Tohoku J. Exp. Med., 2007, **211**, 353-358

High Incidence of Pertussis among Schoolchildren with Prolonged Cough in Turkey

Fatma Nur Aksakal,[1] Nilay Çöplü,[2] Mustafa N. Ceyhan,[1] Cemile Sönmez,[2] Seçil Özkan,[1] Berrin Esen,[2] Mustafa N. Ilhan[1] and Remzi Aygün[1]

[1]Department of Public Health, Gazi University Faculty of Medicine, Ankara, Turkey
[2]Communicable Diseases Research Department, Refik Saydam National Hygiene Center, Ankara, Turkey

Aksakal, F.N., Çöplü, N., Ceyhan, M.N., Sönmez, C., Özkan, S., Esen, B., Ilhan, M.N. and Aygün, R. *High Incidence of Pertussis among Schoolchildren with Prolonged Cough in Turkey.* Tohoku J. Exp. Med., 2007, **211** (4), 353-358 —— Older children, adolescents and adults with prolonged cough should be screened for pertussis, because they can be a major source of transmission for infants who are not yet fully immunized. This study aimed to estimate the incidence of pertussis among schoolchildren of 6-14 years old with prolonged cough attending two government primary schools in central Ankara. A questionnaire and an informed consent form were sent to the parents of 1,859 schoolchildren. The former comprised questions regarding sociodemographics, and the presence and duration of cough. Parents of 1,698 (91.3%) schoolchildren returned the questionnaire. Three hundred and eighty three (22.6%) of the schoolchildren had cough for more than two weeks, and 307 (80.2%) of them, whose parents gave consent, were included in the study. Their blood samples were collected twice, with a two-week interval. Anti-pertussis toxin IgG levels were measured by enzyme-linked immunosorbent assay (ELISA). Fifty-one children (16.6%) had evidence of recent pertussis infection documented by either a titer ≥ 100 ELISA Unit (EU)/ml in one of two serum samples or seroconversion in paired sera. None of the schoolchildren who had attended health care facilities for cough had been diagnosed as pertussis by a physician. In conclusion, pertussis is evident among older children and adolescents in Turkey, and in order to control the disease, priority should be given to strengthen primary immunization services along with the implementation of booster vaccinations beyond childhood. ———— pertussis; incidence; school children; Turkey
© 2007 Tohoku University Medical Press

Bordetella (*B.*) *pertussis* causes 50 million infections and 300,000 deaths annully, with an estimated fatality rate of 4% in infants (WHO 2003). In Turkey, in 2004, the surveillance system for pertussis was renewed, using both clinically-suspected and laboratory-confirmed case definitions (Guideline for Standardized Diagnosis, Surveillance and Laboratory 2004). In 2005, there were 200 clinically-suspected cases and 72 laboratory-confirmed cases, for overall incidence of approximately 0.01 per million population (The Annual Statistics for 2005 [2006]). However, the exact figure is estimated to be higher, since clinicians and general practitioners may overlook per-

Received January 16, 2007; revision accepted for publication February 26, 2007.
Correspondence: Dr. F. Nur AKSAKAL, Dostlar Sitesi C-Blok No:26, 06520 Balgat, Ankara, Turkey.
e-mail: naksakal@gazi.edu.tr, nuraksakal@gmail.com

tussis cases or fail to report them (Cagney et al. 2005; Dworkin 2005).

Pertussis rarely is considered and clinically diagnosed in older children, adolescents and adults but transmission of infection to infants causes considerable fatality (Cherry 2005; Wirsing von König et al. 2005; Hu et al. 2006). The waning of vaccine-induced immunity after 5-10 years is considered to be a major cause of an immunity gap within these age groups (Cherry 2005). It is argued that individuals with prolonged cough of more than two weeks should be screened for pertussis at any age (Robertson et al. 1987; Hu et al. 2006).

The aim of the current study was to estimate the incidence of pertussis among schoolchildren of 6-14 years old with prolonged cough attending two primary schools in Ankara.

Materials and Methods

Study population and design

The study was conducted in November 2004 in two government primary schools in central Ankara with a total of 1,859 students of 6-14 years old. A questionnaire was sent to parents of the children, along with an informed consent form, asking questions about the sociodemographics, the presence of an infant younger than six months in child's home, vaccination status against pertussis, history of chronic illness, and the presence and duration of cough. From the two schools, 862 (93.2%) and 836 (89.5%) questionnaires were returned by parents, for an overall total of 1,698 (91.3%). The parents of 207 and 176 students from the first school (24.0%), and second school (21.0%) respectively, reported that their child was suffering from cough for more than two weeks with a total of 383 (22.6%). Out of these children, 25 could not be located at school, 33 children's parents did not give consent, 9 were excluded because of insufficient serum samples, and 9 were excluded because of chronic illness. As a result, a total of 307 children (80.2% of 383) with prolonged cough whose parents signed the consent form were enrolled in the study and their blood samples were collected at the time of the first visit. In order to detect the seroconversion, two weeks later, a second visit was performed to schools. The second blood samples were drawn, and a second questionnaire was sent to parents including questions about persistance of the cough, attendance at any health-care facility and any diagnoses for cough made by a pyhsician.

Prior to all data collection, ethics approval for the study was obtained from the Gazi University Medical Faculty Ethics Committee and from the provincial health and education directorates of Ankara province.

Laboratory Tests

Serum IgG antibody levels against pertussis toxin (anti-PT) were measured by in-house enzyme-linked immunosorbent assay (ELISA) at the Refik Saydam National Hygiene Center, in the Communicable Diseases Research Department as described by Vatansever et al. (2005). The reference antisera was included in each test run as quality control (Çöplü et al. 2005; Vatansever et al. 2005). The limit of detection for anti-PT IgG was 1.0 ELISA Units (EU)/ml.

Table 1. Distribution of anti-PT antibody titers

		Anti-PT antibody				
		First samples				
Age group (n)	GMT*	< 10.0 *n* (%)	10.0-50.0 *n* (%)	50.1-100.0 *n* (%)	100.1-200.0 *n* (%)	≥ 200 *n* (%)
6-8 (150)	24.67	30 (20.0)	77 (51.3)	24 (16.0)	11 (7.4)	8 (5.3)
9-11 (90)	40.83	10 (11.1)	44 (48.9)	20 (22.2)	10 (11.1)	6 (6.7)
12-14 (67)	25.54	7 (10.4)	46 (68.7)	9 (13.4)	5 (7.5)	-
Total	31.25	47 (15.3)	167 (54.4)	53 (17.3)	26 (8.5)	14 (4.5)

% Row percent.
*$p = 0.083$, **$p = 0.281$.

Evaluation of immune status and case definition

A case of pertussis was considered when four-fold or greater increase in anti-PT antibody level was detected in paired sera (WHO 2003; Versteegh et al. 2005a). Anti-PT levels ≥ 100 EU/ml was selected as predictive of recent infection with *B. pertussis* arbitrarily.

Statistical analysis

For the description of subjects' characteristics, median and percents were calculated. For evaluation of antibody titers; Geometric Mean Titers (GMTs) were calculated and titers were compared between age groups by analysis of variance (ANOVA) after logarithmic transformation.

Results

The prevalence of cough for more than two weeks was found to be 24.0% (207/862) and 21.0% (176/836) at the two schools, with overall prevalence of 22.6% (383/1,698). The median duration of cough was two weeks (range, 2 to 5 weeks). The numbers of children in 6-8, 9-11 and 12-14 years age groups were 150 (48.9%), 90 (29.3%), and 67 (21.8%), respectively (Table 1). The median age of the evaluated children was 9 years, and 139 (45.3%) of them were male. The vaccination status was unkown for 148 (51.4%) children, whereas 81 (28.1%) children were vaccinated and 59 (20.5%) children were not vaccinated due to the family history.

All of the 307 students initially tested were accessed and their blood were sampled during the second visit. Cough was persisting in 166 (54.1%) of the students. The distribution of anti-PT antibody titers at the time of the first and second visits were shown in Table 1.

A total of 49 (15.3%) children had anti-PT antibody levels ≥ 100 EU/ml in one or both samples. Children with an antibody level ≥ 100 EU/ml in one or both samples in 6-8, 9-11 and 12-14 years age groups were 14.7, 23.3 and 8.9%, respectively. There was no statistically significant difference between GMTs with respect to age group for both samples. The rate of seroconversion was 1.6% (5/307).

Fifty-one children (16.6%) had evidence of acute/recent infection (Table 2). Male to female ratio among children with recent infection was 24/27. Children with acute/recent infection in 6-8, 9-11, and 12-14 years age groups were 24 (47.0%), 21 (41.2%), and 6 (11.7%), respectively. The vaccination status of 47 (92.2%) of the cases was documented, amongst whom only 5 (10.6%) had a positive history of vaccination.

Among the 307 evaluated children, 81 (26.6%) had attended a health care facility. None of them was diagnosed as pertussis by a physician. Median age, gender distribution and clinical diagnoses of evaluated children with or without evidence of recent infection was shown in Table 2.

in first and second samples by age groups.

titers (EU/ml)					
	Second samples				
GMT**	< 10.0 *n* (%)	10.0-50.0 *n* (%)	50.1-100.0 *n* (%)	100.1-200.0 *n* (%)	≥ 200 *n* (%)
26.39	28 (18.7)	77 (51.3)	23 (15.3)	15 (10.0)	7 (4.7)
41.38	11 (12.2)	43 (47.8)	17 (18.9)	10 (11.1)	9 (10.0)
28.21	7 (10.4)	43 (64.2)	11 (16.4)	5 (7.5)	1 (1.5)
32.27	46 (15.0)	163 (53.1)	51 (16.6)	30 (9.8)	17 (5.5)

TABLE 2. Median age, gender distribution and clinical diagnoses of evaluated children with or without evidence of recent infection.

	Children with evidence of recent infection (n = 51)	Children without evidence of recent infection (n = 256)
Median age (years)	9	9
Gender (male/female)	24/27	115/141
Clinical diagnoses (n = 59)		
Common cold	7	23
Bronchitis	2	12
Sinusitis	2	7
Pneumonia	1	2
Adenoid infection	-	2
Acute Otitis Media	-	1

DISCUSSION

Cough is a symptom commonly encountered in daily life, with overall prevalence estimated between 15 and 26% (Chang and Powell 1998; Faniran et al. 1998; Cagney et al. 2005). The overall prevalence of cough was found as 22.6% in this study which is consistent with prior reports.

B. pertussis infection can be diagnosed reliably by means of anti-PT levels ≥ 100 EU/ml by ELISA as well as documentation of seroconversion in paired sera (de Melker et al. 2000; WHO 2001; Versteegh et al. 2005a). We selected the same ELISA level as predictive of recent infection with *B. pertussis* in this study. However, the drawback of this study is that the reference sera were not calibrated against United States-The Food and Drug Administration standard. In addition, an equivalent titer for cut-off (≥ 100 EU/ml) as reported by European Sero-epidemoiolgy Network (ESEN) group (Giammanco et al. 2003) was not set.

In this study, acute/recent infection with *B. Pertussis* was documented by either a high level of antibody titer (≥ 100 EU/ml) in one sample or the presence of seroconversion in paired serum samples. Around 17% of the children with prolonged cough were found to have laboratory evidence of acute/recent infection with these criteria. A study conducted in Edirne, Turkey, among 12-17 years old adolescents reported recent infection incidence of 22.5% (Vatansever et al. 2005). This figure is slightly higher than the incidence reported here but the difference may be attributed to the younger age range of our study population. In another study performed in a healthy population of ages between 6 months to over 60 years from three different locations in Turkey, the distributions of antibody levels ≥ 100 EU/ml in 6-7, 8-9 and 10-19 years olds were 13.7%, 17.5% and 9.3%, respectively (Esen et al. 2007). These results are similar in general to our findings, except that children with antibody titers ≥ 100 EU/ml in 9-11 age group is found to be higher than the figure reported for 8-9 year olds by Esen et al. (2007). This may be because the schoolchildren were encountering with the infection in older ages when they were around 10-11 years old.

The children recently infected with *B. pertussis* in this study did not have young infants in their home, but there was a baby younger than 6 months in the home of 3 children with very low levels of anti-PT antibodies (< 10 EU/ml), who therefore are susceptible and are at risk for pertussis infection in the near future.

The efficacy of the pertussis vaccine is more than 80% when at least three doses of vaccine are administered in proper conditions, but the vaccine-acquired immunity wanes in 5-12 years (WHO 2003; Cherry 2005). In Turkey, a whole-cell pertussis vaccine is administered at 2nd, 3rd

and 4th months of life, with a booster dose at 16-24 months, together with diphtheria and tetanus toxoids (DTwP). No additional dose beyond this age is present in the schedule. In this study, the vaccination status of 47 (92.2%) of the pertussis cases was documented, amongst whom only 5 (10.6%) had a positive history of vaccination. Although the family history of vaccination may be subject to recall bias, this result suggests that the failure in primary vaccination may be a significant contributory factor of high pertussis incidence among this age group. The pertussis vaccination coverage (three doses) has reached a level of about 80% among children following the National Vaccination Campaign of 1985 and was maintained around this level for the following years with improvement in vaccination practices by the Ministry of Health of Turkey (The Annual of Statistics for 2001 [2002]; The Annual of Statistics for 2005 [2006]) and waning of vaccine-induced immunity is also likely to be the other cause which was reported in other studies as well (WHO 2003; Cherry 2005).

About one third of the evaluated children had attended a health care facility. Most of them have been diagnosed with common cold, followed by bronchitis and sinusitis. Versteegh et al. (2005b) have reported the most common pathogens in prolonged cough as rhinovirus (32%), *B. pertussis* (17%), and respiratory syncytial virus (11%). The frequency of mixed infections was also reported to be high. The other pathogens could not be reported in our study but the incidence of pertussis was found to be similar to the figure reported although none of the cases were diagnosed with pertussis. This argues that, even though prolonged cough is a classic symptom of pertussis (WHO 2003; Guideline for Standardized Diagnosis, Surveillance and Laboratory 2004; Versteegh et al. 2005b), likely many cases of pertussis are missed by physicians similar to what other investigators reported (Robertson et al. 1987; Chang and Powell 1998; Faniran et al. 1998; Cagney et al. 2005; Dworkin 2005; Hu et al. 2006). Another contributory factor for underdiagnosis may be the lack of access to laboratory confirmation.

Global Pertussis Initiative recommends main strategies to control pertussis which might be adapted to countries' specific needs as the reinforcement of implementation of current immunization schedules, and the addition of extra dose of vaccine to current schedules for preschool children, adolescents or some other risk groups as health care workers. Poor recognition of disease in adolescents and adults is listed in the main obstacles against acceptance of these strategies (Forsyth et al. 2005; Wirsing von König et al. 2005).

Our results have demonstrated that pertussis does occur among schoolchildren in Turkey. In order to control the disease, the priority should be given to strengthening of the current routine immunization services and increasing pertussis vaccination coverage along with the consideration of implementation of booster vaccinations beyond childhood. Increasing awareness among health-care workers and the public, and improving access to laboratory confirmation of diagnosis and evaluating the feasibility of introduction of additional booster doses are also needed for an effective control.

Acknowledgments

This research was funded by a grant from the *Gazi University Scientific Research Projects Fund;* project number TF 01/2005-64.

References

Cagney, M., MacIntyre, C.R., McIntyre, P., Torvaldsen, S. & Melot, V. (2005) Cough symptoms in children aged 5-14 years in Sydney, Australia: non-specific cough or unrecognized pertussis? *Respirology*, **10**, 359-364.

Chang, A.B. & Powell, C.V. (1998) Non-specific cough in children: diagnosis and treatment. *Hosp. Med.,* **59**, 680-684.

Cherry, J.D. (2005) The epidemiology of Pertussis: a comparison of the epidemiology of the disease pertussis with the epidemiology of *Bordetella pertussis* infection. *Pediatrics*, **115**, 1422-1427.

Çöplü, N., Esen, B., Kurtoglu, D., Gözalan, A., Miyamura, K. & Yoshida, I. (2005) Standardization of in-house ELISA in pertussis serology and its application in a seroepidemiological study. *Mikrobiyol. Bul.*, **39**, 281-289.

de Melker, H.E., Versteegh, F.G.A., Conyn-Van Spaendonck, M.A., Elvers, L.H., Berbers, G.A.M., van Der Zee, A. & Schellekens, J.F.P. (2000) Specificity and sensitivity of high levels of Immunoglobulin G antibodies against pertussis toxin in a single serum sample for diagnosis of infection with *Bordetella pertussis*. *J. Clin. Micobiol.*, **38**, 800-806.

Dworkin, M.S. (2005) Adults are whooping, but are internists

listening? *Ann. Int. Med.*, **142**, 832-835.

Esen, B., Çöplü, N., Kurtoglu, D., Gözalan, A., Miyamura, K. & Akın, L. (2007) Prevalence of high antibody titers of pertussis in Turkey; a reflection of circulating microorganism, which is a threat to infants. *J. Clin. Lab. Anal., in press*.

Faniran, A.O., Peat, J.K. & Woolcock, A.J. (1998) Persistent cough: is it asthma? *Arch. Dis. Child.,* **79**, 411-414.

Forsyth, K., Nagai, M., Lepetic, A. & Trindade, E. (2005) Pertussis immunization in the Global Pertussis Initiative International Region. Recommended strategies and implementation considerations. *Pediatr. Infect. Dis. J.,* **24**, 93-97.

Guideline for Standardized Diagnosis, Surveillance and Laboratory (2004) Notification and Reporting System for Communicable Diseases. General Directorate of Primary Health Care of Ministry of Health of Turkey, Ankara, Turkey.

Giammanco, A., Chiarini, A., Maple, P.A.C., Andrews, N., Pebody, R., Gay, N., Ölander, R.M., Fivet-Groyne, F., Baron, S., Tischer, A., Swidsinski, S., Schellekens, J. & Reizenstein, E. (2003) European sereoidemiology network: standardization of the assay results for pertussis. *Vaccine,* **22**, 112-120.

Hu, J.J., Lu, C.Y., Huang, C.H., Chou, C.C., Huang, F.Y., Lee, C.Y. & Huang, L.M. (2006) Survey of pertussis in patients with prolonged cough. *J. Microbiol. Immunol. Infect.,* **39**, 54-58.

Robertson, P.W., Goldberg, H., Jarvie, B.H., Smith, D.D. & Whybin, L.R. (1987) *Bordetella pertussis* infection: a cause of persistent cough in adults. *Med. J. Aust.,* **146**, 522-525.

The Annual of Statistics for 2001 (2002) General Directorate of Primary Health Care of Ministry of Health of Turkey, Ankara, Turkey.

The Annual of Statistics for 2005 (2006) General Directorate of Primary Health Care of Ministry of Health of Turkey, Ankara, Turkey.

Vatansever, Ü., Çöplü, N., Öner, N., Sönmez, C., Karasalihoglu, S., Kurtoglu, D., Esen, B. & Ekuklu, G. (2005) Seroprevalance of *Bordetella pertussis* antibodies among healthy adolescent girls in Edirne. *Swiss Med. Wkly.*, **135**, 531-536.

Versteegh, F.G.A., Mertens, P.L., de Melker, H.E., Roord, J.J., Schellekens, J.F.P. & Teunis, P.F. (2005a) Age-specific long-term course of IgG antibodies to pertussis toxin after symptomatic infection with *Bordetella pertussis*. *Epidemiol. Infect.,* **133**, 737-748.

Versteegh, F.G.A., Weverling, G.J., Peeters, M.F., Wilbrink, B., Veenstra-van Schie, M.T.M., van Leeuwen-Gerritsen, J.M., Mooi-Kokenberg, E.A.N.M., Shellekens, J.F.P. & Roord, J.J. (2005b) Community-acquired pathogens associated with prolonged coughing in children: a prospective cohort study. *Clin. Microbiol. Infect.*, **11**, 810-807.

WHO-Pertussis surveillance, A global meeting, Geneva, 16-18 October 2000 (2001) Department of vaccines and biologicals. World Health Organisation. Geneva. WHO/V&B/01.19.

WHO-Recommended standards for surveillance of selected vaccine-preventable diseases (2003) Department of vaccines and biologicals. World Health Organisation.Geneva. WHO/V&B/03.01.28.

Wirsing von König, C.H., Campins-Marti, M., Finn, A., Guiso, N., Mertsola, J. & Liese, J. (2005) Pertussis immunization in the global pertussis initiative European region: recommended strategies and implementation considerations. *Pediatr. Infect. Dis. J.,* **24**, 87-92.