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Epidemiology of pertussis in adolescents and adults in Turkey

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SUMMARY

Two hundred and fourteen patients who had a cough illness lasting at least 2 weeks were studied to investigate *Bordetella pertussis* as a cause of prolonged cough in adolescents and adults. Medical history and nasopharyngeal swab specimens for culture and polymerase chain reaction (PCR) were obtained at presentation. Three (1·4%) patients were *B. pertussis* culture-positive; 15 (7%) were *B. pertussis* PCR-positive (including the culture-positive patients) and 11 (5·1%) were *Bordetella* spp. PCR-positive. Symptom combinations were significantly high both in patients with pertussis and patients with indeterminate results ($P < 0{\cdot}05$). We conclude that *B. pertussis* should be considered among differential diagnoses of prolonged cough in adolescents and adults and PCR and culture should be used to detect these cases and facilitate public health response.

Key words: *Bordetella pertussis*, adolescents, adults, prolonged cough, Turkey.

INTRODUCTION

Pertussis, or whooping cough is an acute infection caused by *Bordetella pertussis.* The disease continues to be a significant health problem worldwide [1]. Pertussis appears with epidemic peaks every 2–5 years [2]. Several factors have been attributed to increasing incidence of pertussis, including waning immunity, increased public health awareness and reporting, and use of more sensitive diagnostics such as PCR assay [3]. Several studies have addressed *B. pertussis* as a common cause of prolonged cough illnesses in adults and have suggested an important role of adults in the epidemiology of pertussis [1, 4–6]. Different symptomatology is possible as a function of time from last immunization, be it a booster or natural infection [7].

The diagnosis of pertussis was related to the number and severity of reported symptoms. Patients with $\geqslant 4$ symptoms suggestive of pertussis are more likely to visit a physician and to be correctly diagnosed [8]. It is well documented that individuals with a primed immune system develop a mild variant of the disease

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characterized by a persistent cough [7]. In Turkey, routine childhood pertussis immunization with whole-cell pertussis vaccine combined with diphtheria and tetanus toxoids (DTwP) was started in 1968. Pertussis vaccine was administered in the second, third and fourth months of life and a booster was administered between 16 and 24 months. In 2007, the cellular pertussis component in DTwP vaccine based on whole bacteria was replaced by the acellular component in DTaP. The current recommended immunization schedule in Turkey is a primary immunization series of three doses of acellular pertussis vaccine in the first year of life and booster doses at ages 15–18 months and 4–6 years. According to Ministry of Health data, Turkey appears to be reaching the WHO target, with a pertussis incidence of <1 case/100 000, with the exception of East Anatolia [4].

The objective of this study was to investigate *B. pertussis* as a cause of prolonged cough in adolescents and adults and its implications for disease control in Turkey.

METHODS

This study was conducted at two hospitals which serve about 2 million people in Antalya, Turkey. In 2010, reported pertussis vaccine coverage was 98% and only one definitive pertussis case was reported to the Ministry of Health from Antalya.

All patients who were admitted to the Departments of Pediatrics and Pulmonary Medicine at Akdeniz University Hospital and to the Department of Pulmonary Medicine at Antalya Research and Training Hospital from 1 October 2010 to 30 May 2011 were included in the study according to the following criteria: (i) they were aged between 10–39 years and (ii) had a cough illness lasting at least 2 weeks (as reported by a health professional). The study excluded those with an underlying medical condition which may cause a prolonged cough such as chronic heart disease, asthma, sinusitis. Each patient's age and sex were noted and the medical history was obtained including information about fever, use of antibiotics, paroxysmal cough, post-tussive vomiting, whooping cough and duration of cough. The case definition for pertussis published by Turkish Ministry of Health was used: (1) *Probable case*: a cough illness lasting $\geqslant$2 weeks with one of the following: paroxysms of coughing, inspiratory whoop or post-tussive vomiting, without other apparent cause. (2) *Confirmed case*: isolation of *B. pertussis* from nasopharyngeal specimen, or probable case criteria with polymerase chain reaction (PCR) positive for pertussis or contact with a laboratory-confirmed case.

Nasopharyngeal swab specimens were collected using rayon nasopharyngeal swabs (Eswab, Copan Diagnostics, Italy) which were placed into modified liquid Amies transport medium. Specimens were inoculated onto charcoal agar with cephalexin (Oxoid, UK) and incubated at 37 °C for 7 days. Suspected *B. pertussis* colonies were identified by biochemical tests, agglutination with specific antisera (Remel, UK) and direct fluorescent antibody staining (Becton Dickinson, USA). After culturing, the specimens were stored at -80 °C until analysis by PCR.

Specimens for PCR testing were extracted using the Magna Pure LC DNA Isolation kit I (Roche, Germany) with Magna Pure LC automatic isolation system according to the manufacturer's instructions. Samples were tested using two PCR assays. First, Bordetella R-gene real-time PCR kit was used to detect the insertion sequence IS*481*. IS*481*-positive specimens were subjected to in-house PCR assay which was used to amplify a 191-bp sequence of the pertussis toxin promoter (*ptx*A-Pr), as reported previously [9]. *PCR interpretation*: The result of PCR assay targeting the insertion sequence IS*481* was considered negative if the cycle threshold (C_t is defined as the cycle number at which the fluorescence passes the fixed threshold) value was $\geqslant$40. A specimen was considered to contain *B. pertussis* when it was positive for both IS*481* and *ptx*A-Pr . If a specimen was positive for IS*481* with a C_t value <35 and negative for *ptx*A-Pr, it was considered to contain *Bordetella* spp. A specimen was considered indeterminate when it was positive for IS*481* with a C_t value between 35 and 40 and negative for *ptx*A-Pr [10].

The study protocol was approved by the Akdeniz University Medical Faculty Ethical Committee of Clinical Research for the Protection of Human Subjects. All subjects provided written informed consent prior to participation in the study.

Data were evaluated with SPSS v. 11·5 (SPSS Inc., USA). The distribution of age, sex, clinical symptoms, use of antibiotics of the cases infected with *B. pertussis* and *Bordetella* spp. were compared with that of 163 cases with negative *B. pertussis* and *Bordetella* spp. results. The same comparisons were made between the cases with indeterminate results and cases with negative results. χ^2 test was used because independent variables were nominal. The mean duration of cough was compared by Student's t test. Combinations of clinical symptoms in patients infected with *B. pertussis*

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Table 1. *Characterics of patients enrolled in the study*

Characteristic	Patients with laboratory evidence of pertussis ($N = 15$)			Patients with laboratory evidence of *Bordetella* spp. ($N = 11$)			Patients with indeterminate results ($N = 25$)			Patients without laboratory evidence of pertussis ($N = 163$)	
	n	(%)	*P*	*n*	(%)	*P*	*n*	(%)	*P*	*n*	(%)
Age, years											
10–19	5	33·3		1	9·1		8	32·0		35	21·5
20–29	8	53·3	0·032*	5	20·0	0·456*	9	36·0	0·277*	49	30·1
30–39	2	13·3		5	20·0		8	32·0		79	48·5
Gender											
Female	7	46·7		6	54·5		12	48·0		93	57·1
Male	8	53·3	0·438*	5	45·5	0·871*	13	52·0	0·396*	70	42·9
Symptoms											
Paroxysmal cough	10	66·7	0·006*	4	36·4	0·726*	20	80·0	<0·001*	51	31·3
Post-tussive vomiting	6	40·0	<0·001*	4	36·4	0·005*	10	40·0	<0·001*	15	9·2
Whooping cough	3	20·0	0·002*	–	0·0	0·719*	3	12·0	0·074*	5	3·1
Fever	3	20·0	0·363*	5	45·5	0·330*	21	84·0	0·118*	51	31·3
Symptom combinations											
Vomiting + cough	6	40·0	0·003*	3	27·3	0·910*	9	36·0	0·001*	15	9·2
Vomiting + whooping	2	13·3	0·007*	–	0·0	–	2	8·0	0·017*	–	0·0
Cough + whooping	3	20·0	0·021*	–	0·0	0·719*	3	12·0	0·074*	5	3·1
Other variables											
Antibiotic usage	11	73·3	0·642*	6	54·5	0·378*	18	72·0	0·652*	110	67·5
Duration of cough (weeks ± S.D.)	4·40 ± 1·06		0·051†	4·09 ± 0·70		0·428‡	4·40 ± 1·32		0·018†	3·83 ± 1·08	

* χ^2 or Fisher's exact test.
† Student's *t* test.

Table 2. *Symptoms and symptom combinations of patients by age group*

	Age group						
	10–19 years (*N* = 49)		20–29 years (*N* = 71)		30–39 years (*N* = 94)		
	n	(%)	*n*	(%)	*n*	(%)	*P**
Symptoms							
Paroxysmal cough	20	40·8	28	39·4	37	39·4	0·984
Post-tussive vomiting	10	20·4	11	15·5	14	14·9	0·679
Whooping cough	2	4·1	4	5·6	5	5·3	0·926
Fever	14	28·6	19	26·8	30	31·9	0·763
Symptoms combination							
Vomiting + cough	9	18·4	10	14·1	14	14·9	0·801
Vomiting + whooping	2	4·1	1	1·4	1	1·1	0·423
Cough + whooping	2	4·1	4	5·6	5	5·3	0·926

* χ^2 test.

and *Bordetella* spp. were compared with 163 *B. pertussis* and *Bordetella* spp. negative cases. The same comparisons were made between the cases with indeterminate results and cases with negative results. Results were analysed by Fisher's exact test. A value of $P < 0{\cdot}05$ was considered to be significant.

RESULTS

Of the 214 cases enrolled in our study, 96 (44·9%) were males and 118 (55·1%) were females.

Patients' ages varied between 10 years and 39 years (mean 26·57 ± 8·55 years). There were 49 (22·9%) cases in the 10–19 years age group, 71 (33·2%) in the 20–29 years age group and 94 (43·9%) in the 30–39 years age group. All patients in our study were aged between 10 and 39 years (born between 1971 and 2000) and had received four doses of DTwP vaccine. Characteristics of patients enrolled in the study are given in Table 1.

Only three (1·4%) specimens were *B. pertussis* culture-positive. These were also PCR positive with both IS*481* and *ptx*A-Pr. Using the interpretation criteria of PCR results; 15 (7%) specimens were positive for *B. pertussis*, 11 (5·1%) specimens were positive for *Bordetella* spp., 25 (11·7%) specimens were interpreted as indeterminate and 163 (76·2%) specimens were considered as negative.

Symptom combinations were significantly high both in patients with pertussis and patients with indeterminate results ($P < 0{\cdot}05$) (Table 1).

By contrast, combinations of symptoms did not show any significant association with *Bordetella* spp. infections. Frequency of symptoms/symptom combinations of patients by age group are given in Table 2.

Antibiotic administration prior to specimen collection was reported in 145 patients. Of the antibiotics used, 89 were β-lactams, 37 were macrolides, seven were fluoroquinolones and one was trimethoprim-sulfamethoxazole. Antimicrobial treatment was not specified in 11 patients. Antibiotic prescription time was not available. Treatment with macrolides or trimethoprim-sulfamethoxazole was reported in five patients with *B. pertussis* infection (one of which was culture positive), in two patients with *Bordetella spp*. infection, in five patients with indeterminate results and in 26 patients with negative results. Symptomatology of the 38 subjects who received appropriate treatment prior to specimen collection was as follows: 28 with cough, 15 with vomiting, three with whooping, 15 with cough and vomiting, three with cough and whooping, and two with vomiting and whooping. Hospitalization was not required for any of these patients.

DISCUSSION

Despite high vaccine coverage pertussis still affects all age groups especially adolescents, adults and young infants in Turkey [2]. *B. pertussis* continues to circulate in populations where high vaccination coverage of infants and children is achieved, because immunity wanes at 4–12 years after vaccination or 4–20 years after natural infection [1].

Pertussis is rarely considered in differential diagnoses of cough in adults and adolescents and is the

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only vaccine-preventable disease with an increasing number of cases and deaths [11].

Many cases remain unrecognized due to inaccurate diagnosis. Physicians' awareness of the infection is very poor.

In our study, the frequency of *B. pertussis* and *Bordetella* spp. infections in adolescents and adults with prolonged cough were 7% and 5%, respectively. Strebel *et al*. [6] studied 212 patients aged between 10 and 49 years who presented with acute paroxysmal cough illness and found at least one positive laboratory result for pertussis infection in 27 (13%) patients. Of these 27 patients, eight (3%) were found to be culture positive, 10 (4·7%) were found to be positive by PCR. Birkebaek *et al.* [5] investigated 201 adult patients with chronic cough. Four (2%) of these patients were *B. pertussis* culture-positive. Eleven (5·5%) of the specimens were *B. pertussis* PCR-positive. Park *et al*. [12] examined 102 adult patients with persistent cough. Three (2·9%) patients had evidence of pertussis infection, and all three were PCR positive but culture negative. Our results are in agreement with Strebel *et al*. and Birkebaek *et al*. The results from several serological studies indicate that between 12% and 32% of adults and adolescents with a cough illness for at least 1 week are infected with *B. pertussis* [13–15]. Although culture is thought to be almost 100% specific, its sensitivity is reduced due to delayed collection of specimens, prior antibotic therapy, increased age or pre-existing immunity [7, 16]. PCR is a rapid, sensitive, and specific technique, but sensitivity of PCR is also reduced by the same factors. However, due to its higher sensitivity, it may be used to diagnose pertussis in vaccinated patients, in patients with prior antibiotic treatment and the late stages of the disease.

In a study from New Zealand neither cough duration nor any individual symptom discriminated between those with and without recent *B. pertussis* infection [17].

Although adolescents and adults can present with atypical disease, several studies suggest that 21–86% of adolescent and adults present with classic pertussis symptoms such as paroxysmal cough, post-tussive vomiting, and whooping cough [18].

We only used insertion sequence IS*481* and *ptx*A-Pr as the target genes therefore we did not discriminate *B. holmesii*, or *B. bronchiseptica* species. Additional PCR targets should be used to improve specificity by differentiating between *Bordetella* spp. [19]. Another limitation of this study was that we did not perform serological tests which are independent of symptoms and may detect asymptomatic cases whose importance for disease transmission is unclear [7]. In the present study, 25 clinical specimens were considered indeterminate which indicates low levels of pertussis DNA. These results may be real positives or false positives due to poor specimen collection, late testing, prior antibiotic treatment or DNA contamination. Interpretation of indeterminate results should be done in conjunction with an evaluation of signs, symptoms and epidemiological information [10].

Our study indicates that pertussis should be considered in the differential diagnoses of prolonged cough in adolescent and adult patients, especially if the cough is associated with paroxysmal cough, post-tussive vomiting, and whooping cough.

As with the rest of the world, booster vaccination against pertussis should be administered to adolescents and adults to develop herd immunity and reduce pertussis-related morbidity in all age groups.

It should be borne in mind that adolescent and adult patients may be a reservoir for *B. pertussis* and transmit the infection to infants. Clinicians should be aware of the ongoing endemic circulation of the organism in the community and PCR and culture will help to detect cases and facilitate an appropriate public health response.

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DECLARATION OF INTEREST

None.

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