Pertussis immunisation during pregnancy: Antibody levels and the impact of booster vaccine

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Abstract

Pertussis (whooping cough) is a highly infectious disease caused by *Bordetella pertussis*. Mothers lacking adequate immunity and contracting the disease represent the biggest risk of transmission to new-borns, for which the disease is often a threat. The aim of the study was to estimate the frequency of pertussis susceptibility among pregnant women, in order to point out the need for a vaccine recall during pregnancy, and to evaluate the antibody response in already vaccinated women. A cross-sectional observational study was conducted in the blood test centre of "St. Anna" Obstetrics and Gynaecology Hospital in Turin (Piedmont, Italy). Eligibility criteria included pregnant women coming to the centre for any blood test, aged 18 or above and with gestational age between 33 and 37 weeks at the moment of the blood draw. The data collection was carried out from May 2019 to January 2020 and the concentration of anti-Pertussis Toxin (anti-PT) IgG was measured through the Enzyme-Linked Immunosorbent Assay (ELISA) technique. Two-hundred women (median age 35) were enrolled: 132 (66%)

had received at least one dose of pertussis vaccine, 82 of which during pregnancy. Recently vaccinated women had significantly higher antibody titres (even 12-15 times as high) compared to those vaccinated more than 5 years before or never vaccinated at all ($p<0.0001$). Moreover, 95.1% of recently vaccinated women had anti-PT IgG levels above 10 IU/ml, and 85.4% above 20 IU/ml, while the same proportions were as low as 37% and 21% (respectively) in the group of women not vaccinated in pregnancy. This study confirmed that the vaccination is greatly effective in ensuring high antibody titres in the first months after the booster vaccine, with considerable differences in anti-PT IgG compared to women vaccinated earlier or never vaccinated at all, and therefore vaccinating pregnant women against pertussis still represents a valuable strategy.

Keywords: pertussis, pregnancy, acellular vaccine, antibody booster, transplacental passive immunity

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Introduction

Pertussis (whooping cough) is a highly infectious disease caused by the *Bordetella pertussis* bacterium. Humans are the only known reservoir of the bacterium, thus the transmission of the disease occurs between people only, and unlike other childhood diseases, the immunity conferred by a first infection is not definitive, but declines over time. In Italy, the introduction of whole-cell pertussis vaccine obtained from *B. pertussis* suspensions (1961) led to a progressive reduction in the incidence of the disease. In 1995, the acellular vaccine was introduced, consisting of inactivated pertussis toxin (PT) and other bacterial components, detoxified by treatment with chemicals or by using genetic engineering techniques from mutant strains of *B. pertussis*. The acellular vaccine was found to induce a good serological response, with an efficacy around 84%[1] and fewer side effects compared to the whole-cell vaccine. As a result, the incidence of pertussis further declined to <5 cases per 100,000 in the 2000s[2].

The Italian vaccination schedule includes acellular pertussis vaccine in a hexavalent formulation (along with diphtheria, tetanus, hepatitis B, inactivated poliovirus and *Haemophilus influenzae* type b) to be administered in three consecutive doses at 3, 5 and 11 months of life: this vaccination is mandatory in Italy since 2017. Moreover, additional booster doses are given at preschool age and during adolescence, along with inactivated poliovirus booster, and a booster dose is then recommended every 10 years throughout adult life[3]. Despite a successful vaccination in childhood, the progressive reduction of immunity in adults and the persistence of circulating infection has led to an increasing pertussis incidence among adults[4].

In Italy, the notification of infectious disease for pertussis is mandatory, but diagnosis is usually clinical; microbiological confirmation is rarely required[5,6]. Moreover, in adults, the disease may have a mild course[4,7]. For this reason, pertussis is often not diagnosed and these cases represent a potential source of infection, especially for infants in the first year of life, when not yet or incompletely immunised[4,8]. Cases of pertussis with severe course, requiring hospitalisation and at high risk of mortality, have been reported in children younger than one-year. Almost all deaths occur in infants less than 3 months of life[9], who are those most at risk beside premature new-borns and children of non-immunised mothers[10]. It is well known that parents are the primary sources of infection for children, along with grandparents[11,12]. In particular, mothers lacking adequate immunity and contracting the disease represent the biggest risk of transmission to the new-borns, not yet immunised[9,10]. Italian parents are often unaware of these risks and the severity of pertussis symptoms in early childhood[2]. Currently, immunisation during pregnancy represents the most cost-effective strategy for preventing neonatal death from pertussis, with an estimated efficacy of around 95%[12,13]. Furthermore, the risk of developing the disease for infants up to 3 months is reduced by more than 90% when the mother is vaccinated compared to children of unvaccinated mothers[10]. The purpose of vaccination during pregnancy is to provide passive protection to new-borns through the transplacental passage of antibodies to protect them from infection during the first months of life, before they can be immunised according to the vaccination protocol and until active immunity is acquired[10]. In Italy, vaccination of pregnant women during the third trimester of pregnancy is recommended and has been included in the 2017-2019 National Vaccine Prevention Plan[3].

The aim of the study was to estimate the frequency of pertussis susceptibility (or low-level immunisation status) among pregnant women, in order to point out the need for a vaccine recall that can work as an antibody booster, and to assess the degree of compliance to

pertussis vaccination in pregnancy. The study was also intended to evaluate quantitatively the antibody response in already vaccinated women.

Methods

Study design and inclusion criteria

A cross-sectional observational study was conducted in the blood test centre of "St. Anna" Hospital in Turin (Piedmont, Italy), which is the regional obstetrics and gynaecology hub belonging to the University Hospital "Città della Salute e della Scienza" (City of Health and Science) of Turin. The research protocol was in conformity with the Declaration of Helsinki (and subsequent revisions) and the Italian (Law 2003/196) and European regulations (GDPR EC/2016/679) on data protection and privacy. All study procedures were reviewed and approved by the Inter-institutional Ethical Review Board of the University Hospital "Città della Salute e della Scienza" (protocol number 2019/0020, file number CS2/1144, formal approval received by the General Director with Resolution 2019/260 on 28 February 2019).

The eligibility criteria included pregnant women coming to the centre for any blood test, as long as their age was 18 or above and their gestational age at the moment of the blood draw was between 33 and 37 weeks. The pregnancy age span was chosen in order to evaluate also the efficacy of the pertussis vaccination campaign, promoted by the Italian Ministry of Health for 28- to 37-week pregnant women, and thus to consider the final immunisation status of women nearing delivery. Women beyond the 37th pregnancy week were excluded because of the chance of distorted serological results due to the physiological immune tolerance mechanisms in the very last weeks of pregnancy[14].

In order to estimate relative proportions with 95% confidence and 10% margin of error, a number of 96 was needed conservatively to estimate the proportions of women with antibody

levels suggestive of liable immunity to pertussis for each of the two groups (vaccinated and unvaccinated). Hypothesising a prevalence of vaccinated women around 50%, the required number was twice as much (i.e. 192) and sample size was set at 200 to account for possible inconveniences during data collection and processing.

Study procedures and laboratory processing

The data collection was carried out from May 2019 to January 2020 on multiple days of the week from Monday to Friday, during the blood test centre's operating times. The researcher in charge of collecting data was informed by the administrative personnel every time a pregnant woman of the required gestational age was registering for the blood drawing procedure. Each candidate participant was informed about the aims and procedures of the study and could then freely choose whether to be enrolled or not. Written and signed informed consent was required to take part in the study. For every enrolled woman, after collecting information about age, nationality and previous vaccination against *Bordetella pertussis*, a 5-ml blood sample was drawn while performing the other blood draws for the routine pregnancy check-ups.

Blood samples were centrifuged and sera were extracted and stored at –20°C until analysis. The concentration of anti-Pertussis Toxin (anti-PT IgG) was measured by a commercial ELISA kit (Technogenetics, Lodi, Italy) in conformity with the manufacturer's protocol. Sera were analysed in a 100-fold dilution, in duplicate, and IgG results were expressed as IU/ml according to the WHO International Standard Pertussis Antiserum (National Institute for Biological Standards and Control, Potters Bar, UK, code: 06/140).

Statistical analysis

Descriptive statistics were presented as median and interquartile range (Q1-Q3) for quantitative variables (age and antibody titre), and as number and percentage for categorical variables (nationality and proportion with corresponding antibody titres). Firstly, women were classified into four groups according to the time since last pertussis vaccination: less than 5 years, more than 5 years, no previous vaccination, or vaccination status unknown. The categorisation was established according to the dynamics of antibody response to vaccination, which was previously found to be evident on average since the first week after vaccination[15] and to wane after a time span varying between 3 and 10 years[16–18]. As the correlation between antibody levels and effective protection is still unclear (some previous analyses found that anti-PT-IgG levels >5 IU/ml might be potentially protective[19], while other studies more conservatively suggested 10[20] or 20 IU/ml[21]), serum IgG levels equal to 10 IU/ml and 20 IU/ml were considered as possible cut-offs to categorise women's immunisation status.

A second classification was made between the women who were vaccinated during pregnancy and those who were not, and the same variables were considered (only women with known vaccination status were considered in this additional classification). Different categories were compared by using the Mann-Whitney U test or the Kruskal-Wallis test (followed by Dunn's post-hoc test for between-group comparison, with Šidák's correction to account for multiplicity[22,23]) for quantitative variables as appropriate, and Fisher's exact test for categorical ones. For all tests, p-values < 0.05 were considered significant. A scatterplot was also drawn to represent antibody titres against time from the last vaccination, with an estimate of the antibody response curve obtained through the Locally Weighted Scatterplot Smoothing technique (LOESS regression)[24]. The statistical software R (version 4.0.3)[25,26] was used to perform all computations and to draw all plots.

Results

Two-hundred women, aged between 19 and 44 (median 35), were enrolled for the study. Among them, 132 (66%) had received at least one dose of acellular vaccine against pertussis: 82 (41%) had been vaccinated during pregnancy, all with a dose of Boostrix® vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium), 4 (2%) before pregnancy but no longer than 5 years before, and 46 (23%) more than 5 years before. Vaccination status was unknown for 37 women (18.5%), whereas 31 (15.5%) had never been vaccinated against pertussis. Sixteen women (8%) reported previous pertussis infection in their early childhood, all belonging to the unvaccinated group. Descriptive statistics for enrolled participants are summarised in Table 1: the majority of women were Italian (79%), followed by other European countries (12%, mostly from Eastern Europe) and by other continents (Northern Africa, Asia and Southern America, 9% altogether).

Among the 200 women, 80 (40%) had an antibody titre below 10 IU/ml, and therefore below the minimum level which is suggestive of previously acquired immunity against pertussis. A detailed analysis of the immunisation status by subgroup showed that, on average, women vaccinated more than five years before or without previous vaccination had antibody titres at a frankly non-protective level (around 6-7 IU/ml), while women vaccinated in the last five years had high levels of antibodies, even 12 to 15 times higher than the other groups. Women vaccinated more than five years before had antibody titres not significantly different from women never vaccinated before ($p=0.9794$), but both the former ($p<0.0001$) and the latter ($p<0.0001$) had significantly lower antibody levels than those recently vaccinated. On the other hand, antibody levels in women with unknown vaccination status were comparable to those in women not vaccinated in the last five years ($p\geq0.7$), thereby providing evidence for considering them as not demonstrably immune.

The distribution of antibody titres in each group with known vaccination status is represented in Fig. 1. Of note, some outliers can be observed in the groups with no recent vaccination, particularly concerning two cases (1%) with antibody titres higher than 100 IU/ml, and 15 (7.5%) with serum titres between 20 and 100 IU/ml.

The comparison between women vaccinated and not vaccinated in pregnancy yielded no statistically significant differences in relation to age (even though vaccinated women tended to be younger) or nationality (a slightly lower adherence to the vaccination campaign was recorded among women from Eastern Europe). On the contrary, the booster vaccine in pregnancy strongly impacted on the immunisation status, with average antibody titres even 15-17 times higher in vaccinated women: levels of serum antibodies indicating likely immunity against pertussis were reached by 85.4-95.1% of women who had received the booster (depending on whether the 20 IU/ml or 10 IU/ml threshold is considered, respectively), compared to 21-37% of women who had not (Table 2).

Considering women with previous vaccination (and whose last vaccination date was known), an increase in the antibody level was observed even for those who had received the booster vaccine in the week before the blood drawing. The peak response was found among women vaccinated around one month before the test, whilst antibody levels tended to decrease progressively over time until reaching baseline levels. A relationship between time since last vaccination and serum antibody titres could be hypothesised via LOESS smoothing (Figure 2), with acceptable goodness of fit (Residual Standard Error, RSE: 0.5099).

Discussion

Whooping cough is still a serious public health problem despite different vaccination schedules worldwide[27]. According to ECDC data, Italy is a low-incidence country, with a

rate, in children under the age of 12 months, varying from 0.8 per 100,000 inhabitants in 2012 to 1.7 per 100,000 in 2016 (Italian general population: 1.8/100,000 Age Standardised Rate in 2018)[28].

Since vaccine-induced protection is limited in time and it is extremely difficult to achieve high vaccination coverage in adults, pertussis keeps circulating and outbreaks occur. Many strategies have been implemented in different countries for prevention of infections among infants, one of which is the so-called "cocoon strategy", consisting in providing a booster dose to all family members in the months preceding the birth, to the mother immediately after the delivery, as well as to all possible contacts with infants (e.g., childcare workers, healthcare workers, teachers, etc.). This strategy has been found to be effective[29–31], but too expensive in a low-incidence context[32]. Other strategies have been proposed, such as neonatal immunisation at birth[33,34] or scheduling of the first dose of DTaP vaccine (diphtheria, tetanus and pertussis) at 6 weeks of age to ensure earlier protection to infants[35,36], but there is conflicting evidence about their effectiveness[37–39] and their impact is likely to be limited[40].

On the contrary, immunisation of women during pregnancy has become a strong recommendation in many countries, including Italy, as evidence emerges of robust effectiveness and safety in protecting young infants and their mothers against pertussis[40–42]. In fact, beside lowering chances of transmission, vaccinating women during the third trimester of pregnancy also protects infants through placental transfer of maternal antibodies, resulting in high antibody levels in the infant at the time of birth and early occurrence of mucosal antibodies due to their presence in breast milk[43,44].

In a study by De Schutter et al. (2015), women vaccinated during pregnancy ($p = 0.012$), or peripartum ($p = 0.001$), had higher levels of secretory IgA anti-PT in breast milk at two months post-partum, compared to women not vaccinated in the 5 years before delivery[44].

The immunogenicity of Tdap vaccination in pregnant women in our study was consistent with that found in previous studies[17,45,46], with noticeably higher pertussis antibody concentrations in women vaccinated in the last 5 years than in women vaccinated earlier or not vaccinated at all (Fig. 1).

In fact, our sample's antibody curve, represented in Fig. 2, shows a dynamic which is consistent with the available literature[17], as the antibody response reaches a maximum between 1-2 months after immunisation and starts to decrease appreciably after the first year, hence progressively declining.

The optimal timing of maternal pertussis immunisation for antibody transfer to the foetus is a critical issue. The initial recommendation in the US was vaccination between 27 and 36 gestation weeks[47], while the UK – from 2012 to 2016 – recommended a window between 28 and 32 gestation weeks (but allowing vaccination up to 38 weeks)[48]. However, since 2016, based on emerging evidence[49], pregnancy vaccination against pertussis has been proposed to women from 16 gestational weeks: this schedule was adopted to gain time to reach more pregnant women, thus reducing missed immunisation opportunities, and to maximise antibody transfer, thereby offering new-borns (especially the pre-term ones) a higher chance to be protected[12]. Indeed, recent observational studies have suggested that higher anti-pertussis antibody concentrations may be achieved in cord blood when mothers are vaccinated earlier: 27– 30 weeks' gestation compared to later[50], 28–32 weeks' compared to 33–36 weeks' gestation[51]**,** or second- vs third trimester immunisation[49]**.**

The Italian Government, consistently with the other countries' schedules, recommends the immunisation between the 28th and the 37th pregnancy week. This strategy appears to be effective as, at 33-37 pregnancy weeks (i.e. 1-8 weeks after the vaccine booster and few weeks before delivery), vaccinated women were found to be generally protected, with a high prevalence of antibody titres above the thresholds advised by the available literature[21] (Table 2) and a noticeable proportion of women with considerably high antibody levels (Fig.

2). In fact, in our experience, 95.1% has anti-PT IgG levels above 10 IU/ml, and the same proportion remains as high as 85.4% if the 20 IU/ml threshold is considered (Table 2). The presence of a majority of vaccinated women with above-threshold IgG titres is strongly encouraging, even with a view to the chance of transplacental passive immunity[52].

Due to immunity waning 9-10 years after booster doses[21], or even before[17,18], the presence of young adults not recently vaccinated against pertussis entails persistence of circulating *B. pertussis*. This implies that pregnant women may be exposed to whooping cough cases, which might be the explanatory ground for the observed women with no recent vaccination against pertussis but with high anti-PT IgG levels: in the absence of a recent booster vaccination, antibody titres higher than 100 IU/ml are likely to be imputed to recent pertussis infection or exposure, whereas serum titres between 20 and 100 IU/ml are possibly ascribable to contact with *B. pertussis* in the previous years[53]. In our experience, the occurrence of women with these characteristics is 1% and 7.5% respectively (Fig. 1).

The importance of pregnancy vaccination against pertussis looks even greater after a more careful inspection of the obtained results, as – within the group of women vaccinated in the last five years (Table 1) – high antibody levels were recorded in particular for women vaccinated in pregnancy, i.e. less than two months before, while lower titres were recorded for those vaccinated 1-5 years before (even though only 4 women fell into this category, Fig. 2). Although these considerations are made on a really small sample, they are corroborated by other studies, where significant decreases in anti-PT antibodies (even if at higher levels compared to pre-booster) were found only few years after the booster dose[17,18].

Following these findings, it is really important to insist on active immunisation against pertussis during pregnancy, as this infection may be extremely harmful on new-borns and infants below the scheduled age for vaccination. Furthermore, the promotion of vaccination campaigns targeted at pregnant women is also endorsed by economic evaluations, since

vaccinating pregnant women with acellular vaccine has been shown to be cost-effective in preventing new-borns and infants from pertussis-associated disease according to the WHO's cost-effectiveness criteria[54].

This analysis has some limitations. First of all, the sample size appeared to be limited, particularly for the low amount of women who had received the last vaccine booster between 1 and 10 years before, which made it impossible to create a robust antibody response curve for those values; however, the obtained estimates are consistent with data reported by available studies on serum titres, thereby reassuring on the reliability of the results and subsequent advice. Moreover, participation in the study was on a voluntary basis, with possible biases due to convenience sampling; though, the refusal rate was not superior to 10-15%, with no appreciable differences for age, pregnancy week or enrolment day, thus ensuring representativeness of the enrolled sample. Eventually, vaccination dates were not systematically retrieved from the vaccine registry (women were simply asked about their last vaccination date): however, positive anamnestic data has been proven to be averagely reliable for the case of vaccinations previously received[55].

Conclusions

First of all, this study found that 8.5% of enrolled women had high levels of antibodies against pertussis despite not being vaccinated, which is indicative of recent infection by *B. pertussis*, and this confirms the relevance of the issue of circulating pertussis among pregnant women, with potential consequences on infants and new-borns. Due to the decrease in antibody protection after a few years, and since in many countries pertussis vaccination has become compulsory only in the last few years, many pregnant women are likely to be susceptible to contracting whooping cough and transmitting it to their babies.

Moreover, our study confirmed that the vaccination is greatly effective in ensuring high antibody titres in the first months after the booster vaccine, with considerable differences in anti-PT IgG compared to women vaccinated earlier than 5 years before or never vaccinated at all. Considering that vaccinating pregnant women has been shown to be impactful and cost-effective in preventing transmission to new-borns, and in light of the available literature in favour of this kind of strategy, these results seem to suggest that pertussis vaccination in pregnancy can bring appreciable benefits, with a possible enhancement of antibody protection for new-borns and a decrease in the occurrence of whooping cough cases among mothers-to-be. However, this strategy can be systematically implemented only in case healthcare professionals (especially gynaecologists and obstetricians, but also general practitioners) are aware of the risk and provide ground for acceptance of pertussis pregnancy vaccination among mothers-to-be.

Conflicts of Interests:
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.

Data Statement:
All data collected and analysed for this study are available upon request to the Corresponding Author of this paper.

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Author Contribution Statement:
Jacopo Garlasco: Conceptualisation, Methodology, Data collection, Formal analysis, Writing - original draft, Writing - review & editing. **Valerio Bordino**: Conceptualisation, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Noemi Marengo**: Conceptualisation, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Erika Rainero**: Conceptualisation, Methodology, Data collection, Writing - review & editing. **Alessandro Scacchi**: Conceptualisation, Methodology, Data collection, Writing - review & editing. **Savina Ditommaso**: Conceptualisation, Methodology, Laboratory analysis, Writing - review & editing. **Monica Giacomuzzi**: Conceptualisation, Methodology, Laboratory analysis, Writing - review & editing. **Fabrizio Bert**: Literature search, Ethical Board procedures, Writing - review & editing. **Carla Maria Zotti**: Conceptualisation, Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **All the Authors approved the final article version to be submitted.**

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FIGURES

Figure 1. Boxplot of antibody titres according to pertussis vaccination status. The plot classifies three groups: never vaccinated (n=31), vaccinated in the last 5 years (n=86), and vaccinated more than 5 years before (n=46). The 10 IU/ml (red line) and 20 IU/ml (blue line) antibody thresholds are also shown in the plot.

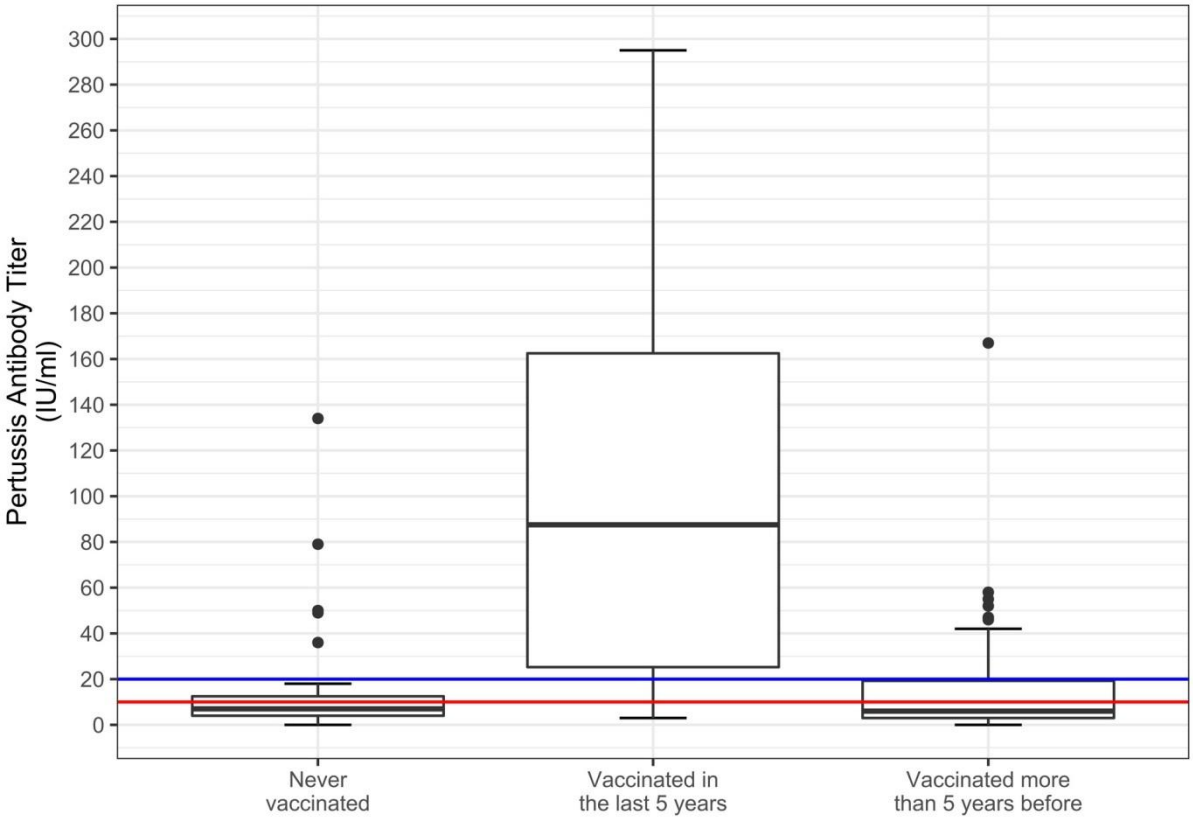
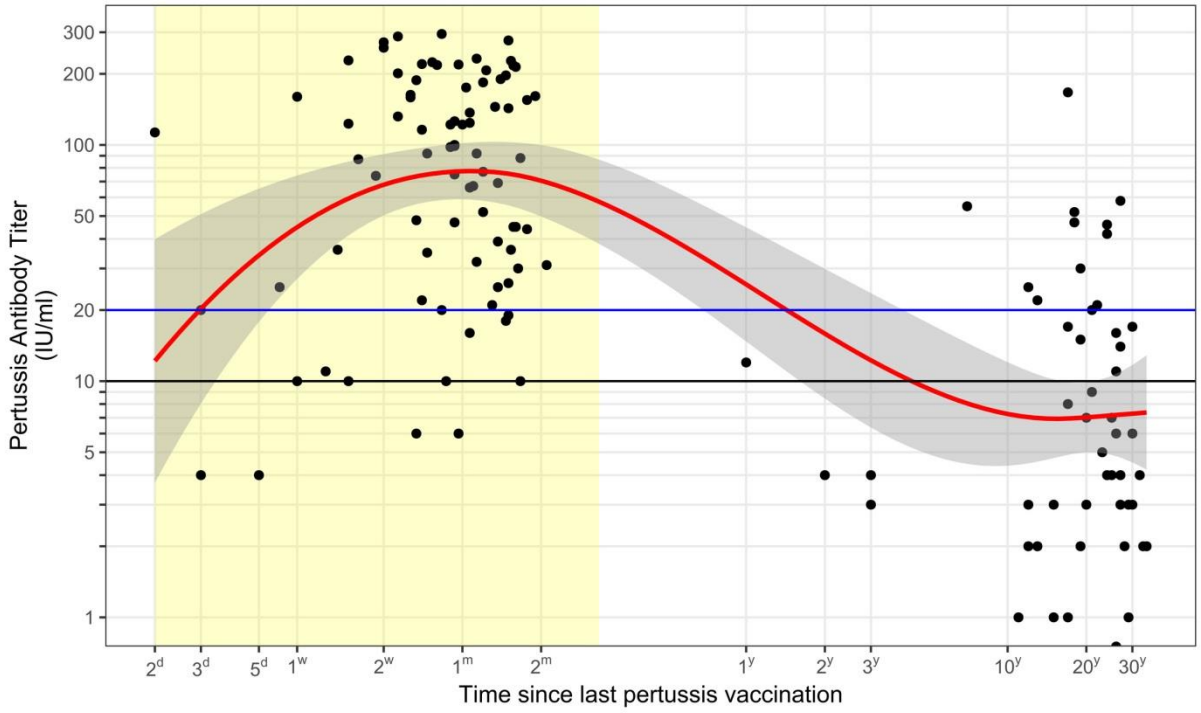


Figure 2. Scatterplot of antibody titres against time since last pertussis vaccination and antibody response curve. Only vaccinated women with known vaccination dates were included in the plot (n=131). Both axes are represented in logarithmic scale. Observations on a yellow background are related to women vaccinated in pregnancy (n=82). The curve (with shaded 95% confidence interval) was elaborated through the LOESS smoothing technique.



TABLES

Table 1. Descriptive characteristics of participants enrolled in the study. Values are expressed as median and interquartile ranges (Q1-Q3) for quantitative variables, and number and percentage for categorical ones.

Characteristics	Never vaccinated (n=31)	Vaccinated in the last 5 years (n=86)	Vaccinated more than 5 years before (n=46)	Unknown vaccination status (n=37)	Total (n=200)
Age	35 (31.5 - 36)	35 (32 - 37)	33 (31 - 36)	34 (31 - 37)	**35 (31 - 37)**
Nationality					
Italian	26 (83.9)	71 (82.6)	35 (76.1)	26 (70.3)	**158 (79.0)**
European (Other)	4 (12.9)	6 (7.0)	6 (13.0)	8 (21.6)	**24 (12.0)**
Other continents	1 (3.2)	9 (10.4)	5 (10.9)	3 (8.1)	**18 (9.0)**
Immunisation status:					
Anti-PT IgG titre [IU/ml]	7 (4 - 12.5)	87.5 (25.3 - 162.5)	6 (3 - 19.3)	5 (2 - 13)	**16 (4 - 70.3)**
IgG titre range					
Titre < 10 IU/ml	20 (64.5)	7 (8.1)	28 (60.9)	25 (67.6)	**80 (40.0)**
10 ≤ titre < 20 IU/ml	6 (19.4)	9 (10.5)	6 (13.0)	7 (18.9)	**28 (14.0)**
Titre ≥ 20 IU/ml	5 (16.1)	70 (81.4)	12 (26.1)	5 (13.5)	**92 (46.0)**

Table 2. Comparison between women vaccinated and not vaccinated in pregnancy for baseline characteristics and immunisation status. Between-group comparisons were made through Fisher's exact test for categorical variables (nationality and IgG titre category) and through the Mann-Whitney-Wilcoxon U test for quantitative variables (age and actual IgG titre in IU/ml). Only women with known vaccination status (n=163) were included in this table.

Characteristics	Not vaccinated during pregnancy (n=81)	Vaccinated between 28th and 32nd week of pregnancy (n=82)	*p*-value
Age	34 (31 - 36)	35 (33 - 37)	0.050
Nationality			0.225
Italian	64 (76.3)	68 (82.9)	
European (Other)	11 (16.1)	5 (6.1)	
Other continents	6 (7.6)	9 (11.0)	
Immunisation status:			
Anti-PT IgG titre [IU/ml]	6 (3 - 17)	92 (31.3 - 172)	**<0.0001**
IgG titre range			**<0.0001**
Titre < 10 IU/ml	51 (63.0)	4 (4.9)	
10 ≤ titre < 20 IU/ml	13 (16.0)	8 (9.7)	
Titre ≥ 20 IU/ml	17 (21.0)	70 (85.4)	