Tampere University

PENELOPE GRACE GRAY

Human Papillomavirus Type Replacement Following the Implementation of HPV Vaccination

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In memory of my dear mother, Karen Anne Elizabeth Gray.

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ABSTRACT

Infection with high-risk human papillomavirus (HPV) is a necessary cause of cervical cancers and an associated cause of several other anogenital and oropharyngeal cancers. The beginning of the era of prophylactic human papillomavirus vaccination, via the implementation of efficacious first-generation vaccines targeting the two most high-risk oncogenic HPV types 16 and 18, offers the opportunity for the control of HPV16/18 infection and elimination of HPV16/18 associated cancers. The first-generation vaccines, however, target only 2 of 12 IARC classified high risk oncogenic HPV types. Previously vaccination of a targeted subset of strains of a pathogen has in some instances led to an increase in the non-targeted strains in a phenomenon known as serotype (or genotype) replacement. Therefore, there has been concern that targeted HPV vaccination might induce HPV genotype replacement by the non-vaccine targeted high risk HPV types, potentially undermining the impact of HPV vaccination.

In this dissertation, we have evaluated and compared the sustainability of vaccine-induced neutralising and cross-neutralising antibody response of the two first generation vaccines, the bivalent and the quadrivalent vaccine, by following up two cohorts of vaccinated Finnish trial participants in the population-representative Finnish maternity cohort serum biobank.

We have then evaluated the degree of HPV16/18 niche clearance, both the direct and indirect impact of vaccination, via active and passive follow-up of the population-based Finnish community randomised trial of HPV vaccination strategy with moderate vaccination coverage (gender-neutral vaccination versus girls only HPV vaccination using the bivalent HPV vaccine).

Subsequently we have evaluated the occurrence of the non-vaccine HPV types among both HPV vaccinated and unvaccinated women via the same active and passive follow-up of the community randomised trial, to assess whether the non-vaccine types increased in occurrence and took advantage of the cleared partially cleared HPV16/18 niche (measured as decreased occurrence) in a manner indicative of type replacement. We further investigated the same, among the high-risk taking core group, where transmission dynamics are greater and early indications of type replacement may be observed.

We found that both the bivalent and the quadrivalent vaccines induce a sustainable neutralising antibody response against HPV16 and HPV18 among women vaccinated with three doses of the respective vaccine. However, among the quadrivalent vaccine recipients 15% of the women were found to be seronegative to HPV18 with no detectable HPV18 neutralizing antibodies, whilst among the bivalent vaccine recipients 100% had seroconverted to HPV18. Notable differences in the cross-neutralising antibodies response were observed between the two vaccinated cohorts, with seroconversion to HPV31, 33, 45, 52, and 58 being higher among the bivalent vaccine recipients in comparison to the quadrivalent vaccine recipients.

Following the community randomised trial, we observed the greatest HPV16/18 niche clearance among the unvaccinated residents of the gender-neural trial arm communities. Significant reduction of both HPV16 and HPV18 prevalence was observed after gender-neutral HPV vaccination. No HPV16 niche clearance in unvaccinated residents was observed in the girls-only vaccination arm communities. Among the HPV vaccinated participants, the prevalence of vaccine targeted HPV16 and 18 was almost negligible, whilst the prevalence of HPV31, 33 and 45 were also markedly reduced in comparison to the HBV vaccinated controls.

When evaluating the occurrence of the non-vaccine HPV types via the active follow-up of the community randomised trial we observed consistent increases in HPV51, and some indications of increased HPV58 among the participants from the interventions. However, these increases generally stemmed from the older birth cohort. Additionally, some sporadic inconsistent increases in HPV39 and 66 were observed. However, during the passive follow up among the unvaccinated women resident in the trial communities pre- and post- vaccination era, none of these findings were replicated. We observed an increase post-vaccination in the seroprevalence (cumulative incidence) of HPV68 (a type not measured in the active follow-up) among the residents from the girls-only intervention communities. However, a similar HPV68 seroprevalence increase was also observed among the unvaccinated women from the control arm communities, suggesting that this increase was unlikely to be due to vaccine-induced type replacement.

Overall, we observed that both first generation vaccines induce a high level of sustainable HPV16/18 neutralising antibodies over time among women, whilst the bivalent vaccine provided a higher prevalence of cross-protective neutralising antibodies as compared to the quadrivalent vaccine. We further found significant niche clearance (also HPV16 niche clearance) following gender-neutral vaccination even with moderate vaccination coverage. However, despite this observed niche clearance, we found no clear, decisive signs of increased non-vaccine occurrence

which could irrefutably be due to vaccine-induced type replacement. These findings were specific to the study setting (vaccination coverage, the vaccine used and the duration of follow-up), thereby, continued surveillance of the non-vaccine HPV types will remain crucial in the future.

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TIIVISTELMÄ

Suuren riskin HPV- (human papillomavirus, ihmisen papilloomavirus) infektio on välttämätön kohdunkaulansyövän syy, ja osasyy lukuisissa muissa anogenitaali- ja suu-nielusyövissä. Ennaltaehkäisevän HPV-rokotuksen käyttöönotto ensimmäisen sukupolven rokotteilla, jotka suojaavat HPV-tyypeiltä 16 ja 18, tarjoaa mahdollisuuden kontrolloida HPV16/18 infektioita ja eliminoida niihin liittyvät syövät. Ensimmäisen sukupolven rokotteet kuitenkin kattavat vain kaksi 12:sta kansainvälisen syöväntutkimuslaitoksen (IARC) luokittelemasta korkean riskin HPV-tyypistä. Rokotusten kohdistuminen vain osaan jonkin patogeenin alatyypeistä on johtanut ei-maalitettujen alatyyppien yleistymiseen – ilmiöön, joka tunnetaan tyyppikorvautuvuutena. HPV-rokotusten aiheuttamasta korkean riskin HPV-tyyppien korvautumisesta ei-maalitetuilla HPV-tyypeillä on nostanut huolen siihen, että mitätöisikö tyyppikorvautuminen HPV-rokotuksen vaikuttavuuden.

Väitöskirjassani olen arvioinut ja verrannut rokottamalla aikaansaatujen neutraloivien ja risti-neutraloivien vasta-aineresponssien kestävyyttä kahden ensimmäisen sukupolven, kaksi- ja nelivalenttisen, rokotteen välillä seuraamalla rokotetrialeihin osallistuneita kahta suomalaista kohorttia väestöpohjaisen äitiysneuvolapohjaisen seerumipankin (FMC) avulla. Havaitsin, että sekä kaksi- että nelivalenttinen rokote saavat aikaan kestävän neutraloivan vasta-aineresponssin HPV16 ja HPV18 vastaan kolme annosta saaneilla rokotetuilla naisilla. Nelivalenttista rokotetta saaneista naisista 15% oli HPV18 seronegatiivisia, joten heiltä ei voitu mitata neutraloivia HPV18 vasta-aineita kun taas 100% kaksivalenttista rokotetta saaneista kehitti HPV18 vasta-aineita. Tämän lisäksi huomattavia eroja näiden kahden rokotekohortin välillä oli havaittavissa: HPV31, 33, 45, 52 ja 58 serokonversio oli yleisempää kaksivalenttista rokotetta saaneilla

Arvioin tämän jälkeen ekologisen HPV16/18 lokeron tyhjentymistä, ja HPV-rokotuksen suoraa ja epäsuoraa suojavaikutusta. Tämä on tapahtunut seuraten aktiivisesti ja passiivisesti suomalaista väestöpohjaista paikkakuntasatunnaistettua tutkimusta, jossa arvioitiin poikien ja tyttöjen verrattuna vain tyttöjen HPV-rokotusstrategioiden vaikuttavuutta. Havaitsin ekologisen HPV16/18 lokeron

tyhjentyneen parhaiten niiden rokottamattomien joukossa, jotka asuivat poikien ja tyttöjen rokotuspaikkakunnilla. Merkitsevä sekä HPV16 että HPV18 esiintymisen lasku oli todettavissa näillä paikkakunnilla. HPV16 lokeron ei voitu todeta tyhjentyneen vain tyttöjen rokotuspaikkakunnilla. HPV-rokotettujen joukossa HPV16 ja HPV18 eivät juurikaan esiintyneet, myös HPV31, 33 ja 45 esiintyminen oli huomattavasti laskenut verrattuna hepatiitti B (HBV)-rokotettuihin verrokkeihin.

Viimeiseksi olen arvioinut ei-rokotetyyppien ilmentymistä sekä HPV-rokotetuilla että rokottamattomilla käyttäen aktiivista ja passiivista saman paikkakuntasatunnaistetun tutkimuksen seurantaa määrittääkseni onko ei-rokotetyyppien ilmentyminen yleistynyt hyödyntäen (osittain tyhjentynyttä) HPV16/18 ekologista lokeroa tavalla, joka merkitsisi tyyppikorvautuvuutta. Havaitsin toistettavia yleistymisiä HPV51 ja jossain määrin HPV58 suhteen. Tämän lisäksi yksittäisiä HPV59 ja HPV66 nousuja oli todettavissa. Mikään näistä havainnoista ei kuitenkaan ollut toistettavissa paikkakunnilla rokotusta ennen tai sen jälkeen asuneiden passiivisessa seurannassa. HPV68 (tyyppi, jota ei mitattu aktiivisessa seurannassa) esiintymisessä havaitsimme nousun rokotuksen jälkeisenä aikana tyttöjen paikkakunnilla. Samankaltainen nousu oli kuitenkin havaittavissa myös rokottamattomilla naisilla kontrollipaikkakunnilla, mikä viittasi siihen, että nousu ei todennäköisesti ollut HPV-rokotuksen aikaansaamasta tyyppikorvautuvuudesta johtuvaa.

Kaiken kaikkiaan havaitsin, että molemmat ensimmäisen sukupolven rokotteet saavat useimmilla naisilla aikaan korkean ja kestävän neutraloivien HPV16/18 vasta-aineiden tason. Kaksivalenttinen rokote sai aikaan useammin ristisuojaavia neutraloivia vasta-aineita kuin nelivalenttinen rokote. Löysin merkitsevän ekologisen lokeron tyhjentymisen (myös HPV16 suhteen) poikien ja tyttöjen rokotuksen seurauksena jo keskikorkealla rokotuskattavuudella. Tästä lokeron tyhjentymisestä huolimatta en löytänyt selviä, yksikäsitteisiä merkkejä ei-rokotetyyppien lisääntyneestä ilmentymisestä, joka olisi kiistatta johtunut HPV-rokotuksen aiheuttamasta tyyppikorvautuvuudesta. Tästä syystä jatkuva ei-rokotteeseen kuuluvien HPV-tyyppien seuranta on jatkossa edelleen tärkeää.

CONTENTS

ABBREVIATIONS

BVR	Bivalent Vaccine Recipients
CI	Confidence Intervals
CIN2	Cervical Intraepithelial Neoplasia grade 2
CRT	Community Randomised Trial
DNA	Deoxyribonucleic Acid
E	Early Protein
EC50	Half Maximal Effective Concentration
EMA	European Medicines Agency
FCR	Finnish Cancer Registry
FDA	United States Food and Drug Administration
FMC	Finnish Maternity Cohort
FUTURE	Females United To Unilaterally reduce Endo/ectocervical disease
FVU	First Void Urine
GEE	Generalized Estimating Equation
GMT	Geometric Mean Titre
GMTR	Geometric Mean Titre Ratio
gG2	Glycoprotein G 2
GDPR	General Data Protection Regulation
GSK	GlaxoSmithKline
HBV	Hepatitis-B Virus
HILMO	Hospital Discharge Registry
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
hr	High Risk
HSV-2	Herpes Simplex Virus Type II
HT-PBNA	High-Throughput-Pseudovirion-Based Neutralization Assay
IARC	International Agency for Research on Cancer
IC	Interaction Contrast
ICC	Intracluster Correlation Coefficient

IU	International Unit/s
L	Late Protein
MALDI-TOF	Matrix-assisted Laser Desorption Ionization-time of Flight
MGP	Modified General Primer
MSM	Men who have Sex with Men
ORF	Open Reading Frame
P	Prevalence
PATRICIA	PApilloma TRIal against Cancer In young Adults
PBNA	Pseudovirion-Based Neutralization Assay
PCR	Polymerase Chain Reaction
pm	Person-Months
PV	Papillomavirus
p53	Tumour Protein p53
PR	Prevalence Ratio
pRB	Retinoblastoma Protein
QVR	Quadrivalent Vaccine Recipients
R	Risk
R0	Basic Reproduction Number
RNA	Ribonucleic Acid
RPR	Ratio of Seroprevalence Ratios
r_s	Spearman's Rank Correlation Coefficient
SD	Standard Deviation
sPR	Seroprevalence Ratio
STI	Sexually Transmitted Infection
URR	Upstream Regulatory Region
VE	Vaccine Efficacy
VLP	Virus-like Particle
WHO	World Health Organisation
WSW	Women who have Sex with Women
WSWM	Women who have Sex with Women and Men

ORIGINAL PUBLICATIONS

Publication I Mariz FC, **Gray P**, Bender N, Eriksson T, Kann H, Apter D, Paavonen J, Pajunen E, Prager KM, Sehr P, Surcel HM, Waterboer T, Müller M, Pawlita M, Lehtinen M. Sustainability of neutralising antibodies induced by bivalent or quadrivalent HPV vaccines and correlation with efficacy: a combined follow-up analysis of data from two randomised, double-blind, multicentre, phase 3 trials. Lancet. Infect Dis. 2021; S1473-3099(20)30873-2.

Publication II **Gray P**, Kann H, Pimenoff VN, Eriksson T, Luostarinen T, Vänskä S, Surcel HM, Faust H, Dillner J, Lehtinen M. Human papillomavirus seroprevalence in pregnant women following gender-neutral and girls-only vaccination programs in Finland: A cross-sectional cohort analysis following a cluster randomized trial. PLoS Med. 2021; 18(6), e1003588.

Publication III **Gray P**, Palmroth J, Luostarinen T, Apter D, Dubin G, Garnett G, Eriksson T, Natunen K, Merikukka M, Pimenoff V, Söderlund-Strand A, Vänskä S, Paavonen J, Pukkala E, Dillner J, Lehtinen M. Evaluation of HPV type-replacement in unvaccinated and vaccinated adolescent females- Post-hoc analysis of a community-randomized clinical trial (II). Int J Cancer. 2018;142(12):2491-2500.

Publication IV **Gray P**, Luostarinen T, Vänskä S, Eriksson T, Lagheden C, Man I, Palmroth J, Pimenoff VN, Söderlund-Strand A, Dillner J, Lehtinen M. Occurrence of human papillomavirus (HPV) type replacement by sexual risk-taking behaviour group: Post-hoc analysis of a community randomized clinical trial up to 9 years after vaccination (IV). Int J Cancer. 2019 Aug 1;145(3):785-796.

Publication V **Gray P**, Kann H, Pimenoff V N, Adhikari I, Eriksson T, Surcel HM, Vänskä S, Dillner J, Faust H, Lehtinen M. (2020). Long-term follow-up of human papillomavirus type replacement among young pregnant Finnish females before and after a community-randomised HPV vaccination trial with moderate coverage. Int J Cancer. 2020; 147(12):3511–3522.

1 INTRODUCTION

There are over 300 types of human papillomavirus (HPV), found in five genera: *alpha-*, *beta-*, *gamma-*, *mu-* and *nu-* (IARC, 2012). About 40 HPV types, mostly belonging to the *Alphapapillomaviridae* are the most common cause of sexually transmitted infections worldwide, commonly being acquired soon after sexual debut. *Alphapapillomaviridae* are also associated with anogenital cancers and cancers of the head and neck. High-risk (hr)HPV infection is a necessary prerequisite (i.e., a necessary component cause) in the acquisition of cervical cancer and is causally associated with a high proportion of anal, oropharyngeal, vaginal, vulvar and penile cancer (Walboomers et al, 1999, IARC, 2012).

The highest burden of HPV infection and correspondingly cervical cancer, lies in developing countries and worldwide in those sections of populations with lower socio-economic status (Agosti & Goldie, 2007). The link between cervical cancer and sexual promiscuity was first inferred by the Italian Rigoni-Stern in 1842, when he noted that cervical cancer comparatively rarely occurred in nuns and virginal women (Rigoni-Stern, 1842). However, it was not until much later in 1975 when Harald zur Hausen suggested a possible association between HPV and cancer of the cervix (zur Hausen, 1975). He and his colleagues showed that the association was specific most notably for HPV types 16 and 18 found in cervical cancer cells (Dürst at al, 1983; Boshart et al, 1984), for which he later received a Nobel prize in 2008. The causality of the association was confirmed in longitudinal serological and DNA studies (Lehtinen et al, 1996; Wallin et al 1999).

Same virus-like particles (VLPs) that enabled the above-mentioned serological studies formed the basis for prophylactic HPV vaccines. The HPV16 vaccines were subsequently shown to be efficacious against cervical infections in 2001 (Koutsky et al, 2002). Since then, three prophylactic vaccines targeting a subsection of the most common high-risk types of HPV have been licensed for use: a bivalent vaccine (Cervarix™), a quadrivalent vaccine (Gardasil®) and a nonavalent vaccine (Gardasil 9®) (EMA,2007; FDA, 2006; FDA, 2014). HPV vaccination offers an invaluable means to greatly diminish cervical cancer burden. Many affluent countries have implemented national HPV vaccination programmes, with Australia and the UK implementing their programmes as early as 2007 and 2008 (Gertig et al, 2013; Markowitz et al, 2012). However, despite the general success story, there have been

concerns raised regarding the possibility of vaccine targeted HPV type replacement with non-vaccine targeted types, once the ecological niche of the vaccine targeted HPV types is vacated due to vaccination (Lehtinen & Paavonen, 2004). The concept of vaccine type replacement by non-vaccine types of pathogen was first hypothesised in relation to *Haemophilus influenzae* and *Streptococcus pneumoniae* vaccination by Marc Lipsitch (1997), when he suggested that bacterial serotype replacement may occur post-vaccination. This was subsequently shown to occur post-implementation of *Streptococcus pneumoniae* vaccination programmes with replacement by non-vaccine *Streptococcus pneumoniae* serotypes by Weinberger et al (2011) and threatens to compromise the public health benefit conferred by *Streptococcus pneumoniae* vaccination.

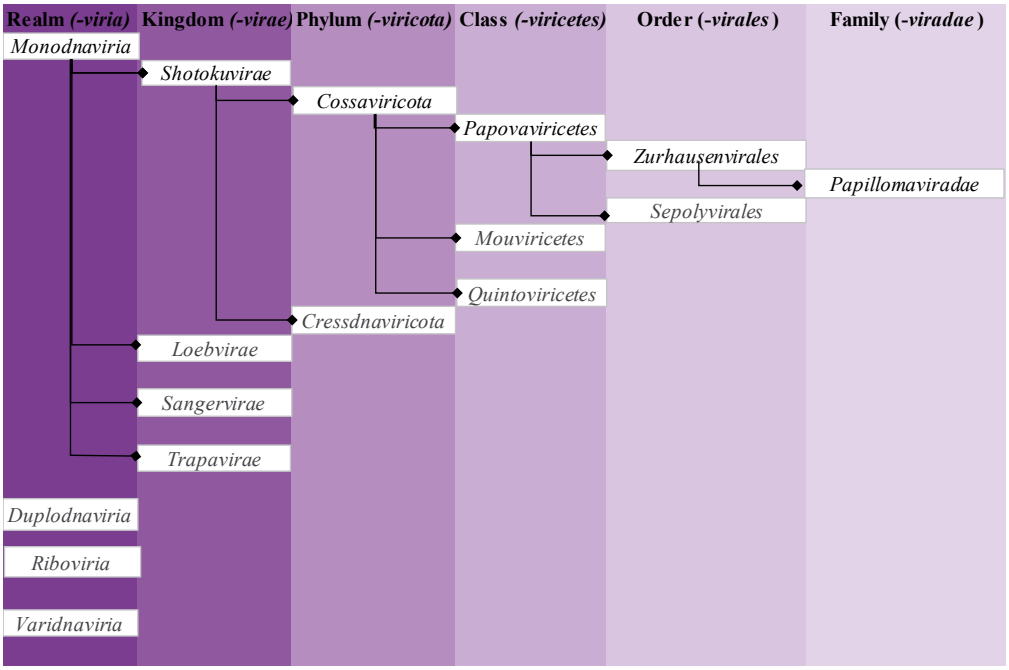
Concerns surrounding the possibility of HPV type replacement have contributed to hesitancy by policy developers and analysts to include HPV vaccination in national vaccination programmes and have provided fodder to the anti-vaccination movement. Therefore, such concerns require to be answered as a matter of urgency. Provided biobanked longitudinal samples of large intervention (vaccinated) and non-intervention cohorts are available there are three main approaches which may be utilised to determine type replacement occurrence; firstly, by comparing pre-vaccination era non-vaccine HPV type prevalences with those post-vaccination, secondly by comparing the odds of non-vaccine HPV types coinfection with vaccine HPV types to the odds of non-vaccine HPV type infections in the absence of vaccine types to assess whether HPV type-type competition occurs, and thirdly using mathematical modelling. This thesis takes an integrated approach to determining whether HPV type replacement by non-vaccine HPV occurs after vaccination with the bivalent HPV vaccine.

2 REVIEW OF THE LITERATURE

2.1 Human papillomavirus, HPV

The *Papillomaviridae* family is from the Order of Zurhausenvirales (named after Harald zur Hausen), which is within the class of *Papovaviricetes* (stemming from the replaced previous "*Papovaviridae*"), which in turn is within the Phylum of *Cossaviricota* (named after Yvonne Cossart, who co-discovered parvovirus B19), within the Kingdom of *Shotokuvirae* (after the Japanese Empress Shōtoku, who in 752AD was the first to document in the written word a viral plant disease), which in turn is from the Realm of *Monodnaviria* (Figure 1)(Koonin et al, 2020).
The *Papillomaviridae* family of viruses is composed of two subfamilies; *Firstpapillomavirinae* (comprising of 52 genera and 132 species) and *Secondpapillomavirinae* (comprised of 1 genus and 1 species).

Figure 1: Papillomavirus taxonomy from realm to family (Koonin et al, 2020).



Of the 53 different known genera, five are known to contain papillomavirus types which infect humans; *Alphapapillomavirus*, *Betapapillomavirus*, *Gammapapillomavirus*, *Mupapillomavirus* and *Nupapillomavirus*. Among the five genera, there appears to be a large disparity in differential success, as to current knowledge the diversity of species and types contained within each genus shows extreme variation, with the *Gammapapillomavirus* being by far the most diverse, followed by the *Betapapillomavirus* genus, then *Alphapapillomavirus* genus, with the *Mupapillomavirus* with only three HPV types and lastly the *Nupapillomavirus* genus with only one known HPV type (Bravo & Féliz-Sánchez, 2015). Of these five genera, *Alphapapillomavirus* is the most notable genus containing papillomavirus types associated with genital and mucosal cancers in humans, including, to differing degrees, cervical, anal, penile, vulvar, vaginal, oral, oropharyngeal and tonsillar cancer (IARC monograph, 2012).

Out of the five genera contain papillomavirus types which infect humans, only *Gammapapillomavirus*, *Mupapillomavirus* and *Nupapillomavirus* genera contain specifically HPVs, whereas the *Alphapapillomavirus* and *Betapapillomavirus* genera contain also papillomavirus types which infect non-humane primates. Each genera is further divided into species (clades) composed of different HPV types. In the *Alphapapillomavirus* genus, the *Alphapapillomavirus* species 5,6, 7 and 9 are most associated with the development of genital and mucosal cancers.

Papillomaviruses are small non-enveloped double stranded DNA viruses (a group I virus type according to the Baltimore classification system). All species contained within the *Firstpapillomaviranae* subfamily contain two late genes (L1 and L2), two early genes (E1 and E2), and at least one accessory gene (E5, E6 or E7) in their genome. Certain species within the *Firstpapillomaviranae* also contain additional early genes (E4 or E8), whereas the species contained within the *Secondpapillomaviranae* subfamily contain only E1, E2, L1 and L2 in their genome.

The genome of papillomaviruses infecting humans, or HPVs, is comprised of a long control region, six overlapping early genes (E1, E2, E4, E5, E6 and E7) and two late genes (L1 and L2) (figure 2) (Doorbar et al, 2015; IARC monograph, 2012). The early and late genes are named as corresponding to the timepoint in the viruses' lifecycle at which the proteins they encode play a role. The E1 and E2 genes are well conserved and encode regulatory proteins. The L1 and L2 genes have well-conserved open reading frames (ORFs) and encode structural proteins.

The L1 protein is the major capsid protein of human papillomaviruses whilst the L2 protein is the minor capsid protein (Buck et al, 2013; Wang et al, 2013). The L1 major capsid protein spontaneously forms into virus like proteins whilst the L2 minor capsid protein plays a role in their assembly to complete the capsomer

structures at the surface of the virion Buck et al, 2013; Wang et al, 2013). High risk *Alphapapillomaviruses* have three oncogenes which encode oncoproteins: E5, E6, and E7. The oncoprotein E5 is the smallest oncoprotein. However, it has numerous functions in the HPV lifecycle, immune evasion (via downregulation of MHC class I molecules), the prevention of apoptosis of infected cells, and interference with normal growth factor receptor signalling (Müller et al, 2015). The E6 oncoprotein plays a pivotal role in the degradations of p53, whilst the E7 oncoprotein interacts with the tumour suppressor, retinoblastoma protein (pRB).

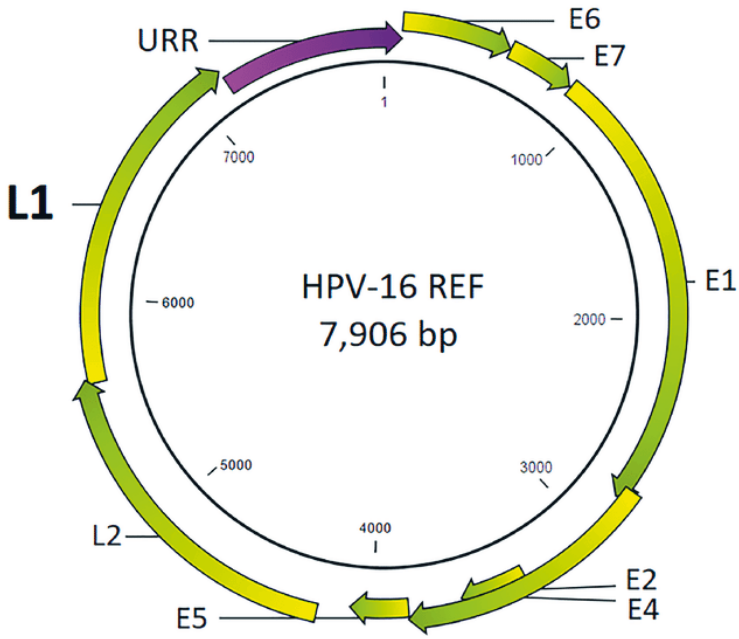


Figure 2: Diagram of the HPV16 genome (from the *Alphapapillomavirus 9* species). URR (upstream regulatory region), bp (base pairs) (Shen-Gunther et al, 2019). Reproduced with permission from Frontiers in Genetics.

2.2 Classification of HPV types

There are currently over 200 different genotypes (hereafter referred to as types) of HPV which have been distinguished based on differences in the L1 ORF of the HPV genome and officially classified. In addition, HPV types which have recently been identified but as of yet not officially been classified bring the total to over 300 distinct HPV types (International HPV Reference Center, 2020; NIH, 2022). To be classified as a distinct type the nucleotide sequence of the L1 gene must be more than ten percent different to other known types (de Villiers, 2013). However, as the divergence of the L1 region in different HPV types has little bearing on the changes in protein expression associated with the differentiation of keratinocytes, it is therefore not the ideal classification marker for HPV, as it may not well distinguish types which are associated with cervical cancer (and other associated cancer types) (García-Vallvé et al, 2005).

As for carcinogenicity, HPV types have been classified into four categories according to their carcinogenic properties by the International Agency for Research on Cancer (IARC); high risk carcinogenic types (group 1), probably carcinogenic types (group 2A), possibly carcinogenic types (group 2B) and types which are not classifiable as carcinogenic (group 3) (IARC, 2012). Twelve *Alphapapillomavirus* types of HPV from four different clades are currently classified as high risk (hr) carcinogens (Table 1). (IARC, 2012)

Table 1: Classification of HPV types as defined by the International Agency for Research on Cancer (IARC), according to genus and clade. Certain HPV types from the Alpha- genus have been associated with cancers of mucosal membranes, whereas certain HPV types of the Beta- genus have been associated with skin cancer (IARC et al, 2012).

	HPV types			
	IARC Classification			
	Group 1	Group 2A	Group 2B	Group 3
Alpha Clade				
5	51		26, 69, 82	
6	56		30, 53, 66	
7	**18**, 45,39, 59	68	70, 85, 97	
9	**16**, 31, 33, 35, 52, 58		67	
10				6, 11
11			34, 73	
Beta Clade				
1			5, 8	

Key
Group 1= carcinogenic
Group 2A= probably carcinogenic
Group 2B= possibly carcinogenic
Group 3= not classifiable as carcinogenic
IARC= International Agency for Research on Cancer
1st generation vaccine HPV types= in bold

2.3 A historical perspective

The discovery and characterization of human papillomavirus as a causative agent in human cancer has mostly materialized over the latter part of the 20th century and the start of the 21st century through the work of many research groups globally (zur Hausen, 2009). However, prior to this, several pieces of the jigsaw had already been noted. Perhaps the foundational stone of this story of discovery could be argued to be the momentous change in thinking of disease causation to a theory of natural causation, from the previous predominating ideologies of disease as a state caused by divine retribution or demons, when Hippocrates stated in reference to the disease of epilepsy that,

… "it appears to me to be nowise more divine nor more sacred than other diseases, but has a natural cause from the originates like other affections"

… around 400BC. This lay the way open to new discoveries, ways of thinking, and the concept of infectious disease epidemiology as we know it today (Winslow, 1980). As early as the ancient Greeks and Romans, genital warts were thought to be associated with sexual promiscuity and therefore infectious, with researchers proving their cause as an infectious agent in the early part of the 20th century. However, many other causative theories and speculations also abounded during this interim. In line with the concurrent theory of humouralism, in 1661, Robert Lovell proclaimed warts of the "neck of the womb" and external genitalia to be caused by "succulent and malignant humours" (Lovell, 1661). Whereas in 1700 the German physician Dürr noted the occurrence of warts on the index and middle fingers in women, which he proclaimed to be due to the individuals partaking in masturbation, an argument more in line with the ongoing theory of disease as caused by divine retribution and not by natural causation (Fleisch & Schneider, 1808).

The first mention of a connection between sexual promiscuity and cervical cancer occurred as early as the nineteenth century, when Rigoni-Stern observed that the prevalence of death due to cervical cancer was higher in women who had been married or prostitutes, than it was in celibate women (Rigoni-Stern, 1842). As early as 1911, Francis Peyton Rous demonstrated the sarcomatous chest cancer in chickens was caused by an invisible infectious agent by the transplantation of cell-free extracts of such tumours into healthy related chickens (Rous, 1911). However, it was not until the 1930s any notable interest in tumour viruses started to emerge in the scientific community (Moore & Chang, 2010). In 1934, studies began to describe the carcinogenic properties of the cottontail rabbit papillomavirus in rabbits (Beard & Rous, 1934; Rous & Beard, 1935), which also contributed to Rous receiving the Nobel prize in Physiology or Medicine in 1966 for the discovery of tumourigenic viruses.

However, it was not until later in the century that papillomaviruses were scrutinized as potential causative candidates in cancer of humans. After failing to successfully locate herpes simplex virus type II DNA in cervical cancer biopsies, in 1972, the research team led by Harald zur Hausen turned their attention to human papillomavirus as a potential candidate (zur Hausen et al, 1975; zur Hausen, 1976; zur Hausen, 1977; zur Hausen, 2009). This led to the discovery of two novel high risk HPV types, firstly HPV16 and secondly HPV18, in cancer biopsies (Dürst, 1983; Boshart, 1984). Eventually Harald zur Hausen received the Nobel prize in Medicine in 2008 for the discovery of the high-risk biological agents (HPV16 and 18) which cause cervical cancer.

The causative link between HPV16 and the development of cervical cancer was substantiated by Lehtinen et al in 1996. These longitudinal biobank-based serological observations were based on the use of virus-like-particles (VLPs) for the type-specific determination of HPV16 antibodies (Lehtinen et al, 1996) and HPV18 antibodies (Dillner, 1997). Whilst, in 1999, the exceptionally strong association between prior HPV infection (as measured via HPV DNA positivity) and subsequent development of cervical cancer was further confirmed also in a case control study conducted in Sweden (Wallin et al, 1999).

The discovery of VLPs also led to the development of two prophylactic HPV vaccines (Cervarix and Gardasil) targeting these two high risk HPV types in the 2000s. These VLPs self-assemble from the major capsid protein L1 (Zhou et al, 1991; Schiller & Hildesheim, 2000; Koutsky, 2002). After licensure (EMA, 2008 & 2009; FDA, 2009) the first-generation prophylactic HPV vaccines were widely implemented in the national vaccination programs in the majority of high-income countries. However, they have yet to be implemented on the same scale in all low- and middle-income countries, due to a combination of factors such as the high cost of the vaccine, lack of political will, lack of resources, other higher priority health concerns competing for the limited resources, and lack of logistical means and infrastructure required for widespread implementation (Kumar et al, 2021).

2.4 HPV Replication

The HPV life cycle is linked to the cellular life cycle of the epithelial cells it infects (Figure 3). HPV initially infects undifferentiated keratinocytes in the basal layer of the epithelium, gaining access via a microabrasion or wound, where it undergoes initial rapid replication, described as the establishment phase where the HPV genome is amplified at a low copy number, during which the E1 and E2 proteins play a large role (Hoffman et al, 2006; McBride, 2008). During the second stage, the maintenance stage, the HPV episome is steadily replicated during the S phase of the host cells genome replication, at the same constant pace at which the host cell divides (McBride, 2008). The third stage of rapid genome amplification occurs in differentiated suprabasal cells, where the viral genome is amplified at a high copy number (McKinney, 2015). (Doorbar et al, 2015)

As a double stranded DNA virus, HPV is deemed to be more stable comparative to RNA viruses.

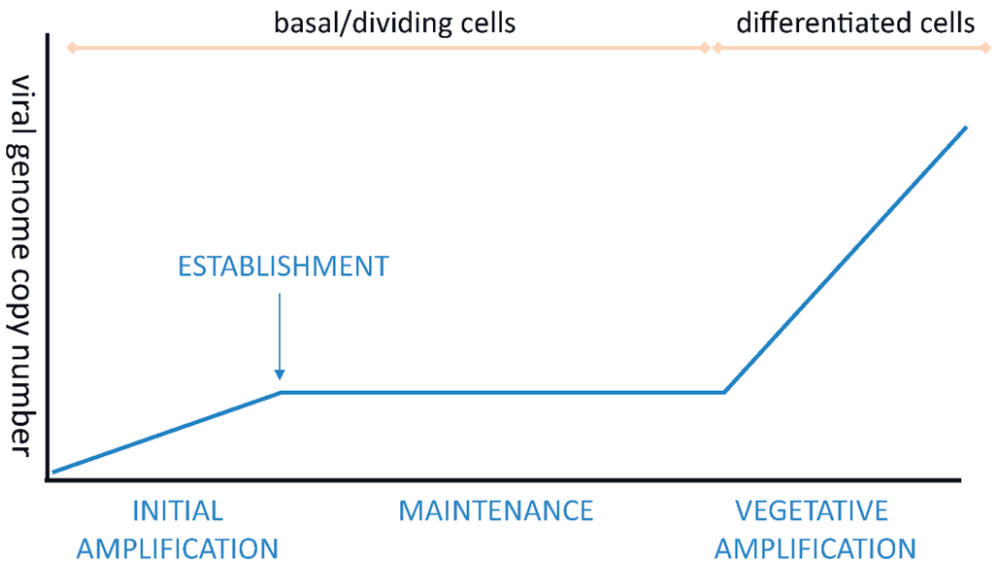


Figure 3: HPV genome copy number according to the stage of the HPV DNA replication (McKinney et al, 2015). Reproduced with permission from MDPI Open Access Journals.

2.5 HPV niche and tissue tropism

Certain low risk HPVs possibly have tropism for the vaginal squamous epithelium in comparison to cervix (Castle et al 2007, Sex transm dis; Castle et al 2006 Cancer Res; Winer et al 2009 JID). On the contrary, there are particularly vulnerable sites, 'special' epithileal sites such as the transformation zone in the cervix, the anal transformation zone and tonsillar crypts (oropharynx). It is likely that high-risk HPV gene expression is not well controlled in these particular sites (Egawa et al, 2015). However, comparison of HPV type specific distribution in the anus versus in the cervix has shown a markedly different distribution. In general, there tends to be a wider range of HPV types observable in the anus (Hernandez et al, 2005). Finally, HPV18 and HPV45 from the alpha 9 genus are known to preferentially infect the endocervical epithelial cells giving rise to cervical adenocarcinoma (Clifford & Franceschi, 2008; Quint et al, 2009).

2.6 Geographic variation in HPV prevalence

There exists considerable variability in HPV type prevalence globally. Globally, the averaged prevalence of HPV in women with normal cytology is approximately 11.7%, however this varies greatly from 35.4% of women in the Caribbean to 1.7% in Western Asia (Forman et al., 2012). Continent-wise, the highest burden of HPV infection lies in Africa (especially in Sub-Saharan Africa), with 33.6% in Eastern Africa, 19.6% in Western Africa, 17.4% in Southern Africa and 9.2 % in Northern Africa in women with normal cytology (Forman et al., 2012). Likewise, the distribution of types which compose each country-wise and continent-wise prevalence estimate also varies greatly. In every continent, HPV16 is the most prevalent HPV type. In Africa and Asia HPV52 is the second most common closely followed by HPV18. However, elsewhere this is different, although HPV18 is in the top most prevalent types globally.

2.7 HPV Epidemiology

2.7.1 Transmission

There are several plausible routes of transmission of HPV types which infect the anogenital and oropharyngeal epithelium. However, the findings of multiple studies implicate direct genital to genital transmission to be the most common and most important transmission route among sexual partners (Burchell et al, 2010; Malagón et al 2019). Studies have also observed a notable difference in the directionality of genital HPV transmission with female to male transmission being more common than the reverse (Widdice et al, 2013; Balaji et al, 2020; Malagón et al, 2021). When taking into account bias due to interval censoring in the data available, female to male transmission rates were 5.6 per 100 person-months (pm) and male to female transmission rate 3.5 per 100 pm (Malagón et al, 2021). Increased susceptibility in men despite previous exposures may continue due to their low incidence of seroconversion following natural infection (Malagón et al, 2021). Findings among adults seem to indicate that genital-to-oral transmission is more likely when the oral sex is being performed on a woman than on a man (D'Souza et al, 2014). Perhaps due to lack of antibodies, hrHPV positivity in oral samples was 6-fold higher among

men than women among 18-69 year-old participants of the NHANES study (Sonawane et al, 2017).

Findings from the HITCH cohort study seem to indicate that the viral load is positively associated with the transmission to an individual's sexual partner (Wissing et al, 2019). As HIV positivity is also associated with increased HPV viral load among co-infected individuals, it is also probable that the transmission probability may be higher when the infecting person is HIV positive, and even more so when not undergoing antiretroviral therapy (Luchters et al, 2010).

There are many plausible routes through which HPV may conceivably be transmitted. The most notable of these are (in decreasing order): genital-to-genital (Malagón et al, 2019), genital-to-oral and vice versa (Dahlstrom et al, 2014), hand-to-genital and vice versa (Widdice et al, 2013; Malagón et al, 2019), genital-to-anal and vice versa (Hernandez et al, 2005; Goodman et al, 2010), hand-to-anal and vice versa, via fomites such as sex toys (Roden et al, 1997; Anderson et al, 2014), oral-to-oral (Dahlstrom et al, 2014), and vertical transmission from mother to child during delivery (Castellsagué et al, 2009).

The most important route of transmission is that from directly from genital-to-genital, with the HPV DNA positivity on a partner's genitals conferring an over 50-fold increased risk of prevalent genital HPV DNA positivity/deposition in the other partner (Burchell, 2010). The prospective HITCH cohort found that transmission primarily occurs via the genital-to-genital route (Malagón et al, 2019). The same study also reported the common occurrence of HPV deposition from one partner's genitals to the other's hands, although HPV DNA positivity was not found to be a predictor of HPV positivity in the persons own genitals, suggesting that hand-to-genital autoinoculation is unlikely to be a major source of HPV transmission (Malagón et al, 2019). Indeed, observations of genital HPV DNA in the fingertips of female university students was found in 14% of the females, and the same HPV type was redetected in the fingertips among 15% of the previously HPV DNA positive females (Winer et al, 2010). It is plausible that some hand-to-genital transmission may occur (Widdice et al, 2013).

Oral sex with a HPV positive partner conveys an increased risk of oral HPV acquisition (Dahlstrom et al, 2014). Concordant HPV type-specific prevalence is highest among men who had frequent oral sex with a female partner with a genital HPV infection (Malagón et al, 2019). In line with this observation, the number of oral sex partners, younger age at first oral sex and also the intensity of self-reported oral sex are all risk factors for the development of HPV associated oropharyngeal cancer (Drake et al, 2021). In addition to the risk of oral HPV infection acquisition

via oral sex, several studies indicate that HPV may also be transmitted directly from one partner's oral cavity to another's via open-mouth kissing. Men whose partner had an HPV positive oral sample had an increased risk of also having an HPV positive oral sample, (Dahlstrom et al, 2014; D'Souza et al,2009). However, these studies did not directly take into account confounding due to the number or intensity of oral sex partners.

Within the same individual there is a high degree of HPV type specific concordance between type-specific HPV infection of the vagina/cervix and that of anus. This suggests that autoinoculation from anus-to-vagina and vice versa may play a role in the transmission of HPV (Hernandez et al, 2005; Goodman et al, 2010). However, evidence from a cohort study of adult women conducted in Hawaii suggests that anal HPV positivity may occur as a result of anal sex behaviours. Self-reported history of anal sex was associated with HPV positivity in the anus specifically among the subgroup of women (representing 14% of the participants) who had an HPV DNA positive anal sample but a negative cervical sample (Hernandez et al, 2005).

In addition, there is also much discussion as to whether genital HPV can be transmitted via the environment and fomites (Liu et al, 2016). Non-genital HPV types, such as HPV1 and 2, are well known to be transmittable via fomites (Rowson & Mahy, 1967). HPV16 can withstand desiccation at room temperature, which would imply that transmission via HPV16 infected fomites is indeed plausible (Roden et al, 1997). In this regard, one study reported that HPV DNA was detectable on 23% of airport toilets (Smelov et al, 3013), and another study found HPV DNA present on over half the sampled sites of treatment rooms and toilets from genitourinary clinics (Strauss et al, 2002; Strauss et al, 2003). Another study found that HPV was detectable both directly after vaginal use of sex toys by females, and 24 hours post cleaning (with an alcohol-based product). Thus, transmission of HPV may at least be feasible via this route (Anderson et al, 2014). Previous observations of genital HPV DNA positivity in reported virgins and young children without history of sexual abuse suggests that non-sexual routes of HPV transmission may occasionally occur (Doerfler et al, 2009; Bumbuliene et al, 2011; Shew et al, 2013; Rock et al, 1986).

Although rare, and not a major transmission source, it is also possible to acquire HPV via vertical transmission, i.e., from mother to child, with transmission postulated to occur during pregnancy, birth or nursing (Cason et al, 1998; Castellsagué et al, 2009; Merckx et al, 2013; Louvanto et al, 2017; Zouridis et al, 2018). The Finnish Family study found that persistent cervical HPV infection in

mothers was associated with an increased risk of oral HPV in their infant (Rintala et al, 2005). In a prospective cohort study conducted in Spain, among children born to mothers who were HPV DNA positive, 20% of the children were found to be HPV DNA positive at follow-up (Castellsagué et al 2009). A rare but highly morbid complication of such vertical transmission is the development of recurrent respiratory papillomatosis, with HPV6 or 11 infecting the larynx (Sinal & Woods, 2005; Bonagura et al, 2010).

At the HPV type-specific level, it is thought that hrHPV and lrHPV types have co-evolved to survive using a trade-off between per-contact transmission probability and persistence of infection (Orlando et al, 2012), due to the existence of two sexual subcultures within a population. The low-risk types have higher transmission probability per sexual contact, whereas high-risk types have longer persistence but a lesser per-contact transmission probability. The former favours transmission in subpopulations with a high turnover rate of sexual partners, characteristic of the core-group, while the latter favours transmission in subpopulations with a smaller turnover rate of sexual partners (Orlando et al, 2012). Whilst findings from modelling studies have suggested that the median per contact transmissibility rate estimate is relatively similar between different HPV types (ranging from 0.72 to 0.76) (Johnson et al, 2012), clearance rates differ significantly according to HPV type with HPV16 having the lowest rate of clearance (Lehtinen & Dillner, 2013).

2.7.2 HPV infection among females

Among females HPV from the alpha genus is typically known to infect the epithelium of the cervix, vagina, anus, oropharynx, and larynx, whilst in certain cases may also be detectable and/or infect other anatomical locations such as in the fingers and nailbed (Castellsagué et al, 2008; Grundmeier et al, 2011; Dillner, 2019). The prevalence of HPV including hrHPV, among HPV unvaccinated females differs markedly by geographical location (Forman et al, 2012), age (Smith et al, 2008; Leinonen et al, 2013), sexual risk group (Vorsters et al, 2016), HPV genotype (Clifford et al, 2005; Howell-Jones et al, 2012; Leinonen et al, 2013), cervical abnormality diagnosis and HIV status (Okoye et al, 2021).

Among healthy women the pre-vaccination era prevalence of HPV was found to range greatly among different populations, with the age-standardised HPV prevalence found to range from 1.4% among Spanish women to 25.6% among Nigerian women in a pooled analysis of a limited number of countries (Clifford et al, 2005). Whilst among women with cytological abnormalities the prevalence of any

HPV type has been reported to be 52.1% among ASCUS cases, 75.2% among LSIL cases, and 85.3% among HSIL cases compared to 12.6% among women with a normal cervix (Bzhalava et al, 2013). Further to this the prevalence of HPV differs greatly by HPV genotype, with a study conducted in Finland among women attending cervical screening aged 25-65 years-old found that HPV16 was the most common (prevalent in 9% of the women) followed by HPV31, HPV52, HPV68, HPV45, HPV58, HPV18, HPV33, HPV51, HPV56, HPV39 and finally HPV35 (1.5%) among women with normal cytology (Leinonen et al, 2013). However, this ranked order of HPV type specific prevalence again differs by disease status of cervical abnormality. In the same study, among cases of ASCUS, HPV31 was the most commonly detected HPV type (prevalent in 10% of cases), followed by HPV16, HPV52, HPV58, HPV51, HPV56, HPV45, HPV18, HPV68, HPV33, HPV39, HPV35 and with HPV59 being the least common of the studied genotypes (prevalent in 2.2% of cases). Whereas among cases of LSIL the ranked prevalence of HPV types was again slightly different, with HPV16 being the most common (prevalent in 28%), followed by HPV31, HPV51, HPV33, HPV56, HPV18, HPV52, HPV58, HPV39, HPV45, HPV35, HPV68 and HPV59 (detectable in 1.8% of cases) (Leinonen et al, 2013).

The prevalence and incidence of HPV are also somewhat different depending on the anatomical location which it is infecting, with the prevalence among women being higher in the anatomical sites of the cervix and vagina, compared to the vulva and oral cavity, whilst the prevalence of anal HPV infection appears to be only slightly lower than the prevalence of cervical HPV infection (Giuliano et al, 2015; Castro et al, 2012). The difference in occurrence may conceivably be due to biological differences of the infection sites, due to the transformation zone of the cervix being particularly susceptible to persistent HPV infection. However, there are only a limited number of studies of anal HPV among women, and among a cohort study conducted in Hawaii, the investigators noted that the risk of a women acquiring an anal HPV infection was as common as their risk of acquiring a HPV infection of the cervix (Goodman et al, 2008). On the other hand, the prevalence of oral HPV infection has been observed to be 10-fold lower than in the cervix (Smith et al, 2004). Studies reporting the duration of infection and/or clearance rate of type specific HPV infection have observed rates not dissimilar between anatomical sites such as the cervix and oral cavity. A longitudinal study reporting the mean time to first clearance event of oral HPV16 and 18 infections among Finnish women observed a mean time of 21 months and 17 months respectively (Louvanto et al, 2013). This is not far removed from observations of the duration of HPV16 and 18 infections of

the cervix, with studies reporting a duration of infection of 18 and 16 months respectively (Insinga et al, 2007). Although it should be noted that the study populations from which these statistics were derived differ. Furthermore, a longitudinal cohort study investigating the persistence of HPV anal infection found that the median duration of anal HPV infection among sexually active women was relatively short (in comparison that observed in cervical HPV infections), with the reported median time to clearance being 4.5 months and 7 months, for HPV16 and 18 anal infections respectively (Shvetsov et al, 2009).

The pattern of genital HPV incidence by age has been observed to differ markedly by the population under study (Franceschi et al, 2006; Smith et al, 2008). In many Western and European countries, the incidence of HPV infection peaks before the age of 25 years-old around the same age as sexual debut (Franceschi et al, 2006; Vuyst et al, 2009). In contrast to this however, in some countries in Asia a relatively flat age-incidence curve as been observed, whilst in several other countries (for example, in Chile, Mexico and Costa Rica), a U-shaped age-incidence curve has been observed with the incidence of new HPV infections initially being highest in those aged under 25, subsequently decreasing, then again increasing in those aged 45 and above (Castle et al, 2005; Franceschi et al, 2006). Such a second "peak" in HPV incidence around the age of menopause has been observed in multiple populations, and has raised many questions as to whether it may reflect either an increase in sexual risk taking behaviours around this time, reactivation of earlier acquired latent infections (see section 2.7.5), or even whether it may be a result of cohort specific effects (Fu et al, 2015; Gravitt et al, 2013; Gravitt & Winer, 2017; Rositch et al, 2012; Winer et al, 2014). Conversely, although there are only a limited number of studies on the subject (and limited to specific countries), the pattern of HPV incidence with age appears to be somewhat different in the case of anal HPV infections and likewise also with oral HPV infections (Hernandez et al, 2005; Gillison et al, 2012). A cohort study of adult women found that the prevalence of anal HPV remained relatively constant with age (Hernandez et al, 2005).

Although HPV infection is extremely common, only a minority of women develop persistent hrHPV infection with progression to HSIL. Multiple risk factors are associated with an increased risk of persistent hrHPV infection, and developing invasive cervical cancer; specifically, HPV viral load, described HIV positivity, tobacco use, lifetime number of sexual partners, the lifetime number of partners of a person's spouse, young age at first pregnancy, parity, early coitarche, *Chlamydia trachomatis* infection, vaginal dysbiosis and although the evidence is somewhat inconsistent, the use of hormonal contraceptives.

The incidence of HPV among HIV positive women has also been reported to be higher than among HIV negative women, with one study finding that the HPV seroprevalence was 93% (Luchters et al, 2010; Liu et al, 2018; Kelly et al, 2018). The risk of acquisition of new HPV infection is higher among HIV positive women, especially those with a lower CD4 count (Liu et al, 2018). Similarly, the rate of clearance of HPV infection is significantly lower among HIV positive women compared to negative women, the rate of persistent HPV16/18 infection thereby being higher, with the HIV positive women subsequently having a higher risk also of developing both LSIL and HSIL (Liu et al, 2018; Thorsteinsson et al, 2019).
Multiple studies have observed that young age at the time of first sexual intercourse among women is associated with an increased risk of developing HSIL (Muñoz et al, 1993; Louie et al, 2009; Ruiz et al, 2012). As HPV is typically acquired very shortly after first intercourse (especially for the most common HPV types in circulation, such as HPV16 in the pre-vaccination era), coitarche is thereby likely a proxy of the age at first exposure to HPV infection, as is likely also young age at first birth (Castellsagué et al, 2014; Edelstein et al, 2009; International Collaboration of Epidemiological Studies of Cervical Cancer, 2009). There have been several suggested hypotheses as to why this may be the case. Firstly, it is possible that this increased risk may be due to unmeasured confounding by sexual risk-taking behaviour (Louie et al, 2009). However, it is also possible that the increased risk reflects a true biological vulnerability of the immature cervix, undergoing squamous metaplasia, to persistent HPV infection (Muñoz et al, 1993).

Historically there has been a preconception that among women who have sex with women (WSW) the risk of HPV infection is lower. However, this assumption is not based on scientific proof. Despite the many studies among sexual minority men, to date there is limited literature regarding the epidemiology of HPV infection among sexual minority women and even less literature among transgender men and non-binary people with a cervix. Furthermore, the studies which have been conducted have a limited sample size. Three separate case studies have reported the cases of HSIL (two CIN2 and one adenocarcinoma), in women reporting to have had exclusively female sexual partners (Ferris et al, 1996; O'Hanlan & Crum, 1996; McGauran & Pendlebury, 2020). Two larger studies conducted using the National Health and Nutrition Examination Survey (NHANES) in the U.S., found that both any HPV infection and hrHPV infection (HPV DNA positivity) was common in the genital samples from sexual minority women aged 20-59 years old (Branstetter et al, 2017; Reiter & McRee, 2017). Upon further stratification according to sexual orientation (as indicated both by measures of sexual identity and behavior), both

studies observed that the prevalence of HPV infection (both any and hrHPV) was markedly higher among bisexual women (43.6% prevalence of hrHPV) as compared to heterosexual women (27.9% prevalence of hrHPV), whilst exclusively lesbian women appeared to have either a slightly lower risk or a risk not dissimilar to that of heterosexual women depending on how sexual orientation was defined (behavior or identity). The increased risk among bisexual women is consistent with earlier smaller studies (Marrazzo et al, 1998; Marazzo et al, 2001). However, the increased relative risk of HPV infection among bisexual women as compared to heterosexual women was found to be attenuated when taking into account sexual risk-taking behavior, suggesting that perhaps a higher incidence of risk factors for HPV infection (such as the number of sexual partners) attribute towards the observed increased risk among this group (Reiter & McRee, 2017).

The fact that hrHPV infection is common among sexual minority women is cause for concern, as studies have also observed that this group is less likely to attend cervical screening, to appear at gynecological clinics and in some countries less likely to be offered opportunistic screening, due to a combination of perceived lower risk and real and perceived stigma (Marrazzo et al, 2001; Bustamante, 2021). Additionally, WSW have also been found to have a 2-fold higher risk of developing bacterial vaginosis compared to heterosexual women (Skinner et al, 1996; Koumans et al, 2007; Evans et al, 2007), which is reported to be a risk factor for HPV infection persistence (Kero et al, 2017), which in turn is a predictor of progression). This all implies that sexual minority women, particularly women who have sex with women and men (WSWM), may be at increased risk of cervical cancer, whilst less likely to participate in secondary prevention measures, without HPV vaccination at early adolescence they may be a marginalised group in terms of HPV-associated cancer risk and prevention.

2.7.3 HPV infection among men

The epidemiology of HPV infection among men differs to that observed in females in several aspects, even if comparable to females. HPV from the alpha genus also commonly cause infection in men in the anogenital and oropharyngeal epithelia. Previous studies have found HPV DNA to be present in the penis from 16 to 69% of men, although this statistic varies widely, e.g., by study population, laboratory method, and sexual orientation (Sichero et al, 2019, Tota et al, 2021). In a

multinational prospective cohort study, the HPV in men (HIM) study, conducted in men aged 18-79 years-old from Brazil, Mexico and the U.S. the prevalence of any HPV type (measured as the presence of PCR detected HPV DNA) was found to be 65.2%, whilst the prevalence of any of the oncogenic types was 12.0% and the prevalence of the most oncogenic type HPV16 was 6.5% at enrolment (Giuliano et al, 2008). The prevalence of HPV found at the combined sites of the coronal sulcus, glans penis, shaft, and scrotum was also found to vary dependent on geographical location, of the three countries included in the HIM study. In the HIM study the prevalence for any HPV type was found to be the highest in Brazil, thus suggesting limited generalisability to countries further afield in different contents. A meta-analysis of studies conducted in men in sub-Saharan Africa, found that that the prevalence of any HPV DNA (as detected via PCR) was 56.4% in HIV negative men, whilst among HPV positive men it was much higher at 84.5% (Olesen et al, 2014). Similarly, the study also found that the prevalence of high-risk HPV types was markedly higher in HIV-positive men than HIV-negative men, 74.2% and 30.5% respectively (Olesen et al 2014).

Contrary to the trend seen in females, the prevalence and incidence of HPV infection in adult males does not display a comparable association with age. The HIM study observed that participants aged 18-70 continued to acquire HPV infections (including that of high-risk oncogenic types) without any obvious age-incidence peak. Additionally, a large, pooled analysis of 64 studies of anal HPV infection, found that the prevalence of anal HPV16 infection rapidly increased around the age of 15-24 years old, and subsequently remained high in those aged 25 years old and older (Wei et al, 2021).

It has been observed that men positive for HPV DNA are fourteen times more likely to be reinfected with the same HPV type after 2 years even when reporting no new sexual encounters, raising the question of possible latency and reactivation of infection and or possible autoinoculation (Ranjeva et al, 2017), but such reinfections among previously infected men have also been shown to be associated with high sexual risk taking (Pamnani et al, 2018). It is also possible that a large proportion of the positive DNA detections (contributing to the age-incidence curve observed), may be depositions rather than true infections. Eventually, however, it is conceivable that seroconversion affects the risk of transmission.

Several factors play a role here. Men are far less likely to seroconvert following a HPV infection (or post detection of HPV DNA) than females (Giuliano et al, 2015). Superficial infection of the keratinised epithelium (for example of the penile epithelium), may be less likely to facilitate ongoing susceptibility among men

(Giuliano et al, 2011). In men who do seroconvert this does not reduce the risk of overall HPV acquisition men may remain susceptible throughout their life course (Lu et al, 2012; Beachler et al, 2018).

Circumcision is associated with less risk of HPV acquisition and reinfection and increased clearance rate in the glans (albeit not in the shaft) (Castellsagué et al 2002; Hebnes et al, 2021; Smith et al, 2021). Similarly, consistent condom usage in sexually active men is associated with a 2-fold decrease in risk of acquisition of HPV infection and also associated with increased probability of clearance of infection (Repp et al, 2012; Nielson et al, 2010; Hariri & Warner, 2013; Pierce Campbell et al, 2013). These findings are in line with the earlier observations that the incidence of cervical cancer was noticeable lower among Jewish women, compared to the incidence in their non-Jewish counterparts (Braithwaite, 1901; Kennaway, 1948).

Likewise to females, both *Chlamydia trachomatis* infection and HSV-2 seropositivity in men has been shown to be associated with increased risk of HPV DNA positivity (Alberts et al, 2013). Also, the lifetime number of sexual partners and recent number of sexual partners have been shown to be a strong risk factor for HPV DNA positivity (Guiliano et al, 2009; Kjaer et al, 2005), and decreased clearance of hrHPV infections in men (Giuliano et al, 2011). Studies have additionally found that current smoking is associated with an increased risk of HPV infection (Schabath et al, 2012; Vaccarella et al, 2008).

Similar to females, in men HPV16 infections have been observed to have a longer duration of infection relative to other HPV types (Giuliano et al, 2011). The HIM study found that the median duration of HPV infection for any HPV type was 7.52 months among men, whilst for HPV16 it was 12.2 months (Giuliano et al, 2011). As mentioned above, men are much less likely to seroconvert following HPV infection, and when they do, the median antibody titre induced by natural infection tends to be much lower than that induced in females (Giuliano et al, 2011). Furthermore, the likelihood of seropositivity appears to be higher when the concordant HPV types are detected in the anal canal as compared to when it is only present in the external genitalia, suggesting that the immune response to HPV infection in males varies depending on the site of infection (Lu et al, 2012). This is further substantiated by the observation that HPV seropositivity in men is strongly associated with lifetime number of anal sex partners (Rahman et al, 2016). However, the clearance of oncogenic HPV infection in men has been observed to be associated with age, which may be associated with increased seroprevalence with age (Giuliano et al, 2011).

Among men anal HPV infection is relatively common, with studies reporting the prevalence of any HPV infection to be approximately 12% among MSW aged 17-70 years old, whilst among MSM and HIV positive men this tends to be higher. A pooled study reported that the prevalence of any hrHPV was 41.2% among MSM (negative for HIV), whilst among MSM also HIV positive the prevalence was 74.3% (Nyitray et al, 2011; Sudenga et al, 2017; Wei et al, 2021). By comparison oral HPV infection in males is relatively rare, with reports observing a ten-fold lower risk of oral HPV positivity compared to anogenital HPV positivity (Kreimer et al, 2011), with no confirmed observable difference in risk between MSM and MSW (King et al, 2016). However, a study conducted among male students aged 18-24 years old, found that oral HPV infections appeared for the most part to transient, which might contribute to the observed lower point prevalence in comparison to anogenital sites of infection (Edelstein et al, 2012).

Persistent HPV infection in males is associated with the development of HPV associated cancer of the penis, anus, oral cavity, tonsil and oropharynx, and is estimated to be responsible for 0.8% of cancers in men worldwide (de Martel et al, 2017). The aforementioned HPV associated cancers, although not all attributable to HPV, were responsible for 36,068 cases (penile cancer), 21,706 cases (anal cancer), 264,211 cases (oral cavity), and 79,045 cases (oropharyngeal cancer) among men worldwide in the calendar year of 2020 (Sung et al, 2021). As compared to the general male population, the risk of developing a HPV associated cancer such as anal cancer, is increased among HIV positive, MSM, men with autoimmune diseases and solid organ transplant recipients (Clifford et al, 2021).

2.7.4 HPV viral load

The viral load found in HPV infected females has been shown to be associated with persistence of infection, inversely associated with clearance, and a predictor of progression to cervical intraepithelial neoplasia and invasive cervical cancer (Ylitalo et al, 2000; Josefsson et al, 2000; Wallin et al, 1999; Trevisan et al, 2013; Marks et al, 2011; Xi et al, 2011; Adcock et al, 2019). These findings, however, appear to be HPV type specific (Ramanakumar et al, 2010; Fu Xi et al, 2017), and also greater in women aged 30 years-old and older (Malagón et al, 2019). In a longitudinal study of Dutch women, the baseline HPV16 and 18 viral loads were found to be substantially higher in females whose infections were persistent over a one-year period than those who cleared the infection (van der Weele et al, 2016).

Although a circular causal chain between viral load and persistent infection, a longitudinal study reported that the median duration of HPV16 infection was longer in those with higher viral loads, regardless of whether the infection was a single type infection or present with other concurrent infections with different HPV types (Trevisan et al, 2013). Further studies investigating the effect of multiple HPV type coinfections on viral load have found that in the specific case where females are coinfected with multiple HPV types including HPV16, the viral load of HPV16 is higher than in single HPV16 infections (van der Weele et al, 2016).

Multiple studies have now confirmed the original findings of Ylitalo and Josefson (Ylitalo et al, 200; Josefsson et al, 2000) that women who have infections with high viral load have an increased risk of developing CIN2+, CIN3+, carcinoma in situ and invasive cervical cancer (Adcock et al, 2019). This association between viral load and lesion progression has been shown to be strongest for HPV16 (Malagón et al, 2019), to exhibit a dose response in the case of HPV16, 18 and 31 infections where the greater the viral load the greater the risk of progression (Malagón et al, 2019), and to display a temporality of events where the high viral load is observed prior to cervical lesion progression (Wang et al, 2013).

The median viral load of HPV infections has been observed to differ depending on the infecting HPV type and sex (Schmitt et al, 2013; Wissing et al, 2019). Furthermore, a unique cohort study following young Canadian couples, the HITCH study, found evidence that the viral load from penile samples was consistently higher than that in the vaginal samples from their partners (Wissing et al, 2019). Although it is possible that diagnostic artefact due to different sampling methods may have biased the results, the finding was replicated in another study which used a different sampling method (Bleeker et al, 2005). Among men, higher viral load was also associated with increased persistence of HPV infection compared to those with lower viral loads (Wissing et al, 2019). The same study, which was conducted among heterosexual couples, found that the type-specific HPV viral load tended to be correlated between sampling time points (in 4-month intervals) within individuals, especially in men (Wissing et al, 2019). The study also observed that the HPV viral load was correlated between couples over time, which the authors suggest may play some role in persistence and clearance of infection in couples (Wissing et al, 2019).

The HIV status of individuals has also been shown modify the HPV viral load among coinfected individuals. Studies investigating the viral load among HIV positive and negative women, among Senegalese women and Kenyan sex workers, found that the viral load was substantially higher in the samples from the HIV positive women in comparison to the negative women, 881 copies/cell in

comparison to 48 copies/cell (Luchters et al, 2010; Hanisch et al, 2014). However, it is noteworthy that a study which examined the anal HPV viral load among MSM, found that there was no observable difference in the HPV viral load from HIV positive MSM as compared to HIV negative MSM (Marra et al, 2017).

A study which looked at the HPV viral load among HPV infections in mid adult women aged 25-65, found that the HPV viral load did not significantly differ between transient or persistent infections, but tended to be lower in midadult women who were observed to intermittent DNA detection of a specific HPV type (Winer et al, 2014). These intermittent DNA detections in midadult may be indicative of latent infections (see section 2.7.5) and of lower risk in terms of progression to cervical cancer (Winer et al, 2014).

2.7.5 Non-eradicated infections post-clearance

The course of a single HPV infection during a person's life course has several possibilities. After initial virus inoculation, a lag period commences prior to the establishment of infection, the duration of which likely depends on the initial inoculating virus titre (Egawa et al, 2021). If the virus establishes infection, a period of viral gene expression within the limits of detection likely will follow, after which either the immune system (likely T-cell mediated) controls the infection leading either to true viral clearance (the eradication of all HPV viral particles), immune control sometimes termed as latent infection, where HPV viral particles may persist at low copy numbers in the basal cells, below the limits of detection, or low-level chronic infection, where in the absence of an effective immune response, persistent infection is established ultimately possibly leading to neoplastic disease progression (Doorbar, 2021, Gravitt & Winer, 2017, Malagón et al, 2021).

An individual who clears the initial infection may subsequently present as HPV DNA negative. However, this may not always be the equivalent of viral clearance as it may only be a subset of individuals who truly clear HPV, whilst those having latent infection may have HPV but below the limit of detection. These individuals may again present with HPV DNA positivity following a loss of immune control and subsequent viral reactivation (Doorbar, 2018). This somewhat ambiguous phenomenon may be partly responsible for the second peak in HPV incidence found in midadult women (described in section 2.7.2) and also for the redetection of HPV in women reporting sexual abstinence or monogamy. However, it is possible for previously HPV infected females who have once tested negative for HPV to test

positive for the same type via a variety of other sources (Moscicki, 2021); from acquisition following recent sexual encounter reported or otherwise (which may result in a new infection or a transitory deposition), from autoinoculation from either another anatomical site (Goodman et al, 2010), or from a partner serving as a viral reservoir, or from a sampling error. More recent biological studies have found evidence suggesting that following initial infection in a proportion of cases a phase of latency or immune control may indeed occur where the virus is not cleared but persists undetected by immune surveillance (Doorbar et al, 2021).

In line with the concept of immune control but not complete clearance, a study looking at *Betapapillomaviruses* found that in immunocompromised individuals, viral gene expression was much higher (Quint et al, 2015). In infections with rabbit papillomavirus, followed by lesion regression and subsequent experimentally induced immune suppression, an increase in viral gene expression to the extent seen in active infections was observed, suggesting that the rabbit had not completely cleared the virus. A change in the immune status resulted in virus reactivation, in line with the concept of a balance between immune control and surveillance and viral gene expression (Maglennon et al, 2014). In a molecular biology study investigating the entire cervices of two women who had had a hysterectomy (in the absence of any cervical disease), the investigators found evidence of a focal non-transforming and non-productive HPV infection in both cervices, one of which was an HPV18 infection (Hammer et al, 2019). In a recent study examining hysterectomy biopsies from 96 midadult females with no evident cervical disease, when using a highly sensitive detection method, hrHPV DNA was found in 17 of the biopsies, in combination with very low levels of E2 expression (as measured using RNA in-situ hybridisation) which the authors concluded to be in line with the expectation of latent infection in a notable proportion of women post-initial (chronic) infection (Doorbar et al, 2021).

Many epidemiological studies have also been conducted to investigate the source of infections detected in mid-adulthood, and to attempt to quantify the proportions due to reactivation of latent infections, autoinoculation, sampling error and acquisition due to recent sexual exposure. A study conducted by Rositch et al (2012) in midadult women aged 35 to 60 years old with semi-annual longitudinal follow-up found that 85% of incident HPV infection were detected among women reporting abstinence or sexual monogamy during the study. The authors further observed that these detections were significantly associated with cumulative lifetime number of sexual partners. This association was found to consistently increase with age, which is supportive of the concept that HPV detection in older women may be due to

redetection of previous infections (Rositch et al, 2012). On the contrary, a study conducted in Denmark among women aged 40-50 years old with a comparison cohort of younger females, found that the number of sexual partners in the last year was associated with new hrHPV infections regardless of age. However, after taking into account recent sexual behaviour, lifetime number of partners was again significantly associated with new hrHPV detections in the older age group (Brogaard et al, 2014).

If the natural history of latency and reactivation of infection is preceded by a drop in immune surveillance, it would be expected that more frequent reactivation of latent infections might occur in immunocompromised individuals. This was indeed the case, with one study reporting CD4 counts greater than 200 cells/microliter among HIV positive women (reporting to be sexually abstinent) to associated with less reactivation of HPV infection in comparison to those with CD4 counts under 200 (Theiler et al, 2010).

A longitudinal study found that the risk of new HPV detections in women attributable to recent sexual behaviour decreased with age and was associated with recent sexual behaviour in those with a seropositive history of the HPV type (Fu et al, 2016). Another study which compared the LCR DNA sequences of HPV16 reinfections to original infections in women who had cleared the infection in the follow-up interim, found that in 83% of the females from the convenience sample, the LCR sequence was identical to that found of their original HPV infection, which the authors suggested may be indicative of redetection of a low-level chronic infection (Ermel et al, 2018). However, given that the authors had no data on HPV infections from the women's sexual partners and only analysed cervical and vaginal samples, it is also possible that they may have been detecting either reinfections from their partner who was serving as a reservoir, and or autoinoculation from another anatomical site, for example, from the individual's anus.

Yet another study among men (specifically MSM) found a nontrivial occurrence of incident hrHPV detections in the anal and penile samples of men reporting no recent sexual exposure in the 6 months prior (Twisk et al, 2018). This might suggest that it is also possible for men to experience reactivations of possible latent infections.

Despite the multitude of studies undertaken to decipher the source of these redetections of HPV DNA, for this study question it is challenging to design epidemiological study devoid of bias. In the HITCH cohort study where the investigators could distinguish whether new detections of HPV were due to new-acquisition/re-infection from their partner or were in the absence of transmission

factors, the investigators concluded that 42.7% of the incident HPV infections observed were attributable to reactivation of a latent infection (Malagón et al, 2021).

2.8 Immune response to HPV infection

Following the acquisition and infection with HPV in the female genital mucosa, the virus employs a range of immune evasion strategies to avoid detection by the immune system for what may be several months (Stanley, 2010). Viral proteins (L1 and L2) and virions by the very nature of the virus life cycle being tied to the life cycle of the target cell it is infecting, avoid detection from immune surveillance by remaining at very low copy numbers until the infected cell has differentiated, and is on the path towards for programmed cell death. Such infections are not cytolytic, and additionally cause no inflammation, nor viraemia, further aiding the virus to remain undetected (Stanley et al, 2010; Stanley et al, 2012; Stanley et al, 2015). Furthermore, high-risk HPVs additionally actively evade the innate immune system via a variety of mechanisms. The E7 protein plays a role in downregulating toll-like receptor 9, suppressing a possible pro-inflammatory response. In addition to which, the E6 and E7 proteins play a role by actively inhibiting interferon signalling, thus delaying the activation of the adaptive immune system. (Stanley, 2010; Stanley, 2012) A minority of hr HPV infections result in chronic persistent infections resulting in the formation of neoplastic lesions. However, in the vast majority of cases hr HPV infections they are acute, with the immune system ultimately surmounting the virus's evasion strategies in order to mount a successful immune response resulting in clearance of the infection. Previous studies have estimated that approximately 80% of women mount some form of immune response resulting in clearance of infection within 30 months from first detection of infection (as measured by HPV DNA negativity) (Rodriguez et al, 2008).

The likelihood of clearance has been shown to be inversely associated with viral load of the HPV infection, with higher viral loads being associated with greater persistence of infection (Muñoz et al, 2009). Studies have found that the successful clearance of an HPV infections tend to be predominantly due to cell-mediated targeting of the early proteins (particularly E2 and E6) (Stanley, 2015; Woo et al, 2010). Following this approximately 50-70% of females also seroconvert to the major capsid protein L1 around 8-9 months after initial infection (in the case of HPV16 infections) (Carter et al, 2000; Tong et al, 2013; Faust et al, 2013). These infection-induced L1 IgG antibodies have been found to be fairly stable over time

and to persist for at least 10 years (af Geijersstam et al 1998; Antonsson et al 2010), although the extent of the antibody titres generated following natural infection have a much lower geometric mean than those instigated via L1 VLP vaccination (Carter et al, 2000). A study by Sherer et al (2016) found that natural infection induced antibodies tend to be non-neutralising and of low avidity. Previous studies have found evidence suggesting that this natural seropositivity may provide some protection, albeit limited, against the acquisition of subsequent infection with the same HPV type. The likelihood of protection showed dose response characteristics, where higher titres appear to confer greater protection against subsequent reinfection (Beachler et al, 2016; Castellegue 2014; Safaeian et al 2010; Wilson et al, 2014).

Although in the majority of women seroconvert following natural HPV infection (Stanley 2012; Rodriguez et al, 2008), the likelihood of seroconverting appears to differ depending on a variety of factors. Firstly, the inherent biological differences of different sites of infection may play a role in the likelihood of seroconversion following infection (Vriend et al, 2013; Pérez-Caraballo et al, 2018). Among men seroconversion is associated with concordant anal infection but not genital infection (Lu et al, 2012; van Rijn et al, 2014; Zou et al, 2016). However, studies examining possible differences in the rate of seroconversion by infection site have been limited among women and longitudinal studies are still lacking. Two cross-sectional studies have nevertheless attempted to investigate this. A cross-sectional study including women, men who have sex with men, MSM and men who have sex with women, MSW aged 16-24 years old, found that the association between site-specific infection was similar across genders, with the association between anal HPV DNA positivity and serum antibody positivity being similar to that of anal/vaginal HPV DNA positivity and serum antibody positivity, which the authors concluded was suggestive of differences in the natural course of infection depending by infection site (Vriend et al, 2013). Another more recent but smaller study among women aged 16-64 years old did not find any association between anal HPV DNA positivity and serum antibody positivity, despite finding a 2-fold increased likelihood of HPV seropositivity in the case of cervical HPV DNA positivity (Pérez-Caraballo et al, 2018). However, this study was both limited in sample size and cross-sectional. Biologically it is conceivable that the distinct differences in the tissue composition of the anal, penile, vaginal, cervical, and oral sites of infection may induce a humoral immune response at different rates. Infection of the keratinized epithelium of the penis may be more likely to be transient in comparison to infection of the transformation zones of the anus and the cervix (in particular) which are more

susceptible to the establishment of persistent infection (Giuliano et al, 2014; Vriend et al, 2013; Doorbar & Griffin, 2019), in combination with the higher accessibility of the lymphatic system in such nonkeratinized mucosal epithelium sites (Pérez-Caraballo et al, 2018; Lewis et al, 2019).

The risk of seroconversion following HPV infection as measured by HPV DNA positivity appears to differ markedly depending on the infecting type of HPV (Vriend et al, 2013; Artemchuk et al, 2019). Although there are limited studies evaluating the rate of waning of natural infection induced antibodies to HPV, one study reported that over a period of three years follow-up 20.5% of women initially seropositive for 1-2 HPV types seroreverted (Kann, 2020). Infection induced HPV antibodies have further been observed to be more stable among women who are seropositive for multiple types of HPV (Faust et al, 2019), with earlier studies also observing that the persistence of infection induced antibodies differed by HPV type, with antibodies to HPV16/18 more likely to persist than that to HPV6 (Carter et al, 2000).

In males, following infection with anogenital HPV the immune response is markedly different to that in females (Giuliano et al, 2015; Beachler et al, 2018). Few males have been found to seroconvert following infection (as measured as DNA positivity)(Giuliano et al, 2015). However, in those that do seroconvert a study in men who have sex with men found comparable HPV16 antibody titres to that found in women (Beachler et al, 2018). In men, even in those with the highest titre category this seroconversion was found to confer little protection against subsequent reinfection HPV16 (Beachler et al, 2018).

2.9 HPV Vaccines

There are currently three prophylactic subunit HPV vaccines which are available and licenced for use to prevent cervical cancer and cervical intraepithelial neoplasia grade two and above, CIN2+, by the prevention of high risk HPV genital infections. One is a bivalent vaccine, targeting HPV16 and 18, and is produced by GlaxoSmithKline, and the two others are a quadrivalent vaccine, additionally targeting low risk HPV6 and 11 (responsible for the majority of anogenital warts in humans), and a nonavalent vaccine additionally targeting hrHPV31, 33, 45, 52 and 58 (the next most common HPV types found in cervical cancer specimens after HPV16 and 18). There are numerous second-generation prophylactic HPV vaccines emerging, for example, using different expression systems or based on the L2 antigen, albeit these are yet not in use (Ahmels et al, 2022; Mariz et al, 2022).

2.9.1 Biology and mechanism of action

All three of the first-generation vaccines contain the HPV (type specific) L1 antigen self-assembled into virus-like particles (VLPs). Early studies in cattle showed that it was possible to protect the animals from infection with bovine papillomavirus (BPV) by immunizing them with inactivated BPV virions (Jarrett et al, 1990). However, to develop a vaccine against a high-risk HPV type or types required a more ingenious approach, as the properties of the virus precluded the use of either an inactivated or live attenuated virion-based vaccine. This is namely due to the fact the HPV genome contains oncogenes and therefore the risk of vaccine-induced carcinogenesis in the healthy target population would be too high. Also secondly, due to HPVs being notoriously hard to culture, meaning that the prospects for large scale manufacture of such a vaccine were poor (Schiller et al, 2018). These problems were abated however by the discoveries by Zhou and Frazer, and by Gissman and Kirnbauer and by Schiller, that the well-conserved major capsid protein, L1, of BPV and HPV16 had the ability to self-assemble into virus like particles (VLPs) (Zhou et al, 1990, Kirnbauer et al, 1992, 1993). The VLPs expose conformationally intact epitopes similar to that of the native infectious virions (Kirnbauer et al, 1992; Kirnbauer et al, 1993).

Early preclinical studies found that VLP vaccination in animal models could provide protection from pseudovirion challenge in cattle, dogs and rabbits. Furthermore, when transferring serum from a vaccinated to an unvaccinated rabbit model, the unvaccinated specimen was also subsequently protected from pseudovirion challenge, suggesting that the vaccine is able to provide protection via neutralising antibodies. However, the mucosal immune system (especially that pertaining to the genital tract) of many animal models is markedly different to that of humans (Mestecky et al, 2011). Furthermore, in these preclinical trials, the neutralising antibodies produced were predominantly genotype specific.

Following the preclinical trials, two pharmaceutical companies went on to develop the first two HPV vaccines, using L1 VLP technology. Although both companies used the L1 VLP technology, the vaccine composition, the adjuvants, the VLP expression system and the number of type specific VLPs included in the vaccines differ (Table 2).

Table 2: Composition of the different HPV vaccines, including the first-generation vaccines licensed for use.

Liscenced Vaccines						
Vaccine Trade Name	**Subunit**	**VLP Type & composition**	**Adjuvant**	**Expression System**	**Pharmaceutical Company**	**Dose Schedule**
Cervarix®	L1	HPV16 (20µg) HPV18 (20µg)	Aluminum hydroxide (500µg), 3-O-desacyl-4'-monophosphoryl lipid A (50µg)	*Trichoplusia ni* Hi 5, *Spodoptera frugiperda* SF9	GlaxoSmithKline	0, 1 and 6 months
Gardasil®	L1	HPV6 (20µg) HPV11 (40µg) HPV16 (40µg) HPV18 (20µg)	Aluminum hydroxyphosphate sulfate (225µg)	*Saccharomyces cerevisiae*	Merck	0, 2 and 6 months
Gardasil9®	L1	HPV6 (30µg) HPV11 (40µg) HPV16 (60µg) HPV18 (40µg) HPV31 (20µg) HPV33 (20µg) HPV45 (20µg) HPV52 (20µg) HPV58 (20µg)	Aluminum hydroxyphosphate sulfate (500µg)	*Saccharomyces cerevisiae*	Merck	0, 2 and 6 months

The quadrivalent vaccine developed by Merck, named as Gardasil®, is composed of HPV6, HPV11, HPV16 and HPV18 L1 VLPs expressed in *Saccharomyces cerevisiae* (yeast) cells and uses a simple aluminium salt as an adjuvant. The subsequent vaccine developed by Merck, the nonavalent vaccine, named as Gardasil9® uses the same adjuvant and VLP expression system but additionally includes L1 VLPs of five additional high risk oncogenic HPV types, HPV31, 33, 45, 52 and 58 (Table 2).

The vaccine developed by GlaxoSmithKline contains HPV16 and HPV18 L1 VLPs expressed in *Trichoplusia ni* Hi 5 and *Spodoptera frugiperda* SF9 insect cell lines and utilises a toll-like receptor agonist, 3-O-desacyl-4'-monophosphoryl lipid A (MPL), combined with simple aluminium salts, aluminium hydroxide, named as the GlaxoSmithKline Adjuvant System 4 (ASO4) (Garçon et al, 2011).

While aluminium salts have been utilised as adjuvants since the 1930s as an effective method to enhance the immune response to the antigen in the vaccine formulation, the addition of toll-like receptor agonists was a relatively new approach. Early studies conducted in animal models found that the use of MLP and aluminium salt combination formulation, known as ASO4, induced higher titres of neutralising L1 HPV16 and 18 VLP antibodies in comparison to only using an aluminium salt adjuvant (Giannini et al, 2006). The use of this adjuvant in the bivalent vaccine, Cervarix®, made it the first prophylactic vaccine to be licensed using a toll-like receptor agonist adjuvant.

Following the successful outcome of phase III clinical trials (FUTURE, 2007; Paavonen, 2007; Giuliano et al, 2011; Palefsky et al 2011), Gardasil®, Cervarix® and

Gardasil9® were all approved and licenced by both the European Medicines Agency (EMA) in 2006, 2007 and 2015 respectively (EMA, 2020), and by the United States Food and Drug administration (FDA) in 2009, 2006, and 2014 respectively (FDA, 2020).

All three vaccines are designed for systemic administration via intramuscular injection in the deltoid region of the upper arm. All three vaccines were originally recommended as a 3-dose regimen, albeit with slightly different vaccination schedules.

2.9.2 Immunogenicity

All the licensed vaccines were extensively studied for the magnitude, stability and longevity of the induced immune response following intramuscular injection in females and to some extent in males. Prior to the development of the first L1 HPV VLP vaccines the immunogenicity and especially the durability of the immune response to previous subunit vaccines (targeting other pathogens) had been limited, typically requiring multiple booster doses to counter waning antibody titres over time. Therefore, the surprisingly robust humoral response and observed efficacy against new persistent infections generated by both of the first generation has been encouraged the design of subsequent vaccines (Schiller & Lowy, 2018). There are several explanations as to why these subunit vaccines have been so effective. Firstly, the specific life cycle of HPVs which infect the anogenital mucosa may make it especially susceptible to vaccine-induced neutralising antibodies in comparison to other viruses infecting anogenital mucosal tissues (Schiller & Lowy, 2012). As described in section 2.4, in order to establish an infection the HPV first has to traverse to the basal layer and bind specifically to the basal epithelial cells. Once the virus reaches the basement membrane it binds to specific heparan sulfate proteoglycans, with subsequent conformational changes in the virus, which then enable the virus to bind to the receptors on the basal epithelial cells. This process is rather slow taking several hours, thus increasing the chances that the virus comes into contact with vaccine-induced neutralising antibodies before establishing (Kines et al, 2009). The resulting antibody-covered virus may thereby be prevented from binding to the basement membrane, the basal epithelial cell and/or be opsonised by phagocytes (which should be present in the area of disrupted epithelia) (Schiller & Lowy, 2018). In order for the virus to reach the basement membrane the first lines of defence, the epithelium, must first be breached. This may occur via

microabrasions or trauma which may for example occur during sexual intercourse. Although the resulting disrupted epithelia enable HPV to reach the basement membrane, it also may make it more susceptible to antibodies via exudation from the surrounding interstitial tissue and capillaries.

The structure of the HPV viral capsid makes it an exceptional target for the activation of naïve B-cells and subsequent generation of large titres of neutralising antibodies. The HPV L1 VLP comprised of 360 L1 molecules is characterised by a dense repetitive epitope display, at the specific spacing in the range of 5-10 nanometres. B-cells are highly responsive to specific antigen epitope spacing that is often found specifically on the surfaces of viruses and bacteria (but not on mammalian cells) (Bachmann et al, 1993). This is so as it corresponds to the spacing of the B-cell receptors when HPV L1 VLP binds to the B cell receptors it stimulates and enables the oligomerisation of receptors, thus amplifying and prolonging the response by preferentially promoting the differentiation of the activated B-cell in the germinal center into long-lived plasma cells (Cardone et al, 2014, Schiller & Lowy, 2018).

Although the primary antibody-mediated protection against infection in mucosal tissues is via IgA, systemic immunisation with HPV vaccines generates high levels of L1-specific serum antibodies, primarily IgG (Olsson et al, 2007; Romanowski et al, 2009; Schiller et al, 2018; Schwarz et al, 2019). Therefore, vaccine studies were primarily done by analysing the antibody titres in serum (Schiller et al, 2018). In the early immunogenicity studies of the first-generation vaccines Cervarix® and Gardasil®, the two pharmaceutical companies used alternative serological assays, precluding comparisons.

One independent head-to-head study found that the anti-HPV16 and anti-HPV18 antibody levels measured with a standard pseudovirion assay were 5- and 18-fold higher respectively among the Cervarix® recipients as compared to Gardasil® recipients seven to twelve years after vaccination with three doses (Artemchuk et al, 2019). In comparison to natural-infection induced antibodies, vaccine-induced anti-HPV16 antibodies were observed to be 73 and 15-fold higher among the bivalent and quadrivalent vaccine recipients respectively (Artemchuk et al). Furthermore, vaccine-induced anti-HPV18 antibody levels were reported to be lower in comparison to HPV16. However, the vaccine-induced anti-HPV18 antibody titres were still multiple fold higher than that induced by natural infection; 80- and 5-fold higher among the bivalent and quadrivalent vaccine recipients respectively (Artemchuk et al, 2019). Among the bivalent vaccine recipients, the seropositivity to HPV16 and 18 was 100%, whilst among the quadrivalent vaccine

recipients seroconversion was only 92% and 82% respectively. Importantly, for the cross-reactive non-vaccine HPV types, seropositivity was consistently higher among the bivalent vaccine recipients as compared to the quadrivalent vaccine recipients for HPV31, 35, 45, 51, 52, and 58 (Kann et al, 2020).

In another study of immunogenicity among 12–15-year-old girls, the vaccine-induced neutralising response was found to be consistently higher for HPV16 in comparison to HPV18 for both vaccines, and also higher among the recipients of the bivalent vaccine as compared to the quadrivalent vaccine (Draper et al, 2013). The cross-neutralizing antibodies detected in the serum against HPV31, 33, 35, 45, 52, and 58 were generally higher among the recipients of the bivalent vaccine as compared to the quadrivalent vaccine recipients (Draper et al, 2013). An independent study conducted using the Pseudovirion-Based Neutralization Assay, PBNA, likewise found that the peak neutralising HPV16/18 antibody levels (seven months after vaccination) and the cross-neutralising HPV31, 45 and 52 antibodies were higher among the bivalent vaccine recipients compared to the quadrivalent vaccine recipients. They also found that the seroprevalence of cross-neutralising HPV33, 45, 52 and 58 antibodies were highest among the bivalent vaccine recipients (Mariz et al, 2020). The cross-neutralising HPV31 and HPV45 antibodies was correlated with the neutralising HPV16/18 antibody response (Mariz et al, 2020).
These studies primarily evaluated the vaccine-induced antibody response in the serum, whilst HPVs infecting the anogenital and oropharyngeal sites are tropic to mucosal tissues. Normally in mucosal sites, the most abundant immunoglobulin species is that of secretory immunoglobulin A, S-IgA. However, in cervical secretions and seminal plasma, the most abundant immunoglobulin type is IgG although it is present in much less quantity relative to the serum (Kuteh et al, 1998; Johansson & Lycke, 2003; Mestecky et al, 2010).

In addition to microtrauma and leakage from the bloodstream there are various mechanisms through which the vaccine specific IgG may get to the cervical secretions in the female genital tract. IgG is thought to bidirectionally transduce from the serum in the surrounding capillaries through the genital epithelial cells by active transportation of the IgG by the neonatal Fc receptor. The transporting ability of the FcRn is dependent on the pH and appears to also be influenced by the estrous cycle, by the regulation of FcRn expression (Li et al, 2011).
Several studies have confirmed that the vaccine-induced anti-HPV16 and anti-HPV18 antibodies in the serum correlate with that in cervicovaginal secretions, although the antibody titre in the cervical secretions is around 10-fold lower than that found in the serum (Petäjä et al, 2010; Schwarz et al, 2010; Draper et al, 2013).

However, in line with the possibility that FcRn expression is sensitive to hormonal fluctuations during the estrous cycle, one study found that the level of vaccine-induced anti-HPV16 IgG fluctuates during the menstrual cycle, decreasing 9-fold during ovulation, before increasing again during the luteal phase and again during the proliferative phase (Nardelli-Haefliger et al, 2003). This finding therefore begs the question as to whether vaccine-induced protection may be lower during ovulation.

There has been much interest in the possibility of reduced doses and in the efficacy of a 1-dose schedule. An interrupted vaccination trial conducted in India, found that the immunogenicity of 2 doses was non-inferior to that of three doses, whilst 1 dose produced inferior albeit detectable antibody titres (Sankaranarayanan et al, 2016). For HPV16 antibodies the avidity is markedly lower among vaccine recipients receiving one dose in comparison to those receiving three doses (Tsang et al, 2022). Furthermore, a prior history of type specific HPV infection prior to vaccination was observed to result in an even lower avidity in comparison to those without prior history of infection (Tsang et al, 2022).

2.9.3 Vaccine efficacy

The vaccine efficacy of the two aforementioned first-generation vaccines, the bivalent vaccine Cervarix and the quadrivalent vaccine Gardasil, were primarily established via four large phase III trials. Vaccine efficacy (VE) is a measure of the direct effect of vaccination comparing the impact among vaccinated individuals in comparison to unvaccinated individuals and is defined by one minus the relative risk of the outcome among the vaccinated individuals compared to the unvaccinated individuals (Halloran et al, 1991). Prior to the phase III trial, a smaller (N=2392 participants) 'proof of principle' study was conducted using a monovalent HPV16 subunit vaccine (Koutsky et al, 2002). This HPV16 L1 subunit vaccine similar to the subsequently licenced quadrivalent vaccine was produced in *Saccharomyces cerevisiae* and included a simple aluminium adjuvant and was given to female participants aged 16 to 23 years old in a 3-dose regimen (at day 0, month 2, and month 6). The results of this proof-of-concept double blind randomised control trial were encouraging, showing that the vaccine had 100% (95% confidence interval 90-100%) vaccine efficacy against incident persistent HPV16 infection among women who were HPV16 DNA and seronegative at baseline, with no cases of HPV16-associated CIN

in the intervention group in two years after vaccination compared to nine such cases in the equally-sized control group (Koutsky et al, 2002).

The VE of the subsequent quadrivalent vaccine Gardasil, was evaluated by two double blinded randomised placebo-controlled trials, FUTURE I and FUTURE II (Females United to Unilaterally Reduce Endo/Ectocervical disease). These large company sponsored phase III efficacy trials were multinational; conducted in study sites located in twenty-four countries between 2002-2007. In total, 17,622 females aged 16-26 years old were randomised to receive either the vaccine or the placebo in a 3 dose regimen (at day 1, month 2 and month 6). Potential participants in the FUTURE II trial with more than four lifetime sexual partners were excluded, to minimise baseline exposure. This threatened to reduce the generalisability of the findings, therefore in Finland an exception was made by omitting the life-time sexual partner exclusion criterium. The two trials were designed to evaluate vaccine efficacy against a composite endpoint of CIN 2/3, adenocarcinoma in situ and HPV16 or HPV18 associated cervical cancer. Such an endpoint measures were deemed appropriate as a surrogate measure of the vaccine's efficacy in preventing cervical cancer, as to have cervical cancer as the sole endpoint would have been unethical (FUTURE II Study Group, 2007; Schiller et al, 2012).

Excluding Finland, these efficacy trials were limited due to the choice of enrolling participants at median age of 21 years old, when the primary target age-group for vaccination programs is early adolescent girls and boys aged 11-13 years old prior to first sexual intercourse. Regardless, the first interim analysis conducted after the accrual of a sufficient number of endpoints reported a high vaccine efficacy of 98% in preventing HPV16 or 18 associated CIN2/3 or adenocarcinoma in situ in the per protocol study population three years after first vaccination. The per-protocol study population included only women who had no deviation from the study protocol and were HPV16/18 seronegative at baseline and HPV16/18 DNA negative at both baseline and during the first month following receiving the first vaccine dose, thus categorising them as susceptible, and providing an estimate of the prophylactic vaccine efficacy in those prior to HPV16/18 exposure (FUTURE II Study Group, 2007; Schiller et al, 2012). In a less strict comparison, conducted in the intention-to-treat study population, i.e., all those participating in the trial subsequent to randomisation irrespective of baseline HPV DNA and serostatus and deviations from trial protocol regarding dosage and dose regimen, a vaccine efficacy of 44% was reported (FUTURE II Study Group, 2007). The same study also reported a low vaccine efficacy of 17% against CIN2+ lesions irrespective of the associated HPV type of the lesion among the same intention-to-treat study population (FUTURE II

Study Group, 2007). These results subsequently formed the basis for the subsequent licensure of the quadrivalent vaccine for use in females and males aged 9 and older for the prevention of premalignant genital and anal lesions, cervical and anal cancers and condyloma acuminata by the regulatory authorities; the United States Food and Drug Administration (FDA) in June of 2006 and the European Medicines Agency (EMA) in September of the same year (Muñoz et al, 2010).

The vaccine efficacy for the bivalent vaccine was evaluated by the international phase III double blind randomised control trial, the PATRICIA trial (PApilloma TRIal against Cancer In young Adults) (Paavonen et al, 2009). This trial commenced in 2004 and randomised women aged 15-26 to receive either the bivalent vaccine (N=8093) or a control Hepatitis A vaccine (N=8069) (Paavonen et al, 2009). All participants received their allocated vaccine in a dosage regimen of 0, 1 and 6 months. In the according-to-protocol analysis of women who were both HPV seronegative and DNA negative at baseline, a vaccine efficacy against HPV16/18 associated CIN2+ of 93% (95% confidence interval 80-98%). Additionally, a vaccine efficacy of 54% (34-68%) was observed against non-vaccine targeted HPV type associated CIN2+ (Paavonen et al, 2009).

Since these vaccine trials were initiated, sufficient time has elapsed to permit the evaluation of vaccine efficacy over a longer time period. From a follow-up study of the Costa Rica Vaccine Trial over a period of 11 years since initiation, the study investigators reported cumulative vaccine efficacies against HPV16/18 associated CIN2+ and CIN3 of 97% and 95% respectively (Porras et al, 2020).

2.9.4 Cross-protective efficacy

During phase III clinical trials the bivalent and quadrivalent vaccines also demonstrated to have a degree of cross-protective efficacy against some phylogenetically closely related HPV types, although the degree of cross-protection is somewhat higher for the bivalent vaccine. Following phase III trial of the bivalent vaccine, for types from the Alpha 9 clade closely related to HPV16, cross-protective efficacy was reported for HPV31 (vaccine efficacy: 77% [95% confidence interval, 67-84%]), HPV33 (43% [19-60%]) and HPV52 (18% [3-32%]) against persistent infection (Wheeler et al, 2012). For types from the Alpha 7 clade, closely related to HPV18, vaccine efficacy was observed for HPV45 (79% [61-89%]) (Wheeler et al, 2012).

Cross-protective vaccine efficacy was also reported following phase III efficacy trials of the quadrivalent vaccine against persistent HPV31 infection (46% [15-66%]) (Brown et al, 2009). No other notable cross-protective vaccine efficacies were reported for the other non-vaccine types (Brown et al, 2009).

2.9.5 Vaccination against a sexually transmitted infection

The successful development and licensure of the first-generation HPV vaccines has resulted in a novel primary prevention instrument in the control of a sexually transmitted infection (STI). Given that this is the first successful and highly efficacious prophylactic vaccine for an infection where the most common transmission route is sexual, and there are differences in disease manifestation by gender, this presents new questions as to the ethical and most beneficial implementation of vaccination.

Traditional vaccination programmes have largely targeted childhood diseases such as measles, mumps and rubella. The pathogens causing such diseases are ideal candidates for vaccination programs as naturally transient infection tends to result in life-long immunity with their persistence in human populations only a result of subsequent generations being born into the susceptible pool, rather than due to factors such as antigenic drift or shift or a non-human reservoir of infection (Garnett, 2005). For sexually transmitted infections such as HPV the situation is quite different, as the susceptible population is more or less limited by the sexual route of transmission and assortativeness of sexual contacts.

Therefore, if a sexually transmitted pathogen utilises a strategy of antigenic drift or immune evasion in order to persist in a population, it makes them poor candidates for producing a vaccine, as is the case for HIV. HPV on the other hand is host specific, has no non-human reservoir of infection, as a double stranded DNA virus is remarkably stable, and in order to persist in a population it has evolved to evade the host's immune system (Harper, 2000). This makes HPV a difficult candidate to be tackled by immune surveillance. Although natural infection with HPV induces some degree of protection among females against subsequent reinfection (Beachler et al, JID, 2016), seroconversion is not universal and a small proportion of women have been observed to serorevert (af Geijersstam et al, 1998; Kann, 2020). However, the first-generation vaccines induce a long-lived sustainable immune response multiple-fold higher than that induced by infection (Artemchuk et al, 2013; Kann et al 2020).

Furthermore, unlike traditional childhood diseases, the heterogeneity among populations in the risk of both acquiring and transmitting HPV due to varying levels of sexual activity also has large implications to the critical vaccination threshold for eradication (Garnett & Waddell, 2000). The critical vaccination threshold for eradication is the proportion of the population which is required to be protectively vaccinated in order for the infection to be eliminated, and it is directly related to the basic reproduction number, R0, of an infectious agent, by 1-1/R0. However, this is assuming within-population homogeneity both in the risk of acquisition and transmission. For a sexually transmitted infection, these parameters differ by age, and there exists considerable heterogeneity within-populations according to sexual risk-taking behaviour, with groups such as the core group, MSM, WSW and the HIV positive all having different risks (Figueroa et al, 1995; Silins et al, 2002; Luchters et al, 2010; Branstetter et al, 2017; Fairley et al, 2017; Reiter & McRee, 2017, Malagón at al, 2018). The core group is defined as the subpopulation within the population with high sexual contact rates, which tends to be assortive by sexual behaviour. However, for a very common STI such as HPV, the risk differences between these groups may be smaller than for example syphilis (Kibur et al, 2000).

Early modelling studies of the vaccination coverage for a hypothetical STD vaccine indicated that the vaccine coverage which would be effective would differ drastically between the general, low risk-taking population and the high sexual risk-taking population. Given that for many STIs the basic reproduction number is already below one in the general population, even a low gender-neutral vaccination coverage should result in a large prevalence reduction (Vänskä et al, 2020), with an effect possible even among the high sexual risk-taking population if the gender-neutral vaccination coverage is high (Garnett, 1998; Garnett & Waddell, 2000). Although HPV is sexually transmitted, it is also very common among the general low risk-taking proportion of the population. Therefore, compared to the "hypothetical STD vaccine" situation HPV is different with the R0 being above 1 also among the general population. Therefore, Scotland the critical vaccination threshold is likely to be higher among the general population, and especially among the high sexual-risk taking group. Finally, given that the HPV vaccine is prophylactic, and it is not possible to pre-identify those who will become the high-sexual risk-taking group prior to the commencement of sexual activity, targeted strategies are unlikely to be effective, thereby demanding higher cohort vaccination coverage among the whole population.

Prior to the implementation of HPV vaccination, there was concern regarding how the general public would accept a vaccination against a STI. This concern was

notable especially given that the vaccination was advocated by the manufacturers primarily to young females pre-sexual debut (Garnett & Waddell, 2000). Given that the vaccine was initially targeted at girls only, there was also concern that the vaccination coverage among males might not be the same as among females (Gottvall et al, 2017). This latter concern appears to have been well-founded given that many countries which have commenced gender-neutral vaccination (following a previous girls-only policy) have observed consistently lower vaccination coverages among males. For example, in Scotland the vaccination coverage among males has been consistently lower compared to that among females across all health boards (Public Health Scotland, 2020). Whilst the former concern appears to have been one of the contributing factors to parental non-consent to vaccinate their children with the HPV vaccine (Widman et al, 2018).

Concern was also raised whether vaccination against a sexually transmitted infection such as HPV may result in an increase in sexual risk taking and or reduced attendance rates to cervical screening programs due to a perceived lower risk of infection (Marlow et al, 2008). Epidemiological studies conducted in Canada and the Netherlands post-HPV vaccination found that sexual behaviour remained relatively constant among adolescents' post-vaccination, with no indications suggesting the occurrence of risk compensation (Ogilvie et al, 2018; Donken et al, 2018).

Furthermore, the implementation of a vaccination against a sexually transmitted virus such as HPV raises several questions in the field of ethics. When choosing the vaccination strategy, it is imperative that attention be paid to the key concepts in ethics, and that the strategy of choice maintains and does not compromise justice, non-discrimination, privacy and non-stigmatization (Malmqvist et al, 2011; Natunen et al, 2011; Malmqvist et al, 2012; Luyten et al, 2014). When deciding whether to implement a publicly funded gender-neutral HPV vaccination policy or a targeted policy (for example, girls only and/or MSM-specific vaccination) it is important to maintain social justice. That being that the vaccination should not contribute to widening social inequalities both on a national level and on an international level. It is crucial that the most marginalized females within society, those who are at increased risk of acquiring HPV infection, who are also less likely to receive HPV vaccination and attend cervical screening and protected only by slow/ineffective second order herd effect are not placed at disproportionate risk to the rest of the population in the port-vaccination world (Vänskä et al, 2020). First order herd effects are defined as the herd effect conferred to unvaccinated men from vaccinated women, whilst second order herd effects are defined as the protection conferred to unvaccinated women via the first order herd effect conferred to the unvaccinated

men (Vänska et al, 2020; Hoes et al, 2021). This can be achieved best by implementing school-based gender-neutral vaccination which provides higher and more equitable vaccination uptake rates than opportunistic vaccination (Wang et al, 2019). Most importantly, the gender-neutral vaccination provides far superior herd effect thus protecting even the marginalized unvaccinated females in the society (Lehtinen et al, 2018a; Lehtinen et al, 2018b; Vänskä et al, 2020).

One of the key issues regarding HPV vaccination program implementation has been regarding the most efficient strategy to implement. In the early days since HPV vaccine licensure the majority of HPV vaccination programs were targeted only at girls, and as this was thought to be the most cost-effective strategy. It would have the greatest direct effect in preventing cervical cancer (the greatest burden of disease due to HPV infection). However, there are multiple caveats in this approach. Firstly, HPV-associated disease burden is not exclusive to females. Men are susceptible to HPV-associated anal cancer, oropharyngeal cancer and penile cancers. Unlike the cervical cancer these HPV-associated cancers are generally attributable to HPV16 infection, and there are no current screening measures in place for these cancers. Thus, cases usually present at a late disease state, where prognosis may be poor. Thereby, males also benefit from the direct effect of vaccination in the prevention of HPV associated disease burden.

The counterargument has been that males may also be protected by the indirect effect of girls-only HPV vaccination, i.e. herd effect readily documented against HPV16/18 with moderate coverage (70%) of girls-only vaccination programs (Pillsbury et al, 2017). Herd effect or herd immunity is the term which describes the indirect effect among the unvaccinated individuals in a population where vaccinated individuals are also present. The problem with this strategy is that in real-life high vaccination coverage is the exception not the rule and is all too susceptible to sudden drops in vaccination confidence (Bruni et al, 2016; Bruni et al, 2021). More realistic vaccination coverages reign in the region of 40-60% in the case of organised school-based vaccination programs, with even lower coverages common place in unorganised/opportunistic programs. Such coverages are not only suboptimal but are insufficient to induce a herd effect against HPV16 as the basic reproduction number has been estimated to be as high as 4 (Vänskä et al, 2020). Thus the critical threshold for elimination is also higher, with coverages of above 90% generally being necessary (modelling estimates calibrated to the Finnish population setting reported an immunity threshold of 95% for HPV16 when girls-only vaccination is the strategy in use, although this figure will differ by population, due to the R0

differing by population, owing to differing sexual networks between populations) (Baussano et al, 2017; Vänskä et al, 2020).

The critical vaccination coverage required for elimination is dependent on the pathogen's basic reproduction number. The basic reproduction number for a given HPV type is dependent on the mean duration of infection and the effective transmission rate, both parameters differ both by HPV type and by population. For example, the mean duration is much longer for HPV16 (Figure 4) (Lehtinen & Dillner, 2013), which is responsible for its higher basic reproduction number and therefore, the higher vaccination coverage required for elimination. Whilst for other HPV types such as HPV45, the mean duration of infection is much shorter, thus eradication is possible at much lower vaccination coverages (Baussano et al, 2017). However, given that HPV16 is well known to be the most oncogenic HPV type of the high-risk types and is responsible for the majority of HPV-associated cancers (Bzhalava et al, 2013), the coverage required for the elimination of this type should guide HPV vaccination policy.

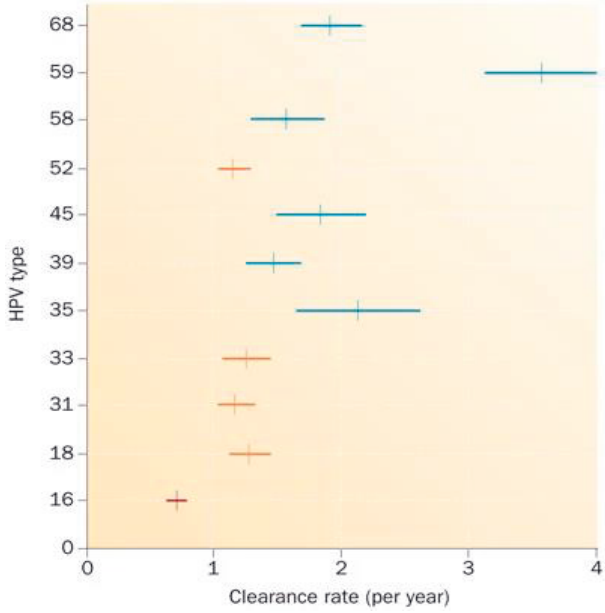


Figure 4: HPV type specific clearance rates per year with 95% confidence intervals (Lehtinen & Dillner, 2013). Reproduced with permission from Nature Publishing Group.

2.10 Vaccine-induced pathogen evolution

For the majority of established pathogens which are endemic within populations their ability to persist is a delicate evolutionary balance against the odds of multiple

survival pressures. A pathogen may have to encounter negative (or positive) pressures, such as interspecies competition, intra species competition, the host immune system, over-virulence resulting in host death, a noninfinite pool of susceptible hosts, limited possibilities for transmission to a new susceptible host, or environmental pressures to name but a few. Thereby, the survival of pathogens at endemic level in a more or less stable ecological equilibrium represents a remarkable feat in fitness honed over time.

There are many ways in which pathogens may adapt or evolve due to the selective pressures of vaccination, including but not limited to vaccine-induced virulence evolution, changes in tissue tropism, epitope evolution, strain replacement (where multiple strains present) and changes in the immunosuppression (Read and Mackinnon, 2007). If and how a pathogen may evolve as a result of targeted vaccination is intrinsically dependent on the biology of that pathogen in question. In the context of HPV, where there are multiple HPV types causing genital HPV infection, there was much concern raised among the scientific community, as to whether the targeted vaccination of only HPV16 and 18, would cause an increase in the occurrence of the other non-targeted HPV types in a process known as genotype (or simply type) replacement (Garnett and Waddell, 2000; Lehtinen & Paavonen, 2004). HPV is a relatively stable virus as a double stranded DNA virus which uses the host cells machinery to replicate, therefore the likelihood of other means of vaccine-induced evolution is unlikely at least in the short term.

2.10.1 Type replacement

The concept of vaccine-induced type replacement or serotype replacement is in the realm of evolutionary ecology. In very basic terms, according to the Gauses's law of competitive exclusion, when one or more species are in competition for the same ecological niche, they cannot stably coexist, and as the dogma goes, we would expect one species to outperform or outcompete the other (Gause, 1934). However, in practise, it is again slightly more complicated than this. There are multiple modes in which a species or strain may interact with one another, be it at the extreme end of symmetrical antagonism (i.e. both species negatively compete with one another) or mutualism (i.e. both species benefit the existence of the other), all the other combinations of interaction in between (commensalism, parasitism or amensalism) or no interaction at all (otherwise known as independence between the two strains or species) (Man, 2021). In ecology independence is the rare exception rather than

the rule. Further to this, the strength of any of these interactions may be non-symmetrical, and/or may involve different modes of interaction through which trade-offs may allow for the coexistence of strains or species.

When we think of the case of vaccination targeting a subset of microbial strains, we are disrupting the ecological equilibrium between a number of strains. Thus, if the non-vaccine targeted strains had been interacting with the vaccine targeted strain to the detriment of the non-vaccine targeted strain, upon removal of the vaccine targeted strain this competitive pressure shall be removed from the non-vaccine target strain. This should result in the increased occurrence (or virulence, viral/bacterial load, transmission, etc.) depending on what the negative consequence of the vaccine targeted stain was on the non-vaccine target strain. Similarly, if the interaction was synergistic, say infection with strain A increased the occurrence of strain B, then upon removal or reduction of strain A owing to targeted vaccination, we should expect strain B to decrease owing to the removal/reduction of this synergistic pressure. If the former case is true, where the vaccine and non-vaccine strains negatively interacted and resulted in increased occurrence post-vaccination, then this phenomenon is termed as vaccine-induced type replacement (be it serotype or genotype replacement).

Prior to the widespread implementation of HPV vaccination, such similar phenomenon had previously been observed following vaccination against selected serotypes of *Streptoccocus pneuomoniae* and strains of *Bordetella pertussis* (causing whooping cough) (Weinberger et al, 2011; Elomaa et al, 2005; Read & Mackinnon, 2007; Kallonen et al, 2011). Following national pertussis vaccination, strains with a pertussis toxin allele not found in the vaccine strains have notably increased among the isolates of from cases, replacing the vaccine strains. A study conducted comparing the findings from four European countries pre- and post- vaccination era found that there were differences in the rate of change between the countries which the authors concluded might be due to vaccine-induced strain replacement (Kallonen et al, 2011).

Therefore, given the fact that there so many non-vaccine targeted HPV types, there was understandably worry that the same might happen following HPV vaccination. HPV as a pathogen is very different from the examples above. However, to understand whether this might be possible, many studies were conducted by many research groups to try to ascertain whether HPV types interact with one another in a manner which might indicate vaccine-induced HPV type replacement to be a possibility. Many different approaches have been and continue to be used, however they can be categorised into those using epidemiological study designs, those from

biological data and those using mathematical modelling to try to disentangle and understand HPV occurrence and whether it is suggestive of HPV type competition.

The studies conducted using epidemiological approaches are based on the assumption that if HPV types compete with one another then the risk of acquiring or clearing a second HPV type will be negatively or positively associated respectively with the positivity status (whether it be seropositivity or DNA positivity) of the first HPV type (Tota et al, 2013). This approach further assumes that in the absence of confounding and interaction between the HPV types then the association between the frequency of one HPV type will not be associated with the other (Tota et al, 2013). To assess this the most aptly designed studies have used a longitudinal cohort design (Plummer et al, 2007; Tota et al, 2016 & 2017). However, many studies have also been conducted using a cross-sectional design where the assumption is that if HPV type competition occurs then DNA positivity for one specific HPV type will be negatively associated with positivity for another type.

When studies have evaluated HPV co-occurrence from cross-sectional data although there have been some sporadic findings of negative associations between certain HPV types (for example between HPV16 and HPV51 [Mejlhede et al, 2010]), generally HPV types have been observed to be positively associated with the occurrence of other HPV types (with most authors reasoning that this may be due to residual confounding owing to sexual risk-taking behaviour), with the few negative findings generally being attributed to diagnostic artefact (Chaturvedi et al, 2005 & 2011; Mejlhede et al, 2010; Vaccarella, 2010, 2011 & 2013; Mollers et al, 2013; Tota et al, 2015).

In longitudinal studies assessing the occurrence, risk of acquisition or persistence/clearance of one HPV type when infected at baseline with another, the overwhelming findings have been that there is an increased risk of acquiring another HPV types when one is positive at baseline for another, although residual confounding owing to sexual risk-taking behavior is likely (Thomas et al, 2000; Liaw et al, 2001; Rousseau et al, 2001; Mendez et al, 2005; Plummer et al, 2007; Tota et al, 2016). With studies assessing the risk or rate of clearance of one HPV type when infected with another generally found no association, suggesting that the rate of clearance with one HPV type is independent of infection with another (Tota et al, 2016). Overall, the authors generally concluded that there was no convincing evidence of HPV competition between the types, and thereby that the risk of HPV vaccination inducing type replacement by non-vaccine HPV types to be low or nil.

Unfortunately, however, there are several potential flaws with these approaches and the underlying assumptions (Murall et al, 2014). Especially with cross-sectional

data the assumption is generally of symmetrical interaction and only includes interaction between two HPV types at a time. It does not take account of the real-life multi-type dynamic ecological system which may be the reality (Murall et al, 2014). Furthermore, especially when interpreting the data from cross-sectional studies, even with advanced statistical techniques and in depth behavioural questionnaires it is essentially impossible to perfectly take account of all possible confounding; such as confounding owing to sexual risk taking behaviour, the common route to acquisition, and bias due to the time of acquisition of one type being correlated with that of another owing to the common transmission route when one has a new infectious contact, i.e. a new or newly infectious sexual partner (Malagón et al, 2016). Such confounding is likely to bias the associations above the null, and such a positive finding is generally interpreted as evidence of no HPV competition or as evidence of synergistic interaction. Furthermore, if infection induced cross-immunity does exist between HPV types (which would be suggestive of the potential for post-vaccination HPV type replacement), then counterintuitively it is possible that HPV positivity with one type will be positively associated with positivity for the other HPV type (Durham et al, 2012; Man et al, 2018). This suggests that the observed increased odds ratios observed among previous cross-sectional studies may be also be indicative of HPV type competition and the potential for type replacement in the long-term (Durham et al, 2012; Man et al, 2018).

2.11 Epidemiological studies of HPV type replacement

To evaluate whether type replacement actually occurs after vaccination several approaches have been taken. Firstly, investigators have directly compared the occurrence of non-vaccine HPV infections between vaccinated trial participants and controls, interpreting negative vaccine efficacy as supporting type replacement, or type replacement potential of that given type. Following vaccination there were some negative vaccine efficacies against persistent HPV infection recorded for example for HPV35 and 58. However, the confidence intervals overlapped the null suggesting that this may have been due to chance and is not convincing evidence of type replacement potential (Wheeler et al, 2012). Whilst similarly although some negative efficacies were reported following the phase III trials of the quadrivalent vaccine, the confidence intervals always overlapped the null (Brown et al, 2009)

In 2016, systematic review and meta-analysis of such studies found some evidence of increased HPV38 and 52 occurrence post-vaccination (Mesher et al, 2016). However, this increase may be due to possible diagnostic artefact owing to unmasking (Tota et al, 2015; Mesher et al, 2016). Whilst a later systematic review and meta-analysis in 2019 found an increase in the prevalence of combined high-risk non-vaccine HPV types post-vaccination (Drolet et a, 2019).

However, it is possible that inherent limitations due to a lack of time since follow up may have precluded the ability of these studies to observe the full potential of HPV vaccination to induce type replacement in the long-term (Man et al, 2021). A mathematical modelling study using a transmission model assuming that HPV type competition occurs via natural infection induced cross-immunity, found that the time from the initiation of HPV vaccination to type replacement occurrence may be a trade-off between the degree of vaccine-induced cross-protection against the non-vaccine type in question and the strength of natural infection-induced cross-immunity (from infection with the vaccine targeted HPV types) (Man et al, 2021). The study found that even when there was potential for type replacement, it might be possible for the vaccine-induced cross-immunity to mitigate the occurrence and or degree of the eventual state of type replacement (Figure 5) (Man et al, 2021). However, if the cross-protection efficacy is not strong enough then it might result in the occurrence of a honeymoon period, where the prevalence or incidence of the non-vaccine type in question first decreases after vaccination then after a time starts to rebound into type replacement (Man et al, 2021). The time to which this rebound effect might take place was shown to depend on multiple factors but might not be observable until at least ten years after vaccination initiation (Man et al, 2021). Thus, the need for continuing monitoring of post-vaccination type replacement occurrence will remain critical for many years to come.

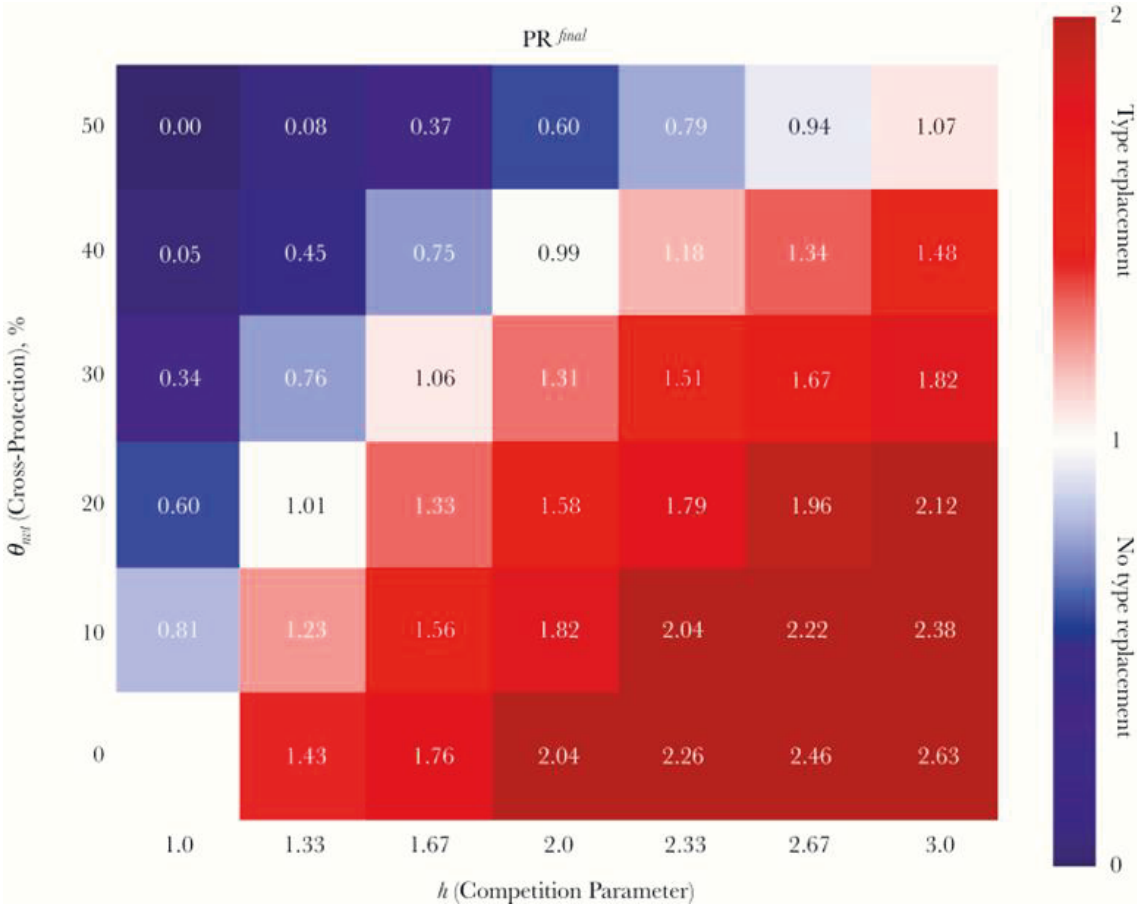


Figure 5: Predicted change in prevalence ratio of non-vaccine HPV types in the postvaccination equilibrium when changing the parameters of vaccine-induced cross-protection and HPV type competition using a SIRS transmission model with the sexual contact structure parameterised based on the Dutch population (Man et al, 2021). Reproduced with permission from Oxford University Press.

2.12 Limitations and bias in HPV epidemiology studies

Epidemiological studies evaluating HPV type replacement are susceptible to a host of various systematic biases and limitations often depending on the study design and/or applied methods. These may give rise both to erroneous results mis-interpretable as the occurrence of HPV type replacement, to results which may falsely be biased towards no change in current non-vaccine HPV type occurrence and to results which may falsely be interpreted as non-potential for future HPV type replacement.

2.12.1 Outcome Misclassification

One common example of such a bias is that of outcome misclassification. When conducting epidemiological studies comparing pre- and post- vaccination HPV type occurrence, there are multiple sources of outcome misclassification which may occur and which should be taken into consideration when interpreting the results.

When the outcome being measured is occurrence of HPV type-specific DNA, as a measure of transitory HPV infection, particular attention should be paid to the PCR method applied. When PCR methods using consensus primers are utilised attention should be paid to the primer sets used. Previous studies comparing the primer sets GP5+/6+, MY09/11 and PGMY09/11, found that in a sample of cervical cancer specimens from Hong Kong Chinese women, that the GP5+/6+, had poor sensitivity for detecting the presence HPV52, due to a prevalent nucleotide substitution in the HPV52 from the samples, resulting in a sequence mismatch with that on the primer binding site, and reduced HPV52 detection (Chan et al, 2005). A further study, comparing the amplification of HPV16, 18, 31, 51 and 52 DNA using PCR with different available primer sets, also found that especially the PCR using consensus primer sets GP5+/6+ and MY09/11, both had poor sensitivity for detecting HPV51 and 52 DNA (Mori et al, 2011). With this in mind, when conducting pre-and post- prevalence studies it is crucial that the same PCR method is used to analyse the samples from both time periods, so as to avoid differences HPV detection by HPV type due to differential [type-specific] sensitivity of the PCR methods used being misconstrued as HPV type replacement.

Further to this, even when using optimal HPV genotyping methodology, HPV DNA positivity has imperfect specificity as a measure of current HPV infection. In reality there are several means in which a female may be measured to be HPV DNA

positive (assuming perfect sensitivity and specificity of measurement): 1) the individual may have an actual HPV infection and free viral DNA, 2) the individual may have acquired a deposition of HPV virions from their partner, 3) the individual may have the presence of infected sperm cells recently acquired via sexual activity, or 4) via recently exfoliated (via sexual activity) HPV infected cells of the partners epithelium (Malagón et al, 2017). A study conducted to attempt to quantify this degree of misclassification of transient infection among a cohort of young women and their male partners, found an excess in concordant partners of 14.1% attributable to vaginal sex in the last week. Further to this, the degree of excess partner concordance 0-1 days since last vaginal sex was found to be modified by condom usage, suggesting that condom usage reduces the occurrence of HPV deposition. The same study also investigated the validity of Y chromosome DNA (detected from the female's vaginal sample) as a biomarker for such misclassification. However, Y-chromosome DNA positivity was not found to be an independent marker for excess partner concordance in HPV positivity (Malagón et al, 2017). On the other hand, a later follow-up of the same cohort study, found that Y-chromosome DNA positivity and especially the quantity of Y-chromosome DNA in women's vaginal samples were strongly associated with recent vaginal sex and non-usage of condoms (Malagón et al 2018).

The majority of studies evaluating HPV vaccination implementation, whether evaluating vaccine efficacy or vaccine effectiveness at preventing vaccine HPV type infection or HPV type replacement, have paid little heed to this inherent outcome misclassification owing to depositions. Vaccine effectiveness is defined as the relative difference in HPV prevalence between the vaccinated and unvaccinated within a population at the same given time (Halloran et al, 1991). The 14.1% estimated by Malagón et al (2017) may be different in different populations and particularly age groups, as well as for individual HPV types (although there is currently neither evidence supportive nor contrary to this supposition). The corresponding 85.9% specificity may likely result in a substantial bias in the estimates of vaccine efficacy/effectiveness and type replacement, towards the null hypothesis when pooling the findings across multiple studies (Jurek et al, 2005). However, it is a fallacy to state that in any one particular study that this assumed non-differential outcome misclassification will bias the estimate towards the null, as it is also a fallacy to assume that in the study population the misclassification will be exactly non-differential (Jurek et al, 2005; Sorahan & Gilthorpe, 1994; Wacholder et al, 1995). By virtue of chance, in any one particular study the real outcome misclassification rates as they pertain to the data may be less than perfectly non-differential between the two

exposure groups, even when the misclassification process would have been expected to be non-differential (Sorahan & Gilthorpe, 1994; Wacholder et al, 1995). As even small variations from perfectly non-differential misclassification may have a large effect on the bias of the final study estimates (Jurek et al, 2008), biasing the estimate away from the null, this should not have been ignored when conducting HPV vaccination studies. Furthermore, even if the outcome misclassification were to be perfectly non-differential, studies evaluating the effect of HPV vaccination on HPV type specific incidence, should be especially careful, as the degree of bias resulting from suboptimal specificity will be more pronounced for rarer disease outcomes (i.e. the rarer HPV types) (Sorahan & Gilthorpe, 1994). Therefore, when conducting studies evaluating the impact of HPV vaccination HPV type specific incidence or prevalence it is necessary to also perform sensitivity analysis to quantify bias owing to outcome misclassification, taking into account error due to both random variation and systematic error, in order to properly interpret the results (Lash et al, 2009; Lash et al, 2014).

2.13 Potential public health impact of type replacement

There is a fear that the selective removal of HPV16/18 (and also HPV6/11) via national HPV immunisation programs may result in HPV type replacement by the non-vaccine targeted HPV types thus undermining the public health impact and effectiveness of HPV vaccination (Lehtinen and Paavonen, 2004). However, it may be that even if HPV type replacement does occur it will not equate to complete disease replacement, due to the fact that the non-vaccine types have a lower oncogenic potential especially in comparison to HPV16. Nevertheless, there are some caveats to this argument. If HPV type replacement does occur at the level of incidence of HPV infection, it may also have some adverse impacts on the performance of HPV-based methods of cervical screening. Overall, it is likely that if type replacement does occur the extent of the impact may not be observed until at least 10 years since HPV vaccination initiation and may likely be mitigated by the either cross-protective vaccine effectiveness and/or multivalent vaccines.

2.13.1 Carcinogenicity of non-vaccine HPV genotypes and disease resurgence

The possibility of HPV type replacement occurring in the post-vaccination era in response to targeted HPV vaccination may have caused much concern among the scientific and medical communities regarding the overall impact of vaccination on HPV-associated cancer reduction. However, it is likely that even in the most extreme scenario, of total type replacement at the level of HPV infection, it will not lead to disease incidence completely replacing the pre-vaccination level; in short type replacement at the level of infection does not equate to disease replacement. This is due to the fact that HPV types dramatically differ in potential to cause both high grade and low grade cervical intraepithelial lesions (Arbyn et al, 2014; Elfström et al, 2015; Smelov et al, 2015; Bzhalava et al, 2013).

The prevalence of many of the non-vaccine hrHPV types is substantially higher among lower grade and intermediate grade abnormalities in comparison to high grade abnormalities (Bzhalava et al, 2013). This suggests that there is a large difference between types in their ability to cause lesions which progress from CIN2/3 to invasive cervical cancer (Bzhalava et al, 2013). This difference in the oncogenic potential between different hrHPV types was further exemplified in two studies conducting using pre-vaccination cohorts participating in a primary HPV screening trial (Smelov et al, 2015; Elfström et al 2015). Among this population the HPV type specific population attributable proportion (PAR) for CIN3+ was highest for HPV16, 45, and 31 (46.9%, 8.9% and 8.8% respectively), whereas for CIN2+ it was highest again for HPV16, but then 31 and 52 (35.9%, 11.0% and 9.3% respectively) (Smelov et al, 2015). Among the same population, the type specific PAR for CIN1 was the greatest for HPV31 (17.3%) followed by HPV16, 52, 18 and 45 (13.4%, 8.7%, 7.2% and 6.8% respectively) which is in line with the low progression potential of CIN1 (Elfström et al, 2015). Increase in the incidence of lower grade lesions due to the replacing non-vaccine targeted HPV types in question, will largely not result in ICC.

Further to this, the bivalent vaccine has been documented to provide cross-protection via cross-neutralising antibodies to certain HPV types (HPV31, 33, 45, 52 and 58) phylogenetically related to HPV16 and 18 (Bogaards et al, 2019; Mariz et al, 2020), whilst the quadrivalent vaccine has been documented to provide cross-protection against HPV31 (Mariz et al, 2020). The types which the bivalent vaccine confers cross-protection against include the HPV types which are the next most prevalent in ICC cases (Bzhalava et al, 2013), and the HPV types most likely the progress to cases of CIN3+ (after HPV16) (Smelov et al, 2015). Thereby it is possible

that disease replacement due to HPV type replacement following implementation of the first-generation vaccines may be impeded and or delayed depending on the strength of cross-protective immunity (Verdenius et al, 2013; Man et al, 2021). Whilst subsequent second-generation vaccines additionally targeting the next most oncogenic HPV types may further prevent such possibilities for disease replacement, due to superior vaccine efficacy conferred in comparison to the differential cross-protective efficacy of the first-generation vaccines (Joura et al, 2015).

The mantra that HPV type replacement will not lead to disease replacement due to the lower risk profile of the non-vaccine HPV types relies on the assumptions that the observed and documented pre-vaccination era HPV type specific cancer risk per infection is firstly universally identical to the true pre-vaccination era HPV type specific cancer risk and secondly, that it is equal to the post-vaccination era risk. Unfortunately, however there are several scenarios in which these assumptions may not be robust.

Firstly, a matched case control study conducted among women failing cervical pre-cancer treatment in Sweden found that the risk of women who were HIV negative was much less than those who were HIV positive, among whom non-vaccine targeted HPV35 the second most prevalent type (after HPV16) (Carlander et al, 2021). Therefore, if type replacement were to occur, the risk (in terms of disease burden) among immunocompromised individuals may be higher than among the immunocompetent.

Secondly, it may be that the true HPV type specific attribution in cervical cancer (or other anogenital cancers) may be misattributed due to misclassification of the causal type in cases of HPV coinfections due to imperfect classification methods (Venetianer et al, 2020; Choi et al, 2012; Tota et al, 2013). The above-mentioned differences in attributable risk according to HPV type has historically been characterized via genotyping of HPV present in cytology samples from the women with cervical pre-cancer. However, it is often the case, that a woman presenting with a pre-cancerous lesion on the cervix may have multiple HPV types detectable in their biological specimen (Gargiulo et al, 2007). In such cases, an oncogenic hierarchical attribution model is often utilised to attribute the causal type, due to a further assumption that only one HPV type should be the causal type responsible for the cervical lesion in question (Choi et al, 2012). This practise may be problematic, as it misclassifies a proportion of cases due to "less oncogenic" HPV types as due to the "more oncogenic" HPV types such as HPV16 (Venetianer et al, 2020). An observation study of biopsies from women with CIN2+ diagnoses which compared the conventional hierarchical attribution model to a gold standard of tissue-based

HPV genotyping and laser-capture microdissection, observed that the use of the hierarchical attribution model resulted in a significant over-attribution of CIN2+ cases as due to HPV16, whilst significantly under-attributing cases as due to HPV18 and HPV35 in particular (Venetianer et al, 2020).

It is conceivable that the reduction in HPV16/18 due to vaccination could indirectly lead to an increase in precancerous lesions due to non-vaccine types (Tota et al, 2013). Although hrHPV is a necessary cause of cervical cancer, it is only one, albeit necessary, part of the causal pie of cervical cancer causation. Individuals which are at risk of developing cervical cancer due to the most oncogenic and widespread types (in the pre-vaccination era) HPV16 and 18, are at increased risk of developing cervical cancer due to non-vaccine types in comparison to their lower risk counterparts in a given population. When an individual therefore receives excisional ablative treatment to remove lesions from their cervix due to HPV16/18, this procedure may also remove early-stage lesions due to non-vaccine types thereby halting their progression. Thus, if HPV16/18 is no longer in widespread circulation, it is conceivable that the same women would not receive the excisional treatment, thereby possibly permitting the progression of the non-vaccine HPV type, resulting in an increase in the incidence of non-vaccine type HPV associated pre-cancers and/or cancer (Tota et al, 2013; Venetianer et al, 2020).

Lastly, it is also possible that the selective removal of specific (low risk) HPV types via vaccination could directly impact the risk associated with non-vaccine or vaccine type HPV in the post-vaccination era. Previous studies have already conclusively documented that co-infection with low risk HPV6/11 has an antagonistic effect on the risk of developing cervical cancer associated with HPV 16 infection and possibly HPV31 (Luostarinen et al, 1999; Luostarinen et al, 2004; Luostarinen et al, 2013; Arnheim Dahlström et al, 2011; Sundström et al, 2015). Therefore, in the populations where HPV vaccination has been implemented targeting also HPV6/11, (for example, in countries using Gardasil, or Gardasil9), it is also plausible that the removal of this antagonistic effect without the benefits of a strong cross-protective effect, could result in an increased risk associated withHPV16 and HPV31 infections. Likewise, if the vaccination coverage and herd effects are suboptimal, it is likely that HPV16 may continue to circulate among the remaining susceptible pool (Baussano et al, 2017), thus if the sub-optimal vaccination coverage has concomitantly resulted in herd effect against HPV6/11, then it is also conceivable that the risk of HPV16 infection among the unvaccinated may be elevated in comparison to the pre-vaccination era when HPV6/11 were widely circulating.

2.13.2 Impact on performance of HPV-based screening

If HPV vaccination does result in type replacement by other non-vaccine targeted HPV types, it may also have an impact on the positive predictive value, and specificity of HPV-based cervical cancer screening. The reduction of HPV16/18 is already expected to result in a decrease in the PPV and specificity of HPV-based screening, due to the fact the hrHPV types detected will be less likely to progress to high grade lesions, thereby also lowering the benefit to harm ratio (El-Zein et al, 2015).

3 OBJECTIVES OF THE STUDY

The objective of this study was to evaluate whether HPV type replacement occurs after the introduction of HPV16/18 vaccination in the Finnish adolescent population, with up to 50% vaccination coverage, by evaluating the degree of niche clearance induced by vaccination, and subsequent niche reoccupation by non-vaccine targeted HPV types after a maximum of 9 years since vaccination.

The specific aims were as follows:

1. To compare and quantify the sustainability of vaccine-induced neutralising/cross-neutralising antibody levels and corresponding vaccine efficacy among two parallel cohorts of vaccinated participants receiving three bivalent or quadrivalent vaccine doses up to 12 years since initial vaccination.

2. To compare HPV type specific prevalence distributions among HPV vaccinated and non-HPV vaccinated 18-year-old female participants of the community-randomised HPV vaccination trial four years' post-vaccination by vaccination strategy (gender-neutral or gender specific vaccination).

3. To evaluate whether HPV type specific occurrence differs by core group membership among vaccinated and unvaccinated female participants of the community-randomised trial four and eight years since vaccination.

4. To detect and evaluate HPV type specific herd effect among unvaccinated pregnant females under the age of 23 years old, by vaccination strategy (gender-neutral vs girls only vaccination), in communities subject to a community-randomised trial of vaccination strategy with moderate vaccination coverage up to 9 years post-vaccination.

5. To evaluate the cumulative incidence of non-vaccine HPV types among unvaccinated females under the age of 23 years old subject to the herd effect via the community-randomised HPV vaccination trial up to 9 years post-vaccination.

4 MATERIAL AND METHODS

4.1 Study designs

The material utilised in this study is comprised of the population-based follow-up of three randomised HPV vaccination trials.

4.1.1 Parallel intervention cohorts

The material for evaluating the first specific aim comes from a health registry follow-up of two intervention cohorts. During 2002 and 2004-5, two international phase III clinical trials were adjoined in Finland to study the vaccine efficacy of the quadrivalent and bivalent vaccines respectively. The first trial was the clinical trial of the quadrivalent HPV6/11/16/18 vaccine and was a part of an international multicentre trial, FUTURE II (ClinicalTrials.gov number, NCT00092534) (FUTURE II Study Group, 2007). A total of 1,745 Finnish females aged 16-17 years old from the 1984-87 birth cohorts were enrolled in the study from the cities of Helsinki, Vantaa, Espoo, Kuopio, Oulu, Tampere and Turku. Participants were randomised to an allocation ratio of 1:1, with 874 participants receiving the quadrivalent vaccine and 875 receiving a placebo. Participants received their allocated 3 vaccine doses according to a 0-, 2- and 6-month schedule. A total of 865 quadrivalent vaccine allocated participants received all three of the allotted doses and are referred to as quadrivalent vaccine recipients (QVR).

The second trial was the PATRICIA trial (NCT00122681) of the bivalent HPV16/18 vaccine (Paavonen et al, 2007). This was also a large international multicentre trial. In Finland, 4808 female participants aged 16-17 years old from the 1986-88 birth cohorts were enrolled into the PATRICIA trial from the cities and townships of Helsinki, Jyvaskyla, Jarvenpaa, Kotka, Kouvola, Kuopio, Lahti, Lappeenranta, Mikkeli, Oulu, Pori, Rauma, Seinajoki, Tampere, Turku and Vaasa. Similarly, participants were randomised to an allocation ratio of 1:1 with 2409 participants receiving the bivalent HPV16/18 vaccine and 2399 receiving the control vaccine, a GSK produced hepatitis A vaccine, Havrix™. In this trial the participants

received their three doses of allocated vaccine in a 0-, 1- and 6-month schedule. A total of 2355 of the HPV vaccinated participants randomized to receive the bivalent vaccine received all three doses of the vaccine and are referred to as bivalent vaccine recipients (BVR).

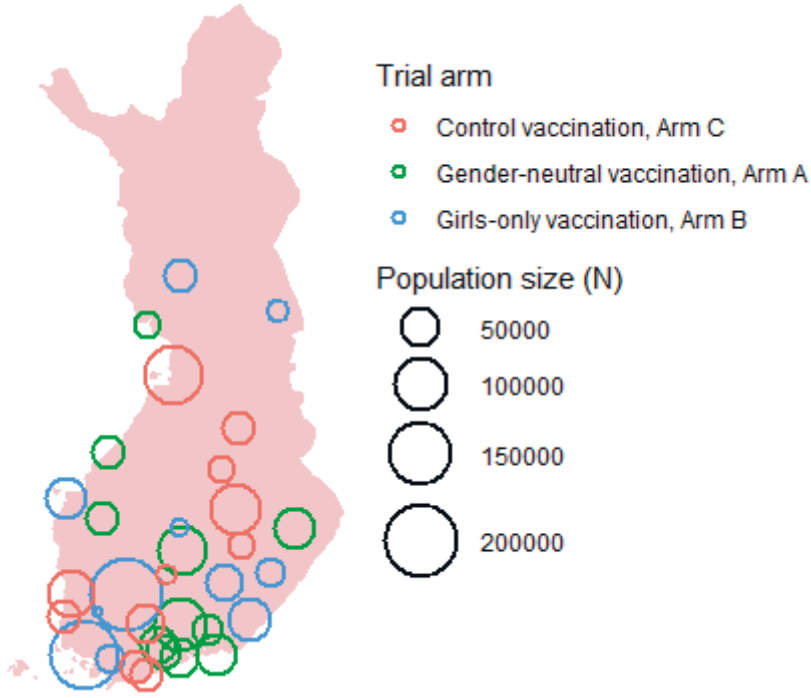
All of the Finnish participants of the FUTURE II and PATRICIA trials consented to long-term health-registry-based follow-up (via the Finnish Cancer Registry, FCR, the Finnish Maternity Cohort biobank, FMC, and the Hospital Discharge Registry, HILMO) using their Finnish social security number. Every person born or residing (even temporarily) in Finland is given a personal social security number (a unique personal identification number), this enabled individual level linkage between different country-wide population and health registries, including biobanks with serum and diagnostic tissue samples.

The study evaluating the sustainability of vaccine-induced neutralising and cross-neutralizing antibody levels (the first aim of this thesis) utilized the follow-up of the FUTURE and PATRICIA participants who had received all three of their allocated intervention vaccine doses in the Finnish Maternity Cohort (FMC). The FMC is a population-based biobank containing 2,059,617 serum samples from 992,695 women to date. The FMC was established by Docent Pentti Koskela in 1983 as a valuable resource for biobank studies. The biobank consists of serum samples given by all pregnant women for mandatory testing for congenital infections (hepatitis-B virus [HBV], human immunodeficiency virus [HIV] and syphilis) during the first trimester of pregnancy. Approximately 96% of pregnant women in Finland have consented to have residual volume of this sample stored in the FMC between 1983 and the end of 2016. (Lehtinen et al, 2017) By the end of 2016, a total of 595 and 1451 post-vaccination serum samples had accumulated in the FMC from the HPV vaccinated FUTURE II and PATRICIA participants respectively. Of these we retrieved and tested 577 serum samples from FUTURE II quadrivalent vaccine recipients and 571 serum samples from PATRICIA bivalent vaccine recipients. The 571 BVR samples were randomly selected from the total available samples in an age-aligned fashion, so that each QVR had an equivalent sample according to the time since vaccination (between 2 to 12 years post-vaccination) in the BVR's cohort.

4.1.2 Risk-stratified community-randomised trial

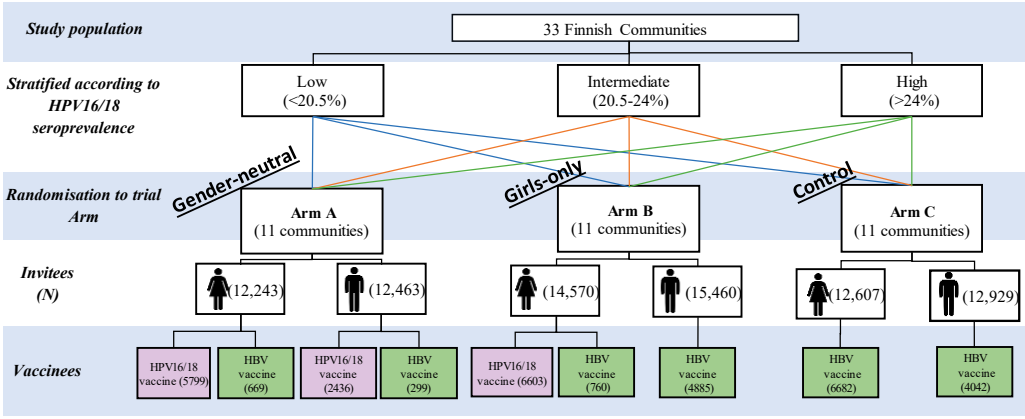
In 2007, a large phase IV population-based community-randomised trial (CRT) on the impact of different HPV vaccination strategies (gender-neutral or girls-only vaccination) was initiated (NCT00534638). From 34 Finnish communities deemed eligible, 33 communities representative of the Finnish population were enrolled into the study. The Helsinki metropolitan area was excluded owing to its substantially different contact patterns to the rest of Finland and due to the expected difficulty of introducing herd effect into a large and heterogeneous population of adolescents. For inclusion the communities had to be a minimum of 50km apart from each other, or 35km in the more populated South of Finland. The 33 communities were stratified by previously ascertained HPV16/18 seroprevalence (Lehtinen et al, 2006; Lehtinen et al, 2015) into those with low (less than 20.5%), intermediate (20.5-24%) or high seroprevalence (greater than 24%). Within each of the three strata, communities were then randomised to one of three trial arms with an allocation ratio of 11:11:11 communities (Figure 6). This yielded a low coefficient of variation, K of 0.13. (Lehtinen et al, 2015)

Figure 6: Map of Finland showing the thirty-three communities participating in the community randomised trial.



The three trial arms were allocated to receive either a gender-neutral HPV vaccination strategy of early adolescents (Arm A), a girls-only vaccination HPV vaccination strategy (Arm B) or no HPV vaccination (Arm C) (Figure 7). All Swedish or Finnish speaking early adolescent males and females from the 1992-95 birth cohorts residing in the 33 trial communities were identified through the Finnish Population registry. A total of 80,272 individuals were identified and subsequently invited to participate in the trial from which a total of 20,513 females and 11,662 males agreed to participate in the trial. Informed consent was received from the participants parent and/or guardian in order to participate, or from the participants themselves if they were aged 15 years old.

Figure 7: Study design of the Finnish community randomised trial of HPV vaccination strategy.



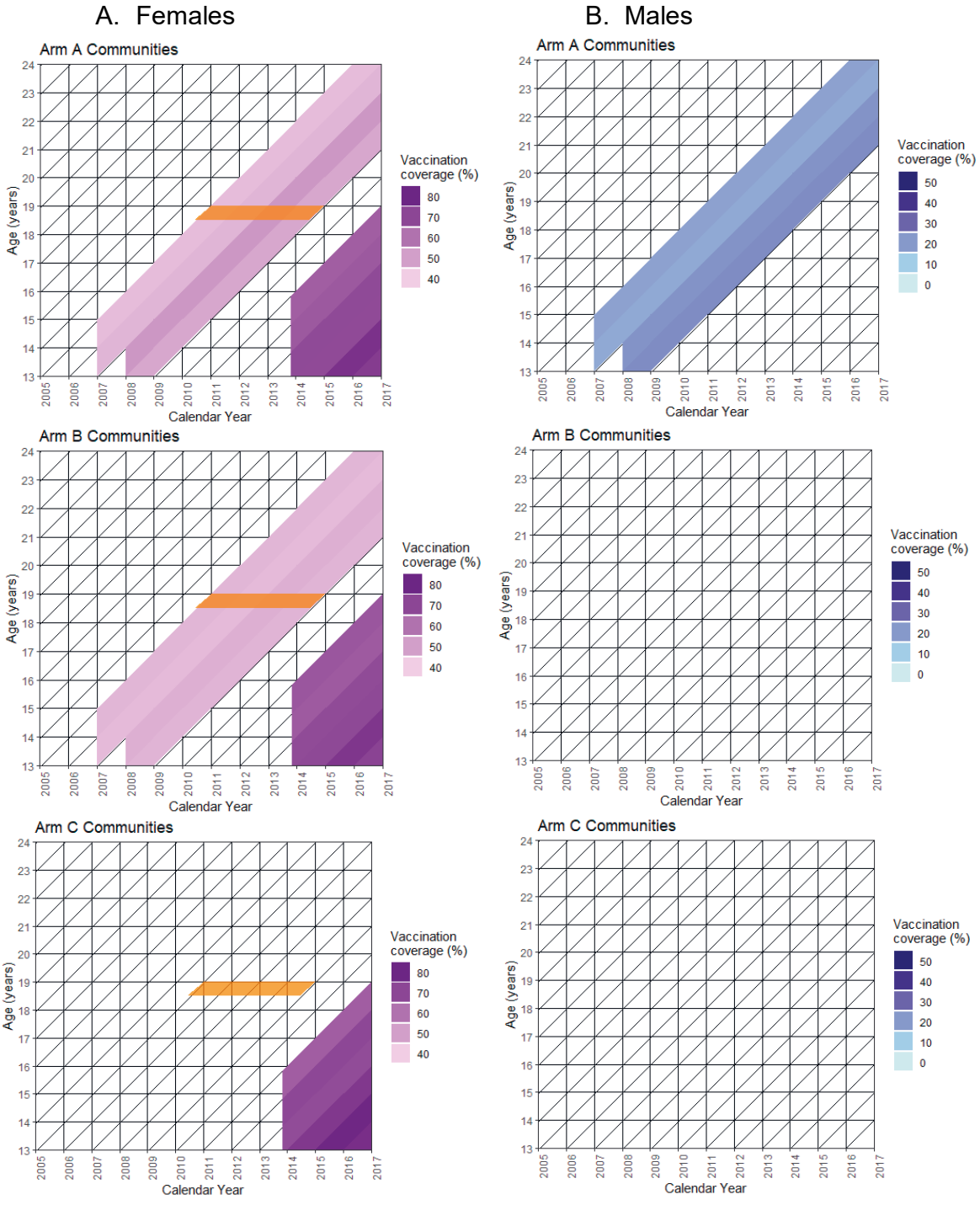
In the gender-neutral vaccination strategy Arm A, 90% of all participants both male and female were randomised to receive the bivalent ASO4-HPV-16/18 vaccine (Cervarix®), with the remaining 10% given the hepatitis B vaccine (Engerix-BTM). In girls-only vaccination strategy Arm B, only 90% of the females were randomly given the HPV vaccine, whilst the remaining 10% of females and all participating males were given the HBV vaccine. In the trial Arm C serving as the control, all participants both male and female were given the HBV vaccine. The participants received their allocated vaccine as early adolescents aged 12-15 years old, during the calendar years 2007-2010 (from October 2007 until April 2010). The vaccine allocation was receiver blinded in Arm A among all participants, and in Arm B only among the female participants. Ninety-nine point four percent of participants received all three of doses of their allocated vaccine. At the age of 18 years old the

participants were offered cross-vaccination with the vaccine (HPV or HBV) which they did not receive initially.

4.1.2.1 Active follow-up of the CRT

During the years 2010-2014, all female residents born between 1992-95 in the 33 CRT communities were invited to attend a follow-up visit at one of the 33 University of Tampere study sites, when they were aged 18 and a half years-old. Females were invited regardless of whether or not they had initially participated in the CRT during 2007-10 (Figure 8). At the follow-up visit they were invited to donate a self-collected cervico-vaginal sample rinsed in first void urine (FVU) as part of an ancillary chlamydia screening trial, to give a cervico-vaginal sample collected by one of the CRT study nurses and to fill-out a questionnaire on behavioural and demographic factors (see Appendix). Participants were requested to abstain from sexual intercourse for one day prior to attending the follow-up visit. Participants were then offered the possibility to receive cross-vaccination, with the HPV vaccine if they were initially HBV vaccinated or the HBV vaccine if they were initially HBV vaccinated. (Lehtinen et al, 2018) In addition to the data collected at follow-up visits, additional information on participant mobility between or outwith the study communities was gathered by individual linkage with the Finnish Population Registry using the PIN unique to each individual.

Figure 8: Lexis diagram depicting the vaccination coverage among the CRT birth cohorts and adjacent cohorts, and the active follow-up (highlighted in orange).



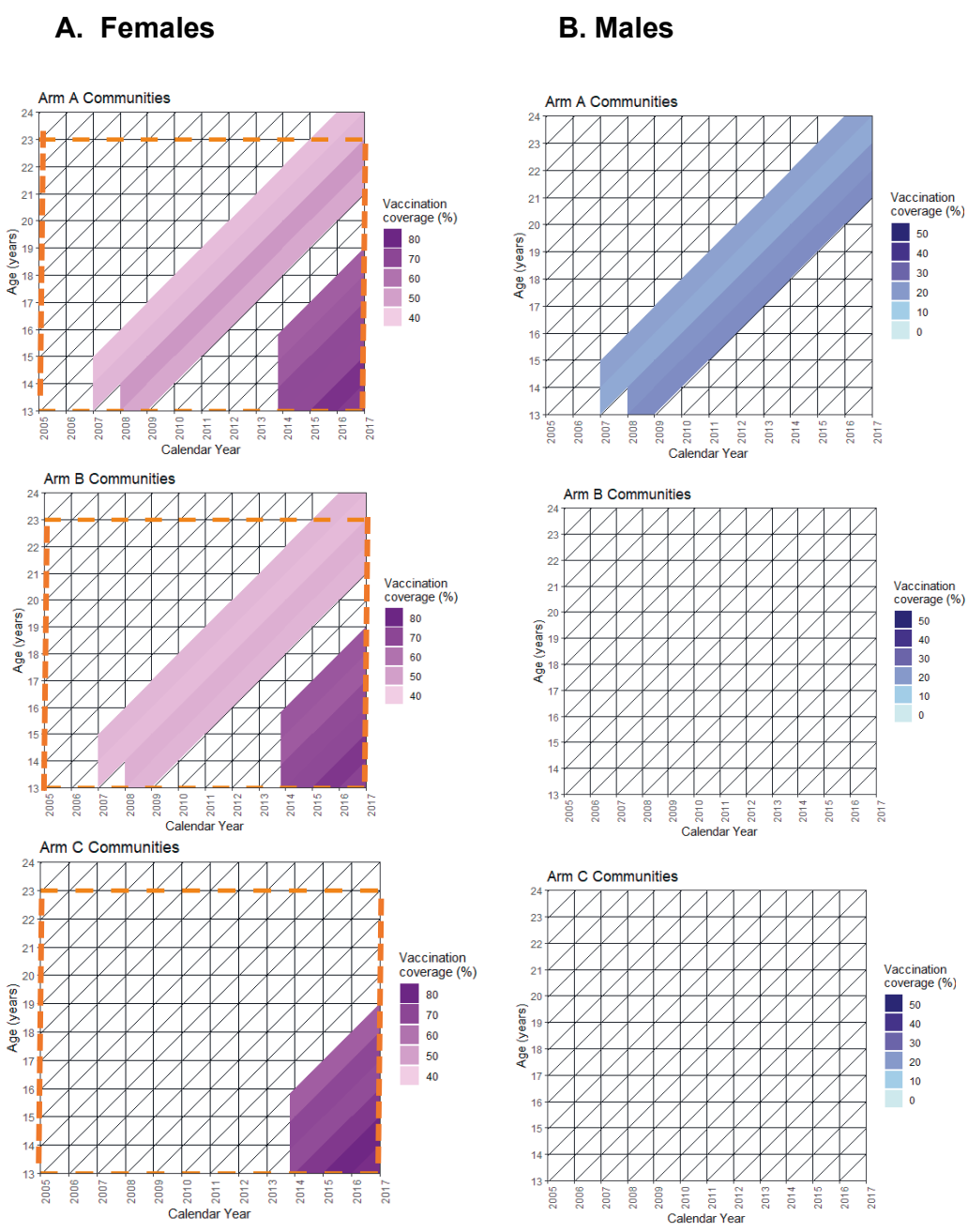
Subsequently, during the years 2014-2018, all the female HPV vaccinated participants (included those vaccinated at initial enrolment and those cross-vaccinated at aged 18 years old) were invited to attend a series of follow-up visits at the ages of 22, 25 and 28 years old as part of a re-randomized trial of different cervical screening strategies among HPV vaccinated women (NCT02149030) (Louvanto et al 2019). Similarly, to the first follow-up visit, participants were invited to donate self-collected cervicovaginal samples rinsed in FVU, and to complete another questionnaire on lifestyle, behaviour and demographics.

4.1.2.2 Biobank based follow-up of the CRT

To evaluate the indirect effects of the implementation of gender neutral or girls-only vaccination via the community randomised trial, a serological survey of strictly HPV unvaccinated females under the age of 23 years-old was conducted covering the calendar years 2005 to 2016 (both pre- and post- HPV vaccination in the CRT) among the females resident in the 33 Finnish communities of the CRT (Figure 9). This was achieved by retrieving all the available serum samples in the FMC by the end of the year 2016 which fitted the inclusion criteria.

To fit the inclusion criteria, the subject donating the sample had to under the age of 23 years old and residing in one of the 33 CRT communities at the time of sample donation (as identified using the postal codes of the maternity clinic at which the subjects donated their samples), they had to be unvaccinated with any HPV vaccine, the sample had to be the first pregnancy sample donated by the subject to the FMC (no serial samples were included) and the sample had to be donated between the 1st of January 2005 to the 31st of December 2016 (Figure 9). The vaccination status of the possibly eligible subjects was ascertained by linking the FMC subjects which fit all the other eligibility criteria with the registry of HPV vaccinated individuals (containing information on those vaccinated via ongoing and past HPV vaccination clinical trials in Finland). The HPV vaccination status of the small number of subjects who were eligible to have received HPV vaccination via the Finnish national vaccination program (the 1998 birth cohort and birth cohorts younger) was assessed by later manual scrutinization of their HPV16 and 18 antibody titres for titres indicative of vaccination (titres multiple fold higher than that reasonable induced from natural infection alone).

Figure 9: Lexis diagrams depicting the vaccination coverage among the female cohorts eligible for sampling for the biobank-based follow-up of the CRT in the Finnish maternity cohort, and their male counterparts. The vaccination coverage among the cohorts is indicated by a coloured gradient, and the biobanked based follow up are indicates as the that within the orange dashed squares.



To gather additional information on general risk-taking behaviour, data was gathered on self-reported smoking among all pregnant females under the age of 23 years-old between 2005 to 2016 in the 33 CRT communities from the Finnish Medical Birth Register. The Finnish Medical Birth Register is a population-based register established in 1987, for statistics and research purposes, and contains data on all live births, on stillbirths (under certain criteria), and on the mothers. The data was retrieved at the community level and was therefore both anonymous and non-linkable at the individual level. The purpose of this information was to compare general risk-taking behaviour between communities to take account of unmeasured confounding owing to possible differences in sexual risk-taking between the communities.

To gather information on the HPV vaccination coverage (to assess the level of indirect exposure to HPV vaccination) among the cohorts represented in the study population in each community, data on was extracted from the HPV trial registry to identify the birth cohort specific coverage among birth cohorts eligible for HPV vaccination via all HPV trials conducted in Finland, and from the National Institute for Health and Welfare's Finnish vaccination register for birth cohorts eligible to have received HPV vaccination via the national immunisation program.

4.2 Ethical considerations

The Pirkanmaa Hospital District Ethical Review Board granted ethical permission for the community randomised trial in 2007 (HPV040 R07113M, 12.6.2007) and for the cervical screening trial of HPV vaccinated females in 2014 (HPV004 R13149, 19.2.2014). The Finnish National Ethical Review Board (Tukija) granted ethical approval for the FUTURE II (015-00 58.04.02, 10.6.2002) and PATRICIA (HPV008 17/04/04, 5.4.2004) trials in 2002 and 2004 respectively and for the future follow-up of both trials with the Finnish registries. Further ethical permissions for compare and quantify the sustainability of vaccine-induced neutralising/cross-neutralising antibody levels the questionnaire and long-term follow-up of unvaccinated controls were granted in 2003 (HPV002-02 50/04/03, 7.4.2003). The ancillary study to the CRT on the effectiveness of *Chlamydia trachomatis* screening was granted ethical permission from the Pirkanmaa ethical review board in 2009 (111/2009).

All the samples comprising the FMC biobank are stored following informed consent by the pregnant female at the time of sample donation, for its storage and future use for research purposes. Since 2016, all individuals who have samples stored

in the FMC, have had the possibility to opt out at any point, and thus have their sample removed from the biobank in accordance with the European Union General Data Protection Regulation. A negligible number of sample donors (<40) have withdrawn their consent in this manner. Male participants of the CRT were offered the opportunity to receive HPV- or HBV- cross-vaccination after the trial follow-up, in 2015-2017.

4.3 Laboratory analyses

4.3.1 HPV neutralization assay

The FMC serum samples collected from the QVR and BVR from the FUTURE II and PATRICIA cohorts respectively to evaluate the first aim, were analysed using a high-throughput-pseudovirion-based neutralization assay (HT-PBNA) to determine the vaccine-induced neutralizing or cross-neutralizing antibody titer for HPV6, 16, 18, 31, 33, 45, 52 and 58 (Sehr et al, 2013). The assay utilises HPV pseudovirions comprised of the L1 and L2 capsid proteins, encapsidating a Gaussia luciferase plasmid. The pseudovirions were produced by co-transfecting HEK293TT cells with plasmids encoding the HPV L1 and L2 genes then purified by ultracentrifugation in an Optiprep gradient (Mariz et al, 2020). In the absence of neutralizing (or cross-neutralizing) antibodies, these pseudovirions bind to the surface proteins of HeLAT cells and enter within, enabling transduction of the Gaussia luciferase reporter plasmid. The expression of Gaussia luciferase may then be subsequently measured and quantified by its luminescent reaction with coelenterazine (a luciferin). However, if neutralizing or cross-neutralising HPV antibodies are present then they will bind to the pseudovirions thus preventing the pseudovirion from infecting the HeLAT cells and transduction of the Gaussia luciferase reporter plasmid. This will then result in a reduced or absence the luminescent reaction in the presence of the Gaussia luciferase substrate of coelenterazine. (Sehr et al, 2013)

The FMC serum samples collected from the QVR and BVR were serially diluted seven times in 3.33-fold increments, to give rise to dilutions of 1:40 to 1:180,000, which were used in the PBNA. The neutralising or cross-neutralising antibody titres were then defined as the serum dilutions which inhibited 50% of the pseudovirion infection as measured by a 50% reduction in the luminescent reaction, these are termed as the EC50 values. Serum which gave rise to an EC50 value greater than

40 were classified as positive for neutralising antibodies (or cross-neutralising antibodies depending on the type). International units (IU) were then calculated for HPV16 and 18 using internal plate standards as bridging sera to the World Health Organisation (WHO) HPV16 and 18 reference standards. Serum samples giving rise to EC50 values above 180,000 were not differentiated any further.

4.3.2 Heparin-bound HPV pseudovirion Luminex serology

The samples collected from the FMC serological survey of unvaccinated women (corresponding to study aims 4 and 5) were analysed for the presence of serum total binding antibodies to the HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 L1 and L2 antigens and to herpes simplex virus type II (HSV-2) using a heparin bound HPV pseudovirion and HSV-2 glycoprotein G 2 (gG2) Luminex assay (Faust et al 2010, Faust et al 2013). The assay panel was extended for this study to include HPV51 and 66. The seventeen HPV pseudovirions were produced by transfecting the HEK293TT cell line with codon optimised plasmids encoding the L1 and L2 genes. The HSV-2 type-specific gG2 antigen was sourced from a commercial company (The Native Antigen Company, Kidlington, United Kingdom). Antibodies to HSV-2 were used as an indicator of sexual risk-taking behaviour.

All the HPV pseudovirions and the HSV-2 gG2 antigen were bound to heparin coated beads. The extension of the panel to include the new antigens was validated using serum samples from women of previously determined HPV DNA positivity status (for HPV51 and HPV66), and with samples of previously determined HSV-2 seropositivity (as determined using a HSV-2 glycoprotein G 2 based ELISA). A negative control serum panel from children under the age of 12 years-old (arithmetic mean age=4.73 years-old, SD=3.11) was used to establish HPV seropositivity cut-off levels.

4.3.3 Modified general primer PCR and MALDI-TOF mass spectrometry

Cervicovaginal samples were collected from the female participants of the community-randomised trial via active follow-up visits of the 1992-95 born when aged 18 and 22 years old. These samples were HPV genotyped using modified general primer (MGP) PCR followed by Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) (Söderlund-Strand et al 2009;

Soderlund-Strand & Dillner, 2013). This allowed for the genotype specific detection of HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.
In brief, all the HPV DNA present in a subject's sample was amplified using consensus MGP PCR. The MGPs used were modified GP5+/6+ primers (Söderlund-Strand et al 2009). Following this, HPV genotype specific mass extension (ME) primers were then used for single base extension. Each of the genotype specific ME primers has a distinct molecular mass, therefore allowing their detection via mass spectrometry. A specific extended ME-primer was interpreted as presence of that HPV genotype in the subject's sample (Söderlund-Strand & Dillner, 2013). Further confirmatory testing for samples which were found to be positive for HPV11 or HPV68 was conducted using the Luminex platform, as this method has been documented to show some cross-reaction between HPV 68 and HPV70, and between HPV11 and 89. (Lehtinen et al, 2017)

4.3.4 *Chlamydia trachomatis* PCR

The female participants of the CRT who participated in the active follow-up at the age of 18, were also invited to participate in a *C. trachomatis* screening trial. The women who consented to participate in this study provided self-collected cervicovaginal samples rinsed in first void urine. These samples were then subsequently tested for *C. trachomatis* DNA positivity using a commercial PCR kit (Abbott, Illinois, U.S.A.). In these studies, *C. trachomatis* DNA positivity is used as a marker of sexual risk-taking, and/or as a surrogate marker for current core-group membership.

4.4 Statistical analyses

4.4.1 Study I Sustainability of neutralising antibodies & correlation with VE

Firstly, to compare the vaccine-induced neutralising antibody response between the bivalent vaccine recipients (from the PATRICIA intervention cohort) and the quadrivalent vaccine recipients (from the FUTURE intervention cohort), the results from the serological analysis with the neutralisation assay, were used to calculate the HPV type-specific seroprevalence (for HPV types 6, 16, 18, 31, 33, 45, 52 and 58)

stratified by the vaccine received. Similarly, the median neutralizing anti-HPV antibody titre and geometric mean titres (and 95% confidence intervals) were also estimated separately among the bivalent and quadrivalent vaccine recipients for HPV6, 16, 18, 31, 33, 45, 52, and 58 using GraphPad Prism 8.3.1.

To further compare the neutralising antibody response induced by the bivalent vaccine compared to the quadrivalent vaccine, GMT ratios (GMTR) were estimated using a linear regression model with the outcome of antibody titre log-transformed.

To investigate the correlation between the vaccine-induced HPV type specific GMT and seroprevalence and priorly reported HPV type specific efficacy the bivalent and the quadrivalent vaccines, Spearman's ranked correlation coefficients were calculated (r_s). The corresponding 95% confidence intervals were calculated using Fisher's Z-transformation. The r_s calculations were completed stratified by the time (since vaccination) in which the seroprevalence or GMT was estimated, into 2-4, 5-7, 8-10, 11-12 years and 5-12 years since vaccination. The GMTRs were estimated using R statistical software (version 3.6.1) with the Epi package (version 2.40).

To visualise the different neutralising antibody responses over time induced by the bivalent vaccine as compared to the quadrivalent vaccine, local regression with a smoothing span of 0.75 was used to plot the neutralising antibody responses for HPV 6, 16, 18, 31, 33, 45, 52, and 58. The locally weighted regression lines and accompanying 95% confidence intervals were plotted using datapoints from vaccine recipients for which the EC50 values were above 40. This was completed using R statistical software (version 3.6.1) with the ggplot2 package (version 3.2.1).

4.4.2 Study II Ecological niche vacation

To study the indirect effects of gender-neutral versus girls only HPV vaccination strategies among the unvaccinated females, firstly the level of exposure (the vaccination coverage) was estimated among the study population of the biobanked follow-up of the CRT (see section 4.1.2.2), and secondly the indirect effects (the herd effect against HPV16 and 18) were estimated among the study population according to trial Arm from the serological results (see section 4.3.2).

The study population consisted of pregnant females under the age of 23 years old residing in each of the study communities from 2005 to the end of 2016. The degree to which they were indirectly exposed to HPV vaccination was assessed by

computing the vaccination coverage stratified by birth cohort, community, gender and year. Using these estimates the yearly community specific vaccination coverage among the study population was computed by weighting the vaccination coverage by birth cohort according to the birth cohort distribution of participants for each community and year.

Secondly the degree of within-cluster correlation of HPV16/18 and HPV16/18/31/33/33/35/45 seropositivity was assessed by estimating the intracluster correlation coefficient, ICC, using Fleiss and Cuzick's estimator (Fleiss & Cuzick, 1979). The accompanying 95% confidence intervals were estimated using Zou and Donner's modified Wald test (Zou & Donner, 2004). To assess whether underlying trends may have been occurring in general STI incidence due to changing sexual behaviour over time, the crude HSV-2 seroprevalence was calculated stratified by trial arm and time (into the "pre-vaccination era", from 2005-2010, and the "post-vaccination era", from 2011-2016). The accompanying 95% confidence intervals for the crude seroprevalences were calculated using the methodology of Agresti-Coull (Agresti & Coull, 1998).

To evaluate the impact of indirect exposure to community-level HPV vaccination via gender-neutral or girls only vaccination strategies the crude seroprevalence of HPV16, 18, 31, 33, 35, 45 (plus 16/18 combined and 31/33/35/35/45 combined) was calculated stratified by vaccination era and trial arm. Likewise, the 95% confidence intervals for the seroprevalence estimates were calculated using the Agresti-Coull method (Agresti & Coull, 1998). To directly evaluate the comparative impact of indirect exposure to HPV vaccination, the HPV type specific seroprevalence ratio was estimated for HPV16, 18, 31, 33, 35 and 45, (plus 16/18 combined), using a log binomial generalized estimating equation (GEE) model with an exchangeable correlation structure comparing the post-vaccination era (2005-10) to the pre-vaccination era (2011-16) stratified by trial Arm. The model estimates were adjusted for community-level self-reported maternal smoking among women under the age of 23-years old as a surrogate for community-level risk taking behaviours, to take account of possible confounding due to baseline differences in this variable irrespective of vaccination coverage. To evaluate whether "ever core-group membership" modified the observed indirect effect of vaccination strategy on HPV type specific seroprevalence, the estimates were further stratified by HSV-2 seropositivity (as a marker for life-time sexual risk-taking).

Finally, to directly assess the impact of HPV vaccination strategy on the herd effect, the between-arm ratio of the with-in arm seroprevalence ratio were calculated comparing the estimates from Arm A or B to the estimates from Arm C

(representing the counterfactual scenario). The 95% confidence intervals for these ratios of seroprevalence ratios were computed using the methodology of Altman and Bland (2003).

Expected systematic non-differential outcome misclassification of the pseudovirion based serological assay to classify HPV type specific cumulative incidence was quantified and accounted for via probabilistic bias analysis (Lash et al, 2009). Estimates of the sensitivity and specificity of the heparin bound HPV pseudovirion based Luminex assay obtained in an earlier validation study for HPV types (Artemchuk et al, 2018) were firstly used assuming a constant probability distribution. In the instance that the previously obtained sensitivity and or specificity estimates were incompatible with the observed data, then a uniform probability density ranging from 0 to 1 was applied. Next, all the possible values for the sensitivity and specificity which were compatible with the data were obtained to give a range of plausible sensitivity and or specificity values for the given HPV type. The range of values were then employed with a uniform probability density. From these sensitivity analyses, the new estimates of the with-in arm seroprevalence ratios were obtained with accompanying 95% confidence intervals accounting for both random error and systematic error (due to outcome misclassification).

4.4.3 Study III Comparison of non-vaccine HPV prevalence among 18-year old CRT participants (active follow-up)

The prevalence of behavioural characteristics of the female CRT participants at age 18 was calculated from the responses given in the behavioural questionnaires when the participants attended the 1st follow-up. The characteristics on sexual and risk-taking behaviour were calculated stratified by Arm and vaccination status, into HPV vaccinated or non-HPV vaccinated including both HBV vaccinated and those who were HPV/HBV unvaccinated who did not initially actively participate in the CRT at early adolescence.

Using the results on the HPV type specific DNA positivity from the laboratory analysis of the active follow up of the female participants of the CRT at the age of 18 years old, the prevalence of each of the measured HPV types was compared in the intervention Arms to the control Arm C for signs of increasing prevalence indicative of type replacement. HPV type specific prevalence ratios were calculated using log binomial regression comparing the prevalence in Arm A to C, and Arm B to C, stratified by birth cohort (into those born in 1992-3 and 1994-5) and

vaccination status (comparing the HPV vaccinated in Arms A or B to the HBV vaccinated in Arm C, and non-HPV vaccinated plus the HBV vaccinated in Arms A or B to those in Arm C).

Earlier follow-up of the CRT participants in order to investigate differential herd effects by arm, found that the effects were strongest in the youngest birth cohorts, thus necessitating the stratification by birth cohort in the analysis for type replacement. Prevalence ratios were adjusted for individual level mobility, community level self-reported smoking to take account of intercommunity variation in general risk-taking behaviour and individual level *C. trachomatis* status as a marker of sexual risk-taking behaviour (Dillner et al, 1996). To take account of participation bias (differential risk-taking behaviour of the non-HPV vaccinated between the initially non-participating unvaccinated who participated attended follow-up at 18 years old and the initially participating HBV vaccinated attendees) steps were taken to ensure that the ratio of unvaccinated to HBV vaccinated females were similar when comparing the HPV prevalence among the non-HPV vaccinated between Arms.

The ratio of unvaccinated (initial non-participants) to HBV vaccinated was initially different in Arm C compared to Arms A and B due to the larger number of HBV vaccinated participants in Arm C. Thus, this ratio was corrected by randomly selecting twenty-one samples from 44 birth year community strata, so that the samples contained one eighth of the HBV vaccinated from Arm C. Each of the 21 samples were combined with all of the unvaccinated women from Arm C. Then the PRs for the non-HPV vaccinated Arm comparisons were estimated by calculating the mean of the 21 random sample-specific PR estimates. The 95% confidence intervals for these non-HPV vaccinated PR estimates were then calculated via a homogenization-based approach (Lehtinen et al, 2018).

To take account of possible clustering of the outcome due to cluster randomised study design and possible changes in the counterfactual Arm C prevalence over time, the HPV prevalence ratios were also estimated using a log binomial GEE model adjusting for smoking and mobility, comparing the prevalence among the 1992-3 or 1994-95 birth cohorts among Arm A or B to the all the birth cohorts combined from Arm C. As a further sensitivity analysis to take account of heterogeneity in vaccination coverage between the communities, the same analysis was then repeated but excluding the outlier communities as per the pre-analysis plan specified in the study protocol. In the CRT study protocol outlier communities had been defined as those communities in Arm A or B which had a vaccination coverage of20% or more

different than the mean of the vaccination coverage among the remaining communities within the trial Arm in question.
To further evaluate changes in non-vaccine HPV type ranked prevalence between the intervention and control arms, Spearman's rank correlation coefficients were calculated. In addition to this, as a sensitivity analysis the odds ratios of type specific HPV positivity among those positive for HPV16 were compared to that in those negative for HPV16 (and the same again but replacing the "exposure" HPV type from HPV16 to HPV18). The ORs were estimated stratified by Arm, vaccination status and adjusted for mobility, smoking and *C. trachomatis* using binomial logistic regression.

4.4.4 Study IV Comparison of non-vaccine HPV prevalence among CRT participants by current core-group membership (active follow-up)

To compare the baseline characteristics of the female CRT participants from the 1992-94 birth cohorts between the intervention and control arms, the prevalence of sexual risk-taking behaviours and general risk-taking behaviours were calculated stratified by age of follow-up visit (18- or 22-years-old), HPV vaccination status (non-HPV vaccinated, HPV vaccinated, HBV vaccinated, or at the age of 22 years old "cross-HPV vaccinated") and trial arm (into intervention Arms A/B combined or control Arm C) using data gathered from the questionnaires completed at the study follow-up visits at the ages of 18- and 22-years-old. Likewise, the prevalence of mobility out of the study community, *Chlamydia trachomatis* and the HPV vaccination coverage was also calculated for the same stratifications.
To investigate the impact of core-group membership on the occurrence of HPV vaccine-induced type replacement, in the following analyses, positivity for *Chlamydia trachomatis* was used as another marker of sexual risk-taking behaviour but unlike HSV-2 antibodies it was a surrogate for "current" core-group membership. Firstly, the HPV type specific prevalence among the 1992-94 born female participants of the CRT was calculated stratified by study Arm, age of follow-up visit and *C. trachomatis* positivity for vaccine targeted HPV16 and 18 and non-vaccine targeted HPV 6, 11, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 (and 6/11 combined [low risk types], 16/18/45 [vaccine targeted plus cross-protected types], 31/33/35 [cross-protected and phylogenetically related HPV types]). Participants with missing data of Chlamydia positivity were excluded from the analyses.

It was possible that participant bias (differences in the risk-taking behaviours) might bias the results among the non-HPV vaccinated; different baseline risk may be expected between those who were initially HBV vaccinated and those who initially did not participate in the CRT at early adolescence but attended the follow-up visit at the age of 18-yearsold. Therefore, to take account of such bias due the ratio of HBV to unvaccinated was balanced between the Arms, when calculating the HPV type specific prevalence by estimating the prevalence from the mean of 21 balanced strata for Arm C, so that the ratio was comparable to that of Arms A/B. The accompanying 95% confidence intervals for these estimates were calculated using the Agresti-Coull method (Agresti & Coull, 1998).

To directly evaluate the impact of HPV vaccination on the occurrence of vaccine HPV types (for niche clearance) and non-vaccine HPV types (for signs of HPV type replacement or cross-protection), HPV type specific prevalence ratios (and accompanying 95% confidence intervals) were estimated via a binomial GEE model with exchangeable correlation structure, comparing the prevalence in the intervention Arms A or B to that in the control Arm C among the 1992-94 born CRT participants. Prevalence ratios were likewise estimated stratified by *C. trachomatis* positivity, age (18 years old or 22 years old) and vaccination status and adjusted for mobility. For the HPV vaccinated PRs among the 18 years old participants, the prevalence among the HPV vaccinated from arms A and B combined was compared to the prevalence among the HBV vaccinated from Arm C, and for the non-HPV vaccinated PRs the HBV plus unvaccinated from Arms A and B combined was compared to the same from Arm C. To ensure that the balance of HBV:unvaccinated was consistent between the comparison Arms, the same method of estimating the PR from mean of the 21 random sample-specific PR estimates as described for study III was utilised, with the 95% confidence intervals likewise being calculated using a homogenization-based approach (see section 4.4.3). For the HPV vaccinated PRs among the 22-year-olds, participants from the intervention Arms A or B were compared to participants from the control Arm C. The former were HPV vaccinated as early adolescents, the latter were HPV cross-vaccinated at the age of 18 years old.

For HPV types for which *C. trachomatis* positivity (core-group membership) was found to result in a notable difference in the PR between the Arms, as defined as non-overlapping confidence intervals between the PR from the *C. trachomatis* positive compared to the *C. trachomatis* negative PR, the possibility of additive and/or multiplicative interaction was evaluated as follows.

To assess interaction on the additive scale, the interaction contrast was calculated (where a value notably different from zero was interpreted as indicative of interaction) as described by Rothman et al (2008):

Interaction Contrast, IC=Risk, R (ArmA/B, *C. trachomatis* positive)
-[R(Arm A/B, *C. trachomatis* negative)-R(Arm C, *C. trachomatis* negative)]
-[R(Arm C, *C. trachomatis* positive)-R(ArmC, *C. trachomatis* negative)]
-R(ArmC, *C. trachomatis* negative)

The 95% confidence limits for the interaction contrast were calculated by firstly computing the standard error,

SE(IC) = sqrt[variance(R11)+variance(R10)+variance(R01)+variance(R00)]

The variance for each proportion (R) was calculated by n*p*(1-p), and then computing the confidence limits associated to the interaction contrast as follows:

95% confidence limits (IC) = IC+/-1.96*SE(IC).

To evaluate interaction on the multiplicative scale an interaction term was added to the HPV type specific model (the same model as previously used to estimate the prevalence ratios but stratified by *C. trachomatis*), this time the estimates were unstratified and *C. trachomatis* was also added into the model as follows:

$$\log(p)=\beta_0+\beta_1\text{Mobility}+\beta_2\text{Smoking}+\beta_3\text{C.trachomatis}+\beta_4\text{Trialarm}+\beta_5 C.trachomatis*\text{TrialArm}$$

To further evaluate the impact of HPV vaccination on the type-specific HPV occurrence and possible type replacement, prevalence ratios of persistent infection (HPV DNA positivity for the same HPV type at both the 1st and 2nd follow-up visits) were estimated using a Poisson regression model with robust standard errors for non-vaccine types HPV39, 51, 52, 56, 58, 59, 66 separately and HPV6/11, 16/18/45, and 31/33/35 combined comparing the prevalence among the HPV vaccinated in Arms A/B combined to the prevalence in the HBV vaccinated in Arm C. These prevalence ratios were likewise adjusted for mobility and stratified by *C. trachomatis* status.

To further investigate the impact of core-group membership (as identified using the *C. trachomatis* positivity as a surrogate) on HPV type occurrence after vaccination, the type specific HPV prevalence was calculated stratified again by the *C. trachomatis* status and Arm.

The statistical analyses of the fourth study were conducted using R statistical software version 3.4.3. with the DescTools package version 0.99.25, the Epi package version 1.2-1, the geepack package version 1.2-1, the ggplot2 package version 2.2.1 and the sandwich package version 2.4-0.

4.4.5 Study V Comparison of non-vaccine HPV seroprevalence among <23-year-old FMC donors (passive follow-up)

The final study evaluating the impact of community-level HPV vaccination on the occurrence of HPV type replacement utilises the same material as study II (the population-based follow-up of the RCT in the FMC over time). The occurrence of non-vaccine targeted and/or protected HPV types among unvaccinated females was evaluated for signs of increasing cumulative incidence (seroprevalence)by increasing HPV vaccination coverage.

Firstly, the degree of clustering within-cluster among the non-vaccine HPV type seropositivity (for HPV39, 51, 56, 58, 59, 66, 68 and 73) was evaluated among the post-vaccination era (serum samples from 2005-2010) by calculating the ICC and accompanying 95% confidence intervals using Fleiss and Cuzick's estimator and Zou and Donners modified Wald test (Fleiss & Cuzick, 1979; Zou & Donner, 2004).

To assess whether non-vaccine type seroprevalence had increased post-vaccination, the crude seroprevalence of the non-vaccine HPV types was firstly calculated stratified by time period (from 2005-2010, the pre-vaccination period and 2011-2016, the post-vaccination period) and trial Arm (A, gender-neutral, B, girls-only or C, control). Likewise, the accompanying 95% confidence intervals were computed using the methodology of Agresti and Coull (Agresti & Coull, 1998).

To further evaluate for the signs of HPV type replacement, the non-vaccine HPV cumulative incidence (seroprevalence) in the post-vaccination was directly compared to the pre-vaccination era by estimating the type specific seroprevalence ratio (sPR) stratified by Arm using a log binomial GEE model with exchangeable correlation structure to take account of within-community clustering due to the study design. To take account of possible confounding due to differences in risk-taking behaviours between the comparison groups, the sPR estimates were adjusted for community-

wise self-reported smoking as a proxy of general-risk taking behaviour. Due to the possible expectation of a time delay between niche clearance and HPV type replacement occurrence (Man et al, 2021), the sPR estimates were further calculated stratified by post-vaccination time period (into the first or second post-vaccination period, 2011-13 or 2014-16 respectively). In line with study IV, the sPR (comparing the entire post-vaccination period to the pre-vaccination period), were also estimated stratified by HSV-2 seropositivity as a surrogate marker of "ever-core group membership", as a possible effect modifier of the initial occurrence of HPV type replacement.

It is possible that any observed changes in non-vaccine HPV type seroprevalence over time may be due to secular trends over time independent of HPV vaccination (Lipsitch, 1999), therefore to evaluate whether any changes in HPV occurrence observed in the intervention Arms over time were truly due to vaccination, the within arm HPV type specific sPRs from intervention arms A and B were directly compared to the respective sPRs from Arm C (serving as the counterfactual estimate in the absence of community-level HPV vaccination). This was achieved by calculating ratios of SPRs using the methods of Altman and Bland (Altman & Bland, 2003).

As a sensitivity analysis to take account of bias due to outcome classification when using HPV seroprevalence as a measure of cumulative infection, the type specific sPRs were recalculated using probabilistic bias analysis to quantify and correct for misclassification. As per study II, the previously estimated values of sensitivity and specificity of heparin-bound HPV pseudovirion Luminex serology as a measure of cumulative incidence were utilised. As the previous validation studies did not include HPV51 and 66, a validation study for these two types was conducted to estimate the specificity and sensitivity for these two types, and the resultant sensitivity and specificity estimates used in the probabilistic bias analysis.

All the statistical analyses for study V were conducted using R statistical software package (version 3.6.0), with the ICCbin package (version 1.1.1), the geepack package (version 1.2-1), the episensr package (version 0.9.5).

5 RESULTS

5.1 Sustainability of vaccine-induced neutralising antibodies

The neutralising antibody response to HPV16 and 18 measured among the vaccine recipients two to twelve years after initial HPV vaccination substantially differed by vaccine received. For HPV types 16 and 18 the locally weighted regression lines of neutralising antibodies as measured in IU over time were significantly and consistently approximately one log higher among the bivalent vaccine recipients as compared to the quadrivalent vaccine recipients by one approximately one order of magnitude for both HPV16 and 18 (Figure 10). This difference in the magnitude of vaccine-induced neutralising antibody response between the two vaccines was further observed in the seroprevalence of neutralising antibodies induced, the median antibody titre and also the geometric mean titre for both HPV16 and 18. For HPV16 and 18 (from the alpha 9 and alpha 7 clades), the seroprevalence among the vaccine recipients 5-12 years after vaccination was 100% (98.8-100%) among the BVRs compared to 95.8 % (92.9-97.7%) among the QVRs for HPV16, and the substantially lower, 84.8% (80.3-88.6%) among QVRs for HPV18.

The median HPV16 antibody titre observed 5-12 years since vaccination was 6-fold higher among the BVRs compared to the QVRs, and the geometric mean titre being multiple fold higher among the BVRs (geometric mean titre ratio, GMTR=5.48, 4.58-6.56). A decline in the HPV16 neutralising antibody titre in the initial years since vaccination was observed only among the QVR, of 45% between 2-4 years and 5-7 years.

For HPV18 (from the alpha clade 7), the vaccine-induced GMT and the median antibody titre were consistently lower among both vaccine recipient groups in comparison to that induced against HPV16. However, the seroprevalence among the BVR was still 100% (98.8-100%) 5 to 12 years since vaccination. In comparison the observed HPV18 neutralising antibody titre was additionally higher among the BVR compared to the QVR, the median antibody titre was 20-fold higher among the BVR, whilst the GMT was 12-fold higher (GMTR=12.4, 10.3-15.0). Likewise, to

HPV16, a decline in the neutralising antibody titre was also observed post-vaccination in the early years, of 29% between 2-4 years and 5-7 years since vaccination.

The vaccine-induced neutralising (among the QVR) or cross-neutralising (among the BVR) anti-HPV6 antibody response was comparably different according to the vaccine received albeit to the opposite direction. The neutralising antibody titre was one-log higher among the QVRs compared to the BVR, although the BVR were found to have a sustainable antibody response consistently above the cut-off for neutralising antibody positivity over the 2 to 12 years of follow-up since HPV vaccination (Figure 10). The proportion HPV6 seropositive, was significantly higher among the QVR compared to the BVR, 99.3% (97.6-99.9) as compared to 86.5% (82.2-90.1). The median anti-HPV6 neutralising/cross-neutralising antibody titre and the GMT were substantially higher among the QVR in comparison to the BVR, 4252 (3352-5023) as compared to 78 (71-89), and 3646 (3081-4315) as compared to 172 (146-203) respectively. Likewise, to HPV16 and 18, an initial decline in the HPV6 neutralising antibody titre was observed among the QVR, after which the antibody titre was found to plateau.

For the non-vaccine HPV types, cross-neutralising antibodies were observed to varying degrees for HPV31, 33, 52 and 58 (those clade A9 types phylogenetically related to HPV16) and HPV45 (phylogenetically related to HPV18) among the vaccine recipients (Figure 10). The seroprevalence of cross-neutralising antibodies differed notably between the two vaccine recipient groups, with the seroprevalence consistently higher among the BVR compared to the QVR (Table 3). Among the BVRs the median cross-neutralising antibody titres were above the EC50 cut-off of 40 for HPV31, 33 and 52, whilst among the QVRs the median cross-neutralising antibody titre was only above the cut-off for HPV52 (Figure 10).

Figure 10: Neutralizing antibody titres induced by the bivalent or quadrivalent vaccines among vaccine recipients over time. IU, International Units, EC50 (half maximal effective concentration). Alpha 7: HPV18, 45; Alpha 9: HPV16, 31, 33, 52, 58; Alpha10: HPV6.

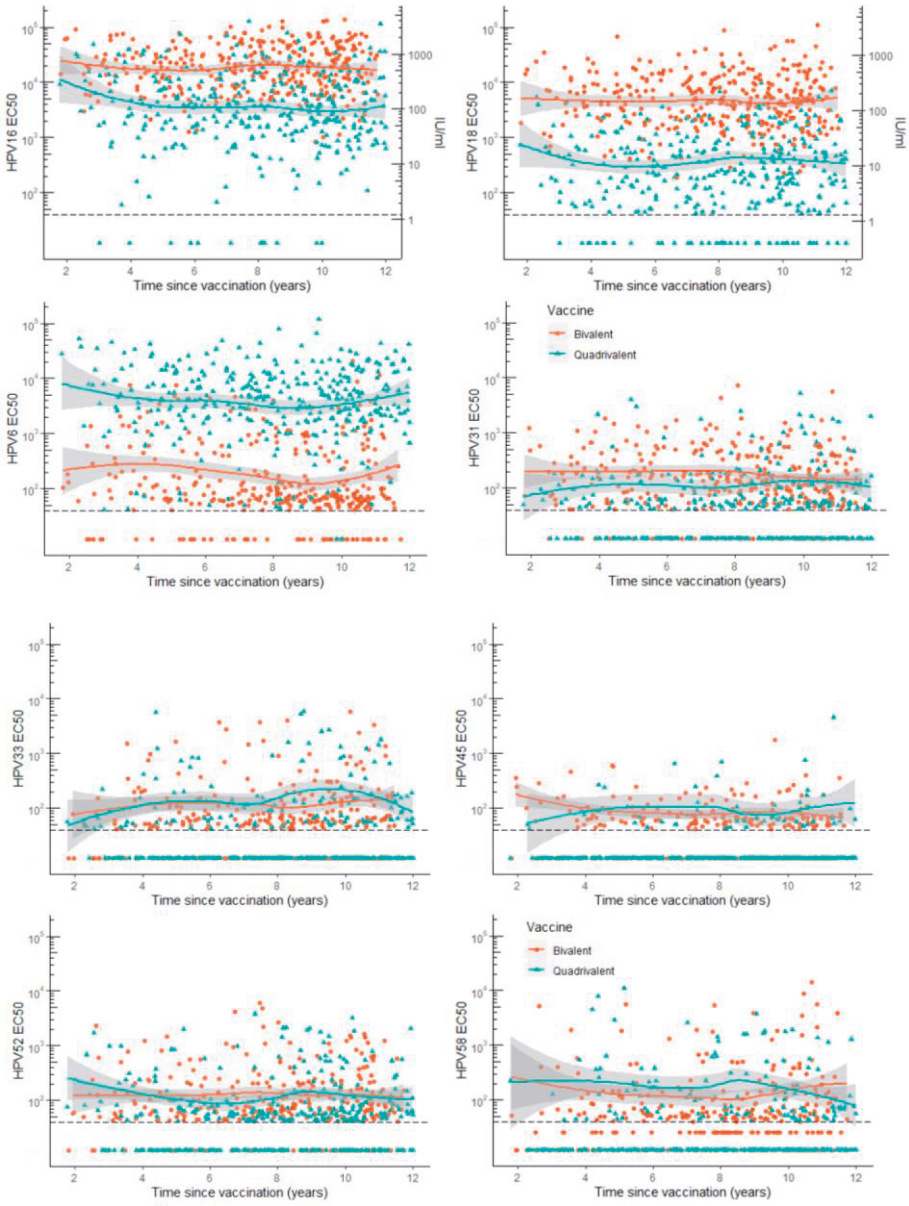


Table 3: Seroprevalence of neutralising anti-HPV antibodies among the bivalent and quadrivalent vaccine recipients 5 to 12 years since HPV vaccination.

	Seroprevalence % (95% CI) 5-12 years post-vaccination	
HPV type	**Bivalent vaccine recipients**	**Quadrivalent vaccine recipients**
HPV6	86.5 (82.2-90.1)	99.3 (97.6-99.9)
HPV16	**100 (98.8-100)**	**95.8 (92.9-97.7)**
HPV18	**100 (98.8-100)**	**84.8 (80.3-88.6)**
HPV31	83.6 (79.0-88.3)	48.4 (42.7-54.1)
HPV33	57.7 (52.0-63.2)	34.8 (29.5-40.4)
HPV45	45.1 (39.5-50.9)	15.1 (11.3-19.6)
HPV52	77.8 (72.8-82.3)	62.9 (57.2-68.3)
HPV58	51.6 (45.9-57.2)	35.1 (29.8-40.7)

5.2 Impact of HPV vaccination strategy on type specific herd effect

Of the 8022 subjects residing in one the 33 CRT communities at the time of sample donation to the FMC and initially thought to be eligible for the study, a further 491 were found to be ineligible upon further scrutiny of the eligibility criteria (see Section 4.1.2). The HPV16/18 and HPV16/18/31/33/35/45 intracluster correlation coefficients, ICCs, among the eligible subjects donating samples during 2005-10 were both very close to zero, 0.0007 and 0.005 respectively.

The characteristics of the study population were comparable between the pre- and post-vaccination era with respect to age distribution, with the mean age being 20 years-old among all arms in the pre-vaccination era, and among Arms A, B and C respectively in the post-vaccination era. The community-level mean prevalence of self-reported smoking, an indicator of general risk-taking behaviour at the community level, was higher in Arm C as compared to Arms A or B in both the pre- and post-vaccination era but remained relatively stable between the pre-and post-vaccination eras. The seroprevalence of HSV-2 was found to be marginally higher in the pre-vaccination era than the post-vaccination era, most so in Arm B, with the seroprevalence increasing from 16.9%, 19.2% and 17.2% to 15.2%, 14.0% and 15.9% in Arm A, B and C respectively. The community-level HPV vaccination

coverage among the birth cohorts which were eligible for the study (standardised to the birth cohort distribution observed in the study population) was negligible in the years 2005-10, with it progressively increasing from the year 2011 to 2016.

When comparing the HPV seroprevalence in the post-vaccination era to that pre-vaccination era in each Arm among the unvaccinated women subject to the community-level vaccination coverage, the seroprevalence of HPV16/18, HPV18 (to some extent) and HPV16 were found to decrease most notably in the Arm A communities (seroprevalence ratio, $sPR_{HPV16/18}$=0.80 [95% confidence intervals, 0.74-0.87], sPR_{HPV18}=0.86 [0.70-1.06], sPR_{HPV16}=0.79 [0.72-0.87]) (Table 4). Taking into account outcome misclassification, the seroprevalence of HPV16/18 was observed to have further decreased among the unvaccinated women in Arm A (seroprevalence ratio, $sPR_{HPV16/18}$=0.66 [0.10-0.85], sPR_{HPV18}=0.72 [0.21-0.96], sPR_{HPV16}= 0.64 [0.09-0.86]) (Table 4).

For the cross-protected HPV types, in the gender-neutral Arm A notable decreases in the seroprevalence were also observed for HPV35, however, this was almost replicated in Arm C, ratio of seroprevalence ratios, $RPR_{[Arms\ A\ vs\ C]}$=0.92 (0.61-1.37). Similar decreases in the seroprevalence of HPV31 were also observed in both Arms A and B (Table 4), but again also observed in the control Arm C, $RPR_{[Arms\ A\ vs.C]}$=1.25 (0.96-1.62) and $RPR_{[Arms\ B\ vs.\ C]}$= 1.20 (0.90-1.59)(Table 5). There was also a marginal non-significant decrease observed in the seroprevalence of HPV45 in the post-vaccination era in the gender-neutral Arm sPR_{HPV45}=0.89 (0.69-1.14). However, a similar decrease was again observed in the control Arm C communities, $RPR_{[Arms\ A\ vs.\ C]}$=0.99 (0.65-1.50).

Table 4: HPV Seroprevalence Ratios comparing the seroprevalence in the post-vaccination era, 2011-16, to the pre-vaccination era, 2005-10 within each CRT arm among the unvaccinated women resident in the CRT communities. *Estimates are adjusted for community-level self-reported maternal smoking.

	Unvaccinated Finnish females aged under 23 years		
	Post- vs. Pre-vaccination era		
sPR (95% CI) accounting for random error*			
HPV type	**Arm A** (N= 1247 vs. 1322)	**Arm B** (N= 1158 vs. 1289)	**Arm C** (N=1211 vs. 1304)
16	**0.79 (0.72-0.87)**	1.09 (0.91-1.32)	1.01 (0.86-1.20)
18	**0.86 (0.70-1.06)**	0.96 (0.74-1.24)	0.89 (0.70-1.13)
16/18	**0.80 (0.74-0.87)**	0.98 (0.85-1.12)	0.91 (0.81-1.03)
31	0.90 (0.79-1.01)	0.86 (0.73-1.02)	0.72 (0.57-0.91)

33	1.05 (0.88-1.26)	0.94 (0.77-1.14)	0.81 (0.63-1.03)
35	0.70 (0.52-0.94)	0.98 (0.64-1.51)	0.77 (0.58-1.01)
45	0.89 (0.69-1.14)	1.01 (0.76-1.36)	0.90 (0.64-1.26)
sPR (95% CI) accounting for random error & systematic error			
16	**0.64 (0.09-0.86)**	1.19 (0.98-3.70)	1.07 (0.89-1.85)
18	**0.72 (0.21-0.96)**	0.89 (0.39-1.12)	0.79 (0.21-1.03)
16/18	**0.66 (0.10-0.85)**	0.92 (0.44-1.07)	0.84 (0.24-1.01)
31	0.79 (0.20-1.00)	0.75 (0.15-0.97)	0.55 (0.07-0.78)
33	1.14 (0.86-2.73)	0.85 (0.31-1.13)	0.66 (0.15-0.94)
35	0.52 (0.07-0.83)	0.90 (0.44-1.25)	0.59 (0.10-0.91)
45	0.73 (0.19-1.06)	1.01 (0.78-1.33)	0.79 (0.25-1.13)

Table 5: Between-arms Ratio of seroprevalence ratios (RPR), comparing the with-in Arm seroprevalence ratios from the intervention Arms A or B to the within arm seroprevalence ratio from the control Arm C (Table 4).

Ratio of within-arm sPR's (95% CI), RPR, accounting for random error		
HPV type	**Arm A vs. C**	**Arm B vs. C**
16	**0.78 (0.64-0.95)**	1.08 (0.84-1.39)
18	0.96 (0.70-1.32)	1.08 (0.76-1.53)
16/18	0.88 (0.76-1.02)	1.07 (0.89-1.29)
31	1.25 (0.96-1.62)	1.20 (0.90-1.59)
33	1.31 (0.96-1.78)	1.16 (0.85-1.60)
35	0.92 (0.61-1.37)	1.28 (0.77-2.13)
45	0.99 (0.65-1.50)	1.13 (0.72-1.76)

When comparing the HPV seroprevalence post-vaccination to that pre-vaccination among the HSV-2 positive unvaccinated women, the seroprevalence of HPV16 was especially decreased in the gender-neutral arm A, sPR_{HPV16}=0.64 (0.50-0.81). In contrast, among the subjects from Arm B and C the seroprevalence of HPV16 showed no noticeable change in the post-vaccination era comparative to the pre-vaccination era sPR=0.94 (0.63-1.42) and sPR=0.98 (0.70-1.38) respectively. For HPV18, when not taking into account outcome misclassification, no notable decreases in HPV18 seroprevalence were observed among the HSV-2 seropositive women in the intervention Arms in the post-vaccination era $sPR_{[Arm\ A]}$=0.95 (0.60-1.51)and $sPR_{[Arm\ B]}$=0.74 (0.34-1.65)(Table 6).

Table 6: With-in Arm HPV type specific seroprevalence ratios (95% confidence intervals) stratified by HSV-2 seropositivity (as a surrogate of "ever core-group membership") comparing the post-vaccination era, 2011-16, to the pre-vaccination era, 2005-10. NA, not available.

Seroprevalence Ratios, sPR (95% CI)			
HSV-2 seropositive			
HPV type	Arm A (N=189 vs. 224)	Arm B (N=162 vs. 247)	Arm C (N=192 vs. 224)
16	**0.64 (0.50-0.81)**	0.94 (0.63-1.42)	0.98 (0.70-1.38)
18	0.95 (0.60-1.51)	0.74 (0.34-1.65)	1.00 (0.68-1.46)
16/18	**0.71 (0.55-0.92)**	0.79 (0.56-1.10)	1.01 (0.74-1.36)
31	0.74 (0.53-1.02)	0.75 (0.48-1.16)	0.64 (0.42-0.98)
33	0.92 (0.62-1.38)	NA	0.77 (0.50-1.19)
35	0.57 (0.37-0.88)	1.12 (0.37-3.38)	1.12 (0.72-1.73)
45	0.64 (0.37-1.08)	1.04 (0.63-1.71)	0.69 (0.37-1.30)
HSV-2 seronegative			
HPV type	Arm A (N=1058 vs. 1098)	Arm B (N=996 vs. 1042)	Arm C (N=1019 vs 1080)
16	**0.85 (0.77-0.93)**	1.15 (0.98-1.35)	1.03 (0.87-1.21)
18	0.84 (0.65-1.08)	1.02 (0.82-1.27)	0.87 (0.66-1.14)
16/18	**0.84 (0.76-0.91)**	1.03 (0.92-1.16)	0.90 (0.78-1.03)
31	0.95 (0.81-1.12)	0.90 (0.77-1.06)	0.75 (0.58-0.99)
33	1.10 (0.88-1.39)	0.89 (0.73-1.10)	0.82 (0.64-1.05)
35	0.75 (0.55-1.02)	0.96 (0.63-1.44)	0.69 (0.51-0.92)
45	0.98 (0.73-1.32)	1.05 (0.77-1.42)	0.98 (0.64-1.50)

5.3 Impact of vaccination strategy on non-vaccine HPV type prevalence

Among the CRT female participants at the age of 18 years-old, the self-reported behavioral characteristics showed no major differences between the study arms or between the HPV vaccinated and the non-HPV vaccinated. However, some minor differences were observed (Study III). The mean age of sexual debut was approximately identical between the arms, 16.4, 16.3 and 16.4 years-old in Arms A B and C respectively among the HPV vaccinated. The prevalence of *C. trachomatis* was marginally but consistently lower in the participants from Arm C as compared to Arms A and B, prevalence, $P_{(Arm\ C)}$=2.96%, $P_{(Arm\ A)}$=3.48%, and $P_{(Arm\ B)}$=3.11% among the HPV-vaccinated, and , $P_{(Arm\ C)}$=3.23% as compared to $P_{(Arm\ A)}$=4.12% and $P_{(Arm\ B)}$=4.13% among the non-vaccinated. Additionally, the prevalence of *C. trachomatis* was also consistently albeit marginally higher among the non-HPV vaccinated as compared to the HPV vaccinated. Likewise, the prevalence of having (self-reported) five or more lifetime sexual partners was consistently higher in Arms A and B as compared to C both in the HPV-vaccinated, HBV-vaccinated and non-vaccinated, $P_{(Arm\ C,\ HBV\ vaccinated)}$=13.4%, $P_{(Arm\ A,\ HPV\ vaccinated)}$=14.9% and $P_{(Arm\ B,\ HPV\ vaccinated)}$=15.7%, and $P_{(Arm\ C,\ non\text{-}vaccinated)}$=13.7%, $P_{(Arm\ A,\ non\text{-}vaccinated)}$=19.2% and $P_{(Arm\ B,\ non\text{-}vaccinated)}$=16.7%. Additionally, the prevalence of self-reported current smoking, an indicator of general risk taking behavior, was somewhat higher among the Arm A participants in comparison to the Arm C participants $P_{(Arm\ A)}$=33.8% versus $P_{(Arm\ C)}$=30.3% among the vaccinated participants, and $P_{(Arm\ A)}$=34.2% versus $P_{(Arm\ C)}$=30.3%, among the non-HPV vaccinated participants.

The intracluster correlation coefficient was low irrespective of HPV type combination or vaccination status of the participants (Table 7).

Table 7: Intracluster correlation coefficient (95% confidence intervals) stratified by HPV vaccination status among the 18-year-old female participants of the community randomized trial. na, not available.

	Intracluster Correlation Coefficient (95% confidence intervals)	
HPV type	HPV vaccinated	Non-HPV vaccinated
16/18	na	0.006 (0.00-0.12)
31/33/45	0.001 (0.00-0.13)	0.0007 (0.00-0.05)
35/39/51/52/56/58/59/66	na	0.005 (0.00-0.07)

Prevalence Ratios evaluating the total effectiveness (the direct plus indirect effect) of HPV vaccination

When comparing the HPV type specific prevalence among the HPV vaccinated participants from the intervention arm communities to the HBV vaccinated participants from the control arm communities, the prevalence of vaccine targeted HPV16 was markedly lower in both Arms A and B, $PR_{(Arm\ A\ vs.\ C)}$=0.09 (95% CI, 0.04-0.19) and $PR_{(Arm\ B\ vs.\ C)}$=0.08 (0.04-0.16) among the 1992-93 born, and $PR_{(Arm\ A\ vs.\ C)}$=0.06 (0.02-0.15) and $PR_{(Arm\ B\ vs.\ C)}$=0.09 (0.04-0.17) among the 1994-95 born. The prevalence of HPV18 was similarly reduced among the vaccinated participants from Arms A and B, $PR_{(Arm\ A\ vs.\ C)}$=0.06 (0.02-0.22) and $PR_{(Arm\ B\ vs.\ C)}$=0.08 (0.03-0.21) among the 1992-93 born, and $PR_{(Arm\ A\ vs.\ C)}$=0.04 (0.01-0.17) and $PR_{(Arm\ B\ vs.\ C)}$=0.05 (0.02-0.16) among the 1994-95 born.

When comparing the prevalence of non-vaccine targeted HPV types among the HPV vaccinated participants from the intervention arm communities to the HBV vaccinated from the control arm communities for signs of increasing prevalence indicative of type replacement, signs of increasing HPV51 and 66 were observed. The prevalence of HPV51 was found to be increased among participants from Arm A as compared to Arm C participants but only among the 1992-93 birth cohorts $PR_{(Arm\ A\ vs.\ C)}$=1.56 (1.11-2.19), and among participants from Arm B the 1994-95 birth cohorts $PR_{(Arm\ B\ vs.\ C)}$=1.57 (1.19-2.08)(Table 8) among the 1994-1995 birth cohorts. The prevalence of HPV51 among the participants from both the intervention Arms as compared to those from the control Arm approximated the null $PR_{(Arm\ A\ vs.\ C)}$=0.85 (0.61-1.18) and $PR_{(Arm\ B\ vs.\ C)}$=0.92 (0.70-1.21)(Table 8). The prevalence of HPV 66 was also found to be increased among the Arm A and B participants in comparison to the prevalence among the Arm C participants. However, similarly to the case of HPV 51, this increased HPV66 prevalence was only observed among the 1992-93 birth cohorts, $PR_{(Arm\ A\ vs.\ C)}$=1.55 (0.98-2.46) and $PR_{(Arm\ B\ vs.\ C)}$=1.53 (1.06-2.22). When observing the crude unadjusted prevalence among Arm C, both HPV51 and 66 were found to have increased in the participants from the counterfactual Arm C communities between the 1992-93 to the 1994-95 birth cohorts, from $P_{(1992\text{-}93\ born)}$=5.2% to $P_{(1994\text{-}95\ born)}$=7.0% for HPV51, and from $P_{(1992\text{-}93\ born)}$=3.1% to $P_{(1994\text{-}95\ born)}$=4.2% for HPV66 (Table 8).

When further comparing the prevalence of the non-vaccine HPV types among the HPV vaccinated from the 1992-93 or 1994-95 birth cohorts from the intervention arms to all the birth cohorts from Arm C, to take account of baseline instability in the HPV prevalence among the Arm C participants, the prevalence of HPV51 was increased among the 1992-93 birth cohorts from the intervention arms, $PR_{(Arm\ A\ vs.\ C)}$=1.33 (1.05-1.68) and $PR_{(Arm\ B\ vs.\ C)}$=1.36 (1.07-1.72)(Appendix Table 1).

Similarly, when taking account bias due to heterogeneity in the vaccination coverage between the arms by excluding the outlier communities (Appendix Table 2), the prevalence of HPV51 was still increased among the 1992-93 birth cohorts, $PR_{(Arm\ A\ vs.\ C)}$=1.40 (1.07-1.82) and $PR_{(Arm\ B\ vs\ C)}$=1.36 (1.07-1.72) (Appendix Table 3).

Prevalence ratios evaluating the indirect effect of HPV vaccination

When comparing the HPV type specific prevalence among the non-HPV vaccinated participants from the intervention arms to the non-HPV vaccinated participants from the control arm decreasing prevalence were observed for vaccine-targeted HPV18 and non-vaccine targeted HPV31 and 33 (from the same phylogenetic clade as vaccine-targeted HPV16) among the younger birth cohorts, those born 1994-95, $PR_{(HPV18,\ Arm\ A\ vs.\ C)}$=0.77 (0.46-1.29) and $PR_{(HPV18,\ Arm\ B\ vs.\ C)}$=0.60 (0.35-1.03), $PR_{(HPV31,\ Arm\ A\ vs.\ C)}$=0.47 (0.26-0.88) and $PR_{(HPV31,\ Arm\ B\ vs.\ C)}$=0.75 (0.46-1.21), and $PR_{(HPV33,\ Arm\ A\ vs.\ C)}$=0.52 (0.23-1.19)(Table 9).

When comparing the prevalence of non-vaccine targeted HPV types among the non-HPV vaccinated participants between the trial arms, similar to the case among the HPV vaccinated participants some signs of increasing prevalence were observed for HPV51 and 66. However, only among the 1992-93 birth cohorts, and only among the participants from the gender-neutral arm A communities, $PR_{(HPV51,\ 1992\text{-}93)}$= 1.46 (1.02-2.11) and $PR_{(HPV66,\ 1992\text{-}93)}$=1.43 (0.97-2.12).

When further estimating the non-vaccine HPV type prevalence ratios among the non-vaccinated from the 1992-93 birth cohorts from Arm A to all the birth cohorts from Arm C the HPV51 prevalence ratio was still marginally increased among the but not the confidence intervals overlapped the null, $PR_{(Arm\ A\ vs.\ C)}$=1.22 (0.87-1.71). When estimating the same prevalence ratio but excluding the outlier communities the HPV51 prevalence ratio was identically marginally increased, but again the confidence intervals were overlapping the null point, $PR_{(Arm\ A\ vs.\ C)}$= 1.22 (0.84-1.78)(Appendix Table 3).

In addition to this, among the participants from the gender-neutral arm A communities some non-consistent increases were observed in the prevalence of HPV39 and HPV45 among the 1992-93 and 1994-95 birth cohorts respectively. Similarly, some non-consistent increases were also observed among the non-HPV vaccinated participants from the girls-only HPV vaccinated Arm B communities compared to the prevalence in participants from Arm C for HPV6, 33, and 52 among the older 1992-93 birth cohorts, $PR_{(HPV6,1992\text{-}93)}$=1.74 (1.19-2.56), $PR_{(HPV33,\ 1992\text{-}93)}$=1.86 (1.05-3.27) and $PR_{(HPV52,\ 1992\text{-}93)}$=1.60 (1.09-2.36) respectively (Table 9).

Ranked distribution of non-vaccine targeted HPV prevalence

When ranking the non-vaccine targeted (and non-cross-protected) HPV types by prevalence stratified by birth cohort and arm, no statistically significant changes were observed between the non-HPV vaccinated participants from the intervention Arms A or B as compared to C, Spearman's rank correlation coefficient, $r_{s(\text{Arm A vs C})}=0.95$ and $r_{s(\text{B vs. C})}=0.91$ among the 1995 birth cohort.

Table 8: HPV type specific Prevalence and Prevalence Ratio among the HPV vaccinated female CRT participants by intervention Arm at the age of 18-years-old.

		HPV vaccinated comparisons				
		Prevalence (%)			**Prevalence ratio (95% CI)**	
HPV type	**Birth cohort**	**Arm A** (N=2929)	**Arm B** (N=3059)	**Arm C** (N=2805)	**A vs C**	**B vs C**
6	**1992-93**	4.3	5.5	4.4	0.96 (0.63-1.46)	1.23 (0.90-1.70)
	1994-95	4.4	3.8	5.2	0.76 (0.51-1.11)	0.72 (0.52-1.01)
11	**1992-93**	0.5	0.8	1.0	0.36 (0.11-1.17)	0.85 (0.39-1.86)
	1994-95	1.0	0.5	0.8	0.89 (0.33-2.37)	0.58 (0.23-1.51)
16	**1992-93**	0.6	0.6	7.1	0.09 (0.04-0.19)	0.08 (0.04-0.16)
	1994-95	0.3	0.6	7.0	0.06 (0.02-0.15)	0.09 (0.04-0.17)
18	**1992-93**	0.2	0.3	3.9	0.06 (0.02-0.22)	0.08 (0.03-0.21)
	1994-95	0.1	0.2	4.0	0.04 (0.01-0.17)	0.05 (0.02-0.16)
31	**1992-93**	0.7	0.9	3.2	0.26 (0.12-0.57)	0.28 (0.15-0.51)
	1994-95	0.8	0.7	3.7	0.26 (0.13-0.53)	0.18 (0.09-0.36)
33	**1992-93**	2.0	1.6	2.6	0.86 (0.49-1.52)	0.60 (0.37-1.00)
	1994-95	1.0	1.1	2.4	0.46 (0.23-0.95)	0.46 (0.26-0.82)
35	**1992-93**	0.7	1.2	1.2	0.65 (0.26-1.63)	1.01 (0.53-1.94)
	1994-95	0.5	0.9	1.5	0.53 (0.21-1.35)	0.61 (0.31-1.20)
39	**1992-93**	3.5	3.1	2.7	1.20 (0.72-2.01)	1.15 (0.75-1.75)
	1994-95	2.8	2.2	3.0	0.93 (0.56-1.53)	0.74 (0.47-1.16)
45	**1992-93**	0.5	0.5	2.1	0.26 (0.11-0.66)	0.25 (0.12-0.56)
	1994-95	0.3	0.3	1.9	0.13 (0.05-0.40)	0.12 (0.04-0.36)
51	**1992-93**	8.0	8.1	5.2	**1.56 (1.11-2.19)**	**1.57 (1.19-2.08)**
	1994-95	6.2	6.4	7.0	0.85 (0.61-1.18)	0.92 (0.70-1.21)
52	**1992-93**	4.1	4.6	4.2	0.96 (0.62-1.49)	1.10 (0.78-1.56)
	1994-95	3.5	3.3	4.4	0.68 (0.44-1.05)	0.75 (0.52-1.08)
56	**1992-93**	3.3	4.7	3.9	0.93 (0.59-1.49)	1.22 (0.86-1.72)
	1994-95	5.2	4.2	5.1	1.05 (0.73-1.53)	0.84 (0.60-1.17)
58	**1992-93**	2.6	2.0	2.8	1.03 (0.61-1.73)	0.72 (0.45-1.15)
	1994-95	2.8	2.1	2.1	1.51 (0.88-2.59)	1.01 (0.61-1.66)
59	**1992-93**	2.9	3.0	2.4	1.25 (0.74-2.11)	1.27 (0.82-1.97)
	1994-95	2.3	2.7	2.7	1.06 (0.63-1.78)	1.04 (0.67-1.61)
66	**1992-93**	4.4	4.6	3.1	**1.55 (0.98-2.46)**	**1.53 (1.06-2.22)**
	1994-95	3.4	3.9	4.2	0.85 (0.54-1.33)	0.92 (0.65-1.32)

Table 9: HPV type specific Prevalence and Prevalence Ratio among the HPV non-HPV vaccinated (includes both those HBV vaccinated and completely unvaccinated) female CRT participants by intervention arm at the age of 18-years-old.

		Non-HPV vaccinated comparisons				
		Prevalence (%)			**Prevalence ratio (95% CI)**	
HPV type	**Birth cohort**	**Arm A** (N=923)	**Arm B** (N=1066)	**Arm C** (N=3375)	**A vs C**	**B vs C**
6	**1992-93**	6.2	6.6	4.2	1.28 (0.81-2.00)	1.74 (1.19-2.56)
	1994-95	4.9	4.6	6.6	0.77 (0.51-1.17)	0.72 (0.48-1.08)
11	**1992-93**	1.1	1.2	1.2	0.80 (0.29-2.20)	1.04 (0.43-2.50)
	1994-95	1.4	0.5	0.7	1.65 (0.62-4.38)	0.84 (0.27-2.67)
16	**1992-93**	6.9	6.0	7.3	0.79 (0.52-1.19)	0.86 (0.59-1.25)
	1994-95	7.4	7.6	8.9	0.91 (0.65-1.27)	0.89 (0.65-1.22)
18	**1992-93**	6.2	5.2	5.7	1.20 (0.81-1.78)	0.91 (0.59-1.38)
	1994-95	3.3	2.7	4.4	0.77 (0.46-1.29)	0.60 (0.35-1.03)
31	**1992-93**	4.6	3.4	3.0	1.47 (0.88-2.43)	0.99 (0.57-1.73)
	1994-95	2.5	3.5	4.6	0.47 (0.26-0.88)	0.75 (0.46-1.21)
33	**1992-93**	3.9	3.2	1.9	1.56 (0.83-2.93)	1.86 (1.05-3.27)
	1994-95	1.8	2.5	2.4	0.52 (0.23-1.19)	1.11 (0.62-1.98)
35	**1992-93**	1.1	1.2	1.8	0.75 (0.32-1.76)	0.63 (0.27-1.51)
	1994-95	1.8	0.9	1.6	0.96 (0.44-2.11)	0.54 (0.21-1.36)
39	**1992-93**	4.1	1.8	2.7	1.84 (1.12-3.02)	0.62 (0.30-1.26)
	1994-95	3.1	2.3	3.1	1.13 (0.67-1.92)	0.71 (0.39-1.30)
45	**1992-93**	2.5	2.2	2.5	0.94 (0.48-1.82)	0.91 (0.48-1.71)
	1994-95	3.3	1.9	2.5	1.70 (1.02-2.82)	0.80 (0.42-1.51)
51	**1992-93**	8.9	7.0	5.7	**1.46 (1.02-2.11)**	1.16 (0.80-1.70)
	1994-95	8.0	7.4	7.4	0.94 (0.65-1.35)	1.00 (0.71-1.40)
52	**1992-93**	5.3	7.0	4.4	0.93 (0.56-1.54)	1.60 (1.09-2.36)
	1994-95	5.7	3.7	4.7	1.21 (0.80-1.85)	0.80 (0.51-1.28)
56	**1992-93**	4.4	4.2	5.2	0.83 (0.51-1.34)	0.82 (0.52-1.30)
	1994-95	4.5	4.8	5.2	0.75 (0.47-1.22)	0.89 (0.59-1.36)
58	**1992-93**	4.6	3.4	3.3	1.47 (0.91-2.39)	1.06 (0.63-1.79)
	1994-95	2.5	3.0	2.2	1.03 (0.53-1.98)	1.45 (0.84-2.52)
59	**1992-93**	3.7	3.4	2.4	1.44 (0.81-2.55)	1.39 (0.80-2.41)

	1994-95	3.3	1.9	3.2	1.24 (0.75-2.06)	0.61 (0.32-1.14)
66	**1992-93**	5.7	4.0	5.0	1.43 (0.97-2.12)	0.73 (0.44-1.19)
	1994-95	3.9	3.4	5.6	0.69 (0.43-1.11)	0.62 (0.39-0.99)

5.4 Impact of HPV vaccination on non-vaccine HPV type prevalence according to current core-group membership

The behavioral characteristics of the CRT participants attending the follow-up visits at the age of 22 was relatively similar to that at the age of 18-years-old. Similar to the findings at the age of 18, the 22-year old's self-reported general and sexual risking-taking behaviour was slightly higher among the participants from Arms A and B as compared to arm C, with the prevalence of current smokers being 28.4% among the HPV vaccinated from Arms A/B compared to 23.2% among the originally HBV vaccinated from Arm C, and the prevalence of having had 5 or more lifetime partners being 42.5 % in Arms A/B as compared to 38.6% among the HBV vaccinated Arm C participants. The prevalence of *C. trachomatis* among the 22-year-olds was higher among the participants from arms A/B, 3.4%, as compared to Arm C, 2.5%.

Prevalence ratios evaluating the direct effect of HPV vaccination by current core-group membership
When evaluating the prevalence of vaccine targeted HPV16 and 18 between the HPV vaccinated intervention and HBV vaccinated control arm participants stratified by *C. trachomatis* positivity (a surrogate marker of current sexual risk-taking core group membership) at the age of 18, the prevalence of both HPV16 and 18 was universally markedly reduced. This was among both the *C. trachomatis* positive and negative Arm A/B participants as compared to those from Arm C, $PR_{(C.tr+)}$=0.00 (0.00-0.00), $PR_{(C.tr-)}$= 0.07 (0.05-0.11) for HPV16, and $PR_{(C.tr+)}$=0.00 (0.00-0.00), $PR_{(C.tr-)}$=0.06 (0.04-0.12) for HPV18 respectively. Similarly, the prevalence of vaccine-cross-protected HPV31/33/35 was also found to have decreased among the vaccinated Arm A/B participants relative to the control arm participants, both among the *C. trachomatis* positive and negative participants $PR_{(C.tr+)}$=0.42 (0.21-0.87), and $PR_{(C.tr-)}$= 0.48 (0.38-0.62).
When evaluating the prevalence of non-vaccine targeted HPV types by *C. trachomatis* positivity among the HPV vaccinated 18-year-old participants for possible signs of increasing prevalence indicative of HPV type replacement, only HPV51 was consistently increased among both the *C. trachomatis* positive and negative

participants, $PR_{(C.\ tr+)}$=1.36 (0.76-2.41) and $PR_{(C.tr-)}$=1.40 (1.11-1.77). Among the *C. trachomatis* negative participants only the Arms A/B prevalence of HPV66 was somewhat increased in comparison to the prevalence among the *C. trachomatis* negative HBV vaccinated participants from Arm C, $PR_{(C.\ tr-)}$=1.32 (1.00-1.75). In contrast among the *C. trachomatis* positives the prevalence of HPV52 was higher in Arm A/B participants, $PR_{(C.\ tr+)}$=1.32 (1.00-1.75) as compared to Arm C participants $PR_{(C.\ tr-)}$=0.82 (0.37-1.84).(Table 10)

Table 10: HPV type specific prevalence ratio (comparing the intervention Arms to the control Arm) stratified by *C. trachomatis* positivity among the **HPV vaccinated** female CRT participants at the age of 18 and 22 years old from the 1992-94 birth cohorts.

	Prevalence Ratio (95% CI), Arms A/B vs. C			
	18 years old		**22 years old**	
HPV type	***C. trachomatis* positive (N=162 vs. 63)**	***C. trachomatis* negative (N=4452 vs. 2079)**	***C. trachomatis* positive (N=87 vs. 20)**	***C. trachomatis* negative (N=2480 vs. 789)**
6/11	0.80 (0.43-1.48)	0.99 (0.81-1.23)	Na	0.81 (0.56-1.18)
16	**0.00 (0.00-0.00)**	**0.07 (0.05-0.11)**	Na	0.69 (0.32-1.48)
18	**0.00 (0.00-0.00)**	**0.06 (0.04-0.12)**	Na	3.01 (0.40-22.9)
39	1.28 (0.44-3.73)	1.01 (0.75-1.37)	Na	0.77 (0.55-1.08)
51	**1.36 (0.76-2.41)**	**1.40 (1.11-1.77)**	0.28 (0.07-1.09)	0.91 (0.69-1.21)
52	**2.45 (0.78-7.67)**	0.83 (0.65-1.07)	Na	0.74 (0.51-1.07)
56	1.05 (0.48-2.29)	0.92 (0.71-1.21)	20.5 (0.39-23.6)	0.81 (0.52-1.25)
58	0.17 (0.05-0.60)	0.96 (0.69-1.34)	0.29 (0.02-4.52)	0.91 (0.55-1.50)
59	0.97 (0.39-2.41)	1.16 (0.84-1.61)	0.42 (0.07-2.43)	1.12 (0.67-1.88)
66	0.82 (0.37-1.84)	**1.32 (1.00-1.75)**	0.28 (0.02-4.11)	1.38 (0.94-2.04)

Evaluating the indirect effect of HPV vaccination on prevalence by current core-group membership
When evaluating the indirect impact of HPV vaccination on vaccine targeted HPV16 and 18 among the non-HPV vaccinated 18-year-old participants resident in the trial communities according to *C. trachomatis* status, the impact of vaccination was found to somewhat differ by *C. trachomatis* positivity. Among the *C. trachomatis* negative non-vaccinated participants, the prevalence of HPV16 was found to be lower among the participants from Arms A/B as compared to those from Arm C, $PR_{(C.\ tr+)}$=0.75 (0.59-

0.96). Whereas no analogous decrease in HPV16 prevalence was observed among their *C. trachomatis* positive counterparts $PR_{(C.\ tr+)}$=1.62 (0.80-3.30). In contrast when evaluating the indirect impact of vaccination on the prevalence of HPV18, especially among the *C. trachomatis* positives the prevalence of HPV18 was lower among the participants from Arms A/B in comparison to those from Arm C, $PR_{(C.\ tr+)}$=0.44 (0.22-0.89), whilst no substantial decrease in the HPV18 prevalence was observed among the *C. trachomatis* negatives $PR_{(C.tr-)}$=0.94 (0.79-1.27).(Table 11)

Likewise to the observations among the HPV vaccinated participants, among the non-vaccinated participants, the prevalence of HPV51 was universally higher among the participants from Arms A/B as compared to C among both the *C. trachomatis* positives and negatives, but especially among the *C. trachomatis* negatives, $PR_{(C.\ tr+)}$=3.78 (1.19-12.1) and $PR_{(C.\ tr-)}$=1.23 (0.96-1.57). Also similar to the case among the HPV vaccinated participants the prevalence of HPV52 was higher among the *C. trachomatis* positives from Arm A/B as compared to the prevalence among Arm C, but the confidence intervals still overlapped the null, $PR_{(C.\ tr+)}$=1.81 (0.89-3.68).(Table 11)

Table 11: HPV type specific prevalence ratio (comparing the intervention Arms to the control Arm) stratified by *C. trachomatis* positivity among the non-**HPV vaccinated** female CRT participants at the age of 18 years old from the 1992-94 birth cohorts.

	Prevalence Ratio (95% CI), Arms A/B vs. C	
	18 years old (non-HPV females)	
HPV type	***C. trachomatis* positive**	***C. trachomatis* negative**
6/11	0.56 (0.27-1.15)	1.23 (0.95-1.59)
16	1.62 (0.80-3.30)	0.75 (0.59-0.96)
18	**0.44 (0.22-0.89)**	0.94 (0.79-1.27)
39	na	1.00 (0.69-1.47)
51	**3.78 (1.19-12.1)**	1.23 (0.96-1.57)

52	**1.81 (0.89-3.68)**	1.10 (0.83-1.47)
56	0.78 (0.33-1.83)	0.82 (0.60-1.12)
58	1.52 (0.50-4.64)	1.32 (0.92-1.90)
59	0.68 (0.31-1.52)	1.50 (1.01-2.25)
66	0.44 (0.17-1.14)	0.84 (0.62-1.13)

Testing for effect measure modification by current core group membership

When quantifying possible risk difference modification on the effect of trial arm by *C. trachomatis* positivity, among the HPV vaccinated participants the interaction contrast, IC for both HPV51 and 52 were departed from the null, however neither departure were unexplainable by random error (Table 12). When quantifying possible risk ratio modification by *C. trachomatis* positivity, among the same HPV vaccinated participants only for HPV52 was *C. trachomatis* positivity found to modify the effect of trial Arm in excess of that expected by the simple multiplication of the risk factors (table12).

Among the non-HPV vaccinated participants of the community randomized trial, when evaluating possible risk difference modification between trial Arm due *C. trachomatis* positivity, the risk among the doubly exposed (*C. trachomatis* positive and from the intervention Arms) exceeded that expected via the simpler addition of the risk due to *C. trachomatis* and trial arm for both HPV51 and 52, albeit similarly to the evaluation on the multiplicative scale. Neither of these findings were unexplainable by random error alone (Table 12).

Table 12: Interaction analyses to assess the effect of *C. trachomatis* status (as a proxy for current core-group membership) on the impact of HPV vaccination on HPV DNA positivity among the female CRT participants at the age of 18 years old (comparing Arms A/B combined to Arm C). *For the non-HPV vaccinated interaction analyses on the multiplicative scale, a range of p-values were calculated for the interaction term, that from the 21 unvaccinated strata (See section 4.4.4). An interaction contrast greater than 1 is interpretated interaction departing from additivity.

HPV vaccinated CRT participants (18-years-old)		
HPV type	**Interaction term** (p-value)*	**Interaction contrast** (95% CI)
51	0.95 (0.89)	3.36 (-37.4, 44.1)
52	2.96 (0.06)	7.73 (-24.0, 39.4)
58	0.18 (0.01)	-9.15 (-33.5, 15.2)
Non-HPV vaccinated CRT participants (18-years-old)		
51	83.1 (range:0.00-0.57)	14.1 (-16.2,44.4)
52	1.64 (range: 0.23-0.79)	9.29 (-18.3, 36.9)
58	1.29 (range: 0.34-0.92)	2.49 (-18.7, 23.7)

5.5 Impact of vaccination strategy on non-vaccine HPV type cumulative incidence

In the study population of pregnant women under 23 years-old, the mean prevalence of self-reported maternal smoking (an indicator of general risk-taking behaviour) between the years of 2005 to 2016 was high among all the subjects in all communities. However, it was consistently elevated among the Arm C subjects in both the pre- vaccination era (years 2005 to 2010) and the post-vaccination era (years 2011 to 2016) in comparison to the mean among the Arm A and B subjects, $P_{[Arm\ C,\ 2005\text{-}10]}$=42.8% (standard deviation, SD, 7.6), and $P_{[Arm\ C,\ 2010\text{-}2016]}$=43.3 (9.3), as compared to $P_{[Arm\ A,\ 2005\text{-}2010]}$=38.4% (6.0), $P_{[Arm\ A,\ 2011\text{-}2016]}$=37.8% (5.3), $P_{[Arm\ B,\ 2005\text{-}2010]}$=36.7% (6.0), and $P_{[Arm\ B,\ 2011\text{-}2016]}$=38.6% (6.4). The seroprevalence of HSV-2 (a surrogate marker of sexual risk-taking behaviour) whilst not being substantially different between the subjects from the different Arms, was found to be higher among the subjects from the pre-vaccination era as compared to those from the post-vaccination era, $P_{[Arm\ A,\ 2011\text{-}16]}$=15.2% as compared to $P_{[Arm\ A,\ 2005\text{-}10]}$=16.9%, $P_{[Arm\ B,\ 2011\text{-}16]}$=14.0% as compared to $P_{[Arm\ B,\ 2005\text{-}10]}$=19.2%, and $P_{[Arm\ C,\ 2011\text{-}16]}$=15.9% as compared to $P_{[Arm\ C,\ 2005\text{-}10]}$=17.2% (Table 13).

When comparing the post-vaccination era seroprevalence of the non-vaccine HPV types, from the years 2011 to 2016, to the pre-vaccination years 2005 to 2010, HPV68 was found to have increased in the later time period. However, this was only observed among the subjects from Arm B sPR=1.28 (0.97-1.68) and was not far removed from the ratio of seroprevalence among the subjects from Arm C

comparing the two time periods. When further investigating the non-vaccine type seroprevalence among the HSV-2 seropositive the HPV68 seroprevalence was further found to have increased among the subjects from Arm B $sPR_{[HSV2+]}$=2.78 (1.23-6.31). However, again the 95% confidence intervals were overlapping that found in the counterfactual seroprevalence comparison in Arm C $sPR_{[HSV2+]}$=1.40 (0.79-2.49)(Table 14).

Upon further evaluation of the seroprevalence by the time since implementing HPV vaccination, the observed increase in HPV68 seroprevalence was found to originate from the later post-vaccination era, 2014-16) among the subjects in Arm B, $sPR_{[Arm\ B]}$=1.54 (1.02-2.34). However yet again, when performing the counterfactual comparison among the subjects from Arm C, the 95% confidence intervals were found to overlap, sPR=1.36 (1.00-1.85) (Table 15).

Table 13: HPV type specific seroprevalence ratio (95% confidence intervals), comparing the seroprevalence among the FMC subjects from the post-vaccination era, years 2011-2016, to the seroprevalence among the subjects among the pre-vaccination era, years 2005-2010.

	Seroprevalence ratio (95% CI), post- vs pre- vaccination era		
HPV type	**Arm A** (N=1247 vs 1322)	**Arm B** (N=1158 vs 1289)	**Arm C** (N=1211 vs 1304)
6	0.78 (0.67-0.92)	0.82 (0.75-0.91)	0.93 (0.82-1.05)
11	0.93 (0.68 -1.28)	0.78 (0.60-1.01)	0.80 (0.58-1.10)
16	0.79 (0.72-0.87)	1.09 (0.91-1.32)	1.01 (0.86-1.20)
18	0.86 (0.70-1.06)	0.96 (0.74-1.24)	0.89 (0.70-1.13)
39	1.04 (0.88-1.22)	1.07 (0.85-1.33)	1.09 (0.92-1.31)
51	0.95 (0.78 -1.17)	1.03 (0.91-1.15)	0.98 (0.80-1.20)
52	0.99 (0.83-1.19)	1.06 (0.90-1.25)	0.96 (0.80-1.16)
56	0.83 (0.73-0.93)	0.93 (0.77-1.13)	0.85 (0.70-1.02)
58	0.70 (0.63-0.79)	0.86 (0.72-1.03)	0.79 (0.65-0.96)
59	0.99 (0.80-1.22)	1.00 (0.82-1.22)	1.00 (0.79-1.26)
66	0.83 (0.73-0.96)	0.89 (0.73-1.08)	1.05 (0.89-1.24)
68	0.94 (0.72-1.23)	1.28 (0.97-1.68)	1.16 (0.90 -1.51)
73	0.74 (0.60-0.91)	0.87 (0.76-1.00)	0.99 (0.85-1.15)

Table 14: HPV type specific seroprevalence ratio (95% confidence intervals) comparing the seroprevalence among the **HSV-2 seropositive FMC subjects** from the post-vaccination era, years 2011-2016, to the seroprevalence among the subjects among the pre-vaccination era, years 2005-2010.

	Seroprevalence ratio (95% CI), post- vs pre- vaccination era		
	HSV-2 seropositive		
HPV type	**Arm A** (N=189 vs. 224)	**Arm B** (N=162 vs. 247)	**Arm C** (N=192 vs. 224)
6	0.61 (0.43-0.87)	0.43 (0.34-0.54)	0.95 (0.64-1.43)
11	0.82 (0.48-1.39)	0.54 (0.21-1.37)	0.96 (0.51-1.81)
16	0.64 (0.50-0.81)	0.94 (0.63-1.42)	0.98 (0.70-1.38)
18	0.95 (0.60-1.51)	0.74 (0.34-1.65)	1.00 (0.68-1.46)
39	0.77 (0.62-0.96)	0.90 (0.50-1.60)	1.10 (0.70-1.75)
51	0.71 (0.48-1.05)	1.04 (0.78-1.38)	1.14 (0.81-1.59)
52	1.03 (0.83-1.27)	0.70 (0.41-1.20)	1.02 (0.65-1.60)
56	0.72 (0.55-0.94)	0.68 (0.43-1.07)	0.65 (0.40-1.05)
58	0.65 (0.44-0.96)	0.79 (0.54-1.14)	0.64 (0.39-1.06)
59	0.90 (0.75-1.09)	0.76 (0.54-1.07)	0.82 (0.57-1.18)
66	0.72 (0.53-0.98)	0.76 (0.44-1.30)	0.79 (0.59-1.06)
68	0.57 (0.23-1.38)	2.78 (1.23-6.31)	1.40 (0.79-2.49)
73	0.45 (0.35-0.59)	0.84 (0.57-1.24)	0.86 (0.53-1.39)

Table 15: HPV type specific seroprevalence ratio (95% confidence intervals, comparing the seroprevalence among the FMC subjects from the first or second post-vaccination eras, years 2011-13 and 2014-2016 respectively, to the seroprevalence among the subjects among the pre-vaccination era, years 2005-2010.

	Seroprevalence ratio (95% CI)					
	1st post-vaccination vs. pre-vaccination era			**2nd post-vaccination** vs. pre-vaccination era		
HPV type	**Arm A** (N=662 /1322)	**Arm B** (N=602 /1289)	**Arm C** (N=650 / 1304)	**Arm A** (N=585 / 1322)	**Arm B** (N= 556/ 1289)	**Arm C** (N=561 / 1304)
6	0.83 (0.68-1.02)	0.98 (0.81-1.19)	1.02 (0.87-1.21)	0.73 (0.61-0.86)	0.66 (0.49-0.90)	0.81 (0.69-0.96)
11	1.04 (0.72-1.50)	0.86 (0.61-1.22)	1.01 (0.72-1.43)	0.80 (0.55-1.15)	0.70 (0.46-1.07)	0.55 (0.33-0.89)
16	0.76 (0.65-0.88)	1.16 (0.95-1.42)	0.96 (0.78-1.17)	0.83 (0.72-0.96)	1.03 (0.83-1.28)	1.07 (0.90-1.27)
18	0.78 (0.59-1.01)	0.92 (0.72-1.18)	0.81 (0.62-1.06)	0.95 (0.75-1.19)	1.01 (0.71-1.43)	0.98 (0.76-1.27)
39	0.99 (0.82-1.20)	1.19 (0.91-1.55)	1.05 (0.84-1.31)	1.08 (0.86-1.36)	0.93 (0.75-1.16)	1.14 (0.91-1.44)
51	1.02 (0.79-1.31)	1.06 (0.90-1.25)	1.03 (0.84-1.28)	0.88 (0.70-1.10)	0.98 (0.82-1.18)	0.91 (0.71-1.17)
52	0.92 (0.68-1.25)	1.14 (0.90-1.46)	0.89 (0.71-1.12)	1.07 (0.94-1.22)	0.99 (0.75-1.30)	1.05 (0.85-1.30)
56	0.83 (0.70-0.99)	1.06 (0.90-1.24)	0.95 (0.73-1.23)	0.81 (0.66-1.00)	0.80 (0.58-1.09)	0.73 (0.56-0.95)
58	0.78 (0.67-0.91)	1.03 (0.86-1.23)	0.89 (0.74-1.07)	0.61 (0.53-0.70)	0.66 (0.53-0.84)	0.66 (0.51-0.87)
59	0.99 (0.73-1.33)	1.13 (0.87-1.46)	1.12 (0.86-1.46)	0.99 (0.75-1.32)	0.86 (0.68-1.09)	0.85 (0.67-1.07)
66	0.85 (0.71-1.01)	0.93 (0.74-1.16)	0.96 (0.77-1.21)	0.82 (0.68-1.00)	0.85 (0.65-1.11)	1.16 (0.91-1.47)
68	0.80 (0.54-1.16)	1.02 (0.71-1.48)	0.99 (0.65-1.52)	1.10 (0.76-1.59)	1.54 (1.02-2.34)	1.36 (1.00-1.85)
73	0.76 (0.60-0.96)	0.96 (0.75-1.23)	1.13 (0.96-1.33)	0.72 (0.55-0.93)	0.78 (0.56-1.08)	0.83 (0.59-1.17)

6 DISCUSSION

6.1 Sustainability of vaccine-induced neutralising and cross-neutralising antibodies

We observed vaccine-induced neutralising HPV16 and HPV18 antibodies among both the cohort of BVR and QVR up to 12 years post-vaccination with three doses. However, whilst among the BVR neutralising antibodies against HPV16 and 18 were observed in 100% of the women, among the QVR 4% of women receiving all three doses were observed to have no neutralising antibodies against HPV16, and a further 15% found to have no neutralising antibodies that are deemed to mediate the vaccine conferred protection against HPV18. Furthermore, similar to earlier studies of total binding antibodies (Artemchuk et al, 2019), the GMT of neutralising antibodies was found to be 5.7-fold and 12.4-fold higher among the BVR in comparison to the QVR for HPV16 and 18 respectively (Mariz et al, 2020).

The GMT of vaccine-induced HPV6 neutralising antibodies among the QVR was found to be much higher than that of the cross-neutralizing HPV6 antibodies among the bivalent vaccine recipients, similar to previous head-to-head studies total binding antibodies (Kann et al, 2021). The finding of cross-neutralising antibodies HPV6 antibodies among the BVR is surprising as HPV6 not being closely phylogenetically related to the vaccine types. Despite the disparity, among both cohorts the neutralising/cross neutralizing HPV6 antibodies were sustained up to twelve years post-vaccination.

Notable differences also in the vaccine-induced cross-neutralising antibodies were also observed between the two vaccinated cohorts, with cross-neutralising antibodies against HPV31, 33, 45, 52, and 58 being more prevalent among the BVRs in comparison to the QVRs. This is in line with what has been reported for the neutralizing HPV31 antibodies following vaccination with the bivalent vaccine. (Godi et al, 2015). Among neither cohort were cross-neutralising antibodies observed among all the women, with the seroprevalence differing by type, similar to earlier studies measuring total-binging antibodies (Kann et al, 2021). In spite of this, among women found to be seropositive for cross-neutralising antibodies among both the vaccinated cohorts the neutralising antibodies were observed to be

comparatively stable up to 12 years post-vaccination. Only for HPV31 was the GMT among those seropositive found to be notably higher among the BVRs in comparison to the QVRs.

The notable differences in the immunogenicity of the two vaccines in the two otherwise comparable cohorts is likely attributable to differences in the vaccines composition and the expression platforms used. The adjuvant incorporated in the bivalent vaccine uses a toll-like receptor agonist MPL, which has been shown to enhance the immune response (Didierlaurent et al, 2009). This may partly explain the higher neutralising antibody titres and the higher seropositivity of cross-neutralising antibody titres among the bivalent vaccine recipients in comparison to those observed among the recipients of the quadrivalent vaccine which uses a simple aluminium salt adjuvant. Additionally, it is possible that the differences in the expression platforms of the L1, the use of yeast cells (the quadrivalent vaccine) compared to insect cells (the bivalent vaccine), may have impacted the conformation of the VLPs, particularly for HPV18 VLPs (Lehtinen & Paavonen, 2012). This is corroborated by differences in the ability of Merck's HPV18 VLP to disassemble into L1 pentamers under certain conditions in comparison to their HPV6 and HPV16VLPs (Mach et al, 2006).

There are several plausible explanations as to the observed sustainability of the cross-neutralising antibodies. Sustainability may be due to natural boosting due to exposure to hrHPV infections as has previously been demonstrated for HPV16 and 18 (Donkin et al, 2019). However, other possibilities may play a role given the disparity in the seropositivity of cross-neutralizing antibodies by HPV type compared to the vaccine efficacy observed in earlier studies (Lehtinen et al, 2018; Wheeler et al, 2012; Villa et al, 2009; Brown et al, 2009). We observed a high seroprevalence of cross-neutralising HPV52 antibodies and a relatively high GMT comparative to that seen for other cross-protected HPV types among recipients of both vaccines. However, vaccine efficacy studies have only reported an efficacy of about 20% against persistent infection for the two vaccines (Wheeler et al, 2012; Brown et al, 2009). Whereas for HPV45 a moderate level of seroprevalence of cross-neutralising antibodies, 47%, among the BVR only was observed despite high previously reported cross-protective vaccine efficacy against persistent HPV45 infection of 79% (Wheeler et al, 2012). This raises the question as to how protective these cross-neutralising antibodies are in preventing persistent infection, and whether the protective antibody level may be different dependent on HPV type. Finally, other possibilities such as vaccine-induced cellular immune responses may play a role in cross-protective vaccine efficacy against non-vaccine types. This would

be in line with reports that the correlation between vaccine-induced cellular immune responses were strongest for those types which were closest to the phylogenetically related vaccine types and with studies reporting that cross-protective efficacy correlates with phylogenetic distance of the non-vaccine HPV type to the vaccine targeted HPV types HV16 and 18 (Bogaards et al, 2019; Pinto et al, 2006). It is possible that in addition to cross-neutralising antibodies, broadly vaccine-induced cross-reactive T-cell mediated responses may play a role (Pinto et al, 2003 & 2006; Toh et al, 2018), which may conceivably contribute to the disparity observed between the immunogenicity of the two vaccines, as the bivalent vaccine adjuvant is known to boost the cellular immune response (Didierlaurent et al, 2009). However, how much vaccine-induced cellular immune response eventually contributes to protection against the infection is an open question (Hildesheim et al, 2007).

This study was strengthened by the use of the Finnish infrastructure facilitating the head-to-head long-term comparison of two identical cohorts of adolescent women in an independent manner. However, the magnitude of the immune response may differ somewhat if the same study would be conducted among women vaccinated at either an older or younger age. Previous studies have shown that the vaccine-induced HPV antibody titres are higher among females vaccinated as early adolescents (Pedersen et al, 2007; Petäjä et al, 2011), whereas the women in this study were vaccinated between the ages of 16-17 years-old. Furthermore, it is likely that our findings may have limited transportability to immunocompromised persons. In addition, our use of the HT-PBNA to measure neutralising and cross-neutralising HPV antibodies, the accepted gold standard of HPV serology assays, is a key strength of this study (Sehr et al, 2013).

6.2 Niche clearance following the community randomised trial

We evaluated the herd effect against the most important vaccine targeted HPV types induced by low to moderate HPV vaccination coverage via the community randomised trial via two approaches: 1) by comparing the HPV seroprevalence among unvaccinated sexually active women resident in the trial communities in the post-vaccination era versus the pre-vaccination era, and 2) by comparing HPV PCR positivity among the women from the trial birth cohorts when they were aged 18 and 22 years old, comparing the prevalence among the women residing in the gender-neutral or girls only arm communities to the prevalence among the women from the control arm communities.

6.2.1 Evaluation of niche clearance at the community level using a measure of HPV16/18 cumulative incidence

The seroprevalence of vaccine targeted HPV16/18 was found to be marginally decreased among the unvaccinated women in the post-vaccination era among the women who were residing in the communities where there was gender-neutral vaccination, whilst no comparable decrease was observed among the women residing in the communities where there had been girls only vaccination or vaccination with the control HBV vaccine. Most notably, the seroprevalence of HPV16 was observed to have decreased among the unvaccinated women resident in the communities where there was gender-neutral vaccination. This is notable as HPV16 is not only the most oncogenic of the high-risk types (Bzhalava et al, 2013), but it is also the most notoriously hard to achieve herd effect against owing HPV16 having a higher R0 relative to other hrHPV types (Baussano et al, 2017). This decrease was still apparent when compared to the HPV16 seroprevalence change among the women in the counterfactual Arm C where there was HBV vaccination, thereby indicating that the reduction is not explainable simply by community-wise changes in sexual behaviour and or HPV epidemiology over time not due vaccination. Also, encouragingly we observed this reduction of HPV16 seroprevalence in the gender-neutral vaccination communities also among the high sexual risk-taking core group, as identified by HSV-2 seropositivity is a marker cumulative sexual risk-taking behaviour. However, yet again no such decrease in HPV16 seropositivity was observed among the HSV-2 seropositive women residing in the communities where there had only been gender neutral vaccination.

The finding of decreased HPV16 seroprevalence indicative of HPV16 herd effect among the unvaccinated women after gender-neutral vaccination, is of critical importance, as in this setting the vaccination coverage was only low to moderate. The World Health Organisation has set the target to eliminate cervical cancer as a public health problem, with the control of hrHPV16/18 infection via vaccination being fundamental in its plan (WHO, 2018; 2020). However, as of yet the strategy being adopted is an unrealistic one, mandating that every country globally achieves 90% vaccination coverage among girls under the age of 15 years old (WHO, 2020; Lehtinen et al, 2022). Without the instigation of unethical mandatory vaccination this is unlikely to occur. Although HPV vaccination coverages differ globally more typical coverages tend to be around 67% coverage, with many countries suffering the impact of low vaccine confidence (such as the case in France) having much lower coverages (Dalon et al, 2021), and other countries suffering the impacts of

programmatic instability due to political and or economic instability (Daniels et al, 2021). Such girls-only vaccination coverages are wholly insufficient to induce HPV16 herd effect sufficient to protect the most vulnerable unvaccinated women and men among vaccinated populations (Lehtinen et al, 2022).

For vaccine preventable infectious agents such as HPV, the vaccination coverage threshold required to result in herd effect among the unvaccinated population, depends on the basic reproduction number of the particular infectious agent. The R0 for HPV16 is particularly high (Vänskä et al, 2020), meaning that the vaccination coverage required to create an HPV 16 herd effect is also high, around 70-80%, although this also differs according to the population in question. However, this aforementioned vaccination coverage threshold is specific to girls-only vaccination. When the vaccination strategy is gender-neutral, modelling studies have suggested that vaccination coverages of 40% may already be sufficient to create some degree of herd effect, with vaccination coverages of 80% providing the potential to eliminate HPV16 infection (Brisson et al, 2016; Vänskä et al, 2020). Our finding further exemplifies this key point, that gender-neutral vaccination facilitates HPV16 herd effect when vaccination coverage is moderate, around 40%, whereas girls-only vaccination does not. Therefore, if HPV16 herd effect and elimination is to be realistically achieved, in the majority of countries it will be key to implement gender-neutral vaccination.

In the active follow-up of this community randomised trial, when following up the female participants from the trial birth cohorts (those born in 1992-95) only, no second order HPV16 herd effect was observed among the non-HPV vaccinated participants (Lehtinen et al, 2018; Gray et al, 2018). However, these previous studies differed to this study in several key aspects, not only was the study population restricted to the trial birth cohorts but also the study outcome HPV DNA as measured via PCR measured current HPV infection as compared to this current study where the study population also includes adjacent birth cohorts to the 1992-95 born and uses HPV serology as a measure of cumulative incidence of HPV infections.

Furthermore, the finding of HPV16 herd effect among the ever-core group members, as measured by HSV-2 seropositivity, under the indirect effects of gender-neural vaccination is an important and reassuring finding. As previous authors had suggested that it may be hardest among the core-group to achieve herd effect due to the higher contact rate and R0 among this group (Garnett, 2005). A concern which is warranted, as this is also the subpopulation at highest risk for HPV-associated disease (Dillner et al, 1996). In order to ensure equitable delivery in the prevention

of HPV-infection and associated disease at a population level, gender-neutral vaccination is more effective.

In this study, a sizeable number of clusters (i.e., communities) were randomised, N=33 (Lehtinen et al, 2015). However, despite this we still observed some baseline differences in the seroprevalence of HPV16/18 prior in the pre-vaccination era. Most notably HPV16/18 seroprevalence was somewhat lower among the women resident in the control arm communities. This finding precluded the direct comparison of the seroprevalence between the arms in the post-vaccination era, as any finding may have been confounded by these baseline differences in cumulative HPV risk. This source of bias was tackled by the adoption of a difference of differences/ratio of ratios approach, by assuming a common trend between the comparison arms in the absence of HPV vaccination (Lechner, 2011). The application of this method allowed the direct comparison of any changes to the intervention arm to that observed in the counterfactual control arm to assess whether the changes in seroprevalence for explainable by changes in HPV epidemiology over time due to factors other than vaccination.

As the transmission of HPV moves in the direction of the older to the younger birth cohorts, it is therefore plausible and expected that the vaccination of the 1992-95 birth cohorts may have had some first and second order herd effects on the birth cohorts immediately younger and adjacent those born in 1996-7 (Vänskä et al, 2020; Elfström et al, 2016). This study may also have been limited by its design due to the selection criteria being defined by the women's age and the time period, and not by the birth cohort, thereby precluding the direct comparison of birth cohorts. A study directly comparing the seroprevalence among the pre- trial, trial, and post-trial birth cohorts may have allowed for greater interpretation of the comparative indirect effects of gender-neutral versus girls-only HPV vaccination.

The use of two different measures of HPV infection (current and cumulation infection) in the follow-up of this community randomised trial, is an important and purposeful one. Both measures are prone to outcome misclassification, to differing degrees in their ability to identify current HPV infection (HPV DNA positivity) or cumulative HPV infection (HPV seropositivity). Such outcome misclassification is likely to have biased the observed relative risk in both this and the previously published studies, with the degree of misclassification likely being different according to HPV type and the measure used (serology or HPV-DNA positivity). When HPV-DNA is used as a measure of current infection, it cannot distinguish established persistent infection from that of a transient deposition, decreasing the specificity of the measure of infection. Whilst HPV seropositivity does not suffer

from this impediment, it does suffer from imperfect sensitivity as a measure of cumulative infection owing to the fact that not all women seroconvert following infection. Albeit this presents a 'chicken or egg' type of paradox, due to fact the rate of seroconversion following infection in many studies has been determined where the gold standard of infection status was determined via HPV DNA positivity. Thus, the reported rates of seroconversion may also be an underestimate.

With regard to the limitations of the heparin bound HPV pseudovirion based Luminex method used in this study to assess type specific HPV seropositivity, previous validation studies reported a particularly low specificity and sensitivity for HPV18 (Artemchuk et al, 2018). Therefore, it was expected that the degree of bias in the estimates comparing HPV18 seroprevalence among the study population in the post- compared to pre-vaccination era would greater. Indeed, we found that in the estimates only taking into account of random error although among the women residing in the gender-neutral arm communities the estimate was slightly decreased, the confidence limits were overlapping the null. Whereas once we had taken account of systematic error due to outcome misclassification in addition to random error, the estimate relative seroprevalence (comparing the post- to pre vaccination era) was found to be decreased among the women residing in the gender-neutral Arm, with the upper 95% confidence intervals also below the null, indicative that the decrease was not explainable by random or systematic error due to outcome misclassification. Although, these findings themselves may conceivably still be subject to systematic error not due to outcome misclassification (such as confounding owing to changes in sexual behaviour over time). Our study may also be limited in its generalisability owing the inclusion of only pregnant women under the age of 23-years-old. This study population may likely represent a subpopulation with on average higher sexual risk-taking behaviours than that of the general Finnish population, as the average age at which a women had her first pregnancy in Finland was 29 years old at the time the study was conducted.

In spite of these limitations, the observed decrease in HPV cumulative infection, provides evidence that the HPV16 may have been partially cleared from its niche among the unvaccinated women. Such a decrease necessitates the monitoring of the non-vaccine HPV type occurrence for possible signs of HPV type replacement.

6.2.2 Evaluation of niche clearance by measuring community-wise prevalence of HPV16/18 post-vaccination

The degree of HPV niche clearance, i.e., reduction in HPV16/18, was examined among the women from the 1992-95 birth cohorts of the community randomised trial. The communities were directly compared for HPV16/18 prevalence among the intervention arms to that from the controls arm. There was a marked clearance of the HPV16/18 niche among the HPV vaccinated women from both intervention arms, as well as a notable reduction in the prevalence of the cross-protected HPV types HPV31, 33 and 45 (Gray et al, 2018; Lehtinen et al, 2018; Vänska et al, 2020). The relative prevalence of HPV16 and 18 among the vaccinated participants of the intervention arms compared to control arm was negligible but not nil. However, in addition to early infections with HPV16/18 (before age 16) it is entirely possible that the few women who were observed to be HPV16 or 18 possible may have been due to HPV16 or 18 depositions not resulting in infection (Malagón et al, 2017). This may be expected if the HPV16/18 were still circulating at the population level (Malagón et al, 2017).

The reduction of prevalence among vaccinated women alone (e.g., in a trial) may not be sufficient to replicate the real-life ecological pressure expected when a national vaccination program is implemented. In comparison to individually randomised trials such as those used to evaluate vaccine efficacy, the community-randomised trial by design is expected to simulate the true community-level ecological pressure within the intervention arm communities, as entire birth cohorts were eligible and participated in the school-based vaccination at rates similar to that of national vaccination programs in an affluent country. Among the non-HPV vaccinated participants of the community randomised trial no decrease in HPV16 PCR prevalence which could be attributed to community-wise vaccination was observed among either intervention arm among the general population. A decrease in the prevalence of HPV18 DNA was observable in both the non-HPV vaccinated women from Arm A and from arm B when compared to their counterparts from Arm C. These decreases in HPV18 were both observed in the youngest birth cohorts (the 1994-95) only, which is in line with what would be expected as the first (in Arm A) and second (in both arms A and B) order herd effects of vaccination should be greater in the younger trial birth cohorts.

Among the non-HPV vaccinated trial participants, the prevalence of HPV31 and 33 were found to have decreased (two types which are closely phylogenetically related to HPV16), a decrease that was notable only among the women from the

gender-neutral arm communities. This decrease in unvaccinated women when gender-neutral bivalent HPV vaccination is implemented is in line with the observations of vaccine efficacy against these types and the cross-neutralising antibodies found against these types induced by the bivalent vaccine (see section 5.1.) (Mariz et al, 2020). Mathematical models suggest that for such non-vaccine types even imperfect vaccine efficacy, when combined with gender-neutral vaccination or very high coverage girls only vaccination, may result in notable herd effect (Baussano et al, 2017; Lehtinen et al, 2022). This is possible as HPV types such as HPV31 have a lower basic reproduction number relative to HPV16, and the vaccination coverage threshold for their elimination is consequently lower than for HPV16. Thereby, this study may have limited generalisability to an hypothetical identical scenario where the quadrivalent vaccine was used, given both the quadrivalent vaccine's lower reported vaccine efficacies against HPV31 and 33 as compared to the bivalent vaccine (46% versus 77%, and 29% versus 43% against persistent HPV31 and 33 infection) and the lower seropositivity (of cross-neutralising HPV31 and 33 antibodies) among quadrivalent vaccinees in comparison to that observed after bivalent vaccination (see section 5.1)(Villa et al, 2006; Wheeler et al, 2012; Kann et al, 2020).

Contrary to the findings among the entire non-HPV vaccinated population, when stratifying by sexual risk-taking behaviour, as defined by *C. trachomatis* positivity, a significant decrease in HPV16 prevalence was observed in the low sexual risk-taking behaviour group (i.e., those who were *C. trachomatis* negative) from the intervention Arms A/B in comparison to C. No decrease in HPV16 was observed among the high sexual risk-taking group from the intervention arms A and B. This is in line with expectations, that it may be hardest to achieve a herd effect for HPV16 among the core-group, due to the higher contact rates among this group, thereby increasing the R0 among this assortative subpopulation. Conflictingly, the prevalence of HPV18 showed opposite effect when stratified by sexual risk-taking behaviour. Whilst no notable relative reduction was observed among non-HPV vaccinated comprising the low-sexual risk-taking behaviour group from the intervention arms, among the high sexual risk-taking behaviour group there was a significant reduction in HPV18 prevalence.

These results should still be interpreted with caution, as the analysis was conducted post-hoc and whilst the combination of the intervention Arms allowed for greater statistical power, the trial was not originally designed with such analysis in mind. Furthermore, as discussed above, the indirect effect of HPV vaccination was expected to cumulate in the youngest birth cohorts, i.e., be strongest in the 1995

born unvaccinated participants, whilst this stratified comparison did not stratify by birth cohort and included only the 1992-94 born. In addition to these caveats, whilst *C. trachomatis* is a known surrogate of sexual risk-taking behaviour (Dillner et al, 1996), the use of this surrogate as a marker for core-group membership may have resulted in degree of effect modifier misclassification.

These findings may have been limited by failure of the randomisation of the communities to correct for all unknown and known confounders. Although, the community randomised trial design is the best design for the evaluating the indirect effects of vaccination of gender-neutral as compared to girls only vaccination strategies, it is limited by the number of units randomised, only 33 communities and only 11 per arm (Lehtinen et al, 2015). Such a small number in comparison to the number of units randomised in typical individual randomised trials, increases the chance of failure of randomisation. We attempted to mitigate this by the initial stratification of the communities according to HPV16/18 seroprevalence, i.e. the baseline risk, prior to randomisation, as supported by the relatively low coefficient of variation reported earlier by Lehtinen et al (2015). However, the findings of a) baseline differences in the HPV serology in the follow-up of the trial in the Finnish maternity cohort reported above, b) the differences in certain non-vaccine HPV types among the non-HPV vaccinated trial participants from the older 1992-1993 birth cohorts between the intervention arms and the control arm, and c) slightly higher self-reported sexual risk taking behaviours among the participants from Arms A and B as compared to C, suggest that the randomisation may not have eliminated all known and unknown confounding. A priori planned adjusted and outlier-free analyses tackled most of these limitations in the estimation of vaccination specific herd effect (Lehtinen et al, 2018, Vänskä et al, 2020).

These finding of limited HPV18, 31 and 33 herd effect but no HPV16 herd effect may be specific to the study setting. It is likely that the first-order and second-order (among the girls only) among the unvaccinated participants of the trial may have been slightly different if it would have been conducted in a different population with a different sexual network structure (such as the Netherlands, Zimbabwe or Oman) (Baussano et al, 2016). Likewise, if more consecutive birth cohorts would have been included in a likewise manner in the trial, it is likely that the indirect effects of vaccination would have had more time to accumulate in the youngest birth cohorts, and a greater relative reduction in the vaccine protected HPV types would have been observed (Elfström et al, 2016). In addition, it is possible that both this study may have some unmeasured effect modification due to different baseline prevalence among the different communities, as this may be an effect modifier of the

production of herd effect by community-wise vaccination (Baussano et al, 2018). Finally, this study is also limited in its scope to evaluate niche clearance among the vaccinated and unvaccinated population as no measurements were made among the male participants of the trial.

Finally, it possible that by the use of point prevalence at a given age among the unvaccinated participants may have resulted in a unique measurement bias, as it may be expected that among the intervention communities the age-incidence curve may have shifted upwards, thus by comparing a specific point (prevalence) on the curve as defined by age (18 years old in this case) this will not be representative of the true infection risk between the comparison groups.

In this active follow-up of the CRT female participants the HPV16 and 18 niche appears to have been effectively cleared and the HPV31, 33 and 45 niches partially cleared among the vaccinated participants, whilst only the HPV18, 31 and 33 niches were partially cleared among the unvaccinated female participants. These findings justified the further study of the non-vaccine HPV types among the study population for possible type replacement.

6.3 Short term type replacement occurrence among the participants of the community randomised trial measured via point prevalence

We subsequently evaluated the occurrence of HPV type replacement, an increase in non-vaccine HPV type prevalence due to vaccination, among the 1992-95 born women participating in the community randomised trial. When comparing the PCR prevalence of non-vaccine types among the participants from the intervention arms to their counterparts in the control arm, the most constant increases were observed for HPV51 and to a lesser degree for HPV58. Some signs of increased HPV66 prevalence were observed among the oldest trial birth cohorts, in addition to sporadic increases in HPV39 and 45 among the unvaccinated women from the gender-neutral arm communities.

The increase in HPV51 was most evident among the vaccinated participants both from the gender-neutral arm A and girls-only arm B. This finding which was still apparent after taking account of variability in the prevalence of HPV51 in Arm C and after excluding the outlier communities. However, the increase was only observed among the oldest birth cohorts, those born in 1992-93. If this increase were due to type replacement one might expect the increase to be most evident in the

youngest birth cohorts, as the impact of both the direct and indirect effect of vaccination on niche clearance accumulates. As an HPV type which is from the Alpha 5 clade and not closely phylogenetically related to either of the vaccine targeted types, HPV51 is of interest. Except for scarce evidence of vaccine efficacy against persistent HPV51 infection in one trial (Wheeler et al, 2012), there has not been well-documented evidence of bivalent-vaccine induced cross-protection against HPV51. In line with our observations, a meta-analysis of observational studies conducted in 2016 found some indications of increasing prevalence of HPV39/51/52 post-vaccination compared to the pre-vaccination period (Mesher et al, 2016).

The prevalence of HPV58 was also found to be higher among both the vaccinated and unvaccinated participants in the gender-neutral arm than in the control Arm. This finding was even more prominent after excluding the outlier communities. Likewise to the increase in HPV51, this increase in the prevalence of HPV58 was most predominant in the older birth cohorts. Unlike HPV51, however, HPV58 is closely related to HPV16, both are from the Alpha 9 clade. Although cross-neutralising antibodies have occasionally been observed among bivalent and quadrivalent vaccinees (see section 6.1), no positive vaccine efficacy against HPV58 infection or -associated disease has been consistently reported (Wheeler et al, 2012; Mariz et al, 2020), which favouring HPV58 as a plausible candidate for type replacement.

Some sporadic increases were observed in the prevalence of HPV39 and 66 among the participants from the intervention Arms. The increase observed for HPV66 was most prominent among the vaccinated participants both in the gender neutral and girls only arms, and upon exclusion of the outlier communities, this increase appeared to be further increased. The low pre-vaccination prevalence of HPV39 (as observed among Arm C) would suggest that it is not that common of a HPV type, thereby when the random error is combined with unmeasured confounding it is possible that the sporadic increase observed for HPV39 may be explainable due to factors other than type replacement. The pre-vaccination prevalence of HPV66 however is not so low. Cross-protection is not expected against HPV66 infection being from the Alpha 5 clade, nor has it been observed priorly (Wheeler et al, 2012). Taken together this suggests HPV66 as a third candidate for type-replacement.

The prevalence of HPV45 was found to be increased among the unvaccinated participants from the gender-neutral communities in comparison to those from Arm C, despite concurrent reductions among their vaccinated counterparts likely due to

cross-protective vaccine efficacy against HPV45 (Wheeler et al, 2012). The increase in HPV45 prevalence was found among the younger birth cohorts, which is in line with expectations should type replacement be the cause. However, upon further sensitivity analysis, to take account of clustering, the 95% confidence intervals overlapped the null, suggesting that this observed increase may be explained by chance.

This study may have some limitations in its ability to measure type replacement due to its design and the specific nature of the study setting. Despite the cluster randomised design being the most pertinent epidemiological design to study type replacement (Lipsitch, 1999), by default of the randomisation unit being a community, there are less units randomised than is feasible for a randomised trial (Donner & Klar, 2004). This may have led to a failure of randomisation to balance the counterfactual prevalence of HPV. However, although some of the self-reported indicators of sexual risk taking appeared to be comparable among the participants from the different trial arms, there was a slightly higher incidence of *C. trachomatis* DNA among the intervention arms compared to the control arm, with the proportion of participants reporting 5 or more life-time sexual partners also being higher among the intervention arms. This suggests that perhaps the baseline risk of HPV may have been higher among the intervention arms as compared to the control arm.

It may have been possible that community-wise variability in the occurrence of HPV (for example in the case of HPV51) may have been accountable for the observed increases in non-vaccine HPV occurrence. It is possible that our trial where four vaccinated consecutive birth cohorts were followed up 4-5 years later, may have had insufficient amount of time since vaccination initiation, to measure type replacement occurrence and final type replacement potential (Man et al, 2021). It may be possible that the non-vaccine HPV occurrence may still be in the honeymoon phase or indeed that niche clearance by the vaccine types, if it is directly related to the occurrence of type replacement, has not had enough time to manifest (Man et al, 2021).

The community randomised trial was strengthened by the inclusion of communities which were geographically distinct, thereby minimising the risk of contamination. However, some degree of contamination may be expected to have occurred by the time the youngest birth cohorts included in the trial became 18 years old, owing to movement among the older birth cohorts. This is unlikely to have had a large impact on HPV occurrence owing to the assortativity of sexual partners by age in the Finnish population. Furthermore, as national girls-only catch-up HPV

vaccination did not commence until November 2013 among the 1998 birth cohort, this is not expected to have impacted the occurrence of HPV among the trial birth cohorts. HPV transmission moves in the direction from the older to the younger birth cohorts (Vänskä et al, 2020).

A further possible limitation of the study might have been the PCR methods used to evaluate HPV type replacement occurrence, due to the potential for unmasking of non-vaccine type HPV as result of diagnostic artefact (Tota et al, 2013 & 2015). However, this is unlikely to have been a possible explanation for any of the observed increases in non-vaccine type HPV occurrence in our study as the PCR technique (MGP-PCR followed by MALDI-TOF MS) does not have this impediment.

Finally, mathematical modelling has suggested that vaccine effectiveness may not be the most suitable measure as an indicator of HPV type replacement depending on the mode of HPV competition and for example whether the vaccine confers cross-protective efficacy against a non-vaccine type with type replacement potential (i.e., a non-vaccine type which was in competition with the vaccine targeted type pre-vaccination) (Man et al, 2021). If HPV type replacement does occur, it may be expected that at the community level the non-vaccine type will also increase among the unvaccinated owing to the indirect impact of vaccination, both in the absence or presence of the cross-protective efficacy among the vaccinated if the cross-protective efficacy is insufficient to mitigate type replacement (Lipsitch, 1999). Thereby, it has been shown that studies surveying the occurrence of non-vaccine HPV types over time (pre- to post-vaccination era) are plausible indicators of type replacement occurrence post-vaccination (Man et al, 2021). However, such studies are subject to bias owing to changes in the incidence of non-vaccine HPV over time that are not due to the impact of vaccination, as even slight changes in the sexual network of a population or sexual risk-taking behaviours may cause a change in the incidence of HPV. Indeed, studies have observed increases in non-vaccine HPV occurrence prior to the impact of vaccination (Pasmans et al, 2020).

Finally, in our evaluation of type replacement where multiple outcomes (i.e., multiple HPV types) have been studied it also increases the likelihood that we may have seen one or more HPV types changing in incidence among the arm comparisons owing to chance, which may feasibly have contributed to the sporadic increases in prevalence observed such as for HPV45.

With our use of a community randomised trial, we are able to mitigate both the potential limitations of characteristic of these two aforementioned study designs. Our Arm C estimates provide a counterfactual estimate of the non-vaccine HPV occurrence in the absence of vaccination taking into account changes in occurrence

due to other factors. Furthermore, the use of a control vaccine in Arm C allows for "vaccine effectiveness" estimates where the non-vaccinated are not subject to the indirect impact of vaccination. In addition, the enrolment of the non-vaccinated combined with HBV vaccinated in the intervention Arms compared to their appropriate counterfactual controls in Arm C allows for the accurate measurement of changes in the occurrence of non-vaccine impact due to the indirect impact of vaccination. This would be otherwise difficult to measure with other study designs.

However, the measure of vaccine effectiveness may also be imperfect depending on the model of competition between HPV types, if HPV types compete via clearance of the other HPV type rather than acquisition, then the use of prevalence may not fully capture the occurrence of type replacement/competitive release (Man, 2021). In this case, a study evaluating type replacement using baseline incidence and trends over time, with an outcome measure such as the incidence of clearance of infection (or conceivably seroprevalence, given that transient infections are less likely to result in seroconversion to that HPV type) may be the most apt to evaluate the occurrence of HPV type replacement and competitive release.

6.4 Short-term type replacement occurrence among the sexual risk-taking core group as measured via point prevalence

Among the sexual risk-taking core group, the conditions may be more permissible for HPV competition should it occur to impact HPV type occurrence and most notably HPV type replacement occurrence. Therefore, to evaluate whether core-group membership modifies the effect of type replacement among the participants of the community randomised trial we evaluated non-vaccine targeted HPV type occurrence stratified by *C. trachomatis* positivity. Whilst HPV51 was similarly increased among the core and non-core vaccinated population, among the non-HPV vaccinated, who were under the indirect effect of vaccination, the increase in HPV51 was more prominent among the core group. It is possible that this may have been due to chance, owing to the smaller sample size among the *C. trachomatis* positive. Interestingly, when stratifying by core group membership, also the prevalence of HPV52 was increased among the core group members from the intervention arms both among the vaccinated and unvaccinated women. The increase in HPV58 prevalence, found earlier among the participants from the intervention arms overall,

was also found when stratifying by core-group membership, but only among the non-HPV vaccinated women.

The effect of core-group membership on the impact of trial arm on HPV51 occurrence was found to be both supermultiplicative and superadditive among the non-HPV vaccinated women under the indirect impact of vaccination. Whilst for HPV52, core-group membership was found to be both supermultiplicative and superadditive among both the vaccinated under the impact of the direct plus indirect impact and non-vaccinated women. These findings suggest that *C. trachomatis* positivity may modify the direct impact of community wise vaccination on HPV52 occurrence, and the indirect impact of community wise vaccination on HPV51 and 52 occurrence.

It is conceivable that among the general population HPV type occurrence may be more limited due to lack of transmission opportunities and stochastic factors rather than due to HPV type interactions. In the group of individuals with the highest sexual risk-taking behaviours HPV types may be more likely to have the opportunity to interact. In the core-group a higher turn-over rate of sexual partners, higher rates of concurrency and assortativity according to sexual risk-taking behaviour, set the stage for HPV type competition to play more of a role in the occurrence of HPV types. Moreover, it may be postulated that among this group, when HPV vaccination opens the possibility for competitive release of the non-vaccine types due to the removal of the vaccine types this is greater among the core-group. Thus, HPV type replacement might have also been more likely to be observed among the core-group.

There are, however, several caveats to the above speculations. Firstly, infectious disease dynamics are complex thereby may defy intuition (Garnett, 2019). Secondly, the core-group is not a stable entity, and people move in and out of it in their lifetime (Humblet et al, 2003). If one assumes that HPV type competition occurs over a longer period for example owing to infection (or vaccination) induced humoral cross-immunity, then it is conceivable that the time in which the HPV types have potential to interact may be longer than the time in which they are a core-group member. Thirdly, the chance of acquiring a new HPV infection with type x is correlated with the time of acquiring type y, also in the general population owing to the common transmission route at the time of acquiring a new sexual partner (Malagón et al, 2016). Thereby for an HPV type, especially the more common types, it is likely that one does not need to be a core group member to acquire multiple HPV type infections, even if this is less common.

Furthermore, although the core-group is by character assortative according to sexual behaviour, it is not completely assortative. There are still network links

between the core and general population via "bridge populations" (Anderson et al, 1991; Aral, 2000). Thereby if HPV type competition impacts HPV occurrence within the core group (or if type replacement itself occurs within the core group) its impact would over time likely leak out into the general population (Anderson et al, 1991; Garnett et al, 1996).

In addition to the above it is also questionable as to whether the niche gets sufficiently cleared among the core group to release the competitive force from the vaccine types especially among the unvaccinated women. Although the prevalence of HPV18 DNA was reduced among the unvaccinated core group members in the intervention arms, the prevalence of HPV16 DNA was not. Thus, if the non-vaccine type occurrence were quenched by HPV16, then we may not have adequately cleared the niche to observe short term HPV type replacement or to give an indication of possible final HPV type replacement potential. However, the non-birth cohort wise increased occurrence of HPV51, 58 and 66 leaves open the impact of niche clearance.

The ability of this study to adequately assess the impact of the effect on non-vaccine type HPV may have been restricted by its limited sample size among the *C. trachomatis* positives. This was partly mitigated by combining the intervention Arms A and B in the arm-wise comparisons. Alas this also limited our ability to observe differences in the occurrence of HPV type replacement between arms A and B, due to greater niche clearance in the gender-neutral Arm. Furthermore, in the *C. trachomatis* stratified analyses, only participants from the three older birth cohorts (the 1992-94 born). This may have precluded our ability to observe HPV type replacement, as it is conceivable that both niche clearance and type replacement (should it occur) were expected to be most evident among the younger birth cohorts which have the greatest cumulative protective (direct plus indirect) effects of vaccination (Vänska et al, 2020). Thus, it is possible that age may be an effect modifier of type replacement occurrence, therefore as this study was limited to women at the age of 18-years-old, with limited data at 22-years-old, we may not have been looking in the most optimal age to observe HPV type replacement (Man, 2021).

Furthermore, whilst *C. trachomatis* serology is a defined marker of sexual behaviour it is likely that our use of current *C. trachomatis* positivity as a marker of current sexual risk-taking behaviour core-group (Dillner et al, 1996), may be subject to a degree of misclassification, both regarding sensitivity and specificity.

Similarly, to the unstratified analysis (as discussed in section 6.3) it is conceivable that changes in non-vaccine type prevalence may have been explainable by differences risk-taking behaviours in the communities from the different Arms, as

the number of self-reported sexual partners and *C. trachomatis* was slightly lower among the participants from Arm C (despite other indicators such as the mean age of sexual debut being almost identical). Whilst our stratification by *C. trachomatis* most probably relieves any of the indirect impacts of such differences, it is still plausible that the indirect effects of increased risk-taking behaviours in the intervention Arms could have contributed as a source of unmeasured confounding in our prevalence comparisons.

6.5 Type replacement by HPV vaccination strategy as measured via cumulative incidence

In comparison to the active follow-up of the community randomised trial, none of the HPV types 51, 58 and 66 which were previously observed with increased prevalence among the 18-year-old non-HPV vaccinated participants, were found to be increasing in seroprevalence in a manner suggestive of type replacement.

When evaluating the non-vaccine HPV cumulative incidence (via seropositivity) among the unvaccinated women under the age of 23 who were residing in the community randomised trial communities, the seroprevalence of HPV68 was found to have increased in the post-vaccination era. This increase was only observed among the girls-only intervention Arm B communities and not in the gender-neutral arm communities. However, as HPV68 is a particularly rare HPV type, this increase was small on the absolute scale but most prominent in the later-post vaccination era (the years 2014-16), as would be suggestive of type replacement. However, the confidence limits were overlapping the HPV68 seroprevalence change in time observed in the counterfactual control Arm C estimates. This suggests that the increase observed in the girls-only arm, may have been due to secular trends and not a result of HPV vaccination. When stratifying the with-in arm seroprevalence ratios by HSV-2 seropositivity, the HPV68 increase among the unvaccinated women from the girls-only Arm B was found to stem from the core-group. Also, among the HSV-2 seropositive core group, the confidence intervals of the with-in arm B estimate overlapped the increase found among the control arm. Therefore, again, the possibility that the HPV68 observation was due to secular trends again cannot be discounted.

Although there are several other plausible reasons for the seroprevalence of HPV68 to be increased among the unvaccinated women resident in the girls-only

Arm B communities between the years 2011-2016, it is still conceivable that it may indicate type replacement. There was a partially cleared HPV18 niche in the girls-only arm B communities. The fact that the HPV68 seroprevalence was most increased in the second time period since vaccination, and in the HSV-2 seropositive population are in line with the proposed honeymoon period between vaccination initiation and type replacement occurrence (Man et al, 2021). It also fits the theory that type replacement may firstly be observable among the core-group. Furthermore, HPV68 is phylogenetically related to HPV18 from the alpha 7 clade, and whilst limited cross-protective vaccine efficacy has been observed against HPV45 (also from the alpha 7 clade), there has not been convincing evidence of cross-neutralising antibodies/vaccine efficacy against any other non-vaccine HPV types from Alpha 7 (Wheeler et al, 2012). Thereby, HPV68 is a plausible candidate type for type replacement.

Modelling studies have suggested that if an HPV type with type replacement potential is under the impact of cross-protective efficacy, then at certain vaccine coverage the resulting competitive release may reach a saturation level at which point any additional coverage will act to mitigate type replacement as it strengthens the cross-protective effect. It is conceivable that in such a scenario HPV type replacement would indeed be stronger in the girls-only trial arm. However, in the case of HPV68, this is unlikely to have been the case, due the lack of any previously observed vaccine-induced HPV68 cross-protection. Although the bivalent vaccine has been shown to produce cross-protective antibodies and vaccine efficacy against HPV types which are phylogenetically related to the HPV16 and 18, the has been less cross-protective efficacy reported against types from the Alpha 7 clade (HPV18 related) than from the Alpha 9 clade (HPV16 related). Furthermore, whilst HPV68 is from the same clade as HPV18 it is not closely located to HPV18 within the clade, thereby it is less likely that HPV vaccination would induce protective cross-reactive neutralising antibodies against this type.

If HPV68 type replacement were to occur, one might expect to see the greatest replacement in the women from the gender-neutral trial arms, where overall niche clearance was the greatest (Vänskä et al, 2020). In conclusion, the fact that the increase stems from the girls only Arm B and not the gender-neutral Arm A is not supportive of HPV68 increase being due to true type replacement, if there is a direct relationship between the extent of niche clearance and type replacement.

Whether the HPV16 and 18 ecological niche was cleared sufficiently and for a sufficient amount of time for the non-vaccine types to take advantage and fill the vacated position remains open. The findings from the unvaccinated women resident

in the 33 communities, show that both HPV16 and 18 have decreased somewhat among the gender-neutral arm communities. However, HPV16 and 18 are still present, which would suggest the competitive release (should it occur) has not of yet reached its full capacity. This would suggest that our study setting, whilst being realistic in terms of national vaccination programs, may not have been able to record the full HPV type replacement potential as would be seen at a HPV vaccination at a higher coverage.

Unfortunately, the randomisation of the 33 risk stratified communities did not manage to perfectly equalise all the baseline differences in HPV seroprevalence between the trial arms (Lehtinen et al, 2018 & 2019). The baseline seroprevalence (among the unvaccinated women from 2005-2010) of some HPV types was higher among the women from the intervention Arm A and B communities in comparison to those from control Arm C. This precluded the possibility of directly comparing the seroprevalence among the women from one Arm to the other. However, in our difference in differences approach (ratio of ratio on the relative scale), comparing the post- to pre- vaccination era with-in the arm and then comparing the with-in arm ratios to between the arms, it was possible to distinguish increases due to secular trends to those likely due to vaccination using the counterfactual control arm C. However, there are still some limitations also to this approach (as mentioned in section 6.2.1), namely the assumption of parallel trends, and the possible impact of later cross-vaccination of the 18-year-old HBV vaccinated women from Arm C. However, the impact of such cross-vaccination at the community level is likely to be limited as notable HPV transmission may already have occurred prior to cross-vaccination at the age of 18.

On the other hand, our approach has several strengths. As described by Man (2021), the impact of cross-protection against non-vaccine HPV types may delay and or mitigate the occurrence of HPV type replacement among those who are HPV vaccinated, whilst among the unvaccinated the non-vaccine HPV types will only be affected by the indirect impact of such cross-protective efficacy. Thus, the use of vaccine efficacy as a measure of type replacement, if the non-vaccine type in question is subject to the impact of cross-protection VE estimates is a poor indicator of type replacement occurrence or potential at the community level (Man et al, 2021; Man, 2021). Whereas in our study comparing the cumulative incidence of non-vaccine HPV among the unvaccinated with appropriate counterfactual controls, was better placed to evaluate type replacement occurrence for such non-vaccine HPV types.

It is also possible that the changes observed in the post-vaccination era in the with-in arm seroprevalence comparisons could have reflected changing sexual risk-

taking behaviours among the study population. We found slightly decreased HSV-2 seroprevalence among the women from post-vaccination era in comparison to the HSV-2 seroprevalence in the pre-vaccination era. It is, however, likely that our use of HSV-2 serology as a marker of "ever core group membership" will have suffered from misclassification. Although, HSV-2 serology may be an improved marker of the core group in comparison to other markers (such as *C. trachomatis* positivity [see section 6.4]), as previous study of the clustering of *C. trachomatis*, gonorrhoea, genital herpes and genital warts found that gonorrhoea and genital herpes cases were the most prone to cluster in a manner characteristic of the core group (Monteiro et al, 2005; Kibur et al, 2000).

Likewise to the second study (see section 6.2.1), it is possible that the biobank based passive follow-up study may have suffered from systematic error due to outcome misclassification. In this study we used type-specific HPV seropositivity to measure cumulative lifetime exposure to a given HPV type. However, HPV serology is an imperfect measure of this due to the fact that i) not all women seroconvert after a HPV infection, ii) some women who have recently acquired the HPV infection prior to giving their blood sample, will not yet have seroconverted (i.e. the outcome is censored) even though they might seroconvert at some later point. On the other hand, it is unlikely that a woman who has had an HPV infection and seroconverted, may again serorevert by the time of blood sampling (af Geijersstam et al, 1998). In this study given the fact the women are under the age of 23 years old the likelihood of seroreversion having biased the results is slim, whilst the possibility that the woman has not yet had time to seroconvert to a recently acquired HPV type may be higher owing to the sampled age group being within the peak of the age/infection-incidence curve.

Overall, our studies of type replacement are limited owing to the fact the outcome measured was only of current and cumulative HPV infection and not of HPV-associated disease. Furthermore, as studies of short-term type replacement within a decade after vaccination initiation, they may be capable of evaluating HPV type replacement potential at the level of HPV occurrence, but certainly incapable of estimating HPV disease replacement. Of the types which were shown to have some indications of increasing in occurrence, HPV51 and HPV68, HPV51 is listed as a high-risk carcinogen, and HPV68 a probable carcinogen (IARC, 2012). However, neither type are commonly found in cervical cancers. If HPV51 and/or HPV68 would in the long-term increase in incidence due to type replacement, this would be unlikely to result in total disease replacement at the level of invasive cervical cancer or other HPV-associated cancers.

7 CONCLUSIONS

In conclusion, there are clear differences in the immunogenicity of the bivalent and quadrivalent HPV vaccines in the induction of neutralising antibodies against the vaccine targeted HPV16/18 and cross-neutralising antibodies against non-vaccine targeted HPV types. Both the neutralising and cross-neutralising antibody responses were higher among women who had received the bivalent vaccine. These disparities in the antibody responses, both width (magnitude) of the protection gained from vaccination against persistent infection with hrHPVs, are of importance in the context of HPV type replacement. It is likely that potential type replacement may be mitigated by the width of the protection. Thus, the likelihood and time to replacement may depend on the vaccine in use.

Parallel to the above, the degree of niche clearance which sets the stage for type replacement will depend on the vaccination strategy and coverage. When the vaccination coverage is restricted to moderate coverage, only gender-neutral vaccination will be able to clear both the HPV16 and HPV18 niches among the unvaccinated women in the population. The HPV18 (and HPV31/33/35) niches may be more easily cleared among unvaccinated, with a degree of niche clearance being observable after both gender-neutral and girls only vaccination. Among the HPV vaccinated the HPV16/18 /31/33/35/45 niches may be almost completely cleared due to the high efficacy of the bivalent HPV vaccine.

Following our community randomised trial with moderate vaccination coverage, some signs of increased HPV51, HPV58 and HPV68 occurrence were observed. The impact of HPV vaccination on the occurrence these non-vaccine types was further found to be modified by sexual risk-taking core group membership. None of these increases were replicable with the different study designs or consistent in a manner indicative of being due to vaccine-induced HPV type replacement. However, it may be that our studies whilst being the optimally designed for detecting type replacement, may suffer from the limited time period between vaccine initiation and outcome measurement. Therefore, and due to our suggestive observations, it is important that in populations where HPV vaccination is implemented there is surveillance beyond 10 years, especially among the unvaccinated for the occurrence of non-vaccine HPV type replacement.

8 APPENDIX

Table 1: HPV type specific Prevalence Ratio among the HPV vaccinated and non-HPV vaccinated female CRT participants by intervention Arm at the age of 18-years-old, comparing the prevalence among the intervention Arms A or B stratified by birth cohort to the prevalence in the entire (unstratified by birth cohort) Arm C. Estimates are computed using a GEE model and are adjusted for smoking and mobility.

		Prevalence ratio (95% CI)			
		HPV vaccinated comparisons		**Non-HPV vaccinated comparisons**	
HPV type	**Intervention arm birth cohort**	**A vs C**	**B vs C**	**A vs C**	**B vs C**
6	**1992-93**	0.84 (0.60-1.18)	1.14 (0.87-1.50)	0.99 (0.66-1.49)	1.07 (0.75-1.53)
	1994-95	0.85 (0.61-1.19)	0.80 (0.57-1.10)	0.94 (0.63-1.41)	0.80 (0.53-1.20)
11	**1992-93**	0.40 (0.16-1.02)	1.00 (0.58-1.72)	0.43 (0.12-1.64)	1.29 (0.58-2.87)
	1994-95	0.76 (0.42-1.38)	0.60 (0.21-1.74)	1.27 (0.54-2.98)	0.44 (0.13-1.48)
16	**1992-93**	**0.10 (0.06-0.19)**	**0.07 (0.03-0.18)**	0.76 (0.52-1.11)	0.89 (0.65-1.21)
	1994-95	**0.05 (0.02-0.14)**	**0.09 (0.06-0.12)**	0.93 (0.67-1.29)	0.95 (0.70-1.29)
18	**1992-93**	**0.06 (0.02-0.21)**	**0.08 (0.04-0.17)**	1.38 (0.96-2.00)	1.04 (0.71-1.52)
	1994-95	**0.04 (0.01-0.15)**	**0.05 (0.02-0.14)**	**0.69 (0.43-1.12)**	**0.53 (0.32-0.89)**
31	**1992-93**	**0.24 (0.11-0.55)**	**0.26 (0.13-0.51)**	1.16 (0.73-1.83)	0.76 (0.47-1.26)
	1994-95	**0.30 (0.20-0.45)**	**0.13 (0.06-0.25)**	0.56 (0.30-1.03)	1.16 (0.76-1.75)
33	**1992-93**	1.01 (0.69-1.49)	0.65 (0.47-0.90)	1.32 (0.74-2.36)	1.35 (0.80-2.28)
	1994-95	**0.47 (0.27-0.82)**	**0.44 (0.30-0.63)**	0.71 (0.34-1.47)	1.40 (0.84-2.35)
35	**1992-93**	0.78 (0.33-1.83)	0.89 (0.58-1.37)	0.84 (0.38-1.86)	0.78 (0.37-1.64)
	1994-95	0.56 (0.21-1.48)	0.69 (0.41-1.19)	0.97 (0.47-1.99)	0.45 (0.18-1.15)
39	**1992-93**	1.14 (0.76-1.72)	1.08 (0.75-1.57)	**1.73 (1.11-2.69)**	0.98 (0.58-1.63)
	1994-95	1.04 (0.67-1.61)	0.79 (0.58-1.07)	1.31 (0.81-2.12)	0.59 (0.31-1.12)

45	**1992-93** **1994-95**	**0.23 (0.10-0.55)** **0.16 (0.06-0.39)**	**0.28 (0.19-0.40)** **0.13 (0.04-0.43)**	1.05 (0.58-1.89) 1.52 (0.94-2.48)	0.90 (0.51-1.60) 0.72 (0.38-1.35)
51	**1992-93** **1994-95**	**1.33 (1.05-1.68)** 0.99 (0.81-1.20)	**1.36 (1.07-1.72)** 1.05 (0.81-1.36)	**1.22 (0.87-1.71)** 1.04 (0.73-1.48)	**1.11 (0.81-1.53)** 1.28 (0.95-1.72)
52	**1992-93** **1994-95**	0.95 (0.71-1.27) 0.72 (0.54-0.96)	1.08 (0.91-1.29) 0.77 (0.62-0.97)	1.07 (0.69-1.65) 1.16 (0.77-1.73)	1.56 (1.12-2.19) 0.84 (0.55-1.31)
56	**1992-93** **1994-95**	0.80 (0.56-1.15) 1.20 (0.88-1.63)	1.05 (0.77-1.43) 0.94 (0.70-1.26)	0.75 (0.47-1.21) 0.84 (0.55-1.30)	0.79 (0.52-1.20) 0.93 (0.63-1.38)
58	**1992-93** **1994-95**	1.30 (0.84-2.02) 1.29 (0.89-1.87)	0.77 (0.62-0.97) 0.88 (0.61-1.29)	**1.65 (1.04-2.64)** 0.81 (0.44-1.50)	1.25 (0.77-2.02) 1.21 (0.75-1.97)
59	**1992-93** **1994-95**	1.32 (0.99-1.76) 1.15 (0.95-1.40)	0.23 (0.05-1.00) 0.84 (0.71-0.99)	1.33 (0.80-2.20) 1.40 (0.87-2.26)	0.91 (0.53-1.56) 0.88 (0.51-1.52)
66	**1992-93** **1994-95**	1.28 (0.99-1.66) 1.12 (0.83-1.52)	**1.38 (1.15-1.65)** 0.98 (0.76-1.27)	1.40 (0.98-1.99) 0.85 (0.55-1.30)	0.69 (0.44-1.07) 0.69 (0.44-1.07)

Table 2: Birth cohort-wise HPV vaccination coverage stratified community and gender among the community randomised trial participants, prior to cross-vaccination at the age of 18 years-old. The outlier communities, those communities with vaccination coverages among the females which are twenty percent or greater different to the mean vaccination coverage among the other communities in the arm, are highlighted in bold.

		Vaccination coverage (%)							
		Females				Males			
		Birth Cohort							
Trial Arm	Community	***1992***	***1993***	***1994***	***1995***	***1992***	***1993***	***1994***	***1995***
A	**Hyvinkää**	**34.8**	**38.4**	**50.2**	**43.4**	13.6	15.4	12.5	18.2
	Järvenpää	**30.8**	**37.7**	**37.9**	**35.4**	8.93	10.5	13.8	16.7
	Joensuu	41.6	42.6	56.1	54.6	16.6	11.9	18.0	25.0
	Jyväskylä	43.5	41.9	50.8	47.7	17.3	20.8	20.5	23.8
	Kemi	**60.3**	**53.2**	**63.6**	**63.5**	21.8	23.5	38.6	35.2
	Kokkola	**30.3**	**31.2**	**37.8**	**32.2**	14.3	10.4	12.1	14.7
	Kotka	50.6	61.6	57.4	56.5	26.9	18.5	30.9	32.5
	Kouvola	29.6	42.6	55.8	53.7	15.0	3.11	15.3	18.0
	Lahti	49.9	51.3	55.7	50.5	19.4	19.7	24.9	19.3
	Porvoo	49.8	44.2	55.8	48.0	23.2	21.2	26.1	20.0
	Seinäjoki	51.8	53.5	47.5	47.1	23.2	23.6	14.1	27.2
B	Kuusamo	49.6	40.9	41.4	46.2	0.00	0.00	0.00	0.00
	Lappeenranta	48.0	42.8	48.1	49.6	0.00	0.00	0.00	0.00
	Mikkeli	33.6	33.1	47.1	43.2	0.00	0.00	0.00	0.00
	Rovaniemi	48.1	47.0	56.5	48.9	0.00	0.00	0.00	0.00
	Salo	46.9	54.6	49.6	43.4	0.00	0.00	0.00	0.00
	Savonlinna	44.0	48.5	46.3	49.2	0.00	0.00	0.00	0.00
	Tampere	47.0	49.3	52.7	51.9	0.00	0.00	0.00	0.00
	Turku	**40.3**	**34.1**	**31.8**	**32.1**	0.00	0.00	0.00	0.00
	Vaasa	48.7	49.2	37.1	39.6	0.00	0.00	0.00	0.00
	Vammala	52.3	48.5	60.0	51.3	0.00	0.00	0.00	0.00
	Äänekoski	43.7	35.3	49.6	52.0	0.00	0.00	0.00	0.00

C	Hämeenlinna	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Iisalmi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Jämsä	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kajaani	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kirkkonummi	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Kuopio	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Lohja	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Oulu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Pori	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Rauma	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Varkaus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3: HPV type specific Prevalence Ratio among the HPV vaccinated and non-HPV vaccinated female CRT participants by intervention Arm at the age of 18-years-old excluding participants residing in the outlier communities, comparing the prevalence among the intervention Arms A or B stratified by birth cohort to the prevalence in the entire (unstratified by birth cohort) Arm C. Estimates are computed using a GEE model and are adjusted for smoking and mobility. The outlier communities were defined as communities where there was a difference in vaccination coverage of 20% or more to the mean vaccination coverage among the other communities of the trial arm.

		Prevalence ratio (95% CI)			
		HPV vaccinated comparisons		**Non-HPV vaccinated comparisons**	
HPV type	**Intervention arm birth cohort**	**A vs C**	**B vs C**	**A vs C**	**B vs C**
6	**1992-93**	0.94 (0.67-1.31)	1.14 (0.87-1.50)	1.01 (0.64-1.59)	1.04 (0.69-1.57)
	1994-95	0.77 (0.55-1.09)	0.80 (0.57-1.10)	0.94 (0.60-1.46)	0.75 (0.48-1.19)
11	**1992-93**	0.41 (0.13-1.34)	1.00 (0.58-1.72)	0.44 (0.10-1.86)	1.47 (0.62-3.48)
	1994-95	0.72 (0.37-1.37)	0.60 (0.21-1.74)	1.27 (0.50-3.20)	0.52 (0.15-1.76)
16	**1992-93**	**0.11 (0.05-0.22)**	**0.07 (0.03-0.18)**	0.70 (0.45-1.08)	1.08 (0.78-1.50)
	1994-95	**0.05 (0.02-0.16)**	**0.09 (0.06-0.12)**	0.93 (0.65-1.33)	1.25 (0.93-1.67)
18	**1992-93**	**0.06 (0.01-0.25)**	**0.08 (0.04-0.17)**	1.38 (0.92-2.08)	1.17 (0.78-1.76)
	1994-95	**0.06 (0.02-0.19)**	**0.05 (0.02-0.14)**	0.69 (0.41-1.18)	0.59 (0.35-1.01)
31	**1992-93**	**0.32 (0.15-0.68)**	**0.26 (0.13-0.51)**	1.61 (1.03-2.49)	0.91 (0.53-1.53)
	1994-95	**0.34 (0.23-0.48)**	**0.13 (0.06-0.25)**	0.56 (0.29-1.09)	1.04 (0.65-1.66)
33	**1992-93**	1.12 (0.73-1.72)	0.65 (0.47-0.90)	1.75 (0.99-3.10)	1.47 (0.83-2.61)
	1994-95	0.45 (0.24-0.84)	0.44 (0.30-0.63)	0.71 (0.32-1.58)	1.79 (1.08-2.96)
35	**1992-93**	0.68 (0.25-1.89)	0.89 (0.58-1.37)	0.60 (0.22-1.66)	0.75 (0.32-1.78)
	1994-95	0.36 (0.10-1.32)	0.69 (0.41-1.19)	0.97 (0.44-2.13)	0.50 (0.19-1.31)
39	**1992-93**	1.07 (0.68-1.67)	1.08 (0.75-1.57)	**1.65 (1.00-2.72)**	0.57 (0.27-1.20)
	1994-95	0.98 (0.58-1.65)	0.79 (0.58-1.07)	1.31 (0.78-2.22)	1.16 (0.69-1.94)
45	**1992-93**	**0.30 (0.13-0.68)**	**0.28 (0.19-0.40)**	1.12 (0.59-2.12)	1.01 (0.54-1.88)
	1994-95	**0.20 (0.09-0.45)**	**0.13 (0.04-0.43)**	1.52 (0.90-2.59)	0.77 (0.40-1.50)
51	**1992-93**	**1.40 (1.07-1.82)**	**1.36 (1.07-1.72)**	**1.22 (0.84-1.78)**	**1.22 (0.86-1.73)**
	1994-95	0.96 (0.76-1.20)	1.05 (0.81-1.36)	1.04 (0.71-1.53)	1.21 (0.87-1.68)

52	**1992-93** **1994-95**	0.98 (0.69-1.39) 0.81 (0.60-1.09)	1.08 (0.91-1.29) 0.77 (0.62-0.97)	1.44 (0.95-2.21) 1.16 (0.74-1.80)	1.65 (1.14-2.39) 0.89 (0.56-1.42)
56	**1992-93** **1994-95**	0.87 (0.58-1.30) 1.32 (0.98-1.77)	1.05 (0.77-1.43) 0.94 (0.70-1.26)	0.78 (0.46-1.31) 0.84 (0.52-1.35)	0.88 (0.56-1.39) 1.15 (0.79-1.69)
58	**1992-93** **1994-95**	**1.56 (1.03-2.36)** 1.33 (0.87-2.02)	0.77 (0.62-0.97) 0.88 (0.61-1.29)	**1.84 (1.12-3.03)** 0.81 (0.42-1.59)	**1.62(0.99-2.63)** 1.06 (0.61-1.85)
59	**1992-93** **1994-95**	1.50 (1.15-1.95) 1.12 (0.89-1.40)	0.23 (0.05-1.00) 0.84 (0.71-0.99)	1.44 (0.84-2.47) 1.40 (0.84-2.36)	1.42 (0.85-2.36) 0.98 (0.56-1.72)
66	**1992-93** **1994-95**	**1.33 (1.00-1.76)** 1.20 (0.88-1.65)	**1.38 (1.15-1.65)** 0.98 (0.76-1.27)	1.20 (0.79-1.83) 0.85 (0.53-1.36)	0.74 (0.45-1.21) 0.56 (0.33-0.95)

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10 PUBLICATIONS

PUBLICATION I

Sustainability of neutralising antibodies induced by bivalent or quadrivalent HPV vaccines and correlation with efficacy: a combined follow-up analysis of data from two randomised, double-blind, multicentre, phase 3 trials

Filipe Colaço Mariz, Penelope Gray, Noemi Bender, Tiina Eriksson, Hanna Kann, Dan Apter, Jorma Paavonen, Emma Pajunen, Kristina M Prager, Peter Sehr, Heljä-Marja Surcel, Tim Waterboer, Martin Müller, Michael Pawlita, Matti Lehtinen

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Tumorvirus-Specific Vaccination Strategies (F C Mariz PhD, M Müller PhD) **and Infections and Cancer Epidemiology** (N Bender PhD, K M Prager PhD, T Waterboer PhD, M Pawlita MD, M Lehtinen MD)**, Deutsches Krebsforschungszentrum, Heidelberg, Germany; Faculty of Social Sciences, Tampere University, Tampere, Finland** (P Gray MSc)**; FICAN-Mid, Pirkanmaan Sairaanhoitopiiri, Research, Development and Innovation Centre Nuorisotutkimusasema, Tampere, Finland** (T Eriksson MSc, M Lehtinen)**; Department of Laboratory Medicine, Karolinska Institute, Stockholm, Sweden** (H Kann MSc, M Lehtinen)**; VL-Medi, Helsinki, Finland** (D Apter MD)**; Department of Obstetrics and Gynecology, University of Helsinki, Helsinki, Finland** (J Paavonen PhD)**; ESIS-EEIG, Luhtaankatu, Tampere, Finland** (E Pajunen MBA)**; EMBL-DKFZ Chemical Biology Core Facility, European Molecular Biology Laboratory, Heidelberg, Germany** (P Sehr PhD)**; Biobank Borealis of Northern Finland, Oulu University Hospital, Oulu, Finland** (H-M Surcel PhD)**; Faculty of Medicine, University of Oulu, Oulu, Finland** (H-M Surcel)

Summary

Background Quadrivalent and bivalent vaccines against oncogenic human papillomavirus (HPV) are used worldwide with different reported overall efficacies against HPV infections. Although protective concentrations of vaccine-induced antibodies are still not formally defined, we evaluated the sustainability of neutralising antibodies in vaccine trial participants 2–12 years after vaccination and the correlation with reported vaccine efficacy.

Methods We did a follow-up analysis of data from the Finnish cohorts of two international, randomised, double-blind, phase 3 trials of HPV vaccines, PATRICIA (bivalent, HPV16 and 18) and FUTURE II (quadrivalent, HPV6, 11, 16, and 18). In 2002 and 2004–05, respectively, Finnish girls aged 16–17 years participated in one of these two trials and consented to health registry follow-up with the Finnish Cancer Registry. The cohorts were also linked with the Finnish Maternity Cohort (FMC) that collects first-trimester serum samples from nearly all pregnant Finnish women, resulting in 2046 post-vaccination serum samples obtained during up to 12 years of follow-up. We obtained serum samples from the FMC-based follow-up of the FUTURE II trial (from the quadrivalent vaccine recipients) and the PATRICIA trial (from corresponding bivalent vaccine recipients who were aligned by follow-up time, and matched by the number of pregnancies). We assessed neutralising antibody concentrations (type-specific seroprevalence) to HPV6, 16, and 18, and cross-neutralising antibody responses to non-vaccine HPV types 31, 33, 45, 52, and 58 from 2 to 12 years after vaccination.

Findings Up to Dec 31, 2016, we obtained and analysed 577 serum samples from the quadrivalent vaccine recipients and 568 from the bivalent vaccine recipients. In 681 first-pregnancy serum samples, neutralising antibodies to HPV6, 16, and 18 were generally found up to 12 years after vaccination. However, 51 (15%) of 339 quadrivalent vaccine recipients had no detectable HPV18 neutralising antibodies 2–12 years after vaccination, whereas all 342 corresponding bivalent vaccine recipients had HPV18 neutralising antibodies.. In seropositive quadrivalent vaccine recipients, HPV16 geometric mean titres (GMT) halved by years 5–7 (GMT 3679, 95% CI 2377 to 4708) compared with years 2–4 (6642, 2371 to 13 717). Between 5 and 12 years after vaccination, GMT of neutralising antibodies to HPV16 and 18 were 5·7 times and 12·4 times higher, respectively, in seropositive bivalent vaccine recipients than in the quadrivalent vaccine recipients. Cross-neutralising antibodies to HPV31, 33, 45, 52, and 58 were more prevalent in the bivalent vaccine recipients but, when measurable, sustainable up to 12 years after vaccination with similar GMTs in both vaccine cohorts. Seroprevalence for HPV16, 31, 33, 52, and 58 significantly correlated with vaccine efficacy against persistent HPV infections in the bivalent vaccine recipients only (r_s=0·90, 95% CI 0·09 to 0·99, p=0·037, compared with r_s=0·62, 95% CI –0·58 to 0·97, p=0·27 for the quadrivalent vaccine recipients). Correlation of protection with prevalence of neutralising or cross-neutralising HPV antibodies was not significant in the quadrivalent vaccine recipients.

Interpretation The observed significant differences in the immunogenicity of the two vaccines are in line with the differences in their cross-protective efficacy. Protective HPV vaccine-induced antibody titres can be detected up to 12 years after vaccination.

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Introduction

The discovery of human papillomavirus (HPV) genotype 16 as a causal factor involved in cervical cancer occurred about 35 years ago,[1] and led to the current understanding of HPV as an established carcinogen in the cervix, penis, vulva, vagina, anus, and oropharynx.[2] About 40 different HPV types are known to infect the anogenital tract and are classified as low-risk and high-risk types according to their

Research in context

Evidence before this study

Both quadrivalent and bivalent human papillomavirus (HPV) vaccines are used worldwide but their efficacy against HPV-associated cancers and cervical intra-epithelial neoplasia grade 3 might differ and is difficult to determine. More than 3000 Finnish women aged 16–17 years were vaccinated in 2002 and 2004 in the PATRICIA and FUTURE clinical trials of the bivalent and quadrivalent HPV vaccines, respectively. The trial participants agreed to long-term follow-up in a population biobanking system (the Finnish Maternity Cohort) where serum samples were collected for up to 12 years. There were no published data on independent, investigator-initiated head-to-head comparisons of the bivalent versus quadrivalent neutralising antibody responses beyond 7 years after vaccination.

Added value of this study

Our data expanded the current understanding on vaccine-induced HPV antibody response over time. Through a comprehensive analysis of the sustainability of neutralising and cross-neutralising antibody responses to low-risk and high-risk HPV types, we showed that there were significant differences between the antibody concentrations induced by the bivalent and quadrivalent vaccines 2–12 years after vaccination. Notably, however, the concentrations of cross-neutralising antibodies to HPV types 31, 33, 45, 52, and 58 were, when measurable, as sustainable as the type-specific concentrations of neutralising antibodies, for both vaccine cohorts. Importantly, seroprevalence to the clade A9-HPV types 16, 31, 33, 52, and 58 strongly correlated with the reported vaccine efficacy in bivalent-vaccine recipients only.

Implications of all the available evidence

The observed significant differences in the immunogenicity of the two vaccines are in line with the differences in their cross-protective efficacy. Our findings indicate that defining protective antibody concentrations following HPV vaccination is the next natural step, which are detectable up to 12 years after vaccination.

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oncogenicity. Worldwide, persistent infection with HPV has been linked to 9% of all cancers in women and 1% of all cancers in men, although there is substantial variation by geographical region and level of economic development.[3] The comprehension of many aspects of HPV disease burden came with the discovery that the major virus capsid protein L1 is able to self-assemble into empty virus-like particles that are highly immunogenic and induce protective antibodies.[4–6] These findings formed the basis of the two pioneering prophylactic HPV vaccines: bivalent (HPV16 and 18) Cervarix (GlaxoSmithKline; Rixensart, Belgium) and quadrivalent (HPV6, 11, 16, and 18) Gardasil (Merck; Durham, NC, USA).

Although these two vaccines were proven to be safe and highly immunogenic and are used worldwide, determining their efficacy against HPV-associated cancers is difficult because of the long time between exposure to the virus and diagnosis of the associated cancer.[7,8] Neutralising antibodies induced upon vaccination are considered to be the primary mechanism of protection from HPV infection. Thus, vaccine-induced neutralising antibodies might be used as a surrogate of vaccine efficacy (ie, correlate markers of protection) against infections with high-risk HPV types and associated precancerous neoplasias.[7,9–11] Some trials[9,12] of the bivalent and quadrivalent vaccines have shown differences not only in their ability to induce neutralising antibodies and cross-neutralising antibodies, but also in the cross-protective efficacy of the two vaccines against cervical intraepithelial neoplasia (CIN) grade 3 (CIN3) and HPV infections (a surrogate measure of CIN3).[13,14] Studies of the sustainability of vaccine-induced total antibodies and neutralising antibodies are ongoing.[11,15,16]

In two international, randomised double-blind trials of the vaccines, PATRICIA (bivalent vaccine)[17] and FUTURE II (quadrivalent vaccine),[18] Finland enrolled 10% and 25% of the participants, respectively. In 2002 and 2004–05, respectively, Finnish girls aged 16–17 years were invited and consented to participate in one of these two trials.[11,18] They also consented to health registry follow-up based on their unique personal identifier.[19] Linkage of these two Finnish trial cohorts with the Finnish Maternity Cohort (FMC), which has been collecting first-trimester serum samples from 96% of all pregnant Finnish women since 1983,[20] provided altogether about 1200 post-vaccination serum samples for the sustainability studies. Although the effect of pregnancy on HPV antibody concentrations is not fully known, current evidence suggests that L1-specific antibody concentrations are not substantially affected during the first trimester of pregnancy.[21] Similarly, HPV antibodies in organ-transplantation recipients are restored to pre-transplantation amounts, even under continuous strong iatrogenic immune suppression.[22] We previously published a head-to-head comparison[15] of the sustainability of HPV16 and HPV18 virus-like particle-binding antibody concentrations induced by the quadrivalent or the bivalent vaccines 7–12 years after vaccination, as well as a proof-of-concept study[23] on the successful use of a high-throughput pseudovirion-based neutralisation assay for detection of cross-neutralising antibodies to HPV6, 31, 33, 45, 52, and 58 induced by the quadrivalent vaccine and the bivalent vaccine 7 months after vaccination. Here, we report our evaluation of the sustainability of neutralising antibody and cross-neutralising antibody concentrations in vaccine trial participants who had received three doses of the bivalent or the quadrivalent vaccine and donated blood samples to the FMC up to 12 years after vaccination. We also assessed the correlation of the neutralising antibody

or cross-neutralising antibody responses with vaccine efficacy against transient and persistent HPV infections.

Methods

Cohorts

In 2002 and 2004–05, two cohorts of 1749 and 4808 girls, respectively, in Finland aged 16–17 years were enrolled by population-based invitations from the Finnish Population Census Register[24,25] to two phase 3 clinical HPV vaccine trials (FUTURE II and PATRICIA).[17,18] The international trials documented the efficacy of the quadrivalent (HPV6, 11, 16, and 18; FUTURE II) and bivalent (HPV16 and 18; PATRICIA) virus-like particle vaccines against HPV16-positive, HPV18-positive, and overall CIN grade 2 (CIN2), CIN3, and CIN of grade 3 or higher endpoints[17] and against persistent HPV infections.[17,26] In the two trials,[17,18] 874 Finnish girls received three doses of the quadrivalent HPV vaccine at 0, 2, and 6 months, and 2409 received three doses of the bivalent HPV vaccine at 0, 2, and 6 months. All participants attended ten follow-up visits with gynaecological examinations and cervical sampling. Opportunistic vaccinations in participants of FUTURE II and PATRICIA have been monitored and avoided through questionnaires.[27,28]

At enrolment, participants also gave informed consent to health registry follow-up (with the Finnish Cancer Registry, the FMC, and the Hospital Discharge Registry) based on their unique personal identifiers.[15,19] At baseline, sexually active girls reported their number of sexual partners and were tested for HPV16 and HPV18 DNA.

Post-vaccination serum samples were retrieved from the FMC Biobank, which was established in 1983 and by the end of 2016 had gathered about 2 million first-trimester serum samples from nearly all pregnant women in Finland. 96% of Finnish pregnant women participate in screening of congenital infections.[20] For the Finnish FUTURE II and PATRICIA cohorts, this practice resulted in the collection of 595 and 1451 post-vaccination serial serum samples, respectively, from consecutive pregnancies during the up to 12 years of follow-up.

We obtained all available serum samples from recipients of the quadrivalent vaccine and used a random number generator to randomly select serum samples from corresponding recipients of the bivalent vaccine who were aligned by follow-up time and matched by the number of pregnancies. To assess the possible effect of the number of pregnancies on the production of vaccine-induced neutralising antibodies before the Finnish median age of pregnancy (29·5 years), we did a sub-cohort analysis stratified by the serial number of pregnancies. In the first sub-cohort, neutralising antibody concentrations were determined in samples collected at the first pregnancy only. In further sub-cohorts, neutralising antibody concentrations were determined in samples from subsequent pregnancies.

The Finnish National Ethical Review Board (ETENE/Tukija) approved the FUTURE (NCT00092534) and PATRICIA (NCT00122681) trials and their registry-based follow-up (diary numbers 52/04/02 and 17/04/04). The FMC samples were collected, based on informed consent, and maintained for scientific research by Finnish law (number 327/2001:1).

Neutralisation assay

A high-throughput, pseudovirion-based neutralisation assay was used to determine neutralising antibodies to HPV6, 16, 18, 31, 33, 45, 52, and 58 as described in a previous study.[29] The assay uses pseudovirions comprising HPV L1 and L2 proteins, which encapsidate a Gaussia luciferase reporter plasmid. Pseudovirions are produced in HEK293TT cells and purified by ultra-centrifugation in an Optiprep gradient. Transduction of the Gaussia luciferase plasmid into HeLaT reporter cells by pseudovirion infection and subsequent expression of Gaussia luciferase is quantified by luminescent reaction with the luciferase substrate coelenterazine. In the presence of neutralising antibodies, pseudovirion infection is blocked and the transduction of reporter genes reduced. Prediluted serum samples were serially diluted seven times in 3·33-fold increments to achieve a final serum dilution in the neutralisation assay of 1:40 to 1:180 000. Antibody titres were calculated as serum dilutions inhibiting 50% of the luciferase activity (EC50 values). EC50 values greater than 40 (for HPV16 and HPV18 corresponding to 1·3 and 1·1 IU/mL, respectively) were defined as neutralising antibody-positive. Titres larger than the last serum dilution (180 000 for HPV16 and HPV18 corresponding to >5818 and >5019 IU/mL, respectively) were not differentiated further. All serum samples were tested in a single-run format to guarantee comparability of neutralising antibody and cross-neutralising antibody titres across all eight HPV types. Sufficiently active HPV11 pseudovirion preparations were not available for this study.

In addition to CIN2 and CIN3, persistent HPV infection is accepted as an appropriate surrogate endpoint for vaccine efficacy.[7,30] By definition, persistent infection results in subsequent detection of the same HPV type in the cervix, vulva, vagina, anus, or oropharynx for 6 months or longer. Conversely, transient infection consists of HPV detection over a period of as little as 3 months. Therefore, we assessed the correlation of the vaccine-induced neutralising antibody and cross-neutralising antibody concentrations with efficacies of the two vaccines against persistent HPV infections.[14]

Statistical analysis

HPV type-specific seroprevalence (proportion of neutralising antibody-positive samples of all serum samples), median anti-HPV antibody titres (in all serum samples), and geometric mean titres (GMT; neutralising antibody-positive serum samples only) with 95% CIs were calculated using GraphPad Prism 8.3.1, and the two-tailed

Spearman non-parametric correlation coefficients (r_s) of type-specific vaccine efficacy and corresponding seroprevalence and GMT were calculated using R (version 4.0.0). 95% CIs for the corresponding two-tailed Spearman correlation coefficients were calculated by applying Fisher's *Z*-transformation. Calculations were stratified for post-vaccination period (2–4, 5–7, 8–10, 11–12, and 5–12 years). These ranked correlation statistics are a robust method of calculating the correlation of neutralising antibody concentrations with vaccine efficacy estimates derived from the Finnish participants of the FUTURE II (10%) and PATRICIA (25%) trials who, at their later pregnancies, who donated serum samples for the high-throughput, pseudovirion-based neutralisation assay done in our study.

Type-specific GMT ratios and their corresponding 95% CIs were calculated comparing the bivalent vaccine recipients with the quadrivalent vaccine recipients using a linear regression model in which the antibody titre outcome was log-transformed. The ratios were calculated using R statistical software (version 3.6.1) with the Epi package (version 2.40).

Local regression with a specified smoothing span of 0·75 was used to compare the GMTs of HPV6, 16, 18, 31, 33, 45, 52, and 58 neutralising antibody responses over time between the bivalent and quadrivalent vaccine recipients. Without assuming any particular data distribution (ie, a relationship between EC50 values and time since vaccination) beforehand, such non-parametric regression analysis is an appropriate method for characterising possible subtleties in the relationships among neutralising antibody concentrations and the number of years after vaccination. The locally weighted regression analysis with calculation of 95% CIs was done using datapoints from vaccine recipients with HPV type-specific EC50 values greater than 40 only (defined as neutralising-positive). For graphical depiction of the data, R statistical software (version 3.6.1) with the ggplot2 package (version 3.2.1) was used.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Up to Dec 31, 2016, we analysed 577 serum samples from quadrivalent vaccine recipients and 568 samples from bivalent vaccine recipients (figure 1). Respectively, nine and 18 serum samples in the bivalent and quadrivalent cohorts were excluded from the analysis because of technical issues. In the first sub-cohort of samples collected at the first pregnancy only, we obtained 339 samples from quadrivalent vaccine recipients and 342 from bivalent vaccine recipients (figure 1). In samples from subsequent pregnancies, neutralising antibody concentrations were determined in 238 quadrivalent vaccine recipients and 226 bivalent vaccine recipients (figure 1, appendix pp 1–5).

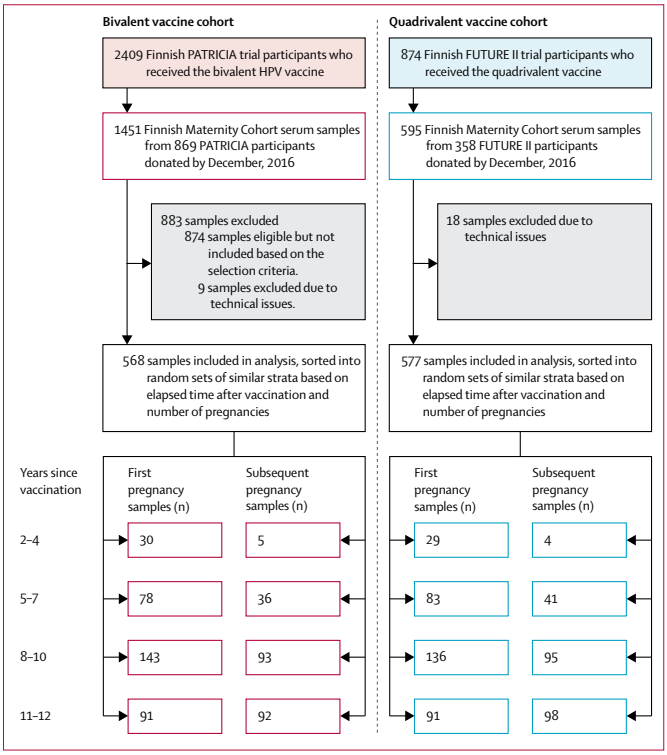

Figure 1: **Flowchart of the study populations and selected samples included in this study**
HPV=human papillomavirus.

See **Online** for appendix

The neutralising antibody response obtained in first-pregnancy samples from each pregnant participant 2–12 years after vaccination are presented in the head-to-head comparison between the two vaccine types. We observed distinguishable (non-overlapping 95% CIs) locally weighted regression lines of neutralising antibodies to HPV16 and HPV18 in the two vaccine cohorts between 2 and 12 years (figure 2). Similar differences in the kinetics of median HPV16 neutralising antibody titres were found within the post-vaccination time intervals (table 1). In quadrivalent vaccine recipients, there was a 45% decline between 2 and 4 years and 5–7 years in median neutralising antibody titres to HPV16 and a decline of 29% in antibody titres to HPV18 (table 1). In bivalent vaccine recipients, the EC50 values of neutralising antibodies to HPV16 and HPV18 did not show an initial decline between 2–4 years (figure 2). In bivalent vaccine recipients, the EC50 values of neutralising antibodies to HPV16 and HPV18 did not show an initial decline between 2–4 years

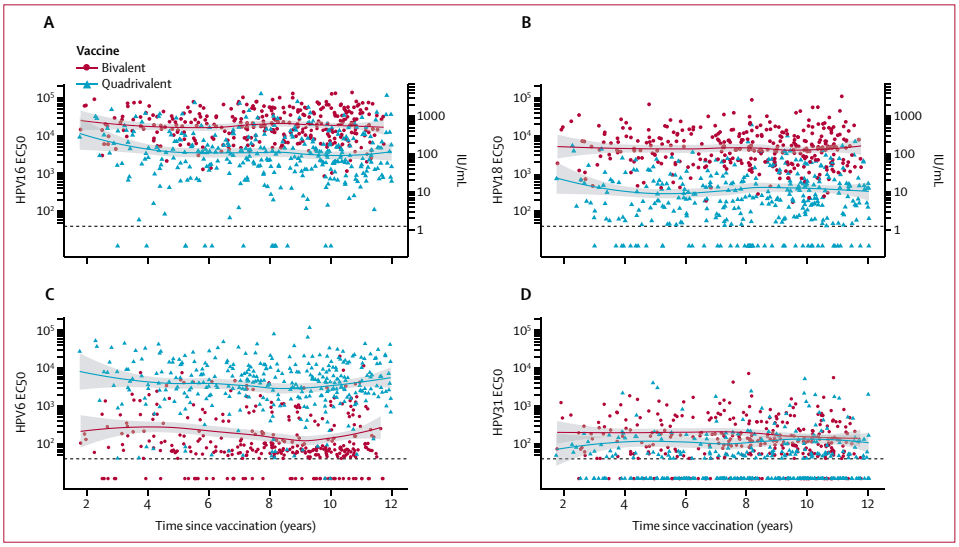

Figure 2: **Locally estimated regression lines of EC50 values to HPV16 (A), HPV18 (B), HPV6 (C), and HPV31 (D) in the first pregnancy sub-cohort 2–12 years after vaccination**

Individual neutralising antibody titres induced by the bivalent and quadrivalent vaccines. Black dashed lines indicate the high-throughput pseudovirion-based neutralisation assay cutoff value (EC50=40). For HPV16 (A) and HPV18 (B), corresponding IU/mLs of neutralising antibodies are also shown in the second y-axis. EC50=half maximal effective concentration. HPV=human papillomavirus.

(figure 2), and were stable for up to 12 years after vaccination (table 1).

For HPV6, neutralising antibodies induced by the quadrivalent vaccine were compared with cross-neutralising antibodies induced by the bivalent vaccine. Sustainable neutralising antibodies to HPV6 were present in almost all (337 [99%] of 339) quadrivalent vaccine recipients up to 12 years after vaccination, whereas about 86% (294 of 342) of the bivalent vaccine recipients were positive for anti-HPV6 cross-neutralising antibodies (table 1, figure 2). Similar to results for HPV16, a 45% decline in the median neutralising antibody titres to HPV6 was noted in quadrivalent vaccine recipients between 2–4 years and 5–7 years after vaccination (table 1). Thereafter, essentially stable regression lines of neutralising antibody and cross-neutralising antibody GMT to HPV6 were observed among seropositive samples in the two vaccine cohorts 5–12 years after vaccination (figure 2). In bivalent vaccine recipients, significantly lower (ie, with non-overlapping 95% CIs) HPV6 cross-neutralising antibody concentrations, compared with the HPV6 neutralising antibody concentrations in the quadrivalent vaccine recipients, persisted for 2–12 years after vaccination (table 1, figure 2).

Of the quadrivalent vaccine recipients, 4% (14 of 339) and 15% (51 of 339) had no detectable HPV16 and HPV18 neutralising antibodies 2–12 years after vaccination, respectively, whereas all 342 bivalent vaccine recipients were positive for neutralising antibodies to HPV16 and HPV18 at all timepoints. The concentrations of neutralising antibodies to HPV16 and HPV18 (as calculated by locally weighted regression) were also notably different in the two vaccine cohorts (figure 2). In quadrivalent vaccine recipients, HPV18 neutralising antibody concentrations were significantly lower than in bivalent vaccine recipients 2–12 years after vaccination (table 1, 2). 5–12 years after vaccination, GMT of neutralising antibodies to HPV16 and HPV18 in bivalent vaccine recipients were 5·67 times and 12·40 times higher, respectively, than in quadrivalent vaccine recipients (table 2) and the corresponding median titres were 6·4 times and 19·9 times higher (table 1). Because of the slight loss of HPV16 neutralising antibody titres in the quadrivalent vaccine recipients in later years after vaccination, the bivalent to quadrivalent median titre ratio and GMT ratio increased over time (tables 1, 2).

For HPV6, significantly different anti-HPV6 seroprevalences between the two vaccines were observed, with 48 (14%) of 342 bivalent vaccine recipients not showing detectable HPV6 cross-neutralising antibodies in the first pregnancy cohort, whereas all quadrivalent vaccine recipients were HPV6 neutralising antibody-positive (table 1). The quadrivalent vaccine induced, on average, 19 times higher GMT of HPV6 neutralising antibodies and 45–55 times higher median anti-HPV6 antibody titres compared with the bivalent vaccine (table 1). The HPV6 cross-neutralising antibody concentrations in bivalent vaccine recipients were close to the cutoff value of 40 (table 1).

	Bivalent vaccine			Quadrivalent vaccine		
	Seroprevalence (95% CI)	Median titre (95% CI)	GMT (95% CI)	Seroprevalence (95% CI)	Median titre (95% CI)	GMT (95% CI)
HPV6						
2–4 years	80·0 (61·4–92·2)	171 (80–286)	284 (164–493)	100 (88·8–100)	7615 (2612–14 647)	4568 (2173–9603)
5–7 years	83·3 (73·1–90·8)	86 (65–158)	232 (160–338)	100 (95·6–100)	4172 (3052–6708)	3836 (2742–5366)
8–10 years	89·5 (83·2–94·0)	75 (68–88)	136 (110–168)	99·2 (95·9–99·9)	4344 (2982–5023)	3099 (2352–4083)
11–12 years	84·6 (75·5–91·3)	77 (67–102)	197 (139–279)	99·0 (94·0–99·9)	4213 (2811–6002)	4442 (3416–5777)
5–12 years	86·5 (82·2–90·1)	78 (71–89)	172 (146–203)	99·3 (97·6–99·9)	4252 (3352–5023)	3646 (3081–4315)
HPV16						
2–4 years	100 (88·4–100)	21 009 (14 451–31 826)	22 970 (15 996–32 985)	93·1 (77·2–99·1)	6642 (2371–13 717)	6141 (3379–11 160)
5–7 years	100 (95·3–100)	18 259 (12 372–23 192)	16 242 (13 076–20 176)	95·1 (88·1–98·6)	3679 (2377–4708)	3337 (2473–4503)
8–10 years	100 (97·4–100)	18 755 (15 666–22 313)	20 113 (17 241–23 464)	94·1 (88·7–97·4)	2906 (2276–3780)	3429 (2701–4355)
11–12 years	100 (96·0–100)	19 443 (14 840–27 110)	18 366 (14 785–22 817)	98·9 (94·0–99·9)	2279 (1876–4190)	3021 (2292–3981)
5–12 years	100 (98·8–100)	19 054 (16v741–21 436)	18 568 (16 660–20 694)	95·8 (92·9–97·7)	2983 (2377–3685)	3276 (2811–3818)
HPV18						
2–4 years	100 (88·4–100)	5746 (3152–8506)	4942 (3218–7591)	86·2 (68·3–96·1)	316 (69–675)	400 (223–717)
5–7 years	100 (95·3–100)	4328 (2711–7098)	4138 (3154–5431)	89·1 (80·4–94·9)	225 (164–398)	316 (240–415)
8–10 years	100 (97·4–100)	4395 (3842–5476)	4508 (3790–5363)	85·3 (78·2–90·7)	236 (183–364)	392 (306–502)
11–12 years	100 (96·0–100)	4929 (4097–5995)	4708 (3667–6046)	80·2 (70·5–87·8)	229 (162–418)	358 (268–479)
5–12 years	100 (98·8–100)	4621 (4108–5232)	4469 (3939–5070)	84·8 (80·3–88·6)	232 (195–344)	360 (308–420)
HPV31						
2–4 years	90·0 (73·4–97·8)	183 (73–290)	205 (134–313)	65·5 (45·6–82·0)	53 (25–93)	102 (66–157)
5–7 years	80·7 (70·2–88·8)	129 (62–192)	189 (144–249)	45·7 (34·7–57·0)	<40	114 (77–169)
8–10 years	85·3 (78·4–90·6)	117 (93–138)	179 (149–216)	46·3 (37·7–55·0)	<40	120 (90–160)
11–12 years	83·5 (74·2–90·4)	97 (63–128)	147 (117–185)	53·8 (43·0–64·3)	44 (25–53)	121 (87–168)
5–12 years	83·6 (79·0–88·3)	110 (92–133)	171 (151–195)	48·4 (42·7–54·1)	<40	119 (98–143)
HPV33						
2–4 years	53·3 (34·3–71·6)	41 (25–59)	108 (63–183)	44·8 (26·4–64·3)	<40	63 (46–86)
5–7 years	58·9 (47·2–69·9)	47 (25–63)	116 (83–164)	36·1 (25·8–47·4)	<40	152 (96–241)
8–10 years	61·5 (53·0–69·5)	51 (43–56)	107 (87–133)	31·6 (23·9–40·1)	<40	186 (120–287)
11–12 years	50·5 (39·8–61·2)	44 (25–59)	154 (104–227)	38·4 (28·4–49·2)	<40	133 (93–190)
5–12 years	57·7 (52·0–63·2)	48 (43–53)	120 (102–142)	34·8 (29·5–40·4)	<40	158 (124–200)
HPV45						
2–4 years	46·6 (28·3–65·6)	<40	124 (80–192)	10·3 (2·1–27·3)	<40	53 (25–112)
5–7 years	48·7 (37·2–60·3)	<40	88 (72–108)	13·2 (6·8–22·4)	<40	97 (53–175)
8–10 years	46·1 (37·7–54·6)	<40	79 (68–92)	13·9 (8·6–20·9)	<40	89 (62–129)
11–12 years	40·6 (30·4–51·4)	<40	74 (60–90)	18·6 (11·2–28·2)	<40	117 (64–216)
5–12 years	45·1 (39·5–50·9)	<40	80 (72–88)	15·1 (11·3–19·6)	<40	101 (76–133)
HPV52						
2–4 years	73·3 (54·1–87·7)	55 (46–97)	117 (72–192)	68·9 (49·1–84·7)	64 (40–183)	157 (92–267)
5–7 years	78·2 (67·4–86·7)	64 (51–89)	122 (93–160)	51·8 (40·5–62·9)	45 (25–56)	105 (77–142)
8–10 years	81·1 (73·7–87·1)	37 (58–84)	120 (99–146)	69·1 (60·6–76·7)	50 (44–57)	119 (93–154)
11–12 years	72·5 (62·1–81·3)	64 (52–83)	140 (106–186)	63·7 (52·9–73·5)	52 (42–63)	106 (81–139)
5–12 years	77·8 (72·8–82·3)	66 (59–72)	126 (110–144)	62·9 (57·2–68·3)	50 (45–55)	112 (96–131)
HPV58						
2–4 years	43·3 (25·4–62·5)	<40	205 (82–514)	17·2 (5·8–35·7)	<40	123 (32–474)
5–7 years	55·1 (43·4–66·4)	45 (25–53)	103 (74–143)	37·3 (26·9–48·6)	<40	212 (122–366)
8–10 years	51·0 (42·5–59·5)	42 (25–49)	118 (89–156)	31·6 (23·9–40·1)	<40	204 (133–312)
11–12 years	49·4 (38·8–60·1)	<40	200 (126–318)	38·4 (28·4–49·2)	<40	105 (71–155)
5–12 years	51·6 (45·9–57·2)	42 (25–48)	132 (108–161)	35·1 (29·8–40·7)	<40	166 (128–216)

HPV antibodies were measured by high-throughput pseudovirion-based neutralisation assay. HPV=human papillomavirus. GMT=geometric mean titre.

Table 1: **Neutralising antibody seroprevalence, median anti-HPV antibody titres, and GMTs of neutralising antibodies per post-vaccination interval against HPV types in samples collected 2–12 years after HPV vaccination in the first pregnancy sub-cohort**

Distinct sustainable cross-neutralising responses to non-HPV vaccine types were observed in the two vaccine cohorts. The long-term GMT to non-HPV vaccine types among seropositive participants were similar in the two vaccine cohorts (table 1, figure 1, appendix p 5), but we noted significant differences in the cross-neutralising antibody seroprevalence. Cross-neutralising antibody seroprevalence to non-vaccine HPV types 31, 33, 45, 52, and 58 were generally lower in quadrivalent vaccine recipients than in bivalent vaccine recipients (table 1). Median cross-neutralising antibody titres that were consistently above cutoff in the quadrivalent vaccine recipients were found only for HPV52, whereas in bivalent vaccine recipients higher and consistent median cross-neutralising antibody titres for HPV31, HPV33, and HPV52 were found (table 1). We did not observe any significant decline in cross-neutralising antibody concentrations to these non-vaccine HPV types over the 2–12 years after vaccination in the two vaccine cohorts (table 1, figure 1, appendix p 5).

For the bivalent vaccine recipients, both the 2–4 year and sustained 5–12 year HPV16, HPV18, HPV31, and HPV33 seroprevalence and vaccine efficacies were similar (table 3). The opposite was true for HPV52 and HPV58, for which early term and long-term seroprevalences were higher than vaccine efficacies. For HPV45, both the 2–4 year and sustained 5–12 year seroprevalences were lower than the vaccine efficacies. For the five types of HPV phylogenetically classified in clade α-9 (A9-HPV types, HPV16, 31, 33, 52, and 58), seroprevalence was significantly correlated with vaccine efficacy against persistent infections with the corresponding HPV types (r_s=0·90, 95% CI 0·09–0·99, p=0·037). The bivalent vaccine-induced HPV6 seroprevalences were moderate 2–4 years and 5–12 years after vaccination but the efficacy against persistent HPV6 infection was low. The opposite was true for HPV45, which showed low seroprevalences and moderate efficacy with the bivalent vaccine (table 3).

For the quadrivalent vaccine, the HPV6 and HPV16 seroprevalence at 2–4 years (100% and 93%, respectively) and 5–12 years (99% and 96%, respectively), and vaccine efficacy against persistent HPV6 (100%) and HPV16 (91·6%) infections were all high (table 3). The corresponding HPV18 seroprevalences were 86% and 85%, and vaccine efficacy against persistent HPV18 infection was 91·6%. Vaccine efficacy against persistent HPV31 and HPV33 infections was 46·2% and 28·7%, respectively, close to the 5–12-year HPV31 and HPV33 seroprevalences (48% and 35%). Despite detectable HPV52 and HPV58 cross-neutralising antibody concentrations, for the five types of clade A9, neither seroprevalence or antibody titres were significantly correlated with vaccine efficacy against persistent infections with the corresponding HPV type (r_s=0·62, 95% CI –0·58 to 0·97, p=0·269; table 3).

	Bivalent versus quadrivalent GMT ratio (95% CI)
HPV6	
2–4 years	0·06 (0·02–0·16)
5–7 years	0·06 (0·04–0·10)
8–10 years	0·04 (0·03–0·06)
11–12 years	0·04 (0·03–0·07)
5–12 years	0·05 (0·04–0·06)
HPV16	
2–4 years	3·74 (1·95–7·18)
5–7 years	4·87 (3·38–7·01)
8–10 years	5·87 (4·45–7·73)
11–12 years	6·08 (4·30–8·59)
5–12 years	5·67 (4·71–6·82)
HPV18	
2–4 years	12·4 (6·27–24·3)
5–7 years	13·1 (8·95–19·1)
8–10 years	11·5 (8·58–15·4)
11–12 years	13·1 (9·02–19·1)
5–12 years	12·4 (10·2–15·1)
HPV31	
2–4 years	2·01 (1·12–3·62)
5–7 years	1·66 (1·05–2·61)
8–10 years	1·49 (1·08–2·07)
11–12 years	1·29 (0·89–1·87)
5–12 years	1·47 (1·19–1·83)
HPV33	
2–4 years	1·71 (0·94–3·1)
5–7 years	0·76 (0·45–1·31)
8–10 years	0·58 (0·38–0·88)
11–12 years	1·16 (0·68–1·96)
5–12 years	0·76 (0·58–1·01)
HPV45	
2–4 years	1·11 (0·55–2·22)
5–7 years	0·78 (0·55–1·11)
8–10 years	1·12 (0·74–1·71)
11–12 years	0·63 (0·39–1·01)
5–12 years	0·81 (0·64–1·03)
HPV52	
2–4 years	0·73 (0·44–1·23)
5–7 years	1·55 (1·08–2·24)
8–10 years	0·79 (0·56–1·13)
11–12 years	1·33 (0·9–1·95)
5–12 years	1·15 (0·93–1·43)
HPV58	
2–4 years	0·53 (0·22–1·28)
5–7 years	0·70 (0·39–1·24)
8–10 years	0·55 (0·32–0·94)
11–12 years	1·91 (1·04–3·5)
5–12 years	0·90 (0·64–1·25)

GMT=geometric mean titre.

Table 2: **GMT ratios comparing bivalent vaccine recipients with quadrivalent vaccine recipients per post-vaccination interval in the first pregnancy sub-cohort**

	Vaccine efficacy against HPV infections		Positive neutralising L1 antibody response (2–4 years after vaccination)		Positive neutralising L1 antibody response (5–12 years after vaccination)	
	Transient	Persistent	n/N (%)	GMT	n/N (%)	GMT
HPV6						
Bivalent	9·8% (–17·8 to 26·9)	34·5% (11·3 to 51·8)	24/30 (80%, 60 to 95)	284 (164 to 493)	274/315 (87%, 3 to 90)	171 (146 to 203)
Quadrivalent	NA	100% (85·0 to 100)	29/29 (100%, 100)	4568 (2173 to 9603)	308/310 (99%, 98 to 100)	3646 (3081 to 4315)
HPV16						
Bivalent	93·1% (88·3 to 96·3)	94·7% (91·8 to 96·7)	30/30 (100%, 100)	22 970 (15 996 to 32 985)	315/315 (100%, 100)	18 568 (16 660 to 20 694)
Quadrivalent	NA	91·6% (73·3 to 98·4)	27/29 (93%, 83 to 100)	6141 (3379 to 11 160)	300/310 (96%, 94 to 98)	3276 (2811 to 3818)
HPV18						
Bivalent	95·5% (81·8 to 98·3)	92·3% (86·5 to 96·0)	30/30 (100%, 100)	4942 (3218 to 8591)	315/315 (100%, 100)	4469 (3930 to 5070)
Quadrivalent	NA	91·6% (43·3 to 99·8)	25/29 (86%, 73 to 100)	400 (223 to 717)	264/310 (85%, 81 to 89)	360 (308 to 420)
HPV31						
Bivalent	75·7% (58·5 to 84·8)	77·1% (67·2 to 84·4)	27/30 (90%, 79 to 100)	205 (134 to 313)	265/315 (84%, 76 to 88)	171 (151 to 195)
Quadrivalent	NA	46·2% (15·3 to 66·4)	19/29 (66%, 48 to 84)	102 (66 to 157)	149/310 (48%, 43 to 54)	119 (98 to 143)
HPV33						
Bivalent	34·8% (0·7 to 58·0)	43·1% (19·3 to 60·2)	16/30 (53%, 34 to 72)	108 (63 to 183)	183/315 (58%, 52 to 63)	120 (102 to 142)
Quadrivalent	NA	28·7% (–45·1 to 65·8)	13/29 (45%, 26 to 63)	63 (46 to 86)	109/310 (35%, 30 to 40)	158 (124 to 200)
HPV45						
Bivalent	77·9% (58·8 to 89·0)	79·0% (61·3 to 89·4)	14/30 (47%, 28 to 66)	124 (80 to 192)	142/315 (45%, 40 to 51)	80 (72 to 88)
Quadrivalent	NA	7·8% (–67·0 to 49·3)	3/29 (10%, 0 to 22)	53 (25 to 102)	47/310 (15%, 11 to 19)	101 (76 to 133)
HPV52						
Bivalent	16·5% (–11·2 to 38·3)	18·9% (3·2 to 32·2)	22/30 (73%, 57 to 90)	117 (72 to 192)	246/315 (78%, 73 to 83)	126 (110 to 144)
Quadrivalent	NA	18·4% (–20·6 to 45·0)	20/29 (69%, 51 to 87)	157 (92 to 267)	195/310 (63%, 58 to 68)	112 (96 to 131)
HPV58						
Bivalent	–8·9% (–59·4 to 24·2)	–6·2% (–44·0 to 21·6)	13/30 (43%, 25 to 62)	205 (82 to 514)	164/315 (52%, 46 to 57)	132 (108 to 161)
Quadrivalent	NA	5·5% (–54·3 to 42·2)	5/29 (17%, 3 to 32)	123 (32 to 474)	109/310 (35%, 30 to 40)	166 (128 to 216)

Data are % (95% CI), n/N (%, 95% CI), or GMT (95% CI). Bivalent vaccine efficacy estimates were reported by Lehtinen et al, 2018[10] (for transient infection) and Wheeler et al, 2012[14] (for persistent infection). Quadrivalent vaccine efficacy estimates (for persistent infection) were reported by Villa et al[15] and Brown et al, 2009.[13] A9=phylogenetic clade α-9, in which HPV16, 31, 33, 52, and 58 are categorised. GMT=geometric mean titres. HPV=human papillomavirus. n=number of positive neutralising antibody responses. N=total number of participants. NA=not appplicable.

Table 3: **Vaccine efficacy against transient or persistent HPV infections and vaccine-induced neutralising or cross-neutralising L1 antibody responses (seropositivity and GMT) in the first pregnancy sub-cohort 2–4 years and 5–12 years after vaccination with a bivalent or quadrivalent HPV vaccine**

Discussion

We found significantly higher sustainable seroprevalences for HPV16, 18, 31, 33, 45, 52, and 58 in bivalent vaccine recipients than in quadrivalent vaccine recipients. HPV16, 18, and 31 neutralising antibody concentrations 5–12 years after vaccination in the bivalent vaccine recipients were significantly higher than in the quadrivalent vaccine recipients. The opposite was true for HPV6, which is included only in the quadrivalent vaccine. Vaccine efficacies against persistent infections—which has been approved as a valid vaccine efficacy endpoint[7]—with the clade A9-HPV types 16, 31, 33, 52, and 58 significantly correlated with the corresponding ranked seroprevalence by type only in the bivalent vaccine recipients. Comparisons between the correlation estimates of the two vaccines should be done carefully, as the 95% CIs observed were wide and indicate that our analysis was not statistically robust. Furthermore, our study shows that, when measurable, cross-neutralising antibody concentrations to HPV types 31, 33, 45, 52, and 58 are as sustainable as type-specific neutralising antibody concentrations, for both vaccine cohorts.

Significant differences in seroprevalence and the neutralising antibody concentrations 5–12 years after vaccination against the three most common oncogenic types HPV16, 18, and 31 were notable. The significant correlation between type-specific vaccine efficacy and the neutralising antibodies induced by the bivalent vaccine to clade A9-HPV types suggests that neutralising and cross-neutralising antibody concentrations probably have a protective threshold, which is different from the technical positivity cutoff level of our neutralising antibody test (EC50 of 40). Protective antibody concentrations, however, still need to be defined.

The observed differences in vaccine-induced neutralising antibody concentrations, which consequently resulted in distinct correlations of vaccine efficacies, is probably due to the different adjuvants used to formulate the bivalent and quadrivalent vaccines.[9,12] Differences in the vaccine-induced immunogenicity might also partly arise from qualitative differences related to the expression platforms used to produce the HPV virus-like particles.[33] Sustainable cross-neutralising antibody concentrations (over 5–12 years) were similar in the quadrivalent vaccine

recipients and in the bivalent vaccine recipients despite the fact that corresponding seroprevalence estimates were substantially higher in the bivalent vaccine recipients. This might reflect a boosting in the immunological memory over time by natural HPV31, 33, 45, 52, and 58 infections that had occurred more often in the quadrivalent vaccine recipients after vaccination. The serological signature of quadrivalent vaccine recipients (ie, the profile of cross-neutralising antibody concentrations by HPV types with the concomitant absence of HPV16 or HPV18 antibodies) suggests that this indeed has been the case. Similar HPV6 seroconversions were seen in the bivalent vaccine recipients, indicative of late HPV6 infections in this cohort. These findings indicate that cross-protection is only partially protective against non-vaccine types, and it probably depends on the vaccine-induced antibody concentrations.

From another perspective, the cases in which cross-neutralising antibody concentrations do not, or only weakly, correlate with vaccine efficacy estimates for a given HPV type have to be considered carefully. These are unexpected and important findings for two main reasons: (1) neutralising antibodies are considered a key mechanism of HPV vaccine-induced protection and (2) antibody-based immunobridging studies have been extensively used to expand vaccination for populations who are not formally targeted in efficacy trials. In this context, it is still to be proved whether cross-neutralising antibodies induced by HPV vaccines are able to elicit protection as effectively as type-specific antibodies. For HPV52 and HPV58, the moderate seroprevalence associated with low vaccine efficacies for both cohorts suggests that cross-neutralising antibodies to these types are not protective. However, the opposite scenario for HPV45 in bivalent vaccine recipients (ie, moderate seroprevalence or antibody concentrations and high vaccine efficacy) suggests that cross-neutralising antibodies are not the sole factor inducing protection. L1-specific T-cell responses, which are also triggered on vaccination with HPV virus-like particles,[34,35] could have a role in cross-type protection. Cellular immune responses are sustainable up to 6 years after HPV vaccination, although the clinical significance of these findings is unclear.[36] Importantly, the bivalent vaccine adjuvant AS04 combines aluminium salt and the toll-like receptor 4 agonist MPL (3-O-desacyl-4'-monophosphoryl-lipid A), which is known to enhance cellular immune response[37] and could make a difference in the immunogenicity of the two vaccines.

The major strengths of our head-to-head comparison are: (1) use of a functional, highly type-specific antibody assay (neutralisation);[29,38,39] (2) inclusion of two identical cohorts of adolescent Finnish girls enrolled 2 years apart in the FUTURE II and PATRICIA trials without sexual risk-taking exclusion criteria;[18,24] and (3) country-wide, population-based follow-up of both cohorts through the FMC Biobank that has collected and stored serum samples from 96% of all pregnant women in Finland since 1983.[15,25] This coverage enabled collection of about 1200 serum samples up to 12 years after vaccination. The HPV16 epidemic that took place in Finland between the 1980s and 1990s[40] had reached a plateau well before the start of the FUTURE II and PATRICIA trials.[41] Furthermore, no measured changes in sexual risk-taking behaviour or smoking had taken place in Finnish adolescents between 2002 and 2004. Lastly, there was no opportunistic vaccination in Finland before or at the time the two licensure trials were done in 2002–07 (FUTURE II) and 2004–09 (PATRICIA). The possibility that quadrivalent vaccine recipients could, after bivalent vaccine licensure in October, 2007, obtain the bivalent vaccine through opportunistic vaccination is negligible.

Although the true disease endpoint for determining HPV vaccine efficacy is cancer, such an endpoint is impractical and obviously unethical. Although CIN2 and CIN3 are the progression states that immediately precede invasive cancer, the consensus reasoning is that persistent infection constitutes the major risk for progression to high-grade CIN and, hence, to cervical cancer. It is, therefore, accepted by WHO as a predictor of high-grade disease and a surrogate of HPV vaccine efficacy. A main limitation of our study is that estimates of vaccine efficacy against persistent HPV6, 16, 18, 31, 33, 45, 52, and 58 infections were available from just two clinical trials from which the vaccine efficacy estimates were derived.[13,17,31] 10% and 40% of the trial participants were Finnish girls. Only for the bivalent vaccine, the vaccine efficacy estimates for transient HPV infections were derived from a 100% Finnish adolescent female trial population.[42] These estimates were similar to the previously mentioned vaccine efficacy against persistent infection, suggesting that the efficacy estimates against persistent HPV infections are generic. Additionally, by not evaluating antibody concentrations to HPV11, a type also included in the quadrivalent vaccine, we had to rely only on HPV6 findings with regard to cross-reactivity of the bivalent vaccine-induced antibodies to the low-risk HPV types. Also, we did not measure cross-neutralising antibody levels to HPV35, a type not included in any of the vaccines, but to which a degree of cross-protective efficacy similar to that of A9-HPV types has been documented for bivalent vaccine recipients.[32] Lastly, a minor limitation included the vaccine cohorts being older than the pre-adolescent girls who are primarily the target group in most of the countries (age 12–13 years) nowadays. Vaccination of younger girls aged 12–13 years has been shown to elicit stronger antibody responses than vaccination of girls aged 16–17 years.[43,44]

In conclusion, our unique head-to-head comparison of sustainable neutralising and cross-neutralising antibody responses in similar cohorts of Finnish bivalent and quadrivalent vaccine recipients revealed significant differences in the immunogenicity of the two vaccines that are sustained over time. Our findings indicate that

protective antibody concentrations following HPV vaccination can be detected up to 12 years after vaccination.

Contributors

FCM, MP, and ML were the principal contributors to the manuscript preparation. FCM was responsible for the production and characterisation of pseudovirion preparations and contributed to the statistical analysis and data interpretation. MM, MP, and ML were responsible for the conception and design of the study. MP was involved in the supervision of laboratory analysis of serum samples, data analysis, and interpretation. ML supervised and contributed to the data analysis and interpretation. PG contributed to the statistical analysis and preparation of figures. NB, TE, HK, and KMP were responsible for study planning and data management. TE, H-MS, EP, NB, and KMP were responsible for sample and data retrieval. PS was responsible for the laboratory analysis, data acquisition, and data analysis. TW was involved in the supervision of laboratory analysis of serum samples, and manuscript review. MM was responsible for the supervision of laboratory analysis of pseudovirion production and characterisation, data analysis, and interpretation. DA and JP were involved in the organisation of HPV clinical trials in Finland. All authors had full access to all the data in the study. FCM, NB, TE, KMP, PS, MP, and ML verified all the data in the study. All authors reviewed and commented on a draft of the manuscript and gave final approval for submission.

Declaration of interests

DA, ML, and JP have received funding for their HPV vaccination studies from Merck and GlaxoSmithKline Biologicals through their employers (Finnish Family Federation, DA; University of Tampere, ML; and University of Helsinki, JP). MM is an inventor of patents related to production of papillomavirus-like particle vaccines owned by Loyola University Chicago (Chicago, USA) and licensed to GlaxoSmithKline Biologicals; a patent related to production of papillomavirus-like particles issued to Deutsches Krebsforschungszentrum (DKFZ); and is an inventor of scaffold-stabilised L2 peptides for HPV vaccines issued to DKFZ. TW serves on advisory boards for Merck Sharp & Dohme. All other authors declare no competing interests.

Data sharing

Data supporting the findings of this study are available upon reasonable request to FCM (f.mariz@dkfz.de) and NB (n.bender@dkfz.de) according to DKFZ data safety protection regulations.

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PUBLICATION II

Human papillomavirus seroprevalence in pregnant women following gender-neutral and girls-only vaccination programs in Finland: A cross-sectional cohort analysis following a cluster randomized trial

Penelope Gray, Hanna Kann, Ville N. Pimenoff, Tiina Eriksson, Tapio Luostarinen, Simopekka Vänskä, Heljä-Marja Surcel, Helena Faust, Joakim Dillner, Matti Lehtinen

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PLOS MEDICINE

RESEARCH ARTICLE

Human papillomavirus seroprevalence in pregnant women following gender-neutral and girls-only vaccination programs in Finland: A cross-sectional cohort analysis following a cluster randomized trial

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Abstract

Background

Cervical cancer elimination through human papillomavirus (HPV) vaccination programs requires the attainment of herd effect. Due to its uniquely high basic reproduction number, the vaccination coverage required to achieve herd effect against HPV type 16 exceeds what is attainable in most populations. We have compared how gender-neutral and girls-only vaccination strategies create herd effect against HPV16 under moderate vaccination coverage achieved in a population-based, community-randomized trial.

Methods and findings

In 2007–2010, the 1992–1995 birth cohorts of 33 Finnish communities were randomized to receive gender-neutral HPV vaccination (Arm A), girls-only HPV vaccination (Arm B), or no HPV vaccination (Arm C) (11 communities per trial arm). HPV16/18/31/33/35/45 seroprevalence differences between the pre-vaccination era (2005–2010) and post-vaccination era (2011–2016) were compared between all 8,022 unvaccinated women <23 years old and resident in the 33 communities during 2005–2016 (2,657, 2,691, and 2,674 in Arms A, B, and C, respectively). Post- versus pre-vaccination-era HPV seroprevalence ratios (PRs) were compared by arm. Possible outcome misclassification was quantified via probabilistic bias analysis. An HPV16 and HPV18 seroprevalence reduction was observed post-

manuscript and supplementary files. All other relevant underlying individual-level data will be returned to Northern Finland Biobank Borealis in accordance with the signed Material Transfer Agreement. Biobank Borealis will subsequently make this individual-level data available researchers in accordance with their data access policies (contact via: biopankkiborealis@ppshp.fi).

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: M.L. has previously received grants from Merck & Co. and GSK Biologicals through his employers the Finnish Institute of Health and Welfare (THL) and the University of Tampere for HPV vaccination studies.

Abbreviations: FMC, Finnish Maternity Cohort; HBV, hepatitis B virus; HPV, human papillomavirus; HSV-2, herpes simplex virus type 2; PR, seroprevalence ratio; RPR, ratio of seroprevalence ratios.

vaccination in the gender-neutral vaccination arm in the entire study population (PR_{16} = 0.64, 95% CI 0.10–0.85; PR_{18} = 0.72, 95% CI 0.22–0.96) and for HPV16 also in the herpes simplex virus type 2 seropositive core group (PR_{16} = 0.64, 95% CI 0.50–0.81). Observed reductions in HPV31/33/35/45 seroprevalence ($PR_{31/33/35/45}$ = 0.88, 95% CI 0.81–0.97) were replicated in Arm C ($PR_{31/33/35/45}$ = 0.79, 95% CI 0.69–0.90).

Conclusions

In this study we only observed herd effect against HPV16/18 after gender-neutral vaccination with moderate vaccination coverage. With only moderate vaccination coverage, a gender-neutral vaccination strategy can facilitate the control of even HPV16. Our findings may have limited transportability to other vaccination coverage levels.

Trial registration

ClinicalTrials.gov number NCT00534638, https://clinicaltrials.gov/ct2/show/NCT00534638.

Author summary

Why was this study done?

- High-risk human papillomavirus (HPV) infection is a necessary cause of cervical cancer in females.
- HPV vaccination targeting high-risk HPV types 16 and 18 has been implemented internationally.
- Achieving herd protection for HPV16 may require greater than 80% girls-only vaccination coverage, a level that has not been achievable in many countries.
- We evaluate whether gender-neutral or girls-only HPV vaccination results in HPV16 and HPV18 herd protection when the vaccination coverage is only moderate (40%–50%).

What did the researchers do and find?

- We implemented a community-randomized trial of gender-neutral versus girls-only versus no HPV vaccination of young adolescents in 2007–2010, with 11 communities in each arm. Vaccination coverage was implemented with moderate coverage (40%–50%) at the community level.
- We evaluated the herd effect created by the different vaccination strategies by measuring the cumulative incidence of vaccine-protected HPV types in 8,022 young unvaccinated pregnant females (under 23 years old), comparing the time periods 2005–2010 (pre-vaccination) and 2011–2016 (post-vaccination).
- An HPV16 herd effect, that is, a reduction in cumulative incidence among the unvaccinated females, was only observed in communities where gender-neutral vaccination had been implemented.

What do these findings mean?

- Achieving a vaccination coverage of above 80%, which is required to achieve herd effect against HPV16, may be unrealistic in some populations. Implementing gender-neutral HPV vaccination provides a solution to this problem as the vaccination coverage threshold required to provide herd effect to unvaccinated females is lower.
- Our study finds that gender-neutral vaccination provides stronger herd effect than girls-only vaccination in the setting of moderate vaccination coverage. However, these findings are limited to this setting and are not readily generalizable to settings with high (>80%) vaccination coverage.

Introduction

The World Health Organization has called for the elimination of cervical cancer as a public health problem [1]. To this end, the WHO has developed a global strategy requiring every country globally to achieve 90% human papillomavirus (HPV) vaccination of girls by the age of 15 years by the year 2030 [2]. However, although some countries such as Scotland have achieved 90% coverage, achieving this globally may be a near impossible challenge [3,4]. Present vaccination coverage levels [5] are notably below the 80% vaccination coverage that is required for the eradication of vaccine-targeted HPV types [6], and herd effect among unvaccinated individuals is needed.

HPV vaccines provide not only strong direct protection but also herd effect/herd protection, also known as herd immunity (i.e., indirect protection to unvaccinated individuals) due to assortative transmission of the HPVs [7–10]. Modeling studies have suggested that already low to moderate vaccination coverage inclusion of boys provides incremental herd effect to unvaccinated girls [6,9,11,12]. In our unique community-randomized HPV16/18 vaccination trial, the herd effect/herd immunity created has been measured as the degree of decrease in HPV incidence/prevalence in unvaccinated women [7,13,14]. We found the predicted herd effect against vaccine-targeted HPV18, and cross-protection against HPV types 31, 33, 35, and 45, when vaccination coverage was approximately 50% [7,13,14]. In populations implementing girls-only vaccination, notable herd effect against HPV16 (the most oncogenic and most common HPV type) has only been observed when the vaccination coverage was high [15–17]. This is probably due to the high basic reproduction number (R0) of HPV16 compared to other HPV types [17], and may depend also on the method of identifying HPV occurrence (one-time PCR positivity or seropositivity, i.e., prevalence or cumulative incidence).

We performed population-based HPV analysis to evaluate the herd effect created by gender-neutral or girls-only vaccination following our community-randomized trial in the instance of low to moderate vaccination coverage. In the previous reports of this trial, the herd effect was evaluated using transitory HPV PCR positivity in study participants when they were aged 18 and/or 22 years; although notable HPV18 herd effect was observed, no HPV16 herd effect was found [12,13,18]. To provide assurance that the lack of HPV16 herd effect was not due to the methodological approach, we then nested a cross-sectional cohort within the community-randomized trial. We then estimated HPV16 seroprevalence (cumulative HPV16 incidence) over time using pre-and post-vaccination-era sera from unvaccinated women under the age of 23 years and resident in the communities with gender-neutral or girls-only vaccination strategies. Possible clearance of an ecological niche by HPV16/18 vaccination is also now

described as the natural counterpart to the serology-based type-replacement study concerning non-vaccine HPV types [18].

Methods

Study design

A population-based, community-randomized HPV vaccination trial was conducted among female and male 1992–1995 birth cohorts between 2007 and 2010 [19]. The trial was originally designed to guide evidence-based decision-making regarding national HPV vaccination policy [20,21], by testing the primary hypothesis of difference in the creation of herd effect by gender-neutral versus girls-only HPV vaccination strategies. Thirty-three geographically distinct Finnish communities located a minimum of 50 km from the next nearest community (or 35 km in the case of the 5 communities from the Helsinki metropolitan area) were included in the trial. To increase study power, the coefficient of variation, Ks (Ks = 0.13), between communities was minimized by first stratifying the communities by previously ascertained HPV16/18 seroprevalence [22] into those with low, moderate, and high seroprevalence. From these 3 strata, the communities were then randomized using a random number generator to 3 trial arms: In Arm A communities, 90% of girls and boys received HPV vaccination, and 10% of girls and boys received hepatitis B virus (HBV) vaccination; in Arm B communities, 90% of girls received HPV vaccination, and 10% of girls and all boys received HBV vaccination; in Arm C communities, all girls and boys received HBV vaccination.

In total, 80,272 Finnish- or Swedish-speaking girls and boys in the 1992–1995 birth cohorts were identified via the Finnish Population Register Centre as being resident in the 33 trial communities. Out of this group, 20,513 girls and 11,662 boys participated in the trial with parental/guardian informed consent. The study was partially blinded to all Arm A study participants and all female Arm B participants. Vaccination took place from 2007 to 2010, when the participants were aged 12–15 years, with 99.4% of participants receiving all 3 doses of the allocated vaccine (the bivalent HPV vaccine Cervarix or the HBV vaccine Engerix-B). The mean community-level vaccination coverage acquired via this vaccination was 47.1% in Arm A communities and 45.8% in Arm B communities among girls from the 1992–1995 birth cohorts (standard deviation [SD] = 9.4% and 6.6%, respectively). In Arm A communities the vaccination coverage acquired among boys from the 1992–1995 birth cohorts was 19.5% (SD = 7.1%) [19,20].

The creation of herd effect by different HPV16/18 vaccination strategies over time was estimated via a nested cross-sectional cohort study [23] of all pregnant women under the age of 23 years who were resident in the 33 trial communities from 2005 until the end of 2016. Their serum samples were extracted from a population-representative biobank, the Finnish Maternity Cohort (FMC) [18,21]. The FMC biobank houses 2 million serum samples obtained from approximately all 1 million pregnant Finnish women between 1983 and 2016 for screening of congenital infections. The participating women provided informed consent at the maternity clinic to have their samples stored for research purposes by the FMC biobank; 96% of women consented.

FMC participants eligible for this study were under the age of 23 years at the time of sample donation, first-time donators to the FMC, resident in 1 of the 33 trial communities, and HPV unvaccinated [18]. In Finland, every citizen (or person resident for greater than 3 months) is given a unique personal identification number at birth (or shortly after arrival into the country). HPV vaccination status was confirmed by linkage via the participants personal identification number with the national HPV vaccination trial registry both prior to and after sample extraction. For the birth cohorts eligible to receive HPV vaccination via the Finnish national

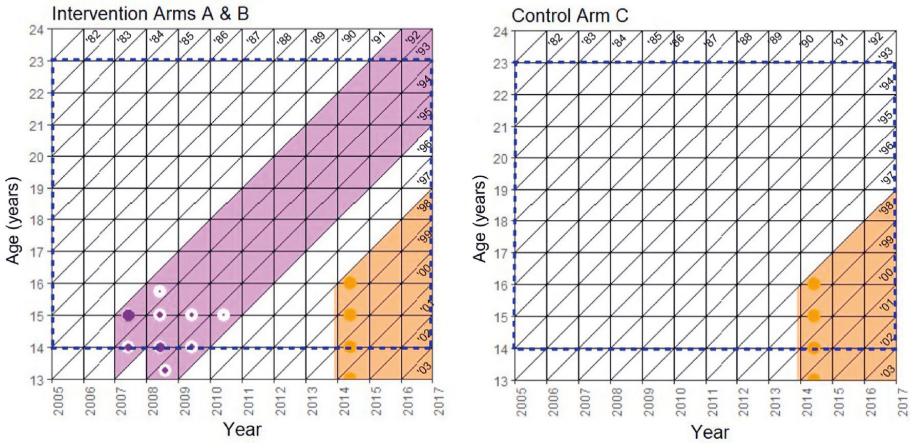

Fig 1. Lexis diagrams depicting the community-level exposure of the adolescent population to direct and indirect effects of the cluster-randomized human papillomavirus vaccination trial by birth cohort and study arm. hite bars represent the birth cohorts with no vaccination, and the purple (trial vaccination) and orange (national vaccination) bars represent post-vaccination birth cohorts. The blue dashed lines indicate the sampling years and ages of this study. The colored fill of the symbols indicates the proportion of each type of vaccination that took place at that time point and age per birth cohort.

https://doi.org/10.1371/journal.pmed.1003588.g001

vaccination program (1998 and younger birth cohorts), HPV vaccination status was ascertained by manually scrutinizing participants' HPV antibody levels for titers indicative of HPV vaccination (i.e., multiple-fold those acquired via natural infection for HPV16 and HPV18).

The eligible participants for the serosurvey came from the 1982 and younger birth cohorts. The 1992 to 1995 birth cohorts were exposed to community-level vaccination via the community-randomized trial intervention, and the 1998 and younger birth cohorts were exposed to community-level HPV vaccination via the Finnish national HPV vaccination program initiated in late 2013 (Fig 1 and S1 Fig). The sampling time frame was divided into the pre-vaccination period (2005–2010) and the post-vaccination period (2011–2016). All the pregnant females under the age of 23 years at the time of sample donation from each of the trial communities were included, totaling 8,022 females.

Data regarding self-reported maternal smoking among women under the age of 23 years and resident in the 33 communities between 2005 and 2016 were collected from the Finnish Medical Birth Register, and used as a surrogate of community-level risk-taking behaviors. To define the core group with high contact rate, we identified herpes simplex virus type 2 (HSV-2) seropositive women [18]. Data on community-specific vaccination coverage over each calendar year were collected from the HPV trial registry for the birth cohorts exposed to the community-randomized trial and from the Finnish vaccination register for the birth cohorts exposed to the Finnish national HPV vaccination program.

This study is reported in accordance to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline (S1 Checklist).

Ethics

The community-randomized HPV-040 study obtained permissions from the Ethical Review Board of Pirkanmaa Hospital District (R07113M 14.6.2007). The FMC steering committee

granted permission for the linkage and use of the serum samples. No harm was caused to the cohorts.

Laboratory analyses

The serum samples were analyzed for the presence of IgG antibodies to HPV types 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73 and HSV-2 using multiplexed heparin-bound pseudovirion (and HSV-2 glycoprotein gG2) Luminex assay [24]. Seropositivity cutoff levels were established with a negative control panel of serum samples from 191 children $\leq$12 years old (mean age = 4.7 years) (S1 Text).

Statistical analyses

The primary hypothesis of this study was that HPV16/18 vaccination created a herd effect (in the HPV-040 and type-replacement study protocols, this was called "indirect effect" or "ecological niche formation") over time. In this study, herd effect is defined and measured as the degree of decrease in HPV cumulative incidence (unattributable to random or systematic error) among unvaccinated individuals in the post-vaccination era. To investigate the herd effect (indirect effect) of increasing community-level HPV16/18 vaccination during the study period (via a gender-neutral or girls-only vaccination strategy), we calculated the absolute seroprevalence of vaccine-targeted HPV types 16, 18, and 16/18 (combined), and vaccine-cross-protected HPV types 31, 33, 35, 45, and 31/33/35/45 (combined). This was calculated for the pre- and post-vaccination eras, 2005–2010 and 2011–2016, respectively. In the case of the former, the participants who had donated the sera were likely to have been unexposed to the indirect effects of HPV vaccination, whereas in the case of the latter, the participants may have been under herd effect [14,19,20].

The degree of clustering of HPV16/18 and HPV16/18/31/33/35/45 seropositivity was assessed by calculation of the intracluster correlation coefficient from the pre-vaccination-era data (from 2005 to 2010) using Fleiss and Cuzick's estimator in combination with Zou and Donner's modified Wald test to compute the 95% confidence intervals [25,26].

The exposure in this study is defined as exposure to the herd effects (indirect effects) of HPV16/18 vaccination due to residing at the time of sample donation in one of the communities of the community-randomized trial. Thus, to evaluate the extent of exposure in the study population of pregnant females under the age of 23 years, the birth-cohort-, community-, and year-specific vaccination coverage was calculated. From this, the community-specific vaccination coverage by year among the study population was then calculated as the birth-cohort-weighted vaccination coverage by gender, weighted by the proportion of participants from each birth cohort found in each year of the study among the study population of pregnant females. We also calculated HSV-2 seroprevalence to assess changes in the occurrence of sexually transmitted infections between the pre- and post-vaccination eras. Calendar-time-specific absolute seroprevalence was calculated stratified by Arms A, B, and C of the community-randomized trial. The accompanying 95% confidence intervals were calculated using the Agresti–Coull method [27].

To further assess the indirect effect of community-level vaccination in the post-vaccination era, we estimated within-arm seroprevalence ratios (PRs) comparing the post- to pre-vaccination HPV-type-specific seroprevalence (for HPV types 16, 18, 31, 33, 35, 45, and 16/18 combined) using a log binomial generalized estimating equation (GEE) model to take account of within-arm clustering. HPV-type-specific seroprevalence was not directly compared between the arms as stated in the pre-analysis plan (S2 Text), as statistically significant differences were found between the arms at baseline prior to any HPV vaccination. To take account of possible

confounding, all estimates were adjusted for community-level self-reported maternal smoking, as a surrogate of community-level risk-taking behaviors. To investigate the effect of core group membership (a possible effect modifier) on the indirect effect, the secondary outcome of the study, we stratified the estimates by HSV-2 seropositivity.

To estimate the overall herd effect (the indirect effect) of gender-neutral and girls-only HPV vaccination compared to the counterfactual scenario, we further calculated the between-arm ratio of PRs, comparing the within-arm PR (adjusted for community-level maternal smoking) of Arm A or B (the intervention arms) to the PR of the control Arm C. The accompanying 95% confidence intervals were calculated according to the methodology of Altman and Bland [28].

Systematic outcome misclassification of the serological assay was quantified and corrected for assuming non-differential bias of the within- and between-arm estimates via probabilistic bias analysis [29]. Previously obtained estimates of test sensitivity and specificity were used at the outset (S1 Table) [30], assuming a constant probability distribution. If these prior estimates proved incompatible with the observed data, then a uniform probability density ranging from 0 to 1 was specified, to obtain all plausible values of the sensitivity or specificity compatible with the observed data. The resultant range of plausible values for the given HPV-type-specific sensitivity or specificity was then assumed, with a uniform probability density ranging from the given minimum to maximum value. The results from this sensitivity analysis were then used to quantify misclassification in the primary analysis.

All statistical analyses were conducted using the R statistical software package (version 3.6.0.).

Results

Baseline characteristics of the study population

In total, all 8,022 HPV-unvaccinated pregnant females under the age of 23 years who were resident in one of the 33 trial communities and had been invited to donate a blood sample to the FMC between the years 2005 and 2016 were identified. An additional 3,498 females were initially found to be ineligible due to being HPV vaccinated. In total, 4,007 participants were from the era preceding completion of vaccination (2005–2010), and 4,015 were from the post-vaccination era (2011–2016). Participants were excluded from the statistical analyses owing to HPV vaccination ($N = 49$) or being aged >22 years at sample donation ($N = 436$). In total, 7,531 women were included: 1,322, 1,289, and 1,304 from the pre-vaccination-era Arm A, B, and C communities, respectively, and 1,247, 1,158, and 1,211 from the same post-vaccination-era communities (Fig 2). The intracluster correlation coefficient was consistently low, at 0.007 for HPV16/18 seropositivity and 0.005 for HPV16/18/31/33/35/45 seropositivity (S2 Table).

The participants' age distributions in the pre-vaccination and post-vaccination eras were comparable, with the majority being 18 to 22 years old (S3 Table). The HSV-2 seroprevalence was materially similar between the arms, but somewhat higher in the pre-vaccination era as compared to the post-vaccination era (17.8% and 15.0%, respectively) (Fig 3). Community-level self-reported smoking was consistently higher in the control Arm C communities than in the gender-neutral vaccination Arm A and girls-only vaccination Arm B communities (S3 Table). The community-specific vaccination coverage among the eligible female birth cohorts for this study was negligible in the pre-vaccination era, from 2005 until 2010, and increased in the post-vaccination era in the intervention arm communities (from 5.6% to 52.5% in Arm A, and from 6.3% to 46.7% in Arm B) (Fig 4).

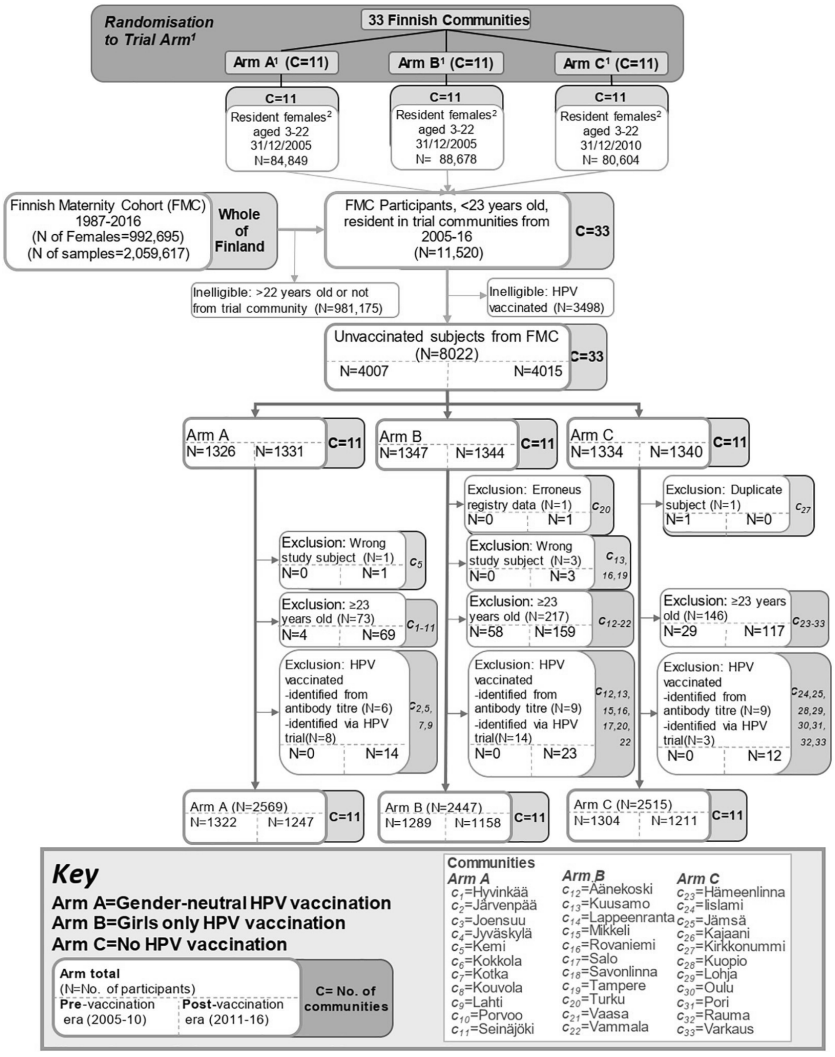

Fig 2. Flow chart of the cross-sectional cohort study nested in the Finnish community randomized human papillomavirus (HPV) vaccination trial with stepwise subsequent exclusions. [1]The arms are the trial arms from the cluster (community) randomized trial of HPV vaccination strategy, conducted in 2007–2010.[2]Includes all females aged 3–22 years who were resident in the communities specified as of the 31 December 2005 (data extracted from Statistics Finland).

https://doi.org/10.1371/journal.pmed.1003588.g002

HPV seroprevalence by vaccination era

In the pre-vaccination era, HPV16/18 seroprevalence was high: 29.7%, 29.6%, and 26.8%, respectively, in the Arm A, B, and C communities. In the post-vaccination era, HPV16/18 seroprevalence was somewhat decreased (23.6%) in the gender-neutral vaccination Arm A

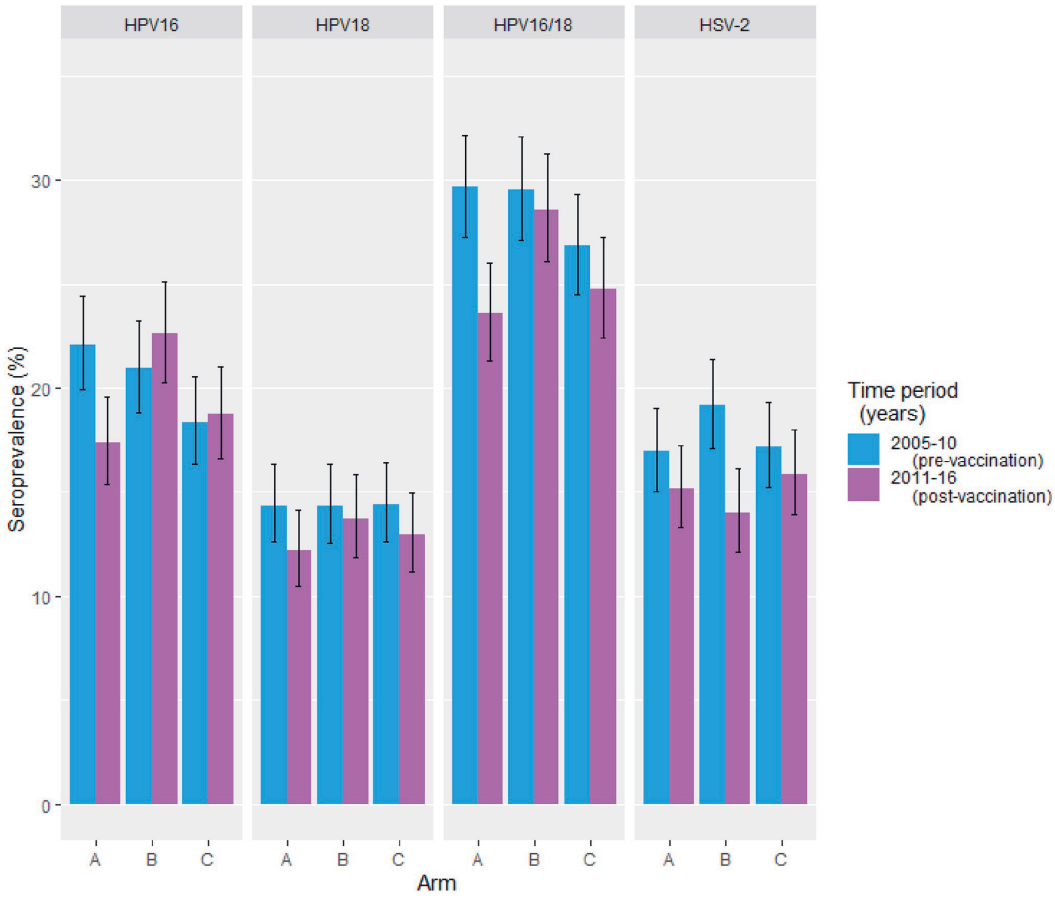

Fig 3. Type-specific human papillomavirus (HPV) and herpes simplex virus type 2 (HSV-2) seroprevalence (%) among unvaccinated females under the age of 23 years by intervention strategy: Gender-neutral vaccination (Arm A), girls-only vaccination (Arm B), and control vaccination (Arm C). Type-specific seroprevalence is stratified by time period of sample donation (pre-vaccination era, 2005–2010; post-vaccination era, 2011–2016).

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communities (Fig 3; S4 Table). Notably, the HPV16 seroprevalence was decreased in the Arm A communities in the post-vaccination era compared to the pre-vaccination era (17.4% versus 22.1%). No decrease in HPV16 seroprevalence was noted in the girls-only vaccination Arm B or control C communities (Fig 3; S4 Table).

Within-arm post- versus pre-vaccination-era HPV PRs

The within-arm HPV16/18 PR comparing the post- to the pre-vaccination era was notably decreased in Arm A. The HPV16/18 estimate was significantly somewhat decreased in the gender-neutral vaccination Arm A ($PR_{16/18}$ = 0.80, 95% CI 0.74–0.87), whereas in the girls-only vaccination Arm B and control Arm C, no significant reductions were noted ($PR_{16/18}$ = 0.98,

Fig 4. Evaluation of human papillomavirus (HPV) vaccination coverage in the study population: Community-specific birth-cohort-weighted vaccination coverage of the consecutive community-randomized trial and national girls-only vaccination program. Exposure to the indirect effects of HPV16/18 vaccination is defined as residing at the time of sample donation in one of the community-randomized HPV vaccination trial communities. Each row represents a trial community, and each column a year of the follow-up period. The community-specific vaccination coverage is calculated for pregnant females under the age of 23 years and includes vaccination of 12- to 15-year-old males and females in 2007–2010, and the national girls-only vaccination program launched in late 2013.

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95% CI 0.85–1.12, in Arm B; $PR_{16/18}$ = 0.91, 95% CI 0.81–1.03, in Arm C) (Table 1). The HPV16 PR specifically was decreased in Arm A (PR_{16} = 0.79, 95% CI 0.72–0.87). No corresponding decrease was observed in Arms B or C (PR_{16} = 1.09, 95% CI 0.91–1.32, in Arm B; PR_{16} = 1.01, 95% CI 0.86–1.20, in Arm C) (Table 1). After applying probabilistic bias analysis to correct for outcome misclassification, the within-arm HPV16/18 and HPV16 PR estimates in Arm A were found to be further decreased ($PR_{16/18}$ = 0.66, 95% CI 0.10–0.85, and PR_{16} = 0.64, 95% CI 0.09–0.86, respectively). Also, the within-arm PR estimate for HPV18 was significantly decreased in the gender-neutral vaccination Arm A after accounting for the error due to outcome misclassification (PR_{18} = 0.72, 95% CI 0.21–0.96) (Table 1).

Table 1. Post- versus pre-vaccination HPV-type-specific adjusted seroprevalence ratio (PR) among unvaccinated Finnish females aged under 23 years.

HPV type	Post- versus pre-vaccination-era PR (95% CI)		
	Arm A (*N* = 1,247 versus 1,322)	Arm B (*N* = 1,158 versus 1,289)	Arm C (*N* = 1,211 versus 1,304)
Accounting for random error			
16	0.79 (0.72–0.87)	1.09 (0.91–1.32)	1.01 (0.86–1.20)
18	0.86 (0.70–1.06)	0.96 (0.74–1.24)	0.89 (0.70–1.13)
16/18	0.80 (0.74–0.87)	0.98 (0.85–1.12)	0.91 (0.81–1.03)
31	0.90 (0.79–1.01)	0.86 (0.73–1.02)	0.72 (0.57–0.91)
33	1.05 (0.88–1.26)	0.94 (0.77–1.14)	0.81 (0.63–1.03)
35	0.70 (0.52–0.94)	0.98 (0.64–1.51)	0.77 (0.58–1.01)
45	0.89 (0.69–1.14)	1.01 (0.76–1.36)	0.90 (0.64–1.26)
Accounting for random error and systematic error			
16	0.64 (0.09–0.86)	1.19 (0.98–3.70)	1.07 (0.89–1.85)
18	0.72 (0.21–0.96)	0.89 (0.39–1.12)	0.79 (0.21–1.03)
16/18	0.66 (0.10–0.85)	0.92 (0.44–1.07)	0.84 (0.24–1.01)
31	0.79 (0.20–1.00)	0.75 (0.15–0.97)	0.55 (0.07–0.78)
33	1.14 (0.86–2.73)	0.85 (0.31–1.13)	0.66 (0.15–0.94)
35	0.52 (0.07–0.83)	0.90 (0.44–1.25)	0.59 (0.10–0.91)
45	0.73 (0.19–1.06)	1.01 (0.78–1.33)	0.79 (0.25–1.13)

Comparisons are between 2 time periods of sample donation (2011–2016, post-vaccination era, versus 2005–2010, pre-vaccination era), stratified by intervention Arm A (gender-neutral HPV vaccination), Arm B (girls-only HPV vaccination), and Arm C (control vaccination), accounting for random error and accounting for random error and systematic error due to outcome misclassification. The estimates corrected for random error only are adjusted for community-level smoking. Corresponding unadjusted estimates are displayed in S5 Table.

https://doi.org/10.1371/journal.pmed.1003588.t001

The HPV35 PR estimate was significantly decreased in the gender-neutral vaccination Arm A (PR_{35} = 0.70, 95% CI 0.52–0.94; Table 1). However, this finding appeared to be essentially replicated in the control Arm C (PR_{35} = 0.77, 95% CI 0.58–1.01; Table 1). No decrease in HPV35 seroprevalence was observed in the girls-only vaccination Arm B (PR_{35} = 0.98, 95% CI 0.64–1.51). HPV31 was non-significantly slightly decreased in both the gender-neutral vaccination Arm A and the girls-only vaccination Arm B (PR_{31} = 0.90, 95% CI 0.79–1.01, in Arm A; PR_{31} = 0.86, 95% CI 0.73–1.02, in Arm B), but was further decreased in the control Arm C with no HPV vaccination (PR_{31} = 0.72, 95% CI 0.57–0.91). HPV33 was not decreased in Arm A (PR_{33} = 1.05, 95% CI 0.88–1.26), while it approximately stayed the same in Arm B (PR_{33} = 0.94, 95% CI 0.77–1.14) and non-significantly decreased slightly in Arm C (PR_{33} = 0.81, 95% CI 0.63–1.03). On the other hand, HPV45 was non-significantly marginally decreased in Arm A (PR_{45} = 0.89, 95% CI 0.69–1.14), approximated the null in Arm B (PR_{45} = 1.01, 95% CI 0.76–1.36), and in Arm C was decreased in a similar manner as in Arm A (PR_{45} = 0.90, 95% CI 0.64–1.26) (Table 1).

The HPV16 PR estimate was also noticeably decreased among the HSV-2 seropositive individuals in the gender-neutral vaccination Arm A (PR_{16} = 0.64, 95% CI 0.50–0.81). Most estimates for vaccine-protected HPV types were also decreased among the HSV-2 seropositive individuals, especially in Arm A (PR_{31} = 0.74, 95% CI 0.53–1.02; PR_{35} = 0.57, 95% CI 0.37–0.88; PR_{45} = 0.64, 95% CI 0.37–1.08), albeit sometimes with borderline statistical significance. The findings for HPV31 and HPV45 were, however, replicated in the control Arm C (PR_{31} = 0.64, 95% CI 0.42–0.98; PR_{45} = 0.69, 95% CI 0.37–1.30) (S6 Table).

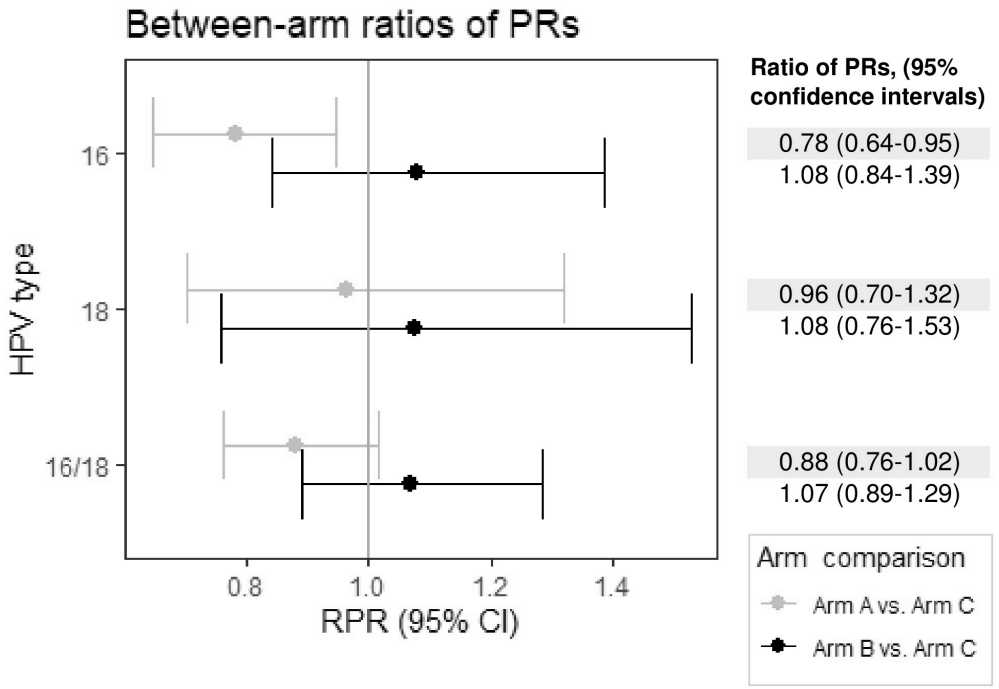

Fig 5. Ratio of human papillomavirus (HPV) seroprevalence ratios (PRs) comparing Arm A/B to Arm C. Arm-specific PRs comprise post-vaccination to pre-vaccination-era HPV PRs among pregnant unvaccinated Finnish females, aged under 23 years, and adjusted for community-level maternal smoking. RPR, ratio of seroprevalence ratios.

https://doi.org/10.1371/journal.pmed.1003588.g005

Between-arm comparison of the post- versus pre-vaccination-era PRs

To account for possible secular trends, between-arm comparisons of the within-arm post- versus pre-vaccination-era PRs were made, comparing the ratios from HPV vaccination arms to the ratios from the control Arm C. The HPV16 ratio of PRs (RPR), remained decreased when comparing the gender-neutral vaccination Arm A to the control Arm C (RPR_{16} = 0.78, 95% CI 0.64–0.95) (Fig 5).

Discussion

We nested a cross-sectional cohort within a population-based, community-randomized HPV16/18 vaccination trial to estimate changes over time in HPV16/18 seroprevalence created by gender-neutral or girls-only vaccination strategies, using pre- and post-vaccination-era sera from unvaccinated women resident in the trial communities. The HPV16 and HPV18 seroprevalence was somewhat decreased in young unvaccinated women after gender-neutral vaccination. This was observed although the vaccination coverage was only moderate to low. Most importantly, a degree of partial herd effect against HPV16 was observed over time within the gender-neutral vaccination arm, when compared to the counterfactual control arm, and within the HSV-2 seropositive core group, representing those with high contact rate. Girls-only HPV

vaccination with moderate vaccination coverage did not result in any notable HPV16 herd effect.

The level of vaccination coverage required for herd effect is a function of a given HPV type's basic reproduction number. This in turn is a function of the effective transmission rate and the mean duration of infection, which for HPV16 is especially long. Thus, the vaccination coverage required to achieve herd effect against HPV16 is expected to be high, higher than for other HPV types [14], and for girls-only vaccination this indeed seems to be the case [16]. Furthermore, the predicted herd effect is 25% to 50% greater for a gender-neutral than for a girls-only vaccination scenario [23]. Our study provides empirical evidence that when vaccination coverage is suboptimal, a gender-neutral vaccination strategy optimizes HPV16 herd effect and thus effectiveness of vaccination.

This observation is of major importance, if the call for action to eliminate cervical cancer is realistically going to be achieved. The gender-neutral vaccination strategy, with its sturdier impact on both HPV16 and HPV18, may assist in overcoming the obstacle of suboptimal girls-only vaccination coverage.

Apart from HPV16 being the most oncogenic HPV type, both dynamic transmission models and randomized trials have suggested that HPV16 is the most difficult to achieve herd protection against [14,17]. The vaccination coverage required to achieve herd protection against a given vaccine-protected type in addition to being strategy-dependent is also population-dependent [17]. The observation of a degree of partial HPV16 herd effect also in the core group following gender-neutral vaccination is reassuring, since modeling studies have suggested that the existence of the core group defies creation of herd effect [17]. When using PCR for the determination of current HPV infections, and concomitant *Chlamydia trachomatis* infection as a proxy of sexual risk-taking behavior, only HPV18 herd effect has been observed among the core group [31]. Whereas the seroprevalence comparisons documented a HPV16 herd effect in those with 'ever' core group membership, with HSV-2 seropositivity as the proxy. Thus, implementing a gender-neutral vaccination strategy is likely to deliver also on targets of equity in eliminating cervical cancer.

Previously, when following up the 1992–1995 birth cohorts of our community-randomized trial, no evidence of HPV16 herd effect among unvaccinated females was observed with PCR-defined endpoints [13]. DNA positivity as identified by one-time PCR-positivity is not a measure of cumulative infection and is also subject to outcome misclassification owing to its inability to distinguish persistent infections from transient depositions [32]. The resulting suboptimal specificity probably biased the previous estimates of HPV16/18 herd effect [13], and likely resulted in an underestimation of the true effects.

Sensitivity analyses assuming that serology has imperfect sensitivity to identify cumulative HPV exposure found that the degree of misclassification was HPV type specific. Furthermore, the previous validation methods [30] may have underestimated the true specificity. The remaining misclassification biased the estimates towards the null point. The previously reported sensitivity estimates were particularly low for HPV18. After quantifying and correcting for this bias, the HPV18 PR estimates were in line with earlier PCR-based observations of HPV18 herd effect after gender-neutral vaccination [13].

This study is limited by the imperfect ability of HPV serology to identify all cumulative HPV infection. However, HPV antibodies are a measure of persistent infection, thus by identifying cumulative infection by seroconversion, we identify women with true persistent HPV infection and exclude the apparent issue of the absence of HPV seroconversion in a proportion of women who have had only an HPV deposition.

Earlier studies were restricted to the birth cohorts that participated in the community-randomized trial [13]. We now also included females from the unvaccinated birth cohorts, 1996–

1997, subsequent to the trial cohorts. Because HPV is a sexually transmitted virus, HPV transmission moves in the direction of the older to younger birth cohorts. Thus, the herd effect was expected to be stronger in the younger vaccinated cohorts [14]. Probably the older vaccinated male and female 1992–1995 birth cohorts have conferred indirect protection to the subsequent birth cohorts in the gender-neutral vaccination arm, due to the disruption in HPV transmission [33].

This is to our knowledge the only serosurvey among unvaccinated females following a community-randomized trial of different vaccination strategies. A previous serosurvey conducted in Australia among unvaccinated males to evaluate the first-order herd effect of girls-only vaccination found somewhat decreased HPV16/18 seropositivity in its post-vaccination era [34]. However, males seroconvert following HPV infections at lower rates than females, thus resulting in a possible underestimation of the true reduction in infection prevalence [35].

This study is strengthened by its utilization of the Finnish infrastructure of population-based intervention cohorts, biobanks, and registries, linkable via unique personal identification numbers. Our extraction of serum samples from all eligible participants in the population-based FMC [21] provided a sufficient population-representative sample size to evaluate vaccination-strategy-specific differences in HPV seroprevalence and account for random error. Further to this, our sampling of only pregnant females under 23 years at the time of sample donation captures the age distribution at which the HPV incidence curve peaks and the demographic most at risk.

This study may be limited in its generalizability, as the average age of mothers at first live birth in Finland is approximately 29 years [36]. It is possible that our study population of pregnant females under 23 years have above-average sexual risk-taking behaviors and lifetime risk of acquiring HPV. Our results may have incomplete transportability to populations that have differing baseline risk and sexual network structures This study is also limited to the setting of moderate vaccination coverage; therefore, the findings are not generalizable to scenarios with greater vaccination coverage. Furthermore, the findings may also have incomplete generalizability to a scenario with more consecutively vaccinated birth cohorts and a longer period of time between vaccination initiation and follow-up. Given our inclusion criteria in this study, it is also possible that participants may have moved between communities between the commencement of sexual activity and sample donation, which may introduce some bias in our estimates. However, given that the participants were all pregnant females under the age of 23 years, it is possible that they are the portion of source population that are least likely to move to a new community.

It may also be possible that changes over time in the sexual network or risk-taking behavior have altered HPV seroprevalence even in the absence of vaccination. The observed changes in HSV-2 seroprevalence could conceivably be interpreted as evidence of this. However, HSV-2 epidemiology has globally been undergoing complex changes in recent decades, with HSV-1 increasing and HSV-2 decreasing as the main cause of genital herpes infections [37]. Therefore, the observed decrease in HSV-2 seropositivity over time is not entirely unexpected and may be independent of any changes in HPV incidence over the time frame. However, with respect to this, our study is strengthened by its design, as the within-arm seroprevalence comparisons in Arm C, where no HPV vaccination was applied, provided us with a counterfactual estimate to tackle such possible secular trends.

Our results suggest that when HPV vaccination coverage is moderate, only gender-neutral vaccination establishes herd effect against HPV16 and HPV18 among unvaccinated females. This finding supports the implementation of a gender-neutral HPV vaccination policy to achieve optimal vaccine effectiveness when obtaining a girls-only vaccination coverage of 90% is impossible.

Supporting information

S1 Checklist. STROBE checklist.
(DOCX)

S1 Fig. Lexis diagrams depicting the vaccinated cohorts and vaccination coverage among the eligible birth cohorts of the study population, by arm and gender. (a) Among females; (b) among males.
(DOCX)

S2 Fig. Type-specific human papillomavirus (HPV) seroprevalence (%) among unvaccinated females under the age of 23 years by vaccination strategy—gender-neutral vaccination (Arm A), girls-only vaccination (Arm B), and control vaccination (Arm C)—and time period of sample donation (pre-vaccination era, 2005–2010, and post-vaccination era, 2011–2016).
(DOCX)

S3 Fig. Ratio of HPV seroprevalence ratios (RPR) comparing Arm A/B to Arm C. Arm-specific PRs comprise post-vaccination to pre-vaccination-era HPV seroprevalence ratios among pregnant unvaccinated Finnish females aged under 23 years old, and are adjusted for community-level maternal smoking.
(DOCX)

S1 Table. Sensitivity and specificity parameters of pseudovirion-based serology in measuring cumulative HPV exposure used in the probabilistic bias analysis.
(DOCX)

S2 Table. Intracluster correlation coefficient (ICC) of any human papillomavirus (HPV) type seropositivity among pregnant females donating sera during the pre-vaccination era, 2005–2010. HSV-2, herpes simplex virus type 2.
(DOCX)

S3 Table. Characteristics of the study population after exclusions owing to ineligibility.
(DOCX)

S4 Table. Absolute HPV-type-specific seroprevalence among unvaccinated pregnant Finnish women stratified by trial arm and vaccination era (2005–2010 is defined as the "pre-vaccination era" and 2011–2016 as the "post-vaccination era"), and additionally by HSV-2 seropositivity.
(DOCX)

S5 Table. Unadjusted HPV-type-specific seroprevalence ratio (PR) among unvaccinated Finnish females comparing the post-vaccination era to the pre-vaccination era. Comparisons are between 2 time periods of sample donation (2011–2016, post-vaccination era, versus 2005–2010, pre-vaccination era), stratified by intervention Arm A (gender-neutral HPV vaccination), Arm B (girls-only HPV vaccination), and Arm C (control vaccination).
(DOCX)

S6 Table. Adjusted seroprevalence ratio (PR) of HPV seropositivity by HPV type among pregnant, unvaccinated Finnish females under the age of 23 years by study arm (gender-neutral vaccination Arm A, girls-only vaccination Arm B, or control Arm C), comparing time period of sample donation (post-vaccination era, 2011–2016, compared to the pre-vaccination era, 2005–2010), and stratified by herpes simplex virus type 2 serostatus. All

estimates are adjusted for smoking. na, not available.
(DOCX)

S1 Text. Supplementary methods (laboratory analysis and statistical analysis).
(DOCX)

S2 Text. Prospective pre-analysis plan.
(PDF)

S3 Text. Trial protocol and report analysis plan (HPV-040 trial).
(PDF)

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PUBLICATION III

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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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

Key words: HPV, type replacement, vaccination, randomized trial
P.G. and J.P. Contributed equally to this work.
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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,

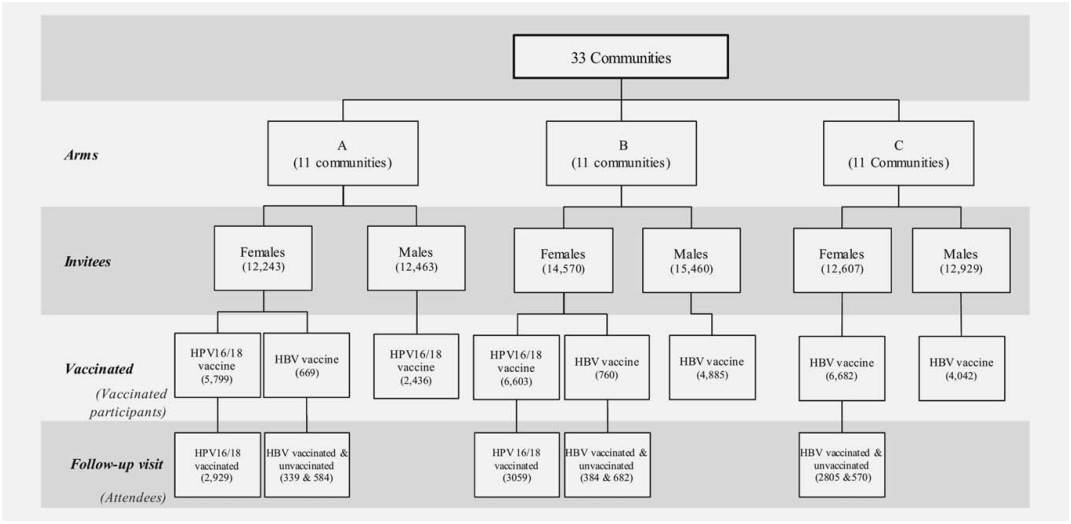

Figure 1. Flow chart of the community-randomized trial. [Color figure can be viewed at wileyonlinelibrary.com]

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).

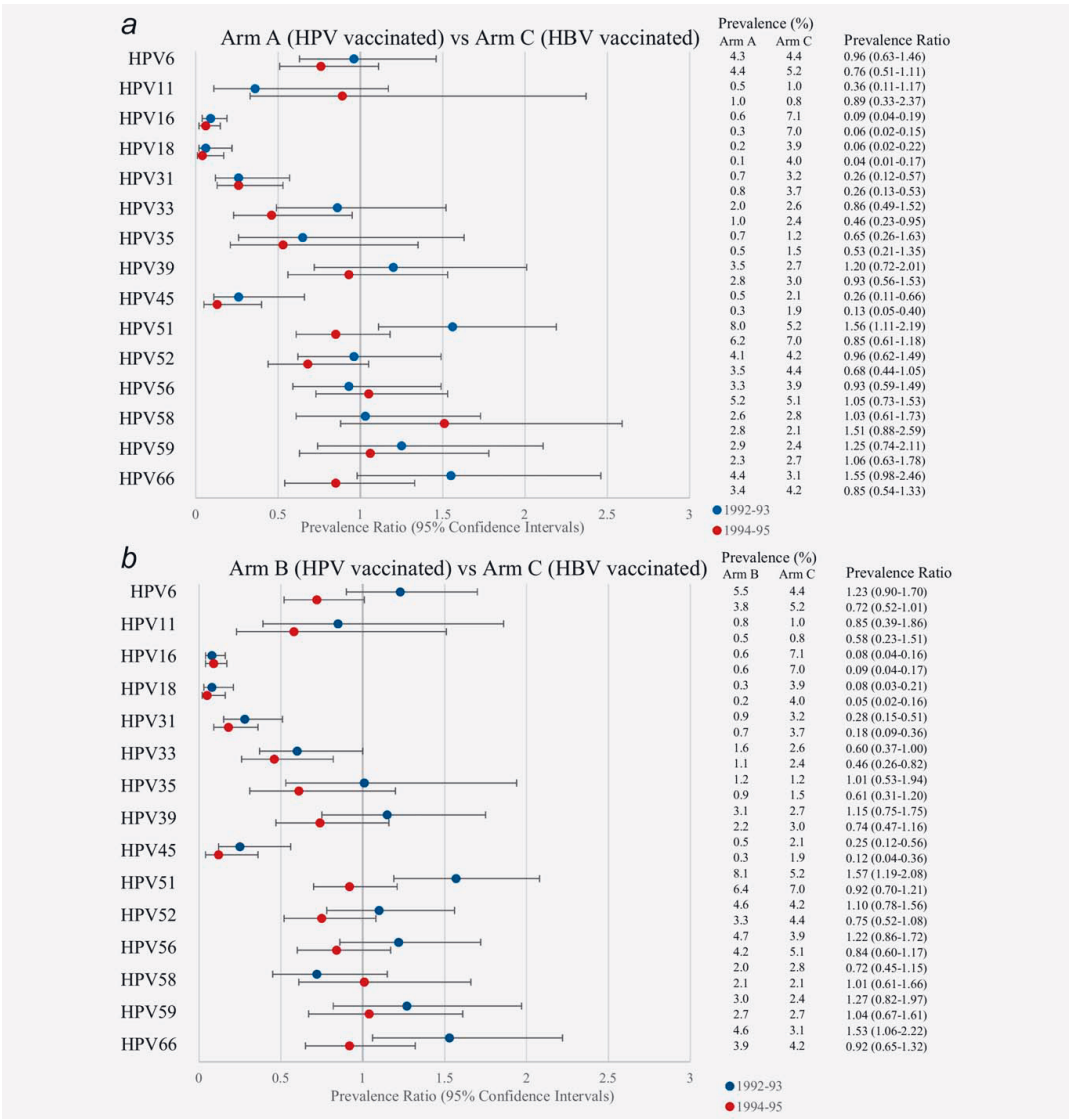

Figure 2. PR (95% CI) estimates of HPV types in HPV16/18 vaccinated, (*a*) and (*b*), and in non-HPV vaccinated, (*c*) and (*d*), stratified by birth cohort and adjusted for mobility, and smoking and *C. trachomatis* status. (*a*) Arm A versus Arm C, (*b*) Arm B versus Arm C, (*c*) Arm A versus Arm C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

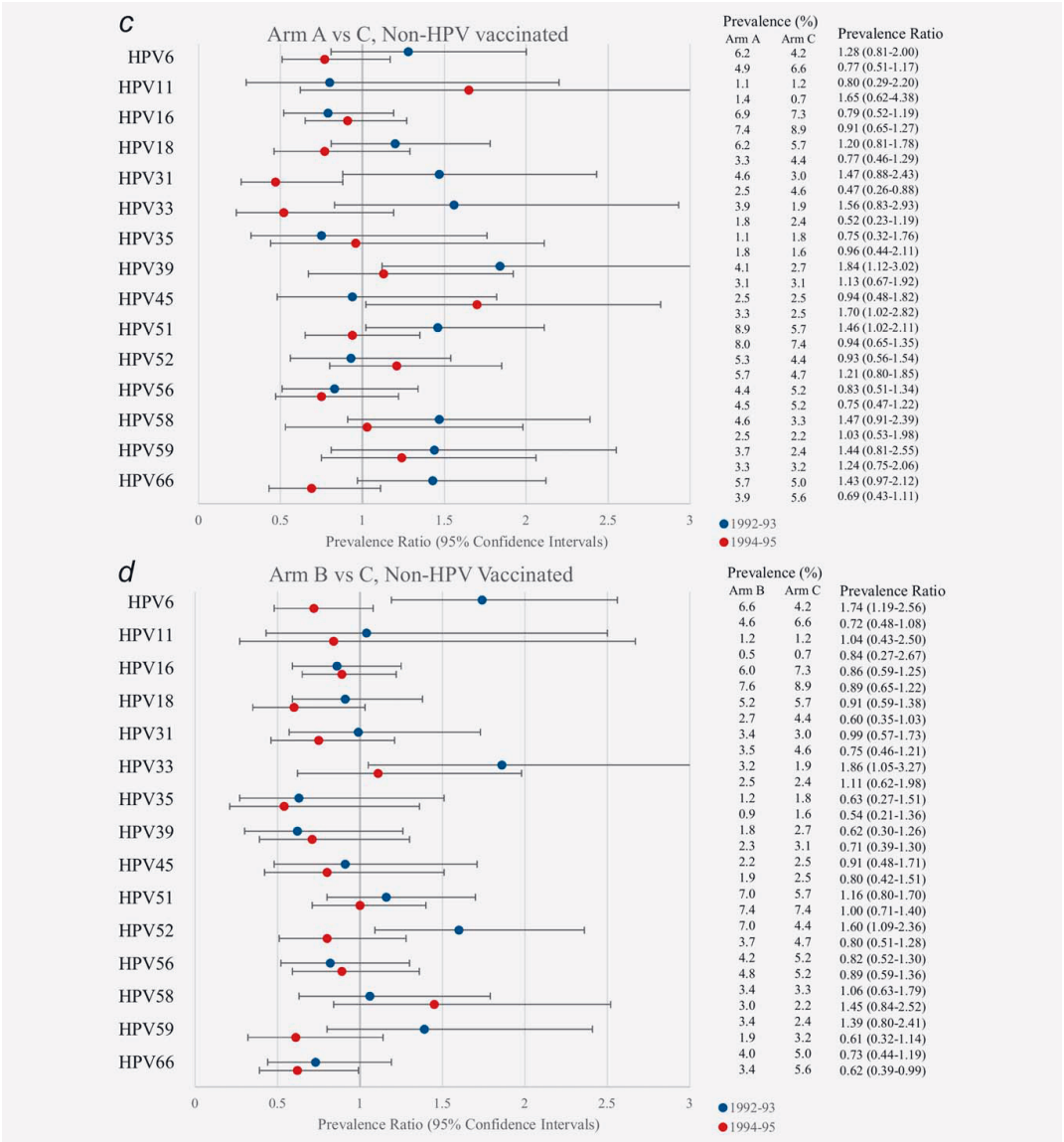

Figure 2. Continued.

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

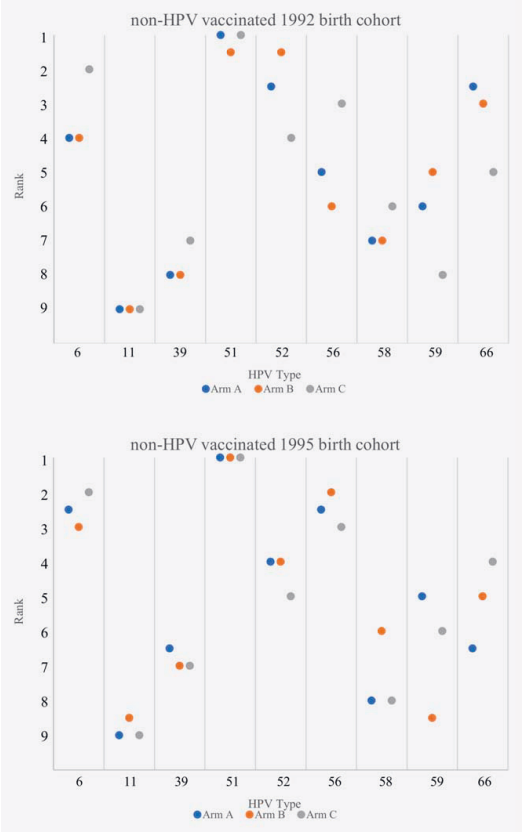

Figure 3. Ranked distribution of non-vaccine HPV types in non-HPV vaccinated women in the 1992 birth cohort [$r_s^{(Avs.C)} = 0.86$, $r_s^{(Bvs.C)} = 0.79$] and 1995 birth cohort [$r_s^{(Avs.C)} = 0.95$, $r_s^{(Bvs.C)} = 0.91$].

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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PUBLICATION IV

Occurrence of human papillomavirus (HPV) type replacement by sexual risk-taking behaviour group: Post-hoc analysis of a community randomized clinical trial up to 9 years after vaccination (IV)

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Occurrence of human papillomavirus (HPV) type replacement by sexual risk-taking behaviour group: Post-hoc analysis of a community randomized clinical trial up to 9 years after vaccination (IV)

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Oncogenic non-vaccine human papillomavirus (HPV) types may conceivably fill the vacated ecological niche of the vaccine types. The likelihood of this may differ by the risk of acquiring HPV infections. We examined occurrence of HPV types among vaccinated and unvaccinated subgroups of 1992–1994 birth cohorts with differing acquisition risks up to 9 years post-implementation of HPV vaccination in 33 Finnish communities randomized to: Arm A (gender-neutral HPV16/18 vaccination), Arm B (girls-only HPV16/18 vaccination and hepatitis B-virus (HBV) vaccination of boys), and Arm C (gender-neutral HBV vaccination). Out of 1992–1994 born resident boys (31,117) and girls (30,139), 8,618 boys and 15,615 girls were vaccinated, respectively, with 20–30% and 50% coverage in 2007–2009. In 2010–2013, 8,868 HPV16/18 and non-HPV vaccinated females, and in 2014–2016, 5,574 originally or later (2010–2013) HPV16/18 vaccinated females attended two cervical sampling visits, aged 18.5 and 22-years. The samples were typed for HPV6/11/16/18/31/33/35/39/45/51/52/56/58/59/66/68 using PCR followed by MALDI-TOF MS. HPV prevalence ratios (PR) between Arms A/B *vs.* C were calculated for *Chlamydia trachomatis* positives (core-group), and negatives (general population minus core group). At both visits the vaccine-protected HPV type PRs did not significantly differ between the core-group and non-core group. Among the vaccinated 18-year-olds, HPV51 occurrence was overall somewhat increased (PR_{core} = 1.4, $PR_{non\text{-}core.}$ = 1.4) whereas the HPV52 occurrence was increased in the core-group only (PR_{core} = 2.5, $PR_{non\text{-}core}$ = 0.8). Among the non-HPV vaccinated 18-year-olds, the HPV51/52 PRs were higher in the core-group (PR_{core} = 3.8/1.8, $PR_{non\text{-}core}$ = 1.2/1.1). The 22-year-olds yielded no corresponding observations. Monitoring of the sexual risk-taking core-group may detect early tendencies for HPV type replacement.

Key words: HPV, randomized trial, sexual risk-taking, type replacement, vaccination, core group
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What's new?

Oncogenic non-vaccine human papillomavirus (HPV) types may conceivably fill the vacated ecological niche of the vaccine types--a phenomenon which could undermine national HPV vaccination programs and warrants surveillance. The high sexual activity core-group, which is permissive for faster transmission dynamics, may be more susceptible to HPV type replacement. Here, the authors report that in the core-group defined by *Chlamydia trachomatis* positivity, among non-HPV vaccinated women high-risk HPV51 and HPV52 were increased four years post-vaccination. In the core-group of HPV16/18 vaccinated women HPV52 only was increased. Monitoring of the sexual risk-taking core-group may detect early tendencies for HPV type replacement.

Introduction

Human papillomavirus (HPV) vaccination is efficacious in preventing persistent HPV infections and high grade squamous intraepithelial lesions (HSIL) caused by a spectrum of vaccine-covered oncogenic HPV types, and also in preventing the ultimate end-point of invasive HPV associated cancers.[1–3] In addition to the vaccine types HPV16/18, vaccination with the bivalent vaccine has been found to be cross-protective against HPV types 31, 33, 35 and 45, and to induce herd effects against HPV18/31/33/35 already with low to moderate gender-neutral vaccination coverage.[4–6] During the last 15 years concern has been raised whether (following the implementation of effective HPV vaccination programs) non-vaccine covered HPV types will take over the niche left exposed by the elimination of the vaccine covered HPV types.[7,8] To this end, a meta-analysis of HPV type-replacement studies identified inconclusive evidence regarding increased prevalence estimates for oncogenic HPV39 and HPV52.[9]

In the general population, due to low sexual risk taking-behaviour, stochastics factors play a dominant role in HPV type occurrence.[10] Studies of HPV type-replacement have mostly assumed that it occurs by chance in the general population with low to moderate vaccination coverage. For HPV type replacement to occur there is a prerequisite that pre-vaccination HPV type occurrence is influenced by HPV type competition. However, in the non-core of the general population, stochastic factors may play the larger role. On the other hand, the core-group, with high sexual risk-taking providing higher transmission possibilities may be more permissible for HPV type competition. Thereby, the core-group is a more likely ground for HPV type replacement to be observed post-vaccination.

While clinical trials have low vaccination coverage and limited follow-up time from HPV vaccination to address the issue of HPV type-replacement,[11,12] community-randomized trials with ample vaccination coverage have been missing until now.[5,6] This may have limited the possibility of observing type-replacement, should it take at least moderate coverage HPV-vaccination induced selective pressure or longer time to manifest.

Cross-sectional approaches and application of substandard/conventional PCR-methodology have assumed symmetrical HPV type-type interactions, which may not be the case.[13,14] The former does not take into account the distinctively longer clearance rate for HPV16.[1] Furthermore, given the high basic reproductive number (R_0) of HPV16, it is likely that the ecological niche of HPV16 may require higher vaccination coverage than the other vaccine-covered HPV types to be effectively cleared over time.[6,15] This is true especially in the subpopulation with increased risk-taking sexual behaviour, and thus with increased R_0.[15] In addition to permissive coverage of vaccination, possibilities for observing HPV type-replacement probably require a population-based cohort approach with ample follow-up time and depend on the ability of the applied PCR-methodology to readily distinguish multiple HPV infections.

In this study, we have addressed HPV type-replacement in subpopulations with low or increased risk taking sexual behaviour (core-group and general population minus the core-group, denoted as the non-core group), determined in terms of *Chlamydia trachomatis* status, using state of the art PCR methodology. A cohort approach based on a very large community randomized trial with moderate HPV vaccination coverage among a few adolescent birth cohorts and up to 9 years of follow-up were applied. This is an ancillary study to the GSK-sponsored HPV-040 randomized trial (NCT00534638) comparing the overall protective effectiveness of gender neutral and girls-only vaccination strategies.

Materials and Methods

Study design and conduct

Two subsequent Finnish trials, a community randomized HPV vaccination effectiveness trial (NCT00534638) and the accuracy of cervical screening trial in HPV vaccinated women (NCT02149030), conducted by the University of Tampere (during the years 2007–2018), provided the material.

A total of 80,272 boys and girls resident in 33 Finnish communities were identified using the Finnish Population Register Centre and invited to participate in the community-randomized trial. Only Finnish or Swedish speaking individuals were invited. Thirty-three Finnish communities were firstly stratified according to HPV16/18 seroprevalence as determined previously using serum samples from the Finnish Maternity Cohort into low (<20.5%), intermediate (20.5–24.9%) and high seroprevalence (>24.0%), as described.[16] Following stratification, the communities from each strata were randomly divided into three study arms (Fig. 1).[16] The vaccination strategy by Arm was as follows: in the gender neutral Arm A 90% of both girls and boys received the HPV vaccine (Cervarix®), and 10% received the hepatitis B (HBV) vaccine (Engerix-B™); in the girls-only Arm B, 90% of girls received HPV vaccination and 10% received HBV vaccination, while all boys received HBV vaccination. In the control Arm C, vaccination was gender-neutral with all participants receiving the

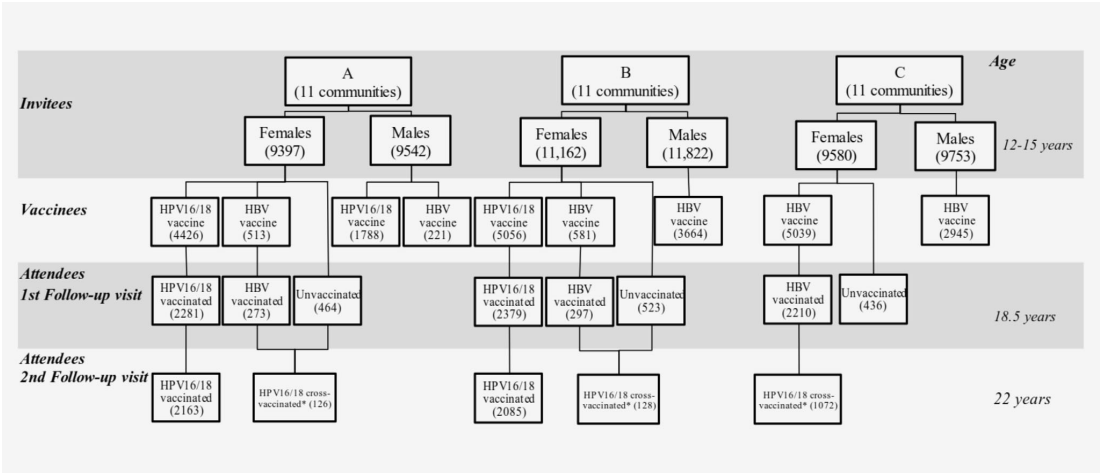

Figure 1. Community-randomized HPV vaccination trial design (1992–1994 birth cohorts).

HBV vaccine. The allocation of the intervention and control vaccinations were receiver blinded in arms A and B.

Of those initially invited, at the study baseline, (during the years 2007–2009), 32,175 participants (20,513 females and 11,662 males) from the 1992–1995 birth cohorts participated in the trial with their own and parental/guardian informed consent. In total, 99.4% of all participants received all three doses of the allocated vaccine. All of the initial visits were conducted at the health care facilities of the junior high schools in the respective communities.

All female residents of the 33 communities were invited to attend a follow-up visit at the age of 18.5 years at the University of Tampere study sites, during the years 2010–2014. They provided a self-collected cervico-vaginal sample rinsed in first void urine (FVU) chlamydia screening samples[17] and offered cross-vaccination with the HPV16/18 if they had not received it earlier.[5,6] Information regarding participant's residential history was collected *via* linkage with the Finnish Population Registry to identify mobility between study communities, and urban or semi-urban community residential status.

Four years later (during the years 2014–2018), HPV vaccinated participants were invited to attend another second follow-up visit at the age of 22 years. Again they provided a self-collected cervico-vaginal sample, rinsed in the FVU chlamydia sample. The participants from the birth cohort of those 1994 born, comprise only of those who both attended the follow-up within the first 12 months since the start of second follow-up commencement for the 1994 birth cohort (N = 1,091). Whereas, the participants attending the second follow-up visit from the two older birth cohorts, those 1992 and 1993 born, represent the entire birth cohort eligible for participation (N = 1,354 and 1,135, respectively).

Ethical permissions for the HPV-040 study (EUDRA-CT-2007-001731-55, NCT00534638) and for the HPV-004 study were granted from the Pirkanmaa hospital district ethical board in 2007 and 2014.

Laboratory methods

The cervico-vaginal samples collected from both the first and second follow-up visits were analysed for presence of HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 using modified general primer (MGP) polymerase chain reaction (PCR) and then Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS).[18,19] HPV DNA was first amplified using consensus MGP PCR. Then type-specific mass extension (ME) primers, each with a unique molecular mass, were used for single base extension in case the correct amplified template was present. In the final MS detection step, all ME-primers were separated by mass. Presence of specific extended ME-primers indicated presence and identity of specific types whereas non-extended ME-primers indicated the absence of that particular type. Since the system shows a slight cross-reaction between HPV 11 and HPV 89, and between HPV68 and HPV70, confirmatory testing of all HPV 11-positive and HPV68-positive samples, respectively, was performed on the Luminex platform.[20]

Statistical analysis

Type-specific prevalence for HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 were calculated at the first follow-up and second follow-up visit for different strata by study arm, vaccination status and *Chlamydia trachomatis* positivity at the first follow-up visit (a surrogate of sexual risk-taking behaviour). Participants with missing *C. trachomatis* data were excluded from the analyses (N = 29/8745, 0.3%). The prevalence in those *C. trachomatis* negative and positive for the unvaccinated Arm C subpopulation was estimated from the mean of 21 strata. The ratio of HBV-vaccinated to originally unvaccinated women in Arm C was balanced to be comparable to that in the non-HPV vaccinated women in Arms A/B. A comparable ratio was attained by keeping all

unvaccinated Arm C women and joining to them a random sample from all community-birth year cohort strata of Arm C women applying sampling fraction of 0.125. Altogether 21 such data sets were created, and HPV type-specific prevalence's by *C. trachomatis* status were estimated in each of them. The mean prevalence's were reported with 95% confidence intervals estimated using Agresti-Coull method.[21]

Mobility adjusted type-specific prevalence ratio (PR) estimates for HPV type combinations previously indicated to have decreased (16/18/45 and 31/33/35), increased (39, 51 and 66), and not been affected (6/11, 52/56/58/59) by vaccination[5,6] were estimated using a binomial generalized estimating equation (GEE) regression model with exchangeable correlation structure. Comparisons were between HPV vaccinated in combined Arm A/B and HBV vaccinated in Arm C to assess the direct impact, and non-HPV vaccinated in Arm A/B and non-HPV vaccinated in Arm C to assess the indirect impact of HPV vaccination. The study populations classified as non-HPV vaccinated incorporated both those HBV vaccinated participants and non-HPV/HBV-vaccinated attendees at the first follow-up visit only. Corresponding PRs for the unvaccinated subpopulations were computed by exponentiating the mean of the natural logarithm of estimates comparing the entire non-HPV vaccinated in Arms A/B to 21 HBV:unvaccinated balanced Arm C strata, so that the ratio of HBV:unvaccinated remained comparable for intervention and control arms.[5] All PR estimates were stratified by *C. trachomatis* status into those positive and those negative.

In similar manner the type-specific prevalence ratios were calculated for HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 comparing HPV vaccinated Arm A/B and HBV vaccinated Arm C participants attending the second follow-up visit at the age of 22 years. HPV type-specific prevalence ratios for HPV 6/11, 16/18/45, and 31/33/35 combinations, 39, 51, 52, 56, 58, 59, 66 and 68 were also calculated comparing the Arms A/B and Arm C attendants.

Finally, type-specific prevalence ratios of persistent infection was calculated using Poisson regression with robust standard errors to adjust for participant mobility for the HPV6/11, 16/18/45, and 31/33/35 combinations and for the HPV types 39, 51, 52, 56, 58, 59 and 66 separately, comparing HPV vaccinated Arm A/B participants and HBV vaccinated Arm C participants at the age of 22 years. Persistent infection was defined as a participant being positive for the given type both at the time of the first and second follow-up visit. Similarly, the HPV type-specific incidence ratio of persistent infection between the first and second follow-up visits was calculated using a Poisson GEE model to adjust for participant mobility, comparing the incidence rate in Arms A/B combined to Arm C, stratified by *C. trachomatis* status.

For HPV types identified as types of interest in the *C. trachomatis* stratified prevalence ratio approach, where the confidence limits were non-overlapping between the *C. trachomatis* positives (core-group) to negatives (non-core group), we included an interaction term into an unstratified log-binomial model to evaluate interaction between the trial arm and the *C. trachomatis* status on the multiplicative scale. Here smoking was defined as community-wise smoking prevalence, and p was the probability of the outcome, as follows:

$$\log(p) = \beta_0 + \beta_1 \text{Mobility} + \beta_2 \text{Smoking} + \beta_3 C.trachomatis + \beta_4 \text{Trial arm} + \beta_5 C.trachomatis^{*} \text{Trial arm}$$

In a likewise manner to the stratified prevalence ratio estimates, for the unstratified model inclusive of the interaction term we used a log binomial GEE model, with exchangeable correlation structure. Furthermore, for HPV types of interest where the inclusion of the interaction term was significantly different from the null, the risk differences were calculated for all four possible combinations of *C. trachomatis* status and trial arm status (Arms A/B or Arm C). The interaction contrast (IC) was calculated and assessed for nonzero-ness to evaluate interaction on the additive scale between the trial arm and *C. trachomatis* status, where R is equal to the risk of prevalence,[22] as follows:

$$\begin{aligned} IC &= R(\text{ArmAB}, C.trachomatis+) \\ &\quad - [R(\text{ArmAB}, C.trachomatis-) - R(\text{ArmC}, C.trachomatis-)] \\ &\quad - [R(\text{ArmC}, C.trachomatis+) - R(\text{ArmC}, C.trachomatis-)] \\ &\quad - R(\text{ArmC}, C.trachomatis-) \\ &= [R(\text{ArmAB}, C.trachomatis+) - R(\text{ArmC}, C.trachomatis+)] \\ &\quad - [R(\text{ArmAB}, C.trachomatis-) - R(\text{ArmC}, C.trachomatis-)]. \end{aligned}$$

In addition, the HPV type-specific total prevalence were calculated for HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 in the females participants at 18 years stratified into *C. trachomatis* positive and negative, and further subdivided into those presenting as single type infections, (defined as positive only for one of the 15 measured HPV types), and those presenting as multiple type infections.

All the statistical analyses were completed using R statistical software version 3. 4. 3. with DescTools package (version 0.99.25), geepack package (version 1. 2-1), Epi package (version 1.2-1), sandwich package (version 2.4-0) and ggplot2 (version 2. 2. 1). (The R Foundation; https://www.r-project.org/).[23,24]

Results

Attendance and risk-taking characteristics at the follow-up visits

Respectively, 8,863 females and 5,574 females from the 1992–1994 birth cohorts, attended in the first follow-up visit, and second follow-up visit (Fig. 1). The number of unvaccinated Arm C participants attending the second follow-up visit was negligible.

Demographic characteristics between the combined Arms A/B and Arm C showed no material differences at the first and second follow-up visits, at the respective ages of 18.5 and 22 years (Table 1). However, somewhat increased risk-taking behaviour in the Arms A/B in comparison to the Arm C was consistent. *C. trachomatis* positivity was 1.2–1.4 times higher, among the 18- and 22-year-olds, respectively, in the Arms A/B

Table 1. Characteristics of 1992–1994 born participants when attending the first follow-up visit at the age of 18 years, and when attending the second follow-up visit at the age of 22 years, 7–10 years since initial vaccination

	(a) 18.5-year-olds (first follow-up visit)				(b) 22-year-olds (second follow-up visit)		
	Arms A/B		Arm C		Arms A/B		Arm C
	HPV vac	Non-HPV vac[1]	HBV vac	Non-HPV vac[1]	HPV vac	Cross-HPV vac	Cross-HPV vac[2]
Characteristic	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)
Lives in study community[3]							
Yes	4995/5699 (87.6)	1252/1567 (79.9)	2306/2687 (85.8)	2576/3059 (84.2)	1607/3331 (48.2)	103/226 (45.6)	398/826 (48.2)
No	704/5699 (12.4)	315/1567 (20.1)	381/2687 (14.2)	483/3059 (15.8)	1724/3331 (51.8)	123/226 (54.4)	428/826 (51.8)
Missing	0/5699 (0.00)	0/1567 (0.0)	0/2687 (0.0)	0/3059 (0.0)	0/3331 (0.0)	0/226 (0.0)	0/826 (0.0)
Mean age at sexual debut[3–5]	-	-	-	-	16.5 (1.9)	16.4 (16.5)	16.6 (1.8)
No. of life-time partners[3]							
0	1263/5699 (22.2)	291/1567 (18.6)	577/2687 (21.5)	632/3059 (20.7)	151/3331 (4.5)	9/226 (4.0)	31/826 (3.8)
1	1485/5699 (26.1)	385/1567 (24.6)	790/2687 (29.4)	906/3059 (29.6)	532/3331 (16.0)	49/226 (21.7)	152/826 (18.4)
2	925/5699 (16.2)	246/1567 (15.7)	429/2687 (16.0)	477/3059 (15.6)	394/3331 (11.8)	21/226 (9.3)	112/826 (13.6)
3	652/5699 (11.4)	159/1567 (10.1)	296/2687 (11.0)	329/3059 (10.8)	399/3331 (12.0)	28/226 (12.4)	96/826 (11.6)
4	451/5699 (7.9)	139/1567 (8.9)	189/2687 (7.0)	228/3059 (7.5)	405/3331 (12.2)	27/226 (11.9)	101/826 (12.2)
5 or more	863/5699 (15.1)	281/1567 (17.9)	366/2687 (13.6)	423/3059 (13.8)	1415/3331 (42.5)	87/226 (38.5)	319/826 (38.6)
Missing	60/5699 (1.1)	66/1567 (4.2)	40/2687 (1.5)	64/3059 (2.1)	35/3331 (1.1)	5/226 (2.2)	15/826 (1.8)
No. of partners in last year[3]							
0	2617/5699 (45.9)	664/1567 (42.4)	1291/2687 (48.0)	1431/3059 (46.8)	1513/3331 (45.4)	115/226 (50.9)	379/826 (45.9)
1	1347/5699 (23.6)	381/1567 (24.3)	608/2687 (22.6)	701/3059 (22.9)	796/3331 (23.9)	47/226 (20.8)	199/826 (24.1)
2	684/5699 (12.0)	180/1567 (11.5)	314/2687 (11.7)	364/3059 (11.9)	360/3331 (10.8)	22/226 (9.7)	82/826 (9.9)
3	437/5699 (7.7)	133/1567 (8.5)	209/2687 (7.8)	245/3059 (8.0)	242/3331 (7.3)	20/226 (8.8)	61/826 (7.4)
4	265/5699 (4.6)	88/1567 (5.6)	103/2687 (3.8)	124/3059 (4.1)	166/3331(5.0)	9/226 (4.0)	44/826 (5.3)
5 or more	309/5699 (5.4)	113/1567 (7.2)	135/2687 (5.0)	165/3059 (5.4)	229/3331 (6.9)	12/226 (5.3)	58/826 (7.0)
Missing	40/5699 (0.7)	8/1567 (0.5)	27/2687 (1.0)	29/3059 (0.9)	25/3331 (0.8)	1/226 (0.4)	3/826 (0.4)
C. trachomatis status[6]							
C. trachomatis positive	162/4623 (3.5)	68/1537 (4.4)	63/2149 (2.9)	(3.8)[7]	87/2569 (3.4)	12/198 (6.1)	20/809 (2.5)
C. trachomatis negative	4452/4623 (96.3)	1461/1537 (95.1)	2079/2149 (96.7)	(95.4)[7]	2480/2569 (96.5)	86/198 (93.9)	789/809 (97.5)
Missing	9/4623 (0.2)	8/1537 (0.5)	7/2149 (0.3)	(0.8)[7]	2/2569 (0.1)	0/198 (0.0)	0/809 (0.0)
Smoking habit[3]							
Never smoked	3406/5699 (59.8)	929/1567 (59.3)	1619/2687 (60.3)	1846/3059 (60.3)	1947/3331 (58.5)	144/226 (63.7)	542/826 (65.6)
Quit smoking	387/5699 (6.8)	114/1567 (7.3)	194/2687 (7.2)	223/3059 (7.3)	400/3331 (12.0)	27/226 (11.9)	86/826 (10.4)
Current	1848/5699 (32.4)	506/1567 (32.3)	847/2687 (31.5)	957/3059 (31.3)	946/3331 (28.4)	52/226 (23.0)	192/826 (23.2)
Current other than cigarettes	9/5699 (0.2)	3/1567 (0.2)	0/2687 (0.0)	1/3059 (0.0)	22/3331 (0.7)	1/226 (0.44)	3/826 (0.4)
Missing	49/5699 (0.9)	15/1567 (1.0)	27/2687 (1.0)	32/3059 (1.1)	16/3331 (0.5)	2/226 (0.88)	3/826 (0.4)
Vaccination Coverage[8]							
<40%	654/4623 (14.1)	243/1537 (15.8)	na	na	na	na	na

(Continues)

Table 1. (Continued)

Characteristic	(a) 18.5-year-olds (first follow-up visit)				(b) 22-year-olds (second follow-up visit)			
	Arms A/B		Arm C		Arms A/B		Arm C	
	HPV vac	Non-HPV vac[1]	HBV vac	Non-HPV vac[1]	HPV vac	Cross-HPV vac	Cross-HPV vac[2]	
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	n/N (%)	
40–50%	2187/4623 (47.3)	732/1537 (47.6)	na	na	na	na	na	
>50%	1782/4623 (38.5)	562/1537 (36.6)	na	na	na	na	na	

Arm A = gender-neutral HPV-16/18 vaccination, Arm B = girls-only HPV 16/18 vaccination, Arm C = originally HBV vaccination or no vaccination.
[1]Non-HPV vaccinated women consist of women who were either HBV vaccinated or unvaccinated as early adolescents.
[2]HPV 16/18 cross-vaccinated after cervical sampling at the first follow-up visit.
[3]Questionnaire data obtained at the age of 18.5–19 years and 22 years.
[4]Standard deviation.
[5]Questionnaire data obtained at the age of 22 years from vaccinated participants only.
[6]Laboratory analysis data obtained at the age of 18.5–19 years
[7]Prevalence estimates the mean from 21 strata prevalence estimates.
[8]Community-wise vaccination coverage.

participants compared to Arm C participants in each corresponding HPV-vaccination status group (Table 1). Also, the corresponding numbers of participants with multiple (five or more) lifetime sexual partners were 1.1 to 1.3 times higher at the first follow-up visit (Table 1). At the second follow-up visit the originally HPV16/18 vaccinated Arms A/B participants had 1.1 times higher numbers of multiple sexual partners than the Arm C participants (Table 1). Among the originally HPV16/18 vaccinated Arm A/B participants, current smoking was somewhat more common than in the Arm C participants both at the age 18 and age 22 years (Table 1). All following comparisons were made between subgroups defined by *C. trachomatis* status.

Prevalence of HPV types

Among the originally HPV16/18 vaccinated 18-year-old study participants, the prevalence of vaccine types HPV16 and HPV18 was remarkably lower in Arm A/B participants as compared to originally HBV vaccinated Arm C participants in both the *C. trachomatis* positives and negatives (Tables 2 and 3). Corresponding prevalence were also decreased for HPV16/18, HPV16/18/45, and HPV31/33/35 combinations, both in the *C. trachomatis* positives and negatives (Tables 2 and 3). On the contrary, HPV51 prevalence were somewhat higher in the Arms A/B as compared to Arm C both in the *C. trachomatis* positives and negatives (Tables 2 and 3). HPV39 and HPV52 prevalence in Arms A/B were increased both among the 18- and 22-year-olds but in the *C. trachomatis* positives only (Tables 2 and 3).

Among the originally non-HPV vaccinated 18-year-olds, HPV18 prevalence was decreased in the Arms A/B participants in comparison to the Arm C participants in both the *C. trachomatis* positives and negatives (Table 3). Again HPV51 prevalence was somewhat higher in Arms A/B as compared to Arm C both in the *C. trachomatis* positives and negatives (Table 3). In the *C. trachomatis* positives, the HPV16 and HPV31/33/35 prevalence were increased in the Arms A/B participants. Also, the HPV39 and HPV52 prevalence were increased in the *C. trachomatis* positive non-HPV vaccinated Arms A/B participants.

Prevalence ratios of HPV types

Among the originally HPV16/18 vaccinated 18-year-old study participants adjusted HPV16/18, HPV16/18/45 and HPV31/33/35 prevalence ratios between Arms A/B *versus* Arm C were decreased both in the *C. trachomatis* positives and negatives (Table 4). Corresponding HPV51 prevalence ratios were similarly increased in both the *C. trachomatis* positives ($PR_{18yrCtrpos}$ = 1.36, CI 0.76–2.41) and *C. trachomatis* negatives ($PR_{18yrCtrneg}$ = 1.40, CI 1.11–1.77; Table 4).

Among the originally non-HPV vaccinated 18-year-olds, the prevalence ratios between Arms A/B *vs.* Arm C were mainly decreased for HPV16/18/45 (Table 5). However, for HPV16 the decrease was found only in the *C. trachomatis* negatives. For the cross-protected HPV types 31/33/35 the prevalence ratio was increased in the *C. trachomatis* positives ($PR_{HPV31/33/35,Ctrpos}$ = 2.19, CI 1.07–4.47). The HPV51 and

Table 2. HPV type-specific prevalence among 18-year-old and 22-year-old vaccinated females according to trial arm and *Chlamydia trachomatis* positivity

n/N (% [95% confidence intervals]) Vaccinated[1] females born 1992–1994								
Arm A and B (HPV vac)					Arm C (HBV vac)			
	18-year-olds		22-year-olds		18-year-olds		22-year-olds[2]	
HPV type	*C. trachomatis* positive	*C. trachomatis* negative	*C. trachomatis* positive	*C. trachomatis* negative	*C. trachomatis* positive	*C. trachomatis* negative	*C. trachomatis* positive	*C. trachomatis* negative
6	21/162 (13.0 [8.6–19.1])	195/4452 (4.4 [3.8–5.0])	2/87 (2.3 [0.1–8.5])	87/2480 (3.5 [2.8–4.3])	11/63 (17.5 [9.9–28.8])	90/2079 (4.3 [3.5–5.3])	0/20 (0.0 [0.0–19.0])	29/789 (3.7 [2.6–5.2])
11	4/162 (2.5 [0.7–6.4])	28/4452 (0.6 [0.4–0.9])	0/87 (0.0 [0.0–5.1])	17/2480 (0.7 [0.4–1.1])	2/63 (3.2 [0.2–11.5])	14/2079 (0.7 [0.4–1.1])	0/20 (0.0 [0.0–19.0])	11/789 (1.4 [0.7–2.5])
16	0/162 (0.0 [0.0–2.8])	24/4452 (0.5 [0.4–0.8])	1/87 (1.1 [0.0–6.8])	19/2480 (0.8 [0.5–1.2])	7/63 (11.1 [5.2–21.5])	150/2079 (7.2 [6.2–8.4])	0/20 (0.0 [0.0–19.0])	9/789 (1.1 [0.6–2.2])
18	0/162 (0.0 [0.0–2.8])	11/4452 (0.2 [0.1–0.4])	1/87 (1.1 [0.0–6.8])	9/2480 (0.4 [0.2–0.7])	7/63 (11.1 [5.2–21.5])	80/2079 (3.8 [3.1–4.8])	0/20 (0.0 [0.0–19.0])	1/789 (0.1 [0.0–0.8])
31	0/162 (0.0 [0.0–2.8])	35/4452 (0.8 [0.6–1.1])	0/87 (0.0 [0.0–5.1])	22/2480 (0.9 [0.6–1.3])	1/63 (1.6 [0.0–9.3])	70/2079 (3.4 [2.7–4.2])	0/20 (0.0 [0.0–19.0])	8/789 (1.0 [0.5–2.0])
33	8/162 (4.9 [2.4–9.6])	69/4452 (1.5 [1.2–2.0])	2/87 (2.3 [0.1–8.5])	42/2480 (1.7 [1.2–2.3])	5/63 (7.9 [3.1–17.7])	53/2079 (2.5 [1.9–3.3])	0/20 (0.0 [0.0–19.0])	16/789 (2.0 [1.2–3.3])
35	5/162 (3.1 [1.1–7.2])	37/4452 (0.8 [0.6–1.1])	3/87 (3.4 [0.8–10.1])	51/2480 (2.1 [1.6–2.7])	6/63 (9.5 [4.1–19.6])	21/2079 (1.0 [0.7–1.5])	0/20 (0.0 [0.0–19.0])	10/789 (1.3 [0.7–2.4])
39	13/162 (8.0 [4.6–13.4])	123/4452 (2.8 [2.3–3.3])	5/87 (5.7 [2.2–13.1])	90/2480 (3.6 [3.0–4.4])	4/63 (6.3 [2.0–15.7])	57/2079 (2.7 [2.1–3.5])	0/20 (0.0 [0.0–19.0])	38/789 (4.8 [3.5–6.6])
45	2/162 (1.2 [0.1–4.7])	20/4452 (0.4 [0.3–0.7])	0/87 (0.0 [0.0–5.1])	23/2480 (0.9 [0.6–1.4])	4/63 (6.3 [2.0–15.7])	44/2079 (2.1 [1.6–2.8])	0/20 (0.0 [0.0–19.0])	4/789 (0.5 [0.1–1.3])
51	37/162 (22.8 [17.0–29.9])	317/4452 (7.1 [6.4–7.9])	5/87 (5.7 [2.2–13.1])	178/2480 (7.2 [6.2–8.3])	11/63 (17.5 [9.9–28.8])	106/2079 (5.1 [4.2–6.1])	3/20 (15.0 [4.4–36.9])	61/789 (7.7 [6.1–9.8])
52	19/162 (11.7 [7.6–17.7])	161/4452 (3.6 [3.1–4.2])	5/87 (5.7 [2.2–13.1])	147/2480 (5.9 [5.1–6.9])	3/63 (4.8 [1.1–13.6])	91/2079 (4.4 [3.6–5.3])	0/20 (0.0 [0.0–19.0])	44/789 (5.6 [4.2–7.4])
56	19/162 (11.7 [7.6–17.7])	172/4452 (3.9 [3.3–4.5])	5/87 (5.7 [2.2–13.1])	152/2480 (6.1 [5.2–7.1])	7/63 (11.1 [5.2–21.5])	87/2079 (4.2 [3.4–5.1])	1/20 (5.0 [0.0–25.4])	34/789 (4.3 [3.1–6.0])
58	3/162 (1.9 [0.4–5.5])	100/4452 (2.2 [1.8–2.7])	4/87 (4.6 [1.4–11.6])	80/2480 (3.2 [2.6–4.0])	7/63 (11.1 [5.2–21.5])	49/2079 (2.4 [1.8–3.1])	1/20 (5.0 [0.0–25.4])	23/789 (2.9 [1.9–4.4])
59	16/162 (9.9 [6.1–15.5])	116/4452 (2.6 [2.2–3.1])	1/87 (1.1 [0.0–6.8])	72/2480 (2.9 [2.3–3.6])	6/63 (9.5 [4.1–19.6])	47/2079 (2.3 [1.7–3.0])	0/20 (0.0 [0.0–19.0])	25/789 (3.2 [2.1–4.7])
66	14/162 (8.6 [5.1–14.1])	181/4452 (4.1 [3.5–4.7])	1/87 (1.1 [0.0–6.8])	104/2471 (4.2 [3.5–5.1])	7/63 (11.1 [5.2–21.5])	64/2079 (3.1 [2.4–3.9])	1/20 (5.0 [0.0–25.4])	24/789 (3.0 [2.0–4.5])
68	-	-	2/86 (2.3 [0.1–8.5])	43/2475 (1.7 [1.3–2.3])	-	-	0/20 (0.0 [0.0–19.0])	12/788 (1.5 [0.8–2.7])
6/11	24/162 (14.8 [10.1–21.2])	217/4452(4.9 [4.3–5.5])	2/87 (2.3 [0.1–8.5])	102/2480 (4.1 [3.4–5.0])	12/63 (19.0 [11.1–30.6])	103/2079 (5.0 [4.1–6.0])	0/20 (0.0 [0.0–19.0])	40/789 (5.1 [3.7–6.8])
16/18/45	2/162 (1.2 [0.1–4.7])	55/4452 (1.2 [0.9–1.6])	2/87 (2.3 [0.1–8.5])	50/2480 (2.0 [1.5–2.7])	17/63 (27.0 [17.5–39.1])	243/2079 (11.7 [10.4–13.1])	0/20 (0.0 [0.0–19.0])	14/789 (1.8 [1.0–3.0])
31/33/35	13/162 (8.0 [4.6–13.4])	140/4452 (3.1 [2.7–3.71)	4/87 (4.6 [1.4–11.6])	112/2480 (4.5 [3.8–5.4])	12/63 (19.0 [11.1–30.6])	134/2079 (6.4 [5.5–7.6])	0/20 (0.0 [0.0–19.0])	31/789 (3.9 [2.8–5.5])

Chlamydia trachomatis was used as a surrogate of sexual risk-taking behaviour.
[1]Arm A and B vaccinated are originally HPV vaccinated (Arm C vaccinated individuals are originally HBV vaccinated) as early adolescents. HPV68 DNA data only available from the laboratory analysis of the 22-year-olds.
[2]HPV 16/18 cross-vaccinated.

Table 3. HPV type-specific prevalence among 18-year-old non-HPV-vaccinated females according to trial arm and *Chlamydia trachomatis* positivity

	n/N (%) originally non-HPV vaccinated[1] females born 1992–1994			
	Arm A/B		Arm C[2]	
	18-year-olds		18-year-olds	
HPV type	*C. trachomatis* positive	*C. trachomatis* negative	*C. trachomatis* positive	*C. trachomatis* negative
6	5/68 (7.4 [2.8–16.5])	83/1461 (5.7 [4.6–7.0])	(14.4 [8.3–23.7])	(4.6 [3.8–5.5])
11	1/68 (1.5 [0.0–8.6])	14/1461 (1.0 [0.6–1.6])	(5.0 [1.6–12.3])	(0.7 [0.4–1.1])
16	14/68 (20.6 [12.6–31.8])	87/1461 (6.0 [4.8–7.3])	(13.4 [7.5–22.5])	(8.0 [7.0–9.1])
18	8/68 (11.8 [5.8–21.8])	63/1461 (4.3 [3.4–5.5])	(28.1 [19.5–38.6])	(4.6 [3.8–5.5])
31	9/68 (13.2 [6.9–23.5])	50/1461 (3.4 [2.6–4.5])	(7.7 [3.4–15.8])	(3.9 [3.2–4.7])
33	5/68 (7.4 [2.8–16.5])	44/1461 (3.0 [2.2–4.0])	(5.3 [1.8–12.7])	(2.2 [1.7–2.9])
35	4/68 (5.9 [1.9–14.6])	16/1461 (1.1 [0.7–1.8])	(2.3 [0.1–8.7])	(1.5 [1.1–2.1])
39	4/68 (5.9 [1.9–14.6])	41/1461 (2.8 [2.1–3.8])	(1.5 [0.0–7.6])	(2.8 [2.2–3.5])
45	4/68 (5.9 [1.9–14.6])	36/1461 (2.5 [1.8–3.4])	(6.2 [2.4–13.9])	(2.5 [2.0–3.2])
51	13/68 (19.1 [11.4–30.1])	101/1461 (6.9 [5.7–8.3])	(3.7 [0.9–10.7])	(5.6 [4.8–6.6])
52	15/68 (22.1 [13.7–33.4])	73/1461 (5.0 [4.0–6.2])	(12.3 [6.7–21.3])	(4.6 [3.8–5.5])
56	7/68 (10.3 [4.8–20.0])	57/1461 (3.9 [3.0–5.0])	(13.5 [7.6–22.6])	(4.7 [3.9–5.6])
58	6/68 (8.8 [3.8–18.3])	47/1461 (3.2 [2.4–4.3])	(5.7 [2.1–13.3])	(2.5 [2.0–3.2])
59	8/68 (11.8 [5.8–21.8])	43/1461 (2.9 [2.2–3.9])	(16.5 [9.9–26.1])	(2.0 [1.5–2.6])
66	5/68 (7.4 [2.8–16.5])	60/1461 (4.1 [3.2–5.3])	(17.0 [10.3–26.6])	(4.9 [4.1–5.8])
6/11	6/68 (8.8 [3.8–18.3])	96/1461 (6.6 [5.4–8.0])	(22.6 [14.9–32.8])	(5.2 [4.4–6.1])
16/18/45	20/68 (29.4 [19.9–41.2])	63/1461 (11.2 [9.6–12.9])	(37.9 [28.2–48.7])	(13.1 [11.8–14.5])
31/33/35	17/68 (25.0 [16.2–36.5])	104/1461 (7.1 [5.9–8.6])	(11.3 [5.9–20.1])	(7.0 [6.1–8.1])

Chlamydia trachomatis was used as a surrogate of sexual risk-taking behaviour.
[1]Includes those originally vaccinated with HBV vaccine and those completely unvaccinated as early adolescents. HPV-cross vaccination of a portion of these participants who consented to cross-vaccination took place at the age of 18.5 years, after the donation of the cervical sample used in the laboratory analysis for HPV DNA typing.
[2]Mean estimates from 21 strata prevalence ratio estimates.

HPV52 prevalence ratios were again increased both in the *C. trachomatis* positives and negatives, albeit with notably higher point estimates in the former ($PR_{HPV51,Ctrpos}$ = 3.78, CI 1.19–12.1, *vs.* $PR_{HPV51,Ctrneg}$ = 1.19, CI 0.96–1.57)($PR_{HPV52,Ctrpos}$ = 1.81, CI 0.89–3.68, *vs.* $PR_{HPV52,Ctrneg}$ = 1.10, CI 0.83–1.47) (Table 5).

To further elaborate the role of sexual risk-taking behaviour, as determined by *C. trachomatis* status, in the post-vaccination occurrence of HPV types 51, and 52, an interaction term was added to the statistical model. Furthermore, on the additive scale the associated interaction contrast (IC) estimate was superadditive among the non-HPV vaccinated (IC_{HPV51} = 14.1, 95% CI −16.2, 44.4) (IC_{HPV52} = 9.3, 95% CI −18.3, 36.9). When the interaction term was added to the model comparing HPV51 prevalence among the non-HPV vaccinated it was supermultiplicative (Interaction term = 83.1, p-value ranging from 0.00 to 0.57). No corresponding findings were made for HPV52 among the non-HPV vaccinated but among the HPV16/18 vaccinated the interaction term was slightly increased (Appendix).

Occurrence of HPV types over time

In the *C. trachomatis* negatives the prevalence of infections persisting from 18 to 22 years of age (between the first and the second visits) was not increased for the majority of the non-vaccine HPV types in the Arms A/B participants when compared to Arm C participants (Table 6). For HPV51, the corresponding prevalence ratio estimates of persistent infections appeared to be below unity (PR = 0.57, CI 0.17–1.92) (Table 6). For HPV52, the corresponding prevalence ratio estimate was increased, albeit not significantly (PR = 1.32, CI 0.28–6.26) (Table 6).

Prevalence of HPV multi-type and single type infections

Finally, the prevalence of HPV (type-specific) infections was compared separately for multiple type and single type infections among originally HPV16/18 vaccinated and among originally non-HPV vaccinated participants.

Among the HPV16/18 vaccinated *C. trachomatis* positive participants, HPV52 multiple type infections were increased in Arms A/B as compared to Arm C ($P_{A/B}$ = 8.64 *vs.* P_C = 3.17, respectively) (Fig. 2*a*). Single type HPV51 and 52 infections ($P_{A/B}$ = 8.02 *vs.* P_C = 4.76, $P_{A/B}$ = 3.09 *vs.* P_C = 1.59, respectively) (Fig. 2*a*). In the *C. trachomatis* negatives corresponding increase was found only for single type HPV51 infections ($P_{A/B}$ = 3.62 *vs.* P_C = 1.49) (Fig. 2*a*).

Among the non-HPV vaccinated *C. trachomatis* positive participants, HPV51 and HPV52 multiple type infections were increased in Arms A/B as compared to Arm C ($P_{A/B}$ = 5.88 *vs.*

Table 4. Type-specific prevalence ratio estimates (95% confidence intervals) comparing intervention Arms A/B to control Arm C in the *Chlamydia trachomatis* positive and negative vaccinated females, who attended the first follow-up visit at the age of 18 years old and second follow-up visit at the age of 22 years-old

Prevalence Ratio (95% confidence intervals) HPV-vaccinated females born 1992–1994				
Arm A/B *vs* Arm C				
	18 years		**22 years**	
HPV type	***C. trachomatis* positive**	***C. trachomatis* negative**	***C. trachomatis* positive**	***C. trachomatis* negative**
6/11	0.80 (0.43–1.48)	0.99 (0.81–1.23)	na	0.81 (0.56–1.18)
16	0.00 (0.00–0.00)	0.07 (0.05–0.11)	na	0.69 (0.32–1.48)
18	0.00 (0.00–0.00)	0.06 (0.04–0.12)	na	3.01 (0.4–22.86)
16/18/45	0.05 (0.01–0.19)	0.11 (0.08–0.14)	na	1.14 (0.67–1.93)
31/33/35	0.42 (0.21–0.87)	0.48 (0.38–0.62)	na	1.14 (0.79–1.65)
39	1.28 (0.44–3.73)	1.01 (0.75–1.37)	na	0.77 (0.55–1.08)
51	1.36 (0.76–2.41)	1.40 (1.11–1.77)	0.28 (0.07–1.09)	0.91 (0.69–1.21)
52	2.45 (0.78–7.67)	0.83 (0.65–1.07)	na	0.74 (0.51–1.07)
56	1.05 (0.48–2.29)	0.92 (0.71–1.21)	20.5 (0.39–23.6)	0.81 (0.52–1.25)
58	0.17 (0.05–0.60)	0.96 (0.69–1.34)	0.29 (0.02–4.52)	0.91 (0.55–1.50)
59	0.97 (0.39–2.41)	1.16 (0.84–1.61)	0.42 (0.07–2.43)	1.12 (0.67–1.88)
66	0.82 (0.37–1.84)	1.32 (1.00–1.75)	0.28 (0.02–4.11)	1.38 (0.94–2.04)
68	na	na	na	1.1 (0.65–1.88)

Estimates adjusted for mobility. *Chlamydia trachomatis* was used as a surrogate of sexual risk-taking behaviour. na = not available. nc = nonconvergence of the model.

Table 5. Type-specific prevalence ratio estimates (95% confidence intervals) comparing intervention Arms A/B to control Arm C in the *Chlamydia trachomatis* positive and negative non-HPV vaccinated females, who attended the first follow-up visit at the age of 18 years

	Prevalence ratio (95% confidence intervals) originally non-HPV-vaccinated[1] females born 1992–1994	
	Arm A/B *vs* Arm C	
	18 years	**18 years**
HPV type	***C. trachomatis* positive**	***C. trachomatis* negative**
6/11	0.56 (0.27–1.15)	1.23 (0.95–1.59)
16	1.62 (0.80–3.30)	0.75 (0.59–0.96)
18	0.44 (0.22–0.89)	0.94 (0.79–1.27)
16/18/45	0.80 (0.51–1.25)	0.85 (0.72–1.02)
31/33/35	2.19 (1.07–4.47)	1.01 (0.80–1.28)
39	na	1.00 (0.69–1.47)
51	3.78 (1.19–12.1)	1.23 (0.96–1.57)
52	1.81 (0.89–3.68)	1.10 (0.83–1.47)
56	0.78 (0.33–1.83)	0.82 (0.60–1.12)
58	1.52 (0.50–4.64)	1.32 (0.92–1.90)
59	0.68 (0.31–1.52)	1.50 (1.01–2.25)
66	0.44 (0.17–1.14)	0.84 (0.62–1.13)

Estimates adjusted for mobility. The estimates are the mean of 21 stratified prevalence ratio estimates. *Chlamydia trachomatis* was used as a surrogate of sexual risk-taking behaviour.
[1]Includes both those originally vaccinated with HBV vaccine and those completely unvaccinated as early adolescents. A portion of these participants received HPV-cross vaccination at the age of 18.5 years, after the donation of the cervical sample used in the laboratory analysis for HPV DNA typing.

P_C = 1.33; $P_{A/B}$ = 13.2 *vs.* P_C = 2.53; $P_{A/B}$ = 17.6 *vs.* P_C = 8.33, respectively) (Fig. 2*b*). Only single type HPV51 infections were increased ($P_{A/B}$ = 5.88 *vs.* P_C = 1.18). In the *C. trachomatis* negatives, both multiple type and single type HPV51 infections were marginally increased ($P_{A/B}$ = 4.45 *vs.* P_C = 3.71; $P_{A/B}$ = 2.46, P_C = 1.90, respectively).

Among the 22-year-olds, there were no significant findings of multiple or single type infections in either the *C. trachomatis* positives or negatives, when comparing Arms A/B to Arm C (data not shown).

Discussion

The subpopulations with increased sexual risk-taking behaviour may be more facilitative of the preconditions for HPV type replacement occurrence after HPV vaccination induced ecological pressure. Among the originally HPV16/18 vaccinated 18-year-old females, the HPV51 occurrence was consistently increased albeit in the originally non-HPV vaccinated the increase was statistically significant in the core-group with high sexual risk-taking behaviour only.

In order for HPV type replacement to occur post-vaccination, a prerequisite of competition between HPV types over the same ecological niche should be fulfilled. However, in the non-core group background, HPV exposure and acquisition may, due to assortativeness of sexual behaviour, be limited simply by stochastic factors.[25] Thus, while in the non-core group HPV occurrence and its changes are due to chance, especially for the rarer HPV types, in the core-group, increased concurrency and high turnover of sexual partners probably facilitate the transmission of

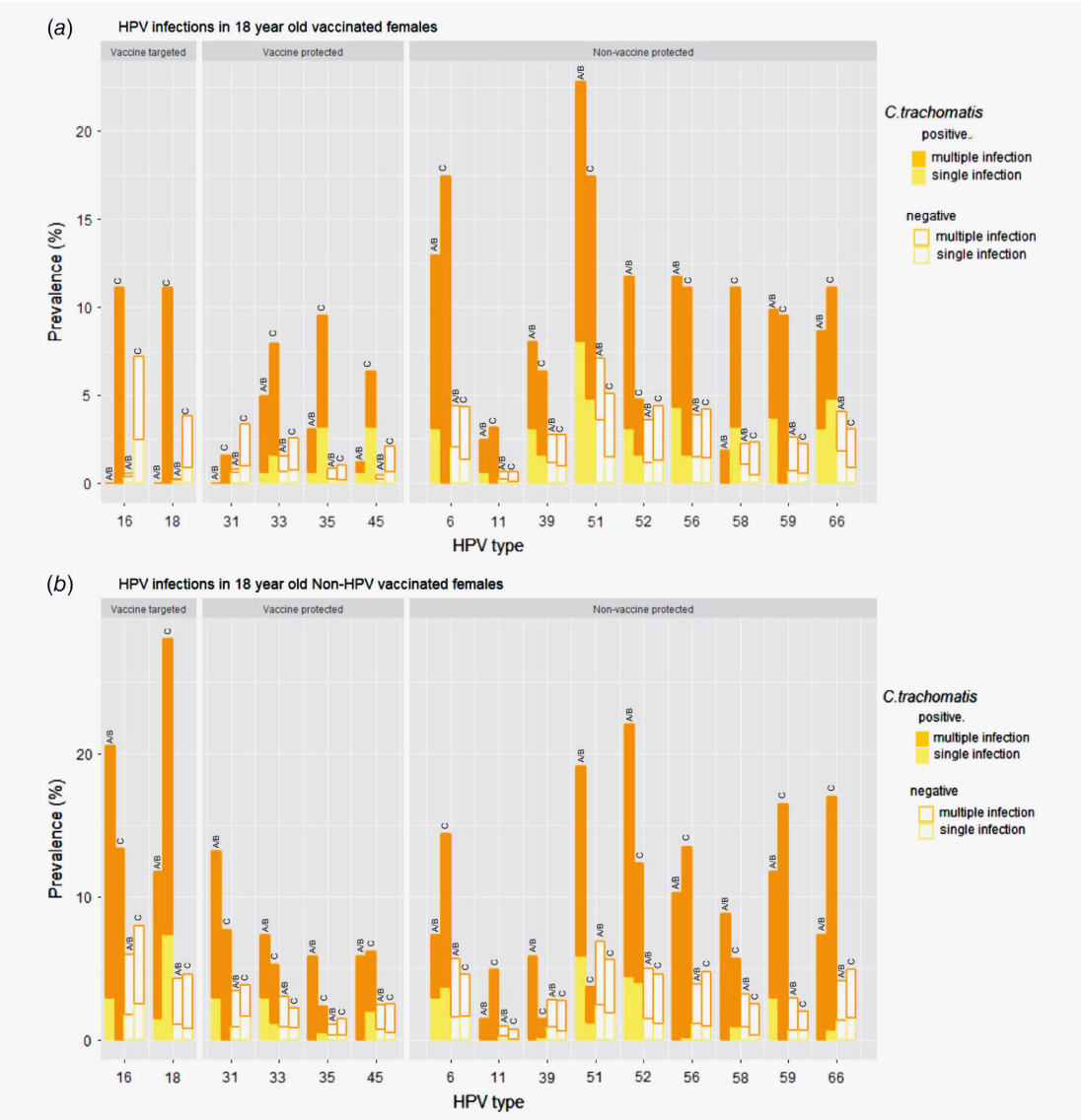

Figure 2. Prevalence of HPV type-specific infection occurring as multiple type infections and single type infections stratified by trial Arm, *Chlamydia trachomatis* positivity, and vaccination status, in participants attending the first follow-up visit, at the age of 18.5 years old, up to 5 years after initial vaccination. Estimates displayed left to right, Arm A/B, Arm C.

HPV types and competition over the ecological niche. Furthermore, due to the assortative sexual mixing behaviour, ecological niches in the core-group may be limited in their capacity to accommodate all the different HPV types. This could create conditions prone to competitive exclusion of HPV types, therefore, following ecological selection pressure already from moderate coverage vaccination, type replacement might be more likely to occur in the core-group than in the non-core group. We show that the effect of vaccination on the occurrence for the two specific HPV types 51 and 52, may differ in the core-group in comparison to the non-core group.

Post-vaccination HPV51 occurrence remained increased both in the core-group and in the non-core group among HPV16/18 vaccinated and non-HPV vaccinated alike. However, in the core-group among the non-HPV vaccinated the HPV51 increase

Table 6. Type-specific adjusted prevalence ratio estimates (95% confidence intervals) of persistent HPV infections comparing intervention Arms A/B to control Arm C in the *Chlamydia trachomatis* positive and negative females who attended both the first and second follow-up visits at the ages of 18.5 and 22 years in the vaccination and screening trials

	Prevalence ratio[1] (95% confidence intervals) HPV-vaccinated females born 1992-1994
	A/B *vs* C
HPV type	C. *trachomatis* negative
6/11	0.41 (0.04–4.29)
16	na
18	1.00 (0.39–2.56)
16/18/45	na
31/33/35	1.00 (0.69–1.45)
52/56/58/59	1.01 (0.32–3.22)
39	0.66 (0.06–6.83)
51	0.57 (0.17–1.92)
52	1.32 (0.28–6.26)
66	Na
All vaccine protected types	$2.92e^{-08}(4.10e^{-09}–2.08e^{-07})$
All non-vaccine protected types	0.90 (0.42–1.92)

Estimates adjusted for mobility. *Chlamydia trachomatis* was used as a surrogate of sexual risk-taking behaviour. The estimates for the *C.trachomatis* positive strata are not shown owing to an insufficient sample size.
[1]Persistent prevalence ratio is defined as the prevalence of specific HPV types at age 22 years in those who were positive for the given types at the age of 18.5 years.

departed from that of the non-core group, regardless of its widespread occurrence. In further analyses, the interaction between sexual risk-taking behaviour and especially HPV51 occurrence shown to be supermultiplicative and superadditive (Appendix). This suggests that sexual risk-taking behaviour in the core-group may modify the HPV51 occurrence following vaccination.

The increase of HPV51 and HPV52 occurrence in the core-groups of both HPV16/18 vaccinated and non-HPV vaccinated was observed both as multiple type infections and single type infections, whereas in the non-core group only HPV51 was increased, and as single type infections only. It is noteworthy that the pre-vaccination prevalence of HPV51 in the core-group was higher than any other measured HPV type barring HPV6. This suggests that in the core-group HPV51 may hold a pre-vaccination niche larger than any other high-risk type, and was readily available to fill the niche(s) vacated. The increased HPV51 and HPV52 multiple type infections in the different core-groups may be reflective of the increased occurrence of multiple non-vaccine HPV types circulating post-vaccination. However, in terms of public health impact it should be noted that previous studies have consistently observed that HPV51 despite being classified as a high risk type, has a very low capacity to cause progression to invasive cervical cancer (ICC), and is more prevalent among low-grade squamous intraepithelial lesions (LSIL) and cervical intraepithelial neoplasia grade 1 (CIN1).[26]

In the non-HPV vaccinated core-group where HPV prevalence exceeded 20% both pre-vaccination and post-vaccination, the only vaccine type significantly decreased was HPV18. Concomitant increase in HPV51 occurrence might suggest that this phylogenetically non-HPV18 related/non vaccine-protected type was taking over the niche of the vaccine protected type HPV18. The prevalence (and incidence, data not shown) of persistent HPV51 infections was, however, consistently lower in the vaccinated arms, which does not support an increase in persistent HPV51 infections.

The identification of increased HPV52 prevalence in the core-groups, in contrast to reported bivalent vaccine induced HPV52 cross-protection,[27] is in line with the previous meta-analyses which reported possible increased HPV52 occurrence post-vaccination.[9]

C. trachomatis status is linearly correlated to the lifetime number of sexual partners and can be used surrogate of sexual risk-taking behaviour.[28] It is likely that the time of acquisition of *C. trachomatis* is correlated with the time of acquisition of HPV infections owing to the shared transmission route. Even if this affects HPV prevalence in the *C. trachomatis* positive group, it should not have biased the Arm A/B *versus* Arm C comparisons.

As for the vaccine covered types the protective impact of HPV vaccination among the vaccinated women was consistent both for the core-group and the non-core group.[5,6] However, among the non-HPV vaccinated women the protective impact of herd effect was not observed for HPV16 or HPV31/33/35 among the core-group. Increased HPV16 prevalence in the non-HPV vaccinated core-group is in line with difficulties in conferring herd effect against this type.[5,6,15] It is open whether the HPV16 occupied niche has in our cohort been cleared effectively enough to observe type replacement. Furthermore, our previous studies,[6] found in the same non-HPV vaccinated population herd effects mostly in the youngest birth cohorts. Thereby it may be that in our current analyses using three of the four trial birth cohorts the ability to detect type replacement in the unvaccinated may have been somewhat suboptimal owing to less efficiently cleared ecological niche.

Although, the combined Arms A/B and 1992–1994 birth cohorts increased sample size may ward off findings due to chance, the stratified analyses may suffer from insufficient power in the core-group for more rare HPV types due to a small sample size. Additionally, although extension of follow-up to the 22-year-olds provides longer follow-up time to observe type replacement, owing to ethical concerns the previously non-HPV vaccinated controls were offered HPV cross-vaccination at the age of 18.5 years. This contaminated the control group, and decreased power to observe the true effect of vaccination on the occurrence of non-vaccine HPV types over time. Furthermore, although the generalizability of this study to the source population is high, the transportability of the findings may be limited

depending on the baseline prevalence of different HPV types, and population dynamics of the population in question.

In conclusion, post-vaccination HPV occurrence differs in the sexual risk-taking behaviour core- group as compared to the non-core group. Our new findings extend those found earlier,[8] backing up that HPV51 and HPV52 require continued post-vaccination surveillance.

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PUBLICATION V

Long-term follow-up of human papillomavirus type replacement among young pregnant Finnish females before and after a community-randomised HPV vaccination trial with moderate coverage

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Long-term follow-up of human papillomavirus type replacement among young pregnant Finnish females before and after a community-randomised HPV vaccination trial with moderate coverage

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Abstract

Large scale human papillomavirus (HPV) vaccination against the most oncogenic high-risk human papillomavirus (HPV) types 16/18 is rapidly reducing their incidence. However, attempts at assessing if this leads to an increase of nonvaccine targeted HPV types have been hampered by several limitations, such as the inability to differentiate secular trends. We performed a population-based serological survey of unvaccinated young women over 12 years. The women were under 23-years-old, residents from 33 communities which participated in a community-randomised trial (CRT) with approximately 50% vaccination coverage. Serum samples were retrieved pre-CRT and post-CRT implementation. Seropositivity to 17 HPV types was assessed. HPV seroprevalence ratios (PR) comparing the postvaccination to prevaccination era were estimated by trial arm. This was also assessed among the sexual risk-taking core group, where type replacement may occur more rapidly. In total, 8022 serum samples from the population-based Finnish Maternity Cohort were retrieved. HPV types 16/18 showed decreased seroprevalence among the unvaccinated in communities only after gender-neutral vaccination ($PR_{16/18A}$ = 0.8, 95% CI 0.7-0.9). HPV6/11 and

Abbreviations: CI, confidence intervals; CRT, community-randomised trial; FMC, Finnish Maternity Cohort; GEE, generalised estimating equation; HBV, hepatitis B vaccine; HPV, human papillomavirus; HSV-2, herpes simplex virus type II; IARC, International Agency for Research on Cancer; ICC, intracluster correlation coefficient; P, seroprevalence; PR, seroprevalence ratio; RPR, ratio of seroprevalence ratios.

HPV73 were decreased after gender-neutral vaccination ($PR_{6/11A}$ = 0.8, 95% CI 0.7-0.9, PR_{73A} = 0.7, 95% CI 0.6-0.9, respectively) and girls-only vaccination ($PR_{6/11B}$ = 0.8, 95% CI 0.7-0.9, PR_{73B} = 0.9, 95% CI 0.8-1.0). HPV68 alone was increased but only after girls-only vaccination (PR_{68B} = 1.3, 95% CI 1.0-1.7, $PR_{core68B}$ = 2.8, 95% CI 1.2-6.3). A large-scale, long-term follow-up found no type replacement in the communities with the strongest reduction of vaccine HPV types. Limited evidence for an increase in HPV68 was restricted to girls-only vaccinated communities and may have been due to secular trends (ClinicalTrials.gov number: NCT00534638).

KEYWORDS

community-randomised trial, core-group, HPV, serosurvey, type replacement

1 | INTRODUCTION

Oncogenic human papillomavirus (HPV), the necessary cause of cervical cancer,[1] is a well-established causal agent of several anogenital and oropharyngeal cancers. There are currently three efficacious HPV-vaccines licensed which target the two most high-risk HPV types HPV16 and 18, (the two first-generation vaccines, Cervarix and Gardasil), or five additional high-risk HPV types, HPV31, 33, 45, 52 and 58 (the nonavalent vaccine, Gardasil9).[2] Since 2007, these vaccines have been gradually implemented in national vaccination programs.[3] However, there are a total of 12 high-risk HPV types which are classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59, with a further eight types classified as possibly (or probably) carcinogenic, HPV26, 53, 66, 68, 67, 70, 73 and 83.[4] As such, concern has been flagged over a decade ago, whether such selective vaccination could induce HPV type replacement to occur.[5] By removing selected HPV types, vaccination may disrupt the dynamic equilibrium among HPV types. Subsequently, the vacant niche may become superceded by one or more of the nonvaccine types. This vaccine-induced evolutionary response in the niche habitation by the nonvaccine types has already been described in analogous situations, for example, following vaccination against *Streptococcus pneumoniae*, and is commonly known as type replacement.[6,7]

Several studies have evaluated the occurrence of HPV type replacement, using differing methodologies; comparison of the HPV prevalence between the postvaccination and prevaccination era,[8-10] between vaccinated and unvaccinated persons in the postvaccination era[11] or via individually randomised HPV vaccination trials.[12] Importantly, a recent meta-analysis of the above-mentioned studies found an increasing trend in the pooled nonvaccine targeted (and noncross-protected) HPV types,[13] further to an earlier meta-analysis which found possible increases in HPV 39 and 52.[14]

However, there are multiple major limitations when evaluating the occurrence of HPV type replacement. When conducting post-vaccination era surveillance and comparing the HPV prevalence prevaccination and postvaccination era, it is difficult to distinguish possible increases due to vaccine induced-type replacement from that due to secular trends. On the other hand, evaluating type replacement by means of negative vaccine effectiveness in the postvaccination era, may be an unsuitable measure for both the identification and prediction of type replacement occurrence. That is, type replacement may be subdued in the vaccinated by vaccine-induced cross-protection, while manifesting with lesser limitation in the unvaccinated due to the indirect impacts of community-wise vaccination.[15] Last but not least, when evaluating type replacement via individually randomised clinical trials, it is likely that any estimates will underestimate the probability of type replacement, as the vaccination-induced selective pressure stems from too small a proportion of the population in comparison to that present after community-wise vaccination.[16]

Further to these, following vaccination against several other pathogen types, there has been a transitory "honeymoon period" immediately following vaccination implementation, before arriving at a new endemic equilibrium.[17,18] In the context of HPV vaccination, a recently published modelling study has found that there may be a HPV type replacement "honeymoon period" following vaccination, wherein nonvaccine types may at first appear to remain stable or even decrease before rebounding due to type replacement after a certain

What's new?

Vaccination efforts have decreased the prevalence of oncogenic HPV types, such as HPV 16/18. This may create space for other types to expand, but it is difficult to distinguish the effect of the vaccine from long-term temporal trends. Here, the authors conducted a community-randomized trial in 33 communities. Each group of 11 communities received either gender-neutral HPV vaccination, girls-only HPV vaccination, or gender neutral hepatitis vaccination. In the girls-only arm, vaccination reduced the prevalence of HPV 6/11, and HPV 68 increased in prevalence. However, this effect may have been due to secular trends.

incubation period since vaccination initiation.[15] Thus, previous inconclusive findings of any HPV type replacement may have been premature to identify type replacement occurrence.

We now evaluate the occurrence of HPV type replacement in a decade following a large population-based community-randomised HPV vaccination trial with close to 50% vaccination coverage (beginning in October 2007), by conducting a survey of HPV seroprevalence in unvaccinated Finnish female community residents over the pretrial and posttrial era. Herpes simplex virus type II (HSV-2) serology is an established marker of sexual risk taking, thereby we now utilise this as a proxy of core group membership,[19] to further investigate the occurrence of type replacement also within the core-group (the assortative subgroup of the population with high sexual contact rates), as it has been suggested that HPV type replacement may first manifest within this group.[20]

2 | MATERIALS AND METHODS

2.1 | Study design and materials

The material of our study comprises of longitudinal population-based biobank follow-up of the Finnish community-randomised HPV vaccination trial evaluating the comparative effectiveness of girls-only or gender-neutral HPV vaccination (NCT00534638).[21-23]

Briefly, in 2007, 33 Finnish communities were stratified according to preascertained HPV16/18 seroprevalence,[24] into those with low, moderate or high seroprevalence. From each seroprevalence strata, the communities were then randomised to one of three trial arms, with an allocation ratio of 11:11:11.[21] All Swedish or Finnish speaking 1992 to 1995 born adolescents who were residing in the trial communities were identified via the Finnish Population Register and were invited in 2007 to 2010 to participate in the trial.[21] Of the 80 272 invited, a total of 20 513 females and 11 662 males participated in the trial from 2007 to 2010, with informed parental, guardian or participant consent (if aged 15 years old). In Arm A, a gender-neutral HPV vaccination strategy was applied to the communities, in Arm B a girls-only HPV vaccination strategy was applied and in Arm C a gender-neutral vaccination strategy was applied using the Hepatitis B (HBV) vaccine, thereby serving as the control Arm.[21,23] No organised HPV vaccination was administered to the birth cohorts following the trial birth cohorts until November 2013, when the Finnish national HPV vaccination program was initiated targeting those born in 1998 and younger.

The indirect effects of the trial were followed up by a population-based serological survey of unvaccinated women under the age of 23-years-old from 2005 until 2016, representing both the periods pre-HPV and post-HPV vaccination.[22] This was achieved by retrieving all the available first pregnancy serum samples from the Finnish Maternity Cohort (FMC), from women under 23-years-old at the time of donation within the sampling time frame, who had been resident in one of the 33 trial communities at the time of sample donation. The Finnish Maternity Cohort is a population-representative biobank housing approximately 2 million samples (the Finnish population is approximately 5.5 million), established in 1983. Since 1983, all pregnant women in Finland have been invited to donate the residual volume of their blood sample after the mandatory testing for congenital infections for future research purposes; approximately 96% consent.[25] The vaccination status of the women was confirmed via linkage of the eligible FMC subjects with the HPV vaccination trial registry for all women, and via manual scrutinization of the subject's antibody titres for those women from the birth cohorts previously eligible for HPV vaccination via the Finnish National vaccination program (those born from 1998 and younger).

Among the 1992, 1993, 1994 and 1995, birth cohorts initially receiving HPV vaccination in the trial arms, community-wise vaccination coverage among females was 44.0%, 45.5%, 51.9% and 48.2% in the gender-neutral vaccination arm, 45.2%, 44.2%, 46.5% and 45.4% in girls-only vaccination arm and 0% in all four birth cohorts among the control arm (Figure 1). Among the same birth cohorts, the community-wise vaccination coverage among the males was 18.3%, 16.9%, 21.1% and 22.2% in the gender-neutral-vaccination Arm, whereas it was 0% among all four birth cohorts in girls-only vaccination and control arms. Among 1998, 1999 and 2000 birth cohorts receiving girls-only vaccination via the Finnish national vaccination program the vaccination coverage among females was 65.1%, 66.8% and 69.0%, respectively, in the communities which were also subject to the community-randomised trial.[26]

Information on self-reported maternal smoking among the pregnant females under the age of 23 from the same communities and sampling years 2005 to 2016 was garnered from the Finnish Medical Birth Registry.[27] Birth cohort-specific vaccination coverages for males and females by community and calendar year were gathered from the Finnish vaccination register.[22,26]

2.2 | Laboratory analyses

The retrieved FMC serum samples were analysed for the presence of serum antibodies to HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and herpes simplex virus type II (HSV-2) using heparin bound HPV pseudovirion and HSV-2 glycoprotein G 2 Luminex assay.[22,28,29] A negative control panel of serum samples from children under the age of 12-years-old was used to calculate HPV type-specific seropositivity cut-off values, by computing the median fluorescence intensities of the negative control serum panel plus three SDs.[22] For this study, the assay panel was extended to include also HPV51, 66 and HSV-2, more details of which are described in the Supporting Information Methods and reference 22.

2.3 | Statistical analyses

The magnitude of within-arm clustering was measured by calculating the intracluster correlation coefficient (ICC) using observations from 2005 to 2010, among all subjects and subjects stratified by HSV-2 seropositivity. The ICC of vaccine targeted and nonvaccine targeted

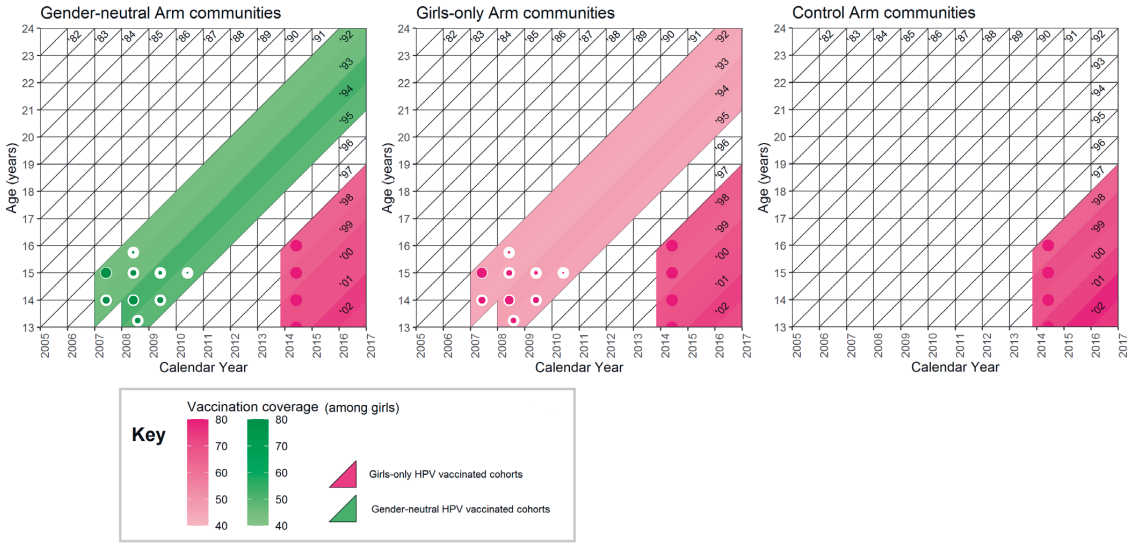

FIGURE 1 Lexis diagrams describing the birth cohorts included in the study population. Each diagonal line represents an individual birth cohort, and the coloured fill the vaccination coverage among each birth cohort. The circles represent the years in which the particular cohorts received vaccination, and the coloured fill, the proportion of the total vaccination received that year

HPV was estimated using Fleiss and Cuzick's estimator and the accompanying 95% confidence intervals estimated using Zou and Donners modified Wald test.[30,31]

To evaluate the occurrence of HPV type replacement, the absolute seroprevalence of nonvaccine protected HPV39, 51, 56, 58, 59, 66, 68 and 73 was calculated among subjects stratified by trial Arm, and vaccination era (from 2005 to 2010 or 2011 to 2016, respectively the prevaccination or postvaccination era). Nonvaccine protected HPV types are defined as HPV types excluding bivalent vaccine targeted HPV16 and 18, and documented cross-protected types 31, 33, 35, 45 and 52. Within-arm HPV type-specific seroprevalence ratios, PR, were then estimated using a log binomial generalised estimating equation (GEE) model with an exchangeable correlation structure, comparing the postvaccination era seroprevalence, 2005 to 2010, to that in the prevaccination era, 2011 to 2016. All estimates were adjusted for era-specific community-wise maternal smoking as a proxy measure of general risk-taking behaviour. To evaluate the occurrence of type replacement among the core group, with-in arm PRs were similarly estimated among subjects who were HSV-2 seropositive (as a proxy measure of core-group membership). To take account for a possible delay between niche clearance and type replacement occurrence, with-in arm PRs were further stratified by the postvaccination era numerator, into the first or second postvaccination era, 2011 to 2013 or 2014 to 2016, respectively, compared to the entire prevaccination era. To assess whether observed increased with-in arm PRs were due to secular trends or type replacement, type specific with-in arm PRs from the intervention arms were compared to the with-in arm PRs from the control arm using the methodology of Altman and Bland.[32]

As a sensitivity analysis, probabilistic bias analysis was used to take account of misclassification owing to the known lack of seroconversion in a proportion of individuals following HPV infection. Previously[33] and currently ascertained (see Appendix for HPV51 and 66) specificity and sensitivity values of this heparin-bound HPV pseudovirion serology to identify cumulative infections were used. As a further sensitivity analysis, the HPV type-specific PRs were additionally estimated stratified by subjects age at the time of sample donation (into those aged 14- to 19-years-old and 20- to 22-years-old).

The statistical analyses were conducted using R software package (version 3.6.0.) with the following packages: ICCbin (version 1.1.1), geepack (version 1.2-1), episensr (version 0.9.5) for the analyses and ggplot2 (version 3.2.1) and LexisPlotR (version 0.3.2) for the graphical presentation of the results.

3 | RESULTS

3.1 | Characteristics of the study

A total of 8022 unvaccinated pregnant subjects from the 33 trial communities were identified who had consented to participate in the Finnish Maternity Cohort during the years 2005 to 2016 while under the age of 23-years-old. Four thousand and seven were identified from the prevaccination years, 2005 to 2010, and 4015 from the postvaccination years, 2011 to 2016. From these subjects, 91 were excluded from the prevaccination years, and 345 from the postvaccination era, due to being over-aged when attending serum

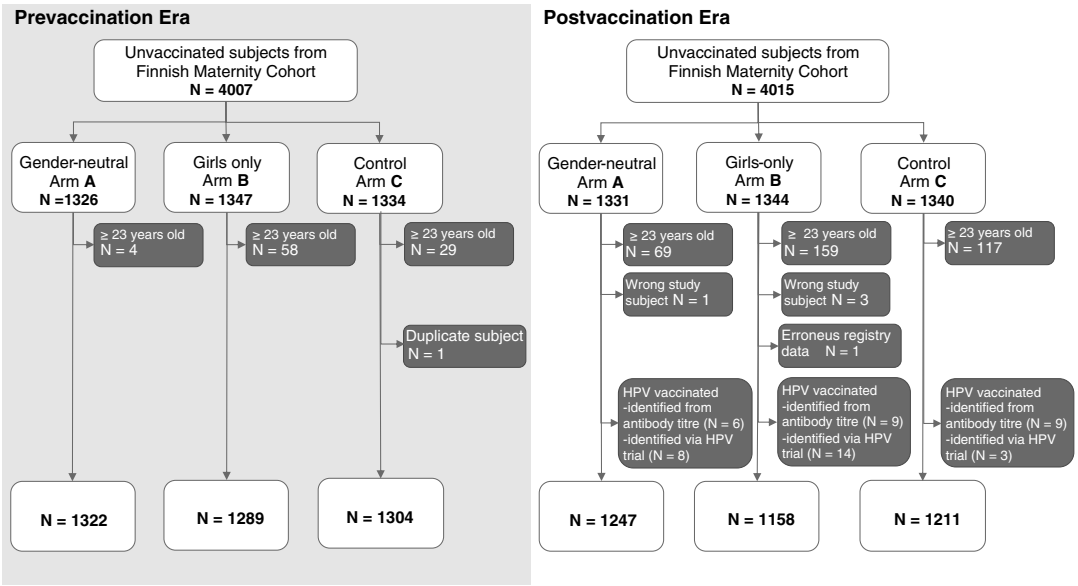

FIGURE 2 Flow chart of the study population and all exclusions stratified by time period [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Characteristics of the prevaccination era and postvaccination era unvaccinated females according to trial Arm (gender-neutral vaccination Arm A, girls-only vaccination Arm B or control Arm C)

	Prevaccination Era (2005-2010)			Postvaccination Era (2011-2016)		
	Arm A (n = 1322) n (%)	Arm B (n = 1289) n (%)	Arm C (n = 1304) n (%)	Arm A (n = 1247) n (%)	Arm B (n = 1158) n (%)	Arm C (n = 1211) n (%)
Birth cohort						
1982-1983	126 (9.5)	115 (8.9)	111 (8.5)	0 (0.0)	0 (0.0)	0 (0.0)
1984-1985	296 (22.4)	317 (24.6)	327 (25.1)	0 (0.0)	0 (0.0)	0 (0.0)
1986-1987	411 (31.1)	374 (29.0)	387 (29.7)	0 (0.0)	0 (0.0)	0 (0.0)
1988-1989	319 (24.1)	311 (24.1)	288 (22.1)	116 (9.3)	116 (10.0)	115 (9.5)
1990-1991	142 (10.7)	146 (11.3)	168 (12.9)	315 (25.3)	302 (26.1)	311 (25.7)
1992-1993	28 (2.1)[a]	26 (2.0)[a]	23 (1.8)[a]	424 (34.0)	409 (35.3)	415 (34.3)
1994-1995				272 (21.8)	231 (19.9)	250 (20.6)
1996-1997	0 (0.0)	0 (0.0)	0 (0.0)	105 (8.4)	89 (7.7)	106 (8.8)
1998-2000	0 (0.0)	0 (0.0)	0 (0.0)	15 (1.2)	11 (0.9)	14 (1.2)
Age (years)						
14-16	28 (2.1)	21 (1.6)	27 (2.1)	19 (1.5)	15 (1.3)	26 (2.1)
17-19	392 (29.7)	390 (30.3)	376 (28.8)	354 (28.4)	305 (26.3)	339 (28.0)
20-22	902 (68.2)	878 (68.1)	901 (69.1)	874 (70.1)	838 (72.4)	846 (69.9)
Herpes simplex virus type 2 (HSV-2)						
Positive	224 (16.9)	247 (19.2)	224 (17.2)	189 (15.2)	162 (14.0)	192 (15.9)
Negative	1098 (83.1)	1042 (80.8)	1080 (82.8)	1058 (84.8)	996 (86.0)	1019 (84.1)
	Arithmetic mean (SD)					
Community-wise self-reported maternal smoking	38.4 (6.0)	36.7 (6.0)	42.8 (7.6)	37.8 (5.3)	38.6 (6.4)	43.3 (9.3)

[a]In the prevaccination era reporting for the 1992-1995 birth cohorts is merged to avoid reporting identifiable data due to small count numbers (n < 5).

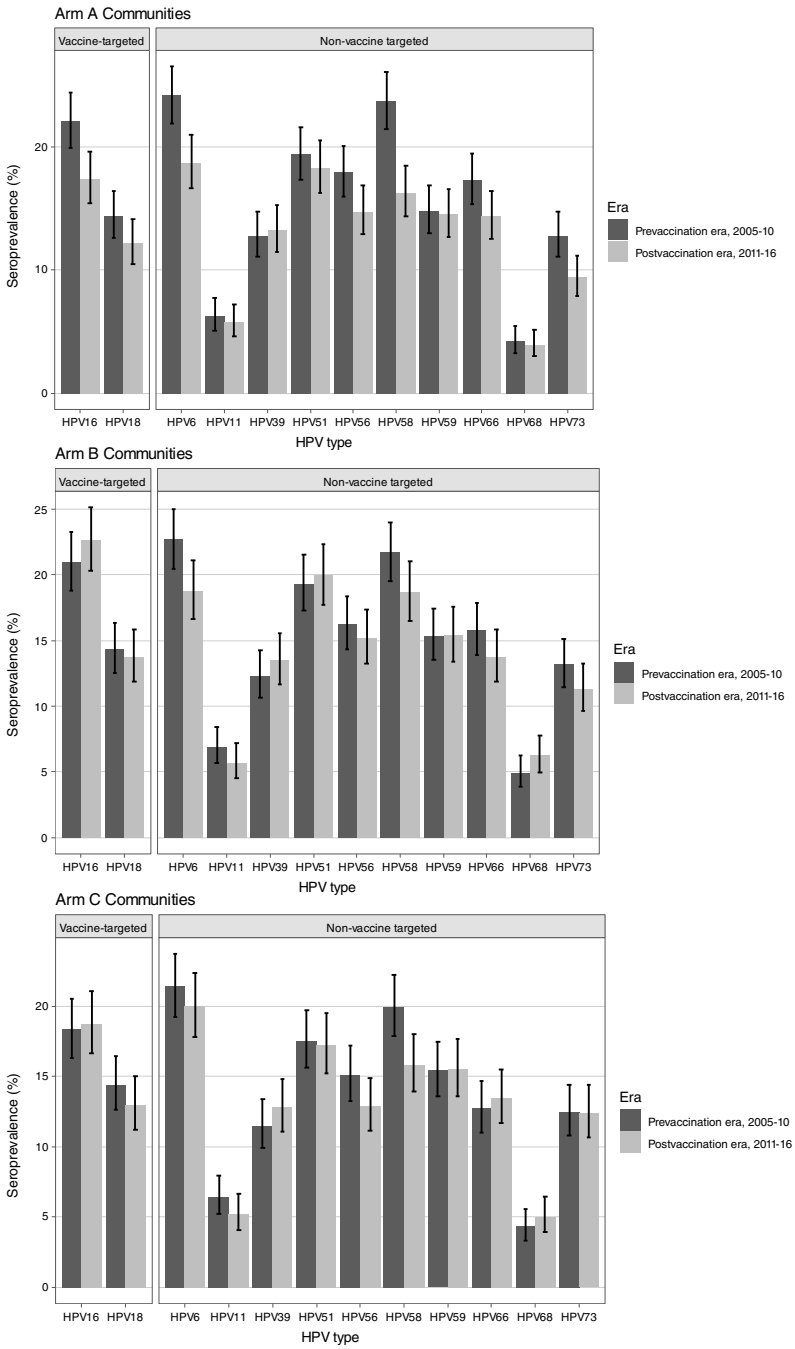

FIGURE 3 HPV type-specific seroprevalence (%) by trial Arm and vaccination era (prevaccination and postvaccination era, 2005-2010 and 2011-2016, respectively). The error bars signify the 95% confidence intervals

sampling. A total of 49 subjects from the postvaccination years were excluded due to being HPV vaccinated (as identified via registry linkage, n = 25, and via characteristically high antibody titres, n = 24; Figure 2). From the initial subjects identified, a total of 3915 were included in the study from the prevaccination era and 3616 from the postvaccination era. The intracluster correlation coefficient, ICC, among the postvaccination era approximated zero for high risk (hr) HPV16/18 (ICC = 0.007, 95% confidence intervals, CI 0.00-0.06), HPV39/51/56/58/59/66/68/73 (ICC = 0.009, 0.00-0.06) and low risk (lr) HPV6/11 (ICC = 0.001, 0.00-0.03).

TABLE 2 Seroprevalence ratios (95% confidence intervals) comparing the seroprevalence in the postvaccination era, 2011-2016, to that in the prevaccination era among, A, all subjects and B, among the core-group (identified by herpes simplex virus type 2 seropositivity)

A			Seroprevalence ratios (95% CI)		
			Unvaccinated Finnish females aged under 23 years		
			Postvaccination vs prevaccination era		
HPV type			Arm A (n = 1247 vs 1322)	Arm B (n = 1158 vs 1289)	Arm C (n = 1211 vs 1304)
Vaccine targeted	hr	16	0.79 (0.72-0.87)	1.09 (0.91-1.32)	1.01 (0.86-1.20)
		18	0.86 (0.70-1.06)	0.96 (0.74-1.24)	0.89 (0.70-1.13)
Nonvaccine targeted	lr	6	0.78 (0.67-0.92)	0.82 (0.75-0.91)	0.93 (0.82-1.05)
		11	0.93 (0.68-1.28)	0.78 (0.60-1.01)	0.80 (0.58-1.10)
	hr	39	1.04 (0.88-1.22)	1.07 (0.85-1.33)	1.09 (0.92-1.31)
		51	0.95 (0.78-1.17)	1.03 (0.91-1.15)	0.98 (0.80-1.20)
		56	0.83 (0.73-0.93)	0.93 (0.77-1.13)	0.85 (0.70-1.02)
		58	0.70 (0.63-0.79)	0.86 (0.72-1.03)	0.79 (0.65-0.96)
		59	0.99 (0.80-1.22)	1.00 (0.82-1.22)	1.00 (0.79-1.26)
		66	0.83 (0.73-0.96)	0.89 (0.73-1.08)	1.05 (0.89-1.24)
		68	0.94 (0.72-1.23)	1.28 (0.97-1.68)	1.16 (0.90-1.51)
		73	0.74 (0.60-0.91)	0.87 (0.76-1.00)	0.99 (0.85-1.15)
			Seroprevalence ratios (95% CI)		
			Unvaccinated Finnish females aged under 23		
			Postvaccination vs prevaccination vaccination era		
B	HPV type		Arm A (n = 189 vs 224) HSV-2 positive	Arm B (n = 162 vs 247) HSV-2 positive	Arm C (n = 192 vs 224) HSV-2 positive
Vaccine targeted	hr	16	0.64 (0.50-0.81)	0.94 (0.63-1.42)	0.98 (0.70-1.38)
		18	0.95 (0.60-1.51)	0.74 (0.34-1.65)	1.00 (0.68-1.46)
Nonvaccine targeted	lr	6	0.61 (0.43-0.87)	0.43 (0.34–0.54)	0.95 (0.64-1.43)
		11	0.82 (0.48-1.39)	0.54 (0.21-1.37)	0.96 (0.51-1.81)
	hr	39	0.77 (0.62-0.96)	0.90 (0.50-1.60)	1.10 (0.70-1.75)
		51	0.71 (0.48-1.05)	1.04 (0.78-1.38)	1.14 (0.81-1.59)
		56	0.72 (0.55-0.94)	0.68 (0.43-1.07)	0.65 (0.40-1.05)
		58	0.65 (0.44-0.96)	0.79 (0.54-1.14)	0.64 (0.39-1.06)
		59	0.90 (0.75-1.09)	0.76 (0.54-1.07)	0.82 (0.57-1.18)
		66	0.72 (0.53-0.98)	0.76 (0.44-1.30)	0.79 (0.59-1.06)
		68	0.57 (0.23-1.38)	2.78 (1.23-6.31)	1.40 (0.79-2.49)
		73	0.45 (0.35-0.59)	0.84 (0.57-1.24)	0.86 (0.53-1.39)

Note: Estimates are HPV type-specific including both low and high-risk nonvaccine targeted HPV types and to ensure niche clearance also vaccine targeted HPV16/18. Estimates are stratified by trial arm (*hr*, high risk HPV type; *lr*, low risk HPV type).

The demographics of the study subjects were comparable between the prevaccination and postvaccination eras. In both eras, the age distribution was left-skewed with the majority of subjects being aged 17 to 22 years old, and only a small minority being aged under 17 (Table 1). The shape of the birth cohort distribution remained the same in the prevaccination to postvaccination era, but with a shift towards the younger cohorts in the postvaccination era (from those born 1992 and younger, to those born in 1988 and younger; Table 1). The HSV-2 seroprevalence was somewhat reduced between the prevaccination and postvaccination eras among subjects from Arm B (19.2%-14.0%), although not notably altered in subjects from Arms A or C (Table 1). The vaccination coverage among the 1992 to 1995 females was comparatively similar among the intervention Arms A and B (Figure 1).

3.2 | HPV seroprevalence, postvaccination vs prevaccination era

Among the subjects, the crude seroprevalence, P, of vaccine targeted HPV16 and 18 was notably high among all three intervention arms

TABLE 3 HPV type-specific seroprevalence ratios comparing the seroprevalence in the postvaccination era, to that in the prevaccination era

			Seroprevalence ratios (95% CI)					
			Unvaccinated Finnish females aged under 23 years					
			First postvaccination vs prevaccination era			Second postvaccination vs prevaccination era		
	HPV type		Arm A (n = 662 vs 1322)	Arm B (n = 602 vs 1289)	Arm C (n = 650 vs 1304)	Arm A (n = 585 vs 1322)	Arm B (n = 556 vs 1289)	Arm C (n = 561 vs 1304)
Vaccine targeted	hr	16	0.76 (0.65-0.88)	1.16 (0.95-1.42)	0.96 (0.78-1.17)	0.83 (0.72-0.96)	1.03 (0.83-1.28)	1.07 (0.90-1.27)
		18	0.78 (0.59-1.01)	0.92 (0.72-1.18)	0.81 (0.62-1.06)	0.95 (0.75-1.19)	1.01 (0.71-1.43)	0.98 (0.76-1.27)
Nonvaccine targeted	lr	6	0.83 (0.68-1.02)	0.98 (0.81-1.19)	1.02 (0.87-1.21)	0.73 (0.61-0.86)	0.66 (0.49-0.90)	0.81 (0.69-0.96)
		11	1.04 (0.72-1.50)	0.86 (0.61-1.22)	1.01 (0.72-1.43)	0.80 (0.55-1.15)	0.70 (0.46-1.07)	0.55 (0.33-0.89)
	hr	39	0.99 (0.82-1.20)	1.19 (0.91-1.55)	1.05 (0.84-1.31)	1.08 (0.86-1.36)	0.93 (0.75-1.16)	1.14 (0.91-1.44)
		51	1.02 (0.79-1.31)	1.06 (0.90-1.25)	1.03 (0.84-1.28)	0.88 (0.70-1.10)	0.98 (0.82-1.18)	0.91 (0.71-1.17)
		56	0.83 (0.70-0.99)	1.06 (0.90-1.24)	0.95 (0.73-1.23)	0.81 (0.66-1.00)	0.80 (0.58-1.09)	0.73 (0.56-0.95)
		58	0.78 (0.67-0.91)	1.03 (0.86-1.23)	0.89 (0.74-1.07)	0.61 (0.53-0.70)	0.66 (0.53-0.84)	0.66 (0.51-0.87)
		59	0.99 (0.73-1.33)	1.13 (0.87-1.46)	1.12 (0.86-1.46)	0.99 (0.75-1.32)	0.86 (0.68-1.09)	0.85 (0.67-1.07)
		66	0.85 (0.71-1.01)	0.93 (0.74-1.16)	0.96 (0.77-1.21)	0.82 (0.68-1.00)	0.85 (0.65-1.11)	1.16 (0.91-1.47)
		68	0.80 (0.54-1.16)	1.02 (0.71-1.48)	0.99 (0.65-1.52)	1.10 (0.76-1.59)	1.54 (1.02-2.34)	1.36 (1.00-1.85)
		73	0.76 (0.60-0.96)	0.96 (0.75-1.23)	1.13 (0.96-1.33)	0.72 (0.55-0.93)	0.78 (0.56-1.08)	0.83 (0.59-1.17)

Note: The prevaccination era is defined as 2005-2010, and the postvaccination era as 2011-2016, which is further divided into the first and second postvaccination eras of 2011-2013 and 2014-2016 respectively. Estimates are stratified by trial arm (*hr*, high-risk HPV type; *lr*, low-risk HPV type).

consistently above 15% for HPV16 and above 10% for HPV18. When comparing the postvaccination era to the prevaccination era, 2011-2016 to 2005-2010, respectively, the seroprevalence of the HPV16 and HPV18 was reduced (without overlapping 95% confidence intervals in the case of HPV16) in Arm A (from P_{HPV16} = 22.1%, to P_{HPV16} = 17.4% and from P_{HPV18} = 14.4% to P_{HPV18} = 12.2%), no comparable reductions were observed in Arm B or C. Among the nonvaccine targeted HPV types the crude seroprevalence of HPV6, 56, 58, 66 and 73 was found to decrease in Arm A (from P_{HPV6} = 24.1% to P_{HPV6} = 18.7%, P_{HPV56} = 17.9% to P_{HPV56} = 14.8%, P_{HPV58} = 23.7% to P_{HPV58} = 16.3%, P_{HPV66} = 17.3% to P_{HPV66} = 14.4% and from P_{HPV73} = 12.8% to P_{HPV73} = 9.3%; Figure 3).

When comparing the postvaccination era to the prevaccination era among all the subjects, the smoking adjusted seroprevalence ratios of both HPV16 and 18 were reduced among Arm A only (Table 2A). Among the nonvaccine targeted HPV types, the PR was decreased for HPV6, 66 and 73 (PR_{HPV6A} = 0.78, 95% CI 0.67-0.92, PR_{HPV66A} = 0.83, 0.73-0.96 and PR_{HPV73A} = 0.74, 0.60-0.91), without any similar decrease in Arm C. In Arm B for the nonvaccine targeted HPV types the PR was decreased for HPV6, 11 and 73 (PR_{HPV6B} = 0.82, 95% CI 0.75-0.91, PR_{HPV11B} = 0.78, 0.60-1.01 and PR_{HPV73B} = 0.87, 0.76-1.00). The PR of HPV68 was found to increase (PR = 1.28, 95% CI 0.97-1.68) only among Arm B, albeit nonsignificantly so (Table 2A). When conducting sensitivity analysis to additionally take into account systematic error due to outcome misclassification HPV68 was found to be further increased (PR = 1.51, 95% CI 1.02-2.44; Table S1). Further sensitivity analysis stratified by age group, found the Arm B specific increase in HPV68 to be more prominent among the younger subjects aged 14 to 19 years old (PR = 1.62, 95% CI 1.01-2.60; Table S2).

When comparing the postvaccination era to the prevaccination era among all HSV-2 seropositive subjects (as a proxy of core-group membership), the vaccine targeted HPV16 PR continued to be decreased in Arm A (PR_{16} = 0.64, 95% CI 0.50-0.81). Among the nonvaccine targeted types, no PRs were found to increase in Arm A. In Arm A, the PRs for HPV6, 66 and 73 were again all decreased (PR_{6A} = 0.61, 95% CI 0.43-0.87, PR_{66A} = 0.72, 0.53-0.98 and PR_{73A} = 0.45, 0.35-0.59), notably with the exception of HPV66 there were no similar decreases in Arm C. In Arm B, the PRs for HPV6, 66 and 73 were also decreased (PR_{6B} = 0.43, 95% CI 0.34-0.54, PR_{66B} = 0.76, 0.44-1.30 and PR_{73B} = 0.84, 0.57-1.24). However, only for HPV6 was the magnitude of the decrease not replicated in Arm C. Among the HSV-2 seropositive subjects of Arm B, the PR was found to be substantially increased solely for HPV68 (PR_{68} = 2.78, 95% CI 1.23-6.31; Table 2B).

When comparing the latest half of the postvaccination era to the prevaccination era, 2014-2016 to 2005-2010, among all subjects irrespective of HSV-2 seropositivity, no significant increases in nonvaccine target PRs were observed in Arm A. Among Arm B, the PR estimate for HPV68 was further increased (PR_{68} = 1.54, 95% CI 1.02-2.34). However, during this era, HPV68 PR was also increased among Arm C (PR_{68} = 1.36, 95% CI 1.00-1.85; Table 3) When conducting sensitivity analysis to account for additional systematic error, HPV68 was again found to be further increased in the second postvaccination era, but not significantly different to that found in Arm C (Table S3).

3.3 | Comparing HPV seroprevalence changes by trial arm

When comparing the within-arm PRs between the Arms A or B to Arm C, we found no notable increases in the nonvaccine HPV types'

ratios of seroprevalence ratios (RPRs; Figure S1). When comparing Arm B to Arm C core-groups, although the HPV68 RPR was increased, the confidence intervals overlapped the null (RPR = 1.98, 0.73-5.40), and for the latest subjects, the RPR approximated 1 (Figure S2, Table S4).

4 | DISCUSSION

Of all the nonvaccine targeted HPV types measured, only the seroprevalence of HPV68 was found to have increased in the postvaccination era in the manner which would be expected if type replacement was occurring. This increase was only observed after girls-only vaccination, with concomitantly reduced HPV6/11 seroprevalence. The magnitude of the postvaccination era increase in HPV68 seroprevalence was particularly pronounced in the core-group (identified using HSV-2 seropositivity). Moreover, this increase was found to stem more from the later postvaccination years, from 2014 to 2016, as would be expected if this increase was as a result of type replacement.

When comparing this postvaccination vs prevaccination increase to that in the counterfactual control Arm C, this increase among Arm B was found to disappear, except in the core-group. This might suggest that the observed increase in Arm B may have been partially due to secular trends rather than type replacement. Furthermore, the core-group observations lacked statistical significance, meaning that the observed increase may have been due to chance.

Although the finding of increased HPV68 may be explainable due to aforementioned reasons other than type replacement, this possibility deserves comment. Previous modelling studies assuming that HPV types compete via the hosts immune system, have demonstrated that the occurrence of type replacement, and the ability to observe it at an early stage of the postvaccination era, is a trade-off between vaccine-induced cross-protection and naturally acquired cross-immunity.[15] HPV68 is from the alpha 7 species, and although phylogenetically related to HPV18 present in the bivalent vaccine, has not been shown following vaccination programs or clinical trials to be cross-protected by the vaccine.[34] Although a large degree of vaccine-induced cross-protection has been demonstrated against types phylogenetically related to vaccine targeted HPV16 and 18, alpha 9 and alpha 7 species, respectively, the vaccine has been much more successful at eliciting cross-neutralising antibodies to HPV types from alpha 9, than it has to HPV types from alpha 7.[35] From the alpha 7 species, only HPV45 has been shown to be cross-protected by the bivalent vaccine.[13] Thus, the HPV68 increase will likely not be mitigated by vaccine-induced cross-protection. Furthermore, the fact that the observed increase in HPV68 after girls-only vaccination stems more from the second postvaccination era, is in line with a proposed honeymoon period postvaccination before type replacement might occur.[15]

We noted a decrease in both the occurrence of low-risk type HPV6, HPV 66 and the possibly high-risk type HPV73. This reduction in HPV6 was found to exactly follow the patterns that would be expected if the bivalent vaccine had induced HPV6 herd effect; the observed reduction was the greatest postgender-neutral vaccination and when comparing to the control Arm C was not explainable due to secular trends. HPV6 is a very common low-risk HPV type, responsible for a large proportion of genital warts, and not phylogenetically related to either of the vaccine-targeted types. Nevertheless, this finding of a possible HPV 6 herd effect postbivalent HPV vaccination, is consistent with previous findings of bivalent vaccine efficacy against HPV6,[36] reported postvaccine era reductions in genital warts when using the bivalent vaccine,[37,38] and also with findings of HPV6 specific vaccine-induced cross-neutralising antibodies among individuals vaccinated with the bivalent HPV vaccine.[35] The finding of decreased HPV73, however, is unexpected. The majority of previous studies monitoring the HPV type distribution postbivalent HPV vaccination via PCR methodology have either not measured HPV73 at all or have been unable to distinguish transitory infection with HPV68 to that due to HPV73, due to limitations of the laboratory method used. This finding of a possible herd effect against HPV73 is therefore reassuring; although HPV73 was officially classified as a possibly high-risk type in the last published version of the IARC monograph pertinent to HPV,[4] a study conducted since its publication found HPV73 to be causally associated with the development of invasive cervical cancer.[39] HPV73 is from the alpha 11 species group, which is of particular interest given that the alpha 11 species group is phylogenetically close to the alpha 9 species, and the degree of cross-protection is correlated to the phylogenetic distance to the vaccine types.[40] Contrary to this, the observed decrease of HPV66 in Arm A was unexpected. Previous studies have not documented any decrease in HPV66 following the induction of the bivalent HPV vaccine. In several such studies, HPV66 was not included in the laboratory assay,[41,42] and where HPV66 was evaluated, there was no notable vaccine efficacy observed against a HPV infection endpoint.[43,44] Thus, the currently observed decrease in HPV66 necessitates further study before any causal relationship may be asserted, to guard against a chance finding among the multiple HPV type comparisons which are commonplace in such HPV type replacement studies.

Even with a decade of follow-up, our study may still be limited in its ability to evaluate HPV type replacement. A recent modelling study found that there may be a honeymoon period of 10 years after the start of HPV vaccination in a population, before HPV type replacement starts to occur.[15] However, this is only considering the scenario where prevaccination competition occurs via naturally acquired cross-immunity,[15] thus if type competition occurs via another mechanism, such as for resources (eg, competition for available micro-abrasions), then it may be that this honeymoon period either does not apply or is altered. Therefore, whether or not our survey is limited by the follow-up may be subject to the biology of HPV type competition.

Furthermore, our study may also suffer from bias due to misclassification of the outcome, cumulative HPV infection. As previously described,[22] when measuring the occurrence of HPV infection in a population, when using either HPV DNA measures or serological measures among unvaccinated individuals, both methods suffer from misclassification of the outcome. When using transitory DNA positivity

as a marker of current infection, the investigator shall also incorrectly identify a proportion of individuals as HPV positive whom have only a deposition of transient HPV and not an actual infection.[45] When using HPV serology, on the other hand, it allows for the identification of those individuals who have had true persistent infections, and is a measure of cumulative HPV infection exposure.[46] Serum IgG antibodies induced by natural infection specific to HPV types have previously been shown to be stable over several years of follow-up among women.[47] However, this method will also incorrectly identify a proportion of individuals as negative who have previously had a HPV infection but have not seroconverted; among a sample of Swedish women with clinically confirmed HPV16 infections only 65% were found to be HPV16 seropositive.[48] Therefore, by using type-specific HPV serology as a measure of cumulative infection our comparative measures of HPV occurrence are likely deviated towards the null.

There are many difficulties in designing a study with the ability to evaluate type replacement of any kind. The community-randomised trial design of our study with both pretrial and posttrial outcome measurements is best placed to evaluate type replacement and avoid many of these common problems.[15] The pretrial and posttrial measurements in the counterfactual control Arm C, makes it possible to distinguish type replacement from secular trends in nonvaccine HPV occurrence, and the community-wise vaccination where entire birth cohorts of early adolescents were identified and invited to participate in the trial, mimics the expected selective pressure to HPV ecology after the application of a national vaccination program. Further to which, the comparison of HPV unvaccinated females in the prevaccination and postvaccination eras allows for the evaluation of type replacement among the population subject to the indirect effects of community-wise vaccination.

Although our study is highly generalizable to the wider pregnant Finnish population under the age of 23 due to the population-based nature of the study, it may be limited in its generalisability to all females under the age of 23 years old. Although the prevalence of maternal smoking (an indicator of general risk-taking behaviour) is high in our study population, it is almost identical to that found in previous studies of pregnant females of similar age over for the total population of Finland.[49] However, it is likely that our study population has above and below average sexual risk-taking behaviours compared to the general population given that the average age of first pregnancy in Finland is currently 29-years-old.[50] Despite the presence of our control Arm C to control for secular trends, our study still may not have been able to completely distinguish the magnitude of the increase in HPV68 due to type replacement from that due to secular trends, for example, if the parallel trend assumption between the Arms does not hold. Finally, although our study with moderate vaccination coverage may mimic typical vaccination coverages achieved in many national vaccination programs, it is limited in its transportability to scenarios with greater vaccination coverage.

In conclusion, no clear indications of type replacement were found, as of yet. Possible increases in HPV68 after girls-only vaccination may have resulted from secular trends. Continued monitoring in the postvaccination era to confirm or refute possible HPV type replacement by HPV68 and all other nonvaccine targeted HPV types is necessary.

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CONFLICT OF INTEREST

M. L. has previously received grants from Merck & Co. Inc. and the GSK group of companies through his then employer (The University of Tampere).

ETHICS STATEMENT

At the time of sample donation, the pregnant females comprising the FMC gave their informed consent for the future use of their samples for research purposes. Ethical permission for the community randomised trial was granted in 2007 from the Pirkanmaa Hospital District Ethical Review Board (R07113M 14.6.2007).

DATA AVAILABILITY STATEMENT

The data that support the findings of our study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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