Evolution of human papilloma virus prevalence in a highly vaccinated region in Belgium: a retrospective cohort study in Flemish women (2010-2019)

Peer-reviewed author version

Huyghe, Evelyne; ABRAMS, Steven; Bogers, John-Paul; Verhoeven, Veronique & Benoy, Ina (2023) Evolution of human papilloma virus prevalence in a highly vaccinated region in Belgium: a retrospective cohort study in Flemish women (2010-2019). In: EUROPEAN JOURNAL OF CANCER PREVENTION, 32 (1) , p. 48 -56.

DOI: 10.1097/CEJ.0000000000000761
Handle: http://hdl.handle.net/1942/39296

Evolution of HPV prevalence in a highly vaccinated region in Belgium: a retrospective cohort study in Flemish women (2010-2019)

Running head

HPV prevalence in a highly vaccinated region

Authors

Evelyne Huyghe[a]; Steven Abrams[a,b]; John-Paul Bogers[c,d]; Veronique Verhoeven[a]; Ina Benoy[b]

Affiliations

[a]Department of Family Medicine and Population Health, University of Antwerp, Wilrijk, Belgium

[b]Data Science Institute, Interuniversity Institute for Biostatistics and statistical Bioinformatics, UHasselt, Diepenbeek, Belgium

[c]Laboratory for Cell Biology and Histology, University of Antwerp, Wilrijk, Belgium

[D]Algemeen Medisch Labo (AML), Antwerp, Belgium

Correspondence

Veronique Verhoeven

University of Antwerp

Department of Family Medicine and Population Health

Doornstraat 331

2610, Wilrijk

Belgium

E-mail : veronique.verhoeven@uantwerpen.be

+3232652518

Conflicts of interest: non declared

Source of funding: non declared

Abstract

Objectives

In order to lower the incidence of cervical cancer, vaccines against high risk types of the human papilloma virus (hrHPV) were approved and brought on the market in 2007, with a partial reimbursement for Belgian citizens younger than 18 years old ([Anon, 2021]). From 2010 onwards, a school-based vaccination program ensures a high vaccination coverage in young women ([Arbyn et al., 2016]). In this study, the impact of the Belgian vaccination program on the prevalence of HPV 16/18 is studied, together with the evolution of the prevalence of other hrHPV types and precancerous lesions.

Methods

Results of HPV typing and cytology in PAP-smears from women aged 20 to 23 years taken between 2010 and 2019 were used. An older, non-vaccinated group of women of [40-45) years old served as a control group.

Results

A significant decrease in prevalence of HPV types 16 and 18 was found in the 20-23 years old women, whereas no decrease was found in the age group [40-45). Alongside this decrease, a significant decrease in prevalence of subtypes 6, 11 and 31 was observed, while type 31 is not included in the administered vaccines. Remarkably there was no decrease in prevalence of cytological abnormalities in the study group during this study. There was even an increase in prevalence of high risk types 53, 58 and 67.

Conclusions

These findings emphasise the need to maintain the screening programs, even in areas with high-vaccination coverage.

Keywords: human papilloma virus, vaccination, cervix cancer

1. Introduction

Worldwide, cervical cancer is in the top four of the most frequent cancers affecting women. In 2018, this type of cancer accounted for approximately 570,000 new confirmed cancer cases, which makes up 6.6% of all the cancer cases in women ([WHO, 2019]). Although this type of cancer is most frequent in low- and middle income countries, 634 women (11/100,000 person years) were diagnosed with cervical cancer in Flanders (Belgium) in 2018 ([Kankerregister, 2021]). A strong association between HPV infections and cervical cancer is proven ([de Martel et al., 2017,Graham, 2017,Araldi et al., 2018]), with even an association of 70.6% for HPV subtypes 16 and 18. Beside this two types, another 11 high risk HPV types are found in cervical dysplasia and cancer ([Arbyn et al., 2014]).

Therefore, the Belgian government has set-up a national vaccination program in 2007 with a partial reimbursement of licensed HPV vaccines Gardasil® (quadrivalent vaccine targeting subtypes 6, 11, 16 and 18) and Cervarix® (bivalent vaccine for subtypes 16 and 18) for girls aged 12-18 years. In 2017, the quadrivalent Gardasil® vaccine was replaced by the nona-valent Gardasil9 vaccine (adding HPV subtypes 31, 33, 45, 52, and 58 as targets). Since 2010 a school-based HPV vaccination program exists in Flanders offering girls in the first year of secondary school (aged 12-13 years) to get the quadrivalent vaccine for free, though on a voluntary basis. The quadrivalent vaccine changed to the bivalent one mentioned above in the school-based vaccination program in 2014 and to the nonavalent vaccine in 2019. Since that same year (2019), boys are also included in the school-based vaccine program. This leads to a school-based vaccination with the quadrivalent, bivalent or nonavalent vaccine for women born in the periods 1998-2001, 2002-2006 or 2007-…, respectively ([Anon, 2021]) (Figure 1).

More than ten years after the introduction of a school-based vaccination program, vaccinated girls reached the age to have them screened against cervical cancer and HPV infections. In this study, we investigate whether the prevalence of HPV 16 and 18 has changed in this

vaccinated cohort of women aged 20-23 years, in comparison with the pre-vaccination era. Furthermore, we study the prevalence of other high-risk HPV (hrHPV) infections. Additionally, a possible evolution in the prevalence of cytological abnormalities (LSIL, HSIL and ASC-H) is studied in our highly vaccinated cohort. To control for effects unrelated to vaccination, we compare the evolution of HPV status with a control group of non-vaccinated women aged [40-45) years.

2. Materials and methods

2.1 Study sample

This is a monocentric study, executed in the Algemeen Medisch Labo (AML) in Antwerp. Here, PAP-smears are collected from contributing clinical centres, gynaecologists and general practitioners located across the entire Flemish region. The screening PAP-smears from females aged [20-23) years (>19,9 years and <23 years) are used in the context of this study. The PAP-smears taken between January 1, 2010 and December 31, 2019 are included. When subjects had more than one PAP-smear a year, only the first one was included. The year 2010 is used as a baseline measurement as we can expect a negligible amount of vaccinations at that point. The year 2019 is chosen as the endpoint of our analysis given the potential impact of the COVID-19 pandemic in 2020 on the observed HPV prevalence and/or HPV vaccination in Flanders.

A group of women aged [40-45) years (>39,9 years and <45 years) will serve as a control group in our study. No subject in this group was part of a vaccine program or received a reimbursement. As a result, a negligible proportion of vaccinated individuals can be expected in this group at any time.

2.2 Vaccination coverage in Flanders

A lexis diagram depicting the vaccination strategy in Flanders is presented in Figure 1. Here, the diagram shows no reimbursement in the study population at the start of the study. Only a

negligible proportion of vaccinated individuals can be expected in these age groups. As the study progresses, the birth cohorts that benefited from the partial reimbursement are increasingly represented (Figure 1). In these birth cohorts, observational studies showed a vaccination rate ranging between 30% and 60%, depending on the birth cohort ([Lefevere et al., 2015,Arbyn et al., 2016]). At the end of the study, there is a considerable part of the study population that is included in the school-based vaccination programs. In these cohorts, a stable vaccination rate of more than 80% is found ([Lefevere et al., 2015,Arbyn et al., 2016]).

2.3 PCR analysis for the detection of HPV

All samples are processed in the department of molecular pathology in AML. Upon arrival at the laboratory, samples in liquid-based cytology medium (Thinprep, Hologic Inc) are split into two parts: one aliquot (2 ml) is used for the HPV genotyping test, the other part (remaining 18 ml) is used for the preparation of the thin layer smear for cytology diagnosis based on the Bethesda classification.

HPV detection and genotyping is performed with the Riatol qPCR HPV test, an ISO certified (ISO 15189), fully automated, clinically validated laboratory developed test ([Micalessi et al., 2011]). In short: DNA extraction is done exploiting standard boom extraction with magnetic beads using the Genfind® DNA extraction kit (Hologic Inc). Subsequently, the DNA is amplified using a series of real-time qPCR reactions on the LightCycler 480 type I (Roche). The presence of 18 different HPV genotypes is determined using TaqMan based real-time PCR reactions targeting type specific sequences of viral genes. The Riatol qPCR HPV test, not only detects 14 hrHPV types (HPV16 E7, 18 E7, 31 E6, 33 E6, 35 E6, 39 E7, 45 E7, 51 E6, 52 E7, 56 E7, 58 E6, 59 E7, 66 E6, 68 E7), but also reports selected potential high-risk or low-risk HPV (lrHPV) types (HPV6 E6, 11 E6, 53 E6, 67 L1). Cellularity control is performed on every sample, by amplification of the beta-globin gene.

2.4 Data collection

The results of the HPV determination from the cases and controls that meet the inclusion criteria are provided by the AML HPV database, together with the cytology results. Per sample, results are reported as negative/positive per individual HPV genotype. Furthermore, genotype results are condensed as HPV 16/18 positive/negative and as hrHPV positive/negative when the sample is positive for one or more hrHPV types. Samples are also categorised in function of the number of infected HPV types: single infection, co-infection (2 HPV types), multiple infection (3 or more HPV types) and no infection. Cytology results are reported according to the Bethesda classification as NILM, ASC-US, AGC, L-SIL, ASC-H and H-SIL.

From each sample, the patient number, date of HPV analysis and date of birth are linked to the results. Data are extracted anonymously from the LIS (laboratory information system) to an excel file and converted to a csv-file for further statistical analyses.

2.5 Statistics

The HPV prevalence data is described using absolute and relative frequencies. Proportions are compared using a Chi-square test. Uncertainty with regard to the estimated prevalence is quantified by means of 95% exact Clopper-Pearson confidence intervals (CIs) or using pointwise 95% asymptotic CIs for model-based estimates. The evolution of the prevalence of HPV types 16 and 18 over time is modelled using a generalized additive model (GAM) ([Wood, 2017]), an extension of the generalized linear model framework, to accommodate non-linear time effects. More specifically, the binary outcome variable (HPV positive or negative) is assumed to follow a Bernoulli distribution and the mean outcome, conditional on year and age group, is linked to the linear predictor by means of a logit-link function. All statistical analyses are performed using the statistical software package R, version 3.1.0 ([Anon, 2019]). All statistical tests are conducted two-sided at a 5% significance level.

The same analysis is done for the group [40-45) years old, with this results compared to those of the group [20-23).

2.6 Ethics statement

During data collection, privacy of the participants was always respected. Permission of the ethical committee of the University of Antwerp and the Antwerp University Hospital was given on the 6th of May 2019. The number of agreement is 19/17/222.

3. Results

In Table 1, we present a summary of the PAP-smear results between 2010 and 2019 in the two age groups [20,23) and [40,45).

3.1 HPV 16/18 prevalence

In Figure 2, we graphically depict the observed prevalence of HPV types 16 and 18 in Flanders over time for age groups [20,23) (black dots) and [40,45) (red dots). In 2010, the HPV 16/18 prevalence was significantly different between young [20-23) and elderly [40-45) women (Table 1; two-sided Chi-square p-value < 0.0001). From the graph, we can clearly observe a decrease in observed prevalence over time in the age group [20,23) including vaccinated individuals. In the unvaccinated group (i.e., [40,45)) the observed prevalence remains quite stable over time. Based on the GAM, a significant non-linear time effect (at the linear predictor scale) is observed in the age group [20,23) (two-sided Chi-square p-value < 0.0001) induced by HPV vaccination. Decomposing the time-effect into linear and non-linear effects, we found a non-significant linear time effect in the age group [40,45) (p-value = 0.700) and a significant non-linear time effect (p-value = 0.0005). Despite such a significant non-linear time effect the HPV16/18 prevalence fluctuates around a constant level over time (Figure 2). The time-dependent odds ratio for age groups [20,23) and [40,45) are presented in Supplemental Digital Content together with bootstrap-based 95% confidence intervals. These estimates clearly show a significant decrease in prevalence in age group [20,23) and no significant temporal

change in HPV 16/18 prevalence in the control group (see Figure, supplementary digital content 1).

3.2 Prevalence of infections with high-risk HPV types

The prevalence of infections with high-risk HPV types (see Supplemental Digital Content 2 for an overview of these types) is significantly higher in age group [20,23) as compared to [40,45) in all years (Table 1; two-sided Chi-square p-values < 0.0001). Although the prevalence of high-risk HPV types in the vaccinated age group [20,23) initially decreased between 2010 (28.4%, 95%CI: 26.8%-30.0%) and 2013 (p-value < 0.0001), the prevalence increased again to a level (in 2019) which is not significantly different from the prevalence in 2010 (p-value = 0.445). In Figure 3, we graphically present the prevalence of HPV infections with high-risk HPV types with 95% Clopper-Pearson confidence limits (as error bars) (Figure 3). As seen in Figure 4, the proportion of single (pink), co- (blue) and multiple (brown) infections with high-risk HPV types (among high-risk positive samples in age group [20,23)) remains stable over time (Figure 4).

3.3 Prevalence of precancerous cells

Here we study the time evolution of the presence of precancerous cells in the group of potentially vaccinated individuals in age group [20,23) as compared to that one in the unvaccinated age group [40,45). In Table 1, the observed proportion of low-grade squamous intraepithelial lesion (L-SIL) and high-grade squamous intraepithelial lesion or atypical squamous cells (H-SIL/ASC-H) are presented. In general, in each age group the proportion L-SIL and H-SIL/ASC-H are very similar over time. Moreover, the prevalence of L-SIL is observed to be higher in [20,23) than in [40,45), whereas no significant differences in prevalence of H-SIL/ASC-H are detected between these age-groups (all pairwise Chi-square p-values > 0.068).

3.4 Replacement of HPV types 16 and 18

Finally, we study the time-varying prevalence of all different HPV types in order to detect which types are emerging as a result of vaccination in the Flemish population. In Figure 5, we show the time-dependent prevalence of HPV infections of a specific subtype in the age group [20,23) observed in a given year. Based on Chi-square tests of differences in proportions in 2010 vs. 2019 with Bonferroni-Holm corrected p-values to account for multiplicity, significantly different proportions of HPV infections are found for HPV types 6, 11, 16, 18, 31 (with a decrease in observed prevalence between 2010 and 2019), and 53 and 67 (with an increase in observed prevalence between 2010 and 2019). In the unvaccinated age group [40,44) no significant differences in prevalence between 2010 and 2019 were observed, except for the prevalence of HPV type 45 (two-sided p-value = 0.003) (Figure 5).

4. Discussion

This study shows a clear impact of the HPV vaccination on HPV 16/18 infections on population level. In the women aged [20-23) years, where a high vaccination level is shown, the positivity ratio decreased from 10.6% in 2010 to 1.9% in 2019. This in contrast to the women aged [40-43) years in which a stable HPV 16/18 ratio is found.

On the other side, the HPV 16/18 prevalence in the [20-23) aged group seems to stabilise around 2% in the last few years which can probably be explained by the stabilization of the vaccination coverage for women born after 1998 (see also Figure, Supplementary Digital Content 1). Elimination of HPV 16/18 infections in Belgium seems impossible when only vaccinating girls, even with very high vaccination coverages of more than 80%. However, mathematical modelling approaches show that elimination of HPV types 16 and 18 is possible when boys are included in HPV vaccination programs and an 80% vaccination coverage is reached and maintained for a longer period ([Brisson et al., 2016]). For the Flemish population, it still has to be investigated whether the inclusion of boys in the vaccination campaigns will be effective in reducing the prevalence of HPV types 16 and 18 even further, potentially leading to almost no new HPV cases of these specific types. Given the fact that administration of the

HPV vaccine in boys was only recently initiated (from 2019 onward), insufficient data is available to date to quantify its impact. Moreover, it is questionable whether vaccination coverages in boys and girls will be comparable, and the effect thereof requires careful consideration in any further analysis.

In our study population, HPV types 6 and 11 follow a similar path as the one that is observed for HPV types 16 and 18. The decrease in prevalence for these HPV types can be explained by the fact that the quadrivalent Gardasil vaccine is used in the school-based vaccination program targeting this age cohort. In 2014, this changed to Cervarix ([Anon, 2021]) and in 2019 to the nonavalent Gardasil ([Anon, 2021]). The cohort that is vaccinated with Gardasil9 is however not present in this study so we cannot yet quantify what the effect is of the change from the bivalent to the nonavalent HPV vaccine. In this study, there is no decrease of the HPV types 33, 45 and 52, which are all included in the nonavalent vaccine, in the vaccinated age group.

Besides the decrease in the HPV viruses included in the quadrivalent vaccine, there is even a decrease in prevalence of HPV type 31 in the Flemish population (significant two-sided Bonferroni-Holm corrected p-value < 0.0001). This finding corresponds to two double-blind placebo controlled trials. In a first clinical trial, Wheeler et al. ([Wheeler et al., 2009]) found a significant reduction of HPV 31 after administration of the quadrivalent vaccine with an efficacy of 33.6% (95% CI: 14.6% to 48.5%). Another clinical trial from Brown et al. ([Brown et al., 2009]) also showed a significant reduction in HPV 31 infections after vaccination with the quadrivalent vaccine with a vaccine efficacy of 46.2% (95% CI: 15.3% to 66.4%). Looking at the phylogenetic tree of HPV viruses based on their L1-gene, HPV 31 appears to be related to HPV 16 (see table, Supplementary Digital Content 2) ([Van Ranst et al., 1992,de Villiers et al., 2004,Harari et al., 2014]). This taxonomy could explain why there is not only a decrease in the types included in the vaccine, but also in type 31.

To the best of our knowledge, we are the first to include samples from women aged [40-45). In Flanders these individuals cannot get a reimbursement for the vaccine, neither inclusion in a vaccination program, which means a negligible vaccination rate can be assumed. As we

found no changes in HPV 16/18 infections as well as other hrHPV (except for HPV 45), we can conclude that there is no effect of methodology in our study. Furthermore, our study samples were taken in a stable time period.

In our age group [20-23) years old, there was an increase of HPV types 53, 58 and 67 over time. This so-called type-replacement is already found in other population-based trials with an increase of HPV types 39 and 51 in the Scandinavian and an increase of HPV types 54 and 56 in the Dutch populations ([Gray et al., 2018,Hoes et al., 2021]). Earlier, in 2016, an increase of types 39, 52, 53, 58 and 73 was observed in a meta-analysis ([Mesher et al., 2016]). The explanation for the increase in these types has been a subject of discussion for years, mostly because no clear pattern can be found. Different explanations for this phenomenon include an artefact or increasing sexual intercourse in young women ([Tota et al., 2013,Drolet et al., 2015]). In fact, no competition could be found as subjects with a HPV infection appear to have less clearance of other HPV types in epidemiological studies ([Rousseau et al., 2001,Mendez et al., 2005]). Furthermore, there are no arguments found to assume clustering between different HPV types ([Mollers et al., 2014]). Man et al. suggested type-replacement could occur when there is no natural protection and cross-immunity fades away several years after vaccination, so that this type replacement would be a rebound-phenomenon ([Man et al., 2021]). However, it remains unclear why there is no consensus on which types increase, while it is on cross-protection with a consensus on types that decrease.

Apart from the discussion whether there is type replacement or not, this alteration in HPV subtype infections could lead to an unmasking phenomenon. Here, non-vaccination HPV types that were previously considered as rather harmless may result in precancerous lesions ([Wheeler et al., 2009]). This is because these so-stated harmless non-vaccination HPV types were detected together with clear carcinogen types in the past. This phenomenon could influence the discussion about screening methods. Further research is needed to investigate the causes and possible consequences of these so-called type replacements.

Another goal of this manuscript was to investigate the effect of the vaccination program on the prevalence of intra-epithelial lesions. Here, we found no significant decrease of L-SIL, H-SIL or ASC-H lesions in our high-vaccinated cohort. As a consequence, we could not prove a time-dependent or vaccination dependent decrease in intra-epithelial cervical lesions on population level. Further investigation is needed to find the reason for this lack of decrease. What this data do show, is that screening must be continued, even in high-vaccinated cohorts.

The strength of this study is that samples collected across the entire Flemish are included in the study. Furthermore, the profiles of the samplers (general practitioners, gynaecologists or other medical doctors) and patients are similar to other centres analysing PAP-smears in the region. Hereby, it could be expected that there was no selection bias in either the profile of the patient, age, region or sampler.

A limitation in this study is that the precise vaccination coverage in our region is not known. Otherwise, authors studying the coverage in some cohorts found a stable vaccination rate of more than 80% ([Lefevere et al., 2015,Arbyn et al., 2016]).

5. Conclusion

In this study, we evaluated the effect of a school-based vaccination program on population based HPV infections. A significant decrease of the vaccine-included types 16 and 18 was observed, as well as types 6, 11 and 31. On the other hand, there was an increase in hrHPV types 45 and 67, together with an overall increase of all the hrHPV other than types 16 and 18. Knowing this, a lowering of screening programs with pap-smears is clearly not yet the case.

6. References

Anon. A language and environment for statistical computing. . In: Computing RFfS editor. Vienna, Austria2019.
Anon. Vaccinatie tegen HPV. In: Gezondheid AZe editor. 2021.
Araldi RP, Sant'Ana TA, Módolo DG, de Melo TC, Spadacci-Morena DD, de Cassia Stocco R et al. (2018). The human papillomavirus (HPV)-related cancer biology: An overview. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 106:1537-1556. doi: 10.1016/j.biopha.2018.06.149.
Arbyn M, Tommasino M, Depuydt C, Dillner J (2014). Are 20 human papillomavirus types causing cervical cancer? The Journal of pathology 234(4):431-435. doi: 10.1002/path.4424.
Arbyn M, Broeck DV, Benoy I, Bogers J, Depuydt C, Praet M et al. (2016). Surveillance of effects of HPV vaccination in Belgium. Cancer epidemiology 41:152-158. doi: 10.1016/j.canep.2015.12.011.
Brisson M, Bénard É, Drolet M, Bogaards JA, Baussano I, Vänskä S et al. (2016). Population-level impact, herd immunity, and elimination after human papillomavirus vaccination: a systematic review and meta-analysis of predictions from transmission-dynamic models. The Lancet Public Health 1(1):e8-e17. doi: https://doi.org/10.1016/S2468-2667(16)30001-9.
Brown DR, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Wheeler CM et al. (2009). The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in generally HPV-naive women aged 16-26 years. The Journal of infectious diseases 199(7):926-935. doi: 10.1086/597307.
de Martel C, Plummer M, Vignat J, Franceschi S (2017). Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer 141(4):664-670. doi: 10.1002/ijc.30716.
de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004). Classification of papillomaviruses. Virology 324(1):17-27. doi: 10.1016/j.virol.2004.03.033.
Drolet M, Bénard É, Boily M-C, Ali H, Baandrup L, Bauer H et al. (2015). Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. The Lancet Infectious diseases 15(5):565-580. doi: 10.1016/S1473-3099(14)71073-4.
Graham SV (2017). The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. Clinical science (London, England : 1979) 131(17):2201-2221. doi: 10.1042/cs20160786.
Gray P, Palmroth J, Luostarinen T, Apter D, Dubin G, Garnett G et al. (2018). Evaluation of HPV type-replacement in unvaccinated and vaccinated adolescent females-Post-hoc analysis of a community-randomized clinical trial (II). International journal of cancer 142(12):2491-2500. doi: 10.1002/ijc.31281.
Harari A, Chen Z, Burk RD (2014). Human papillomavirus genomics: past, present and future. Current problems in dermatology 45:1-18. doi: 10.1159/000355952.
Hoes J, Woestenberg PJ, Bogaards JA, King AJ, de Melker HE, Berkhof J et al. (2021). Population Impact of Girls-Only Human Papillomavirus 16/18 Vaccination in The Netherlands: Cross-Protective and Second-Order Herd Effects. Clin Infect Dis 72(5):e103-e111. doi: 10.1093/cid/ciaa1770.
Kankerregister. Tabellen op jaarbasis. 2021.
Lefevere E, Theeten H, Hens N, De Smet F, Top G, Van Damme P (2015). From non school-based, co-payment to school-based, free Human Papillomavirus vaccination in Flanders (Belgium): a retrospective cohort study describing vaccination coverage, age-specific coverage and socio-economic inequalities. Vaccine 33(39):5188-5195. doi: 10.1016/j.vaccine.2015.07.088.
Man I, Vänskä S, Lehtinen M, Bogaards JA (2021). Human Papillomavirus Genotype Replacement: Still Too Early to Tell? The Journal of infectious diseases 224(3):481-491. doi: 10.1093/infdis/jiaa032.

Mendez F, Munoz N, Posso H, Molano M, Moreno V, van den Brule AJ et al. (2005). Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. The Journal of infectious diseases 192(7):1158-1165. doi: 10.1086/444391.
Mesher D, Soldan K, Lehtinen M, Beddows S, Brisson M, Brotherton JML et al. (2016). Population-Level Effects of Human Papillomavirus Vaccination Programs on Infections with Nonvaccine Genotypes. Emerg Infect Dis 22(10):1732-1740. doi: 10.3201/eid2210.160675.
Micalessi IM, Boulet GA, Bogers JJ, Benoy IH, Depuydt CE (2011). High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. Clinical chemistry and laboratory medicine 50(4):655-661. doi: 10.1515/cclm.2011.835.
Mollers M, Vriend HJ, van der Sande MAB, van Bergen JEAM, King AJ, Lenselink CH et al. (2014). Population- and Type-Specific Clustering of Multiple HPV Types Across Diverse Risk Populations in the Netherlands. American journal of epidemiology 179(10):1236-1246. doi: 10.1093/aje/kwu038.
Rousseau MC, Pereira JS, Prado JC, Villa LL, Rohan TE, Franco EL (2001). Cervical coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. The Journal of infectious diseases 184(12):1508-1517. doi: 10.1086/324579.
Tota JE, Ramanakumar AV, Jiang M, Dillner J, Walter SD, Kaufman JS et al. (2013). Epidemiologic approaches to evaluating the potential for human papillomavirus type replacement postvaccination. American journal of epidemiology 178(4):625-634. doi: 10.1093/aje/kwt018.
Van Ranst M, Kaplan JB, Burk RD (1992). Phylogenetic classification of human papillomaviruses: correlation with clinical manifestations. The Journal of general virology 73 (Pt 10):2653-2660. doi: 10.1099/0022-1317-73-10-2653.
Wheeler CM, Kjaer SK, Sigurdsson K, Iversen OE, Hernandez-Avila M, Perez G et al. (2009). The impact of quadrivalent human papillomavirus (HPV; types 6, 11, 16, and 18) L1 virus-like particle vaccine on infection and disease due to oncogenic nonvaccine HPV types in sexually active women aged 16-26 years. The Journal of infectious diseases 199(7):936-944. doi: 10.1086/597309.
WHO WHO. Cervical cancer. 2019.
Wood SN (2017). Generalized additive models: an introduction with R. CRC press.

7. Table legend

Table 1: Overview of the HPV prevalence data.

8. Figure legend

Figure 1: Lexis diagram visualising HPV vaccination efforts in Flanders with every line representing a single birth cohort. The black rectangle indicates the study population.

Figure 2: Observed prevalence of HPV types 16 and 18 in Flanders over time by age group (black dots for [20,23), red dots for [40,45)) with pointwise 95% Clopper-Pearson confidence intervals as error bars. Estimated prevalence of HPV types 16 and 18 in Flanders based on a generalized additive model depicted as solid lines with 95% asymptotic confidence bands shown as shaded areas.

Figure 3: Observed prevalence of infections with high-risk HPV types in Flanders over time by age group (black dots for [20,23), red dots for [40,45)) with pointwise 95% Clopper-Pearson confidence intervals as error bars.

Figure 4: Distribution of single (pink), co- (blue) and multiple (brown) infections with high-risk HPV types over time in age group [20,23).

Figure 5: Observed time-varying prevalence of all different HPV types in age group [20,23) with shaded areas constructed based on pointwise 95% exact Clopper-Pearson confidence intervals.

9. Supplemental digital content

Supplemental digital content 1 (Figure 1.1 and 1.2): Estimated time-dependent odds ratios (black dots) for a unit increase in year based on the GAM model for age groups [20,23) (Figure 1.1) and [40,45) (Figure 1.2) together with (parametric) bootstrap mean evolution (red solid line) and pointwise bootstrap-based 95% confidence limits (pink shaded area).

Supplemental digital content 2 (Table): Overview and taxonomy of the high risk HPV types

Table 1: Overview of the HPV prevalence data.

Age group	Year	N	HPV 16/18+	hrHPV+	L-SIL	H-SIL/ASC-H
[20,23)	2010	3008	319 (10.6%)	853 (28.4%)	297 (9.9%)	20 (0.7%)
	2011	3496	294 (8.4%)	866 (24.8%)	305 (8.7%)	37 (1.1%)
	2012	3340	242 (7.2%)	907 (27.2%)	366 (11.0%)	29 (0.9%)
	2013	2422	108 (4.5%)	543 (22.4%)	192 (7.9%)	25 (1.0%)
	2014	2517	80 (3.2%)	624 (24.8%)	227 (9.0%)	23 (0.9%)
	2015	2528	72 (2.8%)	616 (24.4%)	250 (9.9%)	23 (0.9%)
	2016	2347	64 (2.7%)	609 (25.9%)	268 (11.4%)	28 (1.1%)
	2017	2254	44 (2.0%)	620 (27.5%)	268 (11.9%)	18 (0.8%)
	2018	2017	47 (2.3%)	587 (29.1%)	275 (13.6%)	14 (0.7%)
	2019	2069	39 (1.9%)	608 (29.4%)	243 (11.7%)	16 (0.8%)
[40,45)	2010	7838	266 (3.4%)	880 (11.2%)	213 (2.7%)	54 (0.7%)
	2011	10280	279 (2.7%)	1016 (9.9%)	276 (2.7%)	74 (0.7%)
	2012	9359	304 (3.2%)	990 (10.6%)	339 (3.6%)	83 (0.9%)
	2013	6137	184 (3.0%)	653 (10.6%)	197 (3.2%)	47 (0.8%)
	2014	7352	174 (2.4%)	594 (8.1%)	173 (2.4%)	51 (0.7%)
	2015	8004	212 (2.6%)	669 (8.4%)	238 (3.0%)	58 (0.7%)

	2016	7380	189 (2.6%)	751 (10.2%)	272 (3.7%)	72 (1.0%)
	2017	7266	175 (2.4%)	814 (11.2%)	272 (3.7%)	57 (0.8%)
	2018	7622	208 (2.7%)	839 (11.0%)	322 (4.2%)	56 (0.7%)
	2019	7493	235 (3.1%)	918 (12.3%)	279 (3.7%)	63 (0.8%)

hrHPV+: infections with high-risk HPV types (see additional data for an overview of these types); L-SIL: low-grade squamous intraepithelial lesion (mild dysplasia); H-SIL: high-grade squamous intraepithelial lesion (moderate to severe dysplasia); ASC-H: atypical squamous cells, cannot exclude a high-grade lesion.

Figure 1

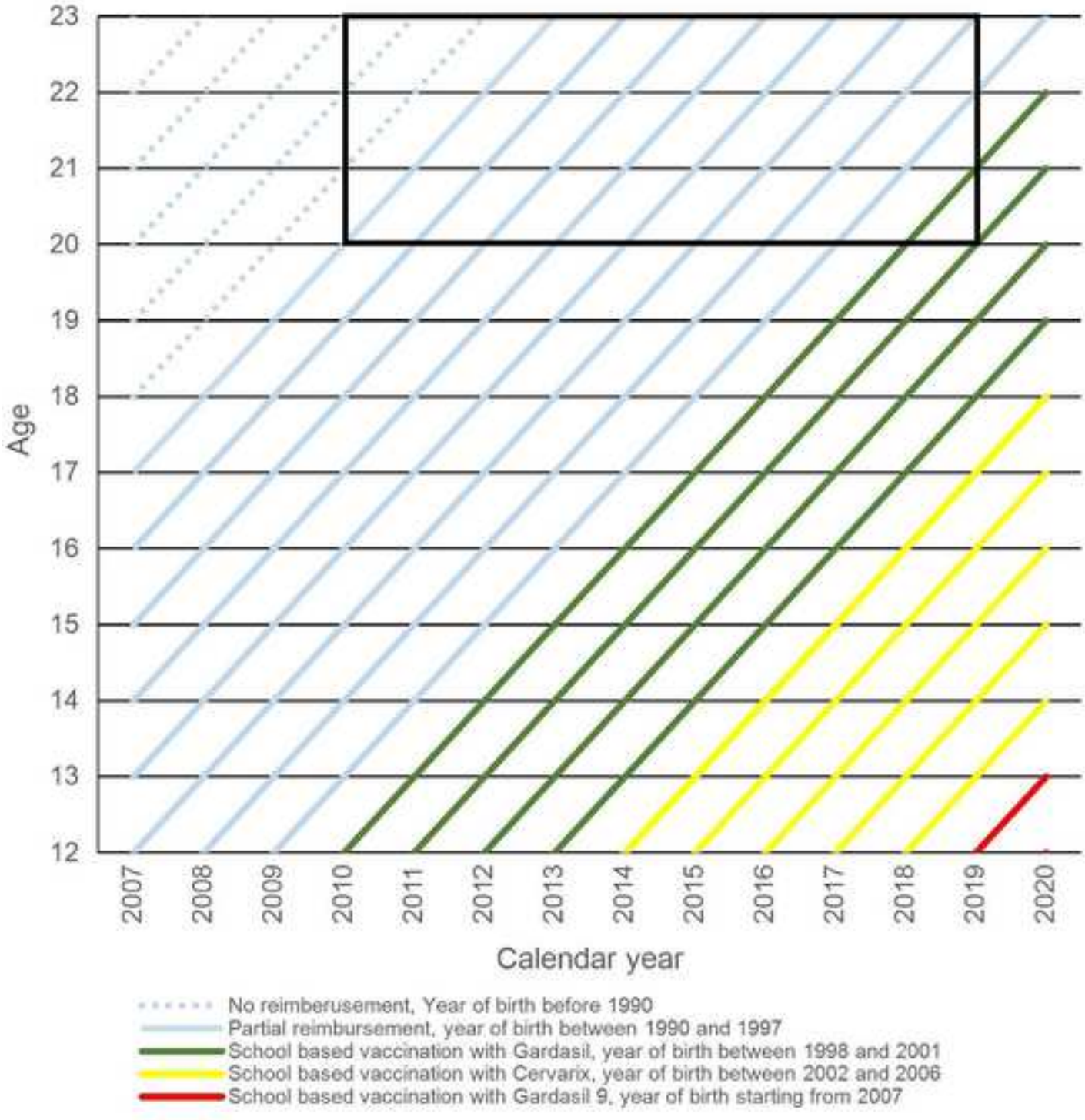


Figure 2

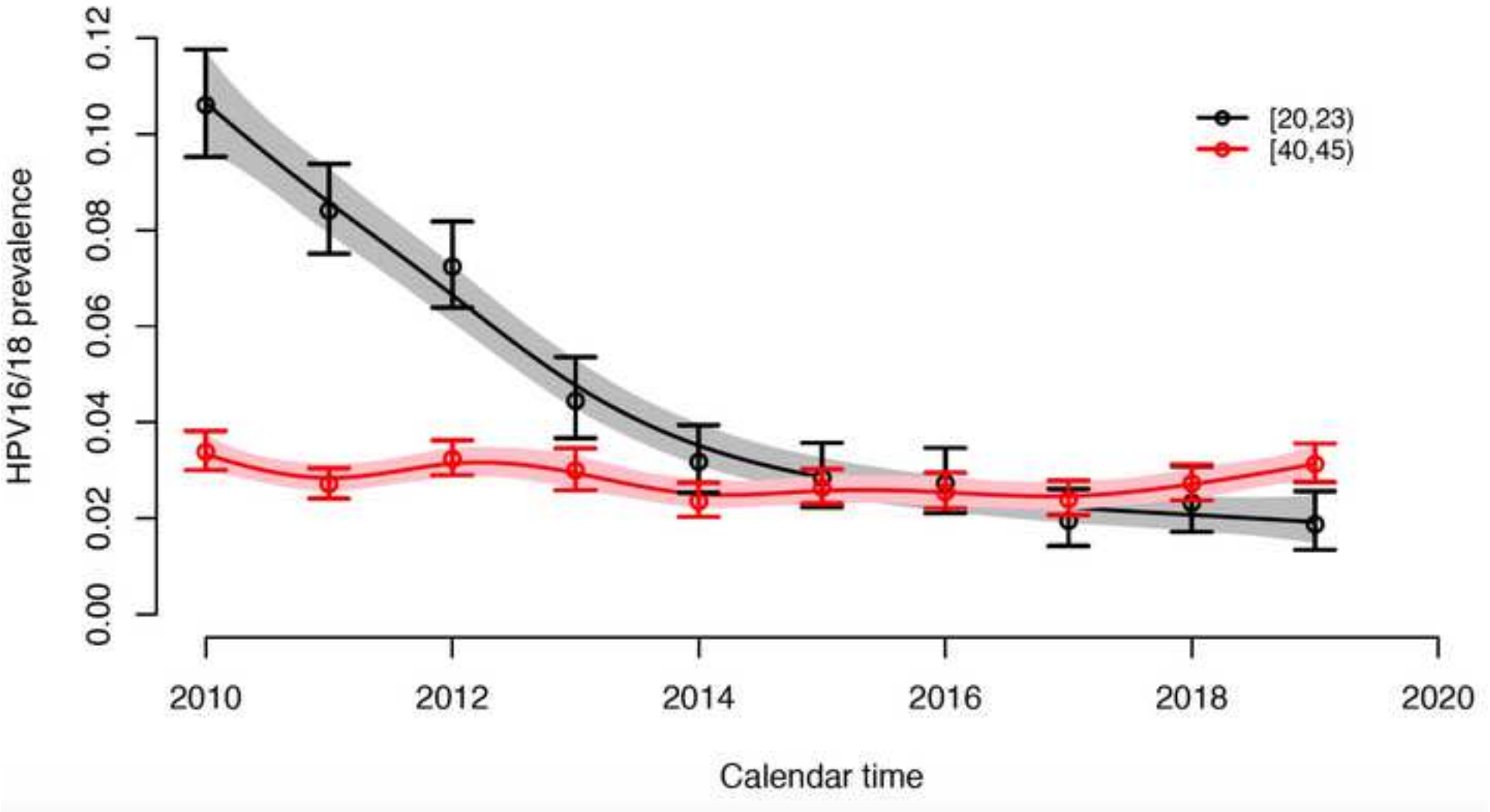


Figure 3

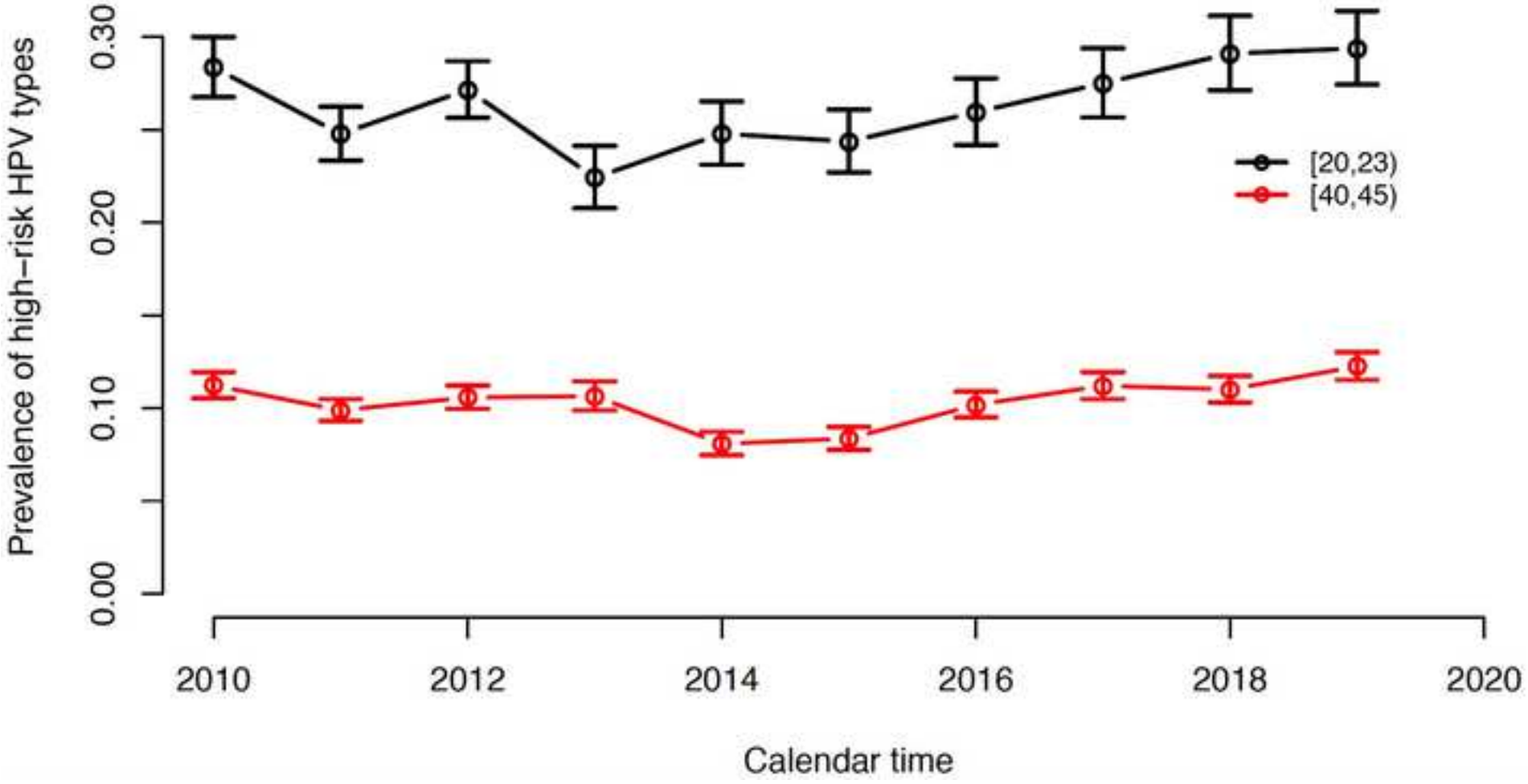


Figure 4

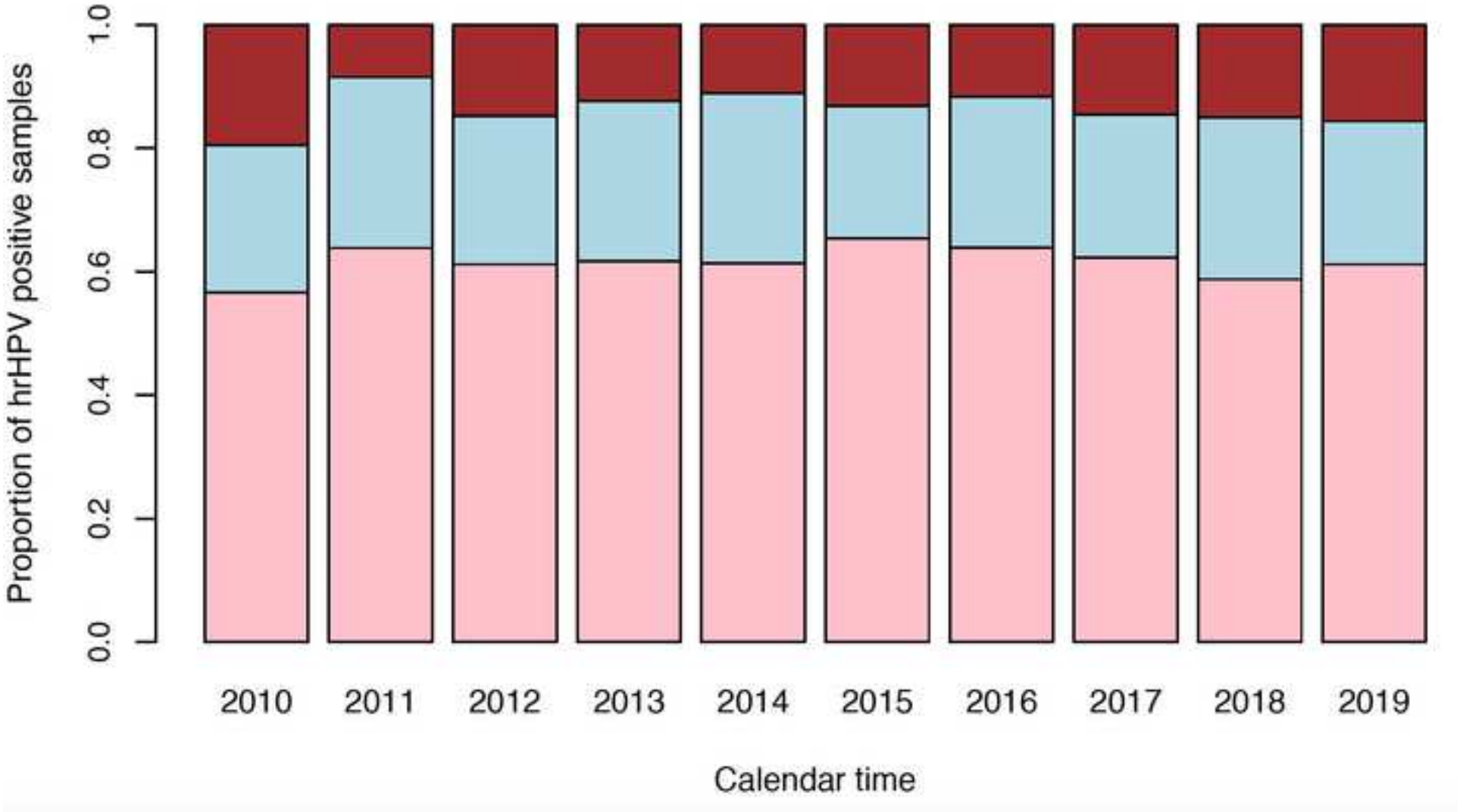
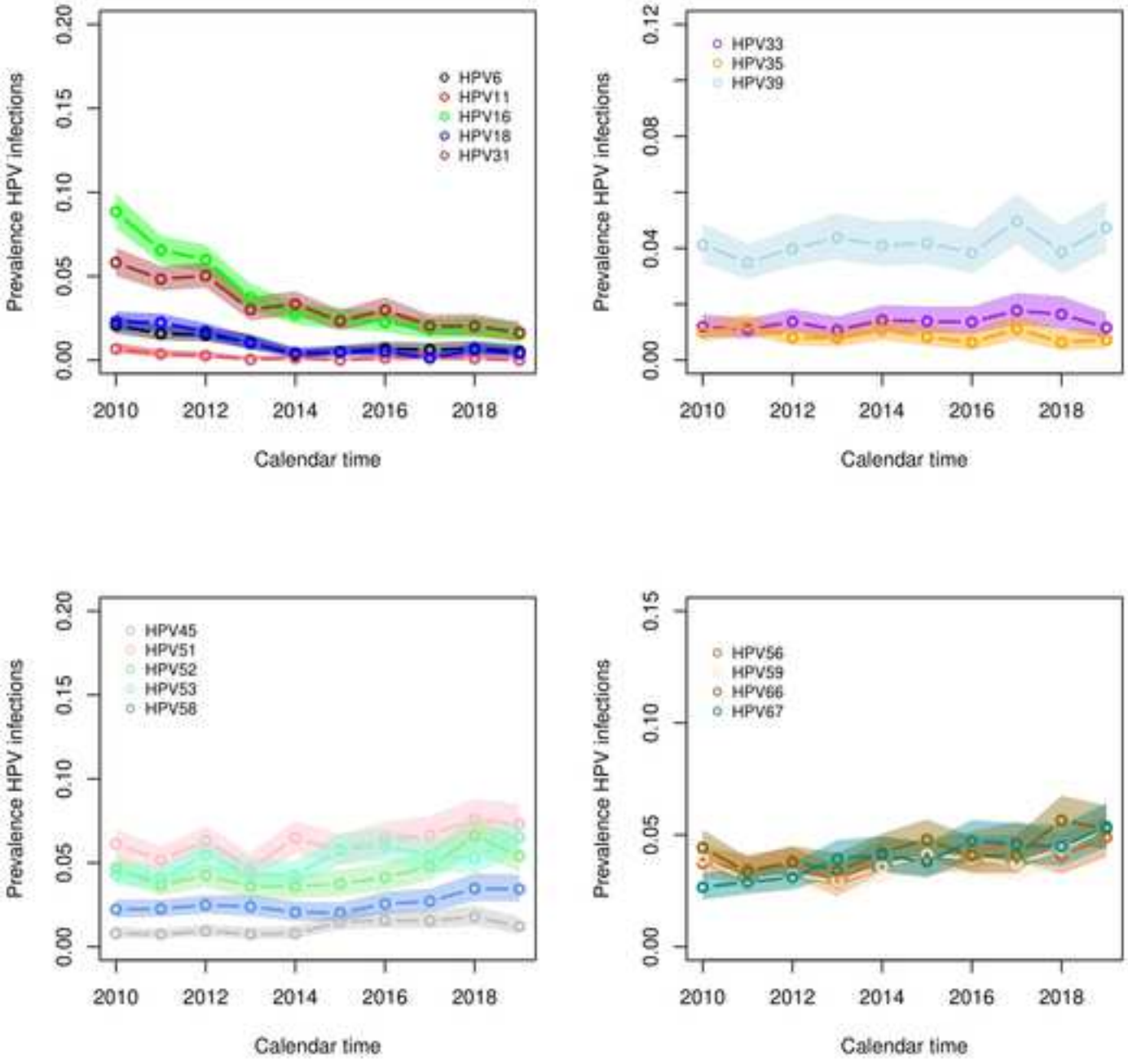
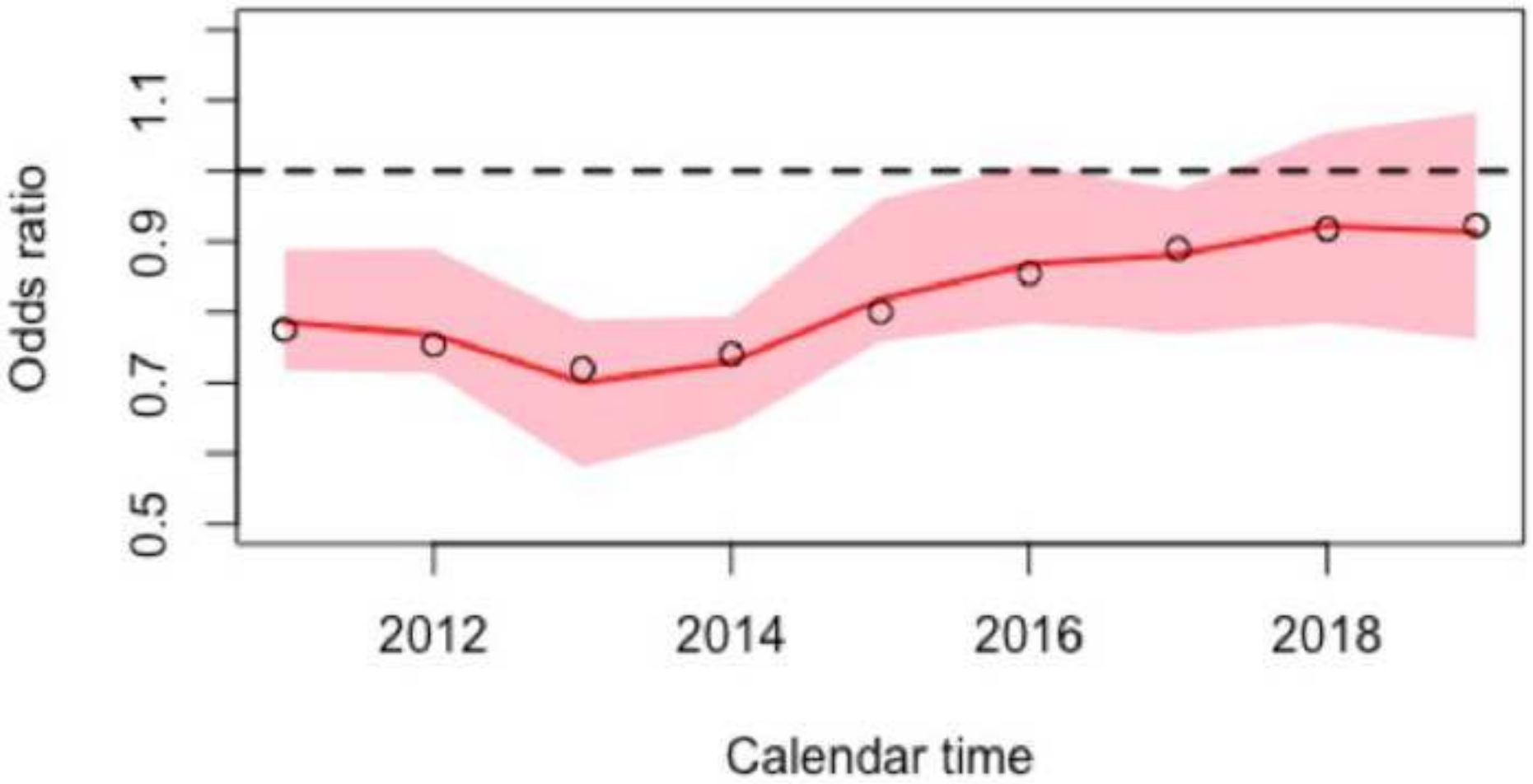
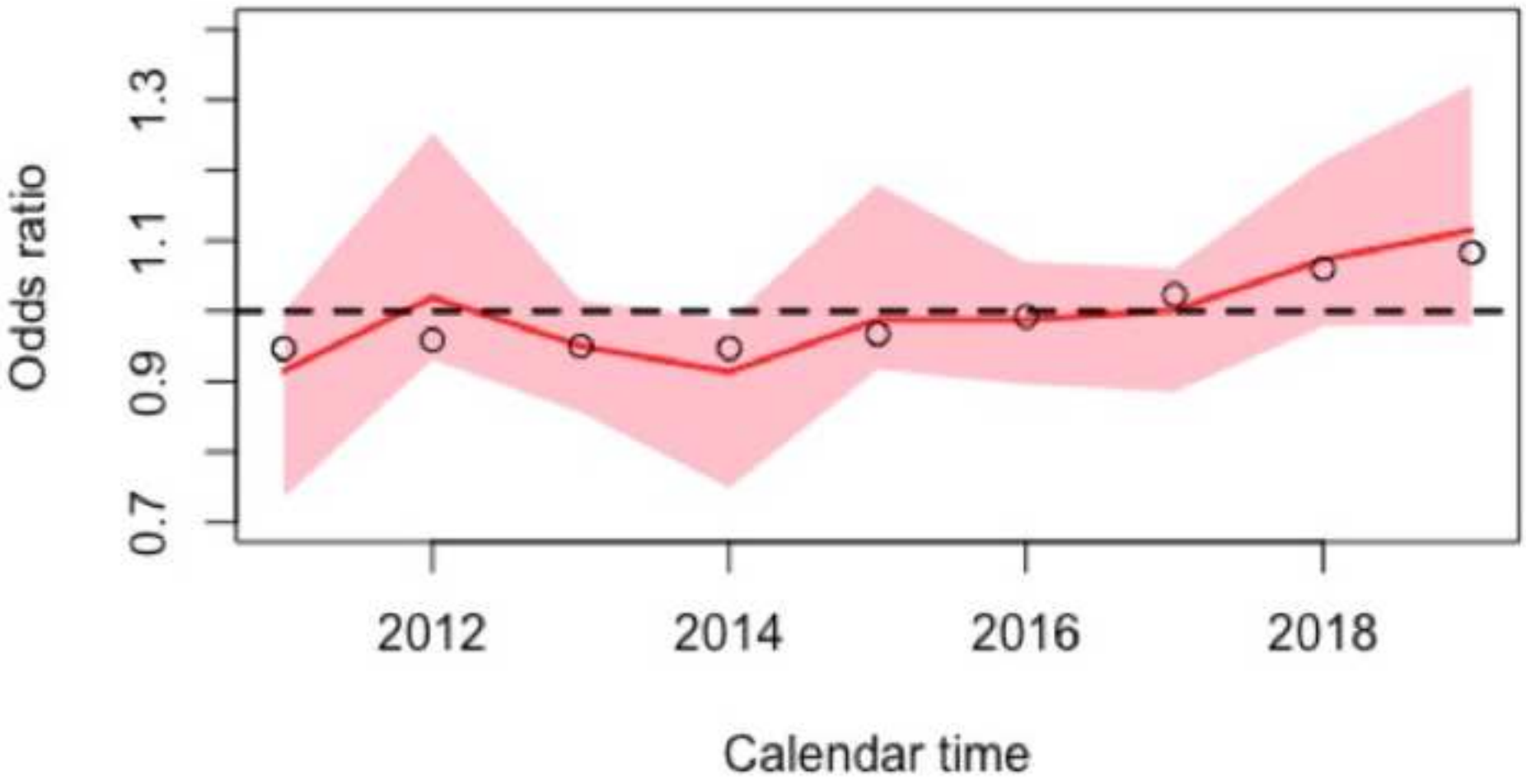


Figure 5







Suppelemntal Digital Content 2

Group	Types
A5	26, 51, 82
A6	53, 56, 66
A7	18, 39, 45, 59
A9	16, 31, 33, 35, 52, 58, 67
A10	6, 11
A11	73