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

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10 *****

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17
18 **Pertussis immunisation during pregnancy:**

19 **antibody levels and the impact of booster vaccine**

20 **Abstract**

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

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

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

45 **Pertussis immunisation during pregnancy:**
46 **antibody levels and the impact of booster vaccine**

47 **Introduction**

48 Pertussis (whooping cough) is a highly infectious disease caused by the *Bordetella pertussis*
49 bacterium. Humans are the only known reservoir of the bacterium, thus the transmission of
50 the disease occurs between people only, and unlike other childhood diseases, the immunity
51 conferred by a first infection is not definitive, but declines over time. In Italy, the introduction
52 of whole-cell pertussis vaccine obtained from *B. pertussis* suspensions (1961) led to a
53 progressive reduction in the incidence of the disease. In 1995, the acellular vaccine was
54 introduced, consisting of inactivated pertussis toxin (PT) and other bacterial components,
55 detoxified by treatment with chemicals or by using genetic engineering techniques from
56 mutant strains of *B. pertussis*. The acellular vaccine was found to induce a good serological
57 response, with an efficacy around 84% [1] and fewer side effects compared to the whole-cell
58 vaccine. As a result, the incidence of pertussis further declined to <5 cases per 100,000 in the
59 2000s[2].

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

69 In Italy, the notification of infectious disease for pertussis is mandatory, but diagnosis is
70 usually clinical; microbiological confirmation is rarely required[5,6]. Moreover, in adults, the
71 disease may have a mild course[4,7]. For this reason, pertussis is often not diagnosed and
72 these cases represent a potential source of infection, especially for infants in the first year of
73 life, when not yet or incompletely immunised[4,8]. Cases of pertussis with severe course,
74 requiring hospitalisation and at high risk of mortality, have been reported in children younger
75 than one-year. Almost all deaths occur in infants less than 3 months of life[9], who are those
76 most at risk beside premature new-borns and children of non-immunised mothers[10]. It is
77 well known that parents are the primary sources of infection for children, along with
78 grandparents[11,12]. In particular, mothers lacking adequate immunity and contracting the
79 disease represent the biggest risk of transmission to the new-borns, not yet immunised[9,10].
80 Italian parents are often unaware of these risks and the severity of pertussis symptoms in early
81 childhood[2]. Currently, immunisation during pregnancy represents the most cost-effective
82 strategy for preventing neonatal death from pertussis, with an estimated efficacy of around
83 95%[12,13]. Furthermore, the risk of developing the disease for infants up to 3 months is
84 reduced by more than 90% when the mother is vaccinated compared to children of
85 unvaccinated mothers[10]. The purpose of vaccination during pregnancy is to provide passive
86 protection to new-borns through the transplacental passage of antibodies to protect them from
87 infection during the first months of life, before they can be immunised according to the
88 vaccination protocol and until active immunity is acquired[10]. In Italy, vaccination of
89 pregnant women during the third trimester of pregnancy is recommended and has been
90 included in the 2017-2019 National Vaccine Prevention Plan[3].

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

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

96

97 **Methods**

98 *Study design and inclusion criteria*

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

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

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

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

122 *Study procedures and laboratory processing*

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

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

139

140 *Statistical analysis*

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

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

165

166

167 **Results**

168 Two-hundred women, aged between 19 and 44 (median 35), were enrolled for the study.
169 Among them, 132 (66%) had received at least one dose of acellular vaccine against pertussis:
170 82 (41%) had been vaccinated during pregnancy, all with a dose of Boostrix[®] vaccine
171 (GlaxoSmithKline Biologicals, Rixensart, Belgium), 4 (2%) before pregnancy but no longer
172 than 5 years before, and 46 (23%) more than 5 years before. Vaccination status was unknown
173 for 37 women (18.5%), whereas 31 (15.5%) had never been vaccinated against pertussis.
174 Sixteen women (8%) reported previous pertussis infection in their early childhood, all
175 belonging to the unvaccinated group. Descriptive statistics for enrolled participants are
176 summarised in Table 1: the majority of women were Italian (79%), followed by other
177 European countries (12%, mostly from Eastern Europe) and by other continents (Northern
178 Africa, Asia and Southern America, 9% altogether).

179 Among the 200 women, 80 (40%) had an antibody titre below 10 IU/ml, and therefore below
180 the minimum level which is suggestive of previously acquired immunity against pertussis. A
181 detailed analysis of the immunisation status by subgroup showed that, on average, women
182 vaccinated more than five years before or without previous vaccination had antibody titres at
183 a frankly non-protective level (around 6-7 IU/ml), while women vaccinated in the last five
184 years had high levels of antibodies, even 12 to 15 times higher than the other groups. Women
185 vaccinated more than five years before had antibody titres not significantly different from
186 women never vaccinated before ($p=0.9794$), but both the former ($p<0.0001$) and the latter
187 ($p<0.0001$) had significantly lower antibody levels than those recently vaccinated. On the
188 other hand, antibody levels in women with unknown vaccination status were comparable to
189 those in women not vaccinated in the last five years ($p\geq 0.7$), thereby providing evidence for
190 considering them as not demonstrably immune.

191 The distribution of antibody titres in each group with known vaccination status is represented
192 in Fig. 1. Of note, some outliers can be observed in the groups with no recent vaccination,
193 particularly concerning two cases (1%) with antibody titres higher than 100 IU/ml, and 15
194 (7.5%) with serum titres between 20 and 100 IU/ml.

195 The comparison between women vaccinated and not vaccinated in pregnancy yielded no
196 statistically significant differences in relation to age (even though vaccinated women tended
197 to be younger) or nationality (a slightly lower adherence to the vaccination campaign was
198 recorded among women from Eastern Europe). On the contrary, the booster vaccine in
199 pregnancy strongly impacted on the immunisation status, with average antibody titres even
200 15-17 times higher in vaccinated women: levels of serum antibodies indicating likely
201 immunity against pertussis were reached by 85.4-95.1% of women who had received the
202 booster (depending on whether the 20 IU/ml or 10 IU/ml threshold is considered,
203 respectively), compared to 21-37% of women who had not (Table 2).

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

211

212 **Discussion**

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

215 rate, in children under the age of 12 months, varying from 0.8 per 100,000 inhabitants in 2012
216 to 1.7 per 100,000 in 2016 (Italian general population: 1.8/100,000 Age Standardised Rate in
217 2018)[28].

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

230 On the contrary, immunisation of women during pregnancy has become a strong
231 recommendation in many countries, including Italy, as evidence emerges of robust
232 effectiveness and safety in protecting young infants and their mothers against pertussis[40–
233 42]. In fact, beside lowering chances of transmission, vaccinating women during the third
234 trimester of pregnancy also protects infants through placental transfer of maternal antibodies,
235 resulting in high antibody levels in the infant at the time of birth and early occurrence of
236 mucosal antibodies due to their presence in breast milk[43,44].

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

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

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

248 The optimal timing of maternal pertussis immunisation for antibody transfer to the foetus is a
249 critical issue. The initial recommendation in the US was vaccination between 27 and 36
250 gestation weeks[47], while the UK – from 2012 to 2016 – recommended a window between
251 28 and 32 gestation weeks (but allowing vaccination up to 38 weeks)[48]. However, since
252 2016, based on emerging evidence[49], pregnancy vaccination against pertussis has been
253 proposed to women from 16 gestational weeks: this schedule was adopted to gain time to
254 reach more pregnant women, thus reducing missed immunisation opportunities, and to
255 maximise antibody transfer, thereby offering new-borns (especially the pre-term ones) a
256 higher chance to be protected[12]. Indeed, recent observational studies have suggested that
257 higher anti-pertussis antibody concentrations may be achieved in cord blood when mothers
258 are vaccinated earlier: 27– 30 weeks' gestation compared to later[50], 28–32 weeks'
259 compared to 33–36 weeks' gestation[51], or second- vs third trimester immunisation[49].

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

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

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

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

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

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

294 This analysis has some limitations. First of all, the sample size appeared to be limited,
295 particularly for the low amount of women who had received the last vaccine booster between
296 1 and 10 years before, which made it impossible to create a robust antibody response curve
297 for those values; however, the obtained estimates are consistent with data reported by
298 available studies on serum titres, thereby reassuring on the reliability of the results and
299 subsequent advice. Moreover, participation in the study was on a voluntary basis, with
300 possible biases due to convenience sampling; though, the refusal rate was not superior to 10-
301 15%, with no appreciable differences for age, pregnancy week or enrolment day, thus
302 ensuring representativeness of the enrolled sample. Eventually, vaccination dates were not
303 systematically retrieved from the vaccine registry (women were simply asked about their last
304 vaccination date): however, positive anamnestic data has been proven to be averagely reliable
305 for the case of vaccinations previously received[55].

306

307 **Conclusions**

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

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

327

328 **Conflicts of Interests:**

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

331

332 **Data Statement:**

333 All data collected and analysed for this study are available upon request to the Corresponding
334 Author of this paper.

335

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349

350 **Author Contribution Statement:**

351 **Jacopo Garlasco:** Conceptualisation, Methodology, Data collection, Formal analysis,
352 Writing - original draft, Writing - review & editing. **Valerio Bordino:** Conceptualisation,
353 Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Noemi**
354 **Marengo:** Conceptualisation, Methodology, Formal analysis, Writing - original draft, Writing
355 - review & editing. **Erika Rainero:** Conceptualisation, Methodology, Data collection,
356 Writing - review & editing. **Alessandro Scacchi:** Conceptualisation, Methodology, Data
357 collection, Writing - review & editing. **Savina Ditommaso:** Conceptualisation, Methodology,
358 Laboratory analysis, Writing - review & editing. **Monica Giacomuzzi:** Conceptualisation,
359 Methodology, Laboratory analysis, Writing - review & editing. **Fabrizio Bert:** Literature
360 search, Ethical Board procedures, Writing - review & editing. **Carla Maria Zotti:**
361 Conceptualisation, Methodology, Formal analysis, Writing - original draft, Writing - review
362 & editing. **All the Authors approved the final article version to be submitted.**

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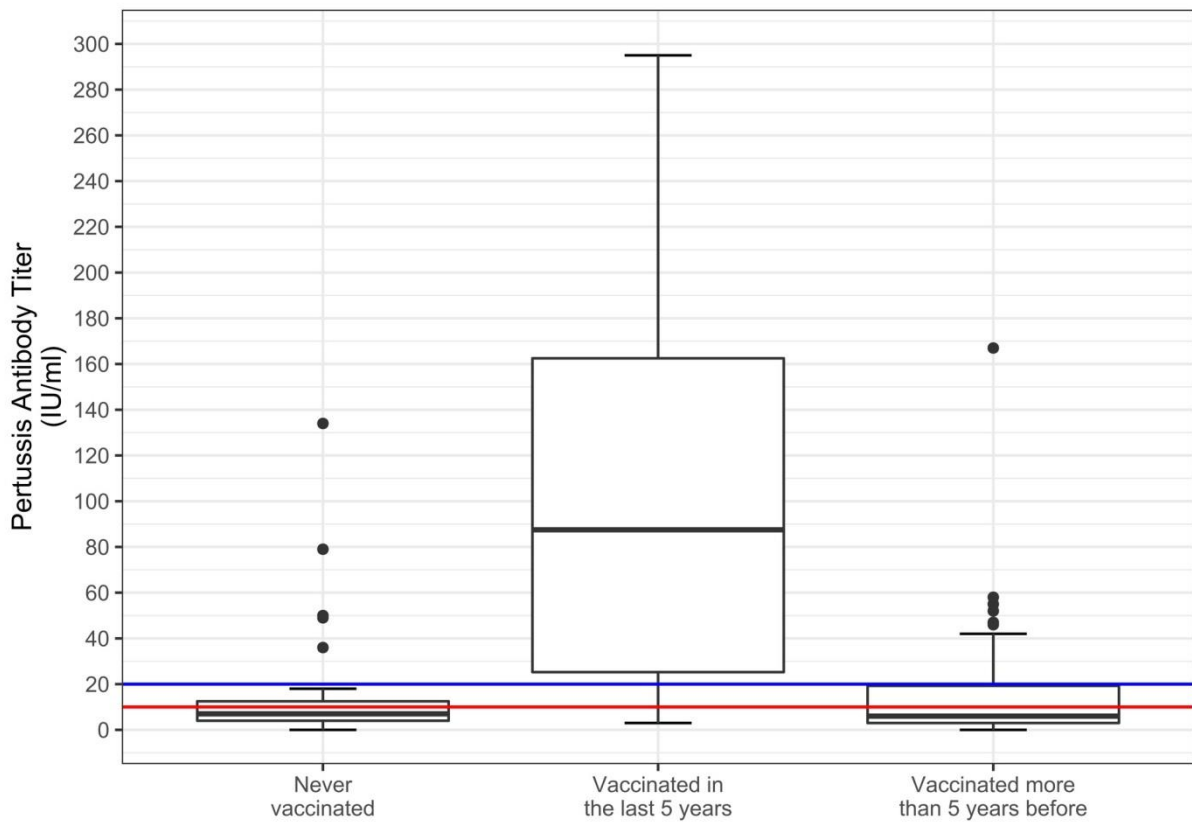
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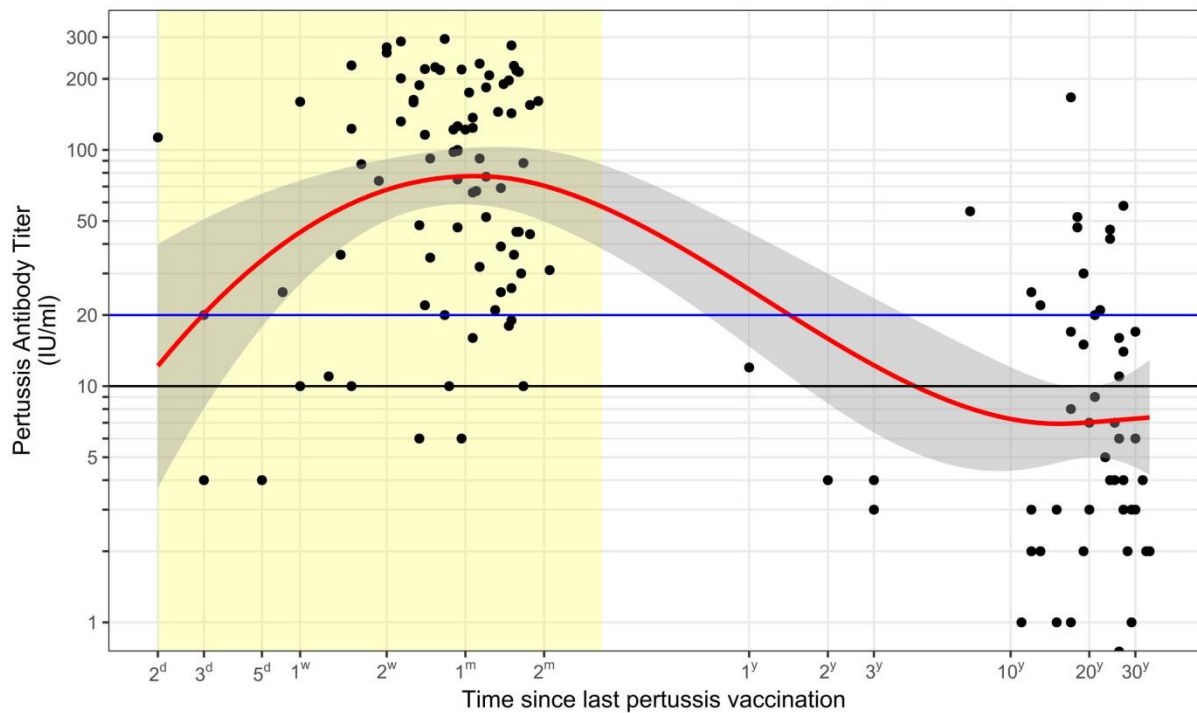
FIGURES

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



545

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



551

552

TABLES

553

554 **Table 1. Descriptive characteristics of participants enrolled in the study.** Values are
 555 expressed as median and interquartile ranges (Q1-Q3) for quantitative variables, and number
 556 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)

557

558

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

Characteristics	Not vaccinated during pregnancy (n=81)	Vaccinated between 28 th and 32 nd 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)	

565

566