PERTUSSIS IN THAI ADULT AND PEDIATRIC PATIENTS PRESENTING WITH PROLONGED ACUTE COUGH

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Abstract. Pertussis is a highly contagious respiratory disease caused by *Bordetella pertussis*. In Thailand, a national passive surveillance system showed a rise in the incidence of pertussis during 2015-2016, especially among infants less than one year of age. In order to investigate the rate of pertussis in children and adult presenting with cough of more than seven days and to evaluate associated factors, a cross-sectional study was conducted at King Chulalongkorn Memorial Hospital, Bangkok and at Phrasaeng Hospital, Surat Thani, Thailand between September 2016 - 2017. Nasopharyngeal swabs were collected from out-patient and hospitalized children and adults who presented with cough of more than seven days. Each specimen was tested for presence of *Berdetella pertussis* by quantitative real-time PCR and further confirmed by conventional PCR and nucleotide sequencing. Of 70 patients presenting with prolonged acute cough enrolled in this study, seven cases were diagnosed with pertussis by confirmed sequencing, comprising four infants younger than one year of age and three adults 27, 38 and 56 years of age. Patients with pertussis have significantly more reported whooping ($p = 0.001$), cyanosis ($p = 0.002$), tachypnea ($p = 0.001$), and chest retractions ($p = 0.009$) compared to the enrolled patients. Our results highlight the presence of pertussis in 10% of patients with prolonged acute cough. Physicians should acknowledge the possibility of pertussis in infants presenting with the abovementioned signs and symptoms.

Keywords: *Bordetella pertussis*, pertussis, prolonged acute cough, Thai patients

INTRODUCTION

Pertussis is a highly contagious respiratory disease caused by *Bordetella pertussis,* a gram-negative bacterium. Pertussis can be severe in infants leading to hospitalization and death (Barger-Kamate *et al*, 2016). Despite the widespread use of pertissis vaccine, the disease remains endemic and in 2016 affected more than 130,000 people worldwide with outbreaks occurring in different regions around the world (WHO, 2017). In the United States, the Centers for Disease

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Control and Prevention in 2014 reported 32,971 cases and 13 deaths from pertussis, with nearly 70% of the fatalities being infants younger than one year of age (CDC, 2014). In Thailand, the number of pertussis cases based on passive surveillance ranges from 0.01 in 2009 to 0.04 cases per 100,000 population in 2014 with no report of major outbreaks (Bureau of Epidemiology, 2014). However, in 2015 and 2016 there was an increased incidence of 51 cases (0.08/100,000) and 72 cases (0.11/100,000), respectively (Bureau of Epidemiology, 2015-2016). In addition, there were two pertussis-related deaths in 2015, and both cases occurred in Thai infants younger than one year of age.

Active surveillance of pertussis has recently been conducted in Thailand. Siriyakorn *et al* (2016) reported 18.4% of Thai adults presenting with cough of more than two weeks between 2010 and 2011 have pertussis, with most of the cases diagnosed by serological assays. Another study reported between 2012 and 2013 a 6.1% prevalence of serologically confirmed pertussis in Thai adults with prolonged cough of more than two weeks (Koh *et al,* 2016). Regarding pertussis in pediatric patients, Suntarattiwong *et al* (2013), in a surveillance of a pediatric tertiary care hospital in Bangkok, found 19% of children presenting with cough of more than seven days plus paroxysm, inspiratory whooping or post-tussis vomiting have pertussis confirmed by conventional PCR (Suntarattiwong *et al*, 2013).

In order to evaluate in a wider age range the prevalence of pertussis, detected using PCR, we conducted a cross-sectional study of pediatric and adult patients presenting with cough of more than one week and analyzed factors associated with contracting pertussis.

MATERIALS AND METHODS

Study population

Patients presenting with cough of more than seven days were recruited. They were identified at the outpatient clinic and inpatient wards of King Chulalongkorn Memorial Hospital (KCMH), Bangkok and Phrasaeng Hospital, Surat Thani, Thailand between September 2016 to September 2017. Data regarding severity and duration of symptoms, history of pertussis vaccination, history of close contact with people currently having cough, complications, and medical treatment were recorded. Results of chest X-ray and complete blood counts (CBC) were also recorded if available.

The study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines (ICH-GCP) (Dixon, 1998) and was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB no. 351/59). Written informed consent was obtained from each participant above 18 years of age and parents/legal guardians of children below 18 years of age after the study protocol had been explained.

Specimen collection

Nasopharyngeal swabs were collected using Dacron swab and individually placed in sterile tubes containing 1 ml of sterile normal saline solution. Samples collected at KCMH were transported to the Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University within 24 hours, and those collected at Phrasaeng Hospital were kept at 2-8°C until shipment to Chulalongkorn University within one week.

Laboratory procedures

Multi-target qPCR. Total nucleic acid was

extracted from 200 μl of a clinical sample using a Ribospin vRD kit (GeneAll, Seoul, South Korea) and subjected to qPCR-based assay of *B. pertussis* as previously described with some minor modifications (Tatti *et al,* 2011). In short, two bacterial genes, namely, IS*481* (encoding insertion sequence 481) and *ptxS1* (encoding pertussis toxin subunit S1) were amplified in a single qPCR. According to the algorithm for the interpretation of multi-target qPCR assay, any specimen with positive IS*481* and *ptxS1* amplification is interpreted as positive for *B. pertussis*.

Conventional PCR and nucleotide sequencing. Semi-nested conventional PCR, which is a more sensitive assay compared to qPCR, was performed on every clinical specimen in order to confirm the result of *ptxS1*. In short, *ptxS1* region was amplified by using primers S1F (5′ TAGGCACCATCAAAACGCAG 3′), S1R (5′ TCAATTACCGGAGTTGGGCG 3′) and S1FM (5′ACAATGCCGGCCGTATCCTC 3′) (Mooi *et al,* 1998). The final *ptxS1* amplicon (400 bp) was nucleotide sequenced. The amplicon was gel-purified using HiYieldTM Gel/PCR DNA Fragments Extraction kit (Arrowtec, Taipei, Taiwan) and sequenced by First BASE Lab (Selangor Darul Ehsan, Malaysia).

Statistical analysis

Demographic data were presented as median with ranges and percentages. Associated factors for contracting pertussis were calculated by a univariate binary logistic regression analysis (Agresti, 2002). A *p-v*alue <0.05 is considered statistically significant.

RESULTS

From September 2016 to September 2017, 70 patients presenting with cough of more than seven days were enrolled, with 51 and 19 cases from KCMH and Phrasaeng Hospital, respectively. The median age was 9.5 years (range: 1 month to 74 years) and the median duration of cough at the time of specimen collection was 10 days (range: seven days to four months) (Table 1). Other common associated symptoms were coryza (66%), fever (34%), anorexia (13%), cough-associated vomiting (11%), whooping (9%), cyanosis after cough (6%), and diarrhea (1%). No participant in this study reported having received a booster dose of tetanus-diphtheria-acellular pertussis (Tdap) vaccine during adolescence or adulthood.

All samples were tested for presence of IS*481* and *ptxS1* by qPCR and the latter gene by conventional semi-nested PCR and sequencing. Multi-target qPCR assay showed that five samples were positive for *B. pertussis* and conventional PCR identified two more *ptxS1* positive (Table 2). The sequences of all seven 400 bp amplicons were identified to be that of *B. pertussis ptxS1* (data not shown), 10% of cases diagnosed with pertussis.

Pertussis was detected in very young infants of less than three months of age and adults above 25 years of age (Table 3). All affected infants were hospitalized and one infant who was intubated had to receive intensive medical care due to aspiration pneumonia. All infants were reported to have at least one household member currently having a cough, but the nasopharyngeal swabs from the household member were negative for *B. pertussis* (data not shown). When the history of close contact with people having cough within one month and clinical symptoms was compared between pertussis and non-pertussis cases, it became apparent the presence of whooping, cyanosis, tachypnea and chest retractions were associated with pertussis (Table 4). Among

Table 1
Descriptive characteristics of participants.

Charactertistic	Number (%) (n = 70)
Study site	
KCMH, Bangkok	51 (73)
Phrasaeng Hospital, Phrasaeng, Surat Thani	19 (27)
Gender	
Male	35 (50)
Female	34 (49)
Data not available	1 (1)
Age group	
0-18 years	39 (56)
18-74 years	31 (44)
Median age (range) (year)	9.5 (1 month to 74 years)
History of previous pertussis vaccination	
Complete childhood vaccination	23 (33)
Incomplete childhood vaccination	26 (37)
Age <4 years (incomplete due to age)	18
Age >10 years	8
Data not available	21 (30)

KCMH, King Chulalongkorn Memorial Hospital.

Table 2
Detection of *Bordetella pertussis* IS*481* and *ptxS1* by multi-target qPCR assay and *ptxS1* by conventional semi-nested PCR assay in clinical samples.

Number of patients	Multi-target qPCR assay		Conventional semi-nested PCR assay	Interpretation
	IS*481*	*ptxS1*	*ptxS1*	
5	+	+	+	*B. pertussis* positive
2	+	-	+	*B. pertussis* positive
6	+	-	-	*B. pertussis* negative[a]
57	-	-	-	*B. pertussis* negative

[a]Possibly *B. holmesii.*

four infant pertussis cases, all reported whooping while 75% reported cyanosis and tachypnea. Fifty percent of infant pertussis cases also had chest retractions. Nevertheless, these symptoms were not detected among adult pertussis cases. There were two one-month old infants in the non-pertussis group who had prolonged acute cough with whooping, one of whom also had whooping with cyanosis after cough; however, nasopharyngeal swabs for *B. pertussis* were negative by

Table 3
Characteristics of pertussis cases in this study.

Age	Case 1 27 years	Case 2 38 years	Case 3 56 years	Case 4 1 month	Case 5 2 months	Case 6 1 month	Case 7 1 month
Study site	KCMH	Phrasaeng	Phrasaeng	KCMH	KCMH	KCMH	KCMH
Comorbidities	None	HIV infection	None	None	None	No	No
Duration of cough at the time of diagnosis (days)	10	30	21	17	10	9	7
Physical examination	Normal	Normal	Normal	Crepitation in both lungs	Normal	Normal	Suprasternal retractions
Chest X-ray	Not done	Normal	Right perihilar infiltration	Perihilar infiltration both lungs	Normal	Infiltration of right lower lung	Normal
Lymphocytes (cell/μl)	No data	No data	No data	24,680	9,360	7,207	27,038
ESR (mm/hour)	No data	No data	No data	2	No data	23	8
Pertussis vaccination	Complete childhood vaccination (No history of Tdap vaccination booster)	Unknown	Unknown	None (no maternal Tdap vaccination during pregnancy or postpartum)	None (no maternal Tdap vaccination during pregnancy or postpartum)	None (no maternal Tdap during pregnancy or post-partum)	None (no maternal Tdap during pregnancy or post-partum)
Hospitalization, duration and type of hospital ward	No	No	No	Yes/14 days (ICU)	Yes/3days (General ward)	Yes/3 days (General wards)	Yes/3 days (General wards)
Treatment	None	None	None	Azithromycin	Azithromycin	Azithromycin	Azithromycin
Close contact with people having cough	No	No	Son (no clinical specimen collected)	Mother (negative PCR for *B. pertussis*)	Mother (negative PCR for *B. pertussis*)	Brother (PCR for *B. pertussis* negative)	Father, mother, grandmother (PCR for *B. pertussis* negative)

ESR, erythrocyte sedimentation rate; KCMH, King Chulalongkorn Memorial Hospital.

Table 4
Characteristics of patients with prolonged acute cough stratified by diagnosis of pertussis and non-pertussis.

Variable	Pertussis case (n = 7)	Non-pertussis case (n = 63)	Odds ratio (95% CI)	p-value
Gender				
Male	4	31	1.3 (0.3-5.5)	1.000
Female	3	31	0.8 (0.2-3.3)	1.000
History of close contact with people having cough	5	23	3.8 (0.8-18.0)	0.107
Fever	2	22	0.8 (0.2-3.7)	1.000
Whooping	4	2	14.2 (4.1-49.2)	0.001
Cyanosis	3	1	12.4 (4.1-37.4)	0.002
Tachypnea	3	0	16.8 (6.5-43.3)	0.001
Chest retraction	2	0	13.6 (5.9-31.6)	0.009

CI, confidence interval.

PCR and the final diagnosis of both cases was parainfluenza virus pneumonia.

DISCUSSION

Our results highlight that *B. pertussis* can be detected by PCR assay in approximately 10% of Thai adults and pediatric patients presenting with cough of more than seven days. Morbidity associated with pertussis often occurred in infants too young to receive the primary series of vaccination. Adult pertussis were also be detected despite having a complete childhood vaccination, suggesting current pertussis vaccination program in Thailand cannot provide lifelong immunity against this disease.

Diagnosis of pertussis is challenging due to many reasons (Cherry *et al*, 2005). Symptoms that occur after *B. pertussis* infection can vary from asymptomatic to fatal disease. In adults and adolescents, symptoms are usually milder compared to children, thereby leading to physician unawareness and improper treatment. The application of PCR-based detection of *B. pertussis* is more sensitive compared to conventional bacterial culture, but the proper period for PCR detection of *B. pertussis* from nasopharyngeal swab is limited (Siriyakorn *et al*, 2016**)**. Although it provides the best results in the first few weeks after the symptom of onset, PCR results can remain positive for a median duration of 58 days (ranging from 40 to 110 days) as reported from a study conducted in USA (Stone *et al*, 2014). It is difficult to extrapolate these data to our population as the age groups, genetic variations, techniques in sample collection and laboratory assay are different. Serological diagnosis defined by anti-pertussis toxin IgG titer >100 IU/ml can be used instead of PCR especially when pertussis is suspected after a long duration from the disease onset (Koh *et al*, 2016); however, there are limited laboratory facilities to perform this test.

Two pertussis active surveillances in

Thai adults presenting with cough of more than two weeks reported pertussis is detected in 18.4% (Siriyakorn *et al*, 2016) and 6.1% (Koh *et al*, 2016) of the cases. Both studies employed mostly serological diagnosis. The study of Siriyakorn *et al* (2016) also tested for *B. pertussis* using a PCR-based assay, but there is only one positive sample among 14 cases, suggesting that the PCR assay has lower sensitivity compared to the serological technique when specimen collected beyond two weeks of disease onset. Although our study used PCR testing in adults presenting with cough of more than one week, the rate of pertussis detection (10%) is similar to the previous surveys.

As regards pertussis in pediatric patients, an active pertussis surveillance in children presenting with cough of more than one week plus symptoms suggestive of pertussis reported 19% pertussis cases using PCR, with 60% of pertussis patients being infants less than one year of age (Suntarattiwong *et al*, 2013). On the other hand, our study demonstrates a rate of pertussis in children presenting with prolonged acute cough of 10% and all were infants younger than three months of age. The higher rate of infection in the previous study might be due to selection of patients with symptoms suggestive of pertussis at recruitment. Nevertheless, both studies demonstrated that infants are at the greatest risk. Pre-existing vaccine-induced maternal *B. pertussis*-specific antibody show low concentrations in newborns, and their presence in infants is short-lived resulting in infant susceptibility to pertussis shortly after birth (Gall *et al*, 2011). Recent studies found pertussis vaccination during pregnancy elicits a high *B. pertussis*-specific antibodies in infant cord sera and these antibodies persist at a high level at two months of age compared to infants born to unvaccinated mothers (Munoz *et al*, 2014; Maertens *et al*, 2016). The effectiveness of Tdap vaccine during pregnancy to prevent infant pertussis has been confirmed in epidemiological studies (Amirthalingam *et al*, 2014; Dabrera *et al*, 2015; Vizzotti *et al*, 2016). Thus, there is a potential benefit of a Tdap booster during pregnancy in expecting mothers to prevent infant pertussis.

The significant association of pertussis with whooping, cyanosis, tachypnea and chest retractions in patients with prolonged acute cough are similar to a previous study that found post-tussive vomiting, convulsion and cyanosis are significantly more common in pertussis compared to non-pertussis cases (Suntarattiwong *et al*, 2013). Another study from 12 European countries found patients with pertussis have longer durations of phlegm production, shortness of breath, disturbed sleep and interference with normal activities or work after presentation of the disease (Teepe *et al*, 2015). A study in Africa that enrolled children with clinically suspected pertussis noted that poor nutritional status, HIV infection and HIV exposure are not associated with pertussis (du Plessis *et al*, 2018). These findings should help guiding physicians in their considerations of pertussis in the clinical setting and encourage collection of nasopharyngeal swabs for laboratory analysis.

This study was performed at KCMH, Bangkok, a tertiary care center in the capital city of Thailand and Phrasaeng Hospital, a community hospital in Surat Thani Province. With regards to the limited sample size and selection of only two study sites, our data can not be comsidered as representive of the whole country. In addition, the total number of patients visiting both hospitals due to prolonged acute cough was not known, so a sam-

pling bias in which pertussis patients were more likely to be recruited might have occurred resulting in the higher frequency of the disease reported in our survey.

In conclusion, the results show pertussis was not uncommon in Thailand despite a high coverage of pertussis vaccines for several decades. Infants too young to receive the first vaccine dose were at the greatest risk, and maternal immunization might help to protect their infants from pertussis during early life.

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