Sero epidemiology of *Bordetella pertussis* Immune Responses in a Healthy Population in Northern Greece

A. Polyzou¹, S. Pournaras¹, U. Dafni², D. Sofianou¹, E. Christeli³,
S. Patrinos², and A. Tsakris^{3*}

¹Department of Microbiology, Hippokration University Hospital, Thessaloniki, Greece

²Department of Statistics, Faculty of Nursing, School of Health Sciences, University of Athens, Athens, Greece

³Department of Microbiology, Faculty of Nursing, School of Health Sciences, University of Athens, Athens, Greece

A seroepidemiological study was conducted on a representative sample of the northern Greek population (healthy individuals, age range = 1 day to 80 years) to assess the prevalence of antibodies to pertussis toxin (PT) and filamentous hemagglutinin (FHA). Antibody concentrations were significantly elevated with age (analysis of variance (ANOVA), $P < 0.001$). In addition, a significant increase in antibody levels was detected in subjects >50 years old compared to children aged 5–10

years (post-hoc Scheffe analysis, $P = 0.007$). These data suggest that pertussis occurs frequently in Greek adults, and that sometimes a fifth booster vaccine dose is not given after the second year of life. Routine revaccination with the acellular vaccine for children >4 years of age, adolescents, and adults should be considered in order to ensure effective protection of the whole population. J. Clin. Lab. Anal. 18:211–214, 2004. © 2004 Wiley-Liss, Inc.

Key words: *Bordetella pertussis*; immunization; seroepidemiology; pertussis toxin; filamentous hemagglutinin

INTRODUCTION

Although the whole-cell pertussis vaccine has been used for over 40 years in developed countries, *Bordetella pertussis* infection continues to be an important cause of prolonged “cough illness” with significant morbidity and complication rates, even in immunized populations (1,2). There has been a significant decrease in the incidence of childhood pertussis since the introduction of widespread vaccinations; however, in recent years the number of cases has increased in older children, adolescents, and adults (1). Moreover, not only has a resurgence of this disease affected these age groups, but a change in the epidemiology (i.e., increased morbidity and mortality in infants infected by adolescents and adults) has also been observed (3).

The resurgence of *B. pertussis* is the result of multiple factors, including vaccine efficacy, tolerance, and coverage, as well as polymorphism of the agent of the disease (2). Thus, the effectiveness of long-term immunity from either immunization or childhood infection is controversial (4), highlighting the importance of continued

booster immunizations in older children and adults. Control of the disease is also complicated by the high transmissibility of the bacterium, and a significant underestimation of its real incidence hinders immunization programs. Understanding the seroepidemiology of *B. pertussis* in a population might provide information about immunity status in various age groups, although protection may be continuous even after antibody levels have waned in immunized children (5). In addition, explanations for the resurgence of this disease vary depending on the countries involved, because they may use different vaccines, vaccination schedules, and diagnostics. In the current study, we assessed the

*Correspondence to: A. Tsakris, M.D., Ph.D., Department of Microbiology, Faculty of Nursing, School of Health Sciences, University of Athens, 123 Papadimantopoulou Street, 11527 Athens, Greece. E-mail: atsakris@med.uoa.gr

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immune level against pertussis by evaluating antibody titers to pertussis toxin (PT) and filamentous hemagglutinin (FHA) in a random sample of apparently healthy children and adults in northern Greece. These antibodies arise from vaccination as well as natural infection, and it is believed that in most cases the immunoglobulin responses are directed against PT and FHA immunogens (2,6).

MATERIALS AND METHODS

Population and Serum Samples

A retrospective survey was carried out on 431 serum samples collected during January to October 2000 from normal healthy persons (age range = 1 day to 80 years). The subjects were randomly selected from individuals who visited Hippokration University Hospital, Thessaloniki, Greece, and health care centers in northern Greece for periodical checkups. The sera were frozen immediately after sampling, and thawed once for testing. The subjects were divided into groups according to the following ages: 1–60 days (32 sera), 2–12 months (32 sera), 1–4 years (34 sera), 5–10 years (34 sera), 11–20 years (58 sera), 21–30 years (72 sera), 31–40 years (74 sera), years 41–50 years old (36 sera), and > 50 years (59 sera).

Serological Testing

IgG class antibodies to *B. pertussis* were detected by means of a commercially available ELISA test (Genzyme Virotech, Rüsselsheim, Germany) (7). As the *B. pertussis* antigen, acellular components of PT and FHA (which are produced only by the *B. pertussis* Tohama Phase I strain) were used. The antigen was highly purified and cross-absorbed to reduce cross-reaction with other organisms, such as *Bordetella* species or *Haemophilus influenzae* (8). The tests were performed manually as directed by the manufacturer's protocol. Antibody levels were expressed in ELISA units (EU) per milliliter, as suggested by the Laboratory of Pertussis, U.S. Food and Drug Administration (FDA), Bethesda, Maryland. According to the manufacturer's instructions, the levels were considered elevated when they were ≥ 9 EU/ml. For the seroepidemiology, we validated the assay by testing control sera corresponding to World Health Organization (WHO) and FDA standards.

Statistical Analysis

A regression analysis model on IgG antibodies in the logarithmic scale, with age as the explanatory variable, was used. Logarithmic transformation of IgG was necessary to satisfy the normality assumption. Age was also used as a categorical variable in the nine categories

described above. An analysis of variance (ANOVA) and post-hoc Scheffe analysis were performed. In addition, a nonparametric Kruskal-Wallis analysis was used, which provided the same results as the ANOVA.

RESULTS

The overall prevalence of elevated antibody concentration to PT and FHA was 59.4%, ranging from 35.3% in the category of 5–10 years to 83.1% in the category of > 50 years (Table 1). Following an antibody increase in the first months of life, there was a decrease of specific antibodies at 5–10 years, followed by an age-specific increase. The log(IGG) levels of pertussis antibodies by age category are shown in Fig. 1. According to the analysis of results from the estimated regression model, log(IGG) was significantly increased with age ($P < 0.001$). When age was treated as a categorical variable with the nine age categories, the ANOVA also

TABLE 1. Age-specific prevalence of IgG antibodies to PT and FHA among the Greek population

Age	No. positive ^a /no. tested	% positive
1–60 days	13/32	40.6
2–12 months	19/32	59.4
1–4 years	21/34	61.8
5–10 years	12/34	35.3
11–20 years	36/58	62.1
21–30 years	40/72	55.6
31–40 years	42/74	56.8
41–50 years	24/36	66.7
> 50 years	49/59	83.1
Total	256/431	59.4

^aSamples were considered as positive when higher or equal to 9 EU/ml.

Fig. 1. Means plots of IgG antibodies to PT and FHA in the logarithmic scale by age category.

showed significant differences in log(IGG) between age categories. According to the Scheffe post-hoc tests, the only significant difference in the log(IGG) levels was between the categories of 5–10 years and >50 years ($P=0.007$).

DISCUSSION

Serodiagnosis of pertussis has been used as evidence to support the need for immunizations in various populations (9–12). However, there are no well-defined criteria for evaluating the results of seroepidemiology of pertussis (5,9,12). Moreover, although cellular immunity lasts much longer than it used to, the titer of antibodies appears to decrease quickly after infection or vaccination, with either whole-cell or acellular vaccines (2,12). Previous studies have shown that antibody levels against PT and FHA antigens are generally parallel (9,11). Furthermore, most serological surveys have not evaluated newborns, infants, or adults for immunity against pertussis (10–12). The aim of this study was to investigate the seroepidemiology of *B. pertussis* infections in a healthy northern Greek population, in subjects ranging from 1 day old to 80 years old. Although pertussis was not epidemic in the population during the study period, sporadic cases are commonly reported in Greek hospitals (data not shown), and it is possible that outbreaks occurred but were missed.

In Greece, vaccination against pertussis usually consists of four doses given at the ages of 2, 4, 6, and 18 months. Occasionally, a fifth dose at the ages of 4–6 years is given (13). In the current study, babies 2–12 months old showed higher titers compared to infants <2 months of age. This may have been due to primovaccination; however, it should be noted that maternal antibodies can also be present in children <6 months old. Antibody levels in children 1–4 years old did not differ considerably from those in the younger age groups, and a relatively low proportion of these children had antibody titers considered to be elevated. This may indicate that humoral immunity of children against pertussis due to basic immunization is inadequate even in the first 2 years of life. A recent study of northern Greek children regarding immunity rates against diphtheria found that their immune response was satisfactory (>95%) (14). Therefore, it is more likely that the licensed whole-cell DTP vaccines have a low immunogenicity. A similar observation was also made in a controlled trial in Italy, in which the efficacy and antibody responses of a whole-cell vaccine were unexpectedly low (15). Furthermore, a recent German study that tested IgG antibodies against a mixture of purified PT and FHA (as in the present study) showed that pertussis immunization in early childhood does not

protect pregnant women adequately against the disease (12).

A study in the United States demonstrated two peaks in antibodies against both PT and FHA (9). One peak occurred at 4–6 years of age due to immune response after the administration of whole-cell pertussis vaccine, and the second occurred at the ages of 13–17 years due to pertussis infection in adolescents. In contrast to that report, in the present study antibody titers were relatively low in the 5–10-year age group. This finding further supports the notion that either a fifth booster pertussis vaccine dose is not given after the second year of life, or that the whole-cell DTP vaccines that were usually administered to children 4–6 years old before and during the study period were not immunogenically effective. The former hypothesis is also strengthened by a recent assessment of vaccine coverage in children of primary or secondary school age in a region in Greece where only 12.4% of the children had received the fifth dose for pertussis (13). As vaccine induced-immunity wanes over time, the observed increasing distribution of antibodies in adolescents and adults suggests that *B. pertussis* infection is widespread in the Greek population. However, the frequent lack of classic pertussis findings in adults may have led to the underestimation of the real incidence of this disease. The influence of the recent introduction of acellular pertussis vaccines in the circulation of *B. pertussis*, and the occurrence of natural infections in the Greek population should be investigated in future seroepidemiological studies.

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