Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Effectiveness of pertussis vaccination in pregnancy to prevent hospitalisation in infants aged <2 months and effectiveness of both primary vaccination and mother's vaccination in pregnancy in infants aged 2-11 months

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ARTICLE INFO

ABSTRACT

Background: PERTINENT is an active hospital-based surveillance system for pertussis in infants. In 2019, four of the six participating European countries recommended pertussis vaccination in pregnancy. Among infants aged <2 months, we measured the vaccine effectiveness (VE) in pregnancy; among infants aged 2–11 months, VE of vaccination in pregnancy and of primary vaccination (PV).

Methods: From December 2015 to 2019, we included all infants aged <1 year presenting with pertussis-like symptoms. Using a test-negative-design, cases were infants testing positive for *Bordetella pertussis* by PCR or culture. Controls were those testing negative for all *Bordetella* species. Vaccinated mothers were those who received vaccine in pregnancy. Vaccinated infants were those who received ≥1 dose of PV > 14 days before symptom onset. We excluded infants with unknown maternal or PV status or with mothers vaccinated ≤14 days before delivery. We calculated pooled VE as 100 * (1-odds ratio of vaccination) adjusted for study site, onset date in quarters and infants' age group.

Results: Of 829 infants presenting with pertussis-like symptoms, 336 (41%) were too young for PV. For the VE in pregnancy analysis, we included 75 cases and 201 controls. Vaccination in pregnancy was recorded for 9 cases (12%) and 92 controls (46%), adjusted VE was between 75% [95%CI: 35–91%] and 88% [95%CI: 57–96%]. Of 493 infants eligible for PV, we included 123 cases and 253 controls. Thirty-one cases and 98 controls recorded both PV with ≥ 1 dose and vaccination in pregnancy, adjusted VE was between 74% [95%CI: 33–90] and 95% [95%CI: 69–99]; 27 cases and 53 controls recorded PV only, adjusted VE was between 68% [95%CI: 27–86] and 94% [95%CI: 59–99].

L. Merdrignac, L. Acosta, A. Habington et al.

Conclusion: Our findings suggest that vaccination in pregnancy reduces pertussis incidence in infants too young for PV. In infants aged 2–11 months, PV only and both PV and vaccination in pregnancy provide significant protection against severe pertussis.

1. Introduction

Pertussis (whooping cough) is a highly contagious acute respiratory infection caused by the bacterial pathogen *Bordetella pertussis*. In 2019, across the 30 European Union/European Economic Area (EU/EEA) Member States reporting pertussis data to the European Centre for Disease Prevention and Control (ECDC), infants aged <1 year were the most affected age group (46.8 per 100,000 population). Three deaths were reported that year in infants, all were too young to have received the first dose of primary vaccination (PV) [1].

Pertussis PV includes three doses in the first year of life and aims to reduce the risk of severe pertussis in infants.

After the introduction in the 1950s of pertussis vaccination with whole-cell (wP) vaccine in children in Europe, pertussis incidence and mortality markedly decreased [2]. Most European countries replaced wP with acellular-pertussis (aP) containing vaccine in the 1990s, which is less reactogenic. After a continued decline, reported cases have progressively increased again in recent years with the last peak incidence in 2012 with >42,000 reported cases in EU/EEA [3]. The World Health Organization (WHO) estimated that pertussis was still responsible for around 63,000 deaths in children aged <5 years worldwide in 2013, despite a global vaccination coverage estimated at 86% in 2014 [4]. Even with immunisation achievements, pertussis remains a major public health concern worldwide.

In September 2012, in response to an increase of hospitalisations and deaths in unvaccinated infants aged <3 months, the United Kingdom recommended for each pregnancy a single dose of aP-containing vaccine between 28 and 32 weeks of gestation. The programme was based on the evidence of transplacental transfer of maternal antibodies known to be maximal from the 34th week of gestation. One year after the programme was introduced, pertussis mortality decreased and VE in pregnancy remained stable around >90% in the following years [56]. Since 2012, an increasing number of EU/EEA countries introduced vaccination in pregnancy: Belgium, Czech Republic, Ireland, Italy, Portugal, Slovenia and Spain [1,7].

However, recent immunological studies suggest that vaccination in pregnancy could interfere with PV and reduce infants' immune response. But little evidence exists about the clinical implications of this potential "blunting effect" of vaccination in pregnancy with infants' PV [8].

From September 2015 to January 2020, ECDC created and funded PERTINENT, "Pertussis in Infants European Network", a multi-country hospital-based active sentinel surveillance system to measure pertussis incidence and VE in infants aged <1 year [3]. For the first time in Europe, a prospective test-negative design (TND) [9] in hospital settings was used to estimate pertussis VE in a multi-country study.

In this study, we estimate VE in pregnancy in infants aged <2 months (i.e., too young to be eligible for PV) and investigate the effect of vaccination in pregnancy and PV in infants aged 2–11 months (i.e., eligible for PV).

2. Methods

2.1. Study sites

In 2019, four of the six European countries participating in PERTINENT recommended pertussis vaccination in pregnancy to protect infants too young for PV: Czech Republic, Ireland, Italy and Spain with two participating regions, Catalonia and Navarra (five study sites, 14 hospitals).

All sites complied with the generic PERTINENT sentinel surveillance and vaccine effectiveness protocol and laboratory guidelines [10] allowing to pool the data across sites.

All sites used the aP-containing vaccine for both PV and vaccination in pregnancy. Even though recommended schedules vary across countries, infants were eligible for the first dose of the primary series from their 61st day of life (2 months of age) in the four participating countries, including the two countries with a 3, 5, 11-month-old schedule.

The Czech Republic and Italy introduced vaccination in pregnancy during the course of the PERTINENT study and were included in both analyses only from that point onwards. Vaccine coverage estimates were not available for these two sites, and ranged from 50% to 90% in the other three sites (Table 1).

2.2. Study population and eligibility criteria

The study population consisted of all infants aged <1 year, likely to be hospitalised in one of the participating hospitals if developing pertussis-like symptoms.

All infants attending one of the participating hospitals and presenting with apnoea or cough associated with at least one of paroxysms, whoop or post-tussive vomiting were tested for pertussis. Infants with any respiratory symptoms and an epidemiological link with a pertussis confirmed case or those not meeting the above clinical presentation but diagnosed as pertussis by a physician were also tested for pertussis.

We included all infants who were tested for pertussis and invited their parents to participate in the study. When required by site-specific ethical committee, infants' legal guardians provided with an informed consent.

2.3. Laboratory methods

We recommended to the hospital laboratories to ensure an accurate identification of the *Bordetella* species using, as much as possible, a triplex quantitative PCR (qPCR): first targeting IS*481* gene (in *B. pertussis*, *B. holmesii*, and some *Bordetella bronchiseptica* strains), pIS*1001* (*B. parapertussis*-specific) and RNase P as the human internal control and two confirmatory singleplex tests for *B. pertussis* (*ptxA-Pr*) and *B. holmesii* (hIS*1001*) if IS*481* was positive. Diagnostic algorithm was detailed in the PERTINENT laboratory guidelines [10].

2.4. Test-negative case control study

We conducted a multi-centre case control study using TND in the 14 participating hospitals.

We defined a laboratory-confirmed *Bordetella pertussis* case as an infant testing positive for *Bordetella pertussis* by PCR (DNA detection of *Bordetella pertussis* using PCR or real-time PCR in a nasopharyngeal aspirate or swab) or culture (isolation of *Bordetella pertussis* from the prior-mentioned clinical specimen) regardless of the clinical criteria. Test-negative controls were those testing negative to all *Bordetella* species by PCR or culture. In the Catalan hos-

Table 1

Characteristics of PERTINENT study sites, vaccination strategy during pregnancy, in adulthood, primary schedule in infants, Europe, 1 December 2015–31 December 2019.

Study sites	Vaccination strategy								Number of participating hospitals
	Pregnancy			Cocooning	Adult	Primary schedule in infants (age in months)			
	Year of introduction	Gestational age (in weeks)	Estimated vaccination coverage	Year of introduction		1st dose	2nd dose	3rd dose	
Czech Republic	2016	28–36	1.6% in 2021 [a]	No	At least once	3 [b]	5 [b]	11–13[b]	6
Ireland	2013	16–36	49.9% in 2017/2018 [11]	2013	No	2	4	6	2
Italy	2017	≥ 27	NA	No	Every 10 years	3	5	11	1
Spain, Catalonia	2014	27–36	82.8% in 2019 [12]	No	No	2 [c]	4 [c]	11[c]	1
Spain, Navarra	2015	27–36	91.1% in 2019 [12]	No	No	2 [c]	4 [c]	11[c]	4

NA: not available.

[a] Estimates from the final report of the project "Monitoring the vaccination of pregnant women against pertussis and influenza, 2020–2021" financed from NIPH Prague internal institutional funds. In this pilot prospective observational hospital-based study in the maternity hospital in Prague, 4617 women (84%) were included in the analysis out of the 5475 women who gave birth in 2021.

[b] Before 2018: doses at 2, 3, 4 and 10 months.

[c] Before 2016: doses at 2, 4 and 6 months.

pital, due to heavy workload, we selected systematically the next three controls per case matched for date of specimen collection.

2.5. Exposures

We defined infants as vaccinated with PV if they had received at least one dose of pertussis vaccine >14 days before symptoms onset. Unvaccinated infants were those who had not received any dose or who had received the first dose ≤14 days before symptom onset.

We defined an infant as having a mother vaccinated during her pregnancy if she had received a pertussis vaccine dose >14 days before delivery. We defined an infant as having a mother not vaccinated if she did not receive any dose during adulthood.

2.6. Exclusion criteria

We excluded all infants with missing information for laboratory results, date of onset, or vaccination status. We also excluded infants sampled >4 weeks after symptoms onset, those testing positive to other *Bordetella* species than *Bordetella pertussis*, those with previous laboratory confirmed pertussis episode and those whose legal guardian did not give consent.

For both analyses, we excluded infants with unknown maternal vaccination status, those whose mothers were vaccinated ≤14 days before delivery or before/after pregnancy or had contra-indication for pertussis vaccination.

2.6.1. Effectiveness of vaccination in pregnancy in infants too young for vaccination (<2 months)

To estimate VE in pregnancy, we restricted the analysis to infants too young to be vaccinated and aged <61 days of life. Additionally, we excluded infants too young to develop the disease and aged <4 days of life (4 days being commonly known as the minimum incubation period for pertussis [2]).

2.6.2. Effectiveness of vaccination in pregnancy and PV in infants (2–11 months)

To explore the effect of both vaccinations, we restricted the analysis to infants eligible for PV and aged 2–11 months. We excluded all infants with unknown PV status or with contra-indication for pertussis vaccination.

2.7. Analysis

For both analyses, we described cases and controls by clinical presentations, severity, risk and protective factors. We used Fisher's exact test to compare those characteristics between cases and controls.

2.7.1. Effectiveness of vaccination in pregnancy in infants too young for vaccination (<2 months)

We compared the odds of vaccination of the infants' mother between cases and controls. We used a logistic regression to model the odds ratio (OR), including study site as fixed effect. We adjusted for time of onset in quarter and age group (4–30 days; 31–60 days). We computed VE as 1 minus the OR, expressed as a percentage.

2.7.2. Effectiveness of vaccination in pregnancy and PV in infants (2–11 months)

We conducted an indicator analysis based on four categories: (1) infants recording no vaccination in pregnancy nor PV (reference category); (2) infants recording PV only (at least one dose); (3) infants recording vaccination in pregnancy only; (4) infants recording both vaccination in pregnancy and PV (at least one dose).

Using infants recording no vaccination in pregnancy nor PV (1) as reference category, we compared the odds of each category of vaccination exposure (2), (3) and (4) between cases and controls and estimated the corresponding OR using logistic regression. We refer to this analysis as the indicator analysis. We included study site as fixed effect in the model and adjusted for time of onset in quarter and age group (2 months; 3–11 months). We computed VE as 1 minus the OR, expressed as a percentage.

2.7.3. Sensitivity analyses

Bordetella species can be isolated from both nasopharyngeal swabs (NPS) or aspirates (NPA). However, a 15% gain in the isolation rate can be obtained by using aspirates in neonates and infants [13].

Additionally, the Czech Republic and Italy encountered difficulties of adherence to the maternal immunisation programme in the first years of its implementation. National vaccine coverage in both sites were assumed to be very low and the mothers of the children enrolled in the analysis were not vaccinated.

Therefore, we conducted sensitivity analyses: (I) excluding the two sites with no mother vaccinated in pregnancy included in

L. Merdrignac, L. Acosta, A. Habington et al.

the study, (II) excluding all infants sampled with NPS, (III) excluding both the two sites and the infants with NPS.

If the number of events per parameter was lower than 10, we conducted an additional sensitivity analysis using Firth's method of penalised logistic regression to assess small sample bias [14].

2.8. Data collection

Using a standardised questionnaire we collected demographic, epidemiological, clinical, and laboratory data, vaccination status of the infant and the mother, risk and protective factors. Hospital teams collected data through the review of clinical case-patient notes, vaccination cards, interviews with parents or legal guardians, and extraction from patient registries.

2.9. Ethical statement

Each site complied with the local ethical procedures. The planning, conduct and reporting of the study was in line with the Declaration of Helsinki [15]. Ethical approval was not needed in Navarra as the PERTINENT study was considered part of the mandatory surveillance system. Other study sites sought ethical approval from a review board according to country-specific regulations (Catalonia: PIC-31-16, Czech Republic: SZU/05992/2019, Ireland: Royal College of Physicians in Ireland REC reference number 16.058 and Gen/499/16, Italy: Bambino Gesù Children's Hospital Ethical Committee: protocol n. 1064_OPBG_2016).

3. Results

From December 2015 to December 2019, 829 infants aged less than one year were tested for *Bordetella pertussis*. Among them, 336 (40.5%) were too young to receive the first dose of PV (aged < 2 months) and 493 (59.5%) were eligible for PV (aged 2–11 months). No death was reported during the study period.

3.1. Effectiveness of vaccination in pregnancy in infants too young for vaccination (<2 months)

After applying the exclusion criteria for VE in pregnancy analysis, we included 276 infants aged <2 months with 75 *Bordetella pertussis* laboratory confirmed cases (27%) and 201 test-negative controls (73%). Among excluded infants, 31 had a missing maternal vaccination status or date of vaccination (Fig. 1).

Twenty-six cases (35%) and 53 controls (26%) were aged 4–30 days (p = 0.181). The median-birthweight was 3320 g for cases (range: 1740–4925; interquartile range (IQR): 800) and 3260 g for controls (range: 1000–5150; IQR: 649) (p = 0.412). The median gestational week at birth was 39 for both cases (range: 29–42; IQR: 2) and controls (range: 28–42; IQR: 2) (p = 0.671).

Information on the type of specimen collection was available for the 75 cases and 199 controls with 18 cases (24%) and 71 controls (36%) only diagnosed based on NPS collection (p = 0.043) (Table 2).

Out of the 75 cases, 20 cases (27%) were both PCR and culture-confirmed, 17 cases (23%) were PCR-confirmed but culture-negative, 37 cases (49%) were PCR-confirmed (no culture result) and one case (1%) was culture-confirmed (no PCR performed). Out of the 201 controls, 6 (3%) were confirmed by culture only.

The proportion of cases and controls by risk and protective factors such as prematurity, delivery type, child care, breastfeeding was similar. Three cases (4%) and no controls had their mother experiencing pertussis in pregnancy. The mothers did not receive pertussis vaccine during their pregnancy.

The median gestational age at vaccination was 30.4 weeks for cases (range: 23–36; IQR: 4) and 30.1 for controls (range: 20–37; IQR: 3.5) (p = 0.741).

Out of the 276 infants too young to be vaccinated, nine cases (12%) and 92 controls (46%) had their mother vaccinated in pregnancy. VE in pregnancy adjusted for study site and time of onset (in quarter) was 76% (95% CI: 38–91) and 75% (95% CI: 35–91) when also adjusted for age group (Table 3).

In the sensitivity analysis excluding infants sampled only with NPS (N = 185), VE adjusted for site and time of onset (in quarter)
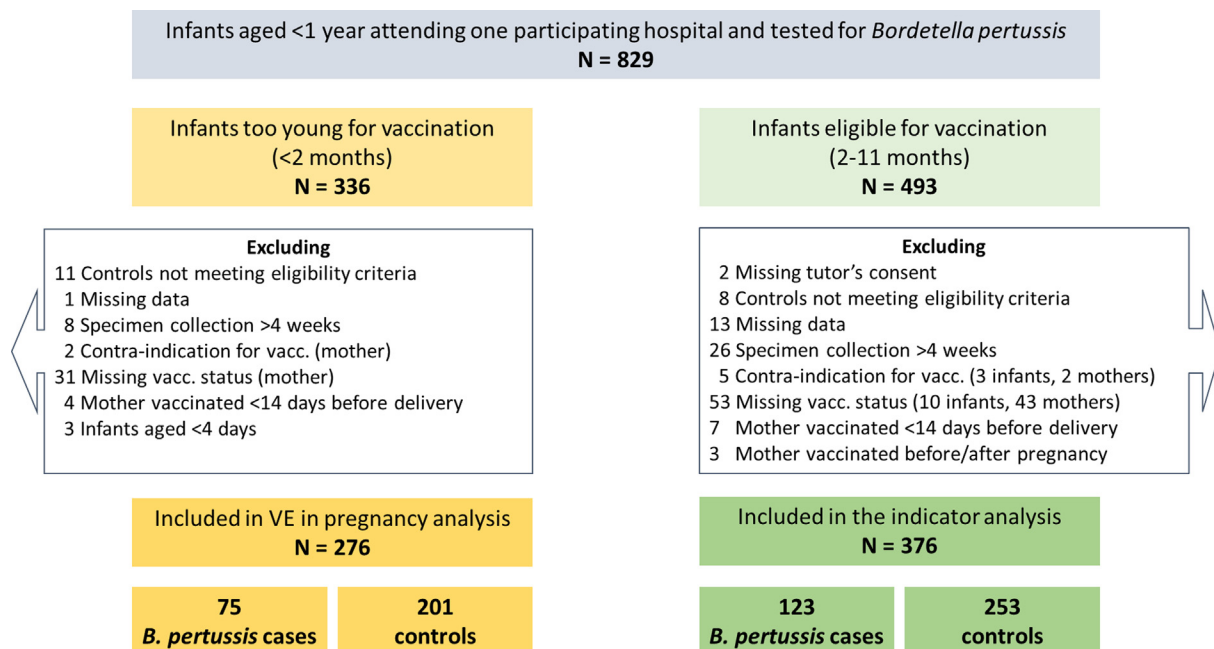

Fig. 1. Flowchart of hospitalised infants aged <1 year inclusion in or exclusion from the analysis of the effectiveness of vaccination in pregnancy, and the analysis of the effectiveness of vaccination in pregnancy combined with primary vaccinations after at least one dose, PERTINENT study, Europe, 1 December 2015–31 December 2019 (N = 829).

L. Merdrignac, L. Acosta, A. Habington et al.

Table 2
Characteristics of *Bordetella pertussis* cases and controls by analysis (left: effectiveness of vaccination in pregnancy analysis in infants aged <2 months; right: vaccination in pregnancy and primary vaccination analysis in infants aged 2–11 months) and by sex, laboratory components, clinical presentation, severity and risk/protective factors, hospitalised infants aged <1 year, PERTINENT study, Europe, 1 December 2015–31 December 2019.

Characteristics		Cases <2mo (n = 75)		Controls <2mo (n = 201)		p value	Cases 2-11mo (n = 123)		Controls 2-11mo (n = 253)		p value
		N	%	N	%		N	%	N	%	
Demographic											
Sex	Female	39	52.0	91	45.3	0.345	57	46.3	126	49.8	0.583
	Male	36	48.0	110	54.7		66	53.7	127	50.2	
Laboratory											
Nasopharyngeal specimen collection	Aspirate or both aspirate and swab	57	76.0	128	64.3	**0.043**	92	75.4	159	62.9	**0.019**
	Swab only	18	24.0	71	35.7		30	24.6	94	37.2	
Clinical criteria											
Cough	Yes	72	96.0	186	92.5	0.415	121	98.4	249	98.4	1.000
	No	3	4.0	15	7.5		2	1.6	4	1.6	
Cough with paroxysms	Yes	64	85.3	120	59.7	**<0.001**	112	91.1	179	70.8	**<0.001**
	No	11	14.7	81	40.3		11	8.9	74	29.2	
Whoop	Yes	35	47.9	36	18.6	**<0.001**	66	53.7	47	18.9	**<0.001**
	No	38	52.1	158	81.4		57	46.3	202	81.1	
Post-tussive vomiting	Yes	39	52.0	77	38.5	0.055	56	45.5	124	49.0	0.582
	No	36	48.0	123	61.5		67	54.5	129	51.0	
Apnoea	Yes	50	67.6	92	46.0	**0.002**	61	49.6	62	25.0	**<0.001**
	No	24	32.4	108	54.0		62	50.4	186	75.0	
Cyanosis	Yes	47	63.5	62	30.8	**<0.001**	53	43.1	49	19.4	**<0.001**
	No	27	36.5	139	69.2		70	56.9	203	80.6	
Epidemiological link	Yes	43	58.9	3	1.5	**<0.001**	64	54.7	10	4.0	**<0.001**
	No	30	41.1	192	98.5		53	45.3	241	96.0	
Diagnosis by a clinician	Yes	71	94.7	74	36.8	**<0.001**	113	93.4	137	54.6	**<0.001**
	No	4	5.3	127	63.2		8	6.6	114	45.4	
Severity											
Death	Yes	0	0.0	0	0.0	NA	0	0.0	0	0.0	NA
	No	75	100.0	199	100.0		123	100.0	249	100.0	
ICU	Yes	26	34.7	24	12.0	**<0.001**	14	11.4	13	5.2	**0.035**
	No	49	65.3	176	88.0		109	88.6	238	94.8	
ECMO	Yes	0	0.0	0	0.0	NA	0	0.0	0	0.0	NA
	No	75	100.0	200	100.0		123	100.0	249	100.0	
Pneumonia	Yes	4	5.3	5	2.5	0.262	3	2.5	14	5.6	0.289
	No	71	94.7	195	97.5		119	97.5	237	94.4	
Encephalopathy	Yes	0	0.0	1	0.5	1.000	0	0.0	0	0.0	NA
	No	75	100.0	199	99.5		122	100.0	251	100.0	
Seizure	Yes	1	1.3	2	1.0	1.000	1	0.8	0	0.0	0.327
	No	74	98.7	198	99.0		121	99.2	251	100.0	
Eating difficulties	Yes	22	29.3	66	33.2	0.566	32	26.2	83	33.2	0.190
	No	53	70.7	133	66.8		90	73.8	167	66.8	
Kidney failure	Yes	0	0.0	2	1.0	1.000	0	0.0	0	0.0	NA
	No	75	100.0	198	99.0		123	100.0	250	100.0	
Dehydration	Yes	6	10.2	5	3.2	0.075	12	10.8	15	6.8	0.208
	No	53	89.8	151	96.8		99	89.2	207	93.2	
Risk factors											
Premature <37 weeks	Yes	7	9.5	23	11.4	0.828	14	11.4	43	17.0	0.170
	No	67	90.5	178	88.6		109	88.6	210	83.0	
Delivery type	Vaginal	53	70.7	145	73.6	0.649	90	73.8	182	74.3	1.000
	C-section	22	29.3	52	26.4		32	26.2	63	25.7	
Episode in pregnancy	Yes	3	4.1	0	0.0	**0.020**	2	1.8	1	0.4	0.233
	No	71	95.9	197	100.0		112	98.2	248	99.6	
Infant going to day care	Yes	5	6.7	6	3.0	0.178	6	4.9	26	10.3	0.114
	No	70	93.3	194	97.0		117	95.1	226	89.7	
Infant with babysitter	Yes	1	1.4	11	7.3	0.109	5	4.5	11	5.7	0.793
	No	69	98.6	139	92.7		105	95.5	182	94.3	
Infant staying regularly with grandparents	Yes	24	32.0	40	20.1	0.054	38	31.9	64	25.5	0.214
	No	51	68.0	159	79.9		81	68.1	187	74.5	
Protective factors											
Breastfeeding	Yes	55	73.3	157	78.5	0.421	84	68.3	174	69.3	0.905
	No	20	26.7	43	21.5		39	31.7	77	30.7	
Mother vaccination in pregnancy	Yes	9	12.0	92	45.8	**<0.001**	40	32.5	136	53.8	**<0.001**
	No	66	88.0	109	54.2		83	67.5	117	46.2	
Vaccinated at least 1 dose	Yes	0	0	0	0	NA	58	47.2	151	59.7	**0.027**
	No	0	0	0	0		65	52.8	102	40.3	
Number of doses	1 dose	0	0	0	0	NA	30	24.4	73	28.9	0.151
	2 doses	0	0	0	0		22	17.9	62	24.5	
	3 doses	0	0	0	0		6	4.9	16	6.3	

Table 3

Adjusted vaccine effectiveness of pertussis vaccination in pregnancy in hospitalised infants too young to be vaccinated (aged < 2 months), PERTINENT study, Europe, 1 December 2015–31 December 2019 (n = 276).

Adjustment variables	Df	N	Cases		Controls		VE (95% CI)
			Vacc.	N	Vacc.	N	
All infants, 5 sites (N = 276)							
Site; Onset quarter	9	276	9	75	92	201	**76 (38–91)**
Site; Onset quarter;	10	276	9	75	92	201	**75 (35–91)**
Age group (4-30d; 31-60d)							
All infants, 3 sites* (N = 165)							
Site; Onset quarter	7	165	9	30	92	135	**76 (39–91)**
Site; Onset quarter;	8	165	9	30	92	135	**75 (35–90)**
Age group (4-30d; 31-60d)							
Infants sampled with NPA, 5 sites (N = 185)							
Site; Onset quarter	9	185	6	57	51	128	**88 (59–96)**
Site; Onset quarter;	10	185	6	57	51	128	**87 (55–96)**
Age group (4-30d; 31-60d)							
Infants sampled with NPA, 3 sites[a] (N = 88)							
Site; Onset quarter	7	88	6	20	51	68	**88 (57–96)**
Site; Onset quarter;	8	88	6	20	51	68	**87 (53–96)**
Age group (4-30d; 31-60d)							

CI: confidence interval; Df: degree of freedom; NPA: nasopharyngeal aspirate; VE: vaccine effectiveness.

[a] Excluding 2 sites due to the absence of vaccinated women.

and VE also adjusted for age group were, respectively 88% (95% CI: 59–96) and 87% (95% CI: 55–96).

The results were similar when excluding the two sites with no infant with mother vaccinated in pregnancy, or when using penalised logistic regression.

3.2. Effectiveness of vaccination in pregnancy and PV in infants (2–11 months)

After applying the exclusion criteria for the effectiveness of both vaccinations analysis, we included 376 infants eligible for PV (aged 2–11 months) with 123 *Bordetella pertussis* laboratory confirmed cases (33%) and 253 test-negative controls (67%). Among excluded infants, 43 had a missing maternal vaccination status or vaccination date and 10 had a missing PV status or vaccination date (Fig. 1).

Thirty-six cases (29%) and 97 controls (38%) were in their third month of life (p = 0.053). The median-birthweight was 3250 g for cases (range: 1160–4780; IQR: 750) and 3200 g for controls (range: 640–4500; IQR: 770) (p = 0.186). The median gestational week at birth was 39 for both cases (range: 28–42; IQR: 2) and controls (range: 24–43; IQR: 2) (p = 0.220).

Information on the type of specimen collection was available for 122 cases and the 253 controls with 30 cases (25%) and 94 controls (37%) only diagnosed based on NPS (p = 0.019) (Table 2). Out of the 123 cases, 32 cases (26%) were both PCR and culture-confirmed, 22 cases (18%) were PCR-confirmed but culture-negative, 65 cases (53%) were PCR-confirmed (no culture result) and four cases (3%) were culture-confirmed (no PCR performed). Two controls (<1%) were confirmed by culture only.

The median gestational age at vaccination was 30.1 weeks for cases (range: 19–36; IQR: 4) and 30.6 for controls (range: 14–36; IQR: 3) (p = 1.000).

Out of the 376 infants eligible for PV, 40 cases (33%) and 136 controls (54%) had their mother vaccinated in pregnancy (p < 0.001), 58 cases (47%) and 151 controls (60%) were vaccinated with at least one dose of PV (p = 0.027) (Table 2). Thirty-one cases (25%) and 98 controls (39%) had received both PV and vaccination in pregnancy, 27 cases (22%) and 53 controls (21%) had received PV only, 9 cases (7%) and 38 controls (15%) had received vaccination in pregnancy only (Table 4).

In the main analysis (N = 376), using unvaccinated infants and mothers as the reference group, VE adjusted for site, time of onset and age group was 74% (95% CI: 33–90) for infants with both PV and vaccination in pregnancy; 68% (95% CI: 27–86) for those with PV only; 36% (95% CI: -85–78) for those with vaccination in pregnancy only (Table 4).

In the sensitivity analysis excluding the two sites with no infants with vaccination in pregnancy (N = 257), VE adjusted for site, time of onset and age group was 90% (95% CI: 64–97) for infants with both PV and vaccination in pregnancy; 92% (95% CI: 69–98) for those with PV only; 63% (95% CI: -29–89) for those with vaccination in pregnancy only. When excluding infants sampled with NPS only (N = 251), VE adjusted for site, time of onset and age group was 88% (95% CI: 62–96) for infants with both PV and vaccination in pregnancy; 81% (95% CI: 46–93) for those with PV only; 44% (95% CI: -109–85) for those with vaccination in pregnancy only. Applying both exclusions (N = 164) provided with similar results (Table 4).

4. Discussion

After four years of PERTINENT data collection in 14 participating hospitals from four EU/EEA countries, we included 276 infants aged <2 months in the VE in pregnancy analysis and 373 infants aged 2–11 months in the indicator analysis of both vaccination in pregnancy and PV. Our results suggest that vaccination in pregnancy reduces the risk of being hospitalised for pertussis by 75–88% in infants aged <2 months too young to be vaccinated with PV. In the indicator analysis, regardless of the recommended schedule, when the infants are aged 2–11 months and eligible for vaccination, at least one dose of PV in infants whose mother had received vaccination in pregnancy would reduce the risk of hospitalisation for confirmed pertussis by 74–95%. Using the same reference group, at least one dose of PV in infants with unvaccinated mother would reduce the risk by 68–94%. Even though those results are based on small sample sizes, they suggest a good VE in pregnancy, consistent with existing literature [16] and also a similarly good VE after at least one dose of PV only and receiving both PV and mother vaccination.

However, these findings need to be interpreted with caution due to some existing limitations. Despite four years of active per-

Table 4

Adjusted effectiveness of three combinations of vaccine exposures in hospitalised infants eligible for vaccination (2–11 months): (1) mother vaccinated in pregnancy and infant vaccinated with at least one dose of PV; (2) infant vaccinated with PV only (at least one dose); (3) mother vaccinated in pregnancy only. PERTINENT study, Europe, 1 December 2015–31 December 2019 (n = 376).

Vaccination status		N	Cases	Controls	VE (95% CI)[a]
Infant	Mother				
All infants, 5 sites (N = 376)					
Unvaccinated	Unvaccinated	120	56	64	Ref.
Vaccinated	Vaccinated	129	31	98	**74 (33–90)**
Vaccinated	Unvaccinated	80	27	53	**68 (27–86)**
Unvaccinated	Vaccinated	47	9	38	**36 (-85–78)**
All infants, 3 sites[b] (N = 257)					
Unvaccinated	Unvaccinated	29	13	16	Ref.
Vaccinated	Vaccinated	129	31	98	**90 (64–97)**
Vaccinated	Unvaccinated	52	11	41	**92 (69–98)**
Unvaccinated	Vaccinated	47	9	38	**63 (−29–89)**
Infants sampled with NPA, 5 sites (N = 251)					
Unvaccinated	Unvaccinated	84	43	41	Ref.
Vaccinated	Vaccinated	90	24	66	**88 (62–96)**
Vaccinated	Unvaccinated	52	19	33	**81 (46–93)**
Unvaccinated	Vaccinated	25	6	19	**44 (−109–85)**
Infants sampled with NPA, 3 sites[b] (N = 164)					
Unvaccinated	Unvaccinated	21	11	10	Ref.
Vaccinated	Vaccinated	90	24	66	**95 (69–99)**
Vaccinated	Unvaccinated	28	7	21	**94 (59–99)**
Unvaccinated	Vaccinated	25	6	19	**61 (−89–92)**

CI: confidence interval; NPA: nasopharyngeal aspirate; PV: primary vaccination; VE: vaccine effectiveness.

[a] Adjusted for site, onset quarters and age group (2; 3–11 months).

[b] Excluding 2 sites due to the absence of vaccinated women.

tussis surveillance, the achieved sample sizes for maternal vaccination studies did not allow for more precise estimates. Increasing data collection in this multicentre study is needed to consolidate our results and to allow additional adjustments for potential confounding factors or stratification by effect modifiers (e.g., breastfeeding, repeated vaccination in pregnancy). Due to this substantial limitation in our study, we could not compute VE in pregnancy by site and estimate sites' heterogeneity. The current sample size also prevented us to explore VE in pregnancy according to time of and since vaccination in pregnancy, VE for one dose of primary series only, VE by dose or VE by time since vaccination. As described by Barug et al. [17], pertussis antibody responses in infants may differ depending on the infant vaccination schedule. Their study suggested a higher immunological effect when PV is starting at 2 months compared with starting at 3 months of age. Interaction between vaccination in pregnancy and PV may also differ according to the number of doses received, the time of and since vaccination of the mother and other additional factors [18].

Implementation and compliance to the maternal immunisation programme was very heterogeneous across sites during the study period. It was very well established in Spain with Catalonia and Navarra regions. Conversely, immunisation programmes for pregnant women were not being fully implemented in Czech Republic and Italy. Vaccine acceptance aspects were documented in Italy [19].

Hospital teams had to test for pertussis and include in the study any infants suspected for pertussis, even though some typical symptoms were missing [20]. However, clinicians may be more likely or less likely to test suspected pertussis cases according to vaccination status leading to selection bias. We believe this bias may had a very limited impact at least on the VE in pregnancy analysis as we assume that clinicians may not have direct access to the mother vaccination status at the infant's admission.

Vaccination status data were obtained by reviewing clinical case notes, vaccination cards, interviews with parents or legal guardians, and extraction from patient registries. The current small sample size did not allow to compare VE estimates by source of

information for the vaccine status. In the VE in pregnancy analysis, 31 infants were excluded due to missing values for mother vaccination status or vaccination date. Out of them, 21 were excluded due to missing vaccination date (2 cases and 19 controls), assuming that the mother was vaccinated. In the indicator analysis, 43 infants were excluded due to missing values for mother vaccination status or vaccination date. Out of them, 35 were excluded due to missing vaccination date (4 cases and 31 controls), assuming that the mother was vaccinated. For both analyses, this suggests that mother's vaccinations may be better documented among cases than among controls, which could lead to underestimation of VE in pregnancy.

A large proportion of the exclusions in the study are due to lack of information on vaccination status and vaccination date from the mother. Even though efforts done for an enhanced data collection at hospital level were successful, more efforts are needed to retrieve information outside of the hospital setting.

In our study population, the clinical case definition was associated with confirmed pertussis. Even if this lends support to the definition used, discussing pertussis clinical presentation was however not part of our study objectives.

To validate our findings, we would need to further study and confirm that TND in hospital settings is a proper study design for pertussis VE estimation in infants. This is the first time that a prospective TND is used in Europe in hospital settings for estimating pertussis vaccine effectiveness in infants. The rationale for TND is that the control group (infants hospitalised for pertussis-like symptoms but with other respiratory disease than pertussis) are representative of the vaccine coverage in the source population of pertussis cases. The risk of hospitalisation for non-pertussis respiratory infections should then be equal between vaccinated and unvaccinated infants. There is a need to validate this assumption using large cohorts in Europe. Unfortunately, we could not compare the proportion of our controls that were vaccinated to the vaccine coverage in the catchment area of the participating hospitals. Aiming to validate TND for pertussis, a recent Canadian study compared their results with a frequency-matched design (FMD) for

L. Merdrignac, L. Acosta, A. Habington et al.

pertussis VE studies estimating waning immunity. In both designs, VE estimates were high and consistent with clinical trials at early stage after vaccination and in early years of life [21].

In both our analyses, we included all infants tested for pertussis and classified them as cases and controls according to PCR or culture results. Although PCR has a high sensitivity, culture sensitivity is only about 60% with the highest among unvaccinated infants [22]. Including false-negative, especially among vaccinated infants, could lead to overestimate VE in both analyses. However, only six controls (3%) aged <2 months and two controls (<1%) aged 2–11 months were confirmed by culture only, which lead us to assume a very minor impact on our results.

Even though our VE estimates for both analyses are consistent with existing literature [23], they tend to be in the lower range. In our study, controls were more likely than cases to have been diagnosed based on the laboratory results of a NPS only (Table 2). Since NPS can be less sensitive than NPA in infants to isolate *Bordetella pertussis* by PCR or culture [13], we cannot prevent inclusion of false-negative among controls. Misclassification of unvaccinated cases as controls would lead to underestimating the corresponding VE. Despite the very low sample size, when excluding infants sampled with NPS, we observed higher VE estimates, closer to existing literature. Overall, our results are in the range of VE observed in other studies reporting VE in pregnancy between 70% and 90% in infants aged <2 months [8,24] and additional protection from vaccination in pregnancy during the first year of life [16]. In our indicator analysis, we also observed a good VE after at least one dose of PV only and after at least one dose of PV in infants whose mother was vaccinated. However, our limited sample size did not allow a robust stratified analysis to investigate whether vaccination in pregnancy modifies VE after at least one dose of PV. It did not allow either to measure the interaction between the two vaccinations. Therefore, even if our results may indicate a similarly good VE of at least one dose of PV irrespective of the vaccination status of the mother, we cannot conclude about the absence of clinical significance of the immunological blunting effect of maternal vaccination in infants' immune response to PV.

5. Conclusion

The PERTINENT network is the only EU/EEA collaboration that allows for large, independent and multi-country pertussis vaccine effectiveness studies.

Despite PV starting at 2 months of age, infants too young to be eligible for vaccination still harbour the highest risk of illness and related deaths.

Our findings suggest that vaccination in pregnancy is an effective strategy to fill the immunisation gap of the first two months of life, when infants are not eligible for vaccination and the disease is the most life-threatening. From 2 months of age onwards, despite existing immunological studies suggesting a possible lower immunological response after PV in infants whose mother had received vaccination in pregnancy [8], our results suggest a good effectiveness of at least one dose PV in infants aged 2–11 months irrespective of the vaccination status of the mother.

In making decisions about vaccination strategies, countries take into account various factors, including cost-effectiveness evaluations. As health economic analyses are sensitive to local circumstances and are not easily generalisable, national health-economic studies may need to be conducted as part of such comprehensive evaluations.

In the up-coming post-acute COVID-19 pandemic times where an increase of vaccine-preventable respiratory infections such as bronchiolitis and pertussis is to be expected [25], consideration should be given to increase disease awareness, to improve pertus-sis surveillance and laboratory diagnosis [3] but, above all, to enhance maternal vaccination in pregnancy, as well as ensuring that these recommendations are effectively implemented in accordance with national guidelines.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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