Pertussis Serosurveillance Study in Izmir, Turkey
by Ebru Türkoglu,¹ Cemile Sönmez,² Zafer Kurugöl,¹ Nilay Çöplü³ and Guldane Koturoğlu¹

¹Department of Pediatrics, Ege University
²Microbiology Reference Laboratories, Turkish Public Health Institution
³Microbiology Department, Diskapi Yıldırım Beyazıt Training and Research Hospital

Correspondence: Microbiology Department, Diskapi Yıldırım Beyazıt Training and Research Hospital. E-mail <nilaycoplu@gmail.com>.

SUMMARY
Pertussis is a life-threatening, vaccine-preventable infection. Adults who can be asymptomatic may infect infants. The aim of this study is to determine the IgG antibody levels against pertussis toxin (PT) and filamentous hemagglutinin from 6 months to 60 years in Izmir, Turkey. A cluster sample design developed by Expanded Programme on Immunization of the World Health Organization was carried out for the selection of the study population, which consisted of 399 healthy subjects. In-house ELISA was studied in Turkish Public Health Institution. Antibody levels of <10 EU/ml, ≥10 EU/ml and ≥100 EU/ml were accepted as non-immune, immune and possible acute/recent infection, respectively. Anti-PT antibody levels were 8.5% <10 EU/ml, 68.2% 10–100 EU/ml and 23.3% ≥100 EU/ml; the latter was correlated with possible acute/recent infection. Results showed that pertussis is endemic, particularly among adolescents and adults, which is a threat for infants who have not completed their primary immunization.

KEYWORDS: Bordetella pertussis, serosurveillance, ELISA

INTRODUCTION
Pertussis caused by Bordetella pertussis is a highly contagious and life-threatening vaccine-preventable respiratory infection. It can cause morbidity and mortality among young infants who are not immunized or have not completed their immunization schedule [1]. In 2013, 7.69/100 000 cases and 9 deaths were reported to Centers for Disease Control and Prevention [2]. In Turkey, acellular pertussis vaccine is being administered in the 2nd, 4th and 6th months of life, in combination with booster doses administered at the 18th months and at the age of 6 years [3]. After participation in the Expanded Programme on Immunization (EPI) of the World Health Organization (WHO), vaccine coverage (three doses) reached at a level of about 80% among children less than 6 years of age. It was found that third-dose vaccination coverage increased from 45% (1986) to 96% (2010) and the incidence of laboratory-confirmed pertussis decreased from 2.03/100 000 in 1986 to 0.008/100 000 in 2007 [4]. According to data of Ministry of Health of Turkish Republic, the number of pertussis cases notified were 72, 57, 52 and 21 for 2005, 2006, 2007 and
2008, respectively, showing a decrease at the year when this study was performed. On the other hand, studies conducted in our country shows that there is evidence of whooping cough infection and it might be higher than the reported cases [5–9]. Because neither infection nor vaccination confers long-lasting immunity to subsequent infection or disease, the infection in adolescents and adults that may be atypical or asymptomatic are often source of the infection for pediatric cases those had not yet completed the primary immunization, and may be complicated for the patient, as well. This fact has given rise to the need for seroepidemiological studies where ELISA is usually a preferred technique. The ELISA test is sensitive, specific, relatively cheap and requires only a small amount of serum. The use of ELISA to quantify anti-pertussis toxin (PT) IgG antibody levels can be performed with paired (acute and convalescent phase) or single serum samples [10].

In this study our aim is to determine the antibody levels against *B. pertussis* toxin (PT) and filamentous hemagglutinin (FHA) in ages from 6 months to ≥60 years in Izmir, Turkey, to evaluate the immune status of the population and the need for assessment for vaccination policy.

**MATERIALS AND METHOD**

**Study population**

This seroepidemiological study was conducted in the third largest city of Turkey, in Izmir, in March 2008. A cluster sample design developed by EPI of the WHO for the surveys of immunization was carried out for the selection of the study population. According to State Institute of Statistics data, 30 clusters of samples for both urban and rural areas were selected. The number of selected samples was determined according to the total population of areas. Selection procedure was carried out by creating a cumulative list of community populations and selecting systematic samples randomly. Subjects were classified into 10 age-groups. A starting household was selected in each community by locating the ward’s center, randomly selecting a house from a list of all houses falling along the line drawn from the ward center to the periphery in the chosen direction. The house was then examined to determine whether the subject of eligible age and sex was living there. Subsequently, the nearest household to the right was visited and the steps repeated until the desired number of persons was obtained. The study population consisted of 399 healthy subjects of 0–2, 3–4, 5–6, 7–9, 10–14, 15–19, 20–29, 30–39, 40–59 and ≥60 years and the number of subjects for each age-group were 30, 31, 37, 42, 37, 35, 48, 50, 49, 38, respectively. In these subjects, 152 were male and 247 were female.

The Human Ethics Committee of Ege University had approved this study.

**Laboratory tests**

Blood samples were collected from each participant and the sera were stored at −80°C until tested. ELISA tests were performed in Public Health Institution of Turkey in 2008. An in-house ELISA test was performed for this purpose and 399 serum samples were tested. The test was conducted by using 96-well flat-bottom plates (Greiner, 655001, Frickenhausen, Germany). Purified PT 10 μg PN/ampoule (JNIH-5, Biken, Japan) and purified FHA 10 mg PN/ampoule (JNIH-4, Biken, Japan) were used for coating the plates (100 ml at 0.1 mg PN/ml for PT and 0.04 mg PN/ml for FHA in 0.05 M carbonate bicarbonate buffer, pH 9.6), which were kept in a refrigerator in humid atmosphere for 48 hours. On the test day, firstly the plates were blocked by adding 125 ml of blocking buffer [phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA)] and incubated for 1 hour at 37°C on an Incubator/shaker (Labsystem iEMS), Helsinki, Finland). This shaker was used for every incubation period. After every step, plates were washed three times with PBS containing 0.05% Tween 20 (PBS-T). Eight 2-fold serial dilutions of test sera and reference serum {anti-Pertussis Reference human sera IgG [250 ELISA Unit (EU) for anti-PT IgG, 400 EU for anti-FHA IgG, Biken, Japan]} in PBS containing 0.5% BSA and 0.05% Tween 80 were added. The following steps, which were Fc-specific alkaline phosphatase-conjugated goat anti-human IgG (Seikagaku, Kogyou, Tokyo, Japan) diluted in PBS-T, P-Nitrophenyl phosphate (Sigma) diluted in diethanolamine buffer (1 mg/ml, pH 9.6) and 3 M NaOH is used for stopping stage. Plates were read at
A 405/630 on an ELISA reader (Labsystem, Multi Skan EX, Helsinki, Finland). The anti-PT and anti-FHA IgG antibody titers were calculated by the parallel line assay (\( p = 0.05 \)). The limit of detection for both antibodies was 1.0 EU/ml [11].

**Evaluation of the results**

According to WHO criteria, anti PT IgG levels \( \geq 100 \) EU/ml is regarded as recent pertussis exposure [10]. Although protective levels have not been established according to WHO, in this study, cases with anti-PT IgG levels \( \geq 10 \) EU/ml were regarded as ‘protected’ arbitrarily, based on the reports where the lowest antibody levels were among children recovering from pertussis [12]. Although antibodies to PT are specific for \( B. \) pertussis according to WHO and so only these antibodies can be used in serosurveys as an estimate of the circulation of \( B. \) pertussis, we have studied anti-FHA antibodies as a supporting evidence, as well. The results are presented by the distribution of the same cutoff values as anti-PT antibodies, to show the accordance between the two antibodies.

**Statistical analysis**

Statistical analysis was performed using SPSS for Windows 15.0. Descriptive statistics was given as number and percentages for categorical variables, mean and standard deviation for numerical variables. (Ratios of categorical variables between groups were tested with chi-square test. A \( p \) value of \( <0.05 \) was taken to be statistically significant.

**RESULTS**

The age of the subjects ranged from 6 months to 85 years, with a mean of 24.1 ± 20.68 years.

**Anti-PT IgG antibody levels**

Of the study population, 8.5% had <10 EU/ml, 68.2% had 10–100 EU/ml and 23.3% had \( \geq 100 \) EU/ml anti-PT IgG antibodies, and the last percentage indicates the recent pertussis exposure. When antibody levels were evaluated according to age-groups, the incidence of recent pertussis exposure (\( \geq 100 \) EU/ml) was lowest (18.4% and 18.9%) among \( \geq 60 \) and 5–6-year-olds, increased in the school age children and was highest (34.3%) among 15–19 years old. On the other hand, the percentage of anti-PT IgG for the levels <10 EU/ml was lowest among 10–14 years (Fig. 1).

**Anti-FHA IgG antibody level**

Two serum samples were insufficient for determining anti-FHA IgG antibody levels; therefore, 397 subjects were evaluated for anti-FHA IgG. Of the total study population, 7.8% had <10 EU/ml, 58.2% had 10–100 EU/ml and 34% had \( \geq 100 \) EU/ml anti-FHA IgG antibodies. When anti-FHA IgG levels were evaluated according to age-groups, it was lowest among 7–9 and 15–19-year-olds for the levels <10 EU/ml. Antibody levels \( \geq 100 \) EU/ml increased from the school age, 7–9 years olds, and was highest (48.6%) among 15–19-year-olds. These findings support anti-PT IgG antibody data (Fig. 2).

**DISCUSSION**

In this study, we determined the age-specific presence and levels of \( B. \) pertussis antibodies based on sero-epidemiological survey among healthy population between 6 months and 85 years of age by using ELISA test. Recent pertussis exposure was found 23.3% of all sera according to anti-PT IgG levels. The incidence of recent pertussis exposure was highest in the 15–19 years age-group (Fig. 1). Besides, presence of below protective level was 8.5% all together, and among <10-year-olds and >40-year-olds, the vulnerable group is higher than the other age-groups. Especially newborns and younger children may have serious infection with complications, and when evaluated together with the frequency of recent pertussis exposure, it is obvious that there is a considerable risk for infection for younger population. This finding is in accordance with other studies conducted in our country [6, 11]. On the other hand, newborns are expected to have protective levels of antibodies, which are acquired from the mothers, but it is something related with the antibody levels of the mothers. The half-life of anti-PT and anti-FHA IgG antibodies are 36 and 40 days, respectively, and transplacental passage of the antibodies is shown to be 150% for both of the antibodies [10, 12, 13]. With regard to these findings the estimated antibody level, which might be protective until the first dose of vaccine, was
shown to be lacking with 31.2% and 42.9% among supposed to be mothers in previous studies in Turkey [6]. In our study the youngest age-group is 0–2 years, who might include children those have had their primary vaccination schedule completed, so 9.7% have not-protective levels of antibodies. Besides this group, 19.4% show recent pertussis exposure, which might be due to vaccination, as well.

Pertussis can be complicated for the adolescents and adults in addition to be a source of transmission for younger age-groups. According to a study performed among 539 adult patients in Turkey who had prolonged cough of more than 14 days, 9.7% of them had anti-PT IgG level $\geq 100$ EU/ml and three of the patients had chronic obstructive lung disease and/or Angiotensin Converting Enzyme inhibitory drug use history [14]. Besides, there are considerable pertussis knowledge gaps among physicians, especially for adolescents and adults, which leads to misdiagnosis like asthma or screening for cancer, which have large health-care cost [15]. Our study showed the serological evidence of presence of pertussis in every age-group pointing out that pertussis should be taken under consideration for every patient with prolonged cough history.
According to the findings of this study, the evidence of recent pertussis exposure and the percentage of vulnerable group points out that there is need for adult vaccination policy including expected to be mothers and/or pregnant women. In this way, young children should be protected from the exposure of \textit{B. pertussis}, which is clearly in circulation in our country.

REFERENCES