HPV is the most common sexually transmitted virus worldwide, and is the causative agent of cervical cancer. Cervical cancer develops via precursor lesions (cervical intraepithelial neoplasia (CIN1/CIN2/CIN3)). Most HPV infections are transient and only 3-5% lead to cervical cancer in 20-25 years, when no therapeutic intervention is performed. Since 2009/2010, HPV vaccination is introduced in the National Immunization Program. The primary goal of an HPV vaccination program will be to reduce cervical cancer. Awaiting the primary outcome of HPV vaccination (i.e. incidence of cervical cancer), surrogate markers such as the incidence of cervical precursor lesions, incidence and prevalence of HPV DNA infections, and serology as a tool for cumulative exposure, can already play a role in monitoring the impact of vaccination. Therefore, this thesis presents results of various studies on the (sero)epidemiology of HPV infection in the pre- and early post HPV vaccination era. These studies mainly aimed to aid in assessing the early effectiveness of an HPV vaccination program in order to inform public policy for prevention and control of HPV. In this Chapter, we will describe what the added value of the results of this thesis is to the monitoring perspective of the HPV vaccination program in the Netherlands.
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HPV DNA prevalence in teenage populations
Pooled- and meta-analyses of age-specific HPV DNA prevalence have demonstrated considerable variability across geographical regions [1,2]. Country-specific knowledge of HPV infections in the pre-vaccine era is needed in order to measure future changes in HPV dynamics in the Netherlands, caused by the introduction of the bivalent prophylactic HPV16/18 L1 VLP vaccine. Repeating these measurements after vaccination are particularly relevant until the age when screening starts (i.e. 30 years in the Netherlands).

In the Netherlands, most data on the prevalence of HPV is from women aged 18 and above. From this age onwards, the highest HPV DNA prevalence is seen in the age group 20-25 years, followed by a decrease in women 25-34 years and an even further decrease in women aged 55-65 years [3,4]. In Chapter 2 of this thesis, we show HPV DNA prevalence data from younger women in the Netherlands. A very low prevalence of HPV16/18 was found among these young girls in the HAVANA study (1% for HPV16/18 at age 15), and fits with the age of initiation of sexual debut (in the Netherlands ~16 years [5]). Therefore, prophylactic vaccination with the HPV16/18 L1 VLP vaccine of women under the age of 16 is an optimal strategy for complete protection. The target age of 12 years as set in the current Dutch vaccination program is adequate to ensure this protection.

After age 15, the prevalence of HPV and the number of girls who become sexually active rises rapidly (77% of Dutch girls report to be sexually active at age 18, compared to 29% at age 15 and 4% at age 12) [5]. The increase with age, although in a study with higher sexual risk behaviour, was also seen among young women (aged 16-29) participating in a Chlamydia screening program (CSI) (Chapter 3) in the Netherlands. A slightly different age pattern was observed when looking at HPV16/18 separately, indicating the importance for type-specific monitoring.

Thus, studies in both the general population, such as the HAVANA study, as well as in high-risk groups, such as the CSI study and STI clinics [6], can contribute to HPV vaccination monitoring. The first, because in this group the real-life effect of vaccination can be studied, and the latter because the expected shift in HPV types after HPV vaccination will become apparent first in these high-risk populations.

HPV serosurveillance as a monitoring tool
To date, the biological working mechanism of naturally derived antibodies is not fully understood [7]. HPV is effective in evading detection by the immune system for long periods and only generates a weak immune response [8,9]. Only 40%–60% of women who test positive for a given HPV type in a cervical smear seroconvert to that HPV type [7,10-13]. When a detectable serum antibody response to the viral major capsid protein L1 of a given HPV type does occur, it remains relatively stable [13]. Therefore, measuring HPV type-specific antibodies can be a useful tool for estimating cumulative HPV type-specific lifetime exposure.

To get more insight into factors that are associated with HPV seropositivity we explored the literature in Chapter 4. We found that persistent HPV DNA infections, increased viral load, high sexual risk behaviour and being immunocompromised increase the likelihood to be seropositive. In addition to these findings, we found that in line with other studies [14,15], HPV seropositivity was higher in women than in men in two population-based studies (Chapter 5 and 6) and differed by the anatomical site that was infected in a cross-sectional study among men who have sex with men (MSM) (Chapter 7). These differences in the anatomical site might be due to differences in the epithelium infected. The risk factors described above should be taken into account when interpreting HPV monitoring studies. For example, when taking sexual behaviour into account, we showed that the differences in the overall HPV seroprevalence in two cross-sectional surveys in the Netherlands (Chapter 6) seem mostly related to an increase in sexual activity between the two periods (1995/1996 and 2006/2007) and hardly due to an increase in certain HPV types.

After introduction of vaccination, serosurveillance should preferably be performed in various study populations. On one hand, large-scale cross-sectional serosurveillance studies, like the ones described in Chapter 5 and 6 are relatively easy to perform compared to HPV DNA prevalence studies via vaginal DNA swabs. At the population-level seroepidemiological studies could also provide us with information about clustering of HPV-susceptible groups, the impact of HPV16/18 vaccination on the prevalence of other HPV
types (either cross-protective or type replacement) and herd-immunity. On the other hand, longitudinal studies, could inform us on the degree, duration and biological mechanism of protection after vaccination. So in the HAVANA study we followed girls from the start of sexual debut, and linked HPV serology data to DNA status and to sexual behaviour (Chapter 2, 9 and 10). For example in Chapter 10, we showed that all girls included in the HAVANA study and vaccinated with the HPV16/18 VLP L1 vaccine seroconverted for HPV16/18. HPV16/18 antibody levels significantly increased compared to pre-vaccination (Chapter 10) and remained constant in the subsequent year. In Chapter 10, we also showed that in about 80% of the vaccinated girls HPV16/18 antibodies were present in the mucosal surface, although at a 50-fold lower level than in serum. In the next few years, this type of information might be used to explore whether possible breakthrough HPV infections are associated with an absence or low antibody levels in cervical mucus.

Although data so far shows that the HPV vaccine is highly efficacious at the serology level, until now, there is no immune correlate of protection against infection or disease. Thus, at which levels antibodies remain functional is unknown. Interestingly in some studies women in whom antibodies against Vaccine-associated HPV types had declined below the limits of detection, are still protected [16]. For monitoring purposes, it is therefore useful to use mathematical models to predict the duration of an antibody response based on the kinetics of the immune response measured at an earlier time point [17].

The effect of HPV vaccination on HPV diversity
It is known that an estimated 20-50% of HPV infected women harbour multiple HPV types [2,18]. Therefore, understanding the possible interactions among HPV types is important for predictions regarding the effects of HPV vaccination [19,20]. The HPV16/18 vaccine has the potential to reduce about 70% of all cervical cancer cases, at least, if other HPV types do not take the ecological niche. An apparent increase in disease might occur when vaccine types are removed and stop ‘masking’ the types not included in the vaccine (unmasking). Model-based estimates for the size of this effect are in the order of a 3-10% diminished reduction in long-term cervical cancer incidence [21,22]. The ecological niche could also be taken through type replacement, which is a viral population dynamics phenomenon and is defined as elimination of some types causing an increase in incidence of other types. Type replacement has been observed following vaccination against other pathogens (e.g. Streptococcus pneumonia) [23], and is plausible whenever genotypically diverse pathogen strains compete for the same hosts. For type replacement to occur, antagonistic interactions are required between vaccine types and those not included in the vaccine [24,25]. In Chapter 8, we have explored these antagonistic interactions. We confirmed previous findings [26-28] by showing that pairwise interactions were apparently non-existent in three cross-sectional studies from the Dutch population. From this data, we conclude that until now, there were no indications to suspect type replacement.

Although empirical studies, like our study described in Chapter 8, can aid our understanding of the process of type-specific interactions prior to vaccination, the real effect of type replacement will only become apparent in due time. Because differentiating between “unmasking” and “type replacement” will be difficult once these girls enter the screening program, and the first vaccinated cohort will enter the screening program no earlier than 2023, various studies starting early after vaccination and monitoring the prevalence of different HPV types are desirable to assess this “unmasking” or “type replacement” effect. Such information is needed because the oncogenic potential of hrHPV types differ, and changes in incidence of HPV-related CIN and cancer are preceded by changes in type-specific HPV prevalence [29].

Vaccine effectiveness against intermediate endpoints
Prevention of cervical cancer is the ultimate goal of HPV vaccination. Trials have used surrogate end points because it takes 20-25 years before cervical cancer develops, requiring unrealistically large and lengthy studies. Therefore, incident CIN lesions and persistent HPV infections have been recognised to serve as surrogate or intermediate endpoints for cervical cancer. In randomized clinical trials (RCTs), both the bivalent and the quadrivalent vaccines have shown an efficacy of >90% in preventing persistent HPV16/18 infection and CIN lesions for at least 9 years after administration [30-32]. Also for non vaccine types, the vaccines
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have demonstrated cross-protective potential for persistent HPV31 and -45 [33,34]. In line with the results for RCTs and some early VE results of other countries [35,36], we found that among Dutch girls eligible for the HPV vaccination, the vaccine effectiveness against incident and 12 months persistent HPV16/18 infections was high (100%) (Chapter 9). In addition, we found that vaccine effectiveness against infections with HPV16, -18 and the cross-protective HPV types 31 and -45 was comparable with the cross-effectiveness of the bivalent vaccine as described in a recent meta-analysis (Chapter 9) [34]. These results are very reassuring, for the effectiveness of the bivalent vaccine program implemented in the Netherlands.

Further follow-up of the girls in the presented cohort will provide more stable vaccine effectiveness data against persistent HPV infections and in due time against CIN lesions.

Uptake of vaccination program

In the Netherlands, about 95% of infants and young children are vaccinated under the universal childhood vaccination program (National Immunization Program (NIP)) [37]. The uptake of HPV vaccination is much lower than these childhood vaccinations and is probably influenced by the novelty of the vaccine, a new age group, a girls-only vaccination, a vaccine targeting a sexually transmitted infection and negative media attention [38]. In the Netherlands, vaccination coverage in the first year after introduction was 56% (birth cohort 1997) with an increase to 58% for the birth cohort 1998 [37]. To estimate the impact on cervical cancer, insight into the characteristics of individuals who are not being vaccinated is relevant. In Chapter 11, we found that HPV vaccination uptake among 16 years old girls was not associated with high sexual risk behaviour. Unvaccinated girls of this age were not at a disproportionally higher risk of being exposed to HPV compared to vaccinated girls. A longer follow-up of these girls is needed to see if this is still the case in the long term. In addition, we show that risk factors associated with non-attendance in the cervical screening program like ethnicity, or socio-economic status, were not associated with vaccination uptake. So in contrast to some other studies [39], we did not find great inequities in vaccination uptake characteristics, between vaccinated and unvaccinated individuals.

The way to increase vaccination uptake remains debated. For example, contradicting results regarding the influence of HPV knowledge on vaccination uptake have been reported [40]. Several studies suggest that increased knowledge of HPV is associated with greater vaccination uptake [41,42] However, in a study in the UK they found that despite general lack of knowledge about HPV and its link with cervical cancer, vaccine uptake rates were generally high for the first year of the HPV vaccination program [43]. In addition, a Dutch study [44] found the opposite (low vaccine uptake but high knowledge of HPV), although this was among women older than the ones targeted for vaccination.

External factors might also influence vaccination uptake, such as the setting in which the vaccine is given. For example, in Australia, Canada and the UK where the HPV vaccine is given in a school-based setting, HPV vaccination uptake is high (>85%). However, it cannot be excluded that this high uptake is influenced by other factors, which do not play a role in the Netherlands.

Currently, the RIVM is setting up a monitoring system to find out peoples intention to be vaccinated. So far, this monitoring system is directed towards infant and early childhood vaccination. Because HPV is different from other vaccines in many ways, it is recommended to target this monitoring system specifically for HPV. Other studies in the Netherlands have already made a start at exploring intentions to be vaccinated with the HPV vaccine. van Keulen et al. 2012 showed that social-psychological factors were more important in explaining HPV vaccination intention than socio-demographic factors. At present, this research group is exploring the possibilities of providing more tailored information for girls eligible for HPV vaccination and the impact of certain factors on the decision to get vaccinated [45].
Conclusion
In this thesis, several studies to monitor the short-term impact of HPV vaccination in the Netherlands have been performed. Prior to vaccination, we have used cross-sectional DNA studies (such as the CSI study or STI clinics) and population-based serology studies (PIENTER 1 and 2), to outline the (sero)epidemiological situation of HPV. When these studies are repeated, the population-effects of vaccination can be explored, offering the opportunity to detect shifts or replacements of HPV types as a result of the vaccination campaign. Next, we have shown a high vaccine effectiveness against intermediate precursors of cervical cancer in a longitudinal study (HAVANA), among 14-16 year old vaccinated and unvaccinated girls. The annual data collection of the HAVANA study will continue with the aim to study the medium- to long-term impact of vaccination in the Netherlands.

These studies are part of a comprehensive monitoring framework to analyse the real-life effectiveness of HPV vaccination against lesions, the duration of antibody protection, the mechanism of protection, and the occurrence of type-replacement.

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