
Investigation of a Pertussis Outbreak in a Daycare Center in
Central Taiwan using Pulsed-Field Gel Electrophoresis

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Abstract

Pertussis is a respiratory disease transmitted by the inhalation of airborne particles of the saliva or phlegm of an affected person. Before the advent of a Pertussis vaccine, both the incidence and mortality caused by pertussis were high, but the disease is now controlled with vaccine administration. Nowadays, epidemics of pertussis are rare, the majority of cases being sporadic or in family clusters. In April 2005, there was an infant pertussis case which occurred in Taichung. Tracing back contacts in the daycare center and in the family, the

grandmother, the director and a nurse of the daycare center were found to be the carriers. In this study, we used pulsed-field gel electrophoresis (PFGE) to analyze strains isolated from the case and the three contacts in order to investigate the epidemiological relationship between them. The four strains were also compared using a DNA map database of *Bordetella* in order to investigate the dynamic changes among strains. The results of PFGE showed that the four strains had identical PFGE maps, indicating that the case and the contacts were infected from the same source. The results of map comparison showed that the strain was present in the year 2000 in cases from central, northern, and eastern Taiwan, suggesting that the strain may cause endemics around the country. Although it is possible contacts are the infection source of the case, there was no significant elevation of IgA/IgM in contacts, suggesting that contacts were infected for the first time and that it is impossible to differentiate the transmission sequence among contacts and the case. This study shows the importance of case tracing in pertussis infection, especially in daycare centers where infants are in a semi closed group. If workers are carriers, it is very dangerous to the infants.

Introduction

Pertussis is a respiratory transmissible disease caused by *Bordetella pertussis*. It is a Category 3 notifiable communicable disease in Taiwan. *Bordetella pertussis* are aerobic Gram-positive bacteria, and humans are their only natural hosts(1). *Bordetella pertussis* is mainly transmitted through inhalation of aerosol particles of the saliva or phlegm of a sick person. There is a seven to ten day incubation period after *Bordetella pertussis* has attached to the cilia epithelium of the respiratory tract. Leukocyte infiltration will gradually increase during this period and thereby cause copious amounts of secretions, leading to tracheal

obstruction and cough. Hence, a severe barking cough is the major symptom of infection(2).

Pertussis is mainly prevented by vaccine administration. Before widespread use of the pertussis vaccine, there were many cases of pertussis, and the annual worldwide mortality was around 350,000 (3). Cases were mainly infants and children. Since the widespread administration of vaccine beginning in 1940, the worldwide incidence of pertussis gradually decreased (4). Pertussis vaccine was administrated since 1954 in Taiwan, and pertussis has been significantly controlled since then. After 1970, only sporadic cases are reported, and family clusters are occasional. According to Lee et al(5), there were small-scale outbreaks which occurred in Hualien in 1992 and in Taipei in 1997. In recent years, there are few suspected pertussis cases reported annually, between 170 ~ 200, and confirmed cases are below 20 cases per year(6). Cases are mainly unvaccinated infants, although there are still cases of vaccinated children and adults.

In April 2005, a two-month-old female infant in Taichung was reported as a case of pertussis. She was apparently normal after birth without any systemic diseases. She was diagnosed as having a common cold initially when she was brought to a hospital for her cough. However, the symptom of cough increased gradually. Cyanosis and barking cough also occurred. *Bordetella pertussis* was isolated two weeks after admission. Before the onset of symptoms, she was usually brought to a daycare center during daytime and taken home at night. Cough was present during that time. After analyzing 20 contacts in her family and the daycare center, *Bordetella pertussis* was isolated from three contacts. Hence, the epidemiological relationship between them needs to be investigated in order to identify the infection source and transmission process.

There are many methods to analyze pathogens in order to identify the epidemiological relationship between cases and contacts. Among them, pulsed-field gel electrophoresis (PFGE) is most commonly used. PFGE is highly effective in typing, has a high reproducibility, and can identify changes in chromosomal structure within a short time. Therefore, it is suitable for investigation of pertussis outbreaks(7,8). In this study, we used PFGE to analyze four strains, compared the relationship of bacteria isolated from the case and three contacts, and investigate their epidemiological relationship as well as the order of infection. The results were also compared with *Bordetella* DNA map database we established in order to identify epidemiology of the strain.

Material and Methods

I. Specimen gathering and laboratory analysis

Regan-Lowe specimen collecting devices (Difco Laboratories, Detroit, MI, USA) were used to collect nasopharyngeal and serum specimens from 20 contacts among her family and workers of the daycare center for bacterial culture and IgA/IgM titer testing (table 1). Nasopharyngeal swabs were applied directly onto Bordet-GengouG) agar and cultured at 35-37°C for 3 to 5 days. Suspected colonies were then isolated and cultured on BG agar. Bacteria were tested by agglutination test with anti-pertussis serum (Difco, BBL) and by PCR for the porin gene according to Li et al(9). *Bordetella pertussis* was identified according to specific agglutination reaction with anti-pertussis serum and positive porin gene detected by PCR. IgA/IgM serum titer testing was done by enzyme colorimetry comparing with positive controls.

II. PFGE analysis

PFGE was done according to Popovic(10) with minor modification. Bacterial

chromosome DNA was digested with restriction enzymes XbaI, SpeI, and AflIII, and analyzed with bio-Rad CHEF Mapper (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis was done in 1% agarose gels (6 V/cm, 120° electrical field angle, and 4~40 sec pulse transition time) for 20 hours. XbaI-digested *Salmonella ser.* Braenderup H9812 fragments were used as size standard.

III. Data analysis

Digitalized PFGE map images were analyzed by fingerprint analyzing software BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium). Maps were also filed into *Bordetella* DNA map database together with data of the cases (gender, age, date of onset, vaccination, etc.). One or more than one differences in DNA fragments were viewed as different PFGE types. PFGE maps of the four strains were also compared with 56 PFGE maps in the *Bordetella* DNA fingerprint map database. Dendrogram was constructed by UPGMA arithmetics of BioNumerics and Dice coefficient.

Results

I. Results of the laboratory examination of specimens

We traced 20 contacts in her family and the daycare center. *Bordetella pertussis* was isolated from her grandmother, the director and a nurse of the daycare center. Other contacts were negative (Table 1). There was no significant IgA/IgM elevation among all contacts and the case. This may be caused by insufficient amount of IgA/IgM formation during such a short timeframe. *Bordetella pertussis* was not isolated from eleven infants in the daycare center nor was significant IgA/IgM elevation was detected among them. They had received at least 3 shots of pertussis vaccine, and should have

had sufficient immunity.

II. PFGE typing

PFGE maps of XbaI, SpeI, and AflIII-digested chromosomal DNA of *Bordetella pertussis* isolated from the case and the three contacts were identical (Fig. 1), suggesting that the four strains were from the same source and that the four people were infected from the same source.

III. Comparison with past strains

To investigate the relationship between the strains isolated this time and past strains, and to establish if they have caused epidemics in the past, we compared their XbaI-PFGE maps with BioNumerics and established their dendrogram. According to the XbaI-PFGE dendrogram (Fig. 2), the 56 strains could be separated into 25 XbaI-PFGE types. Group I to III could be separated when similarity was set at 55%. Group III could be separated into group A and group B when similarity was set at 70%. Group A could be further separated into A1 to A5 when similarity was set at 85% (Fig 2). Group A was mainly formed by A2 and A4. A1, A2, A4, and A5 accounted for 15%, 42.5%, 2.5%, 37.5%, and 2.5% of group A, respectively. The Four strains in this outbreak were group A4 type P018. P018 in the major type in group A4, accounting for 80% of it. According to our *Bordetella* DNA fingerprint database, P018 had emerged in 2000. Four stains of P018 were isolated in central Taiwan, and two in northern and eastern Taiwan, respectively, suggesting that the strain was once epidemic in Taiwan.

Discussion and Suggestion

The number of reported cases of pertussis was between 20 and 40 in central Taiwan in the past 5 years, and confirmed cases were below 10. Few strains were

isolated from those cases, indicating that the incidence of pertussis was very low in central Taiwan over the past five years. Most cases were unvaccinated infants from family units, and their infection sources were from caregivers. This was also the case in the outbreak in the daycare center in Taichung. The case is a two-month-old infant, and her infection source was possibly her caregivers in the family or at the daycare center. Tracing her contacts, her grandmother and the director and a nurse of the daycare center were carriers (Table 1), suggesting caregivers were the infection source. Because four of them had no elevated IgA/IgM, they might have been infected recently, and it is impossible to infer the sequence of infection from our current laboratory results. Nevertheless, this outbreak still outlined the importance of disease follow-up and tracing. Preventive measures could be taken to prevent spreading to others from the carriers.

According to epidemiological statistics, epidemic pertussis infection occurs every 3 to 5 years, and vaccination is still the most effective way of prevention. Although vaccination can effectively prevent spreading of pertussis, periodic epidemics can still occur(11). Previous results also show that pertussis epidemics are seasonal(12), occurring mainly between spring and summer. This outbreak also occurred between spring and summer. PFGE analysis showed that the strain isolated was type P018, which was once epidemic in central Taiwan in 2000(Fig. 2), but disappeared between 2001 and 2004. Whether its re-emergence suggests the possibility of another epidemic requires further attention. Since type P018 can become latent for years and re-emerge, the mechanisms of latency requires further investigation.

Pertussis tends to occur in children under 5, especially in unvaccinated infants. The case in this outbreak was a 2-month-old who had received only one shot of the vaccine. She might not have had sufficient immunity and thus became

infected. Most other infants complete four shots of the vaccine. Some of them had only completed three shots because of non-compliance to the schedule, but they might have possessed sufficient immunity to prevent infection. The grandmother of the case and the director and a nurse of the daycare center were positive for *Bordetella pertussis*, but they were neither symptomatic nor transmitting the disease to others. Their immunity might decrease due to prolonged intervals since last vaccination(13, 14). Recent studies have suggested that the incidence of infection has increased among vaccinated adolescents and adults(15). Carriers in this outbreak were all adults, suggesting that adult carriers may be an important infection source.

In this study, we used PFGE for strain typing in order to investigate the epidemiological relationship between the case and contacts. PFGE is currently the most effective typing method. If PFGE maps of AflII, SpeI, and XbaI-digested chromosomes are identical, we can infer that they are the same strains(16). The *Bordetella* DNA database gathers PFGE maps of most of strains isolated in Taiwan in the past as well as profiles of their hosts. Since PFGE analysis has been standardized, this database can function as the basis for long-term pertussis surveillance in Taiwan and for investigation of evolution of *Bordetella pertussis*.

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Table 1. Test results of nasopharyngeal swabs and serum IgA/IgM from the case and contacts in the family and the daycare center in this pertussis outbreak

Relationship	Age	Nasopharyngeal Swab	IgA/IgM	Vaccination
Case	2 months	<i>Bordetella pertussis</i>	—/—	1
Father	27	Negative	—/—	4
Mother	28	Negative	—/—	4
Grandfather	58	Negative	—/—	Unvaccinated
Grandmother	56	<i>Bordetella pertussis</i>	—/—	Unvaccinated
Nurse	28	<i>Bordetella pertussis</i>	—/—	4
Director	48	<i>Bordetella pertussis</i>	—/—	4
Worker 1	28	Negative	—/—	4
Worker 2	23	Negative	—/—	4
Worker 3	23	Negative	—/—	4
Infant 1	5	Negative	—/—	4
Infant 2	4	Negative	—/—	4
Infant 3	3	Negative	—/—	3
Infant 4	4	Negative	—/—	3
Infant 5	3	Negative	—/—	3
Infant 6	3	Negative	—/—	3
Infant 7	4	Negative	—/—	4
Infant 8	3	Negative	—/—	4
Infant 9	3	Negative	—/—	3
Infant 10	4	Negative	—/—	4
Infant 11	3	Negative	—/—	3

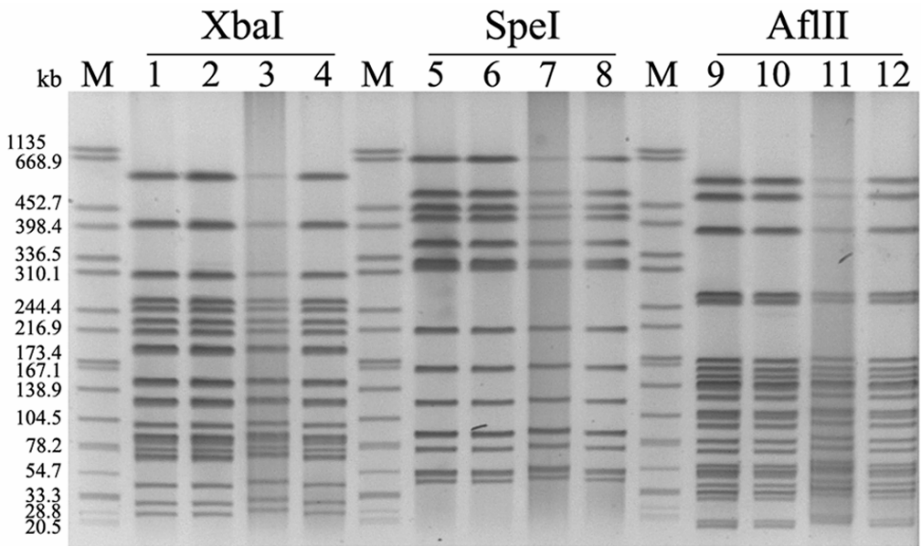


Fig 1. XbaI, SpeI, and AflIII-digested PFGE maps of 4 *Bordetella pertussis* strains from the case and contacts. M: XbaI-digested *Salmonella* ser. Braenderup H9812 chromosomal DNA as the marker. Lanes 1, 5, 9: PFGE maps of the strain isolated from the case; Lanes 2,6,10, lanes 3, 7, 11, and lanes 4, 8, 12 are PFGE maps of the strains isolated from 3 contacts.

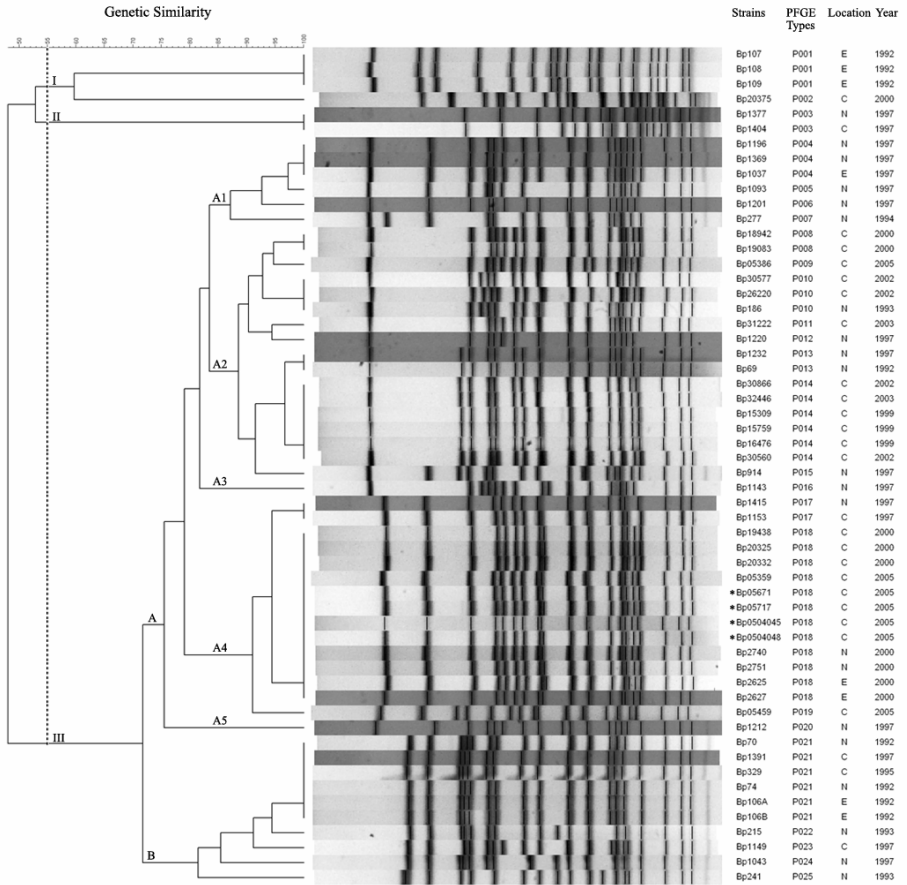


Fig. 2. Dendrogram of PFGE maps of *Bordetella pertussis*

XbaI-digested chromosomal DNA fragments were used to construct PFGE maps. Dendrogram was constructed with UPGMA of BioNumerics software (2% optimization and 0.7% tolerance). *The strain isolated in this outbreak. N, E, and C: northern, eastern, and central Taiwan, respectively.