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Evaluation of HPV type-replacement in unvaccinated and vaccinated adolescent females—*Post-hoc* analysis of a community-randomized clinical trial (II)

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Efficacy of human papillomavirus (HPV) vaccines promises to control HPV infections. However, HPV vaccination programs may lay bare an ecological niche for non-vaccine HPV types. We evaluated type-replacement by HPV type and vaccination strategy in a community-randomized trial executed in HPV vaccination naïve population. Thirty-three communities were randomized to gender-neutral vaccination with AS04-adjuvanted HPV16/18 vaccine (Arm A), HPV vaccination of girls and hepatitis B-virus (HBV) vaccination of boys (Arm B) and gender-neutral HBV vaccination (Arm C). Resident 1992-95 born boys (40,852) and girls (39,420) were invited. 11,662 boys and 20,513 girls were vaccinated with 20–30% and 45–48% coverage, respectively. HPV typing of 11,396 cervicovaginal samples was performed by high throughput PCR. Prevalence ratios (PR) between arms and ranked order of HPV types and odds ratio (OR) for having multiple HPV types in HPV16 or 18/45 positive individuals were calculated. The ranked order of HPV types did not significantly differ between arms or birth cohorts. For the non-HPV vaccinated 1992–1993 birth cohorts increased PR, between the gender-neutral intervention versus control arms for HPV39 (PR_A 1.84, 95% CI 1.12–3.02) and HPV51 (PR_A 1.56, 95% CI 1.11–2.19) were observed. In the gender-neutral arm, increased clustering between HPV39 and the vaccine-covered HPV types 16 or 18/45 ($OR_{A16} = 5.1$, $OR_{A18/45} = 11.4$) was observed in the non-HPV vaccinated 1994–1995 birth cohorts. Comparable clustering was seen between HPV51 and HPV16 or HPV18/45 ($OR_{B16} = 4.7$, $OR_{B18/45} = 4.3$), in the girls-only arm. In conclusion, definitively consistent postvaccination patterns of HPV type-replacement were not observed. Future occurrence of HPV39 and HPV51 warrant investigation.

In clinical phase III trials the three licensed human papillomavirus (HPV) vaccines have been very efficacious (92–100%) against persistent cervical infections and high-grade squamous intraepithelial lesions (HSIL) caused by the vaccine included HPV types.[1,2] The bivalent HPV16/18 and quadrivalent HPV6/11/16/18 vaccines have been shown to protect

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What's new?

Vaccination against high-risk human papilloma virus (HPV) strains is efficacious, but possible resurgence of non-targeted viral strains is a concern. The authors performed a community-randomized study with 20–50% vaccination coverage in 1992–95 birth cohorts of 80,000 adolescents. They compared gender-neutral or girls-only HPV16/18 vaccination or hepatitis B-virus vaccination in 11 communities, and a consistent pattern of HPV type-replacement was not found. However, occurrence of HPV39 and HPV51 types warrants further observation in the future.

also against HSIL associated with high-risk HPV types 31 and HPV types 31, 33 and 45, respectively,[3,4] presumably due to cross-neutralizing antibodies induced by the vaccines.[5]

A concern has, however, been raised, that following implementation of national HPV vaccination programs the resulting reduction in the prevalence of vaccine-covered HPV types could clear the ecological niche for the non-targeted HPV types.[6] In agreement, type-replacement has been observed in a number of countries following pneumococcal vaccination.[7] However, thus far HPV type-replacement has not been observed at the individual level among HPV vaccinated females with low vaccination coverage in the target population.[8,9] A prerequisite of HPV type-replacement, that is, competition between HPV types in unvaccinated populations, has not been commonly found.[10–12] Implementation of national HPV vaccination programs, however, tends to increase prevalence of some non-vaccine covered HPV types suggesting type-replacement.[13]

HPV epidemiology of vaccination-naïve populations shows that multiple HPV type infections are rare in women with normal cytology (<3%),[14] very common in women with precancer lesions (15–41%)[15] and again rare in women with HPV-related cervical cancer (<12%).[16] In this study, we explored the possible signs of type-replacement by comparing occurrence of single and multiple HPV types up to five years after community-randomized introduction of gender-neutral or girls-only HPV vaccination with moderate vaccination coverage.

Materials and Methods

Study design

The material was obtained from the community randomized trial of the ASO4-HPV-16/18 vaccine (Cervarix®) sponsored by GlaxoSmithKline.[17,18] Briefly, all 80,272 resident Finnish or Swedish speaking boys and girls as identified using the population register were invited in 33 Finnish communities randomly assigned to one of three study arms (Fig. 1): Arm A was gender-neutral, Arm B was gender-specific and Arm C served as the control arm. In the Arm A, 90% of all the participants in each community were randomly selected to receive Cervarix® and 10% to receive Engerix-B™. In the Arm B, 90% of the female study population in each community were randomly selected to receive Cervarix® and 10% to receive Engerix-B™, whilst the males all received Engerix-B™. In the Arm C, all of the participants were given Engerix-B™. The vaccination status of all the participants in Arm A and all the female participants in Arm B were receiver-blinded.

In total, 32,175 participants born in 1992–1995 were initially recruited with informed parental/guardian consent in to the 3 study arms (20,514 females and 11,661 males) at the study baseline, in 2007–2009. Ninety-nine point four percent received three vaccine doses. All females from the study communities, both participants and non-participants were invited to a follow-up visit between the years 2010–2014, at the age of 18.5–19 years, within 3–5 years from vaccination. A total of 14,518 females attended this follow-up visit: 4,922 from Arm A, 5,247 from Arm B and 4,349 from Arm C. During the follow-up session, a self-collected cervicovaginal sample was obtained and a cervical sample was obtained by a study nurse. The participants consented to take part in *Chlamydia trachomatis* screening, and participants completed a questionnaire about life-style factors, mobility and their sexual health.[17] Residential history data was also available from the population registry.

The ethical committees for the Pirkanmaa and Pohjois-Pohjanmaa hospital districts granted the HPV-040 study (EUDRA-CT-2007–001731-55, NCT00534638) approval in 2007, and the ancillary *C. trachomatis* screening study (111/2009) approval in 2009, respectively.

Laboratory analysis

The samples collected at the first follow-up visit were analyzed using modified general primer (MGP) PCR followed by matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry (MS).[19,20] The MGP PCR used type-specific consensus primers to replicate specific types of HPV DNA. Every type-specific consensus primer used had a particular molecular weight, which was detected using MALDI-TOF MS to identify the presence of HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66.[20] Confirmative analysis of samples positive for HPV 11 was performed using MGP PCR followed by Luminex,[19] due to interactions between HPV11 and 89 for the HPV11 primer, in order to correctly distinguish between HPV11 and HPV89.

Statistical analysis

The prevalence ratios [PR with 95% confidence interval (CI)] of specific HPV types (HPV6, 11, 16, 18, 31, 33, 35, 39, 45,

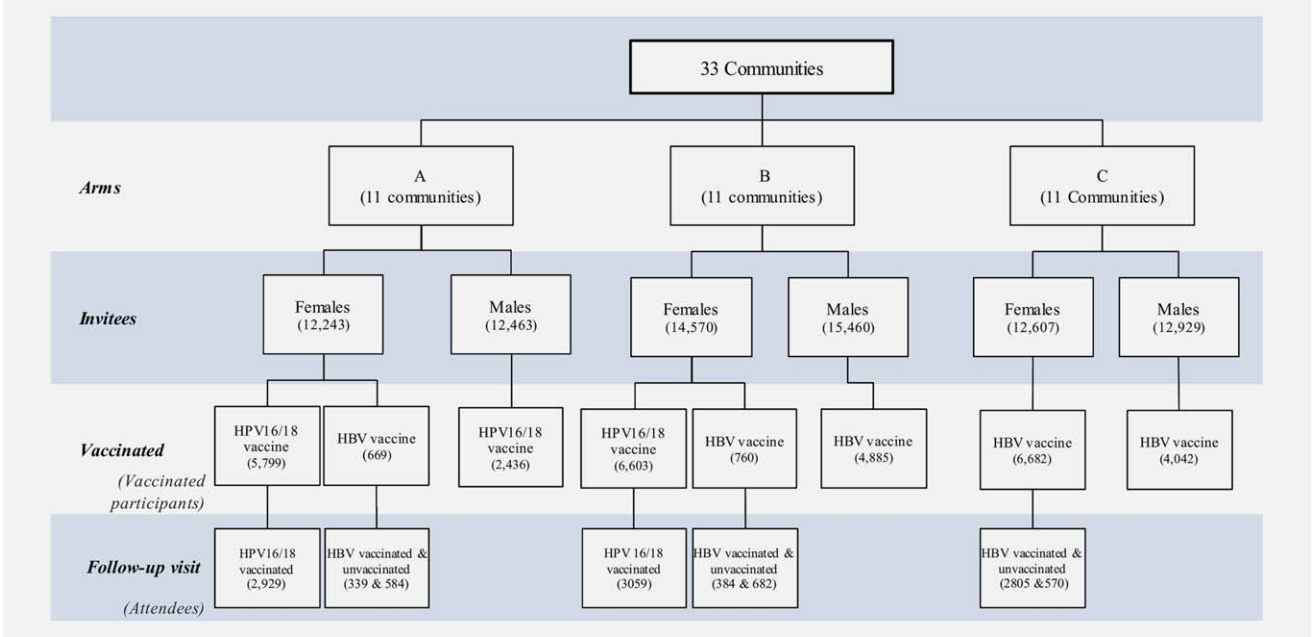

Figure 1. Flow chart of the community-randomized trial. [Color figure can be viewed at wileyonlinelibrary.com]

51, 52, 56, 58, 59 and 66) between Arm A and C, and Arm B and C were estimated by log-binomial regression.

As a sensitivity analysis odds ratios (OR with 95% CI) of having another HPV type among those positive for the vaccine HPV types 16 or 18, or the strictly vaccine covered type HPV45 were estimated by arm using those negative for HPV16 or for HPV18/45 as the reference group applying binomial logistic regression. The PR and OR estimates were adjusted for participant mobility, for community-level prevalence of regular smoking and for individual level *C. trachomatis* status, reflecting the risk-taking behavior.[18]

The between-arms relationships of HPV type prevalence ranks were assessed by comparing Spearman's rank correlation coefficients.

In our first impact of vaccination strategies article,[18] herd effects at the follow-up of age 18.5 years were observed to be stronger in the later than earlier age cohorts. Thus, all the above-mentioned estimates were computed separately for 1992–1993 and 1994–1995 cohorts.

Both participants who had migrated from control arm communities to Arm A or B, and those who had migrated from Arm A or B communities to control Arm C, were excluded from the analysis ($N = 92$). However, participants moving from Arm A to B ($N = 31$), and Arm B to A ($N = 34$) were included. Due to the follow-up invitations of non-participants, the HBV vaccinated:unvaccinated ratio was different in Arm C compared with Arm A and Arm B in non-HPV-vaccinated women. The difference was corrected for in the PR and OR estimation (Fig. 2 and Table 3): Twenty-one samples, one eighth of HBV vaccinated Arm C women each, were randomly selected from 44 birth year-community strata. Each sample was joined with all Arm C unvaccinated women. The PR estimate was the mean of 21 random sample-specific estimates. The 95% CIs were estimated by a homogenization-based approach, described in Ref. 18.

The statistical analyses were conducted using SPSS statistical software version 23.0 (IBM Corp, Armonk, NY) and R statistical software version 3.3.2 with Epi package (version 2.15, The R Foundation; https://www.r-project.org/). The values of trigamma functions for homogenization-based CIs were calculated using SAS 9.4 (SAS Inst. Inc., Cary, NC).

Results

Demographics and behavioral characteristics of the trial arms

Entire 1992–1995 Finnish and Swedish speaking female and male birth cohorts of the 33 randomized communities were invited to the trial on the effectiveness of HPV vaccination strategies (Fig. 1). Participation rates at the study baseline and attendance rates to the follow-up visit at study end were equal in the different groups of vaccination participants and attendees of the cytological sampling (Fig. 1).

Demographic and risk-taking behavior characteristics of the study arms revealed no major differences between the HPV vaccinated and non-HPV vaccinated women (Table 1). However, more non-HPV vaccinated women had 5 or more life-time partners (19.2 and 16.7%) in intervention Arms A and B with gender-neutral and girls-only HPV vaccination, respectively, than in the control Arm C (13.7%) with HBV vaccination. In addition, more non-HPV vaccinated Arm A participants were current smokers (34.2%) in comparison to the non-HPV vaccinated Arm C participants (30.3%) (Table 1).

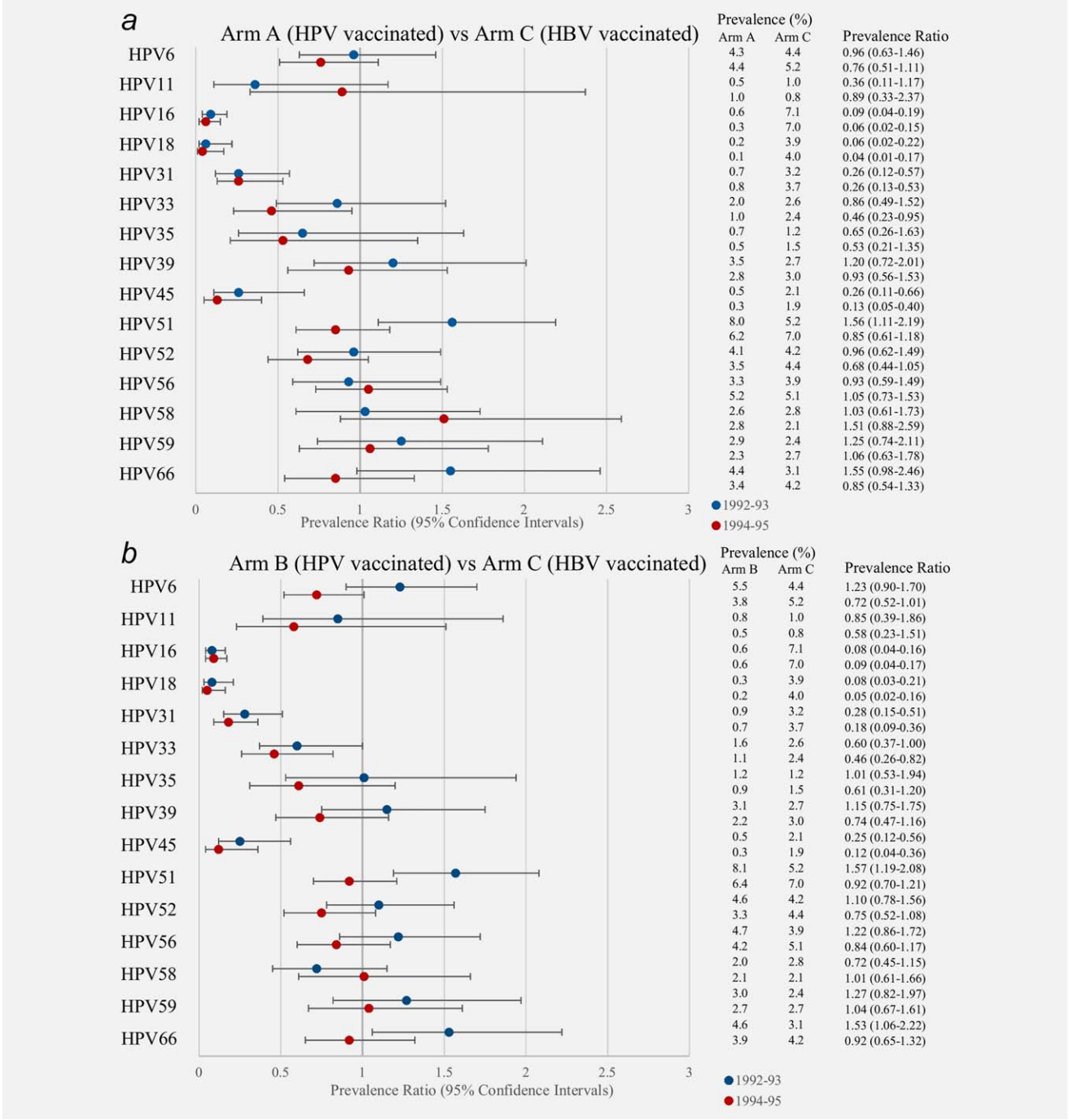

Figure 2. PR (95% CI) estimates of HPV types in HPV16/18 vaccinated, (*a*) and (*b*), and in non-HPV vaccinated, (*c*) and (*d*), stratified by birth cohort and adjusted for mobility, and smoking and *C. trachomatis* status. (*a*) Arm A versus Arm C, (*b*) Arm B versus Arm C, (*c*) Arm A versus Arm C and (*d*) Arm B versus Arm C.

HPV PRs in HPV vaccinated and non-HPV vaccinated women

The effect of HPV vaccination in the HPV16/18 vaccinated 1994–1995 birth cohorts extended over five HPV types: 16/18/31/33/45 in the PR analysis (Figs. 2*a* and 2*b*). As for the nonvaccine covered HPV types, no major differences in the PRs between the intervention Arms A or B and the control Arm C were observed in the non-HPV16/18 vaccinated women (Figs. 2*c* and 2*d*). Amongst increased PRs there, however, was a pattern of 3 out of 4 significantly increased HPV51 PRs in both vaccinated and non-HPV vaccinated 1992–1993 birth cohorts (Figs. 2*a*–2*c*).

In the 1992–1993 birth cohort the PR between the non-HPV vaccinated participants of the gender-neutral

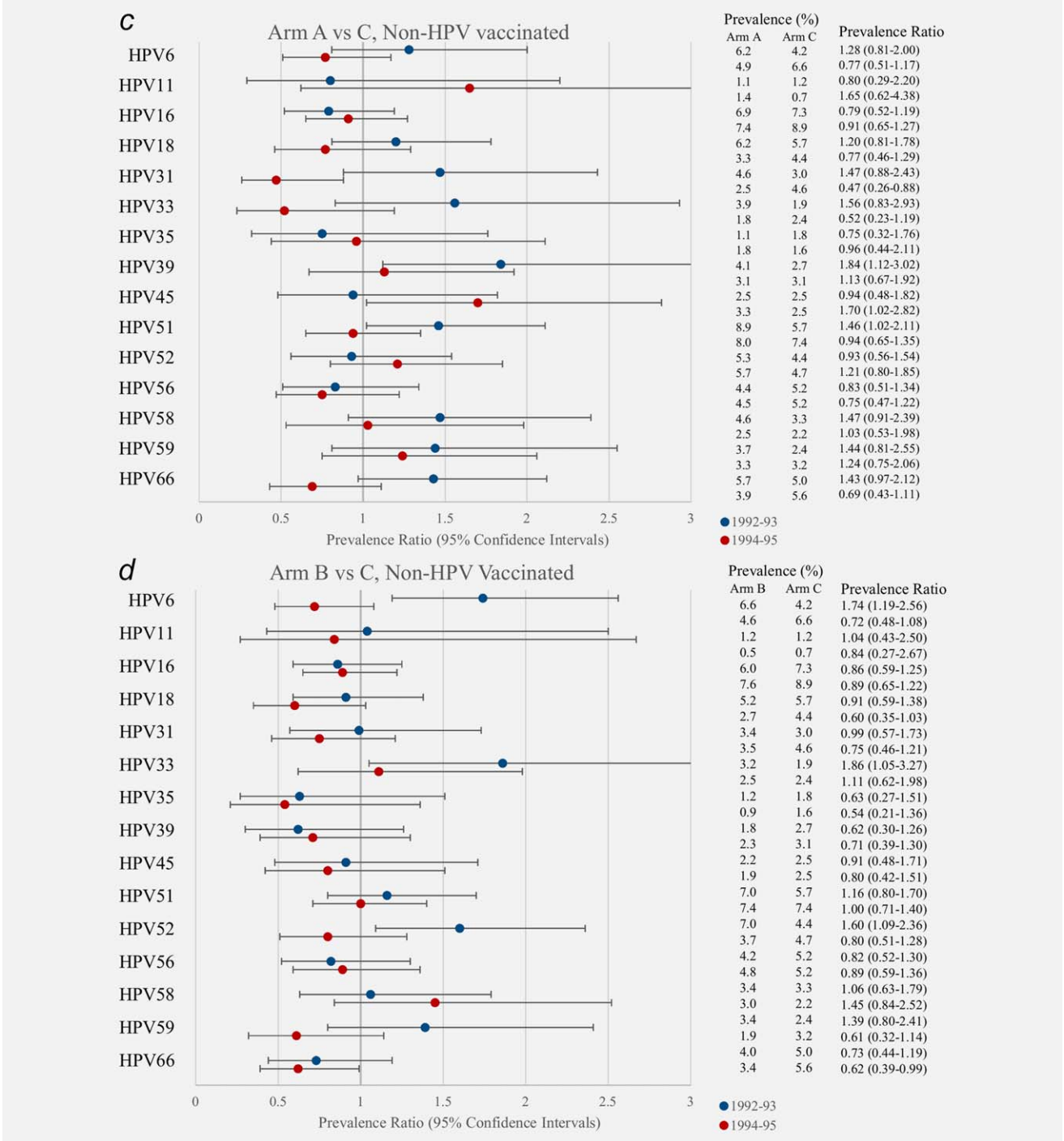

Figure 2. Continued.

intervention Arm A versus the control Arm C for HPV39 (PR_A 1.84, 95% CI 1.12–3.02) was significantly different from unity (Fig. 2*c*). In the girls-only intervention Arm B versus the control Arm C PRs for HPV6 (PR_B 1.74, 95% CI 1.19–2.56) and for HPV52 (PR_B 1.60, 95% CI 1.09–2.36) also were increased (Fig. 2*d*). In addition, in the 1992–1993 birth cohorts between the non-HPV vaccinated participants the PRs for HPV33 tended to be increased in both intervention arms (PR_A 1.56, 95% CI 0.83–2.93, PR_B 1.86, 95% CI 1.05–3.27; Figs. 2*c* and 2*d*).

With the exception of HPV45 (PR_A 1.70, 95% CI 1.02–2.82 vs. PR_B 0.80, 95% CI 0.42–1.51), no notable discrepancy in the PRs were observed between the intervention Arms A and B, and control Arm C in the 1994–1995 birth cohorts.

Table 1. Characteristics of 1992–1995 born participants attending the first follow-up visit at 18.5 years of age by study arm and vaccination status

Characteristic	Arm A HPV vac 1992–1995 *N* (%)	Non-HPV vac[1] 1992–1995 *N* (%)	Arm B HPV vac 1992–1995 *N* (%)	Non-HPV vac[1] 1992–1995 *N* (%)	Arm C HBV vac 1992–1995 *N* (%)	Non-HPV vac[1] 1992–1995 *N* (%)
Lives in study community[2]						
Yes	3,257 (90.6)	814 (79.0)	3,432 (92.7)	975 (82.6)	3,154 (92.0)	3,584 (89.9)
No	287 (7.99)	203 (19.7)	251 (6.78)	188 (15.9)	246 (7.18)	372 (9.33)
Missing	49 (1.36)	14 (1.36)	19 (0.51)	17 (1.44)	28 (0.82)	32 (0.80)
Mobility[3]						
Semi-Urban citizen at study entry	2,573 (87.9)	869 (94.1)	2,455 (80.3)	874 (82.0)	2,407 (85.8)	2,926 (86.7)
First follow-up community different	356 (12.2)	54 (5.9)	604 (19.7)	192 (18.0)	398 (14.2)	449 (13.3)
Mean age at sexual debut[4]	16.4 (1.7)[5]	na	16.3 (1.7)[5]	na	16.4 (1.7)[5]	na
No. of life-time partners[2]						
0	838 (23.3)	202 (19.6)	811 (21.9)	231 (19.6)	776 (22.6)	871 (21.8)
1	884 (24.6)	228 (22.1)	989 (26.7)	309 (26.2)	989 (28.9)	1,146 (28.7)
2	572 (15.9)	161 (15.6)	587 (15.9)	174 (14.7)	546 (15.9)	614 (15.4)
3	435 (12.1)	103 (9.99)	407 (11.0)	122 (10.3)	367 (10.7)	418 (10.5)
4	289 (8.04)	92 (8.92)	292 (7.89)	98 (8.31)	243 (7.09)	303 (7.60)
5 or more	534 (14.9)	198 (19.2)	583 (15.7)	197 (16.7)	458 (13.4)	546 (13.7)
Missing	41 (1.14)	47 (4.56)	33 (0.89)	49 (4.15)	49 (1.43)	90 (2.26)
C. trachomatis status[6]						
C. trachomatis positive	102 (3.48)	38 (4.12)	95 (3.11)	44 (4.13)	83 (2.96)	109 (3.23)
C. trachomatis negative	2,822 (96.3)	881 (95.4)	2,959 (96.7)	1,017 (95.4)	2,715 (96.8)	3,254 (96.4)
Missing	5 (0.17)	4 (0.43)	5 (0.16)	5 (0.47)	7 (0.25)	12 (0.36)
Smoking habit[2]						
Never smoked	2,108 (58.7)	587 (56.9)	2,296 (62.0)	733 (62.1)	2,111 (61.6)	2,442 (61.2)
Quit smoking	232 (6.46)	80 (7.76)	253 (6.83)	90 (7.63)	250 (7.29)	301 (7.55)
Current	1,215 (33.8)	353 (34.2)	1,115 (30.1)	342 (29.0)	1,038 (30.3)	1,208 (30.3)
Current other than cigarettes	7 (0.19)	2 (0.19)	6 (0.16)	2 (0.17)	0 (0)	3(0.08)
Missing	31 (0.86)	9 (0.87)	32 (0.86)	13 (1.10)	29 (0.84)	34 (0.85)
Vaccination coverage[7]						
<40%	334 (11.4)	na	516 (17.2)	na	169 (6.00)	na
40–50%	919 (31.4)	na	1,659 (55.3)	na	691 (24.6)	na
>50%	1,676 (57.2)	na	826 (27.5)	na	1,945 (69.3)	na

[1]Non-HPV vaccinated women consist of both HBV vaccinated and unvaccinated women.
[2]Questionnaire data obtained at the age of 18.5–19 years.
[3]Residential history data obtained from Finnish Population Registry.
[4]Questionnaire data obtained at the age of 22 years.
[5]SD.
[6]Laboratory analysis data obtained at the age of 18.5–19 years.
[7]Community-wise vaccination coverage.
Abbreviations: Arm A = gender-neutral HPV-16/18 vaccination; Arm B = girls-only HPV-16/18 vaccination; Arm C = HBV vaccination.

Multiple infections in HPV vaccinated and non-HPV vaccinated women

The PR observations of note were further elaborated by a sensitivity analysis on clustering of specific HPV types. Clustering of a number of HPV types with the vaccine or vaccine-covered HPV types 16 and HPV18/45 was evaluated as OR of being positive for another HPV type, for HPV16 or HPV18/45 positive versus HPV16 or HPV18/45 negative women. ORs for those HPV types with significantly increased PRs (HPV6/33/39/45/51/52/66) were calculated in HPV vaccinated women (Table 2) and non-HPV vaccinated women (Table 3). We observed occasional, nonsignificant, clustering

Table 2. OR (95% CI) estimates of HPV type 16 (a) or HPV18/45 (b) coinfections with other HPV types in HPV vaccinated females (Arms A and B) and HBV vaccinated females (Arm C) by vaccination strategy [gender neutral (arm A), girls-only (arm B) and no HPV vaccination (arm C)] stratified by birth cohort and adjusted for mobility, smoking and *C. trachomatis* positivity using HPV16 PCR negatives (a) and HPV18/45 PCR negatives (b) as reference groups

	OR (95% CIs)					
	Arm A		Arm B		Arm C	
HPV Type	1992–1993	1994–1995	1992–1993	1994–1995	1992–1993	1994–1995
(a) 16 (neg) as reference group						
6	na	13.1 (2.04–84.1)	2.30 (0.28–18.7)	3.22 (0.39–26.5)	4.42 (2.34–8.37)	4.80 (2.64–8.7)
18	na	na	na	na	4.18 (2.12–8.27)	5.93 (3.14–11.2)
33	na	na	na	na	1.19 (0.36–3.94)	4.42 (1.96–9.95)
39	na	na	na	5.27 (0.63–44.3)	2.09 (0.79–5.50)	3.85 (1.77–8.37)
45	na	na	na	na	2.17 (0.74–6.37)	2.33 (0.77–7.04)
51	na	2.90 (0.29–28.8)	3.62 (0.74–17.7)	na	2.02 (0.97–4.20)	3.20 (1.80–5.67)
52	na	na	2.75 (0.34–22.5)	3.49 (0.42–29.0)	3.90 (1.99–7.65)	4.14 (2.17–7.90)
66	2.84 (0.35–23.1)	5.62 (0.56–56.3)	na	3.29 (0.40–27.0)	3.32 (1.49–7.43)	3.12 (1.53–6.36)
(b) 18/45 (neg) as reference group						
6	2.39 (0.30–19.1)	3.22 (0.37–28.2)	1.28 (0.16–10.2)	4.63 (0.51–42.1)	5.51 (2.86–10.6)	2.26 (1.07–4.78)
16	na	na	na	na	3.89 (2.14–7.06)	4.79 (2.69–8.52)
33	na	na	na	na	4.62 (2.01–10.6)	1.94 (0.65–5.79)
39	3.19 (0.40–25.6)	na	9.65 (2.52–37.0)	na	2.99 (1.19–7.51)	1.23 (0.37–4.12)
51	1.25 (0.16–9.85)	na	3.19 (0.85–12.0)	2.44 (0.26–23.2)	5.41 (2.91–10.1)	2.75 (1.45–5.19)
52	na	na	6.50 (1.67–25.4)	5.60 (0.61–51.3)	4.27 (2.06–8.86)	4.16 (2.08–8.32)
66	5.13 (1.08–24.3)	3.98 (0.44–35.7)	na	na	4.29 (1.94–9.49)	3.57 (1.74–7.35)

between HPV39 and the vaccine-covered HPV16 or HPV18/45 both in the non-HPV vaccinated Arm A and Arm B women ($OR_{A16/92–93} = 4.9$, $OR_{A16/94–95} = 5.1$, $OR_{A18/45/94–95} = 11.4$, $OR_{B16/94–95} = 4.6$ and $OR_{B18/45/94–95} = 3.9$), and HPV vaccinated Arm B women ($OR_{B16/94–95} = 5.3$ and $OR_{B18/45/92–93} = 9.7$). As for HPV51, we observed significant clustering with HPV16 in the girls-only Arm B births cohorts 1994–95 ($OR_{B16/94–95} = 4.7$, 95% CI 2.10–10.5, vs. $OR_{C16/94–95} = 1.2$, 95% CI 0.64–2.07). The model did not always converge, and the CIs overlapped those of the control Arm C estimates.

For HPV33, no consistent pattern of increased ORs was observed in the HPV vaccinated women or in the non-HPV vaccinated women (Tables 2 and 3). Likewise, no consistently increased ORs were observed for HPV6 or HPV52 in the non-HPV vaccinated women or in the HPV vaccinated women (Tables 2 and 3).

Post vaccination HPV type-distributions by vaccination strategy

To further evaluate the impact of HPV vaccination generated herd effect on the HPV population biology we compared the ranked distribution of non-vaccine covered HPV types in the non-vaccinated female 1992-birth cohort (first vaccinated birth cohort) and 1995-birth cohort (last vaccinated birth cohort). Among both the 1992 and 1995 birth cohorts the correlation coefficients of HPV type distributions between Arms A (gender-neutral vaccination) and C, Arms B (girls-only vaccination) and C were high (0.79 to 0.95) and statistically indistinguishable (Fig. 3).

Discussion

Sporadic HPV39 and 51 occurrence but, no patterns suggestive of type-replacement following vaccination with the bivalent HPV16/18 vaccine and up to 20% coverage in boys and 50% coverage in girls were found in our population-based, community-randomized trial.

Despite of HPV16 epidemic documented in Finland in the 1980s and 1990s,[21,22] the prevalence rates of HPV types are relatively stable with HPV16 dominating.[14,23] This seemed to be true also for the ranked order of oncogenic HPV type prevalence rates, especially in our control Arm C, devoid of any intervention. We have previously shown significant herd effect against HPV18, 31 and 33 in our community randomized trial.[18] We now used the community randomized trial setting to discover changes in the HPV type specific occurrence in the vaccinated communities (Arm A and Arm B) subject to moderate vaccination coverage. Due to vaccine induced direct and cross-protection,[4,24] the overall prevalence of HPV types 16/18/31/33/35/45 decreased in trial Arms A and B. Comparing the oldest and the youngest birth cohorts

Table 3. OR (95%CI) estimates of human papilloma virus (HPV) type 16 (a) or HPV 18/45 (b) coinfections with other HPV types in non-HPV vaccinated females by vaccination strategy [gender neutral (arm A), girls-only (arm B) and no HPV vaccination (arm C)] stratified by birth cohort and adjusted for mobility, smoking and *C. trachomatis* positivity using HPV16 PCR negatives (a) and HPV18/45 PCR negatives (b) as reference groups

	OR (95% CIs)					
	Arm A		Arm B		Arm C	
HPV Type	1992–1993	1994–1995	1992–1993	1994–1995	1992–1993	1994–1995
(a) 16 (neg) as reference group						
6	3.40 (1.14–10.1)	4.48 (1.62–12.4)	2.35 (0.75–7.38)	3.06 (1.08–8.65)	2.02 (1.03–3.99)	4.22 (2.89–6.19)
18	5.75 (2.07–16.0)	3.44 (0.92–12.9)	1.62 (0.42–6.24)	8.86 (2.69–29.1)	4.61 (3.02–7.03)	3.36 (1.99–5.67)
33	4.42 (1.29–15.2)	5.27 (1.16–24.1)	2.35 (0.48–11.5)	5.32 (1.55–18.2)	2.65 (1.08–6.53)	4.44 (2.27–8.68)
39	4.91 (1.52–15.9)	5.14 (1.54–17.2)	na	4.64 (1.17–18.4)	na	1.60 (0.72–3.56)
45	7.79 (1.93–31.4)	4.59 (1.36–15.6)	3.42 (0.68–17.2)	1.10 (0.14–8.88)	1.89 (0.78–4.55)	na
51	1.12 (0.34–3.64)	2.26 (0.85–6.01)	0.70 (0.15–3.30)	4.69 (2.10–10.5)	1.79 (0.99–3.22)	1.15 (0.64–2.07)
52	0.75 (0.15–3.74)	2.94 (1.03–8.41)	2.79 (0.97–8.02)	4.35 (1.48–12.8)	3.39 (1.98–5.81)	5.16 (3.31–8.04)
66	1.68 (0.43–6.52)	5.29 (1.77–15.8)	2.42 (0.52–11.2)	4.18 (1.27–13.8)	4.58 (2.95–7.13)	2.99 (1.87–4.77)
(b) 18/45 (neg) as reference group						
6	3.70 (1.36–10.1)	7.48 (2.66–21.0)	4.37 (1.69–11.3)	4.40 (1.39–14.0)	4.55 (2.61–7.93)	3.75 (2.46–5.71)
16	6.08 (2.42–15.3)	3.46 (1.19–10.0)	2.36 (0.80–6.92)	4.30 (1.60–11.6)	4.26 (2.92–6.22)	2.04 (1.29–3.20)
33	2.76 (0.73–10.4)	2.54 (0.30–21.8)	1.82 (0.38–8.86)	3.95 (0.82–18.9)	5.65 (2.62–12.2)	2.17 (0.90–5.27)
39	0.54 (0.07–4.36)	11.4 (3.52–36.9)	1.73 (0.20–15.3)	3.87 (0.79–18.9)	3.07 (1.44–6.55)	2.83 (1.40–5.70)
51	1.67 (0.58–4.75)	1.56 (0.44–5.48)	4.26 (1.69–10.8)	4.26 (1.60–11.3)	4.54 (2.99–6.89)	2.68 (1.71–4.21)
52	2.90 (0.97–8.66)	6.37 (2.28–17.7)	4.58 (1.82–11.5)	na	3.55 (2.06–6.12)	4.24 (2.58–6.95)
66	3.15 (1.06–9.37)	2.24 (0.49–10.4)	3.38 (0.92–12.5)	5.41 (1.41–20.8)	4.68 (2.95–7.43)	2.68 (1.57–4.55)

no major differences in PRs between arms or the ranked order of prevalence rates for non-vaccine covered HPV types were observed in non-HPV vaccinated women. With the latter approach, we identified changes on *C. trachomatis* serotypes over decades due to population movements.[25] In this study, post-vaccination follow-up time may, however, have been too short and the sample size limited, to observe changes in the distribution of nonvaccine oncogenic HPV types.

A recent meta-analysis of 9 studies found slightly increased prevalence rates for HPV types 39 and 52 when comparing pre- and post-vaccination type-distributions.[13] Comparing intervention communities of the gender-neutral and girls-only arms with the non-intervention communities of the control arm was a comparable approach. We found significantly increased HPV39 and HPV51 PRs in the gender-neutral Arm A communities among the 1992–1993 birth cohorts, and found some evidence suggesting increased clustering of HPV39 in HPV16 or HPV18/45 positive women in the gender-neutral intervention communities among the 1994–1995 born. As for HPV51, comparable clustering was observed only in the girls-only arm. The risk of being HPV39 or HPV51 positive among HPV16 or HPV18/45 positive women was adjusted for mobility, smoking and *C. trachomatis* positivity (a surrogate of risk-taking sexual behavior) but the fact that the observations were not birth cohort or intervention arm specific may not support type-replacement. Furthermore, comparable Arm A specific clustering in the 1994–1995 born women was observed for HPV52 which showed a PR increase in the girls-only Arm B communities and 1992–1993 birth cohort only.

Post-vaccination evidence of type replacement occurring in the clustering of multiple types approach is defined as increased clustering in the intervention arms as compared with the controls arms, as the replacing type gains the competitive advantage over the vaccine protected type. Furthermore, clustering of multiple HPV infections is one of the most sensitive ways of looking for changes in the population biology of HPV types although subject to bias based on behavioral differences (risk-taking factors) and possible correlation of the occurrence of HPV types.[26,27] Thus, we used it but only as a sensitivity analysis to achieve more insight into *a priori* hypothesized prevalence rate ratio differences. Additionally, despite the large sample size, the number of some rarer HPV type-specific infections were relatively few in some cases, therefore limiting the power of this analysis. As for HPV16, its unique clearance characteristics make it difficult to compare the different HPV specific clustering

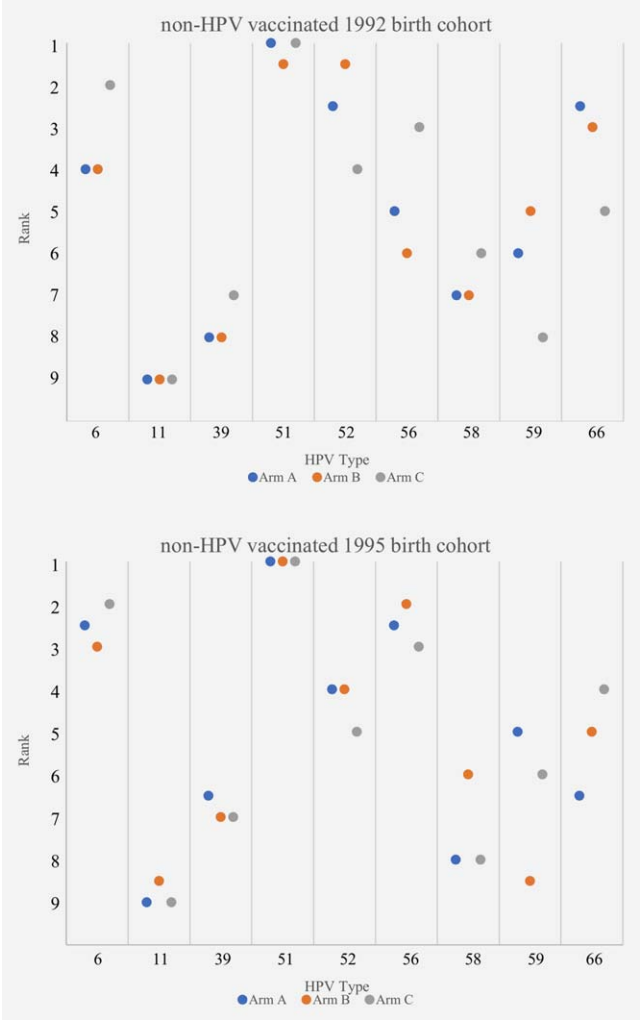

Figure 3. Ranked distribution of non-vaccine HPV types in non-HPV vaccinated women in the 1992 birth cohort [$r_s^{(Avs.C)} = 0.86$, $r_s^{(Bvs.C)} = 0.79$] and 1995 birth cohort [$r_s^{(Avs.C)} = 0.95$, $r_s^{(Bvs.C)} = 0.91$].

observations.[1] Taken together findings from the PR and clustering analyses were not definitively consistent for any HPV type.

Finally, technical unmasking of HPV52 DNA amongst a number of other HPV type DNAs following removal of, for example, HPV16 DNA by vaccination has been described as one potential bias of surveillance studies provided the PCR methodology suffers from the "unmasking" phenomenon.[26,28] Thus, it is possible that following HPV vaccination the increased prevalence of non-vaccine HPV types are not caused by type replacement but rather by unmasking. Concerning oncogenic HPV types our MALDITOF PCR, however, does not suffer from such problems.[20]

Previous studies among non-vaccinated population samples have concluded that HPV type co-infections occur at random in normal cytology, in cervical pre-cancer and in cancer lesion patients.[12,28–36] Indeed, if significant clustering of HPV types was observed it was regarded as a bias stemming from the unspecific genotyping of different HPV types.[28,29] Taken together, and in agreement with the previous studies, despite of sporadic clustering of some high risk HPV types in HPV16 or HPV18/45 positive women no definitively consistent patterns suggestive of type-replacement with non-vaccine HPV types were observed at the population level approximately up to 5 years post vaccination in this real-life community-randomized trial with up to 50% vaccination coverage by community.

In conclusion, our vaccination coverage has been high enough to observe vaccine efficacy and herd effect of HPV vaccination on the occurrence of HPV types, other than HPV16.[18] As for all other HPV types, the study probably had ample power to study type-replacement under the selective pressure from different vaccination strategies. No conclusive signs of type-replacement were observed, but HPV39 and HPV51 occurrence warrants further investigation.

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