Original Article

Analysis of Bordetella pertussis Agglutinin Titers during an Outbreak of Pertussis at a University in Japan

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SUMMARY: In 2007, a large outbreak of pertussis occurred at a university in Japan. Initially, a student, suffering from nocturnal cough and post-tussive vomiting for 3 weeks was diagnosed with pertussis. In the subsequent outbreak, 361 university students and staff members presented with a primary complaint of a cough. The evidence obtained in the present study suggests that the pertussis outbreak in adolescents and adults at the university was caused by Bordetella pertussis. Therefore, the bacterial agglutination assay against strain Yamaguchi may be a useful tool for diagnosis of adolescent and adult pertussis, especially in young adults, when an agglutinin titer cutoff value of ≥160× is used in combination with clinical symptoms and other clinical laboratory tests.

INTRODUCTION

In Japan, more than 100,000 cases of pertussis were reported every year before the 1950s. Whole-cell pertussis vaccine was introduced in 1950 in Japan, followed by a dramatic decrease in the number of pertussis cases (1). In the 1970s, it was reported that the whole-cell pertussis vaccine caused encephalitis in Japan, and the pertussis vaccination rate in Japan then decreased, followed by an increase in the number of pertussis cases (1). A safer purified diphtheria-tetanus-acellular pertussis (DTaP) vaccine was introduced in Japan in 1981. Consequently, the prevalence of pertussis decreased and Japan was reported to reach the WHO pertussis target of <1/100,000 persons (2), although this conclusion was questionable. In 2007, there was a large-scale pertussis outbreak in adolescents and adults at a university in Japan, during which we were able to analyze Bordetella pertussis agglutinin titers. To the best of our knowledge, this is the first report of an analysis of Bordetella pertussis agglutinin titers among adolescents and adults during a pertussis outbreak.

PATIENTS AND METHODS

Serological tests: Analysis of B. pertussis agglutinin titers was performed on people with a cough during a pertussis outbreak at the university (outbreak group: students and staff members n = 310; 176 male, 134 female; age range 18–55 years; average age 23.7; median age 21). Bacterial agglutinin titers against B. pertussis strains Yamaguchi (epidemic strain, agglutinogens 1, 3, 6, 7, and 13) and Tohama (vaccine strain, agglutinogens 1, 2, 4, 7, and 13) were measured using the Bordetella agglutination test ‘SEIKEN’ N (Denka Seiken, Tokyo, Japan) according to the manufacturer’s instructions (3). The cut-off point for a positive result was taken as an agglutinin titer ≥40×. This criterion is used for serological diagnosis for infants and children in Japan. To determine the cut-off criterion for adult pertussis, we compared data from the 2007 university outbreak group with data from healthy people without a cough during the same season (4) (control group n = 246; 156 male, 90 female; age range 21–60 years; average age 40.3; median age 39.5).

Genetic tests: Molecular diagnosis of B. pertussis infections was performed using the loop-mediated isothermal amplification (LAMP) method (5). Nasopharyngeal swab specimens were collected from 60 patients with suspected pertussis, and total DNA was extracted using QIAamp DNA Micro Kits (Qiagen, Hilden, Germany). The DNA samples (2 μl) were analyzed by the LAMP assay.

Case definition: A “probable case” was defined as a person who had a cough and a B. pertussis strain Yamaguchi agglutinin titer ≥40× between May 17 and July 4, 2007. A “definite case” was defined as a person who had a cough, a B. pertussis strain Yamaguchi agglutinin titer ≥40×, and a positive LAMP assay result between May 17 and July 4, 2007.

Statistical analyses: The Mann-Whitney U-test, chi-square test, and t test were performed. The level of significance was P < 0.01.

RESULTS

Progression of the pertussis outbreak: On May 17, 2007, a student complained about a prolonged severe cough and was diagnosed with pertussis based on the clinical findings at a university hospital. On the following day, another stu-
A student complained about a severe cough that had persisted for 4 weeks and was diagnosed with pertussis based on the clinical findings at a general hospital. On the same day, 10 students complained of a persistent cough and, on May 24, 8 of the 10 students were diagnosed with pertussis. Also on May 24, the mass media reported a pertussis outbreak at the university. Starting the next day, many students and staff members began to complain of a persistent cough, and the number of pertussis patients continued to increase. It was agreed that an outbreak was spreading throughout the entire university, and it was decided to close the university for 2 weeks. Patients diagnosed with pertussis were treated with macrolides. The last patient presented on July 4 (Fig. 1). From May 17 to July 4, 361 people (285 students, 76 staff members) had a primary complaint of a cough, and 290 of these (231 students, 59 staff members) were serologically-positive: 270 were diagnosed as “probable cases” and 20 as “definite cases.” Chemoprophylaxis with macrolides was provided to 1,163 persons who had contact with pertussis patients and who would come in contact with infants due to hospital or educational practices (6,7). We analyzed the symptoms and life-styles of students and staff members with severe cough and found that these patients’ contacts clustered around three specific places: a dormitory, a club, and an office.

**Cases in the dormitory:** In the dormitory, a student complained of a nocturnal cough, paroxysmal cough, dyspnea, and post-tussive vomiting for 3 weeks. Five students in the dormitory subsequently complained of a paroxysmal cough...

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**Fig. 1.** Calendar showing duration of cough in patients during pertussis outbreak in a university. Patients (n = 128) with probable pertussis were asked for the date of cough onset during May to July 2007. Length of a bar shows the duration of cough until the date of serological analysis.
and were diagnosed with pertussis. A total of 9 students had a primary complaint of cough. These students shared a dining room and a kitchen in the same dormitory and frequently watched TV and played video-games together in the same room.

**Cases in the club:** In the club, a student complained of a paroxysmal cough for more than 2 weeks. Four students in the club subsequently complained of a persistent cough and were diagnosed with pertussis. A total of 8 students had a primary complaint of cough. All members of this club had dinner with the initial patient after a club activity, and also lived together in a training camp.

**Symptoms of the patients:** In most cases, the main symptom of pertussis has been reported to be a protracted cough (6,8). For the pertussis cases in this study, paroxysmal cough occurred in 53.9% of patients and nocturnal cough in 50.7% of patients (Table 1). Since some patients with a strong positive agglutinin titer were not conscious of their prolonged light cough, it is possible that patients with very mild symptoms transmitted pertussis to other people.

**Serological tests:** The bacterial agglutinin titers against the strain Yamaguchi of the outbreak group (median = 160×, upper quartile = 320×) were significantly higher than those of the control group (median = 40×, upper quartile = 80×) (P < 0.001) (Fig. 2A). Therefore, significantly more cases in the outbreak group had Yamaguchi agglutinin titers ≥160× (178/310, 57.4%) compared to the control group (74/246, 30.1%) (P < 0.001). In contrast, there was no significant difference in Tohama agglutinin titers between the outbreak and control groups (median = 80×, upper quartile = 160×) (Fig. 2B).

**Relationship between the Yamaguchi agglutinin titers and the duration of cough:** We analyzed the relationship between the Yamaguchi agglutinin titers and the duration of cough in the students. Of the 93 student probable cases, 28 had a Yamaguchi agglutinin titer ≤80× and a cough for 3.8 ± 2.5 days, while 65 had a Yamaguchi agglutinin titer ≥160× and a cough for 11.1 ± 23.3 days. Therefore, the duration of cough was longer in student probable cases with Yamaguchi agglutinin titers ≥160× than in cases with Yamaguchi agglutinin titers ≤80× (Table 2A). Of the 17 students in the dormitory and the club, 7 had a Yamaguchi agglutinin titer ≤80× and a cough for 3.8 ± 6.0 days, while 10 had a Yamaguchi agglutinin titer ≥160× and a cough for 10.3 ± 6.3 days. Therefore, the duration of cough was longer in students in the dormitory and the club with Yamaguchi agglutinin titers ≥160× than in cases with Yamaguchi agglutinin titers ≤80× (Table 2B).

**Genetic tests:** Molecular diagnosis of *B. pertussis* infection was performed using the LAMP method (5). Of the 60 patients with suspected pertussis infection, 20 (33.3%) were positive by the LAMP assay, indicating that the outbreak was caused by *B. pertussis* infection. Interestingly, all positive

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**Table 1. Symptoms of the pertussis patients in this study**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxysmal cough</td>
<td>53.9</td>
</tr>
<tr>
<td>Nocturnal cough</td>
<td>50.7</td>
</tr>
<tr>
<td>Whooping cough</td>
<td>14.7</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>17.5</td>
</tr>
<tr>
<td>Post-tussive vomiting</td>
<td>6.3</td>
</tr>
<tr>
<td>Speaking induced cough</td>
<td>23.8</td>
</tr>
</tbody>
</table>

Probable cases (n = 63).

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**Table 2. Analysis of the relationship between the Yamaguchi agglutinin titer and duration of cough in students**

(A) Students probable cases (n = 93)

<table>
<thead>
<tr>
<th>Agglutinin titer</th>
<th>≤80×</th>
<th>≥160×</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of cough (d)</td>
<td>3.8 ± 2.5</td>
<td>11.1 ± 23.3</td>
</tr>
<tr>
<td>(n = 28)</td>
<td>(n = 65)</td>
<td></td>
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</tbody>
</table>

Result of Mann-Whitney U-test; P = 0.078.

(B) Students in the dormitory and the club (n = 17)

<table>
<thead>
<tr>
<th>Agglutinin titer</th>
<th>≤80×</th>
<th>≥160×</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of cough (d)</td>
<td>3.8 ± 6.0</td>
<td>10.3 ± 6.3</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(n = 10)</td>
<td></td>
</tr>
</tbody>
</table>

Result of Mann-Whitney U-test; P = 0.012.
samples had been collected from patients (n = 34) at an early stage (before June 4) of the outbreak (positive rate 20/34, 58.8%). No positive samples were collected at the late stage (after June 5). The difference in the number of positive samples between the early and late stages may have been due to antibiotic prophylaxis or therapy. In fact, no early-stage patients received antibiotics before the LAMP assay, whereas at least 6 of 26 late-stage patients had received antibiotics.

**DISCUSSION**

The index case of the pertussis outbreak in this study was probably a university student living in the dormitory. An infection in such a small, semi-closed population can spread like a wave throughout the population, leading to a mass outbreak. In the university, to control further spread of the pertussis outbreak, the cooperation of students and staff members who had complained of a persistent cough was necessary. Prescription of macrolides as chemoprophylaxis to the club, or the office seemed to be effective in controlling the outbreak.

The 2007 pertussis outbreak suggests that a pertussis vaccination system should be reconsidered in Japan. The pertussis outbreak in this study was an increase in the antibody against agglutinogens 3 and 6 of 26 late-stage patients had received antibiotics. However, the duration of effectiveness of vaccination system has been administered to 86% of pertussis patients (9,10). However, the duration of effectiveness of vaccination-induced pertussis immunity is 4–12 years (9,10). Attenuation of immunity is thought to be due to a decrease in the number of plasma cells producing specific antibodies and specific CD8+ cells, although the number of memory B and T cells does not decrease (9,10). Currently, in Japan, the DTaP vaccine for pertussis vaccination is administered to infants in 4 doses: 3 doses during an infant’s first year and once at 1 year. In contrast, in the USA, DTaP vaccine is administered in 5 doses (at 2, 4, 6, and 15–18 months, and at 5 years), and an additional tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine dose is recommended at 11–18 years, for a total of 6 pertussis vaccine doses (11). It has been reported that Tdap vaccine prevents *B. pertussis* infection in adolescents and adults (12,13). Therefore, since there is a significant risk of pertussis outbreaks in adolescent and adult populations, pertussis vaccination of adolescents and adults should be considered in Japan.

In this study, we demonstrated that the bacterial agglutination assay against strain Yamaguchi might be a useful tool for diagnosis of adolescent and adult pertussis, especially in young adult patients (18–39 years old) when a cut-off value of ≥160× is used to determine positive agglutination results (Table 2A, B, Fig. 3A, B). The major agglutinogens of *B. pertussis* Tohama are agglutinogens 1, 2, and 4, while those of the Yamaguchi strain are agglutinogens 1, 3, and 6 (3,14, 15). In the outbreak group, the Yamaguchi agglutinin titers were significantly higher than in the control group, but the Tohama agglutinin titer was not statistically different between these two groups. This observation strongly suggests that there was an increase in the antibody against agglutinogens 3 and 6 in outbreak group patients, although *B. pertussis* could not be isolated.

It has been reported that anti-pertussis toxin (PT) IgG of 100–125 units/ml in a single serological test is diagnostic of a *B. pertussis* infection in the previous 2-3 weeks in Europe (16–19). Surprisingly, it has also been reported that a *B. pertussis* culture-positive infant was positive by the Yamaguchi agglutinin assay but negative for anti-PT IgG at 7 days after hospital admission (20). These observations indicate that the Yamaguchi agglutinin titer might not always be in agreement with that of anti-PT IgG. Therefore, both anti-PT IgG and Yamaguchi agglutinin titers should be measured to avoid an anti-PT IgG false-negative and to yield a more accurate diagnosis.

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**REFERENCES**


