Prevalence of Markers of Exposure to *Bordetella pertussis* Among Italian Young Adults

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Titers of serum antibody and cell-mediated immunity (CMI) to *Bordetella pertussis* antigens were assessed in a cohort of Italian military school students for whom the coverage of pertussis vaccination was low. The overall prevalence of IgG antibody above the minimum level of detection (MLD) was 71.6% for pertussis toxin (PT), 81% for pertactin (PRN), and 99% for filamentous hemagglutinin (FHA). Levels of IgA antibody to PT above the MLD were detected in 15.9% of the study participants. CMI to FHA, PRN, and PT was positive in 97%, 100%, and 82% of tested individuals, respectively. Only 9.7% of the participants had neither antibody nor CMI specific to *B. pertussis* antigens. In the 5-month clinical, microbiological, and serological follow-up conducted during a high-risk period of pertussis, no cases of pertussis were detected. These data, in particular CMI, demonstrate that most Italian young adults are specifically primed against *B. pertussis*, which should be taken into consideration when future policy on pertussis vaccination is being made in Italy.

Although pertussis is generally considered a childhood disease [1], an increased incidence among adolescents and adults has been recently reported in the United States [2]. Recent studies suggest that adults may represent a natural reservoir of *Bordetella pertussis*, thus contributing to its transmission even in a population for whom the coverage of childhood vaccination is high [3–9]. Moreover, a recent study carried out in Germany [10] indicated that *B. pertussis* infections are also common in adults who were infected with pertussis in childhood.

Since the 1960s, when the whole-cell pertussis vaccine was first made available in Italy, pertussis immunization of infants has only been voluntary (while immunization with diphtheria and tetanus toxoids and live oral poliovirus vaccine has been mandatory) and is not actively offered in all Italian regions. The recommended ages at which the pertussis vaccine is administered are 3, 5, and 7 months, with booster doses at 2 and 6 years. Vaccination coverage for pertussis has been unsatisfactory in most of the birth cohorts over the last 25 years, despite the availability of the whole-cell vaccine [11, 12]. Data from national immunization surveys show that pertussis vaccination coverage declined during the 1970s, ranging from 16% for the 1974 birth cohort to 11% for the 1981 birth cohort. Coverage remained low in the early 1980s and increased to an estimated 40% in 1991. According to pertussis notifications, the disease is mostly confined to the very young population, with the highest attack rate at 5 years of age [13]. However, pertussis in adults is often characterized by a mild clinical presentation and can go unrecognized.

The main objective of this study was to obtain evidence of exposure to *B. pertussis*, as inferred from immunologic markers assessed in a closed community of healthy young male adults belonging to birth cohorts for whom the vaccination coverage was low. Cell-mediated immunity (CMI) was also included among the immunologic markers studied. In addition, the secondary objective of the study was to conduct an intensive surveillance for pertussis in the study population over a 5-month period during a time of high risk for pertussis.

**Methods**

**Study Design**

In Italy, pertussis follows a cyclic pattern; epidemics take place every 3–5 years, and most cases occur in spring and early summer [14]. The study, which began in March 1995, included an initial cross-sectional investigation to determine the prevalence of immunologic markers of exposure to *B. pertussis* and of factors potentially related to pertussis. A 5-month follow-up for clinical pertussis was subsequently carried out to assess the transmission of *B. pertussis* in the study population during the expected high-risk seasonal period (i.e., from March to July) and during a year of expected peak incidence (in 1995, 14,359 cases were reported in the country). The length of follow-up was limited to the duration of military training in the two schools (5 months); there was a complete turnover of students after July.
Study Population

The study population included healthy males attending military boarding schools in the year 1994 to 1995 in the towns of Viterbo (central Italy) and Caserta (southern Italy). The age of the students ranged from 17 to 25 years, and they originated from different parts of Italy. For admission to the school, students must pass a medical and laboratory examination for fitness. In 1994, 450 students were enrolled in the school in Caserta, and 250, in the school in Viterbo. In March 1995, all students in the two schools were invited to participate in the present study. Participation was voluntary, and written informed consent was required.

Cross-sectional Study

At the beginning of the study, each participant answered a questionnaire that included basic demographic data, history of pertussis vaccination and disease, history of cough episodes in the previous 12 months, and smoking habits. A sample of venous blood was taken to assess the presence of antibodies to B. pertussis. CMI to B. pertussis antigens was also assessed for a subsample of 62 individuals (15% of the total) who were randomly chosen from the participants.

Follow-up Study

In the 5 months after the cross-sectional study, active surveillance of cough episodes was performed by a nurse who was based at the boarding school and was responsible for detecting and reporting cough episodes. For each episode lasting at least 7 days, a nasopharyngeal aspirate for B. pertussis culture was taken. A convalescent-phase serum sample was collected 6–8 weeks after the onset of cough, according to established procedures [15], to detect if the antibody titer increased significantly from that measured in the initial cross-sectional study.

Laboratory Studies

Serology

Serum samples collected in the cross-sectional study were stored at −20°C. All ELISAs were performed at the Department of Hygiene and Microbiology in the Microbiology Unit of the University of Palermo. The samples were tested for B. pertussis antigens by using a reference serum calibrated against reference serum samples provided by the U.S. Food and Drug Administration (Bethesda, MD) (serum lot 3 for pertussis toxin [PT] and filamentous hemagglutinin [FHA] and lot 4 for pertactin [PRN]). A standardized ELISA was used to evaluate IgG and IgA antibodies to PT and IgG antibodies to PRN and FHA. The three antigens were provided by SmithKline Beecham (Rixensart, Belgium).

ELISA units (EUs) were computed from the absorbance measured through a standardized software (Unitcalc [1992 version]; Biosys inova, Stockholm) providing results according to both the original parallel line method and the reference line method. The two methods provided comparable results at low titers, but the agreement was lower at higher antibody concentrations. On the basis of a recent international evaluation [16], the reference line method was chosen to determine EU.

The minimal level of detection (MLD) was set at 2 EU/mL for IgG antibodies to PT and FHA, at 3 EU/mL for IgG antibody to PRN, and at 10 EU/mL for IgA antibody to PT [17]. Evaluation of intraassay variability was performed by computing the daily coefficient of variation of positive control sera, which did not exceed 23%. A serological assay was considered positive when the antibody titer exceeded the MLD defined for each determination. Seroprevalence of humoral markers was defined as the proportion of individuals with a specific antibody titer above the MLD.

Assessment of CMI

Another sample of venous blood (at least 5 mL) was collected from 62 participants for CMI assessment by the lymphocyte proliferation assay of peripheral blood mononuclear cells (PBMCs), as described elsewhere [18, 19]. PBMC proliferations induced by mitogens (phytohemagglutinin and IL-2) were used as the positive controls. CMI to B. pertussis antigens was considered to be positive when the difference between the antigen-stimulated PBMC culture and the unstimulated control culture was at least $3 \times 10^3$ counts per minute (cpm). Data are reported as the stimulation index (SI), that is, the ratio of cpm of the stimulated culture to the cpm of the unstimulated culture. Since the cpm of unstimulated PBMC cultures were always $\leq 1,000$, the above-mentioned definition of positivity corresponds to an SI of $\geq 4.0$.

CMI to common recall antigens, such as tetanus toxoid and mannoprotein from Candida albicans, were also included as controls. Purified B. pertussis antigens and tetanus toxoid were provided by Chiron-Biocine (Sienna, Italy); mannoprotein was an in-house preparation [18].

Nasopharyngeal Aspirates

Nasopharyngeal aspirates—collected by means of an 8 French De Lee suction catheter (Sherwood Medical, St. Louis)—from individuals with a cough lasting at least 7 days were cultured on charcoal agar containing 10% defibrinated horse blood and 20 mg of cephalaxin/L (lot CM119; Unipath, Milan, Italy).

Statistical Analysis

Information from questionnaires that was obtained during the cross-sectional study and results of laboratory studies were
Table 1. Prevalence and GMTs of serological markers of exposure to *Bordetella pertussis* in 416 male military school students in Italy.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. of individuals with IgG titer above MLD/total no. of individuals (%)</th>
<th>GMT (95% CI) of IgG above MLD</th>
<th>Total GMTs (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG to PT</td>
<td>298/416 (71.6)</td>
<td>14.2 (12.5–15.5)</td>
<td>7.7 (6.5–8.6)</td>
</tr>
<tr>
<td>IgG to FHA</td>
<td>412/416 (99.0)</td>
<td>45.1 (40.3–50.4)</td>
<td>44.6 (39.9–49.9)</td>
</tr>
<tr>
<td>IgG to PRN</td>
<td>337/416 (81.0)</td>
<td>26.0 (23.3–29.1)</td>
<td>16.1 (14.0–18.4)</td>
</tr>
<tr>
<td>IgA to PT</td>
<td>66/416 (15.9)</td>
<td>25.0 (21.2–29.4)</td>
<td>2.9 (2.5–3.2)</td>
</tr>
</tbody>
</table>

NOTE. FHA = filamentous hemagglutinin; GMT = geometric mean titer expressed in ELISA units/mL; MLD = minimum level of detection; PRN = pertactin; PT = pertussis toxin.

recorded on a computerized database. Descriptive analyses were performed by using Epi-Info Version 5.01 (Centers for Disease Control and Prevention [Atlanta] and World Health Organization [Geneva]). \( \kappa \) was used as the measure of agreement among different humoral markers of exposure in each participant. Geometric mean titers (GMTs) were calculated for all individual antibody titers that were increased by the arbitrary constant value of 0.5 and logarithmically transformed. The constant value was then subtracted from the antilog of the mean. Differences were considered statistically significant at an \( \alpha \) value of .05.

**Results**

In both schools, ~60% of the students agreed to participate in the study (259 of 450 students in Caserta and 157 of 250 in Viterbo). Overall, 416 individuals were enrolled in the study. The mean age of the participants was 21.7 years (median, 22 years; range, 17–25 years), which was similar to the age distribution for the entire school population. The number of household members in the students’ families ranged from two to 10 (mean ± SD, 4.36 ± 1.14; mode, 4).

The place of birth was northern Italy for 8.1% of the participants, central Italy for 26.3%, and southern Italy for 55.1%; 2% of the participants were born abroad, and 9.5% did not provide this information. Only 4.1% of the participants reported vaccination against pertussis in childhood; 29.3% reported that they had not been vaccinated, and 66.7% did not provide this information. A history of pertussis was reported by 32.1% of the participants. No documentation of history of vaccination or infection was available. Smoking was reported by 21.1% of the participants. History of cough in the previous year was reported by 65% of the participants, and for 12%, the cough duration was >7 days.

**Seroprevalence**

GMTs of the antibodies to the tested antigens are reported in table 1. The proportion of individuals with titers of IgG antibody to PT exceeding the MLD was 71.6%; the GMT for these individuals was 14.2 EU/mL. The proportion of participants with titers of IgA antibody to PT above the MLD was 15.9%; the GMT for these individuals was 25.0 EU/mL. Similarly, the proportion of participants with titers of IgG antibody to FHA above the MLD was 99%; the GMT for these individuals was 45.1. The prevalence of IgG antibody to PRN was 81%, with a GMT of 26.0. Only one individual was negative for all serological markers investigated.

![Figure 1. Reverse cumulative curves of distribution of male military school students in Italy by serostatus for IgG antibodies to PT, FHA, and PRN.](https://example.com/figure1.png)

In figure 1, the reverse cumulative curves of titers of IgG antibodies to the three tested antigens show the proportional distribution of the participants according to the magnitude of antibody responses. Approximately 60% of participants had a titer of IgG antibody to PT below 10 EU/mL; only 4% had a titer above 100 EU/mL. Relatively few participants (25% and 8%, respectively) had titers of antibodies to FHA and PRN above 100 EU/mL. The values on the curves, corresponding to each MLD, represent the proportion of individuals with antibody titers equal to or above the MLD for each specific marker.

There was low agreement among the humoral markers. For 114 individuals, serology for FHA was positive; however, no detectable antibodies to PT were found, and \( \kappa \) between IgG antibody to PT and IgG antibody to FHA showed an agreement of 4.7%. \( \kappa \), computed in the same way, between IgG antibody to PT and IgG antibody to PRN showed an agreement of only 3.4%.

Individual serological results did not correlate with demographic data, age, or history of vaccination or disease. Of the 134 individuals who reported a history of pertussis, 73.9% had IgG antibody to PT, which was similar to the overall prevalence among the entire study population. Of the 17 individuals who
Table 2. Correlation between prevalence of IgG and IgA antibodies to PT and a reported history of pertussis and vaccination for male military school students in Italy.

<table>
<thead>
<tr>
<th>History</th>
<th>IgG to PT −</th>
<th>IgG to PT +</th>
<th>IgA to PT −</th>
<th>IgA to PT +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pertussis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (29.7)</td>
<td>99 (33.2)</td>
<td>22 (33.3)</td>
<td>112 (32)</td>
</tr>
<tr>
<td>No</td>
<td>52 (44)</td>
<td>114 (38.3)</td>
<td>15 (22.7)</td>
<td>151 (43.1)</td>
</tr>
<tr>
<td>Not known</td>
<td>31 (26.3)</td>
<td>85 (28.5)</td>
<td>29 (44)</td>
<td>87 (24.9)</td>
</tr>
<tr>
<td>Total</td>
<td>118 (100)</td>
<td>298 (100)</td>
<td>66 (100)</td>
<td>350 (100)</td>
</tr>
<tr>
<td>Vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (4.2)</td>
<td>12 (4)</td>
<td>2 (3)</td>
<td>15 (4.3)</td>
</tr>
<tr>
<td>No</td>
<td>38 (32.2)</td>
<td>84 (28.2)</td>
<td>14 (21.2)</td>
<td>108 (30.9)</td>
</tr>
<tr>
<td>Not known</td>
<td>75 (63.6)</td>
<td>202 (67.8)</td>
<td>50 (75.8)</td>
<td>227 (64.8)</td>
</tr>
<tr>
<td>Total</td>
<td>118 (100)</td>
<td>298 (100)</td>
<td>66 (100)</td>
<td>350 (100)</td>
</tr>
</tbody>
</table>

NOTE. MLD = minimum level of detection; PT = pertussis toxin; + = titer greater than MLD; − = titer less than or equal to MLD.

reported vaccination against pertussis, 70.6% had a titer of IgG antibody to PT above the MLD (table 2).

**CMI**

CMI to PT, FHA, and PRN was positive in 51 (82%), 60 (97%), and 62 (100%) of 62 participants, respectively. The SI for individuals with responses was lower after stimulation with PT than after that with the other antigens tested. The prevalence of CMI to FHA and PRN was similar to that of CMI to tetanus toxoid (as expected, considering that all participants receive a booster dose of tetanus toxoid) as well as that of CMI to mannanprotein from *C. albicans*, a commensal microorganism to which most humans are naturally sensitized (table 3). Of the 51 individuals with CMI to PT, 35 also had titers of IgG antibody to PT above the MLD. The remaining participants had antibodies to FHA and PRN. Of the 11 participants without CMI to PT, five had IgG antibody to PT. The GMTs of antibodies to each antigen did not correlate with the magnitude of the CMI.

**Surveillance of Cough Episodes**

Ten cough episodes lasting ≥7 seven days were detected during the 5-month study period. The average duration was 10 days per episode, and the cough, mainly productive and without spasms, required antibiotic treatment in two cases. None of these episodes were laboratory-confirmed pertussis. In all 10 episodes, microbiological culture of the nasopharyngeal aspirate was negative. No significant increase in antibody titers was detected in any of the convalescent-phase serum samples taken 6 to 8 weeks after the onset of cough. Pre- and postillness GMTs of antibodies to FHA and PT for participants with cough were slightly lower than the corresponding values observed for the entire study group in the cross-sectional part of the study.

**Discussion**

The resurgence of pertussis in countries with high vaccination coverage and the increase in the proportion of adult cases have suggested a shift in the occurrence of the disease to older age groups; this shift is explained by a waning of the protection conferred by immunization in infancy and by the persistence of transmission in the community. A study carried out by Jenkinson [20] showed that the efficacy of the whole-cell pertussis vaccine gradually decreases beginning 1 year after immunization. This finding suggests that in a country with limited transmission of *B. pertussis* in childhood because of high vaccination coverage, routine booster doses for adults could be beneficial in controlling transmission of pertussis among both adults and children [5, 21]. In Italy, where pertussis is common because of low vaccination coverage, a seroepidemiological survey conducted by Giammanco et al. [22] in 1988–1989...
showed that by the age of 19 years the overall prevalence of IgG antibody to PT in unvaccinated individuals is >80%.

The present study provides further information on the prevalence of antibodies to PT and other antigens of B. pertussis in adults as well as CMI to these antigens. CMI has been recently shown to persist longer than titers of antibody to B. pertussis antigens in vaccinated children [18], suggesting that CMI is a more stable marker than humoral response of a state of immune priming against B. pertussis. In our study, titers of circulating antibodies to PT above the MLD were found in 71.6% of the young adults. Of the 62 individuals included in the CMI assessment, 82% had proliferative cellular response to PT, of whom 68.6% had antibodies above the MLD to PT; only 9.7% were found to be negative for both humoral and cellular immunity. Thus, a total of 90.3% of the participants were found to be carriers of a marker of previous exposure to B. pertussis, confirming the high prevalence previously observed. The poor agreement among the different serological markers of infection (particularly PT, FHA, and PRN) is probably in part due to the induction of an immunologic response to FHA and PRN by microorganisms other than B. pertussis, as was also pointed out by Isacson et al. [23].

The magnitude of humoral response in Italian recruits is similar to that described in German recruits [24], although this comparison may be affected by the use of different methods and by the interlaboratory variability observed (even under standardized conditions) [25]. It is interesting that if the percentage of individuals with IgA antibody to PT is computed taking into account only individuals with titers above the MLD, the proportion is lower among Italian recruits (15.9%) than among those in Germany (63%) and young adults in the United States (67%) [24]. By contrast, when all levels of IgA antibody to PT are considered, the percentages are surprisingly similar. In light of these results, it would be interesting to conduct a comparison between Italian and German data on titers of antibody above the MLD (i.e., excluding the low titers, which may be critically influenced by interlaboratory variability) [25].

The proportion of individuals with CMI to the tested antigens was very high. In particular, the proportion of individuals with CMI to FHA and PRN equaled that of individuals with CMI to common recall antigens, such as tetanus toxoid and C. albicans mannanprotein; the magnitude of CMI was also similar. This finding may in part be due to exposure to microorganisms with antigens that cross-react with B. pertussis, but this exposure alone could probably not explain the high proportion of individuals with CMI to PRN and PT. The above-mentioned findings clearly imply that exposure to B. pertussis in adulthood is a very common event (largely independent of overt disease) and that CMI itself has a long persistence (as was also inferred in a recent study involving children [19]).

Although the level and type of antibodies and/or CMI necessary to protect against clinical pertussis or to prevent transmission are not known [1, 3, 26, 27], the present data show that most adults examined, in a country with low vaccination coverage, had acquired a consistent priming of immunity to relevant B. pertussis antigens. The low occurrence of clinically identified pertussis in Italian adults is supported by data from the official notification system and is suggested by the follow-up performed in the present study, although the length of observation was limited because of feasibility constraints in the closed community. More precise evaluation of the incidence of pertussis will require extensive active surveillance of a larger population. Whether the high frequency of this priming justifies a booster dose of vaccine is currently being debated; nonetheless, in Italy, priority must still be given to increasing pertussis vaccination coverage among infants and thus to reducing the occurrence of most of the preventable cases.

Acknowledgments

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References