseline HPV-16 or HPV-18 PCR positivity was associated with an incident cervical HPV type 6, 11, 16, 18,

31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68/73 infection identified by PCR at the study visits.

We calculated incidence rates per 1,000 person years, and used a Poisson regression model[19] to calculate incidence rate ratios (IRR) with 95% CI. This was done to estimate the risk of a new infection with the above-mentioned HPV types after stratification by the initial baseline infection indicated by PCR positivity for at least one vaccine HPV type (HPV-16 or HPV-18) compared to those negative for the vaccine HPV types. A deviance test was used to evaluate the fit of the main effect Poisson models. We repeated the crude IRR analyses to evaluate the possible association of community vaccination coverage (under or over 15%) and *Chlamydia trachomatis* status at baseline. In the adjusted analyses and interaction models, only variables with two-sided p-value of <0.07 according to Wald χ^2 test for individual regression coefficient were included. Finally, the Poisson regression models were fitted to adjust for confounding factors, that is, vaccination coverage and risk-taking sexual behaviour (*C. trachomatis* PCR screening was performed annually, and positive result was a surrogate for risk taking behaviour). All the statistical analyses were performed using PASW 18.0 (SPSS, Chicago, IL) and the Genmod procedure of SAS 9.1 (SAS Inst, Cary, NC).

Results

A total of 24,046 adolescents under 18 years of age (born in Q2/1986-Q1/1988) from Finland were invited to participate in the 4-year PATRICIA trial (Fig. 1)[22–24] from 18 study-site communities (of which two adjacent communities were combined after enrolment in 2005) between May 2004 and April 2005. A total of 4,808 adolescents participated. Vaccination coverage varied considerably by community: from 2 to 44%, HPV-16/18 vaccination coverage was half of this. Three communities had HPV-16/18 vaccination coverage above 15%. Retention in the 4-year follow-up was 93% with minimal variation between the communities.

First, we studied the occurrence of non-vaccine covered hrHPV types in baseline HPV DNA-negative HPV-16/18-vaccinated women as compared to baseline HPV DNA-negative HAV-vaccinated women. No increased risk (as indicated by OR) of overall hrHPV occurrence in 1,708 baseline HPV DNA-negative HPV-16/18-vaccinated women was observed (Table 1). The risk of HPV-35 occurrence (OR 0.6, 95% CI = 0.4–0.9) was significantly decreased in the HPV-16/18-vaccinated women.

We then studied the acquisition of HPV types in baseline HPV-16- or HPV-18-positive women, who have increased likelihood of multiple infections, as compared to baseline HPV-16- or HPV-18-negative women by the vaccination status. HAV-vaccinated baseline HPV-16-positive women (n = 56) appeared to have an increased risk to acquire HPV-6 and HPV-11 (IRR 1.7, 95% CI = 0.96–2.8) as compared to baseline HPV-16-negative women. Corresponding baseline HPV-18 status was associated with an increased risk of acquiring high-risk HPV types in the clade A7 (IRR 1.8. 95% CI = 1.01–3.1, Table 2). Following adjustment for *C. trachomatis*, the HPV-16/18-vaccinated women showed no excess risk for acquisition of lr or hrHPV types in the baseline HPV-16-positive women (n = 53) as compared to baseline HPV-16-negative women (Table 2).

Table 1. Risk of occurrence of high-risk HPV types not covered by the HPV-16/18 vaccine in HPV-16/18-vaccinated baseline HPV DNA-negative individuals (N_1 = 1,708) compared to HAV-vaccinated baseline HPV DNA-negative individuals (N_2 = 1,749) among Finnish PATRICIA participants followed up by cervical sampling every 6 months for 4 years[1]

Follow-up findings	HPV-16/18 vaccinated	HAV vaccinated	OR (95% CI)	p-Value
HPV35	33	60	0.6 (0.4–0.9)	0.004
HPV39	129	140	0.9 (0.7–1.2)	0.3
HPV52	232	253	0.9 (0.8–1.1)	0.2
HPV58	100	110	0.9 (0.7–1.2)	0.3
HPV59	97	100	1.0 (0.7–1.3)	0.5
HPV68	181	217	0.8 (0.7–1.0[2])	0.05

[1]Crude OR with 95% CI. [2]1.03.

Next, we determined the adjusted IRRs for the hrHPV types (16/18/31/33/45/51), at least partially covered by the HPV-16/18 vaccine induced cross-protective immune response, in the baseline HPV-16- or HPV-18-positive women compared to baseline HPV-16- or HPV-18-negative women among the HPV-16/18 and HAV-vaccinated women (Table 3). Again among the HAV vaccinated, but not HPV-16/18 vaccinated, baseline HPV-18-positive women appeared to have an increased risk of acquiring HPV type 45 (IRR 2.5, 95% CI = 0.98–6.4). In the HPV-16/18 vaccinated, the adjusted IRR estimates for risk of acquiring the other hrHPV types at least partially covered by HPV-16/18 vaccine showed no excess risk (Table 3).

Finally, we determined the IRRs for the hrHPV types not covered by the HPV-16/18 vaccine induced immune response in originally HPV-16- or HPV-18-positive women by vaccination status (Table 4). No excess risk of acquiring HPV types (35/39/52/58/59/66/68), for which the bivalent vaccine has not been reported to induce cross-protective immune response, was observed when baseline HPV-16- or HPV-18-positive and -negative women were compared. Between the HPV-16/18 vaccine and the HAV vaccine recipients, these estimates were similar.

Discussion

We found no evidence of type replacement among adolescents enrolled in a phase III HPV-vaccination trial with low-vaccination coverage. HAV-vaccinated baseline HPV-18-positive women had an increased risk of acquiring HPV clade 7 (HPV39/45/59/68) types during 4 years.

The question whether less common HPV types will increase once oncogenic HPV types 16 and 18 are reduced by HPV vaccination is important.[27] As predicted, 15 years

Table 2. Risk of acquiring HPV clade A5, A7, A9 or A10 types in baseline HPV16- or HPV18-positive individuals compared to baseline HPV16/18-negative individuals among HPV-16/18 or HAV-vaccinated Finnish PATRICIA participants with at least one positive HPV DNA finding ($N = 242$ and 250, respectively) followed up by cervical sampling every 6 months for 4 years[1]

	Follow-up findings							
Baseline status	HPVA5 Pos[2] IRR (95% CI)	Adjusted[3] HPVA5 Pos[2] IRR (95% CI)	HPVA7 Pos[2] IRR (95%CI)	Adjusted[3] HPVA7 Pos[2] IRR (95% CI)	HPVA9 Pos[2] IRR (95% CI)	Adjusted[3] HPVA9 Pos[2] IRR (95% CI)	HPVA10 Pos[2] IRR (95% CI)	Adjusted[3] HPVA10 Pos[2] IRR (95% CI)
HPV16/18 vaccinated								
HPV16/18 neg ($n = 165$)	(65) 1.0	1.0	(68) 1.0	1.0	(76) 1.0	1.0	(50) 1.0	1.0
HPV16 pos[4] ($n = 53$)	(21) 0.9 (0.6,1.5)	1.0 (0.6,1.6)	(20) 0.9 (0.5,1.4)	0.9 (0.5–1.4)	(27) 1.2 (0.7,1.8)	1.2 (0.8,1.9)	(7) 0.4 (0.2,0.9)	1.0 (0.6,1.7)
HPV18 pos[4] ($n = 24$)	(9) 1.2 (0.6,2.3)	1.1 (0.5,2.2)	(8) 0.9 (0.4,1.8)	0.9 (0.4–1.8)	(8) 0.8 (0.4,1.7)	0.9 (0.4,1.8)	(7) 1.3 (0.6,2.9)	1.2 (0.6–2.7)
HAV vaccinated								
HPV16/18 neg ($n = 168$)	(54) 1.0	1.0	(75) 1.0	1.0	(107) 1.0	1.0	(38) 1.0	1.0
HPV16 pos[4] ($n = 56$)	(21) 1.2 (0.7,1.9)	0.9 (0.5,1.6)	(32) 1.3 (0.9,1.9)	1.3 (0.8,1.9)	(35) 0.9 (0.6,1.3)	0.9 (0.6,1.3)	(21) 1.7 (1.0^5,2.9)	1.7 (1.0^5,2.8)
HPV18 pos[4] ($n = 26$)	(11) 1.3 (0.7,2.6)	1.0 (0.4,2.2)	(15) 1.7 (1.0^5,2.9)	1.8 (1.0^6,3.1)	(15) 1.0 (0.6,1.6)	0.9 (0.5,1.6)	(6) 1.0 (0.4,2.4)	1.1(0.5,2.6)

[1]Crude/adjusted incidence rate ratios (IRR) with 95% CI. [2]Number ( ) of positives for A5 = HPV51, or A7 = any of HPV39/45/59/68, or A9 = any of HPV31/33/35/52/58, or A10 = any of HPV6/11. [3]Poisson regression analysis: adjusted for *C. trachomatis* and community vaccination coverage. [4]Baseline positives for HPV16 or HPV18 only. [5]<1.0. [6]>1.0.
The *n* numbers in parentheses represent the number of cases.

ago[1] *S. pneumoniae* type-replacement has taken place in the 12 countries where mass vaccination, with one of the multivalent pneumococcal conjugate vaccines, has been implemented, usually within <5 years.[2] With low-vaccine coverage also, the increased incidence of invasive pneumococcal diseases has become visible.[2] Multiple infections with hrHPVs are common and cluster[14–19] also over time[19,28] but the nature of competition between HPV types (co-infection *vs.* super-infection) is not fully understood.[19–21,29–34] On the other hand, the extent of cross-protection against a number of related hrHPV types varies considerably following vaccination with the quadrivalent HPV-6/11/16/18 or bivalent HPV-16/18 vaccines.[11–13] This makes the situation even more challenging. Given that type replacement has been reported in most pneumococcal vaccination studies within 5 years of vaccination,[1,2] the 4 years of follow-up and up to 22% vaccine coverage among three birth cohorts justified evaluation of type replacement following HPV vaccination in our 17 study-site communities. Virtually, no opportunistic vaccination took place during the follow-up time 2004–2009 (<1,000 doses/year) in Finland.

As expected,[23] the occurrence of HPV-16/18 vaccine covered hrHPV types 16, 18, 31, 33, 45 and 51 was significantly reduced in HPV-16/18 vaccinated as compared to HAV-vaccinated women (data not shown). After noting that there is no difference except for HPV-35, which is partially covered by the HPV-16/18 vaccine,[13,23] in the occurrence of non-vaccine-covered hrHPV types in these two groups of baseline hrHPV DNA-negative women, we evaluated the risk of acquiring non-vaccine HPV types over time among baseline HPV-16/18 DNA-positive women. Due to risk-taking behaviour women, with HPV-16/18 infections have a risk of acquiring new HPV infections as shown both by PCR and by the less sensitive serology.[15–19,28,29] This was the subpopulation where type replacement might have been noted in a close follow-up.

It was reassuring that we found no evidence of increased risk for new infections with genital non-vaccine HPV types during bi-annual liquid-phase cytology/PCR 4-year follow-up. Previously, an antagonistic interaction between HPV-6/11 and HPV-16 in early stages of cervical carcinogenesis has been reported.[35,36] In the unadjusted analyses, we found a borderline significant increased risk (IRR = 1.7) for HPV-6/11 in HAV vaccinated, but a decreased risk (IRR = 0.4) for HPV-6/11 in HPV-16/18 vaccinated originally HPV-16-positive women. In the absence of HPV-16/18 vaccine protection, occurrence of HPV-16 and HPV-6/11 infections may cluster in sexually active women. The decreased risk of acquiring HPV-6/11 among HPV-16/18 vaccinated, however, vanished following adjustment for a surrogate of sexual risk-taking behaviour and vaccination coverage. The possibility of a change observation due to multiple comparisons must be noted.

Lehtinen *et al.* have previously shown strong clustering of HPV-16 and HPV-18 seropositivity in pregnant women aged 23–31 years.[28] In addition, the Finnish research group also

Table 3. Risk of acquiring HPV types at least partially covered by the HPV-16/18 vaccine in baseline HPV16- or HPV18-positive individuals compared to baseline HPV16/18-negative individuals among HPV-16/18 or HAV-vaccinated Finnish PATRICIA participants with at least one positive HPV DNA finding ($N = 242$ and 250, respectively) followed up by cervical sampling every 6 months for 4 years[1]

					Follow-up findings			
Baseline status	HPV16/18/31/33/45/51[2] IRR (95% CI)	Adjusted[3] HPV16/18/31/33/45/51 IRR (95% CI)	HPV31/33/45/51[2] IRR (95% CI)	Adjusted[3] HPV31/33/45/51 IRR (95% CI)	HPV31/33[2] IRR (95% CI)	Adjusted[3] HPV31/33 IRR (95% CI)	HPV45[2] IRR (95% CI)	Adjusted[3] HPV45 IRR (95% CI)
HPV16/18 vaccinated								
HPV16/18 neg ($n = 165$)	(96) 1.0	1.0	(87) 1.0	1.0	(67) 1.0	1.0	(7) 1.0	1.0
HPV16 pos[4] ($n = 53$)	(30) 0.9 (0.6,1.4)	1.0 (0.6,1.4)	(26) 0.9 (0.6,1.4)	0.9 (0.6,1.4)	(11) 0.5 (0.3,1.0[5])	1.0 (0.5,2.1)	(0) 0.0 (0.0)	0.0 (0.0)
HPV18 pos[4] ($n = 24$)	(17) 1.5 (0.9,2.6)	1.6 (0.9,2.7)	(11) 1.0 (0.5,1.9)	1.0 (0.5,1.8)	(3) 0.3 (0.1,1.1)	0.8 (0.2,2.5)	(0) 0.0 (0.0)	0.0 (0.0)
HAV vaccinated								
HPV16/18 neg ($n = 168$)	(118) 1.0	1.0	(100) 1.0	1.0	(67) 1.0	1.0	(16) 1.0	1.0
HPV16 pos[4] ($n = 56$)	(38) 0.9 (0.6,1.3)	0.9 (0.6,1.3)	(34) 1.0 (0.7,1.4)	1.0 (0.7,1.5)	(26) 1.1 (0.7,1.7)	1.1 (0.7,1.7)	(1) 0.2 (0.0,1.2)	0.2 (0.0,1.3)
HPV18 pos[4] ($n = 26$)	(20) 1.4 (0.9,2.3)	1.4 (0.9,2.3)	(18) 1.4 (0.9,2.3)	1.4 (0.9,2.4)	(11) 1.1 (0.6,2.2)	1.1 (0.6,2.2)	(6) 2.5 (1.0[5],6.3)	2.5 (1.0[5],6.4)

[1]Crude/adjusted IRR with 95% CI. [2]Number ( ) of positives for HPV16/18/31/33/45/51, or HPV31/33/45/51, or HPV31/33, or HPV45. [3]Poisson regression analysis: adjusted for *C. trachomatis* and community vaccination coverage [4]Baseline positives for HPV16 or HPV18 only. [5]<1.0.
The *n* numbers in parentheses represent the number of cases.

Table 4. Risk of acquiring HPV types not covered by the HPV-16/18 vaccine in baseline HPV16- or HPV18-positive individuals compared to baseline HPV16/18-negative individuals among HPV-16/18 or HAV-vaccinated Finnish PATRICIA participants with at least one positive HPV DNA finding ($N = 242$ and 250, respectively) followed up by cervical sampling every 6 months for 4 years[1]

			Follow-up findings			
Baseline status	HPV39/59/68[2] IRR (95% CI)	Adjusted[3] HPV39/59/68 IRR (95% CI)	HPV35/52/58[2] IRR (95% CI)	Adjusted[3] HPV35/52/58 IRR (95% CI)	HPV35/39/52/58/59/66/68[2] IRR (95% CI)	Adjusted[3] HPV35/39/52/58/59/66/68 IRR (95% CI)
HPV16/18 vaccinated						
HPV16/18 neg ($n = 165$)	(64) 1.0	1.0	(72) 1.0	1.0	(96) 1.0	1.0
HPV16 pos[4] ($n = 53$)	(20) 0.9 (0.6-1.6)	1.0 (0.6,1.6)	(26) 1.1 (0.7,1.7)	1.1 (0.7,1.8)	(35) 1.2 (0.8,1.7)	1.2 (0.8,1.8)
HPV18 pos[4] ($n = 24$)	(8) 1.0 (0.5–2.0)	0.9 (0.5,2.0)	(10) 1.2 (0.6,2.3)	1.2 (0.6,2.4)	(11) 0.9 (0.5,1.7)	0.9 (0.5,1.8)
HAV vaccinated						
HPV16/18 neg ($n = 168$)	(70) 1.0	1.0	(84) 1.0	1.0	(104) 1.0	1.0
HPV16 pos[4] ($n = 56$)	(31) 1.4 (0.9–2.1)	1.4 (0.9,2.1)	(33) 1.3 (0.9,1.9)	1.3 (0.9,2.0)	(40) 1.2 (0.9,1.8)	1.3 (0.9,1.8)
HPV18 pos[4] ($n = 26$)	(12) 1.3 (0.7–2.3)	1.3 (0.7,2.5)	(11) 0.8 (0.4,1.6)	0.9 (0.5,1.6)	(15) 1.0 (0.6,1.7)	1.0 (0.6–1.8)

[1]Crude/adjusted IRR with 95% CI. [2]Nnumber ( ) of positives for HPV39/59/68 or HPV35/52/58 or HPV35/39/52/58/66/68. [3]Poisson regression analysis: adjusted for *C. trachomatis* and community vaccination coverage. [4]Baseline positives for HPV16 or HPV18 only.
The *n* numbers in parentheses represent the number of cases.

reported an increased risk for seroconversion by another hrHPV type in HPV-infected women, especially for subsequent incident infection (confirmed by seroconversion) with HPV-16 in HPV-18 seropositive women, and *vice versa*.[19,29] These observations favour a co-infection model rather than superinfection model in the unvaccinated population due to risk-taking sexual behaviour. Recently, however, observed HPV-33 occurrence in the unvaccinated population was associated with competitive advantage over the other hrHPV types, a possible indication of superinfection potential.[19] Moreover, in the follow-up of a large placebo vaccinated cohort, HPV-33 had an equal likelihood of reappearance as HPV-16 and HPV-18, higher than that of any other non-vaccine hrHPV type, even if its overall incidence was respectively six and two times lower.[37] Thus, it is worth noting that in our study we did not find an increased occurrence of HPV-31/33 (HPV-33, data not shown) or HPV-45 (which totally vanished) in the HPV-16/18 vaccinated women during the 4-year follow-up. On the contrary, in the HAV vaccine recipients significant clustering of HPV-18 and other clade A7 (HPV39/45/59/68) infections was found. The HPV-45 observation is probably due to high cross-protection against HPV type 45 in the HPV-16/18 vaccine recipients.[13,38]

The strengths of our study were population-based nature of the cohorts, low drop-out rate, 4 years active follow-up with repeat cytological sampling and the use of sensitive SPF10-PCR methodology. Adjustment for *C. trachomatis* serostatus, a surrogate of sexual risk taking behaviour and for HPV16/18 vaccination coverage not interacting with baseline HPV status (data not shown) were also important.

As a limitation, the vaccination coverage exceeded 20% in only a few communities. Although our phase III trial enrolment in 2004–2005 was followed by licensure of the HPV6/11/16/18 and the HPV16/18 vaccines in Finland in 2006 and 2007, respectively, and a large community randomized trial involving 33 communities, which largely overlapped with the 17 study-site communities started in 2007[38] the coverage (overall or by birth cohorts) may have been too low to create an ecological niche for type replacement. An additional limitation of our study is that the follow-up time was too short to evaluate invasive disease end points; however, these will be captured in the long-term follow-up.[22] In any case, the present analysis is the first attempt to evaluate the presence of type replacement in a random setting approaching HPV vaccination program. Surveillance of HPV type distribution is ongoing in the large Finnish community randomized trial cohorts with up to 50 and 35% HPV-16/18 vaccination coverage in girls only or girls and boys, respectively, until 2015[39] (in preparation).

In conclusion, our study suggests that HPV type replacement does not take place following mass vaccination. However, surveillance of community randomized trial cohorts and other populations in countries which have implemented HPV vaccination programs immediately after licensure of the vaccines, with a focus on vaccination coverage rates are warranted.

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