Pertussis in children in the İstanbul Faculty of Medicine: results for four years

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Received: 24 March 2014, Revised: 4 April 2014, Accepted: 20 May 2014

SUMMARY: Öksüz L, Hançerli S, Somer A, Salman N, Gürler N. Pertussis in children in the İstanbul Faculty of Medicine: results for four years. Turk J Pediatr 2014; 56: 632-637.

We investigated the frequency of pertussis among children in the İstanbul Faculty of Medicine Hospital during a period of four years. Clinical specimens were obtained from children who exhibited symptoms of whooping cough; a portion of the cases were confirmed microbiologically by PCR as pertussis. A total of 410 nasopharyngeal aspirates were taken for detection of *Bordetella pertussis/parapertussis*. The age groups of the patients were 0-4 months (n=201), >4 -12 months (n=49), 1-4 years (n=79), 5-9 years (n=46), 10-14 years (n=27) and >15 years (n=8). 106 (26%) of all samples were positive for *B. pertussis/parapertussis* by the PCR method. The Bordetella PCR positivity rates were 36% in 2010, 29% in 2011, 15% in 2012 and 15% in 2013. Due to administration of the DTaP-IPV vaccination at seven years of age starting in 2010, pertussis was not detected in the 5–9 age group after that year. According to this result, the five doses of pertussis vaccination administered as the national vaccine scheme are effective in protecting against the infection. A booster dose for adolescents at 14 years of age as well as a cocoon strategy might also be considered in our country.

Key words: pertussis, PCR, Turkey.

Pertussis (whooping cough) is an acute infectious illness of the respiratory tract caused by *Bordetella pertussis* and, less frequently, by *Bordetella parapertussis*. The illness occurs worldwide and affects all age groups, but it is recognized primarily in children; it is most serious in young, unimmunized infants[1-4].

The World Health Organization (WHO) suggests that in 2008 about 16 million cases of pertussis occurred worldwide, 95% of which were in developing countries, and that about 195,000 children died from this disease[5]. Effective whole-cell pertussis vaccines became available in the 1940s, and the rate of pertussis was reduced dramatically in countries in which universal immunization of infants and children was implemented. At present, acellular vaccines are in use in many countries throughout the world[1]. Vaccination plays an important role in protection against pertussis. However, the vaccine does not result in lifelong immunity[6]. Even with vaccine use, occasional local epidemics still occur. Pertussis epidemics in the prevaccine era occurred at 2- to 5-year intervals, and these cycles have continued in the vaccine era. Although immunization has controlled the disease, it has not reduced transmission of the organism in the population. Today, pertussis in adolescents and adults is an important source of *B. pertussis* infection in unimmunized and partially immunized children[1]. In Turkey, the pertussis vaccine had been administered in the 2nd, 4th, and 6th months of age, in combination with a booster dose administered in the 18th month, according to national vaccine scheme of the Ministry of Health. In 2010, the number of pertussis cases was 48, the incidence of pertussis was 0.07 per 100,000 population and the mortality rate of pertussis was 0.0 per

This study was presented as a poster at the 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 10-13 May 2014 in Barcelona, Spain.

1,000,000 population in Turkey[7]. Since 2010, a single-dose diphtheria, tetanus toxoid, acellular pertussis and inactive polio vaccine (DTaP-IVP) has also been administered to children at seven years of age.

The aim of this study is to determine the frequency of pertussis in children in the Istanbul Faculty of Medicine Hospital, Turkey. Clinical specimens were obtained from children who exhibited symptoms of whooping cough; a portion of the cases were confirmed as pertussis by PCR.

Material and Methods

Patients

Patients who were admitted to the Pediatric Department of the Istanbul Faculty of Medicine between 2010 and 2013, with pertussis complaints such as coughing with either paroxysms or whooping for 14 days or longer were studied and evaluated by physicians on the basis of the case definition of the US Centers for Disease Control and Prevention[8]. Thus, the aim of this study was to confirm the presence of pertussis according to the CDC clinical definition. Accordingly, 410 children, whose ages ranged from 1 month to 17 years, were included in the present study. Symptoms such as cough or vomiting followed by cough or fever and cyanosis were criteria used in preliminary diagnosis of whooping cough in neonatal infants; a cough persisting for two weeks was an important criterion in the other age groups.

Sample collection:

A total of 410 nasopharyngeal aspirate samples (178 in 2010, 42 in 2011, 40 in 2012 and 150 in 2013) were taken from the posterior nasopharynx for detection of *B. pertussis/parapertussis* by culture and by polymerase chain reaction (PCR) analysis. A pertussis-specific transport medium (Copan, Italy) was used for this purpose. The *Bordetella pertussis/parapertussis* cultures and PCR were done by the same individual in the Medical Microbiology Laboratory of the Istanbul Faculty of Medicine.

B. pertussis/parapertussis *culture*:

The cultures were made on Bordet-Gengou medium. Nasopharyngeal samples inoculated onto the medium were incubated in aerobic conditions at 37°C for seven days.

B. pertussis/parapertussis *PCR*:

The PCR was done directly from the swabs using the rapid identification kit, GenoQuick® *Bordetella* Ver 2.0 (HAIN Lifescience, Germany). Chromosomal regions targeted by PCR in this test included the repetitive insertion sequences *IS481* for *B. pertussis* and *IS1001* for *B. parapertussis*.

Statistical analysis:

Statistical analysis was performed using SPSS Version 10.0 for Windows, and a P value of 0.05 was considered to be statistically significant. The chi-square test and Fisher's exact test were used to examine the differences between groups. Fisher's exact test was used to detect differences between age groups in 2011, 2012 and 2013 because *Bordetella* PCR positivity was found in only two age groups in those years.

Results

The mean age of the patients was 2.4±4.4 years and the age range between 1 month and 17 years. Ninety-nine (24%) of the patients were hospitalized in pediatric intensive care units. The patients were divided into six age groups:

Group 1: 0-4 months (n=201)

Group 2: >4 -<12 months (n=49)

Group 3: 1-4 years (n=79)

Group 4: 5-9 years (n=46)

Group 5: 10-14 years (n=27)

Group 6: >15 years (n=8)

A total of 410 nasopharyngeal aspirate samples were evaluated by culture and PCR in this study. The number of nasopharyngeal samples by month of the year is shown in Figure 1.

The *Bordetella* PCR positivity rates were 36% in 2010, 29% in 2011, 15% in 2012 and 15% in 2013. Although the rate of positivity was low (n=5; 1.4%) by culture, 106 (26%) out of 410 nasopharyngeal aspirate samples were positive for *B. pertussis/parapertussis* by the PCR method. One of the five positive strains detected by culture was identified as *B. parapertussis* using antiserum. Six of ten adults (four physicians, six parents) were also found to be positive for *B. pertussis* by the PCR method. Through analyzing pertussis positivity by age group, high positivity rates were detected for all age groups in 2010, but only for Group 1 (36%) and Group 3 (43%) in 2011. No *Bordetella* PCR positivity was found in >1-year-old children in 2012 or >10-year-old children in 2013

(Fig. 2). Total *Bordetella* PCR positivity rates by age group were 26% in Group 1, 22% in Group 2, 24% in Group 3, 26% in Group 4, 30% in Group 5 and 50% in Group 6. No significant differences were detected between unimmunized or partially immunized children and immunized children in any age group (χ^2=7,068; p=0.315). *Bordetella* PCR positivity rates were significantly higher in 2010 than in other years (χ^2= 21.84; p<0.001). Statistically significant differences were not found among the *Bordetella* PCR positivity rates of the age groups by year (χ^2=4.18, p=0.524 for 2010; p=1.00 for 2011; p=0.637 for 2012; p=1.00 for 2013).

Discussion

In Turkey, routine childhood pertussis immunization with whole pertussis (wP, DTP) has been implemented since 1968. Vaccination coverage remained constant (78% to 83%) between 1991 and 2005[9]. The morbidity rate of pertussis decreased from 7.58/100,000 in 1975 to 0.10/100,000 in 2005 and the mortality rate from 0.62/1,000,000 in 1975 to 0.0/1,000,000 in 2005 as a result of the vaccination program in our country[10]. Acellular pertussis vaccine (acP) has been administered since 2007[11,12]. The vaccination rate in the 0-1 age group has reached over 90% in our country[20]. The immunization coverage rates of DaPT 1, 2 and 3 were 98%, 98% and 97%, respectively, in 2011[10]. It was reported that incidence of pertussis in Turkey, except for the East Anatolia region, appeared to have reached the WHO target, with an incidence of <1 case per 100,000[13].

According to the results of our study, the positivity rates for *B. pertussis* in all age groups were quite high in 2010. This might be linked to a possible outbreak during this period. The most remarkable positivity rates were in the 4-12-month (47%) and >15-year age groups (67%) in that year. High positivity rates were also detected in the 0-4-month and 1-4-year age groups in 2011: 36% and 43%, respectively. *Bordetella* PCR positivity was found only in children under 1 year (20%) in 2012 and only in children under 10 years (<1 year, 1-4-year and 5-9-year age groups: 17%, 7% and 9%, respectively) in 2013 (Fig. 2). In total, half of children aged over 15 years, one-third of children aged 10-14 years and approximately one-fourth of the other age groups were positive for *Bordetella* by PCR. The overall incidence of pertussis among all patients was 26%. Two hundred and fifty (61%) of the patients were <1 year of age (Fig. 2) and had not completed the three-dose primary immunization series (unimmunized, n=142; partially immunized, n=108). This situation may explain the high positivity rates in the present study. This study found the incidence of pertussis in children under 1 year of age to be 25%. It was reported that in 2005, the incidence for the same age group was 29% in Turkey[10].

Immunity to pertussis following natural infection or vaccination wanes after 5 to 12 years. As a result, *B. pertussis* is a significant cause of respiratory disease in older children and adults[14]. This is confirmed by the high positivity rates of children aged >15 years in this study. Even though only up to 6.5 % of cases were ≥15 years of age prior to 2005, in that year 16.9 % of cases occurred in this age group. Consequently, it could be seen that the four doses of infant pertussis vaccination administered in Turkey were not sufficient to provide lasting protection against the infection. A large number of schoolchildren, adolescents and adults remained susceptible to pertussis infection[11]. Therefore, in 2010, a single booster of aP vaccine in combination with tetanus toxoid, reduced-dose diphtheria and inactive polio (DTaP-IPV) vaccination started to be given at seven years of age (in the first year of primary school) in Turkey. According to the results of the present study, despite the high rates of pertussis found in this age group in 2010, pertussis was not detected in the same group in subsequent years (Fig. 2).

In the past, epidemic pertussis generally had no seasonal pattern. However, in the present

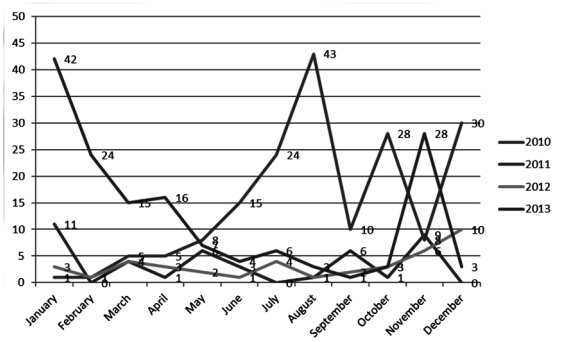

Fig. 1. Number of nasopharyngeal samples by month (n).

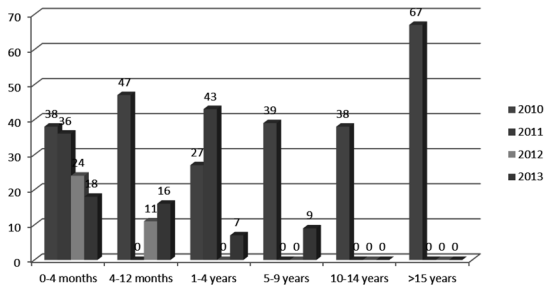

Fig. 2. Positivity rates for *Bordetella* PCR by age group (%).

vaccine era in North America, pertussis usually occurs in the summer and fall[1, 15]. We also detected some linkage between pertussis and the seasons, with the rates high in August (43%) and December (30%) in 2010 and in January (42%) in 2013 (Fig. 1). Similarly, high pertussis rates in January, April and December have been reported for other cities in our country[10]. In European countries, reported pertussis cases did not display seasonal patterns in 2011. The lowest numbers were reported in April; numbers then gradually increased, reaching a high in December[16].

The laboratory diagnosis of pertussis can be made by culture, by direct fluorescent antibody (DFA) testing, by PCR, and by serological methods[1, 17]. The greatest sensitivity is obtained when culture is supplemented by PCR and serologic testing[17]. Culture provides the most specific diagnosis of pertussis. The sensitivity of culture varies greatly depending on patient factors (including antibiotic therapy, duration of symptoms, age and vaccination status), specimen transport conditions, the type and quality of media used and other conditions. While PCR testing is also affected by these factors, culture is affected to a greater degree because of its requirement for viable microorganisms. While culture is virtually 100% specific, its sensitivity is often low due to poorly collected specimens, long specimen transport time and patient factors. PCR generally offers greater sensitivity than does culture. PCR has frequently been shown to provide the specific diagnosis of pertussis when culture is negative[14]. PCR has consistently been reported to be more sensitive than DFA or culture. Because of its greater sensitivity and its ability to detect dead organisms, PCR remains positive longer during the course of disease than does culture, and is also more likely to remain positive following antibiotic therapy[14,18]. Therefore, in the present study, definitive diagnosis was confirmed by the PCR method in all patients with pertussis-like clinical clues. The detection of *B. pertussis/parapertussis* was made by using a rapid identification kit, reported as being a fast, easy and valid test for diagnosis of pertussis[19]. According to the validation data of the manufacturer, the sensitivity of the rapid identification kit is 100%, and the specificity is 98%. In this study, the positivity rates of *B. pertussis* obtained by culture were very low compared to PCR results. Out of 410 samples, a positive result for *Bordetella* spp. was obtained in only 5 cultures (1.4%), versus 106 positive results (26%) using PCR. Based on the data of this study, the *Bordetella* PCR method was 20-fold more sensitive than culture in the diagnosis of pertussis. Internal and external controls for PCR testing were performed in our study.

The fact that the isolation of *B. pertussis* in culture was low in this study may be due to inappropriate sample transport conditions, to inadequate samples being obtained or to difficulties in growing the bacterium. These results are consistent with those of many other studies. Yildirim et al.[20] studied the frequency of pertussis in children in Turkey using culture and PCR, and could not isolate any *B. pertussis* in culture. They diagnosed all cases by PCR and/or serology[20]. In a prospective study in which swabs for PCR and culture were obtained from 555 patients, the use of PCR increased the identification of *B. pertussis* infection almost fourfold, from 28 to 111[1].

In many studies carried out in our country, it has been reported that cases of pertussis among children, adolescents and adults have increased[11,13,20-23]. Most of these studies were performed using the ELISA method. Aksakal et al.[21] found that 51 of 307 schoolchildren (16%) were positive with anti-pertussis toxin IgG. The authors suggested that priority should be given to extending primary immunization and implementing booster immunization[21]. In a study of 997 healthy students aged 9-17, Duranoglu et al.[24] reported that antibody titers against pertussis decreased in the younger age groups, resulting in an increased of number of pertussis cases. Inandi et al.[25] revealed that the seroprevalance of pertussis was 30% in healthy children aged 0-71 months. The authors suggested that the current vaccination program

did not provide adequate protection[25]. Esen et al.[22] reported that up to half of expectant mothers did not have a sufficient level of antibody titers against pertussis. The authors recommended revaccination with acellular vaccine for schoolchildren and pregnant women[22]. Cevik et al.[26] investigated IgG antibodies to *B. pertussis* in 550 serum samples from subjects aged 4-24 years to determine the optimal age for booster immunization. They suggested that booster doses should be administered at the ages of 7 and 15 years[26]. In a study that detected the frequency of pertussis using culture, PCR and serology, it was found that the overall incidence of pertussis was 16.9% [20].

In infants, the source of infection cannot be identified in 30% to 69% of cases. In cases in which the source of infection can be traced, half of the children have been infected by their parents, usually by the mother. Older siblings are another frequent source of infection even if they have been vaccinated, because their immunity will have waned in the absence of a booster vaccination. Studies have shown that in older children, a single dose of vaccine with reduced antigen content induces a sufficient immune response in 90% of cases[4]. The increased number of cases reported in adolescents and adults are attributable to waning immunity. The changing epidemiological pattern presents challenges for disease control strategies. The most widely accepted strategies are universal adolescent immunization and the "cocoon" strategy (immunization of family members and close contacts of the newborn)[27].

According to the Annual Epidemiological Report 2013 of the European Centre for Disease Prevention and Control (ECDC), in 2011 the number of cases of pertussis increased for the first time since 2008. Notification rates varied widely among countries, ranging from 0.2 to 89.5 per 100,000, with northern European countries (Estonia, Norway, the Netherlands and Finland) and central European countries (Slovakia and Slovenia) displaying higher case rates. In 2011, the notification rate of pertussis decreased with increasing age. The age group most affected in countries with higher case rates was 5–14-year-olds (15 cases per 100,000 population); however, when taking into account all countries, 0–4-year-olds were the most affected age group (15.8), as a few less-affected countries exhibited significantly higher rates in this age group (Denmark, Ireland and Spain) [16].

Guiso et al.[6] reported that circulation of the bacterium had not been controlled in the adult population, and that universal adult booster immunization could be possible using pertussis acellular vaccines, which target the virulence of this bacterium. Pertussis experts emphasize the need to vaccinate adults. Recommendations for adult vaccination have already been established in North America and some European countries[6]. Booster vaccination at 4 to 6 years or 11 to 12 years is administered in many countries[11,15]. DTaP-IPV administration started in 2010 in our country. Acellular booster doses are licensed in Australia, Austria, Canada, France, and the USA for administration to adolescents or adults[1]. In the present study, adults who were in direct contact with patients diagnosed with pertussis were investigated if they reported coughing for two weeks or more. Six of ten adults (four physicians, six parents) were also found to be positive for *B. pertussis* by the PCR method. Future studies need to investigate the incidence of pertussis in adults in Turkey.

It is important to be aware that pertussis infection is no longer solely a pediatric infection; case numbers are on the rise in adolescents, adults and children too young to be vaccinated. Waning immunity after vaccination and an absence of natural boosters is contributing to a lack of immunity in adolescents and adults, despite high vaccination coverage in younger age groups. This may create a pool of susceptible individuals who can act as a source of transmission and contribute to rising incidence rates and outbreaks. Vaccine strategies should be revisited and consideration given to adolescent and adult boosters, as well as to vaccinations for healthcare workers and pregnant women, as these measures are essential for prevention[16].

Consequently, although pertussis vaccination is part of the routine vaccination protocol in our country, whooping cough still remains an important health problem. The five doses of pertussis vaccination administered in Turkey are not sufficient for long-lasting protection against the infection. A large number of schoolchildren, adolescents and adults are susceptible to pertussis infection, and therefore improvement in the vaccination procedures in

our country is necessary. In order to protect infants from pertussis, parents, siblings and other individuals who come in contact with affected infants must have complete immunity against pertussis. In addition to sustaining a high rate of pertussis vaccine coverage in infants, a booster dose for adolescents (in the eighth year of primary school) and a cocoon strategy might be considered in our country.

Acknowledgement

The authors would like to thank to Sevda Özel, PhD, from the Department of Biostatistics and Medical Informatics of the Istanbul Faculty of Medicine for her assistance with statistical analysis.

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