Developing, validating and testing non-vaccine-preventable human papillomavirus to control for differences in sexual behaviour when evaluating HPV vaccination

Running title: Non-vaccine-preventable HPV as a marker of sexual behaviour

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Abstract (249/250 words)

Background Evaluating impact/effectiveness of human papillomavirus (HPV) vaccination generally assumes stability in factors driving transmission, which might not be valid. We aimed to develop, validate, and test a grouping of non-vaccine-preventable HPV (NVP-HPV) types as a molecular indicator associated with sexual behaviours to control for changes in HPV transmission risk.

Methods We used data from the National Surveys of Sexual Attitudes and Lifestyles (Natsal-2, 1999-2001, N=1,849; Natsal-3, 2010-2012, N=2,407) to validate the association of NVP-HPV (26/53/66/70/73) with self-reported sexual behaviours. We calculated NVP-HPV-adjusted HPV16/18 vaccine impact/effectiveness estimates in two real-world scenarios: 1) Natsal-2/Natsal-3 (sexually-experienced women in Britain, 18-44yrs) and 2) England's HPV surveillance (women 16-24yrs) (2008, N=3,539; 2010-2020, N=24,707). Samples (urine/vulvo-vaginal swabs) were tested for 21 HPV genotypes (6/11/16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/68/70/73/82) using an in-house multiplex PCR and Luminex-based genotyping assay.

Results NVP-HPV infection was strongly associated with sexual behaviours (e.g., younger age sexual debut, partner numbers). In Natsal data, adjusting for NVP-HPV did not change vaccine impact estimates (unadjusted prevalence ratio (PR: 0.50 (0.27-0.95), adjusted PR: 0.45 (0.25-0.82)). In the second scenario, adjusting for NVP-HPV did not change the prevalence ratio for HPV16/18 comparing 2020 to 2010 (0.07 (0.030.15), unadjusted and adjusted PR). In both scenarios, prevalence of NVP-HPV did not change over time.

Conclusions We have demonstrated proof-of-concept that NVP-HPV is strongly associated with sexual behaviours. Adjusting for NVP-HPV in two datasets found that original estimates were robust.

Impact NVP-HPV might be used to control for changes in HPV transmission risk over time and between groups when evaluating vaccination impact/effectiveness.

Introduction

Globally, the introduction of HPV vaccination programmes in the past 16 years has been critical in reducing human papillomavirus (HPV) prevalence and progressing toward the World Health Organization's (WHO) Cervical Cancer Elimination goals.(1) Monitoring of vaccination programmes remains important to improve routine adolescent vaccination and plan modifications to cervical cancer screening, as well as ensure equitable outcomes in cervical cancer prevention.

However, background changes in sexual behaviour over time, as well as differences in sexual behaviour between the vaccinated and unvaccinated groups, might bias assessment of vaccination impact and effectiveness (i.e., percentage reduction in disease cases due to vaccination). For example, a reduction in HPV 16/18 could be attributable to vaccination but might also be attributable to a reduction in sexual activity or changes in sexual networks resulting in reduced infection incidence; changes in transmission risk could therefore result in overestimated or underestimated vaccination effects.

Population studies, including the National Surveys of Sexual Attitudes and Lifestyles (Natsal) surveys (administered in Britain in 1990 (Natsal-1), 2000 (Nastsal-2), 2010 (Natsal-3) and 2023 (Natsal-4, data forthcoming)), have demonstrated that sexual behaviours do change over time, which should be considered especially when comparing cross-sectional HPV prevalence estimates over time (e.g. with decennial population surveys), as well as longitudinal surveys and routine surveillance.(2)

Thus, it would be ideal to control for changes in sexual behaviour in a population when evaluating HPV vaccination impact and effectiveness to mitigate bias in any estimates. Some population surveys and surveillance have adjusted for self-reported sexual behaviours and

chlamydia test results when estimating vaccination effectiveness,(3–5) yielding lower adjusted vaccine effectiveness in some instances,(5) but such behavioural data are not always available. A molecular indicator associated with sexual behaviour but not affected by vaccination, such as non-vaccine-preventable HPV (NVP-HPV) infection, has potential as a better indicator of transmission risk not subject to recall bias and incorporating sexual network risk. The association between sexual behaviour and HPV infection is well documented, though HPV vaccination reduces the strength of this association for vaccine-preventable infection. Some HPV vaccine clinical trials have therefore adjusted for changes in NVP-HPV infection as an indicator of differences in sexual behaviour between control and intervention groups,(6,7) since these types are unaffected by HPV vaccination. Some real-world population studies have also applied this adjustment,(8,9) as well as presented data showing stability in NVP-HPV over time.(10,11) However, this method has never been formally evaluated. Analysis of these NVP-HPV types might provide a molecular measure to control for differences in sexual behaviours. This approach is likely to be valid because, although some genotypes (including many vaccine-preventable types) are more strongly associated with progression to cervical disease than others, all HPV genotypes (vaccine-preventable and NVP-HPV) share the same sexual transmission route, are often co-transmitted, and have similar early natural history (i.e. infection), limiting the potential for differential confounding by HPV genotype.(12)

The purpose of this analysis is to develop a grouping of non-vaccine-preventable (i.e. not directly targeted or not cross-protected by vaccines) HPV types, validate this grouping as a molecular indicator associated with sexual behaviours in Natsal, and then apply this indicator to two exemplar scenarios as “proof of concept”: vaccine effectiveness estimates from population surveys (Natsal-2 and Natsal-3) and vaccine impact estimates from English

national surveillance data.(3) Our aim is to develop, validate, and test an indicator suitable for use in analyses of HPV vaccination effectiveness to control for differences in sexual behaviour at population-level over time and between vaccinated and unvaccinated individuals and groups, which could be applied in a variety of scenarios.

Materials and Methods

Development of a non-vaccine-preventable (NVP-HPV) grouping

Urine samples from Natsal-2 and Natsal-3 and residual vulvo-vaginal swabs from English national surveillance data were tested by the UK Health Security Agency (UKHSA, or its predecessors).(13–15) An in-house multiplex PCR, Luminex-based genotyping assay was used for detection of HPV types (6/11/16/18/26/31/33/35/39/45/51/52/53/56/58/59/66/68/70/73/ 82). The assay is used for research and surveillance purposes only. The sensitivity of the assay when applied to urine for HPV 16/18 detection was 75% and 84% for any high-risk HPV type.(16) For the NVP-HPV grouping (described below), sensitivity was 76.7% (95%CI 61.4% - 88.2%) and specificity was 95.5% (91.8 - 97.8%). In this study, urine is used as a population-based surveillance sampling tool (not a diagnostic specimen) for which high specificity and a good sensitivity is appropriate. Additional assay characteristics have been previously reported.(16) The laboratory follows a process of internal and external quality assurance of the assay, as well as internal validation with a commercial assay (Roche Linear Array ®).

HPV infection groupings were created for analysis (Supplementary Table 1). Individuals were coded as 'infected' for any group if they tested positive for at least one of the HPV types in that grouping. The high-risk HPV grouping (HR-HPV) (16/18/31/33/35/39/45/51/52/56/58/59/68) was defined according to the WHO

International Agency for Research on Cancer definition.(17) HPV 16/18 are included as a vaccine-preventable grouping which was directly targeted by vaccination in Britain at the time of Natsal-3 data collection. HPV 6/11/31/33/35/45 were excluded from the non-vaccine preventable type groupings due to direct or cross-protective vaccine effects.(18) (19) HPV 68 and 82 were also excluded from groupings due to low assay performance (inter rater agreement between Luminex HPV Genotyping Assay and Roche Linear Array® test, kappa statistic $\kappa < 0.5$, Table 2). High-risk types and vaccine-preventable types (6/11/16/18) and types cross-protected (31/33/35/45) by vaccines used in the UK (see below) as of Natsal-3 (i.e., bivalent and quadrivalent vaccines) (HPV 39/51/52/56/58/59) were excluded from the final non-vaccine preventable HPV measures that were robust against future vaccines with higher valency. Although not included in any vaccines as of 2024, HPV 39/51/56/59 were excluded due to being high risk(20) and potential candidates for inclusion in future vaccines.

The final non-vaccine-preventable HPV (hereafter referred to as NVP-HPV) infection grouping used in the analysis included HPV 26/53/66/70/73 infection. Sensitivity analysis confirmed that the chosen grouping had similar associations with sexual behaviours as other non-vaccine-preventable HPV types (Supplementary Table 2). Although this NVP-HPV grouping was determined to be most appropriate for our study purposes and characteristics of the in-house assay, different non-vaccine-preventable groupings may be used in other studies dependent on available vaccines and assays.

Validating NVP-HPV grouping as a measure of sexual behaviour

To validate the NVP-HPV grouping as a measure of sexual behaviour, data from Natsal-2 and Natsal-3 were used to determine associations between NVP-HPV infection and self-reported sexual behaviours. Natsal-2 (fieldwork 1999-2001) and Natsal-3 (fieldwork 2010-2012) were

household-based, representative, probability sample surveys focusing on sexual and reproductive health in the British population.(14,21) These cross-sectional surveys used a multistage, clustered and stratified probability sample design. Full details of study methodology, including details on recruitment and response rate, have been previously published.(21,22) Participants completed an extensive questionnaire in their homes, which asked about sociodemographic characteristics and detailed sexual behaviours. For both these surveys, sexually experienced participants aged 18-44 years (Natsal-2) and 16-44 years (Natsal-3) were invited to provide a urine sample for sexually transmitted infections (STI) testing.(13–15)

Data from Natsal-2 and Natsal-3 for all women aged 18-44 years who provided an adequate urine sample for HPV testing were extracted: complex survey analysis(23) functions were used to incorporate weighting and stratification.

Descriptive analyses explored type-specific HPV infection groupings and reported sexual behaviours by survey, and differences between the two surveys. Differences were assessed by chi-square tests. Relative percent changes between the two surveys were also calculated and presented with 95% confidence intervals (95% CIs). Logistic regression was used to calculate age-adjusted odds ratios (aORs) to investigate sociodemographic characteristics and sexual behaviours associated with NVP-HPV infection. Models were initially run separately for Natsal-2 and Natsal-3. In an age-adjusted model, associations were similar between surveys, and interaction term analysis testing for heterogeneity in the odds ratios between surveys demonstrated no differences between surveys (Supplementary Table 3), so Natsal-2 and Natsal-3 data were combined for regression analysis to improve precision of estimates (Supplementary Table 4).

Testing the NVP-HPV grouping in two exemplar UK-based scenarios

In the UK, the school-based HPV vaccination programme was introduced to girls aged 12-13 years using the bivalent vaccine (HPV 16/18) in 2008 (including a catch-up programme in older teens until 2010), changing to the quadrivalent vaccine (HPV16/18/6/11) from 2012, and the 9-valent vaccine (HPV 16/18/6/11/31/33/45/52/58) from 2021 (Figure 1).(24) Initially a three-dose schedule, the programme changed to a two-dose course from 2014, and a single dose from 2023.(25) Coverage has been consistently high since the start of the routine vaccine programme (over 80%).(26)

We adjusted for NVP-HPV in two real-world scenarios to evaluate the vaccination programme in the UK:

Scenario 1: NVP-HPV in a cross-sectional, population-based, decennial probability survey (Natsal-2 and Natsal-3)

Generalised linear models with a log link function were used to calculate age-adjusted prevalence ratios for HPV 16/18 infection among all vaccine-eligible women in Natsal-3 compared to same-aged women in Natsal-2 (methods of Natsal surveys described above). Vaccination impact was calculated by subtracting the prevalence ratio from 1. The same methodology was used to calculate vaccine effectiveness between unvaccinated and vaccinated women in Natsal-3 only, using self-reported vaccination status as the comparator rather than survey. Analyses were then repeated adjusting for age and NVP-HPV infection status.

Scenario 2: NVP-HPV in an English national surveillance dataset

The UKHSA collects residual vulvo-vaginal swab samples from women aged 16-24 years receiving chlamydia screening in England and tests them for type-specific HPV infection using the same in-house multiplex PCR and Luminex-based genotyping test as the Natsal studies. This surveillance began in 2008, and annual collections have been tested from 2010 to 2020.(27,28) Samples were sent to the UKHSA Virus Reference Department by 10 different laboratories in 7 regions across England. Demographic variables collected as part of chlamydia surveillance were linked to the samples prior to removal of identifiers prior to testing.

Data from UKHSA surveillance were used to create HPV-grouping variables to match those for Natsal. Descriptive analyses of HPV prevalence were conducted by age group (16-18, 19-21, and 22-24 years) for all samples from women aged 16-24 years in 2008, and then from 2010 to 2020. Individual-level data on vaccination status are not available in the UKHSA surveillance dataset, so tables include estimated vaccination coverage based on birth cohort for each age group. Percentage change in HPV prevalence and p-values for trend from 2010 to 2020 were calculated. Prevalence ratios of, and vaccine impact on, HPV 16/18 infection comparing 2010 and 2020 were calculated in the same way as in the Natsal datasets. Separate models were performed to evaluate prevalence ratios based on different adjustment variables: 1) unadjusted, 2) adjusted for chlamydia positivity only, 3) adjusted for NVP-HPV prevalence only, and 4) adjusted for chlamydia and NVP-HPV. All adjusted models were further adjusted for age and chlamydia screening venue. All analyses were conducted in STATA 17.0 (RRID:SCR_012763).

Ethics approval

Natsal-2 obtained ethical approval from University College Hospital, North Thames Multicentre, and all local research ethics committees in Britain. Subsequently, HPV testing of stored urine samples was approved by St. Mary's Research Ethics Committee (ref 07/Q0403/9). The Natsal-3 study, including HPV testing, was approved by the Oxfordshire Research Ethics Committee A (ref 10/H0604/27).

Data Availability

Natsal-2 and Natsal-3 datasets used in this analysis are available via the UK Data Archive (serial numbers SN 5223/SN 7799) (RRID:SCR_014708). Datasets can be accessed through registration with the UK Data Service. National surveillance data is not publicly available. In order to make our analyses more transparent, example code for deriving the NVP-HPV grouping and running the analysis is available on a GitHub repository (https://github.com/emilycdema/Natsal-Non-Vaccine-Preventable-HPV-analysis.git).

Results

Validation of NVP-HPV as a measure of sexual behaviour using Natsal-2 and Natsal-3 data

Overall, 1,849 women aged 18-44 years from Natsal-2 (1999-2001) and 2,407 women aged 18-44 years from Natsal-3 (2010-2012) provided adequate samples for HPV testing and were included in the analysis (Supplementary Table 5). Both surveys were broadly representative of the British general population.(14,21) Characteristics of importance for this analysis are shown in Table 1. The prevalence of all HR-HPV types was similar between the two surveys (15.9% (95% CI: 14.1-17.8) in Natsal-2, 15.9% (14.3-17.6) in Natsal-3). However, NVP-HPV types slightly increased from Natsal-2 to Natsal-3 (5.1% (4.0-6.5) to 6.3% (5.2-7.4), p=0.17).

In the combined analysis of 4,256 women aged 18-44 from Natsal-2 and Natsal-3, vaccine eligibility (aged 18-20 years vs. 21-44 years), ethnicity, area-level deprivation, education, and previous STI diagnoses were not associated with NVP-HPV infection ($p>0.05$; Supplementary Table 4). Those who reported sexual health clinic attendance in the past year had higher odds for infection with NVP-HPV compared to non-attendees [1.98 (1.31-2.98).

Sexual behaviours were strongly associated with NVP-HPV infection, as was HPV 16/18 in the absence of vaccination (aOR for NVP-HPV infection among those with 16/18 infection compared to those without in Natsal-2; 3.52 (1.86-6.68), $p<0.001$)) (Figure 2). Compared to women who reported their first sexual experience after 20 years of age, women with an earlier sexual debut had higher odds for NVP-HPV infection. This association was strongest for women with the earliest sexual debut (13-15 years v 20+ years) [2.08 (1.18-3.67))]. Other reported sexual behaviours associated with NVP-HPV infection included condomless sex with two or more partners in the past year compared to no condomless sex [2.53 (1.55-4.14)], more new sexual partners in the past year, and more total partners in the past year and past five years. NVP-HPV infection was not associated with reporting a same-sex partner in the past five years.

Scenario 1: Applying NVP-HPV to a population probability survey (Natsal)

Natsal-2 was conducted prior to the HPV vaccination programme in the UK, so none of these women were vaccinated. In Natsal-3, 331 women aged 18-20 years were eligible for vaccination through the catch-up programme. Of these, 170 (52.0%) reported being fully vaccinated (3 doses), and 22 (6.0%) reported receiving one or two doses (Table 1). Among 149 women in Natsal-2 and 331 in Natsal-3 aged 18-20 years (i.e., vaccine-eligible age groups), the prevalence of HPV 16/18 infection decreased from 11.1% to 5.8%, respectively

(Table 2). There was an increase in NVP-HPV infection in this age group from 6.0% to 9.5%. Prior to adjusting for NVP-HPV, the prevalence ratio was 0.50 (0.27-0.95), reflecting a vaccination impact of 50.0% (5.0%-73.0%) (Table 2).(13) Adjusting for the indicator decreased the prevalence ratio to 0.45 (0.25-0.82), reflecting an increase in vaccination impact to 55.0% (18.0%-75.0%). Due to the small denominators (n=331 and n=149) and wide confidence intervals, the analysis lacked statistical power to assess whether the estimates differed.

Among 552 women aged 16-20 years in Natsal-3, HPV 16/18 prevalence was 2.8% (1.5%-5.3%) among vaccinated and 7.4% (4.5%-12.0%) among unvaccinated participants (Table 2). Between these two groups, there were negligible differences in NVP-HPV infection (9.0% (6.0%-13.2%) among unvaccinated vs. 9.9% (6.3%-15.3%) among vaccinated), and adjustment for the indicator did not change the vaccine effectiveness estimate [59.0% (8.0%-81.0%)] [prevalence ratio of 0.41 (0.19-0.92) for both].

Scenario 2: Applying NVP-HPV to a surveillance dataset

Samples tested for type-specific HPV from 24,707 women aged 16-24 years attending chlamydia screening in England in 2008 and from 2010 to 2020 were included in analysis. Among this group, HPV 16/18 prevalence was 13.8% in 2008 and 13.1% in 2010, falling to 0.8% by 2020 (Table 3, Supplementary Figure 1). Chlamydia positivity also decreased over this time, though less markedly, from 7.6% (2008) and 8.3% (2010) to 2.3% (2020) (Table 3). There was a trending increase in NVP-HPV prevalence in the 16-24 year age group between 2010 (15.4%) and 2020 (17.0%) (p for trend=0.02). Unlike HPV 16/18 prevalence, the prevalence of NVP-HPV was not impacted by vaccination and did not change over time,

except for an increase in the oldest age group (22-24 year olds, p<0.0001) (Table 3, Supplementary Figure 2).

The age-adjusted prevalence ratio for HPV 16/18 among 16–24-year-olds in 2020 versus 2010 was 0.07 (0.03-0.15) reflecting a 93% vaccine impact (Table 3). Adjusting for NVP-HPV did not change the prevalence ratio [0.07 (0.03-0.15)] (i.e., 93% vaccine impact). Adjusting for chlamydia, rather than NVP-HPV, gave similar adjusted prevalence ratios [0.07 (0.03-0.15)] as did the model which adjusted for both chlamydia and NVP-HPV [0.07 (0.03-0.15)]. The adjustments performed in similar ways across all three age groups (Table 3).

Discussion

In this paper, we have developed, validated, and tested a molecular method to adjust for (sometimes unmeasured) changes in sexual behaviour that might bias estimates of HPV vaccine impact and effectiveness when analysing real-world population data. To do this, we identified a group of HPV types unaffected by vaccination (NVP-HPV) and validated that these are strongly associated with a range of self-reported sexual risk behaviours, as well as indicators of sexual activity (e.g., sexual health clinic attendance) in population-based probability sample surveys, Natsal-2 and Natsal-3, which have detailed and robust data on sexual behaviour. As proof of concept, we tested this method in two real-world scenarios: a population-based research study dataset (Natsal) and a national surveillance dataset. In both scenarios, our findings suggest that the original estimates were likely to be robust and not affected by unaccounted bias from changes in sexual behaviour, since these did not alter substantially after adjusting for NVP-HPV. This method could be applied more widely in studies where sexual behaviour differs more substantially, if type-specific HPV testing is feasible (Box 1).

Strengths and limitations

While other population surveys have adjusted for self-reported sexual behaviours or chlamydia infection when estimating vaccination effectiveness,(3–5) and some clinical trials and real-world studies have adjusted for NVP-HPV infection between control and intervention groups for HPV vaccination,(6,8,9) or shown stability in NVP-HPV over time,(10,11) this work represents the first formal evaluation of adjusting for NVP-HPV in a population-level survey. NVP-HPV is likely to be a more robust measure of sexual behaviour when estimating HPV vaccine impact and effectiveness than chlamydia or other STIs because it has the same epidemiology as HR-HPV, as well as a higher prevalence in the population (compared with other rarer STIs such as gonorrhoea), and is unaffected by testing and treatment. It is perhaps surprising that the trend seen for chlamydia positivity was not seen for NVP-HPV, however, interpretation of the chlamydia positivity variable is complicated by factors relating to where, and to whom, chlamydia screening is conducted, as has been explored and discussed in more detail elsewhere.(3) Indeed, this trend in the surveillance collection was a motivation for exploring a less biased measure of the risk of exposure to HPV. The Natsal datasets benefit from the inclusion of self-reported sexual behaviour data, in addition to urine samples as an objective measure of HPV infection. Additionally, the Natsal studies are large population-based probability sample surveys with individual-level data, conducted with similar methodology and the same HPV assay, which facilitates analysis of this molecular indicator in the general population over time. We have also demonstrated an application of this methodology to a large, national HPV surveillance dataset which uses the same assay.

Although strongly associated with sexual behaviours, not all individuals reporting sexual risk behaviours will be infected with NVP-HPV, therefore reducing the sensitivity of the NVP-HPV measure to determine sexual activity. However, infection provides an indication of differences in sexual behaviour across the population and over time. Urine is also a suboptimal specimen for detection of HPV in women, with a sensitivity of 75% for HPV-16/18 and 84% for any HR-HPV.(29) However, specificity for HPV 16/18 and NVP-HPV, which are the two most important markers, was quite good at 98% and 96%, respectively. Trends over time and associations with sexual behaviours are unlikely to be substantially affected by the lower sensitivity.

The use of an in-house assay for this analysis may limit the wider applicability; however, similar groupings are likely be valid for other genotyping assays. The non-vaccine-preventable HPV groupings used in previous research vary (e.g., HPV 35/39/56/58/59/66/68(8) or HPV 26/35/39/40/42/43/44/51/53/54/56/59/61/66/68/69/70/73/82(9)), so the specific groupings used in future work should be based on vaccines in use in the study country, as well as characteristics of the assay and the association with sexual risk of exposure to HPV.

In terms of the application of NVP-HPV to surveillance data, surveillance is conducted among a population of sexually-active young people who are at higher average risk for HPV infection,(30) so prevalence estimates are not generalisable to the entire British population of that age group.

A key consideration in the analysis of NVP-HPV and changes in population-level sexual behaviour is duration of infection with different HPV types. Most HPV infections are cleared by an individual's immune system within 12 to 24 months, so NVP-HPV likely provides an

indication of more recent sexual behaviour, rather than over the lifetime.(31) It is also possible that some increases in NVP-HPV could be the result of unmasking, whereby infections with these NVP-HPV types when together with vaccine types may not have been detected (were out competed) as co-infections but are now more likely to be detected in the absence of the vaccine types. Vaccine-induced type replacement is also a possibility, but current evidence is weak.(32–34) Future research should consider the base of evidence around type replacement when determining types to include in a non-vaccine-preventable HPV grouping, since it is currently unclear which types would fill any potential ecological niche. Unmasking and/or type replacement could result in small increases in NVP-HPV detection related to reduction in vaccine types and unrelated to sexual behaviour changes.

Conclusion

We have defined an NVP-HPV variable that is sufficiently common, strongly associated with sexual behaviour risks for HPV transmission, and not impacted by HPV vaccination. Therefore, where available, NVP-HPV might be used as a molecular indicator to adjust for differences in sexual behaviour over time and between populations, thus improving estimates of HPV vaccination impact and effectiveness.

Supplementary data

Supplementary materials are provided.

Author contributions

The paper was conceived by ED, NF, KS, ARK and PS. ED wrote the first draft, with further contributions from JZS, MC, SB, DL, MS, CH, KS, NF, ARK, and PS. Statistical analysis was done

by ED, with support from JZS, SB, KS, and MC. All authors contributed to data interpretation, reviewed successive drafts and approved the final version of the manuscript.

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Conflicts of interest

None declared

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Tables

Table 1. Demographics and prevalence of sexual behaviours among women aged 18-44 in Natsal-2 (1999-2001; n=1849) and Natsal-3 (2010-2012; n=2407)

	Natsal-2		Natsal-3				
	%	95% CI	%	95% CI	Chi-square p value	Relative % change	95% CI
Denominator (unweighted, weighted) [a]	1849, 1525		2407, 2109		-		
Age (years)					p=0.154		
18-19	6.1	[4.9,7.5]	5.8	[5.0,6.8]		-4.9	[-9.3,2.0]
20-24	15.2	[13.1,17.6]	17.5	[16.0,19.2]		15.1	[9.1,22.1]
25-34	40.0	[37.4,42.6]	36.9	[34.7,39.2]		-7.8	[-8.0, -7.2]
35-44	38.8	[36.1,41.5]	39.7	[37.1,42.3]		2.3	[1.9,2.8]
HPV vaccination status[b]							
Fully vaccinated	0	-	52.0	[46.1,57.9]	-	-	-
Partially vaccinated (1-2 doses)	0	-	6.0	[3.6,9.8]			
Age at first sex (years)[c]					p=0.099		
13-15	21.9	[19.8,24.1]	26.1	[24.1,28.1]		19.2	[16.6,21.7]
16-17	43.9	[41.2,46.7]	43.2	[40.7,45.8]		-1.6	[-1.9, -1.2]
18-19	22.5	[20.2,24.9]	19.4	[17.3,21.6]		-13.8	[-13.3, -14.4]
20+	11.7	[10.0,13.6]	11.3	[9.6,13.3]		-3.4	[-2.2, -4.0]
Condomless sex with partner, past year [d]					p=0.378		
0 partners	16.8	[14.9,18.8]	18.5	[16.5,20.7]		10.1	[10.1,10.7]
1 partner	75.1	[72.9,77.2]	73.2	[70.9,75.5]		-2.5	[-2.2, -2.7]
2+ partners	8.1	[6.9,9.5]	8.3	[7.2,9.4]		2.5	[-1.1,4.4]
Total new partners, past year [d]					p=0.257		

0 partners	79.8	[77.5,81.9]	78.1	[76.1,79.9]		-2.1	[-2.4, -1.8]
1 partner	13.6	[11.9,15.4]	13.8	[12.3,15.5]		1.5	[0.7,3.4]
2+ partners	6.7	[5.5,8.1]	8.1	[7.0,9.4]		20.9	[16.1,27.3]
Total partners, past year [d]					p=0.119		
0 partners	6.4	[5.3,7.8]	6.2	[4.9,7.7]		-3.1	[-1.3, -7.6]
1 partner	79.8	[77.6,81.8]	77.9	[75.8,79.9]		-2.4	[-2.3, -2.3]
2 partners	7.7	[6.4,9.2]	7.6	[6.5,8.9]		-1.3	[-3.3,1.6]
3-4 partners	3.8	[3.0,4.8]	4.5	[3.8,5.3]		18.4	[10.4,26.7]
5+ partners	2.3	[1.7,3.1]	3.8	[3.0,4.8]		65.2	[54.8,76.5]
Total partners, past 5 years [d]					p=0.016		
0 partners	2.8	[2.0,3.7]	1.4	[0.9,2.4]		-50.0	[-35.1, -55.0]
1 partner	58.1	[55.5,60.6]	57.4	[54.9,59.8]		-1.2	[-1.3, -1.1]
2 partners	15.1	[13.4,16.9]	14.2	[12.7,15.8]		-6.0	[-6.5, -5.2]
3-4 partners	12.8	[11.2,14.7]	12.8	[11.4,14.4]		0.0	[-2.0,1.8]
5+ partners	11.3	[9.6,13.2]	14.2	[12.8,15.6]		25.6	[18.2,33.3]
2+ partners and condomless sex, past year [d]					p=0.126		
No	94.7	[93.5,95.7]	93.7	[92.6,94.6]		-1.1	[-1.2, -1.0]
Yes	5.3	[4.3,6.5]	6.3	[5.4,7.4]		18.9	[13.8,25.6]

[a] Sexually experienced women aged 18-44 years who provided a urine sample for HPV testing

[b] HPV vaccination reported only among those who were eligible for vaccination (aged 18-20 years), vaccination first introduced in 2008, so was not available in Natsal-2. Unweighted/weighted denominators: Natsal-2, 149/152; Natsal-3, 331/199

[c] Participants who have not had sex yet or had their first sexual experience before age 13 are excluded from analysis of this variable

[d] Includes both partners of the opposite sex and same-sex partners

Table 2. Type-specific HPV infection prevalence and vaccine impact/effectiveness among women aged 18-20 in Natsal-2 (1999-2001; n=149) and Natsal-3 (2010-2012; n=331)

HPV type	Natsal 2	Natsal-3	Prevalence ratio HPV 16/18 (95% CI) (N-3 v N-3)		Vaccine impact (%) against HPV 16/18 (95% CI) (N-3 v N-2)		Vaccine effectiveness (%) against HPV 16/18 (95% CI) (compared to unvaccinated in N-3)	
	Prevalence of infection % (95% CI)	**Prevalence of infection % (95% CI)**	**Unadjusted**	**Adjusted for NVP-HPV**	**Unadjusted**	**Adjusted for NVP-HPV**	**Unadjusted**	**Adjusted for NVP-HPV**
All 18-20 years old[a]	n=149	n=331	-	-	-	-	-	-
[HPV vaccination coverage]	0	52.0 (46.1-57.9)	-	-	-	-	-	-
Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	6.0% (2.8-12.5)	9.5% (6.7-13.2)	1.54 (0.68-3.48)	-			-	-
HPV 16/18 (Cervarix)	11.1% (6.8-17.7)	5.8 (3.9-8.6)	**0.50 (0.28-0.95)**	**0.45 (0.25-0.82)**	**50.0 (5.0-73.0)**	**55.0 (18.0-75.0)**	-	-
All 16-20 years old (Natsal-3 only)	-	n=552[b]	-	-	-	-	-	-
Vaccinated 16-20 years old (Natsal-3 only)	-	n=334	-	-	-	-	-	-
Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	-	9.0 (6.0-13.2)	0.85 (0.43-1.65)	-	-	-	-	-
HPV 16/18 (Cervarix)	-	2.8 (1.5-5.3)	**0.41 (0.19-0.92)**[c]	**0.41 (0.19-0.92)**	-	-	**59.0 (8.0-81.0)**	**59.0 (8.0-81.0)**
Unvaccinated 16-20 years old (Natsal-3 only)	-	n=185	-	-	-	-	-	-
Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	-	9.9 (6.3-15.3)	-	-	-	-	-	-
HPV 16/18 (Cervarix)	-	7.4 (4.5-12.0)	-	-	-	-	-	-

[a]16-17 year olds not included in Natsal-2 analysis

[b] Odds ratios compared fully vaccinated versus unvaccinated – those with partial vaccination are excluded (n=33)

[c] Prevalence ratio comparing vaccinated to unvaccinated in Natsal-3

Table 3. Type-specific HPV infection prevalence and vaccine impact among women aged 16-24 receiving opportunistic chlamydia screening in England 2008, 2010-2020 (n=24,707)

HPV type	2008	2010-2011	2012-2013	2014-2015	2016-2017	2018-2019	2020	p-value[a]	Prevalence ratio HPV 16/18 (95% CI) (2010-2020)				Vaccine impact (%) against HPV 16/18 (95% CI) (2010-2020)			
									Unadjusted	Adjusted for chlamydia only	Adjusted for NVP-HPV only	Adjusted for chlamydia and NVP-HPV	Unadjusted	Adjusted for chlamydia only	Adjusted for NVP-HPV only	Adjusted for chlamydia and NVP-HPV
16-18 years old	n=1417	n=1128	n=2094	n=1954	n=1311	n=1061	n=233									
[Estimated HPV vaccination coverage]		60%	77%	84%	85%	86%	85%		-	-	-	-	-	-	-	-
Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	13.3%	18.0%	16.8%	18.5%	17.0%	16.1%	13.7%	0.195	0.71 (0.49-1.04)	-	-	-	29 (-4-51)	-	-	-
HPV 16/18 (Cervarix)	14.4%	8.2%	3.2%	1.8%	1.1%	0.7%	0.9%	<0.001	**0.10 (0.02-0.39)**	**0.10 (0.03-0.42)**	**0.10 (0.02-0.41)**	**0.11 (0.03-0.43)**	**90 (58-98)**	**90 (57-97)**	**90 (59-98)**	**89 (57-97)**
Chlamydia	8.5%	9.8%	7.6%	6.8%	5.3%	1.8%	2.6%	<0.001	**0.25 (0.11-0.58)**	-	-	-	75 (42-89)	-	-	-
19-21 years old	n=1375	n=1704	n=2892	n=737	n=1607	n=1869	n=462									
[Estimated HPV vaccination coverage]		0%	49%	79%	83%	84%	86%			-	-	-	-	-	-	-
Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	12.8%	16.5%	21.2%	23.2%	18.3%	19.3%	18.2%	0.919	1.10 (0.87-1.41)	-	-	-	-10 (-41-13)	-	-	-
HPV 16/18 (Cervarix)	13.8%	14.0%	8.1%	2.6%	1.1%	0.7%	0.6%	<0.001	**0.05 (0.01-0.14)**	**0.05 (0.02-0.15)**	**0.05 (0.01-0.14)**	**0.05 (0.02-0.15)**	**95 (86-99)**	**95 (85-98)**	**95 (86-99)**	**95 (85-98)**
Chlamydia	7.2%	9.8%	7.2%	5.4%	4.1%	2.4%	1.7%	<0.001	**0.18 (0.09-0.36)**	-	-	-	82 (64-91)	-	-	-
22-24 years old	n=747	n=1212	n=2267	n=120	n=0	n=414	n=103									
[Estimated HPV vaccination coverage]		0%	7%	25%		82%	83%		-	-	-	-	-	-	-	-

Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	10.0%	11.4%	15.3%	14.2%		20.0%	19.4%	<0.001	**1.66 (1.03-2.67)**	-	-	-	-66 (-167—3)	-	-	-
HPV 16/18 (Cervarix)	11.8%	16.4%	15.9%	7.5%		1.7%	1.0%	<0.001	**0.06 (0.01-0.43)**	**0.06 (0.01-0.43)**	**0.06 (0.01-0.43)**	**0.06 (0.01-0.43)**	**94 (57-99)**	**94 (57-99)**	**94 (57-99)**	**94 (57-99)**
Chlamydia	6.7%	4.6%	3.7%	1.7%		1.7%	3.9%	0.01	0.72 (0.26-2.01)	-	-	-	28 (-101-74)	-	-	-
16-24 years old	n=3539	n=4044	n=7253	n=2811	n=2918	n=3344	n=798									
[Estimated HPV vaccination coverage]		27%	44%	80%	84%	85%	86%		-	-	-	-	-	-	-	-
Non-vaccine-preventable low-risk HPV (final grouping, NVP-HPV, 26/53/66/70/73)	12.4%	15.4%	18.1%	19.6%	17.7%	18.4%	17.0%	0.021	1.11 (0.92-1.33)	-	-	-	-11 (-133-8)	-	-	-
HPV 16/18 (Cervarix)	13.8%	13.1%	9.1%	2.3%	1.1%	0.8%	0.8%	<0.001	**0.07 (0.03-0.15)**	**0.07 (0.03-0.15)**	**0.07 (0.03-0.15)**	**0.07 (0.03-0.15)**	**93 (85-97)**	**93 (85-97)**	**93 (85-97)**	**93 (85-97)**
Chlamydia	7.6%	8.3%	6.2%	6.2%	4.6%	2.1%	2.3%	<0.001	**0.26 (0.16-0.42)**	-	-	-	74 (58-84)	-	-	-

[a]P for trend between 2010 to 2020

Box 1. Potential applications of NVP-HPV as an indicator of sexual behaviour

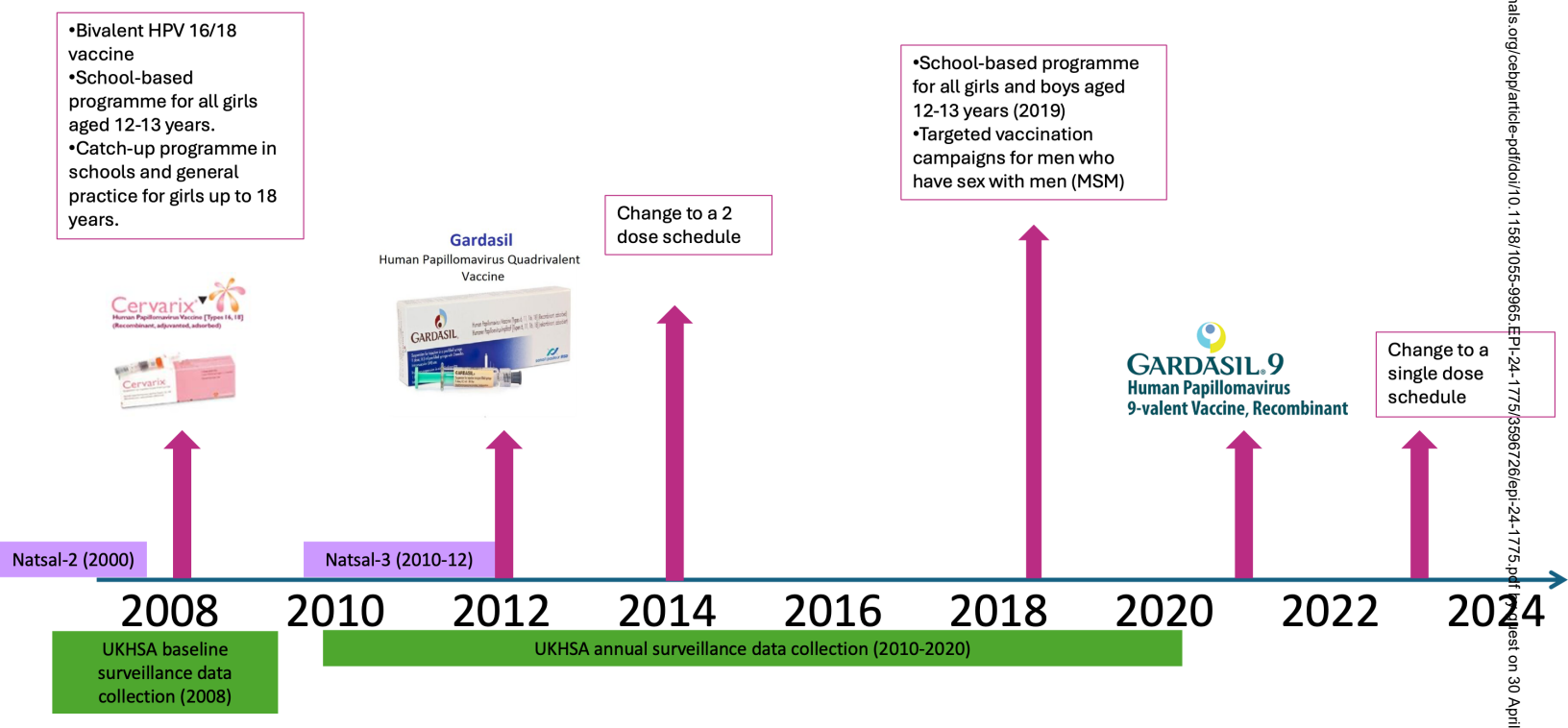
- **Adjusted estimates of HPV vaccine effectiveness and impact in population surveys**; *Although some population surveys have adjusted estimates based on self-reported sexual behaviour or chlamydia infection,- before this analysis, none have adjusted based on a molecular indicator associated with sexual behaviour, which accounts for not only individual sexual activity, but also sexual networks and prevalence of HPV in the population (i.e. exposure risk).*
- **Adjusted estimates of HPV vaccine efficacy in randomized clinical trials;** *This measure could also be applied in clinical trials to adjust for differences in sexual behaviour between control and exposure groups for STI-related interventions, such as HPV vaccines (as previously published[7]), vaccines against other STIs, or preventative treatments against STI infection (e.g. drugs similar to PrEP).*
- **Estimating sexual activity in surveys where self-reported sexual behaviours are not available**; *Current HPV surveillance in the UK is not able to account for underlying changes in population-level risk behaviours, or sampling variations, because sexual behaviour data are not collected, which may lead to bias in estimated changes in vaccine-preventable HPV types due to vaccination. Surveillance, as well as similar studies which collect only HPV infection status could benefit from this measure.*
- **Harmonizing survey data from different modes of data collection**; *NVP-HPV may also be useful as a tool to assess and correct for selection bias when harmonising survey data from different modes of data collection or different sample populations.*
- **Monitoring changes in sexual behaviour among surveillance samples**; *Although adjusting for NVP-HPV did not change vaccination impact estimates in the surveillance data analysed here, given the minimal change in NVP-HPV over time, if HPV surveillance changes in the future to a more targeted group, this variable could give an indication of the risk profile of that group compared to the general population.*
- **Monitoring trends in other STIs (e.g. *Chlamydia trachomatis*)**; *The NVP-HPV molecular indicator associated with sexual behaviour might also be applied to monitoring trends in other STIs, if samples are also tested for NVP-HPV. For example, surveillance of Chlamydia trachomatis (CT) could adjust for trends in NVP-HPV to determine whether increases in CT are due to underlying increases in sexual risk amongst those tested for CT or a true increase in CT prevalence.*

Figure and box legends

Figure 1. Timeline of UK HPV vaccination programme implementation. Key milestones in the UK HPV vaccination programme from 2008 to 2024 are displayed, alongside the data collection timeframes for Natsal-2, Natsal-3, and UK Health Security (UKHSA) surveillance.

Figure 2. Characteristics associated with non-vaccine-preventable HPV infection. Sexual behaviours associated with non-vaccine preventable HPV infection ((NVP-HPV, i.e. HPV 26/53/66/70/73) among women aged 18-44 in Natsal-2 and Natsal-3 (n=4256). Age-adjusted odds ratios (aOR) are presented. Odds ratios are also adjusted for survey, except where the variable was available only in the Natsal-3 dataset. [a]

1



2