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# The seroepidemiology of *B. pertussis* infection in Catalonia, Spain

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## SUMMARY

A survey of the seroprevalence of pertussis antibodies in a representative sample of the population from Catalonia was carried out. Ninety-seven municipalities and 30 schools were randomly selected to recruit the 2126 subjects who participated in the study. A serum sample was obtained from all individuals participating in the study in order to determine levels of pertussis toxin (PT) and filamentous hemagglutinin (FHA) antibodies by ELISA test. Sociodemographic data were collected for all subjects. The prevalence of PT antibodies was 75% and that of FHA antibodies 89%. Significant increments were observed with age, both in the prevalence of PT ($P < 0{\cdot}0001$) and of FHA ($P = 0{\cdot}018$). Of the sociodemographic variables studied, only urban habitat was significantly associated to PT antibodies. The agreement observed among the two types of antibodies studied was weak ($\kappa = 0{\cdot}264$). Routine revaccination with the acellular vaccine in children over 7 years of age, in adolescents and adults seems a reasonable strategy to prevent the appearance of cases of pertussis in the community.

## INTRODUCTION

Pertussis or whooping cough is an acute bacterial disease produced by *Bordetella pertussis* that affects the bronchial tree [1]. In small children and infants over 6 months the characteristic clinical picture is paroxysmal cough. In adolescents and young adults the clinical picture is more variable, with subclinical forms, that hinder the surveillance of the disease, being frequent [2–6]. Although the whole-cell pertussis vaccine has been administered in Catalonia since 1965 and coverage with four doses is high, in the last 5 years an average incidence rate of 3·9 per 100000 person year has been registered in Catalonia, an autonomous region in Northeast Spain with an area of 32000 km² and 6 million inhabitants. The capital is Barcelona, with 1·5 million inhabitants. The ratio urban to rural population is 3·9. Probably the real incidence of the disease is higher than reported, bearing in mind the diagnostic difficulties of the disease and the under-reporting which exists with respect to the cases diagnosed. The high transmissibility of the disease and the fact that the immunity conferred by the vaccination only lasts a few years also complicates control.

The availability of an acellular vaccine that is less reactogenic than the whole-cell pertussis vaccine, especially in children over 1-year of age, has changed the panorama of prevention of this disease. On one hand, some countries that had abandoned routine vaccination with the whole-cell vaccine, such as Sweden which ceased vaccination in 1979, have begun vaccinating again with the acellular vaccine [7]. On the

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Table 1. *Prevalence of pertussis antibodies PT and FHA according to age. Catalonia, 1996*

| | Antibodies to PT | | Antibodies to FHA | | | |
|---|---|---|---|---|---|---|
| Age (years) | % | 95% CI | % | 95% CI | *n* | $\kappa$ statistics* |
| 5–9 | 54·2 | 45·3–63·1 | 84·2 | 79·8–88·6 | 260 | 0·199 |
| 10–14 | 58·7 | 65·0–72·4 | 91·7 | 89·5–93·9 | 617 | 0·179 |
| 15–24 | 76·1 | 68·4–83·8 | 87·2 | 81·1–93·2 | 117 | 0·410 |
| 25–34 | 69·8 | 63·7–75·9 | 86·5 | 81·9–91·1 | 215 | 0·267 |
| 35–44 | 80·5 | 75·3–85·7 | 90·0 | 86·0–93·9 | 221 | 0·238 |
| 45–54 | 82·3 | 77·3–87·3 | 90·5 | 86·6–94·4 | 220 | 0·049 |
| 55–64 | 82·1 | 77·1–87·1 | 92·9 | 89·5–96·3 | 224 | 0·324 |
| > 64 | 73·4 | 67·9–78·9 | 90·1 | 86·4–93·8 | 252 | 0·230 |
| Total | 75·0 | 72·9–77·1 | 89·0 | 87·7–90·3 | 2126 | 0·264 |

* Agreement between PT and FHA antibodies.
CI; Confidence interval.

other hand, periodic booster vaccinations beginning at 7 years of age remain a possibility to be considered in the future if the clinical and epidemiological situation warrants them.

The present study presents the results of a sero-epidemiological survey carried out in Catalonia to determine the level of pertussis antibodies in the population.

## SUBJECTS AND METHODS

The study was carried out using representative samples of the school population 5–14 years of age and of the adult population ($\geqslant$ 15 years of age) obtained in 1996 by means of a cluster sampling procedure. In the first stage of the sampling of the adult population, 97 municipalities in Catalonia were randomly selected, after stratification into urban ($\geqslant$ 10000 inhabitants) and rural habitat ($<$ 10000 inhabitants). In the second stage, individuals were selected randomly from the municipal census, after stratification for age and gender according to the Catalonian population. In the first stage of the sampling of the school population, 30 schools were selected randomly from the list of the Department of Education; and in the second stage all students between ages 5-7 years, 10-11 years and 13-14 years of the selected schools were included in the study.

Informed consent was obtained from both school-children and adults included in the study.

The sample size was calculated for an alpha error of 5%, an assumed prevalence of 50%, and a precision of $\pm$0·025 for the sample of the adult population and of $\pm$0·055 for the sample of the school population. In accordance with these criteria, the necessary sample size was 1600 individuals for the adult sample and 330 individuals for each school year.

A venous blood sample was obtained from all individuals included in the study. The sera were frozen to $-20$ °C until the serologic analysis was carried out. The presence of antibodies to pertussis toxin (PT) and filamentous hemagglutinin (FHA) was determined by ELISA (Pertusscan, Eurodiagnostica, Malmö, Sweden) according to the manufacturer's instructions.

The assay consists of 2 microwell-strip plates coated with highly purified fractions of FHA or PT as antigen. According to the manufacturer's instructions, absorbances above 0·3 indicate the presence of antibodies. Elevated absorbance values of IgG to PT with or without elevated IgG to FHA indicates previous infection or immunization. The $\geqslant$ 1·0 limit represents aproximately 100 EU/mL.

Sociodemographic information was obtained from all participants. The variables investigated in the adult population sample were age, gender, place of birth, place of residence, educational levels, and occupation (valued according to the English classification in five social classes) [8]. The prevalences of PT and FHA antibodies and the corresponding 95% confidence intervals (CI) were calculated. The overall prevalence of antibodies against *Bordetella pertussis* was obtained using the technique of direct standardization, with the 1994 population of Catalonia as the standard population [9]. The prevalences were compared by means of the $\chi^2$ test and the calculation of the odds ratio (OR). A value of $P < 0{\cdot}05$ was considered statistically significant. To measure the agreement between the positivity for the PT and FHA antibodies in each

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Table 2. *Prevalence of pertussis antibodies PT and FHA according to sociodemographic variables in the school and adult population. Catalonia, 1996*

| | School population | | | | | Adult population | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Antibodies to PT | | Antibodies to FHA | | | Antibodies to PT | | Antibodies to FHA | | |
| Variable | % | 95% CI | % | 95% CI | *n* | % | 95% CI | % | 95% CI | *n* |
| Gender | | | | | | | | | | |
| Male | 63·3 | 58·8–67·7 | 89·5 | 86·7–92·3 | 449 | 78·0 | 74·7–81·3 | 90·6 | 88·3–92·9 | 605 |
| Female | 65·7 | 61·2–70·2 | 89·5 | 86·7–92·5 | 428 | 76·9 | 73·6–80·1 | 89·0 | 86·6–91·4 | 644 |
| Habitat | | | | | | | | | | |
| Urban | 66·6* | 63·0–70·2 | 90·8 | 88·6–93·0 | 649 | 77·4 | 74·9–79·9 | 89·6 | 87·7–91·5 | 1050 |
| Rural | 58·3 | 51·9–64·7 | 86·0 | 81·5–90·5 | 228 | 77·4 | 71·6–83·2 | 90·5 | 86·4–94·6 | 199 |
| Place of birth | | | | | | | | | | |
| Catalonia | 64·6 | 61·3–67·9 | 89·3 | 87·2–91·4 | 326 | 77·8 | 75·1–80·4 | 89·9 | 88·0–91·8 | 947 |
| Other | 60·9 | 46·8–75·0 | 93·5 | 86·4–100 | 46 | 76·2 | 71·4–81·0 | 89·4 | 85·9–92·9 | 302 |
| Educational level | | | | | | | | | | |
| ⩽ Primary studies | 64·5 | 60·2–68·8 | 90·6 | 88·0–93·2 | 470 | 77·1 | 73·7–80·5 | 90·8 | 88·5–93·1 | 590 |
| > Primary studies | 64·4 | 59·7–69·0 | 80·2 | 87·4–89·0 | 407 | 77·1 | 74·5–80·9 | 88·8 | 86·4–91·2 | 659 |
| Social class | | | | | | | | | | |
| I–III | 66·0 | 61·8–70·2 | 89·3 | 86·5–92·0 | 485 | 78·7 | 75·0–82·4 | 89·3 | 85·2–93·4 | 460 |
| IV–V | 61·4 | 56·0–66·8 | 90·5 | 87·3–93·7 | 316 | 76·6 | 72·9–80·3 | 89·5 | 86·8–92·1 | 513 |

* $P < 0{\cdot}05$ OR = 1·42 (1·05–1·94).

Table 3. *Distribution of reported cases of pertussis according to age groups and rural or urban habitat. Catalonia, 1997–9*

| Age (years) | No. of cases | Incidence rate per 100000 persons year |
|---|---|---|
| < 1 | 105 | 63·6 |
| 1–4 | 17 | 2·5 |
| 5–14 | 36 | 5·7 |
| 15–24 | 8 | 0·8 |
| 25–34 | 5 | 0·5 |
| 35–44 | 5 | 0·6 |
| 45–54 | 1 | 0·1 |
| 55–64 | 1 | 0·1 |
| > 64 | — | — |
| Total | 177 | 0·97 |
| Habitat | | |
| Urban (⩾ 10000 inh.) | 143 | 0·98 |
| Rural (< 10000 inh.) | 36 | 0·97 |

individual, Cohen's kappa statistic $\kappa$ was calculated [10].

Although pertussis has been a reportable disease since 1982 in Catalonia, only since 1997 has this report included age. Distribution of reported cases in the last 3 years according to age groups and rural or urban habitat (less or more than 10000 inhabitants) was studied.

## RESULTS

The study enrolled 2126 individuals, 887 schoolchildren and 1249 adults. Participation rate was 82%. The distribution of both the child and adult samples was similar in age, gender and socioeconomic status to that of the Catalan population.

The overall prevalence of antibodies to *B. pertussis* was 75% (Table 1). The prevalence of PT antibodies increased significantly with age ($P < 0{\cdot}001$). The prevalence in subjects < 35 years of age, was 66·5%, 79·4% in subjects above this age ($P < 0{\cdot}00001$).

The overall prevalence of FHA antibodies was 89% (distribution by age groups is shown in Table 1). An increment was observed with age ($P = 0{\cdot}018$). The prevalence of these antibodies in subjects < 35 years of age and in those above this age was 88·7% and 90·8% respectively, differences that were not significant ($P = 0{\cdot}13$).

The distribution of the prevalences of PT and FHA antibodies according to sociodemographic variables are shown in Table 2. Only urban habitat was

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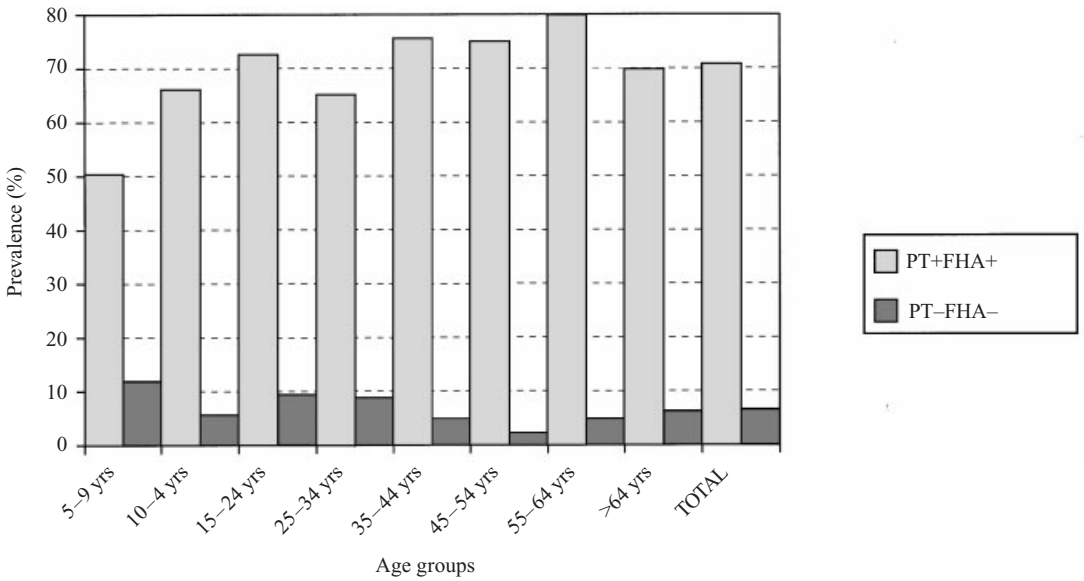


**Fig. 1.** Distribution of the prevalence of positivity and negativity jointly to PT and FHA by age groups. Catalonia, 1996.

statistically associated to a higher prevalence of PT antibodies in the school sample.

Figure 1 shows the prevalences of positivity and negativity coincident with both antibodies (PT and FHA) according to age groups. The value of Cohen's kappa statistic for the agreement of these antibodies was 0·264.

Distribution of reported cases of pertussis in the period 1997–9 according to age groups and habitat is shown in Table 3.

## DISCUSSION

Pertussis vaccination began in Catalonia in 1965, and so it can be assumed that when the survey was carried out there was no vaccinated subject above 35 years of age. With respect to schoolchildren, 90% of those included in this study had received some dose of pertussis vaccine. However, bearing in mind that the vaccine antibodies begin to wane 3 or 4 years after the last dose [11–13], and that the last dose of pertussis vaccine is administered in Catalonia at 18 months, it should be considered that most antibodies detected are due to natural infection with *B. pertussis*.

It is interesting that the prevalence of PT antibodies was lower than that of FHA antibodies in all age groups. This is in agreement with the results obtained by other authors [14].

Rota et al. [15], in a study of 700 students between 17 and 25 years of age carried out in two Italian cities, also obtained higher prevalences for FHA antibodies (99%) than for PT antibodies (71·6%), with the value of $\kappa$ for the agreement between both markers being 0·047 which indicates only slight agreement.

A study by Cattaneo et al. [16] carried out with 585 subjects of 1–65 years of age who had participated in a flu vaccine trial in Vanderbilt (USA) also shows that the titres of FHA are higher than those of PT and that a correlation exists between the geometric means of the titres of both markers.

Figure 1 shows that the maximum prevalence of negativity of both markers in our study corresponds with the youngest age group (5–9 years). This reinforces the idea that, at the age when vaccination protection still exists, few infections are produced and that there are, therefore, fewer infection markers.

In this respect, it is worth pointing out that in our study the value of the $\kappa$ statistic obtained in all age groups was always $< 0{\cdot}4$. This supposes that in the present study, the agreement not explainable by chance can be considered weak or slight.

Our data support the position of some authors [14, 17, 18] that, unlike the PT antibodies which would be specifically produced after contact with *Bordetella pertussis*, FHA antibodies not only appear after contact with *B. pertussis* but could also be induced after contact with different agents such as *Bordetella parapertussis* and *Haemophilus influenzae*, among others.

Therefore, the lack of concordance between both markers and the fact that PT antibodies are specific to the immune response to *Bordetella pertussis* suggest that this is probably the marker of choice for seroprevalence studies.

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With respect to the distribution of PT antibodies according to age, two aspects are worthy of comment. First, the increase of prevalence with age: in the 5–9 years age group the prevalence was 54% while in those over 64 years of age it was 73%. Second, while the prevalence of antibodies according to age showed a rising trend, this increase was greatest between 35 and 64 years of age. The first increment, until 35 years of age, could be explained by the fact that although vaccination protection diminishes substantially in 6 years [19, 20], some protection remains in the 5–9 year and 10–14 year groups. The greater increase that takes place in Catalonia after 35 years of age could be explained partly because none of these subjects had received the vaccine and partly by the contact of adults with their children. In this respect, Baron [21] points out that in half the cases of pertussis transmission is through the parents and in the other half through siblings. Jenkinson [22] suggests that the increase of cases at 30 years of age is because the parents of children with pertussis usually become infected.

Since the disease does not give permanent protection, the increase of PT antibodies with age that other authors have also observed [18, 23] would seem to show that the bacteria circulates extensively in Catalonia during childhood and adulthood.

Although the rates of reported morbidity are very similar in both urban and rural habitats, the higher prevalence of PT antibodies in school children in urban habitats, could be explained because the lifestyle of large cities facilitates the circulation of the bacteria and therefore the appearance of new infections.

Our data would justify routine revaccination with the acellular vaccine in children over 7 years of age, in adolescents and in adults, as is performed against tetanus and diphtheria. Alternatively, some authors [24] support selective revaccination of those adults who have the greatest risk of contracting the disease due to their profession. However, we believe that this strategy would not assure effective protection of the whole population. Routine revaccination should be seen as a possible strategy to control a disease that does not confer permanent immunity [12, 25, 26].

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