16. Matar GM, Rahal E. Inhibition of the transcription of the *Escherichia coli* O157:H7 genes coding for shiga-like toxins and intimin, and its potential use in the treatment of human infection with the bacterium. *Ann Trop Med Parasitol* 2003; **97**: 281–287.
17. Murphy JR, Michel JL, Teng M. Evidence that the regulation of diphtheria toxin production is directed at the level of transcription. *J Bacteriol* 1978; **135**: 511–516.
18. Dickgiesser N, Wallach U. Toxic shock syndrome toxin-1 (TSST-1): influence of its production by subinhibitory antibiotic concentrations. *Infection* 1987; **15**: 351–353.
19. Koszczol C, Bernardo K, Kronke M, Krut O. Subinhibitory quinupristin/dalfopristin attenuates virulence of *Staphylococcus aureus*. *J Antimicrob Chemother* 2006; **58**: 564–574.
20. Jacqueline C, Navas D, Batard E *et al*. *In vitro* and *in vivo* synergistic activities of linezolid combined with subinhibitory concentrations of imipenem against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2005; **49**: 45–51.

RESEARCH NOTE

Seroprevalence of IgG antibodies against *Bordetella pertussis* in healthy individuals aged 4–24 years in Turkey

M. Cevik[1], *U. Beyazova*[2], *A. L. Aral*[3], *A. Duyan Camurdan*[4], *S. Ozkan*[5], *F. Sahin*[2] *and C. Aybay*[6]

[1]Department of Family Practice, Ankara Numune Research and Education Hospital, [2]Department of Social Pediatrics, [3]Department of Immunology, [4]Department of Social Pediatrics, [5]Department of Public Health and [6]Department of Immunology, Gazi University School of Medicine, Ankara, Turkey

ABSTRACT

The distribution of IgG antibodies to *Bordetella pertussis* was investigated in serum samples from 550 subjects, aged 4–24 years, to determine the optimal age for booster immunisation. Levels of antibody to *B. pertussis* antigens were determined using an ELISA that measures a mixture of pertussis toxin, filamentous haemagglutinin and lipopolysaccharide. Geometric mean titres of anti-pertussis antibodies in subjects aged 4–6 years were significantly lower than those in other age groups, which reflects waning immunity following vaccination. High positive titres in older children and adolescents suggested acquired *B. pertussis* infection, and booster doses at the ages of 7 and 15 years are therefore suggested.

Corresponding author and reprint requests: A. Duyan Camurdan, Devlet mahallesi, Sapka Devrimi Caddesi, Yesiloz sitesi 2/5, Eryaman 06374, Ankara, Turkey
E-mail: aysucamurdan@yahoo.com

Keywords Antibodies, *Bordetella pertussis*, ELISA, immunity, seroprevalence, vaccination recommendations

Original Submission: 10 May 2007; **Revised Submission:** 13 September 2007; **Accepted:** 18 October 2007

Clin Microbiol Infect 2008; **14:** 388–390
10.1111/j.1469-0691.2007.01926.x

Even if the cellular and humoral immune responses are both involved in conferring protection against *Bordetella pertussis* [1], determination of the seroepidemiology of pertussis makes possible the evaluation of patterns of pertussis immunity in a given population, and helps define the target population for pertussis booster vaccination [2,3]. The aims of the present study were to determine the distribution of IgG antibodies to *B. pertussis* among different age groups in Turkey, to evaluate the rate of decrease in vaccine-acquired immunity, and to determine the optimal age and frequency for booster immunisations.

Antibody levels to *B. pertussis* antigens were measured in serum samples obtained from 550 (305 male, 245 female) healthy subjects, aged 4–24 years, who visited the Gazi University Medical School well-child clinic, or the paediatric and adolescent health examination clinics, for check-up between April and June 2006, and who did not have a prolonged history of coughing in the preceding month. All study subjects had received whole-cell pertussis vaccine three times in the first year of life, followed by a booster at the age of 18 months. The whole-cell pertussis vaccines used in Turkey for the last 20 years have been obtained from several different foreign companies, and most recently from the Serum Institute (Pune, India). Each single 0.5-mL dose contains diphtheria toxoid ≤25 Lf, tetanus toxoid ≥5 Lf and *B. pertussis* ≥4 IU, adsorbed on aluminium phosphate ≥1.5 mg, with thiomersal 0.01% w/v as preservative (http://www.seruminsitute.com/content/products/product_list.htm). Informed

consent was obtained from all study participants, and the Ethical Committee of the university approved the study.

Serum samples were analysed using a commercial ELISA kit (IBL-Hamburg GmbH, Hamburg, Germany), with a manufacturer's stated sensitivity of >95%. According to the manufacturer's classification, values of <16 U/mL, 16–24 U/mL, and >24 U/mL were considered as negative, equivocal and positive, respectively. Data were analysed using SPSS v.10.0 (SPSS Inc., Chicago, IL, USA), with $p < 0.05$ considered to be significant. Antibody values for the different age groups were analysed by Kruskal–Wallis variance analysis and post-hoc Mann–Whitney *U*-tests. Comparisons of antibody levels for each age group according to gender were done using the Mann–Whitney *U*-test. Antibody levels were given as geometric mean titres (GMTs) according to age group. Percentage distributions of antibody titres in the ranges <16, 16–24, 25–50, 51–125, and >125 U/mL were also calculated for each age group.

The study population had a mean age of 12 ± 5.95 years (range 4–24 years). The population was stratified into four groups according to age: pre-school children (aged 4–6 years, $n = 87$); elementary school children (aged 7–12 years, $n = 162$); high school children (aged 13–18 years, $n = 156$); and young adults (aged 19–24 years, $n = 145$).

GMTs of anti-pertussis antibody in subjects aged 4–6 years were significantly lower than in all other age groups (p 0.0001). The GMT values increased steadily in subjects who had started elementary school, to 17.73 U/mL in the group aged 7–13 years, with the highest value of 51.14 U/mL being in the group aged 13–18 years. The values in the group aged 19–24 years were a little lower (36.27 U/mL), but the difference among these three groups was not statistically significant ($p > 0.05$) (Fig. 1).

Antibody levels ranged between 0.00001 U/mL and 313.58 U/mL. The antibody values were not associated with gender ($p > 0.05$), and analysis of age-stratified data according to gender also revealed no significant difference ($p > 0.05$). The frequency of anti-pertussis IgG titres for the different age groups is shown in Fig. 2. Over half (51.7%) of the children in the group aged 4–6 years were negative for pertussis antibodies, and high-titre positivity was very low (8.1%) in this age group. With increasing age, high-titre positivity increased three-fold in the group aged 7–12 years, reaching 24.7%, and was at its peak frequency (28.6%) in the group aged 13–17 years.

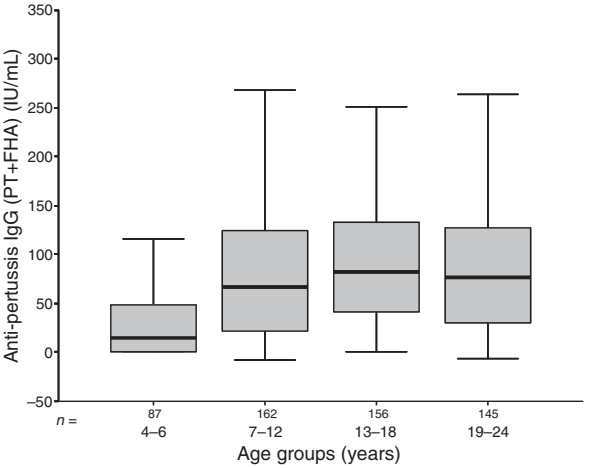

Fig. 1. Occurrence of *Bordetella pertussis* (mixture of pertussis toxin (PT) and filamentous haemagglutinin (FHA)) IgG antibodies according to age group.

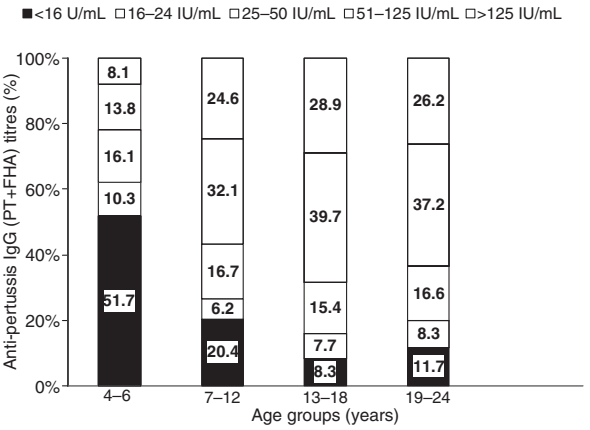

Fig. 2. The frequency of anti-pertussis IgG titres (mixture of pertussis toxin (PT) and filamentous haemagglutinin (FHA)) in different age groups.

Information concerning age-specific antibody levels against pertussis is required to target the appropriate population for booster vaccination [2]. A high degree of protection persists for 3 years after completion of infant immunisation, but the vaccine antibodies begin to decrease 3–4 years after the last dose [2,4–6]. For this reason, a booster dose is given in many countries at the age of 3–6 years [3,7–9]. In Turkey, the final dose of pertussis vaccine is administered at the age of 18 months, and the results of the present study indicate that vaccine-induced immunity decreases after 3–4 years.

The present study has several limitations. First, the sample was based on the population attending a large university hospital and therefore does not represent the entire community. However, the serological trends observed were consistent in many respects with international serosurveys performed with healthy individuals. Second, the ELISA used in this study does not discriminate between responses for pertussis toxin and for filamentous haemagglutinin; positive results could also be obtained if cross-reactive antibodies to filamentous haemagglutinin produced by other bacteria were present in the sera. Nevertheless, this study revealed that the GMT of pertussis antibodies increased after the age of 6 years, with the fastest increase being in the group aged 7–12 years. As there is no booster against pertussis at this age, this increase reflects the acquisition of natural immunity following the beginning of elementary school at the age of 7 years, when children join a new crowded community with a high probability of communicable disease. These children can then become a source of infection for neonates and young infants who have not yet completed their vaccination schedules [10].

In Turkey, diphtheria toxoid is administered in the first and eighth classes of elementary school, when children are aged 7 and 15 years, respectively. As the easiest way to reach older children and adolescents is in schools, addition of pertussis to the school vaccination schedule may be the best means of administering booster vaccination if this is logistically feasible. However, before finalising decisions concerning late childhood and adolescent immunisation against pertussis, evaluation of the health benefits, risks, costs and cost-effectiveness of pertussis vaccination in older children and adolescents in Turkey is necessary.

ACKNOWLEDGEMENTS

This study was supported by GSK Pharmaceuticals, Istanbul, Turkey, who supplied the commercial ELISA kits. The authors have supplied no other information concerning the existence or absence of conflicting or dual interests.

REFERENCES

1. Cherry JD. Immunity to pertussis. *Clin Infect Dis* 2007; **44**: 1278–1279.
2. Van der Wielen M, Van Damme P, Van Herck K, Schlegel-Haueter S, Siegrist CA. Seroprevalence of *Bordetella pertussis* antibodies in Flanders (Belgium). *Vaccine* 2003; **21**: 2412–2417.
3. Cagney M, MacIntyre CR, McIntyre P, Puech M, Giammanco A. The seroepidemiology of pertussis in Australia during an epidemic period. *Epidemiol Infect* 2006; **134**: 1208–1216.
4. Cattaneo LA, Reed GW, Haase DH, Wills MJ, Edwards KM. The seroepidemiology of *Bordetella pertussis* infections: a study of persons aged 1-65 years. *J Infect Dis* 1996; **173**: 1256–1259.
5. Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study. *BMJ* 1988; **296**: 612–614.
6. Dominguez A, Vidal J, Plans P, Salleras L. The seroepidemiology of *B. pertussis* infection in Catalonia, Spain. *Epidemiol Infect* 2001; **126**: 205–210.
7. Wirsing von Konig CH, Campins-Marti M, Finn A, Guiso N, Mertsola J, Liese J. Pertussis immunization in the global pertussis initiative European region: recommended strategies and implementation considerations. *Pediatr Infect Dis J* 2005; **24** (suppl 5): 87–92.
8. Tan T, Halperin S, Cherry JD *et al*. Pertussis immunization in the global pertussis initiative North American region: recommended strategies and implementation considerations. *Pediatr Infect Dis J* 2005; **24** (suppl 5): 83–86.
9. Torvaldsen S, McIntyre PB. Effect of the preschool pertussis booster on national notifications of disease in Australia. *Pediatr Infect Dis J* 2003; **22**: 956–959.
10. Cherry JD, Grimprel E, Guiso N, Heininger U, Mertsola J. Defining pertussis epidemiology: clinical, microbiologic and serologic perspectives. *Pediatr Infect Dis J* 2005; **24** (suppl 5): 25–34.