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Original Article

Seroprevalence of Immunoglobulin M/A/G antibodies against Bordetella pertussis and Bordetella parapertussis among asymptomatic children at 6-8 of age in Turkey

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Abstract. *Bordetella pertussis* infection is a vaccine preventable disease, but immunity following the vaccination is not life-long. Moreover parapertussis may share similar clinical presentation with pertussis and mostly recognized in prolonged bronchitis. Although the serology of pertussis has been well studied, those of parapertussis have not. We herein investigated antipertussis and antiparapertussis serology in asymptomatic healthy children. We examined IgM, IgA and IgG antibodies against *B. pertussis* and *B. parapertussis* among 100 asymptomatic children aged from 6 to 8 years who got regular vaccination. The antibody titers were measured by indirect immunofluorescence test (IFA). 10% of them had IgA titers of > or = 100 EU/ml, 33% had IgM titers of > or = 320 EU/ml who could be considered as acute or recent pertussis infection and 89% had IgG titers > or = 100 EU/ml as a protective level of pertussis. *Bordetella parapertussis* antibody levels of IgG, IgA and IgM were detected in 33%, 17%, and 11% respectively. We suggest 2 explanations for the acquisition of pertussis and parapertussis antibodies in our children: (1) asymptomatic pertussis and parapertussis infections are common; (2) Although higher values of IgG observed, acute infection markers still persisted, and one problem in this regard may be waning immunity against pertussis. Of the strategies considered, the addition of a preschool booster is therefore a priority in Turkey.

Key words: Asymptomatic children, Bordetella parapertussis, Bordetella pertussis, seroprevalence

1. Introduction

Pertussis is a highly contagious respiratory tract infection characterized by severe coughing spasms often associated with vomiting. The complications of pertussis are also troublesome and can be fatal especially in infants.

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Fortunately, pertussis is a vaccine preventable disease and following the widespread use of whole-cell pertussis vaccine in 1940s, incidence of the disease decreased by 1980 (1-4). Whole-cell pertussis vaccines combined with diphtheria and tetanus toxoids were administered children at 2nd, 3rd and 4th months of age with a booster dose between the 18th and 24th months in Turkey. After "National Vaccination Campaign" in 1985 in Turkey, incidence of the disease decreased from 21/100,000 in 1970 to 0.27/100,000 in 2001 (5,6). Despite the incidence of *B. pertussis* infections had decreased significantly in the vaccine era, the number of reported cases has gradually increased recently in school children, adolescents, and adults (7). Therefore, these groups are potentially

a major source of pediatric infections, despite the current immunization program (1,8). These facts led to discussions focus on epidemiology of pertussis, sources of infection, and trends in incidence pattern and vaccination strategies. Because immunity provided by whole cell pertussis vaccines appears to persist for at least 3-5 years and then progressively decline in 6-10 years after vaccination, one problem in this regard is waning immunity against pertussis (8-10).

Bordetella parapertussis is diagnosed less commonly and is milder and of shorter duration than disease caused by *B. pertussis*. Similarity of these microorganisms in both clinic presentation and virulence factors is well known, but *B. parapertussis* infections may be underestimated in prolonged bronchitis in clinical practice. Indeed, as emphasized by some investigators, *B. parapertussis* is a more common etiologic agent of mild respiratory tract infections than is generally recognized (11,12).

In this study, we investigated antipertussis and antiparapertussis immunoglobulin M, A and G antibodies in healthy children at age of 6-8 years in which period that resurgence of the diseases mostly occurs. We aimed to supply information about pertussis immunity after several years from the last dose of pertussis vaccine and high-lightened *B. parapertussis* serology which has similar clinical presentation with *B. pertussis* in order to provide our local epidemiological data.

2. Material and methods

The cross-sectional study was conducted among 100 healthy children whose ages ranged from 6 years to 8 years at Pediatric Departments of Gulhane Military Medical Academy Haydarpasa Training Hospital in 2006. Study population included normal volunteers as siblings and patients with minor noninfectius acute conditions attending pediatric outpatient clinic. The study protocol was approved by the ethics and research committee and written informed consent was obtained from every parent. All individuals had received whole-cell pertussis vaccines at 2^{nd}, 3^{rd}, 4^{th} and 18^{th} months of age. Vaccination status was verified from the children's health books. The serum samples were collected from 48 girls and 52 boys living in Istanbul. Blood samples were obtained from participants and the serum samples were stored at -20°C until assayed by indirect immunofluorescence test (IFA). IgG, IgA and IgM antibodies against pertussis and parapertussis antibody titers were measured by IFA Euroimmun (Euroimmun, Germany) and reference ranges were as follows: IgA of 1:100 or higher and IgM of 1:320 or higher were accepted as acute or recent infection and minimum antibody level for IgG of 1:100 was accepted to be protective level (13).

The questionnaires included name, date of birth and some demographic data such as medical history (any chronic or atopic illness or pertussis diagnosis by physician were excluded) and vaccination status of the children was evaluated with family consult and all had 4 doses of cellular pertussis vaccines combined with diphtheria and tetanus toxoids; the others not fully vaccined were not included the study.

3. Results

Reference cut-off levels of IFA Euroimmun were as follows; IgA of > or =1:100 and IgM of > or =1:320 EU/ml were accepted as acute or recent infection for pertussis and IgG of > or =1:100 EU/ml were accepted as protective levels for pertussis. IgA of > or = 1:100 and IgM of > or = 1:320 EU/ml were accepted as acute or recent infection for parapertussis and IgG of > or =1:100 EU/ml were accepted as protective levels for parapertussis (13). The following antibody prevalences were determined using samples from 100 healthy children; 10% of them had pertussis IgA titers of > or = 100 EU/ml, 33% had pertussis IgM titers of > or = 320 EU/ml who were accepted as acute or recent pertussis infection and protective IgG-antibody rate of pertussis was 89%. Parapertussis IgG-antibody rate was 33% and 17% of individuals had parapertussis IgA titers of > or = 100 EU/ml, 11% of them had parapertussis IgM titers of > or = 320 EU/ml. The evaluation of the results of antibody levels of *B. pertussis* and *B. parapertussis* were shown in Table 1. The distribution of pertussis and parapertussis IgA, IgG and IgM titers by sex were shown according to antibody titers of IgA and IgG of > or = 100 EU/ml, and IgM titers of > or = 320 EU/ml in Figure 1.

Table 1. Antibody prevalences of *B. pertussis* and *B. parapertussis* of 100 healthy blood donors

	IgG	IgM	IgA
Bordetella pertussis	% 89	% 33	% 10
Bordetella parapertussis	% 33	% 11	% 17

*The percentage represents the rate of antibody titers of IgA and IgG of > or = 100 EU/ml, and IgM titers of > or = 320 EU/ml.

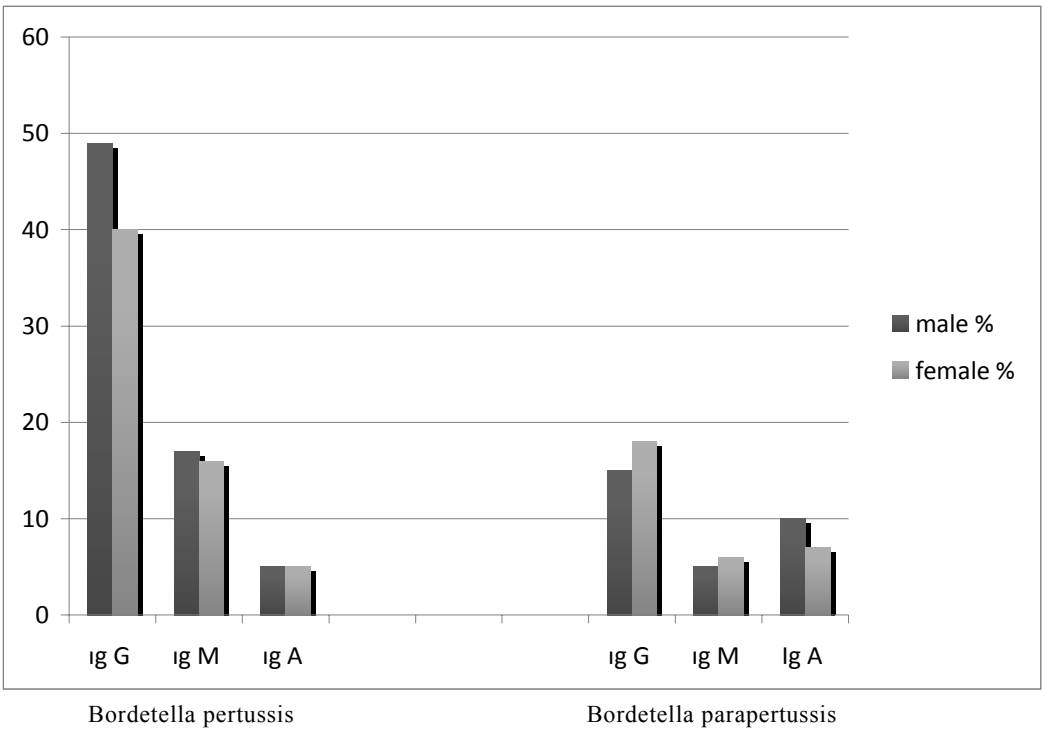

Fig. 1. The distribution of pertussis and parapertussis IgA, IgG and IgM titers (%) by sex among 100 healthy children (48 girls and 52 boys) aged 6 to 8 in the province of Istanbul in 2006. The antibody titers of IgA and IgG of > or = 100 EU/ml, and IgM titers of > or = 320 EU/ml were displayed.

These subjects had no history of either having a pertussis infection or a chronic disease diagnosed by a physician. SPSS® for Windows® (SPSS Inc., Chicago, IL, USA) was used to analyze the results. Statistical analysis was summarized as percentage for qualitative variables.

4. Discussion

Although *B. pertussis* infection is a vaccine preventable disease, immune response against pertussis following the vaccination or natural disease is not life-long. Large number of studies emphasizes an increase in the incidence of pertussis in school children, adolescents and adults (14-16). Whereas the high rates of vaccination, the key factor underlying a continuing endemic *B. pertussis* infection in countries is both vaccine-induced and naturally acquired immunity wane without boosting (8,17).

We determined to examine the distribution of antibody levels against pertussis as a first step of the study in healthy 6-8 age group, since *B. pertussis* antibody-titer changes over time and duration of immunity after vaccination is about 2-5 years (5,8,18). Although higher values of IgG observed in our study as protective efficacy for pertussis, acute infection markers still persisted, and one problem in this regard may be waning immunity against pertussis. Vatansever et al (5) declared the ratio of protection against pertussis is above 94% in 12-14 age groups in Turkey, but this ratio was mostly attributed to recent infection in accordance with our study. This change seems to be due mainly to the lack of vaccine and natural boosters. Furthermore, it may also be due to a decrease in the efficiency of the pertussis vaccine used, a decrease in coverage, or a change in the circulating isolates (19).

It's emphasized that pertussis in older children may be characterized by short duration of spasmodic cough in vaccinated people, so it should be taken into account in the clinical evaluation of patients with suspected pertussis (20). In particular, our data figured out that asymptomatic or unrecognized pertussis infections still persist in our society and this may be the significant hurdle in the way of the resurgence and transmission of pertussis disease. If current vaccination schedules shift towards an increasing burden of disease in older ages, then these unrecognized or asymptomatic infections in older childhood and adults may be the real source in resurgence of pertussis (21). Gustafsson L et al. (16) declared that a concomitant increase in incidence among infants was most likely attributed to infected older siblings and

recommended a booster dose of acellular pertussis vaccine in 5 to 7 years of age. Our data and the researches for our population indicated that pertussis infections still survey in our community and current immunization strategies inadequately control the circulation of *B. pertussis* in our country (22-24). We urgently suggest for the addition of childhood booster dose(s) of pertussis vaccine to the standard schedule after completion of the current four-dose immunization schedule in our vaccination policy.

Whooping cough can be caused by either *B. pertussis* or *B. parapertussis* is on the rise in young children and adolescents. *B. parapertussis* and *B. pertussis* are closely related species and have similar symptoms in clinical practice. *B. parapertussis* in humans can cause unrecognized infection, mild pertussis, or classic pertussis, and mostly parapertussis infections may present with milder and of shorter duration than disease caused by *B. pertussis*; so the real incidence is likely to be greatly underestimated (14). It was notable that the serologic markers related to recent infection of both diseases were higher than we expected in our healthy subjects and this issue urged us that parapertussis infections are also common in our population. However, little attention has been paid to this bacterium because it causes a milder illness than *B. pertussis* and the rate of detection is low, recent studies have revealed high rates of detection in patients (12,25). In our study, rather than classical presentation of both diseases, we thought that unrecognized infections may persist and be underestimated; eventually may cause serious outbreaks as a health problem in front of us in this vaccine era.

Although our sample size is not enough to extend the results to the entire community, the data from present research would probably be of importance regarding asymptomatic pertussis and parapertussis infections are considerably common in our population. This study also stresses the importance of laboratory diagnosis for pertussis and parapertussis to build our vaccination policy. Because whooping cough varies worldwide and is still a significant public health concern, all countries should ensure the highest possible coverage rates to consider expanding existing strategies. In order to control pertussis incidence, of the strategies considered, the addition of a preschool booster is therefore a priority in Turkey.

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