Newly introduced vaccines: effectiveness and determinants of acceptance

Gefenaite, Giedre

Chapter 4. Q fever vaccine effectiveness

Effectiveness of the Q fever vaccine: a meta-analysis

Gefenaite G

Munster JM

Van Houdt R

Hak E

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Abstract

In the Netherlands, the number of notified human Q fever cases showed a steep increase over the last three years and is not expected to disappear in the next few years. Since vaccination might be an option to prevent Q fever cases in the general population, evidence is needed about its effectiveness. We therefore conducted a meta-analysis to determine the evidence base for effectiveness for Q fever vaccination in human populations. We calculated Mantel-Haenszel risk ratios and we used the following formula to calculate the vaccines effectiveness: (1-mhRR)*100%. Although individual and the pooled estimates showed a high effectiveness of Q fever vaccine, conclusions for the general population cannot be confidently drawn about vaccine effectiveness due to potential flaws in the design of the studies and the selected group of study participants.

Introduction

In the Netherlands, the number of notified human Q fever cases, caused by *Coxiella burnetii*, showed a steep increase over the last three years, with 168 versus 2357 new cases in 2007 and 2009 respectively [1]. Despite many measures being taken to prevent further transmission in the Netherlands, it can be expected that Q fever cases will occur in the next few years [1]. This is a serious hazard not only for those at high occupational risk to get the disease, but also to other vulnerable groups, such as pregnant women, immunocompromised persons and those with pre-existing cardiac valve- or vessel defects [2].

Currently only one Q fever vaccine (Q-vax, Commonwealth Serum Laboratories Limited) is available for humans. This vaccine is registered in Australia and is there used in the population that has the highest occupational risk (mainly abattoir workers). Since vaccination with Q fever vaccine might be an option to prevent symptomatic and asymptomatic cases of Q fever in the general population, evidence is needed about its effectiveness. In 2007, a paper discussing the effectiveness of human Q fever vaccine was published [3]. However, although this study gave a good overview of literature, it did not aim to conduct a systematic analysis of current evidence for Q fever vaccine effectiveness.

We therefore conducted a meta-analysis to determine the evidence for the effectiveness of Q fever vaccination in humans in a systematic way. Furthermore, as studies on the effectiveness of Q fever vaccination were often small and probably biased, we aimed to assess bias by using the assessment criteria for randomized controlled trials and observational studies.

Methods

A review of literature was done by searching PubMed and the references of included papers. Our search was limited to human studies in the English language. The search strategy was: ((Q fever OR *Coxiella burnetti* OR *C. burnetti*) AND (vaccination OR vaccine OR immunized OR immunisation)). First we pre-screened the titles and the abstracts;

afterwards the eligibility of the studies was judged by reading the full-text. Only the studies that used Q fever vaccine in human and gave information about the clinical outcome and reported the raw data were included in the analysis. The final analysis was performed on the effectiveness of Q-Vax (CSL Limited) vaccine.

The design and possible limitations of the studies were assessed using criteria for randomized control trials [4] and longitudinal non-randomized observational studies [5]. As the main possible limitations we considered bias because of information, selection or confounding, which may lead to the over- or underestimation of the vaccine effectiveness.

The Mantel-Haenszel risk ratio (mhRR) was calculated after pooling the raw data by using Episheet by K. Rothman [6;7]. Vaccine effectiveness was calculated by the following formula: (1-mhRR)*100%.

Results

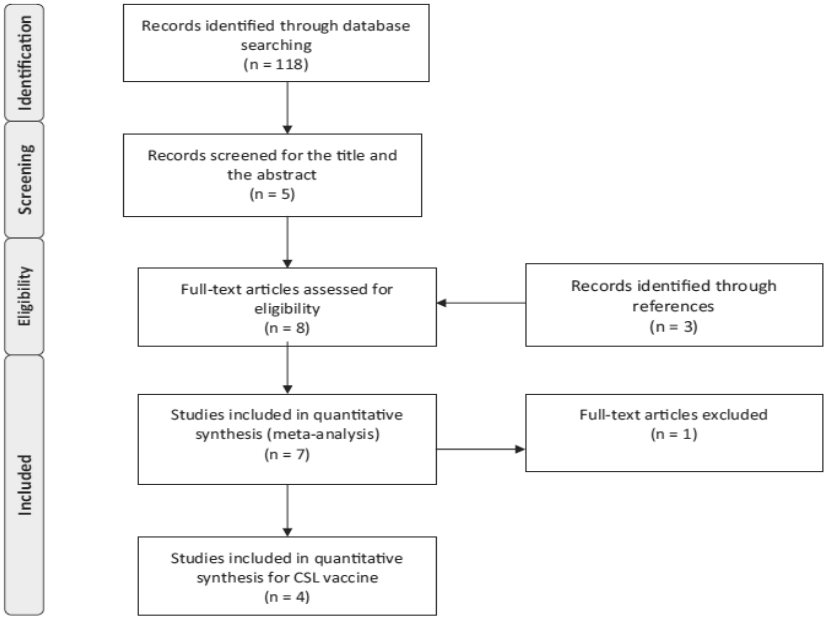
Results of the search

The first search resulted in more than a hundred hits. Only five articles met our inclusion criteria, and three extra papers were included after screening the references (Figure 1). We had to exclude one paper [8] that described an interim analysis as we included the complete study in our meta-analysis [9]. Finally, our search resulted in seven studies containing the raw data about the effectiveness of the Q fever vaccine [9-15]. Four of them contained the raw data about the effectiveness of Q-Vax (CSL Limited) [9,10,13,15].

We included three retrospective cohort studies [10,13,14], one prospective cohort study [9], one randomised controlled trial [15] and two experimental studies [11,12]. Except for the volunteers in the experimental studies, the study population consisted of persons who are at risk to get Q fever due to their profession, mostly abattoir workers and laboratory staff.

The summary of the included studies can be found in Table 1.

Figure 1. Flow diagram**.**



Assessment of vaccine effectiveness

All of the studies showed a protective effect of the vaccine against Q fever (ranged between 91-100%). The overall effectiveness of the vaccine as calculated after pooling the raw data was 97% (95% confidence interval 94% to 99%).

The incubation time of Q fever is around 15 days. Therefore, those who developed clinical signs and symptoms of Q fever within 15 days after vaccination could be considered to be vaccinated within the incubation time of a natural infection. After excluding those cases, the vaccine effectiveness increased to 99% (95% confidence interval 96% to 99.7%).

The effectiveness of Q-Vax (CSL Limited) vaccine was 98% (95% confidence interval 94% to 99%), and reached 100% after excluding the cases that occurred within 15 days after vaccination.

Table 1. Description of studies included into meta-analysis.

	Ackland et al., 1994 [10]	Benenson, 1959 [11]	Gilroy et al., 2001 [13]	Marmion et al., 1990 [9]	*Philip [14]	*Richard B. Hornick [12;14]	Shapiro et al., 1990 [15]
Used Q-fever vaccine and dosage	Q-Vax, CSL (3 batches of 30 µg and 1 batch of 20 µg)	Formalin-killed Ether-extracted Henzerling strain Q-fever vaccine (3 x 1 ml)	Q-Vax, CSL	Q-Vax, CSL (1X 30µg)	Q58-A (1X 22µg 1ml)	Q-Vax, CSL (1X 30µg)	Q-Vax, CSL (1X 30µg)
Study design	Retrospective cohort study	Experimental study	Retrospective cohort study	Prospective cohort study	Retrospective cohort study	Experimental study	RCT, double blind, crossover
Intervention for control group	-	-	-	-	-	-	Flu-vax .05 ml
Setting, study population	3 Australian abattoirs, workers	USA, men volunteers	1 Australian abattoir, workers	1 Australian abattoir, workers	Laboratory staff	USA, volunteers	3 Australian abattoirs, workers
Exclusion and inclusion criteria for vaccinees	Exclusion: positive serology (CF titer >=2.5) or skin test positive (presence of induration at 5-7 days); with a few exceptions	None	Inclusion: negative serology (CF titer <2.5) and skin test negative (7 days after the test)	Inclusion: negative serology (CF negative at <2.5) and skin test negative	Inclusion: skin test negative	Inclusion: negative serology	Volunteers; Exclusion: positive serology and skin test positive
Exclusion and inclusion criteria for nonvaccinees	Not given, but most likely both, who have positive and negative markers for Q-fever	None	None	Both; but possibility to see the raw data with the same inclusion criteria as for cases	Inclusion: skin test negative	Inclusion: negative serology	Volunteers; Exclusion: positive serology and skin test positive
Case definition	"the pattern of symptoms and signs conformed to the description of clinical Q-fever" and "serological evidence indicating current or quite recent infection with C. burnetii"	"developing clinical disease"; "showing complement-fixing antibodies"	Confirmed case: >=4 increase in antibody titer to phase II antigen (AG) by CFT[c] or a positive IgM titer (>=80) to phase II AG by IFT[d]. Suspected case: At least 4 of the following symptoms: fever, sweats, rigorous, fatigue, headache, myalgia, arthralgia, cough; serological tests negative or not available.	Not given	Not given	Not given	Suspected Q fever cases tested by CFT, IFT
Number of cases among vaccinees	2*/2553	2/27	0/19	2*/690	0/282	2/83	0/98
Number of cases among nonvaccinees	55/1365	8/10	7/68	7/61	2/37	5/6	7/102
Effectiveness (RR, CI 95%)	98% (92%-99%)	91% (64%-98%)	100%	97% (88%-99%)	100%	97% (88%-99%)	100%
Effectiveness [b]	100%	-	-	100%	-	-	-
Limitations	Vague definition of cases Exceptions in inclusion/exclusion of cases No sufficient description of the baseline characteristics of vaccinees and nonvaccinees	Vague definition of cases No sufficient description of the baseline characteristics of vaccinees and nonvaccinees No randomization or allocation procedures described No pre-vaccination screening	No description of baseline characteristics of vaccinees and nonvaccinees	No case definition The allocation procedure between vaccinees and nonvaccinees not described	Insufficient case definition No information about the baseline characteristics of vaccinees and nonvaccinees No thresholds for skin tests	Insufficient case definition No information about the baseline characteristics of vaccinees and nonvaccinees Inclusion criteria are not sufficiently described	No information about the baseline characteristics Allocation procedure is not described Case definition is not sufficiently described Exclusion criteria are not sufficiently described

[a] These studies are described in review papers by other authors [12;14]. [b] After excluding those who got ill within 15 days after receiving Q-fever vaccine. [c] Complement Fixation Test. [d] Immunofluorescence Test.

Assessment of bias

One of the problems in the reviewed studies was possible bias due to the inclusion and exclusion criteria of vaccinees and nonvaccinees. In six of the reviewed studies the subjects were excluded from receiving Q fever vaccination when they had a positive antibody titre (CF titre >= 2.5) and/or positive skin test [9,10,12-15]; however there were exceptions and in some cases the thresholds of serological and/or skin tests were not given [10-12,14]. In three studies the inclusion and exclusion criteria for nonvaccinees was not given or it was different from the criteria used for vaccinees [10,11,13]. The inclusion of skin- and/or seropositive nonvaccinees might have led to underestimation of vaccine effectiveness as persons with positive markers are thought not to be at risk for Q fever infection.

Furthermore, vague or even absent case definition might have led to both under- and overestimation of vaccine effectiveness due to lack of objective assessment. Only in one of the reviewed studies Q fever case definition was properly described and included both a list of clinical symptoms and the cut-off values for serological markers [13]. Three studies also used serological markers to confirm suspected Q fever cases [10,11,15]; however the detailed description, including the list of symptoms and the cut-off points of serological markers was missing. A couple of studies did not provide any case definition. Only one of the reviewed studies was a blinded study [13].

The absence of the description of the baseline characteristics of both vaccinees and nonvaccinees might have led to bias as well. The description of baseline characteristics, such as gender or age, of vaccinees and nonvaccinees was poor or absent in six studies [10-15]. For example, according to the National Q fever management program in Australia, the incidence and vaccination against Q fever is higher in males than in females [16]. There is already some evidence from animal studies that females are less susceptible to Q fever infection than males due to female hormones [17]. Such differences in the distribution of gender between vaccinees and nonvaccinees at baseline therefore might lead to bias. Only one of the reviewed studies provided a sufficient description of baseline characteristics [9].

Discussion

Individual studies showed that the effectiveness of the vaccine against Q fever is very high, without exceptions [9-15]. The same high vaccine effectiveness was found after pooling the raw data. Even when cases that occurred within 15 days after vaccination were included, the vaccine effectiveness was very high. However, the designs of the included studies had some potential flaws.

Different inclusion and exclusion criteria for vaccines and nonvaccinees, inclusion of seropositive nonvaccinees, vague or absent Q fever case definition, and differences in baseline characteristics of vaccinees and nonvaccinees might have led to biased results of Q fever vaccine effectiveness.

Another major problem was the selected study sample: there were two studies performed on volunteers, four of the studies focused on abattoir workers and one study focused on laboratory staff. Although information about the demographic characteristics was limited, the study sample was relatively young. At least in three of the reviewed studies the mean age was around 30 years [9,10,13]. Furthermore, the authors of the reviewed studies did not give information about the health status of the study participants. Still, as the study subjects were abattoir workers, laboratory staff and volunteers, it seems likely that they were relatively healthy. This creates problems to generalize the results in different populations. Additionally, it is unclear for how long the vaccine is protective against Q fever, and whether this protection is the result of vaccination in combination with a constant exposure to *Coxiella burnetii*. It was shown that the number of Q fever cases decreased with longer employment at the abattoir [10].

Conclusion

In all, the vaccine effectiveness in groups with a high risk for Q fever seems to be very high. However, due to the selected study population and the absence of a proper description of the studied samples and study procedures, it is not possible to generalize our results and draw conclusion about the effectiveness of Q fever vaccine in the

general population or in specific groups of patients. One of the important goals for the future should be decreasing Q fever incidence and prevention of related complications in persons who are not at constant exposure, but might be more vulnerable, such as pregnant women, immunocompromised persons or those with pre-existing cardiac valve- or vessel defects.

It seems likely that the vaccine against Q fever might decrease the incidence of Q fever in these specific groups and in the general population as well. However more blinded, randomized and unbiased research about its effectiveness is needed.

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Chapter 5. Acceptance of HPV vaccine

Why did the HPV vaccination program among 13-year old girls fail? Insights from a behavioural survey among parents

Gefenaite G

Smit M

Nijman HW

Tami A

Drijfhout IH

Pascal A

Postma MJ

Wolters BA

van Delden JJM

Wilschut JC

Hak E

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Abstract

Background The Dutch Human Papillomavirus (HPV) catch-up vaccination program in 2009 appeared less successful than expected. We aimed to identify the most important determinants of refusing the vaccination.

Methods Two thousand parents of girls born in 1996 targeted for HPV vaccination received an invitation letter to participate in a questionnaire study. Two study groups were defined: the first group consisted of parents of girls who had accepted the vaccine and already received the first dose of HPV vaccination. The second group consisted of parents whose daughters were not vaccinated. The questionnaire consisted of a broad spectrum of possible determinants that were revealed after literature search and discussions with the stakeholders.

Results Four hundred sixty nine questionnaires (24%) were returned, 307 (31%) from those who accepted and 162 (16%) from those who declined the vaccine. The decision not to accept the vaccine was largely determined by: (i) perception that the information provided by the government about the vaccine was limited or biased (OR 13.27); (ii) limited trust that the government would stop the vaccination program if there were serious side effects (OR 9.95); (iii) lack of knowledge about the effectiveness of the vaccine (OR 7.67); (iv) concerns about the side effects of the vaccine (OR 4.94); (v) lack of conviction that HPV can be extremely harmful (OR 3.78); (vi) perception that the government is strongly influenced by vaccine producers (OR 3.54); and (vii) religious convictions (OR 2.18).

Conclusions This study revealed several determinants for HPV vaccination uptake after implementation of the HPV vaccine for adolescent girls. These determinants should be taken into consideration in order to successfully implement HPV vaccination into National Immunization Programs.

Introduction

Based on the recommendations by the Dutch Health Council (DHC) in March 2008 [1], the Dutch government approved implementation of the Human Papillomavirus (HPV) vaccination as part of the National Immunization Program (NIP). The vaccine to be used was Cervarix™ (GlaxoSmithKline), which is a bivalent vaccine against HPV16 and HPV18, and consists of three doses administered at baseline, one and six months [2]. The vaccine was mainly targeted at 12-year-old girls (1997 birth cohort) and a catch-up vaccination program was planned for 13- to 16-year-old girls (1993–1996 birth cohort) [1]. The catch-up vaccination campaign started in March 2009, and the regular vaccination campaign began in 2010. For all targeted girls the vaccination campaign was free of charge. The girls received a personal invitation letter with an information leaflet and were invited to visit local vaccination sessions [3]. Although no permission of the parents was required, the girls were advised to discuss the information and their decision regarding HPV vaccination with parents or other family members [4].

Ultimately, slightly above fifty percent (52.3%) of the targeted catch-up cohort received the HPV vaccine [5]. The vaccination rates varied by age, from 49.0% in 1993 birth cohort to 54.2% in1996 birth cohort [5]. By February 2011, similar vaccination rates (52.5%) were observed in the 1997 birth cohort [5]. One suggested reason for such a low HPV vaccination uptake, as compared to the normal attendance rates for the regular vaccinations of the NIP of 90% [5], was critical reporting in the media, which accused the government of emphasizing the advantages rather than the potential disadvantages of the vaccine. Other reasons, such as the relative novelty of the vaccine, the fact that it was the first vaccine for a sexually transmitted infection and the first vaccine for girls only, and its unknown effectiveness in preventing cancer could have engendered distrust in the parents as well. Another possible factor in declining HPV vaccination or vaccinations in general, could be religious beliefs. In the Netherlands, the overall vaccination coverage is much lower in the so-called Bible-belt region where relatively many people decline vaccinations because of their religious convictions (http://www.rivm.nl/en/infectious-diseases/topics/nip/).

Several Dutch studies have already assessed the knowledge about and the willingness to receive the HPV vaccine. These studies have shown that 56%–88% of the respondents would be willing to receive or have their daughter receive the HPV vaccine [6,7]. Several studies from other countries that assessed parental attitudes towards HPV vaccination showed similar rates of intention to vaccinate (70–80%) [8-10]. Since actual behaviour may be different from intentions, we designed a study which aimed to assess a broad range of demographic, behavioural, and organizational determinants, and knowledge and concerns that were influential in parents' decision to either accept or decline the vaccination for their daughters during the HPV vaccination campaign. Although in the catch-up vaccination campaign 13- to 16-years-old girls were invited to receive HPV vaccinations, only the girls of 13 years of age were invited to our study as with regard to age their parents resembled the parents of the 12-years-old girls the best.

Methods

In July 2009, four months after the HPV vaccination catch-up program for 13- to 16-year-old girls was initiated, we randomly selected parents of girls born in 1996 who had received a call for the HPV vaccine in the Northern provinces of the Netherlands. Two study groups were defined. The first group consisted of parents of girls who had accepted the vaccine and already received the first dose of HPV vaccination. The second group consisted of vaccine decliners. The two groups, including 1000 parents each, were randomly sampled from the vaccination register. On behalf of the researchers, the Institute for Public Health and the Environment (RIVM) sent 2000 invitations to eligible parents to ask if they would be willing to participate in the study. Parents who agreed to enrol in the study returned the response cards with a positive reply and parents who were willing to participate received a paper questionnaire in Dutch (the English version added in Appendix 2). After three weeks a reminder to fill out the questionnaire was sent.

The study was conducted in accordance with the Dutch Law for the Protection of Personal Data (Wet Bescherming Persoonsgegevens) and the Declaration of Helsinki

(http://www.wma.net/e/policy/b3.htm). No medical ethical committee approval was required.

We used an anonymous, self-administered questionnaire to identify the determinants for not accepting the HPV vaccination. In a stakeholders meeting with professionals involved in the HPV campaign, (a gynaecologic oncologist, a sexologist, a doctor of the Municipal Health Service, a regional manager of coordination programs at the center for infectious disease control, an epidemiologist, and municipal health advisors), possible determinants for the uptake of the HPV vaccination were explored. In addition, a review of the literature, a stakeholder analysis, and questionnaires previously developed by our and other research groups [9] were used to construct a new questionnaire. The parents were asked to fill out the questionnaire (in Dutch) on behalf of their daughter who had received a call for the HPV vaccination. Parents could also express their attitudes towards HPV vaccination in a free text.

The demographical determinants of the parents included gender, age, marital status, educational level (none, primary, prevocational, secondary, pre-university, higher-professional, university), religion (Catholic, Protestant, Islam or other) and country of birth. We also assessed the participation in a cervical screening program. We asked whether a participant knew someone with an abnormal cervical smear or cervical cancer in his/her family or circle of acquaintances. Items reflecting behavioural determinants were based on health behaviour criteria according to the "Health Belief Model" and "Behavioural Intention Model" [11,12]. We formulated questions based on five of the six domains in the Health Belief Model; perceived susceptibility, perceived severity, perceived benefits, perceived barriers and cues to action. We posed questions on two more domains that exist in the Behavioural Intention Model; attitudes and social influences. We also posed questions about parents' knowledge concerning HPV and cervical cancer, information services and sources that influenced decision-making, trust in the government, vaccination concerns, age-related items, financial issues, intention to accept the vaccination later, involvement of their daughter in the final decision and future acceptance of other vaccines.

We dichotomized the following variables: education (high (university, higher professional)/other), being religious (yes/no), country of birth (the Netherlands/other), knowing someone with an abnormal cervical smear or cervical cancer in his/her family or circle of acquaintances (yes/no), and participation in cervical screening program (yes/no). The variables assessed with the four- and five-point Likert scale were dichotomized according to the degree of agreement with the proposition, (4–5 [agree - strongly agree]) for the indicator group, and (1–3 [strongly disagree, disagree and disagree nor agree]) for the reference group.

Knowledge about HPV and HPV vaccination was assessed by using 10 statements (true/false/does not know), see Appendix 1. The mean knowledge score was calculated.

The primary outcome was the uptake of the first dose of the HPV vaccine obtained from RIVM.

All determinants with a p-value lower or equal to 0.10 in the univariate analyses were used in the multivariate analysis. We used all determinants with a p-value of 0.05 or lower to construct a final logistic regression model. We calculated odds ratios (OR) and 95% confidence intervals (95% CI) as measures of associations. The area under the curve (AUC) for the receiver operating characteristic (ROC) with its 95% CI was calculated. The statistical analysis was performed using SPSS for Windows (version 16.0; SPSS, Inc Chicago Illinois).

Results

Of the 2000 parents approached by the administrative offices of the RIVM, 863 parents responded and 609 parents were willing to co-operate. Four hundred sixty nine parents returned the questionnaire. Overall the response rate was 24% (469/2000), 31% (307) in the group who received the vaccine versus 16% (162) in the group who did not. Of the respondents 93% (435/468) were female, and mean age was 44 years (range 35–55 years). The mean score for knowledge about HPV and cervical cancer was 5.65/10 correct answers, 5.54 and 5.88 ($p = .09$) in those accepting and refusing the vaccine respectively.

The HPV vaccine was accepted by 66% of the respondents' daughters while 34% (162/469) declined it. The majority (96%) of the girls received the second dose.

When the parents could express their attitudes towards the HPV vaccination, no new determinants of refusing the vaccine were encountered.

Determinants associated with not accepting the HPV vaccination

The results from the univariate analysis to determine the association between the demographic determinants and not accepting the HPV vaccine are shown in Table 1. Being religious was a strong demographic determinant for declining HPV vaccination.

Table 1. Univariate analysis: demographical determinants and declining the HPV vaccination.

Determinants	Vaccinated n = 307	Not vaccinated n = 162	Odds ratio (95% CI)	p-value
Educational level, high	74/305 (24.3%)	51/162 (31.5%)	1.43 (0.94–2.19)	0.09
Religious, yes	152/306 (49.7%)	113/162 (69.8%)	2.34 (1.56–3.50)	<0.001
Country of birth, the Netherlands	298/306 (97.4%)	158/162 (97.5%)	1.06 (0.31–3.58)	0.93
Knowing someone with an abnormal cervical smear or cervical cancer in his/her family or acquaintances, no	181/306 (59.2%)	83/162 (51.2%)	0.73 (0.50–1.06)	0.10
Participation in cervical screening, no	23/306 (7.5%)	10/162 (6.2%)	0.81 (0.37–1.75)	0.59
Regular NIP vaccinations, no	0/304 (0%)	21/162 (13%)	.32 (.28–.36)	<0.001

Several behavioural determinants were associated with declining the HPV vaccination (Table 2). These included the conviction of the parents that their daughter would not get infected with HPV, lack of belief that HPV can be extremely harmful, judgment that it would be unlikely that their daughter might get cervical cancer in the future, perception that vaccinations are not effective in preventing disease, and conviction that HPV is not sufficiently serious to warrant vaccination.

Many determinants regarding the knowledge, and concerns about the safety of the vaccine and organizational issues related to government and information services were associated with declining HPV vaccination (Table 3).

The results from the multivariate analyses indicate that the strongest determinants of not accepting HPV vaccination were: limited information about the vaccine provided by the government, limited trust that the government would stop vaccinations if there were serious side effects and concerns related to vaccine safety, effectiveness and religion (Table 4). The AUC for the final model, including all 7 determinants, was 0.96 (95% CI 0.94–0.97).

Table 2. Univariate analysis: behavioural determinants and declining the HPV vaccination.

Determinants	Vaccinated n = 307	Not vaccinated n = 162	Odds ratio (95% CI)	p-value
It's not likely that my daughter gets infected with HPV some day	81/306 (26.5%)	59/162 (36.4%)	1.59 (1.06–2.39)	0.03
I don't believe HPV can be extremely harmful	37/304 (12.2%)	39/162 (24.1%)	2.29 (1.39–3.77)	0.001
It's not possible that my daughter gets infected with HPV some day	27/305 (8.9%)	13/160 (8.1%)	0.91 (0.46–1.82)	0.79
I don't believe HPV can cause cervical cancer	24/305 (7.9%)	7/161 (4.3%)	0.53 (0.22–1.26)	0.15
It's not possible that my daughter gets cervical cancer in the future	15/306 (4.9%)	3/162 (1.9%)	0.37 (0.10–1.28)	0.10
I don't believe that cervical cancer is a serious disease	4/305 (1.3%)	1/162 (0.6%)	0.47 (0.05–4.22)	0.49
It's not likely that my daughter gets cervical cancer in the future	80/306 (26.1%)	62/160 (38.8%)	1.79 (1.19–2.69)	0.01
Cervical cancer is not something I'm worried about right now for my daughter	117/305 (38.4%)	60/162 (37%)	0.95 (0.64–1.40)	0.78
Vaccines aren't effective in preventing diseases	12/303 (4.0%)	25/153 (16.3%)	4.74 (2.31–9.72)	<0.001
HPV is not that serious to get vaccinated for	6/302 (2%)	24/155 (15.5%)	9.04 (3.61–22.63)	<0.001

Table 3. Univariate analysis: knowledge, concerns, organisational determinants and declining the HPV vaccination.

Determinants	Vaccinated n = 307 (65.5%)	Not vaccinated n = 162 (34.5%)	Odds ratio (95% CI)	p-value
I'm very worried about the side effects of the HPV vaccination	79/301 (26.2%)	140/160 (87.5%)	19.67 (11.53–33.56)	<0.001
We know way too little about the effects of the vaccine	172/298 (57.7%)	158/161 (98.1%)	38.58 (12.03–123.71)	<0.001
We don't know a lot about the side effects of the vaccine	179/298 (60.1%)	155/161 (96.3%)	17.17 (7.36–40.09)	<0.001
It's not very important that my children receive all their vaccinations	10/305 (3.3%)	51/156 (32.7%)	14.33 (7.02–29.25)	<0.001
I won't do everything to prevent my daughter getting cervical cancer	7/306 (2.3%)	9/159 (5.7%)	2.56 (0.94–7.02)	0.06
There are already too many vaccines in the Dutch vaccination program	14/306 (4.6%)	26/160 (16.3%)	4.05 (2.05–8.00)	<0.001
I would had have more information to make a good decision	143/305 (46.9%)	136/160 (85.0%)	6.42 (3.94–10.47)	<0.001
I feel I didn't get enough information to make a good decision	93/303 (30.7%)	95/161 (59%)	3.25 (2.18–4.84)	<0.001
I think the information about the vaccine provided by the government was very limited/biased	115/301 (38.2%)	150/154 (97.4%)	60.65 (21.88–168.17)	<0.001
I think the information about the vaccine provided by the government was not very clear	119/304 (39.1%)	138/160 (86.3%)	9.75 (5.88–16.17)	<0.001
I don't believe/trust that the government would stop vaccinations if there was evidence of serious side effects	14/305 (4.6%)	87/156 (55.8%)	26.21 (14.06–48.84)	<0.001
The government is strongly influenced by the vaccine producers	49/284 (17.3%)	126/153 (82.4%)	22.38 (13.34–37.54)	<0.001
There weren't enough locations to get the vaccination	9/306 (2.9%)	6/158 (3.8%)	1.30 (0.46–3.73)	0.62
It wasn't very clear when my daughter could get the HPV vaccine	12/306 (3.9%)	6/158 (3.8%)	0.97 (0.36–2.63)	0.95
Doctors do not take parents seriously regarding the side effects of vaccinations	35/295 (11.9%)	64/155 (41.3%)	5.22 (3.25–8.41)	<0.001
I think it's good that the HPV vaccine exists, but not at this age	31/303 (10.2%)	79/152 (52.0%)	9.50 (5.82–15.49)	<0.001
I don't think my daughter is very capable to make her own decision about accepting the vaccination	208/302 (68.9%)	112/158 (70.9%)	1.10 (0.72–1.68)	0.66
I would get my daughter vaccinated if the vaccine wasn't only for girls but also for boys	141/303 (46.5%)	12/155 (7.7%)	0.10 (0.05–0.18)	<0.001
Girls who had the HPV vaccination would be more likely to have unprotected sex	5/306 (1.6%)	19/155 (12.3%)	8.38 (3.07–22.92)	<0.001
Having the HPV vaccination might make girls more likely to have sex	21/305 (6.9%)	34/161 (21.1%)	3.62 (2.02–6.49)	<0.001
I would strongly disapprove if my daughter would be sexually active at this age	290/305 (95.1%)	151/162 (93.2%)	0.71 (0.32–1.58)	0.40
Other girls might be vaccinated, but my daughter won't	7/306 (2.3%)	12/157 (7.6%)	3.54 (1.36–9.17)	0.01

Table 4. Multivariate analysis: determinants of declining the HPV vaccine.

Determinants	Vaccinated n = 307 (65.5%)	Not vaccinated n = 162 (34.5%)	OR (95% CI)	p-value
I think the information about the vaccine provided by the government was very limited	103/273 (37.3%)	141/145 (97.2%)	13.74 (3.82–49.46)	<0.001
I have no trust that the government would stop the vaccinations if there was evidence of serious side effects	13/273 (4.8%)	81/145 (55.9%)	10.13 (4.06–25.60)	<0.001
We know way too little about the effects of the vaccine	153/273 (56%)	143/145 (98.6%)	8.34 (1.41–49.50)	0.02
I'm very worried about the side effects of the HPV vaccination	69/273 (25.3%)	127/145 (87.6%)	4.71 (2.13–10.44)	<0.001
I don't believe HPV can be extremely harmful	34/273 (12.5%)	38/145 (26.2%)	4.19 (1.63–10.82)	0.03
The government is strongly influenced by the vaccine producers	47/273 (17.2%)	120/145 (82.8%)	3.60 (1.74–7.47)	0.001
Religious conviction	133/273 (48.7%)	101/145 (69.7%)	2.17 (1.09–4.40)	0.03

Discussion

In our study we aimed to identify the determinants among parents associated with refusal of HPV vaccination for their daughters. We found that, according to reports of parents, limited information provided by the government was the strongest predictor for declining the HPV vaccinations. Although the HPV vaccination campaign included the distribution of invitation letters to families with daughters in the target group and recruitment was supported by a nationwide information campaign targeting health care professionals and the general public [13], these efforts were not sufficient to persuade people to accept the vaccine. Possible explanation, as mentioned before, could include critical media reports that accused the government of emphasizing the advantages rather than the disadvantages of the vaccine. However, it has also been shown that the content of media reports sometimes may lack important information related to the vaccination or the disease [14] which could be misleading.

Another strong determinant for declining the HPV vaccination was a lack of trust that the government would stop vaccination if there were serious side effects. This concern might partly be a consequence of reports about potential associations between certain vaccines and serious adverse events, such as pandemic influenza vaccine and Guillain-

Barré syndrome [15]. This suggests that providing information about the management of vaccine side effects may improve the trust in the government as well.

Concerns about the HPV vaccine effectiveness and safety were associated with refusal of the HPV vaccination. One Dutch study showed that those who were not willing to have their children vaccinated said that they would agree to do so after the vaccine had been used for several years [7]. Secondly, it has also been found that 88% of parents said they would be willing to have their children vaccinated if the government approved the vaccine [7]. Although the HPV vaccine was approved by the Dutch government, which means that a list of criteria such as acceptable safety and effectiveness profiles had to be met [16], the actual behaviour was not consistent with parents' reported intentions. Only slightly above 50% of the target population accepted the HPV vaccine. Differences between the intentions and the actual behaviour regarding the uptake of the HPV vaccinations may therefore be of interest for future research.

Our findings indicate that parents of vaccinated and unvaccinated girls hold very different views on the severity of HPV infection and the likelihood that their daughters might acquire an HPV infection or cervical cancer and on the question as to whether the information about HPV vaccination was adequate. Parents whose daughters were not vaccinated perceived less risk associated with HPV and cervical cancer. Interestingly they felt, more often, that information about HPV vaccination was not sufficient to make a good decision. Although the entire target group received the same information about the HPV vaccination, these findings suggest that the attitudes towards HPV vaccination were largely influenced by more subjective reasons.

The results of our study show that religious respondents were less likely to accept the vaccine. Another Dutch study also found that voters for religious parties are less likely to accept HPV vaccination [3]. This observation is consistent with the general refusal of childhood vaccination by a group of reformed orthodox living in the Netherlands (http://www.rivm.nl/en/infectious-diseases/topics/nip/;
http://www.zorgatlas.nl/preventie/vaccinaties-en-screening/hpv-cohort-1997-per-

gemeente-2010/#breadcrumb). The findings from other countries however provide only limited evidence about the association between religion and HPV vaccine uptake [10, 9].

Moreover, preliminary results from other countries show that HPV vaccination coverage largely depends on the type of vaccination program that is implemented. A school –based approach was superior to vaccination programs on –demand through health professionals, the latter being implemented in the Netherlands [13]. The three-dose vaccination coverage in Scotland in the 1996 birth cohort via a school-based HPV vaccination campaign was 86% [17]. It therefore appears that apart from a need for clearer and more transparent messages to the public, different approaches to reach the target population should also be considered.

From the discussions with the stakeholders at the beginning of our study and during the start of the HPV vaccination campaign in the Netherlands it appeared that parents have much influence on the decision as to whether or not to vaccinate their daughters. However, vaccination coverage rates may also be influenced by the girls' opinions themselves. It has been shown that different information sources may be preferred by girls of different age [18,19]. For example, information provided by health care professionals and mass media (television, the internet) seemed to be a preferred source of information among older teenage girls (15- to 18- or 19-years-old) while younger teenagers (11- or 12- to 14-years-old) had more trust in schools, teachers and family [18,19]. Guidance about HPV and HPV vaccination could therefore be provided through the preferred sources of information for different age groups.

Some limitations of the study need to be addressed. The overall response rate was not high (24%). One of the possible explanations is the two-step response process we employed in which parents were first asked to return a card for participation and only then received a questionnaire. Importantly, the response rate was twice as high among those who received the vaccine versus those who did not (31% versus 16%). This might have introduced bias because those with more positive attitudes towards vaccination were better represented in our study. We expect that the bias introduced more contrast between positive and negative attitudes than in the general population, which agrees

with the exceptionally high discriminative value of the predictive model (AUC-value of 0.96). This means that in the general population the role of these determinants is likely to be less important than what we observed in our study. On the other hand, given the fact that some baseline characteristics were comparable to the general Dutch population the sample seemed to mirror the source population of the Netherlands. Our study also assessed a broad spectrum of possible determinants associated with declining HPV vaccination.

The incidence and mortality from cervical cancer in the Netherlands is one of the lowest in Europe. However, since it is the second most common cancer in 18- to 44-years-old women [20], efforts should be made to prevent it. If 70% of the cervical cancers can be prevented by the currently registered HPV vaccines, it could largely reduce physical and psychological disease burden for the females and their families.

Conclusions

We identified several determinants for the low HPV vaccination uptake. Modifying these determinants might be essential during planning, implementation and continuation the HPV vaccination programs inside and outside the Netherlands. Furthermore, openness and discussion about the pros and cons of HPV vaccination as well as the use of a variety of communication strategies may be helpful for a more successful implementation of HPV vaccination programs.

Abbreviations

HPV, Human Papillomavirus; NIP, National Immunization Program; RIVM, Institute for Public Health and the Environment

Authors' contributions

MS conducted the study, collected, analyzed the data and drafted the first version of a manuscript. GG re-analyzed the data and drafted the final version of the manuscript. HWN and ATG contributed to the design of the study and critically reviewed the manuscript. IHD and BW participated in the qualitative assessments and critically

reviewed the manuscript. MJP, JCW and JJMvD critically commented on the manuscript. EH contributed to the analysis of the study and critically commented on the manuscript. All authors read and approved the final version of a manuscript.

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Appendices

Appendix 1. Knowledge about HPV infection.

1. Often HPV does not present with visible symptoms (true).
2. A cervical smear induces cervical HPV infection (false).
3. HPV usually disappears without treatment (true).
4. More sexual partners increase the risk to get HPV infection (true).
5. It is possible to have HPV for a long time without knowing (true).
6. HPV can be transmitted during the sexual contact (true).
7. HPV can cause the cervical cancer (true).
8. Most of the sexually active people at some point get HPV (true).
9. A condom provides 100% protection against HPV (false).
10. If you have HPV, you always know it (false).

Appendix 2. Questionnaire "Comparatively low attendance during Human Papillomavirus catch-up vaccination program among teenage girls in the Netherlands: Insights from a behavioural survey among parents".

General instruction
Please tick off the boxes as clearly as possible with a blue or black pen. For every question you can fill out one option, unless stated otherwise.

A. General questions	
What is your gender?	Man/Woman
What is your age?	_____ Years
What is your marital status?	Single/ Married/ Living together/ Divorced or separated/ Widowed
What is the highest education that you completed with a certificate?	No education completed / Primary education / Lower vocational education or Initial professional education / Lower general secondary education / Intermediate vocational education / Higher general secondary education or Pre-university education / Higher vocational education or Higher education (university)
What is your religion?	None / Christian Catholic / Protestant/ Muslim / Other, namely
What is your country of birth?	The Netherlands / Other European country / Other, namely
Do you know someone with an abnormal cervical smear or cervical cancer in your family or acquaintances?	Yes / No / Don't know / Do not wish to answer
Do you/ your partner participate in cervical screening (making of cervical smears)?	Yes / No / Not applicable / Do not wish to answer

B. Vaccinations in general (While responding the questions we want to ask you to think about ***your daughter*** who received a ***call for the HPV vaccination***)	
Did your daughter receive the regular/ recommended vaccination of the National Institute Program?	Yes, all of them Yes, not all of them No Don't know
Did your daughter receive the MMR vaccination at the age of 9 years?	Yes No Don't know
Have any of your children ever had a **bad reaction/ side effect** after vaccination with one of the vaccines of the National Institute Program?	No Yes, mild side effect (for example local reaction, painful arm, fever) Yes, severe side effect (for example seizure, severe anaphylactic reaction)
Have you ever **regretted** a decision to get one of your children vaccinated?	No / Yes, reason: …

Please read each of the statements below and show how much you agree or disagree with the statement by ticking the appropriate box.					
	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
It's likely that my daughter gets infected with HPV some day					
I believe HPV can be extremely harmful					
It's possible that my daughter gets infected with HPV some day					
I believe that HPV can cause cervical cancer					
It's possible that my daughter gets cervical cancer in the future					
I believe that cervical cancer is a serious disease					
It's likely that my daughter gets cervical cancer in the future					

C. Questions about HPV and HPV vaccination (While responding the questions we want to ask you to think about ***your daughter*** who received a ***call for the HPV vaccination***)			
	True	False	Don't know
Often HPV does not present with visible symptoms			
A cervical smear induces cervical HPV infection			
HPV usually disappears without treatment			
More sexual partners increase the risk to get HPV infection			
It is possible to have HPV for a long time without knowing			
HPV can be transmitted during the sexual contact			
HPV can cause the cervical cancer			
Most of the sexually active people at some point get HPV			
A condom provides 100% protection against HPV			
If you have HPV, you always know it			

Please read each of the statements below and show how much you agree or disagree with the statements about HPV vaccination by ticking the appropriate box.				
	Strongly disagree	Disagree	Agree	Strongly agree
I'm very positive about the HPV vaccine				
I want to be sure and didn't get my daughter vaccinated				
Cervical cancer is not something I'm worried about right now for my daughter				
I feel I had enough information to make a good decision				
I would strongly disapprove if my daughter would be sexually active at this age				
I'm very worried about the side effects of the HPV vaccination				
Vaccines are very effective in preventing diseases				
HPV is not that serious to get vaccinated for				
Doctors do not take parents serious about what they state about side effects of vaccinations				
There are already too many vaccines in the Dutch vaccination program				
I don't believe/ trust that the government would stop vaccinations if there was evidence of serious side effects				
I think the information about the vaccine provided by the government was very clear				
We know way too little about the effects of the vaccine				
It's very important that my children receive all their vaccinations				
We already know a lot about the side effects of the vaccine				
I think the HPV vaccination is unnecessary because there already is a screening program				
Other girls might need the vaccination, but my daughter won't				
I will do everything to prevent my daughter getting cervical cancer				
I would had have more information to make a good decision				
Having the HPV vaccination might make girls more likely to have sex				
The government is strongly influenced by the vaccine producers				
There were enough locations to get the vaccination				
Girls who had the HPV vaccination would be more likely to have unprotected sex				
I think it's good that the HPV vaccine exists, but not at this age				
It was very clear when my daughter could get the HPV vaccine				
I think the information about the vaccine provided by the government was very limited/ biased				
I think my daughter is very capable to make her own decision about taking the vaccination				
I would get my daughter vaccinated if the vaccine wasn't only for girls but also for boys				

D. Information services *Caution! Question 16 & 17 are the same, question 16 is about **your** decision, and question 17 is about **your daughter's** decision!*	
Which of the sources written below was most important in ***your*** decision? *Please arrange the sources written below in the way of importance (number 1 till 12 where 1 was most important and 12 least important for the decision)*	
	Arrangement 1- 12
Your daughter	
TV (news, opinion broadcasting)	
Internet (website information)	
Facebook, myspace (peer groups)	
Family	
Friends/ acquaintances	
(House) doctor	
School	
Brochure GGD/ RIVM	
Information meeting	
Newspaper	
Radio	

CAUTION! *This question is about **your daughter's** decision*	
Which of the sources written below was most important in ***your daughter's*** decision? *Please arrange the sources written below in the way of importance (number 1 till 12 where 1 was most important and 12 least important for the decision) (you may do this in agreement with your daughter)*	
	Arrangement 1- 12
You/ your partner	
TV (news, opinion broadcasting)	
Internet (website information)	
Facebook, MySpace (peer groups)	
Family	
Friends/ acquaintances	
(House) doctor	
School	
Brochure GGD/ RIVM	
Information meeting	
Newspaper	
Radio	

CAUTION! *Question 18 & 19 are the same, question 18 is about websites **you** used for gathering information, and question 19 is about websites your daughter used for gathering information!*
Name several websites ***you*** used for gathering information (max. 3)
• I didn't use any website
• Yes, I used following websites: …
Name several websites ***your daughter*** used for gathering information (max. 3)
• My daughter didn't use any website
• Yes, following websites: …

E. Ethical and economic considerations					
As of which age you feel comfortable discussing the following subjects with your daughter?					
	Before the age of 9years	As of 9 years	As of 12 years	As of 15 years	Never
Cancer in general					
Cervical cancer					
Sex in general					
Sexual transmitted diseases					
Purpose of vaccinations in general					
Human papillomavirus (HPV)					
HPV-vaccination					
Please indicate at which age you think girls should be vaccinated against HPV	… ….___years				
How much would you be prepared to pay for the set of 3 vaccinations (total price) if these were not for free?	_____euro				
Did you consider vaccinating your older daughter(s) on own expenses (outside the National Immunization Programme)?	Yes, I already did this Yes, I did not do it yet but I'm planning to do this Yes, if it was for free No, even if it was for free Not applicable (I don't have older daughter(s))				

F. Finishing questions	
Did your daughter receive the HPV vaccination?	Yes No Not yet, we are considering to do it I don't know
Who took the final decision to take or not take the vaccine?	Your daughter You/ your partner Together
Did you agree with the final decision of your daughter?	Yes No I don't know
Did your daughter already receive her second injection?	Yes No I don't know
Would you vaccinate your daughter for other diseases that are sexually transmitted in the future if they would come available (for example Hepatitis B, HIV/AIDS)?	Certainly yes Certainly not I'm not sure yet

Space for comments: …

Chapter 6. General discussion

The aim of this thesis was to contribute to the current knowledge about newly introduced vaccines and vaccination campaigns with respect to their effectiveness and determinants of vaccination acceptance. We presented a number of observational and literature studies assessing the effectiveness of seasonal and A(H1N1)pdm09 pandemic influenza vaccines in different populations, including the description of influenza seasons' metrics in the WHO European Region, the demographical and clinical differences between the seasonal and A(H1N1)pdm09 influenza cases and the determinants of influenza vaccine acceptance among health care workers (HCWs) **(Chapter 2)**. We also assessed pneumococcal vaccination campaign effectiveness in young children by using a time-series design **(Chapter 3)** and synthesized the evidence on Q fever vaccine effectiveness into a meta-analysis **(Chapter 4)**. Finally, we examined the reasons of low HPV vaccination acceptance in the Netherlands **(Chapter 5)**. In this final chapter we summarize the main findings, discuss them in the relevant context and give perspectives for future research.

Influenza

Typical influenza season: timing, duration and spread

In **Chapter 2.1** we described seasonal influenza with regard to its timing, duration and spread. We used the World Health Organization (WHO) European Influenza Surveillance Platform (EuroFlu) euroflu.org that contains data on 53 WHO Regional Office for Europe Member States, 48 of which reported the data that could be used in the analysis. We found that the average length of a seasonal influenza epidemic was 9 weeks and not substantially different across the WHO European Region. Subregional analysis showed that the influenza season first peaked in northern, western Europe and central Asia in week 5 and peaked last in eastern Europe in week 8. We observed moderate to strong patterns of west to east geographical spread, and weak patterns of south to north spread. We also found that the agreement between virological and clinical influenza data was high (72.1%). When we limited the analysis to EU/EEA countries only, the agreement between virological and clinical data increased by 5%.

High agreement between virological and clinical data confirms that when virological laboratory-confirmed influenza outcomes are not available, clinical outcomes such as influenza-like illness or acute respiratory infections (ILI/ARI) might be used. Nevertheless, higher agreement between virological and clinical data in the EU/EEA than non-EU/EEA countries in the Region likely indicates that there are differences in influenza case definitions or reporting systems. Systematic assessments of potential differences, i.e. in case definitions, between 'high agreement' and 'low agreement' countries would contribute to better understanding and possibly improvement of influenza surveillance in the Region.

The results of this study can also be integrated into influenza preparedness plans. Taking into account different timing of influenza epidemics across the Region in combination with potentially waning influenza vaccine-induced immunity over time [1], adjusting vaccination schedules according to influenza seasons' timing might contribute to higher vaccine effectiveness.

Different time and spatial prediction methods might be of use to validate the patterns of influenza spread. Although it has been shown that including climatic variables increased the prediction of influenza transmission models [2], a connection between influenza seasonality and climate is still not very clear and needs further investigation [3]. As the WHO European Region is rather diverse, taking temperature, humidity or other climatic and environmental variables and population migration patterns into account might reveal influenza spread patterns in the Region more accurately.

Seasonal influenza vaccine effectiveness in community-dwelling elderly: bias-adjusted meta-analysis

In **Chapter 2.2** we presented a meta-analysis of 14 cohort studies with the main aim to quantify bias and incorporate any uncertainties into the estimates of seasonal influenza vaccine effectiveness in seniors. Without bias adjustment, seasonal influenza vaccine appeared effective in preventing ILI, hospitalization from influenza and/or pneumonia and all-cause mortality among individuals aged ≥60 years living in the community. Although the estimates pointed towards effectiveness, the reduction in laboratory-

confirmed influenza was not statistically significant. After bias-adjustment the overall vaccine effectiveness estimates for the study outcomes decreased and the confidence intervals widened reflecting uncertainty in the estimates due to potential biases. The between-study heterogeneity reduced considerably after the bias adjustment with the largest reduction for all-cause mortality from I^2=91% (p-value<0.01) to I^2=4% (p-value =0.39).

Before the recommendations of what should be included in a complete and accurate report of observational studies were developed by The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) initiative, the quality of reporting confounding in observational studies was found to be rather poor [4, 5]. Poor reporting and failure to adjust for confounding leads to poorer quality of meta-analyses incorporating observational data. Missing information makes it even more difficult to judge the quality of individual studies. In our meta-analysis we also found that quite a few of the included studies did not properly report baseline characteristics and failed to adjust for potential confounders. It is therefore not so surprising that after the adjustment for biases the confidence intervals around the vaccine effectiveness estimates widened indicating more uncertainty. Additionally, high heterogeneity before the adjustment for biases could at least partly be explained by inadequate adjustment for potential confounders. Besides that it is important to further develop the techniques to assess bias and confounding, ideally the individual studies should be able to deal with those at the study design and data analysis phases [6].

Seasonal and A(H1N1)pdm09 influenza vaccine effectiveness: analysis of 2008-2009, 2009-2010 and 2012-2013 influenza seasons

We continued influenza vaccine effectiveness assessment in **Chapters 2.3** and **2.4**, where we presented cohort and case-control studies. A cohort and a matched case-control study **(Chapter 2.3)** were conducted in the Dutch adult population targeted to receive annual influenza vaccination because of age or underlying medical conditions. In the matched case-control study we found that during the 2009-2010 pandemic vaccine effectiveness in the target population was 98% (95% CI 84% to 100%). Based on the

results from the cohort study, after adjustment for measured confounders, the vaccine was effective against influenza and/or pneumonia in subjects aged ≥60 years only (VE 49%; 16% to 69%). Moreover, when we estimated the vaccine effectiveness during the reference period when no effect of the vaccine was expected (OR=1), we identified some unmeasured confounding in the 18-50 years old group, but not in subjects aged ≥60 years. In a test-negative case-control study conducted in adult hospitalized patients in Lithuania we assessed the effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza during the 2012-2013 seasonal influenza season **(Chapter 2.4)**. The adjusted vaccine effectiveness was 86% (95% CI 19% to 97%).

It is important to note, that in the abovementioned observational studies we aimed to adjust for potential confounders as much as possible. We used matching and adjusted for measured as well as unmeasured confounding. Some of the vaccine effectiveness estimates decreased after adjustments, which indicates overestimation of the true vaccine effectiveness if unadjusted estimates are used. This confirms the importance of adjustment in influenza vaccine effectiveness estimation studies.

When we compared the characteristics between 2008-2009 seasonal and 2009-2010 pandemic A(H1N1)pdm09 ILI cases in the general population, we found similar vaccination effectiveness during both seasons, but risk associated with age was much stronger during the pandemic than the seasonal period **(Chapter 2.5)**. During the 2008-2009 seasonal influenza season age ≥60 years decreased the odds of ILI by approximately 52%, and during the A(H1N1)pdm09 pandemic – by 81%. During the 2009-2010 pandemic season we found the vaccine to be more effective in those aged ≥60 years than younger individuals **(Chapter 2.3)**. During the seasonal influenza in 2012-2013 there was no difference in vaccine effectiveness estimates between 18-59 and ≥65 years old subjects from the general population **(Chapter 2.4)**. One of the explanations of the detected differences in vaccine effectiveness estimates and age effects in different influenza seasons might be related to the pre-existing immunity. Due to previous exposure to H1N1 influenza individuals born after 1950 might have been better protected against the novel virus [7, 8, 9], which made age to appear as a protective factor during the 2009-2010 pandemic season. However, as we were not able to include

previous exposure to H1N1 as a predictor in our models, we cannot distinguish between the pre-existing immunity and age effects when estimating vaccination effectiveness. Also, it is unclear why there was a protective age effect during the 2008-2009 epidemic. Still, our results do suggest some patterns that might explain the role of age. We found that in the general population during the 2009/2010 A(H1N1)pdm09 pandemic older age was protective. However, we did not find any age effects during the 2012-2013 influenza season. The 'fading age effect' observed throughout the years could be explained by (continuous) exposure to A(H1N1)pdm09. During 2009-2010 season, at least one fifth of the population was estimated to have been affected by the A(H1N1)pdm09 pandemic [10]. Due to continuous circulation and dominance of the 2009-2010 pandemic strain, it is very likely that by now even larger proportion of the population is affected and possibly immune to this virus. As exposure to A(H1N1)pdm09 in general is expected to be random, i.e. irrespective of age, previously seen age effects could not anymore be observed. This hypothesis, however, should still be confirmed by future studies.

Influenza vaccine acceptance among hospital health care workers

In **Chapter 2.6** we assessed the attitudes of hospital health care workers (HCWs) towards the acceptance of seasonal influenza vaccine. Knowing that the vaccine is effective, willing to prevent influenza transmission, believing that influenza is highly contagious, believing that influenza prevention is important and having a family that is usually vaccinated were statistically significantly associated with a twofold higher vaccine uptake. We therefore recommend targeting these predictors when developing new influenza vaccination implementation strategies for hospital HCWs.

Vaccinating staff has been shown to prevent mortality, consultations with general practitioners and hospital admissions for ILI in institutionalized subjects [11]. Still, despite substantial amount of evidence about the benefits of indirect protection and recommendations by WHO [12][13], the vaccination rates among HCWs remain low [14]. It appears challenging to increase vaccination rates among HCWs even when multi-faceted interventions targeting a number of the most important predictors for vaccine

acceptance as previously identified in **Chapter 2.6** are implemented [15]. Thus, besides that identifying the most important predictors for accepting or declining vaccinations is the first step before further actions directed at increasing vaccine acceptance can be taken, the way to actually reach the target populations and change their attitudes and behaviour remains an obstacle. In the future, identifying the most effective and relatively resource- and cost-saving components of the multi-faceted interventions might be helpful. Moreover, since knowledge and perception about the vaccine safety and effectiveness appeared among the most important predictors when making a decision about accepting vaccinations **(Chapter 2.6 & 5)**, continuous vaccine safety and effectiveness monitoring followed by evidence-based public information campaigns seems to be necessary.

Pneumococcal vaccination campaign effectiveness

In **Chapter 3** we measured the effectiveness of recently introduced 7- and 10-valent pneumococcal conjugate vaccination (PCV7 and PCV10) programmes in preventing respiratory antibiotic use prescribed for acute otitis media and/or pneumonia in young children in the Netherlands. We assessed the effects of the 7-valent and 10-valent PCV vaccination campaigns by using time-series analysis. We found that the introduction of PCV7 statistically significantly reduced antibiotic prescription proportions in 3 and 4 years old children (by 2.8% [95% CI 0.2% to 5.3%] and 5.6% [95% CI 2.3% to 8.8%], respectively). When PCV10 was added to the models, it reduced the proportion of antibiotic prescriptions by 24.5% (95% CI 6.0% to 39.3%], but only in 1 year old children. Due to a relatively recent introduction of PCV10 the number of data points was limited and therefore the conclusions about the effectiveness of the latter campaign are preliminary. In a few years, repeating these analyses might provide a more valid estimate of PCV10 effectiveness.

One of the methods to assess large scale public health interventions, such as population-based interventions, is using time-series analysis. This is a quasi-experimental design to assess longitudinal time-delimited interventions in non-randomized settings. It requires the outcome measure to be collected continuously and equally spaced over time and

expressed as averages, proportions or rates [16]. The minimum of 24 to 50 data time points is required depending on the time-series analysis method [16]. Also, to assess the intervention effect we need to know the characteristics of the intervention, for example, not only the time point when the intervention was introduced, but also when it actually took an effect, whether the intervention effect was continuous or abrupt, and several others. The advantage of time-series method to assess large-scale vaccination programs is that the existing population (clinical and/or pharmacy-dispensing) databases can be used, even though usually they have limited, if any, information on the vaccination status. Collecting vaccination status information, if it is even feasible, is a resource-consuming process. Being able to model the intervention of interest without having the data on the individual vaccination status therefore becomes a good alternative. In recent years, the use of time-series to assess vaccination campaigns and other large-scale public health interventions has been increasing [17]. Nevertheless, comparison of individual and aggregated data based methods to assess the effects of population-based vaccination programs is still missing. Such studies would lead to deeper insights of the effects of the vaccination campaigns as well as methodology.

Q fever vaccination effectiveness

Our meta-analysis demonstrated that Q fever vaccine effectiveness to prevent the disease was very high, and reached 97% (95% CI 94% to 99%). However, the studies included in the meta-analysis were mostly conducted in relatively young and healthy populations, i.e. abattoir workers, who were at constant exposure to *Coxiella burnetii*. It is therefore not clear whether the same high vaccine effectiveness would be achieved in populations that are not constantly exposed to *C.burnetii* or are suffering from high-risk conditions such as cardiovascular diseases. Nevertheless, partly based on the results of this analysis, after three years of Q fever outbreak with more than 3500 cases and assessment of potential Q fever prevention strategies, the Dutch Health authorities decided to offer vaccination to patients at high risk for chronic Q fever living in high Q fever incidence areas [18,19]. In spring 2011, the Dutch Q fever vaccination campaign among individuals with risk factors for chronic Q fever was started [20,19]. The high risk patients included those who have endocarditis, prosthetic heart valves, important

congenital heart anomalies, including those that required grafts, structural defects of the aortic or mitral valve, known aneurysm of aorta, vascular grafts and severe peripheral vascular disease (such as Burger's disease) [18,21]. Implementation of the vaccination program, however, was challenging because previously it was assessed only in relatively healthy subjects **(Chapter 4)**, the vaccine was not registered in any of the European countries [21]. Additionally, because of the latter reason, vaccination could only be provided after the patient's physician signed a medical awareness statement and the patient signed an informed consent form [21].

Such a large-scale Q fever vaccination campaign in a relatively homogeneous patient group sets good grounds to further assess its clinical effectiveness. The study conducted in elderly individuals already suggested lower immune response after vaccination as compared to after past Q fever infection, which suggests decreased vaccine immunogenicity in this high-risk population [19]. As we already discussed in **Chapter 4**, also clinical effectiveness of the Q fever vaccine in these risk groups might be lower than in the study populations that have been assessed up to now. Still, more evidence is needed to confirm or reject this hypothesis.

Although the number of Q fever notifications in the last few years is rather low and further decreasing (81, 66 and 18 cases in 2011, 2012 and 2013, respectively)[22], chronic Q fever infection remains a concern. Vaccine effectiveness studies should also assess the role of vaccination in chronic Q fever prevention, especially because the number of chronic Q fever cases is expected to further increase in the Netherlands.

Attitudes towards HPV vaccination in the Netherlands

As the Dutch Human Papillomavirus (HPV) catch-up vaccination program in 2009 appeared less successful than expected, we conducted a study to identify the most important determinants of refusing the HPV vaccination (**Chapter 5**). Parental decision not to accept the vaccine was largely determined by perception that the information provided by the government about the vaccine was limited or biased; limited trust that the government would stop the vaccination program if there were serious side effects; lack of knowledge about the effectiveness of the vaccine; concerns about the side

effects of the vaccine; lack of conviction that HPV can be extremely harmful; perception that the government is strongly influenced by vaccine producers; and religious convictions. We concluded that these determinants should be taken into consideration in order to successfully implement HPV vaccination into Dutch National Immunization Program (NIP).

Slightly above fifty percent of girls targeted for HPV catch-up vaccination campaign actually accepted it in the first years of introduction in the Netherlands [23]. Apparently, intentions with regard to HPV vaccination acceptance did differ from the actual behavior. Before the HPV vaccine was introduced to the NIP, 88% of the parents said they would have their children vaccinated [24], which appeared not to be true once the vaccine was actually introduced.

HPV vaccination rates in some countries are almost twice as high as in the Netherlands [23,25,26]. Several reasons, including different vaccination strategies, information sources, perceptions of harmfulness or concepts of disease, might be responsible for this.

With some exceptions, school-based approaches of HPV vaccination are more successful than vaccination programs delivered through health professionals [25,27]. Also, the girls of different ages seem to prefer different information sources about HPV and the vaccine [28,29], which also likely contributes to the perception of how necessary it is to accept the vaccine.

In different countries the harmfulness perception of HPV and related outcomes might not be the same. Perceiving HPV as not very harmful was one of the strongest determinants related to vaccine refusal in the Netherlands (**Chapter 5**). In addition to the Dutch HPV vaccination program, every five years women between 30 and 60 years of age are also eligible and invited for a free cervical cancer screening program. In contrast, in countries with limited (access to) health care and absence of other population-based prevention strategies such as cervical cancer screening, HPV and its consequences might be perceived as more harmful and therefore the vaccine against HPV might be better accepted.

Another explanation for different HPV vaccine acceptance could be related to the concept of the disease against which the vaccination is recommended [30]. A question might be raised of whether HPV vaccination program is effective against sexually transmitted disease (STI) or against cervical cancer, the latter having less strong sexual connotation and perceived as a bigger hazard. Vaccinating girls at a very young age before their sexual debut against a sexually transmitted disease could be perceived as unnecessary, stigmatizing or promoting sexual activity, although no evidence for the latter was found [25]. Preventing cervical cancer, on the other hand, would likely raise fewer concerns and could potentially be a "better" reason to accept vaccinations. For the above mentioned arguments, the way we address disease against which the vaccination campaign delivers the benefits is also important, especially for persons belonging to certain ethnic or religious groups.

There is a number of studies that assess attitudes towards HPV vaccination and determinants associated with its acceptance. As indicated by a recent review, better knowledge was associated with HPV vaccine acceptance in the developed countries, while in the low and middle income countries poor knowledge was not an obstacle for high vaccine acceptance [25]. In all countries, the recommendations to accept the vaccine by the health professionals were important for better HPV vaccine acceptance [25]. However, there are no studies systematically assessing the differences in the determinants in populations demonstrating different vaccination behaviours. Studies focusing on the HPV vaccination acceptance reasons in a broader context taking cultural, religious and/or socioeconomic differences into account, possibly across countries, are therefore needed. Assessing attitudes, intentions and behaviour with regard to HPV vaccination as against an STI or cervical cancer might also provide us with better understanding about the reasons of HPV vaccination programs' successes and failures. More research as well as more convincing vaccination strategies will be needed to be able to move towards higher HPV vaccination acceptance in the existing vaccination schedules as well as successful implementation of HPV vaccination campaigns where they are still absent.

Two prophylactic vaccines are currently available against the Human Papilloma Virus (HPV). The bivalent vaccine protects against the two high oncogenic types HPV16 and -18 [31], while the quadrivalent vaccine additionally protects against the two low oncogenic types responsible for anogenital warts [32], namely HPV6 and -11. The data from the clinical trials have demonstrated that both, the bivalent and the quadrivalent HPV vaccines are safe and effective [33, 34], and even cross-protective against the non-vaccine HPV types [34, 35]. Quadrivalent HPV vaccine effectiveness has recently been confirmed by population time-series studies, where decreasing trends in genital warts after the introduction of the vaccination program were found [36][37]. Additionally, the quadrivalent vaccination campaigns seemed to decrease the incidence of genital warts not only in young women, but also in young men, especially when boys where included in the target population to receive HPV vaccine [37]. In the Netherlands, the NIP includes the bivalent vaccine. It was used in 12-year-old girls (1997 birth cohort) and a catch-up vaccination program was planned for 13- to16-year-old girls (1993-1996 birth cohort) [38]. The catch-up vaccination campaign started in March 2009, and the regular vaccination campaign began in 2010. Although in the literature there is some evidence about HPV vaccine effectiveness, another barrier of accepting HPV vaccine found in our study and recognized in several others was lack of knowledge about HPV vaccine effectiveness (**Chapter 5**) [25]. Adding more evidence about HPV vaccine effectiveness to the vaccination campaigns might thus contribute to a more coherent health message, which in turn would lead to a better informed decision regarding HPV vaccination acceptance.

Almost five years have passed since the first cohort of girls was vaccinated in the Netherlands. Although the (severe) clinical outcomes caused by HPV take years to develop, it is about time to start with vaccine effectiveness assessments of the HPV vaccination program on the population level.

General remarks and conclusions

This thesis explored and assessed the effectiveness and determinants of acceptance of newly introduced vaccines. We discussed the main findings in the relevant contexts and suggested some topics for future research.

We provided the description of a typical seasonal influenza season in the WHO European Region; we suggested that to be able to better predict influenza (pandemics), more research focusing on influenza spread should be performed using different climatic and environmental parameters.

Adjusting for confounding remains a crucial issue in vaccine effectiveness assessments. Although we explicitly discussed bias and confounding in the chapters dedicated for influenza and Q fever, it is definitely relevant for all observational vaccine assessment studies, whether using non-individual or individual outcome and exposure data.

Furthermore, we described pneumococcal vaccine effectiveness by using a rather novel method in health research – the time-series approach. We briefly discussed the advantages and potential challenges of using it. We encouraged conducting the studies comparing both, the individual and non-individual data based approaches, as this might result in more resource-saving vaccine effectiveness assessments.

Finally, we reflected on the reasons of relatively low HPV vaccination coverage in the Netherlands as compared to other vaccines provided as part of the NIP for children. Due to the relatively new vaccine and probably because its profile is different from the other vaccines within NIP, more research to identify the reasons of such low vaccination uptake is needed, but then conducted in broader contexts, such as comparing different vaccination provision strategies accross countries.

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