

## Outbreak of Pertussis in a University Laboratory

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### Abstract

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**Objective** Analysis of an outbreak of *Bordetella pertussis* infection in a university laboratory. To prevent and control the outbreak, we conducted a survey of the laboratory staff and their family members, and we investigated the clinical features of adult pertussis.

**Patients and Methods** During the outbreak, four out of the 10 laboratory staff and five out of 16 family members had a primary complaint of cough. Seven of nine patients were diagnosed as definitive *B. pertussis* infection using serology and PCR.

**Results** Clinical findings and laboratory data in adult patients with *B. pertussis* infection demonstrated non-specific cough and normal WBC and lymphocyte count. The patients who received clarithromycin prior to 14 days after clinical onset demonstrated a shorter duration of cough symptoms than patients who received clarithromycin at 14 days or more after clinical onset (duration of cough after administration of clarithromycin:  $17.8 \pm 6.48$  days versus  $35.3 \pm 5.38$  days; duration of total cough after clinical onset:  $24.8 \pm 6.65$  days versus  $56.8 \pm 6.50$  days).

**Conclusion** The clinical findings of adult pertussis are different from pertussis in children. The efficacy of macrolide therapy clearly differed between the catarrhal phase and paroxysmal phase. Physicians should consider *B. pertussis* in the differential diagnosis of an outbreak of non-specific respiratory infection even in adult populations.

**Key words:** *Bordetella pertussis*, outbreak, adult, cough, macrolide, serology

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### Introduction

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*Bordetella pertussis* is a small Gram-negative coccobacillus with exclusive affinity for the mucosal layers of the human respiratory tract (1). This organism adheres to respiratory tract epithelial cells, mainly the lining hair cells, produces toxins, and causes a prolonged intensive cough. It is transmitted from infected to susceptible individuals through droplets. Adolescents and adults are at risk of *B. pertussis* infection because of waning immunity following childhood vaccination (2-4). A number of studies have documented that between 13% and 32% of adolescents and adults with an illness involving a cough of six days' duration or longer have serologic evidence of infection with *B. pertussis* (2-7). Outbreaks of *B. pertussis* have occurred in closed popula-

tions including among families, university students, military trainees, in schools and in hospital. However, the diagnosis of pertussis is frequently missed, often because of a misconception that whooping cough is solely a pediatric illness that has been controlled by routine childhood immunizations and that immunity resulting from pertussis diseases or immunization is lifelong. In addition, residual immunity from prior vaccination may modify the clinical presentation of pertussis in adolescents and adults; making the diagnosis even more difficult.

In contrast to Western countries, the available data of pertussis in Japanese adolescent and adult populations are limited. Thus, the Infectious Disease Surveillance Center (National Institute of Infectious Diseases) developed the "Pertussis Database" for the prevention of *B. pertussis* infection in 2008 and is currently collecting information about pertus-

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sis (8). Recently, we encountered an outbreak of *B. pertussis* infection in a university laboratory. Here, we report the clinical findings of an outbreak of *B. pertussis* infection in adults. In addition, we focused on the clinical course after antibiotic administration. It seems that antibiotics rarely affect the course of the disease when they are given in the paroxysmal phase (2). However, the available data of antibiotic efficacy are limited. In this study, we specified the kinds of drugs, including antibiotics, and the duration of administration in all patients and compared the efficacy of antibiotic therapy between the catarrhal phase and paroxysmal phase.

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## Patients and Methods

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### Outbreak of acute respiratory illness

The university is located in Kurashiki City and the laboratory in which the outbreak of acute respiratory illness (ARI) occurred was separated from the main building. Ten persons worked in the laboratory and they did not have contact with university students during the outbreak. Previously, a healthy 49-year-old female complained of cough in late May, 2010. Her symptoms persisted for more than one week and sputum production appeared. She visited a medical clinic in early June and was treated with pentoxyverine citrate (60 mg/day), serrapeptase (15 mg/day), and PL granules (general cold syndrome treatment preparations: salicylamide 270 mg, acetaminophen, 150 mg, anhydrous caffeine 60 mg and promethazine methylenedisalicylate 13.5 mg/1 g; 3 g/day) for one week, but her symptoms worsened. She visited our hospital on June 9 (day 14 after onset). No microorganisms were detected in the sputum by Gram stain and culture. A chest X-ray showed no infiltrative shadows in the lung fields. In history taking, a fellow laboratory member, a 46-year-old female, had complained of cough in mid-May and her symptoms had persisted (Table 1, index case, patient no. 1). The present patient's desk in the laboratory was next to the index case and they were always in close contact with each other during the working day. Six out of eight other staff worked in the same room but their desks were separated from the index case (two further staff worked in a separate room). Characteristics of cough (paroxysmal and nocturnal cough) and laboratory data were identical to the index case. Thus, post-infectious cough was suspected.

Clarithromycin (400 mg/day) was administered for suspected respiratory infection with atypical pathogens or *B. pertussis* because the index case had persistent cough for more than 3 weeks and we did not consider viral infection as a likely cause. After administration of clarithromycin, her symptoms improved slowly. This case was diagnosed as *B. pertussis* infection, because seroconversion of bacterial agglutination antibody measured using a *B. pertussis* antigen agglutination test was observed in paired serum samples (Table 1, patient no. 2). A survey of the other laboratory staff and their family members was conducted. The labora-

tory staff included 10 persons; three males and seven females. Informed consent was obtained from all subjects and the study protocol was approved by the Ethics Committee at Kawasaki Medical School.

### Epidemiologic and clinical investigation

Respiratory illness surveillance was carried out by the medical doctors in our hospital until August 30, 2010, and microbiologic laboratory tests were done in our laboratory. The surveillance findings were reviewed to determine the extent of the outbreak. In the case of those patients who had a cough during the outbreak period, their charts and hospital medical records were also reviewed for diagnosis, chest X-ray findings, and laboratory results. Paired serum samples were collected at intervals of at least four weeks after onset from all patients with ARI. Nasopharyngeal swab specimens or sputum samples were obtained from all laboratory staff and their family members who lived together in their home. Urine samples were also obtained from all patients with ARI. We carried out laboratory tests within 4 hr after collection.

### Microbiologic laboratory tests

Microbiologic laboratory tests were performed as described previously (9, 10). Serum samples were tested for antibodies to influenza A and B viruses, adenovirus, respiratory syncytial virus, cytomegalovirus, and parainfluenza viruses (types 1, 2 and 3) using complement fixation tests. Antibodies to *Mycoplasma pneumoniae* were measured by a passive agglutination test (Serodia-Myco II kit, Fujirebio, Tokyo, Japan), *Legionella* species by a microagglutination test (detection of *L. pneumophila* serogroups 1-6, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, and *L. micdadei*), *B. pertussis* Yamaguchi strain and Tohama strain by an agglutination test 'SEIKEN' N' (Denka Seiken, Tokyo, Japan) (11) and pertussis toxin (PT) and filamentous hemagglutinin (FHA) using a ball ELISA method (Wako Chemicals, Tokyo, Japan) (12), and *Coxiella burnetii* by an indirect immunofluorescence test. A microimmunofluorescence test was used for the titration of IgG and IgM antibodies against *Chlamydia* or *Chlamydophila* species, using formalinized elementary bodies of *C. pneumoniae* KKpn-15, *C. trachomatis* L2/434/Bu, and *C. psittaci* Budgerigar-1 strains as antigens. Rheumatoid factors were absorbed with GullSORB (Meridian Bioscience Inc., Cincinnati, OH, USA) before IgM titrations.

When sputum was available, Gram staining and a quantitative cultures were performed. Sputum data were only evaluated when the Gram stain test revealed numerous leukocytes (>25 in a 100× microscopic field) and few squamous epithelial cells (<10 in a 100× microscopic field). Nasopharyngeal swab specimens or sputum samples were tested for influenza A and B viruses using a direct enzyme immunoassay (EIA) and for culturing of *M. pneumoniae* and *Legionella* species on pleuropneumonia-like organism broth (Difco, Detroit, MI, USA) and buffered charcoal-yeast extract alpha agar, respectively. Cultures for *C. pneumoniae*

**Table 1. PCR and Serological Results of Patients who Had a Primary Complaint of Cough during the Outbreak**

Patient No., Age and gender	Onset of cough Symptom	<i>B. pertussis</i> PCR results	Bacterial agglutinin titer acute/convalescent		Antibody titer (EU/mL) acute/convalescent	
			Yamaguchi	Tohama	Anti-PT	Anti-FHA
Laboratory staff						
1. 46 F (index case)	May 15	-	1,280/1,280	1,280/1,280	>100/>100	>100/>100
2. 49 F	May 27	-	40/1,280	160/1,280	42/>100	36/>100
3. 56 F	June 5	-	80/1,280	320/320	<1/<1	77/98
4. 24 F	June 14	+	160/160	20/20	21/36	40/37
Family member						
5. 46M	May 29	-	640/640	320/220	>100/>100	35/21
6. 21 M	May 27	-	320/320	640/640	>100/>100	>100/>100
7. 49 M	June 3	-	80/1,280	160/1,280	37/29	36/>100
8. 28 M	June 12	+	40/320	20/160	3/29	8/10
9. 24 F	June 13	+	80/160	80/80	7/>100	77/>100

and *C. psittaci* were performed using cycloheximide-treated HEp-2 cells grown in a 24-well cell culture plate (9, 10). All specimens were passaged twice. Culture confirmation was done by fluorescent-antibody staining with *C. pneumoniae* and *C. psittaci* species-specific and genus-specific monoclonal antibodies. These specimens were also used for polymerase chain reaction (PCR) of *M. pneumoniae*, *Chlamydomphila* species, *B. pertussis*, and *Legionella* species. The target sequences were a region of the P1 cytoadhesin gene for *M. pneumoniae* (13), the 53-kDa protein gene for *C. pneumoniae*, the major outer membrane protein gene for *Chlamydomphila* (14), the insertion sequence IS481 for *B. pertussis* (15), and the nucleotide sequence of the 5S-ribosomal DNA for *Legionella* (16). These assays were performed as described previously (9, 14). In addition to serology, antigen detection, culturing, and PCR assays, a urinary antigen test (Binax NOW, Portland, ME, USA) was used for detection of *Streptococcus pneumoniae* and *L. pneumophila*.

#### Criteria for determination of microbial etiology

Microbial etiology was classified as “definitive”, “presumptive”, or “unknown” as described previously (9). A clinical diagnosis of *B. pertussis* infection was made if one of the following conditions was present: 1) paroxysmal cough; 2) whooping cough; and 3) post-tussive vomiting (17). A definitive *B. pertussis* infection was defined if one of the following conditions was present in addition to the above clinical symptom: 1) a four-fold increase in an agglutinin titer; 2) nasopharyngeal swab PCR assay result positive; and 3) an agglutinin titer of  $\geq 1 : 1,280$  for Yamaguchi strain in either an acute-phase or convalescent-phase serum sample. A presumptive *B. pertussis* infection was considered if one of the following conditions was present in addition to the above clinical symptoms: 1) an antibody titer

of  $\geq 1 : 320$  for Yamaguchi strain in either an acute-phase or convalescent-phase serum sample (17); and 2) an antibody titer of  $>100$  EU/mL for PT in either an acute-phase or convalescent-phase serum sample (18).

#### *B. pertussis* agglutinin titers in adult healthy subjects

The pertussis agglutinin titer was investigated among 196 adult healthy volunteers without ARI, consisting of 89 males and 107 females with a mean age of 38.0 years (range 22 to 56 years). These asymptomatic healthy volunteers included university staff who were assessed between June and August 2010. Subjects were excluded from the study if they reported a history of infection with *B. pertussis* or if they reported having had a clinical syndrome compatible with pharyngitis, sinusitis, bronchitis, or pneumonia within the preceding 12 months before enrollment.

## Results

The outbreak occurred between mid-May and mid-June, 2010, with four out of 10 laboratory staff and five out of 16 family members reporting a primary complaint of cough (Table 1). After June 14, no respiratory illness was noted. Definitive cases of acute *B. pertussis* infection were found in seven out of nine patients, four showed seroconversion, four showed high agglutinin titer against Yamaguchi strain with  $1 : 1,280$  and three were PCR positive (Table 1). The remaining two patients (patient nos. 5 and 6) were diagnosed as presumptive *B. pertussis* infection. No other respiratory pathogens were detected by isolation, PCR, antigen detection assay, or serology in all cases. Among the asymptomatic laboratory staff ( $n = 6$ ) and their family members ( $n = 11$ ) who had direct or indirect contact with the patients,

**Table 2. Clinical Characteristics and Laboratory Data**

Characteristics	<i>B. pertussis</i> infection (n = 9)
Age mean (range), years	38.1 (21 – 56)
Male:Female*	4:5
Smoking history*	3
Co-morbid illness*	2
Symptom*	
Paroxysmal cough	7
Wake up at night for cough	7
Chest pain	4
Scratchy throat	3
Post-tussive vomiting	3
Dyspnea	2
Apnea after cough	1
Whooping cough	1
Laboratory data	
WBC mean, /mm <sup>3</sup>	6,530± 2,090
Neutrophil (mean, /mm <sup>3</sup> )	54–77% (4,110 ± 1,980)
Lymphocyte (mean, /mm <sup>3</sup> )	15–39% (2,060 ± 710)
Monocyte (mean, /mm <sup>3</sup> )	2–11% (290 ± 190)
Eosinophil (mean, /mm <sup>3</sup> )	1–4% (120 ± 150)
CRP mean, mg/dL	1.17 ± 0.98

\*Data represent the numbers of patients.

**Table 3. Clinical Course of Adult Patients with *B. pertussis* Infection**

Patient No., Age and gender	Days after cough onset before appropriate antibiotic use (day)	Duration of cough after administration of CAM* (day)	Duration of total cough after clinical onset (day)
Laboratory staff			
1. 46 F	30	36	66
2. 49 F	14	42	56
3. 56 F	6	27	33
4. 24 F	7	12	19
Family member			
5. 46 M	20	34	54
6. 21 M	22	29	51
7. 49 M	9	22	31
8. 28 M	7	13	20
9. 24 F	6	15	21

\*CAM = clarithromycin. Clarithromycin 400 mg/day was administered for 7 days.

five (29.4%) were PCR positive. All these asymptomatic subjects were administered azithromycin 500 mg/day for 3 days.

Table 2 shows the characteristics of the nine patients with definitive and presumptive *B. pertussis* infection. In most cases, paroxysmal cough and nocturnal cough were observed in this study. However, whooping cough was observed only one patient. The laboratory data demonstrated that the mean WBC count and mean lymphocyte count were normal.

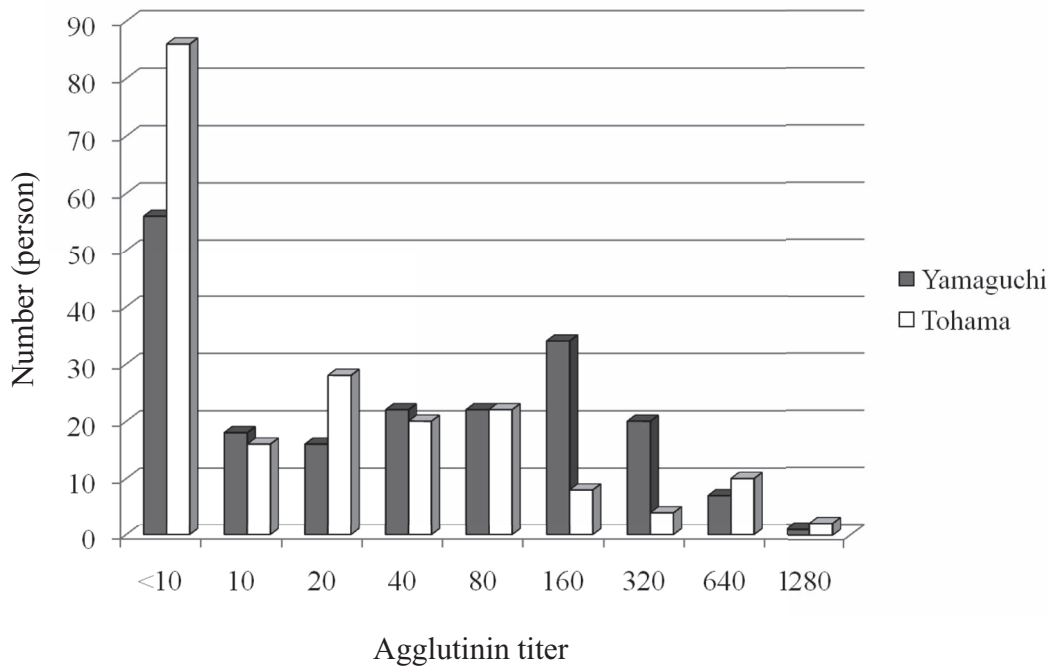
All patients were treated with clarithromycin (400 mg/day) for seven days and tulobuterol (2 mg/day) for 14 days. In the index case (patient no. 1), clarithromycin was administered 21 days after clinical onset and the total duration of cough symptom was the longest (66 days) among all patients (Table 3). The patients who received clarithromycin prior to 14 days after clinical onset demonstrated a shorter duration of cough symptoms than the patients who received clarithromycin at 14 days or more after clinical onset (duration of cough after administration of clarithromycin: 17.8 ± 6.48 days versus 35.3±5.38 days; duration of total cough af-

ter clinical onset: 24.8 ± 6.65 days versus 56.8 ± 6.50 days).

The frequency distribution of agglutinin titer against *B. pertussis* in 196 healthy volunteers is shown in Fig. 1. When the cut-off point for a positive result was taken as an agglutinin titer ≥1:40 (17), the positive rates for the agglutinin antibody against the Yamaguchi and Tohama strains were 54.0% and 33.6%, respectively. When a Yamaguchi agglutinin titer of ≥1 : 320 was taken as a diagnosis of acute pertussis (17), the positive rate was 14.2% among the healthy asymptomatic population.

## Discussion

There has been a change in the age-related epidemiology of pertussis with an increased proportion of cases reported among adolescents and adults in the United States, Canada, France, and Australia, which all have longstanding vaccine-induced pertussis control (19). This epidemiologic shift is probably multifactorial, comprising elements such as waning immunity following childhood vaccination, increased recog-



**Figure 1.** Frequency distribution of *B. pertussis* agglutinin titers in healthy volunteers. *B. pertussis* agglutinin titers against the Yamaguchi strain (shaded bar). *B. pertussis* agglutinin titers against the Tohama strain (open bar).

dition of adult pertussis, and the development of new diagnostic methods (20). With whole-cell pertussis vaccine, immunity begins to diminish after 3 to 5 years, and there is no demonstrable protection by 10 to 12 years. The duration of protection after acellular vaccines is not yet established, but immunity apparently begins to decline after 4 or 5 years, suggesting that a 10-year dose interval will also be appropriate with these vaccines (19). Thus, adolescents and adults are at risk for *B. pertussis* infection not only in the above countries but also in Japan.

In 2007, a large-scale outbreak of *B. pertussis* occurred at a university in Japan (21). During the subsequent outbreak, 361 university students and staff members presented with the primary complaint of cough. From this large outbreak, adolescent and adult pertussis has been recognized in Japan. However, the diagnosis of *B. pertussis* infection is difficult and most cases were diagnosed by serology with a single serum sample. In the present study, we diagnosed *B. pertussis* infection as definitive cases by serology using paired serum samples and a PCR assay. Clinical findings and laboratory data in our adult patients with *B. pertussis* infection demonstrated non-specific cough and normal WBC and lymphocyte counts. These findings were consistent with previous Japanese reports, in which the clinical findings of adult pertussis were different from pertussis in children (21, 22).

Macrolide antibiotics can improve clinical symptoms of pertussis when they are given early, that is, during the incubation period or in the early catarrhal phase (1 to 2 weeks after onset) (1, 2). When prescribed later, there is less benefit (2). It seems that adolescents and adults with sporadic cases frequently present during the paroxysmal phase, which

occurs at least a week after the onset of symptoms, and in such cases, antibiotics rarely affect the course of the disease (2). The present study demonstrated that the efficacy of macrolide therapy clearly differed between the two groups. The patients who received clarithromycin prior to 14 days after clinical onset demonstrated a shorter duration of cough symptoms than patients who received clarithromycin 14 days or more after clinical onset. Although the present results supported previous reports, the sample size was too small in our study to determine statistical differences. In contrast to our results, Nogami reported that macrolide antibiotics could improve cough even if given during the paroxysmal phase in many patients (22). The mechanism of antibiotic efficacy is unclear.

Most cases of pertussis in adults are diagnosed by serological tests. In Japan, bacterial agglutination antibodies against the *B. pertussis* Yamaguchi and Tohama strains are frequently measured for the serodiagnosis of pertussis (strain Yamaguchi: epidemic strain, agglutinogens 1, 3, 6, 7, and 13; strain Tohama: vaccine strain, agglutinogens 1, 2, 4, 7, and 13) (11, 21, 23), in part because historically, a whole-cell pertussis vaccine using the Tohama strain was used until 1975 in Japan. Although a four-fold rise in agglutination titer using paired serum samples is a definitive criterion, the collection of paired serum samples requires more than four weeks; therefore, single-serum agglutination titers are used instead. When only a single serum sample is used, a Yamaguchi agglutinin titer  $\geq 1 : 320$  is recommended for diagnosis of adult pertussis in Japan (17). Recently, Kamano et al demonstrated that the bacterial agglutination assay against strain Yamaguchi might be a useful tool for the diagnosis of

adolescent and adult pertussis when a cut-off value of  $\geq 1:160$  is used to determine positive agglutination results (21). They also demonstrated that there was no significant difference in Tohama agglutinin titers between patient and healthy adult groups (21). Using these criteria with single-serum agglutination titers, our results demonstrated that 14.2% (cut-off value of  $\geq 1:320$ ) and 31.6% (cut-off value of  $\geq 1:160$ ) of asymptomatic healthy subjects were recorded as pertussis infections. Thus, we believe that paired serum samples should be used when the acute serum samples are collected within two weeks after clinical onset for definitive diagnosis of pertussis in adults. If the acute serum samples were not collected within two weeks, the serum samples should be used more than four weeks after clinical onset (2) because the antibody titer may reach a plateau.

Using the criterion for a single antibody of more than 3 standard deviations greater than geometric mean of the control group (4, 6), our results demonstrated the single Yamaguchi agglutinin titer  $\geq 1:1,280$  was defined as having an acute pertussis infection. In the present study, we used the criteria with the cut-off value of  $\geq 1:1,280$  in combination with clinical symptoms for the diagnosis of acute pertussis infection. However, using this criterion with single-serum agglutination titers, only four of nine patients with pertussis were diagnosed as acute pertussis infection in this outbreak. The diagnostic sensitivity was low (but specificity was high) and many pertussis cases might be missed when an agglutinin titer of  $\geq 1:1,280$  is used. Further studies to determine the criteria for suspicion and to pick up possible cases with pertussis (agglutinin titer  $\geq 1:160$  or  $\geq 1:320$ ) are needed.

Antibodies against PT and FHA are widely used for diagnosis of *B. pertussis* infection in the Western countries (1-6). It is well known that anti-FHA antibodies cross-react with *Bordetella parapertussis* and other bacteria such as *M. pneumoniae*, *C. pneumoniae* and *Haemophilus influenzae* (1). Thus, a high titer of anti-PT antibody is the primary serodiagnostic choice for pertussis cases (3-7, 18). Higa et al comparatively evaluated the titers of the bacterial agglutination antibody and anti-PT antibody during a possible outbreak of pertussis and found that the association between bacterial agglutination and anti-PT antibody titers was weak, and the bacterial agglutination antibody titers were not found to be useful for predicting anti-PT antibody titers (23). It has also been reported that a *B. pertussis* culture-positive infant was positive for the Yamaguchi agglutinin titer but negative for anti-PT antibody at 7 days after hospital admission (25). The present results also demonstrated that the bacterial agglutination antibody titers were not in agreement with anti-PT antibody titers. These results indicate that the Yamaguchi agglutinin titer might not always be in agreement with that of anti-PT antibody titers. Thus, both anti-PT and Yamaguchi agglutinin titers should be measured to avoid an anti-PT false-negative and to yield a more accurate diagnosis.

An IS481-based PCR assay has been reported to detect fewer than 10 organisms, and thus the test has significantly

greater sensitivity than culture methods (15). There has been concern about the specificity of detection of *B. pertussis* because of sequence identity with *B. holmesii* (26). However, *B. holmesii*-specific sequences were not detected in any of more than 1,000 clinical retested samples (15). Thus, Riffelmann et al and the Pertussis PCR Consensus Group suggested that IS481-based PCR assay may be sufficiently specific for the laboratory diagnosis of *B. pertussis* (15). In Japan, loop-mediated isothermal amplification (LAMP) for the detection of *B. pertussis* is now being evaluated (27). When culture was used as the reference method, the specificities of IS481-based PCR and LAMP were 82% and 82%, respectively. IS481-based PCR and LAMP results showed 93% agreement (27).

In outbreak situations, asymptomatic carriage has been observed in up to approximately 50% of individuals tested (1). Using a PCR assay, the present study also demonstrated that there are many cases of asymptomatic carriage. Because pertussis is highly contagious, chemoprophylaxis is recommended to control outbreaks (24). A 14-day erythromycin regimen has been the treatment choice; shorter courses of macrolide antibiotics (e.g., azithromycin and clarithromycin) may be as effective with fewer adverse effects and better adherence to therapy. In the present study, chemoprophylaxis with azithromycin 500 mg/day for 3 days was provided to 17 asymptomatic subjects who had contact with pertussis patients. No cases of cough were observed among these subjects. Prescription of macrolides as chemoprophylaxis to people who had contact with pertussis cases seemed to be effective in controlling the outbreak.

Cough is a quite common symptom and is treated symptomatically without a definitive diagnosis. Viruses, *M. pneumoniae*, and *C. pneumoniae* are well recognized causes of outbreaks in closed communities in Japan. In the present findings, an outbreak of *B. pertussis* infection occurred in university laboratory and their family members among younger adults. Therefore, physicians should consider *B. pertussis* in the differential diagnosis of an outbreak of respiratory infection even in adult populations.

**The authors state that they have no Conflict of Interest (COI).**

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