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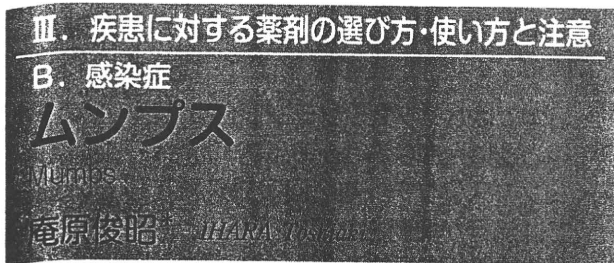

Ⅲ．疾患に対する薬剤の選び方・使い方と注意

B．感染症

ムンプス

Mumps

庵原俊昭* IHARA Toshiaki

1．疾患の概要

ムンプスはパラミクソウイルス科ルブラウイルス属に属するムンプスウイルスの全身性感染症である。上気道で増殖したウイルスが，ウイルス血症により全身の親和性臓器に運ばれ，そこで増殖して臨床症状が出現する（**図**）。潜伏期間は通常16～18日間である。

ムンプスの特徴的な臨床像は48時間以上持続する急性耳下腺腫脹である。多くは顎下腺，舌下腺も腫脹する。全年齢の顕性感染率は70%であるが，3歳以下では不顕性感染率が高く，4歳を超えると顕性感染率が90%以上となる[1)]。一般に年少児が罹患すると軽症だが，思春期以降に罹患すると耳下腺腫脹期間が長くなり，合併症の発症率が高くなる。ムンプスの合併症には，髄膜炎，脳炎，精巣炎，卵巣炎，乳腺炎，めまい，難聴，頸部前胸部腫脹などがある（**表1**）。精巣炎，卵巣炎，乳腺炎は思春期以降に発症する合併症である。脳炎と難聴は予後の悪い合併症である。妊婦が第1三半期にムンプスに罹患すると，約25%に自然流産が認められるが，ムンプスウイルスと関連する先天奇形は認められていない。ときに，ムンプス流行時に唾液腺腫脹を伴わずにムンプスウイルスによる髄膜炎や難聴を発症することがある。

表1　ムンプス自然感染の症状とワクチンの副反応

症状	自然感染	ワクチン
耳下腺炎	70%	3%
無菌性髄膜炎		
細胞増多	50%	不明
症候性	3～10%	1/1,000～10,000
脳炎	0.02～0.3%	0.4/1,000,000
難聴	1/400～20,000	1/6,000,000 ～8,000,000
睾丸炎	25%**†	ほとんどなし*
両側腫脹	10%**	ほとんどなし
乳腺炎	15～30%**	ほとんどなし
卵巣炎	5%**	ほとんどなし
膵炎	4%**	ほとんどなし

第1三半期の妊婦が罹患すると1/4は自然流産する。
*詳細な頻度は不明，**思春期以降の頻度（小児ではまれ），†ムンプス睾丸炎発症者の1.5%に睾丸癌発症
（庵原俊昭：日本臨牀 65：s380，2007を引用一部改変）

2．確定診断はどのように行うか

ムンプス流行時期に急性耳下腺腫脹を認めるときは多くはムンプスであり，非流行時期に認める急性耳下腺腫脹はムンプス以外の原因によるものである。ムンプスの診断には疫学的関連性が大切である。

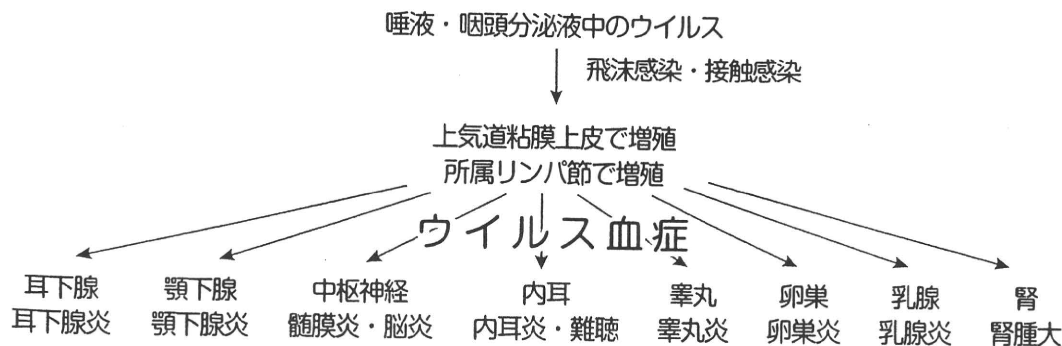

図　ムンプスウイルスの体内での増殖動態

Key words：ムンプス，ムンプスワクチン，急性耳下腺腫脹，ワクチン不全，髄膜炎

*国立病院機構三重病院小児科　［〒514-0125　津市大里窪田町357］
TEL 059-232-2531　FAX 059-232-5994　E-mail：ihara@mie-m.hosp.go.jp

ムンプスの確定診断（実験室診断）には，酵素免疫法（EIA）を用いて血清 IgM 抗体を測定する。ムンプス初感染の多くは，耳下腺腫脹時期にはムンプス IgM 抗体も IgG 抗体も検出されるが，ムンプス IgG 抗体は低値である。ムンプスワクチン後の 2 次性ワクチン不全や再感染では，ときに IgM 抗体が検出されることはあるが，EIA-IgG 抗体は 25.8 EIA 価以上の高値である[1]。唾液からのウイルス分離や，polymerase chain reaction（PCR）法や loop-mediated isothermal amplification（LAMP）法によるムンプスウイルス RNA の検出も診断に有用である。髄膜炎を合併したときは髄液からのウイルス分離やウイルス RNA 検出が診断に有用である。

急性期と回復期（発症 2 週以降）の血清を用いて，IgG 抗体の陽転化または有意上昇も診断に有用である。有意上昇の基準は測定誤差以上の上昇であり，EIA-IgG 抗体では 2 倍以上，赤血球凝集抑制法（HI）や中和法（NT）では 2 管（4 倍）以上の上昇である。

表 2　日本のムンプス流行とムンプス対策

ムンプス流行
- 日本ではムンプスワクチンが任意接種のため，4 年ごとに大きな流行がある。
- ムンプスワクチンを 2 回定期接種をしている国では流行が抑制されているが，ときに大学生や成人に流行を認める。
- 現在欧米，日本で主に流行している野生株の遺伝子型は G である。

ムンプスワクチン
- Jeryl-Lynn 株の遺伝子型は A，Urabe 株，星野株，鳥居株の遺伝子型は B である。
- 日本のムンプスワクチンの有効率は 80～90%であり，株による差は認められていない。
- 家族内でのムンプス患者接触後の緊急接種の効果は高くない。
- ムンプスワクチン後 3 週間以内に急性耳下腺腫脹を発症した場合は，唾液からのウイルス分離またはウイルス核酸の検出を行い，由来株を同定する。

ムンプス抗体
- ムンプスの免疫状態を調べる抗体測定方法は EIA-IgG である。
- ムンプス再感染，ワクチン不全を疑うときは，EIA-IgM 抗体と EIA-IgG 抗体を測定する。

EIA：酵素免疫法

3．ファーストラインの治療戦略

ムンプスウイルスに効果がある抗ウイルス薬はないので，保存的に治療する。耳下腺の痛みが強いときは鎮痛剤を使用し，痛みのために水分の摂取が困難なときは輸液を行う。髄膜炎合併により頭痛や嘔吐が強いときは，髄液を採取し，圧を下げると症状が軽快することがある。精巣炎に対しては，鎮痛剤の投与と局所の冷却が行われている。

ムンプスによる登校停止期間は，日本では耳下腺腫脹が消失するまでであるが，米国での登校停止期間は耳下腺炎出現後 5 日間である[3]。なお，片側が腫脹して 7 日後ごろに反対側の耳下腺が腫脹することがあり，この時も唾液からムンプスウイルスが分離されるので，反対側耳下腺腫脹出現後も 5 日間は登校を停止すべきである[4]。

4．ファーストラインの予防戦略

1．予防の流れと効果のエビデンス（表 2）

ムンプス予防対策の基本はムンプスワクチン接種である。日本では星野株と鳥居株の 2 種類が市販されている。わが国のムンプスワクチンの有効率は 80～90%であり，株による差は認められていない[1]。日本では任意接種であるため 4 年ごとに流行を認めているが，ムンプスワクチンの 1 回定期接種が行われている国では患者数が 90%減少し，2 回定期接種を行っている国では患者数が 99%減少している。欧米の多くの先進国は 2 回接種である。なお，現在欧米や日本で流行している野生株の遺伝子型の多くは G である。

ムンプス患者と接触後の発症予防対策については，ムンプスワクチンの緊急接種は有効率が低く，免疫グロブリンの投与も無効である。ムンプスワクチン接種により特異的免疫が誘導される時期が，麻疹ワクチンや水痘ワクチンよりも遅いこと，免疫グロブリンに含まれるムンプス抗体価が麻疹抗体価よりも低値であることが関係していると考えられている。

2．実際の投与方法

1歳以降の小児を対象に0.5 mL 皮下接種する。保育園や幼稚園などの集団生活に入る前に接種が勧められる。また，ムンプス既往歴およびワクチン歴がない思春期以降の人には，男女にかかわらず接種が勧められる。ワクチン接種前に免疫の有無を確認するために抗体測定を希望する場合は，EIA-IgG で測定する。ほかのムンプス抗体測定方法の感度は EIA に比べて低率である。成人に接種しても副反応が増加することはないし，免疫がある人に接種しても副反応は増加しない。

3．投与後の経過観察

ムンプスワクチンによる耳下腺腫脹（3%）や髄膜炎（1/1,000～10,000 接種）の合併は，ワクチン接種後 18～21 日に認められるので，少なくとも接種後 21 日間の経過観察が必要である。ムンプスワクチン後の精巣炎，脳炎，難聴はきわめてまれである（表 1）。なお，ムンプス流行時期にムンプスワクチンを接種し，15 日以内に耳下腺腫脹を認めたときは，野生株の感染による臨床症状である[2]。唾液を採取し，耳下腺腫脹をきたした由来株を同定すべきである。

4．判定効果の時期と判定のしかた

ワクチン接種 4～6 週後に血清抗体価を EIA-IgG で測定すると，ムンプスワクチン接種による効果が判定できるが，ムンプスワクチン接種後の抗体陽転率は 90～95%であり，一般に接種後の抗体価測定は行っていない。

ワクチンの臨床上の効果は，園や学校においてムンプス流行に遭遇したときムンプスが発症するかどうかで判定される。ワクチンの有効率は（1－ワクチン接種者の発症率/ワクチン非接種者の発症率）×100 で算出される。ムンプスワクチンの有効率は 80～90%である。なお，ムンプスワクチンを接種していた人がムンプスを発症したとしても，耳下腺腫脹期間はワクチン非接種者の腫脹期間よりも短く，髄膜炎の合併率も低率である[1]。ワクチンの効果を検討するためには，流行時の疫学的検討が大切である。

5．ワクチン投与時の問題点

ムンプスワクチンは生ワクチンであるので，免疫不全者および妊婦は接種不適当者である。また，37.5℃以上の発熱者も接種不適当者である。ムンプスワクチンはニワトリ胎児細胞を用いてウイルス増殖が行われているが，含まれるオボアルブミンの濃度は 1 ng 以下である。卵によるアナフィラキシーを発症させる濃度は 600 ng/dose 以上であるので，現行のムンプスワクチンを卵アレルギーの人に接種してもアナフィラキシーを発症させる危険性はない。

ムンプスワクチンはムンプスが流行すると接種希望者が増加するため，ムンプス流行時のほうがワクチン接種後の耳下腺腫脹者の頻度が増加する[2]。ワクチン後の臨床反応を認めたときは，接種したワクチンのメーカーと相談し，臨床症状を出現させた由来株を同定すべきである。

5．ファーストラインが無効・効果不十分の時の予防戦略

ムンプスワクチンの接触後の発症予防効果は，麻疹ワクチンや水痘ワクチンと比較すると効果は乏しいが，園や学校でのムンプス流行時に流行を早期に抑制する唯一の方法は，ムンプス既往歴およびワクチン歴がない児にムンプスワクチンを接種することである。

世界で多く使用されているムンプスワクチン株である Jeryl-Lynn 株は少し病原性を減弱させすぎ（overattenuation）の可能性が指摘されている。実際 2 回の定期接種を受けた成人においてもムンプス発症が認められている[5]。わが国で開発された Urabe 株は，Jeryl-Lynn 株と比べると免疫原性は優れているが，無菌性髄膜炎の副反応発症率は Jeryl-Lynn 株よりも高率である[6]。わが国で使用されているムンプスワクチン株の免疫原性は Urabe 株と同等である。

地域でのムンプス流行排除を考えるならば，90%以上の接種率による 2 回接種が必要である。わが国は先進国のなかでムンプスワクチンの定期接種が行われていない唯一の国である。なお，世界でムンプス野生株の排除を宣言した国はフィンランドだけである。

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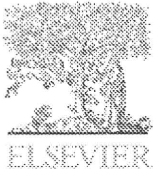

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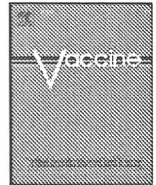

Safe and effective booster immunization using DTaP in teenagers

Kenji Okada[a], Takako Komiya[b], Akihiko Yamamoto[b], Motohide Takahashi[b], Kazunari Kamachi[b], Takashi Nakano[c], Takao Nagai[d], Nobuhiko Okabe[e], Hitoshi Kamiya[c], Tetsuo Nakayama[f,*]

[a] *National Fukuoka Hospital, Department of Pediatrics, Japan*
[b] *National Institute of Infectious Diseases, Department of Bacteriology II, Japan*
[c] *National Mie Hospital, Department of Pediatrics, Japan*
[d] *Nagai Pediatric Clinic, The Society of Ambulatory and General Pediatrics of Japan, Japan*
[e] *National Institute of Infectious Diseases, Infectious Disease Surveillance Center, Japan*
[f] *Kitasato Institute for Life Sciences, Laboratory of Viral Infection I, Kitasato University, Shirokane 5-9-1, Minato-ku, Tokyo 108-8641, Japan*

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FHA

ABSTRACT

The incidence of reported cases with pertussis has increased in young adults in Japan and the lack of additional booster immunizations containing pertussis components is suspected to be one of the causal reasons. Instead of DT immunization at 11–12 years of age, safety and immunogenicity were investigated using 0.2 ml and 0.5 ml of DTaP. 176 subjects in DTaP 0.5 ml, 178 in DTaP 0.2 ml, and 197 in DT 0.1 ml groups were enrolled in clinical trial. The relative risk of local reactions in the DTaP 0.2 ml group compared to the DT 0.1 ml group was 1.13 (95% CI: 0.97–1.30), and that of the DTaP 0.5 ml to the DT 0.1 ml group was 1.34 (95% CI: 1.18–1.53). The relative risks of local pain and heat were 1.62 (95% CI: 1.33–1.98) and 1.59 (95% CI: 1.19–2.13), respectively, in the DTaP 0.5 ml group compared to the DT 0.1 ml group. Seropositive rates against PT and FHA were 54% and 82% before immunization and increased to >95% for both after vaccination with no significant difference in GMT. Instead of the scheduled DT program, 0.2 ml of DTaP was acceptable and demonstrated efficient immunogenicity.

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1. Introduction

Pertussis is still a serious illness in young infants, causing whooping cough, apnea, cyanosis, chocking, and encephalopathy [1]. In Japan, whole cell pertussis vaccine was developed in 1950s and combined with diphtheria and tetanus toxoids (DTwP). DTwP became accepted, resulting in a reduction of reported cases of pertussis [2,3]. Approximately 10% of recipients experienced a febrile illness, with 50–60% showing redness and 20% induration [2]. In 1974–75, two accidental deaths after DTwP administration were reported and, thereafter, DTwP was discontinued for a while. It was re-introduced for children at 2 years of age, but the number of pertussis patients increased because of low vaccine coverage [2,3]. In 1981, a new type of acellular pertussis was developed, and combined vaccine (DTaP) was introduced into recommended immunization practice. Principally, two types of DTaP vaccines were developed: the B-type consisted of two major antigens, pertussis toxin (PT) and filamentous hemagglutinin (FHA) and the T-type contained pertactin and fimbrie besides PT and FHA [4–6]. Nationwide monitoring of clinical adverse events demonstrated low reactogenicity and sufficient antibody responses similar to natural infection. Since 1981, the number of pertussis patients decreased after the acceptance of DTaP. The incidence of pertussis in adults has been increasing gradually from 2002 in Japan, and several outbreaks on college campuses, and in high schools and offices have been reported [7,8]. In addition, the incidence in young infants less than 1 year of age increased as well as adult cases in 2009.

Pertussis is principally an infectious children's illness causing whooping and prolonged cough and the Advisory Committee on Immunization Practices (ACIP) recommended a 5-dose DTaP schedule, at ages 2, 4, 6, and 15–18 months and 4–6 years, instead of the previous DTwP in the US in 1997 [9]. In 1990s, the incidence of pertussis in older age increased in many countries because of waning immunity after primary childhood immunization and antigenic change of pertussis, and adolescent pertussis was identified as the source of transmission of pertussis to young infants through enhanced surveillance studies [10–16]. In 2005, tetanus toxoid, and a reduced concentration of diphtheria toxoid combined with reduced acellular pertussis (Tdap) vaccine was licensed, and the ACIP recommended that adolescents aged 11–18 years should receive a single dose of Tdap for booster immunization [17]. It was now recommended for all generations from 19 to 64 years [18].

It takes several years to obtain a license to introduce a new vaccine from foreign countries into Japan, even though Tdap is

* Corresponding author. Tel.: +81 3 5791 6269; fax: +81 3 5791 6130.
E-mail address: tetsuo-n@lisci.kitasato-u.ac.jp (T. Nakayama).

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used worldwide. The immunization schedule in Japan incorporates no booster dose of pertussis components after the completion of the initial primary immunization (three times over 3 months of age and additional dose after approximately 12 months after the third dose), and vaccine containing pertussis components should be scheduled to cope with an outbreak of pertussis. In this study, safety and immunogenicity were investigated in clinical trials using 0.2 and 0.5 ml of DTaP at the age of 11–12 years, in comparison with 0.1 ml of DT.

2. Subjects and methods

2.1. Subjects

The study was conducted from September 2008 to August 2009, involving 29 pediatric outpatient clinics and departments of pediatrics of regional public and university hospitals. Subjects of this study included 555 children, 11–18 years of age, mostly 11–12 years of age, who had completed primary immunization of more than three doses of DTaP and had not undergone DT immunization. The study protocol was checked by the ethical committee of National Mie Hospital as a central organization and also checked by ethical committee of each hospital. Written informed consent was obtained from their parents or guardians. A total of 555 children were enrolled, but four children were excluded: three did not complete the primary immunization (one or two doses of DTaP), and one had already been immunized with DT. They were divided into two study groups: group 1 consisted of 266 subjects undergoing serological examination: 29 receiving 0.1 ml of DT, 119 for 0.2 ml of DTaP, and 118 for 0.5 ml of DTaP. Group 2 comprised 285 immunized without serological examinations, and totaling 551 subjects, with 197 receiving 0.1 ml DT, 178 for 0.2 ml of DTaP, and 176 for 0.5 ml of DTaP, were examined for safety. They are summarized in Fig. 1.

2.2. Vaccines

Five brands of DTaP were on the market in Japan, and the components of each antigen were different for each brand, as shown in Table 1. Subjects were allocated equally to each brand. The B-type (Biken and Kaketsu) vaccine consisted of PT and FHA and the T-type (Takeda, Denka, and Kitasato) contained other components, and the composition of pertussis antigens differed from the brands of DTaP available abroad [5,6,19]. The PT antigen contents varied from 3 to 23.5 μg/dose, and FHA from 23.5 to 51.5 μg/dose, but the amount of diphtheria and tetanus toxoids was 15 and 2.5 Lf/dose, respectively, without a difference among DTaP brands. 0.2 ml of DTaP contained 1.2–9.4 μg of PT, 9.4–20.6 μg of FHA, 6–6.6 Lf of diphtheria toxoid, and 1.0 Lf of tetanus toxoid. Antigen contents of FHA and diphtheria toxoid were slightly higher in 0.2 ml of DTaP than Tdap, Boostrix and Adacel (2.5–8 μg of PT, 5–8 μg of FHA, 2–2.5 Lf of diphtheria toxoid, and 5 Lf of tetanus toxoid) [17]. A 0.2-ml volume of DTaP contained similar amounts to Tdap. The antigen content of tetanus toxoid was lower in 0.2 ml of DTaP than Tdap available abroad, similar to 0.1 ml of DT.

2.3. Study design

The study was designed as a randomized open trial. Subjects were allocated randomly to DT 0.1 ml, DTaP 0.2 ml, and DTaP 0.5 ml groups. They were observed for 30 min for the appearance of anaphylaxis. To assess the safety afterwards, they were asked to check their body temperature and for adverse clinical events based on the healthcare diary every day for 7 days after immunization. In study group I, paired sera were obtained immediately before immunization and principally 4–6 weeks after immunization and kept at −20 °C. The paired sera were divided into two aliquots and transferred to the National Institute of Infectious Diseases, Department of Bacteriology II to examine antibodies against diphtheria and tetanus toxoids and to Kitasato-Otsuka Bio-Medical Assay Laboratories for the examination of pertussis antibodies (PT and FHA).

2.4. Serology

Antibodies against tetanus toxoid were determined with a KPA kit (Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) [20]. The kit comprised polypeptide artificial carrier particles stained with Reaction Blue solution, sensitized with highly purified tetanus toxoid (3000 Lf/mg PN), and provided in lyophilized form. The test was performed as instructed by the

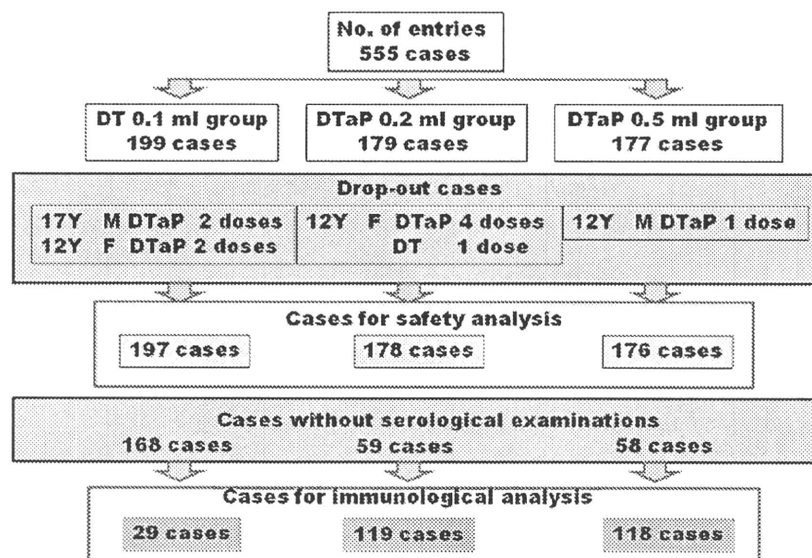

Fig. 1. Number of subjects in the study. A total of 555 subjects were enrolled, of whom four were excluded. Therefore, 551 subjects were evaluated regarding safety. Among the 551, 197 were immunized with 0.1 ml of DT, 178 with 0.2 ml of DTaP, and 176 with 0.5 ml of DTaP. Study group 1 consisted of 266 subjects for serological examination: 29 with 0.1 ml of DT, 119 with 0.2 ml of DTaP, and 118 with 0.5 ml of DTaP.

Table 1
Contents of PT, FHA, and diphtheria and tetanus toxoids.

DTaP/DT (manufacturers)	PT (μg)	FHA (μg)	Pertactin	Fimbrie	D (Lf)	T (Lf)
DTaP 0.5 ml (Kaketsu)	8	32			≦16.7	≒2.5
DTaP 0.5 ml (Biken)	23.5	23.5			≦15	≦2.5
DTaP 0.5 ml (Takeda)	3	34.5	7.5	1	≒15	≒2.5
DTaP 0.5 ml (Denka)	9	32	15	1	≦15	≦2.5
DTaP 0.5 ml (Kitasato)	6	51.5	5	1	≒15	≒2.5
Adacel (Aventis)	2.5	5	3		2	5
Boostrix (GSK)	8	8	2.5		2.5	5
DTaP 0.2 ml	1.2–9.4	9.4–20.6			6–6.6	1.0
DT 0.1 ml					3.2	0.7

Table 2
Background of the subjects.

	DTaP 0.2 ml (N = 178)	DTaP 0.5 ml (N = 176)	DT 0.1 ml (N = 197)	Total (N = 551)
Gender				
Male	93 (52.2%)	95 (54.0%)	113 (57.4%)	301 (54.6%)
Female	85 (47.8%)	81 (46.0%)	84 (42.6%)	250 (45.4%)
Age				
11 years	97 (54.5%)	95 (54.0%)	73 (37.1%)	265 (48.1%)
12 years	68 (38.2%)	68 (38.6%)	111 (56.3%)	247 (44.8%)
Others	13 (7.3%)	13 (7.4%)	13 (6.6%)	39 (7.1%)
Mean age ± SD	11.6 ± 0.8	11.6 ± 0.8	11.8 ± 0.8	11.6 ± 0.8
Median age	11.0	11.0	12.0	12.0
Range (min–max)	(11–15)	(11–15)	(11–17)	(11–17)
DPT history				
I-1	178 (100.0%)	176 (100.0%)	197 (100.0%)	551 (100.0%)
I-2	178 (100.0%)	176 (100.0%)	197 (100.0%)	551 (100.0%)
I-3	172 (96.6%)	172 (97.7%)	193 (98.0%)	537 (97.5%)
I-boost	172 (96.6%)	168 (95.5%)	191 (97.0%)	531 (96.4%)

manufacturers. Antibodies against diphtheria toxoid were examined using the micro cell-culture method with Vero cells, and diphtheria antitoxin titers were expressed as international units (IU)/ml [21]. Antibodies against PT and FHA were examined using enzyme-linked immunosorbent assay (EIA) kits (Wako Chemicals, Japan) as instructed by the manufacturers. Positive levels were defined as ≥0.1 IU/ml for antibodies against diphtheria toxoid, ≥0.01 IU/ml for those against tetanus toxoid, and ≥10 EU/ml for those against PT and FHA [22,23].

2.5. *Statistical analysis*

The sero-positivity rate and the incidence of solicited adverse events (fever as systemic reaction, and redness, swelling, pain, heat, and itching as local reactions) were compared by using Fisher's Extraction test. Geometric mean titers (GMTs) of antibodies before and after immunization were compared by converting to a logarithmic scale using Wilcoxon rank test. The *t* student Welch method was employed to evaluate significance and the significant level was set at $p < 0.05$.

3. Results

3.1. *Background of the subjects*

The subjects included 555 children aged 11–18 years of age, as shown in Fig. 1. A total of 555 subjects were enrolled, but four were excluded. Therefore, 551 subjects were evaluated for safety. Among the 551, 197 were immunized with 0.1 ml of DT, 178 with 0.2 ml of DTaP, and 176 with 0.5 ml of DTaP. The backgrounds of the subjects are shown in Table 2. A total of 301 (54.6%) were male, and the gender ratio was similar among the three groups with no significant differences in ages, which ranged from 11 to 17 years. They had all completed their primary immunizations (three or four doses of DTaP), confirmed by checking their immunization records.

3.2. *Incidence of adverse events*

The incidences of adverse events are summarized in Table 3. Febrile reactions were noted in 8 (4.1%) of 197 in the DT 0.1 ml group, 7 (3.9%) of 178 in the DTaP 0.2 ml group, and 7 (4.0%) of 176 in the DTaP 0.5 ml group, and the relative risks in DTaP

Table 3
Incidence of clinical adverse events.

Adverse events	DTaP 0.2 ml (1)	DTaP 0.5 ml (2)	DT 0.1 ml (3)	Risk ratio (95% CI)		
	(N = 178)	(N = 176)	(N = 197)	(2) vs. (1)	(1) vs. (3)	(2) vs. (3)
Fever	7 (3.9%)	7 (4.0%)	8 (4.1%)	1.01 (0.36,2.82)	0.97 (0.36,2.62)	0.98 (0.36,2.65)
Local reactions	123 (69.1%)	145 (82.4%)	121 (61.4%)	1.19 (1.06,1.34)	1.13 (0.97,1.30)	1.34 (1.18,1.53)
Redness	95 (53.4%)	109 (61.9%)	92 (46.7%)	1.16 (0.97,1.39)	1.14 (0.93,1.40)	1.33 (1.10,1.60)
Swelling	90 (50.6%)	95 (54.0%)	76 (38.6%)	1.07 (0.87,1.30)	1.31 (1.04,1.65)	1.40 (1.12,1.75)
Pain	83 (46.6%)	116 (65.9%)	80 (40.6%)	1.41 (1.17,1.71)	1.15 (0.91,1.45)	1.62 (1.33,1.98)
Heat	50 (28.1%)	74 (42.0%)	52 (26.4%)	1.50 (1.12,2.00)	1.06 (0.76,1.48)	1.59 (1.19,2.13)
Itching	81 (45.5%)	83 (47.2%)	75 (38.1%)	1.02 (0.82,1.28)	1.21 (0.95,1.54)	1.24 (0.98,1.57)

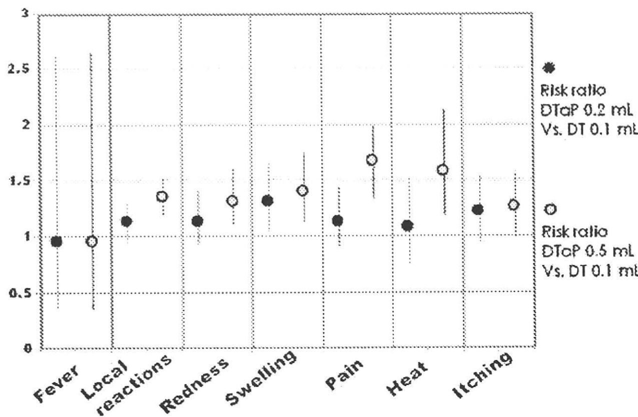

Fig. 2. Summary of the risk ratio regarding the incidence of adverse reactions. The relative risks of the incidence of adverse reactions after immunization with 0.2 ml (●) and 0.5 ml (○) of DTaP in comparison with those observed after immunization with 0.1 ml of DT are summarized. Vertical lines represent 95% CI.

0.2 ml and DTaP 0.5 ml groups were 0.97 and 0.98, respectively, in comparison with that observed in the DT 0.1 ml group. The relative risk of local reactions after immunization with DTaP at 0.2 ml was 1.13 (95% CI: 0.97–1.30) in comparison with the incidence after immunization with DT at 0.1 ml, and that of the DTaP 0.5 ml compared to the DT 0.1 ml group was 1.34 (95% CI: 1.18–1.53). Relative risks of redness, swelling, local pain, heat, and itching in the DTaP 0.2 ml group compared to the DT 0.1 ml group were 1.14 (95% CI: 0.93–1.40), 1.31 (95% CI: 1.04–1.65), 1.15 (95% CI: 0.91–1.45), 1.06 (95% CI: 0.76–1.48), and 1.21 (95% CI: 0.95–1.54), respectively. However, the relative risks of redness, swelling, local pain, heat, and itching in the DTaP 0.5 ml group compared to the DT 0.1 ml group were 1.33 (95% CI: 1.10–1.60), 1.40 (95% CI: 1.12–1.75), 1.62 (95% CI: 1.33–1.98), 1.59 (95% CI: 1.19–2.13), and 1.24 (95% CI: 0.98–1.57), respectively. The relative risks of the adverse reactions after immunization in the DTaP 0.2 ml and 0.5 ml groups in comparison with those observed after immunization in the DT 0.1 ml group are summarized in Fig. 2. Thus, the incidence of local reactions after immunization with 0.2 ml of DTaP was similar to that observed after immunization with 0.1 ml of DT, but those observed after immunization with 0.5 ml of DTaP were higher than after immunization with 0.1 ml of DT, notably regarding the incidences of local pain and heat, demonstrating the relative risks: 1.62 (95% CI: 1.33–1.98) and 1.59 (95% CI: 1.19–2.13), respectively.

3.3. Onset of adverse reactions

The immunization day was defined as day 0. The onset of adverse reactions was examined, and the results are shown in Fig. 3. Febrile reactions were noted from days 0 to 7 without any case accumulation, but the incidence of local reactions peaked on days 1 and 2. Systemic adverse events were reported sporadically: headache in 25 (9 in DT 0.1 ml group, 9 in DTaP 0.2 ml group, and 7 in DTaP 0.5 ml group), fatigue in 11 (3 in DT 0.1 ml group, 4 in DTaP 0.2 ml group, and 4 in DTaP 0.5 ml group), rhinorrhea in 10 (1 in DT 0.1 ml, 2 in DTaP 0.2 ml, and 7 in DTaP 0.5 ml group), sore throat in 8, cough in 7, and nasal obstruction in 7. Three subjects with urticaria eruption were reported: two on day 0 (one for each DT 0.1 ml and DTaP 0.5 ml group) and one on day 1 in DTaP 0.5 ml group. Generalized eruption was reported on day 1 in DTaP 0.5 ml group. The relative risk of local reactions on day 0 after immunization with 0.2 ml of DTaP compared to that observed after 0.1 ml of DT was 1.08 (95% CI: 0.74–1.58), 1.18 (95% CI: 0.96–1.44) on day 1, 1.09 (95% CI: 0.91–1.30) on day 2, 1.19 (95% CI: 0.97–1.47) on day 3, 1.3 (95% CI: 0.99–1.71) on day 4, 1.56 (95% CI: 1.09-2.23) on day 5, 1.42 (95% CI: 0.87–2.29) on day 6, and 1.54 (95% CI: 0.87–2.72) on day 7. The incidence of local reaction for each day after immunization with 0.2 ml of DTaP was similar to that observed after 0.1 ml of DT. The incidence of local reactions after immunization with 0.5 ml of DTaP was higher than that observed in the DT 0.1 ml group, especially on days 1 and 2, with a relative risk of 1.61 (95% CI: 1.35–1.92) on day 1, and 1.33 (95% CI: 1.13–1.92) on day 2. Most local adverse reactions appeared on day 1 and continued for 3–4 days, but those observed in the DTaP 0.5 ml group became prolonged, showing a relative risk of 2.15 (95% CI: 1.39–3.33) on day 6.

In this study, the extents of redness and swelling were monitored when they appeared and the degree of adverse reactions was evaluated (Fig. 4). There was no significant difference in the incidence of redness and swelling of <2.0 cm and 2–5 cm among the

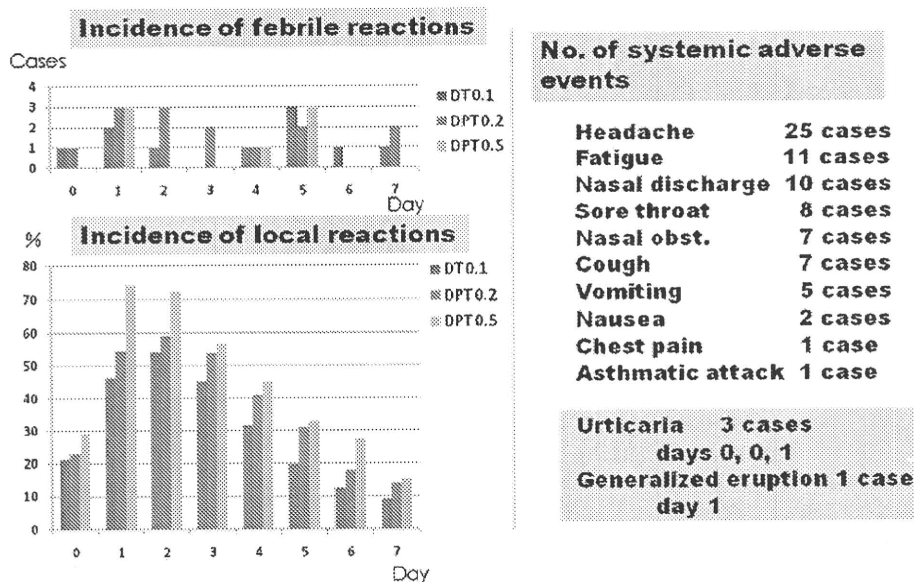

Fig. 3. Onset of febrile and local reactions within 7 days after immunization and the no. of cases with systemic adverse events.

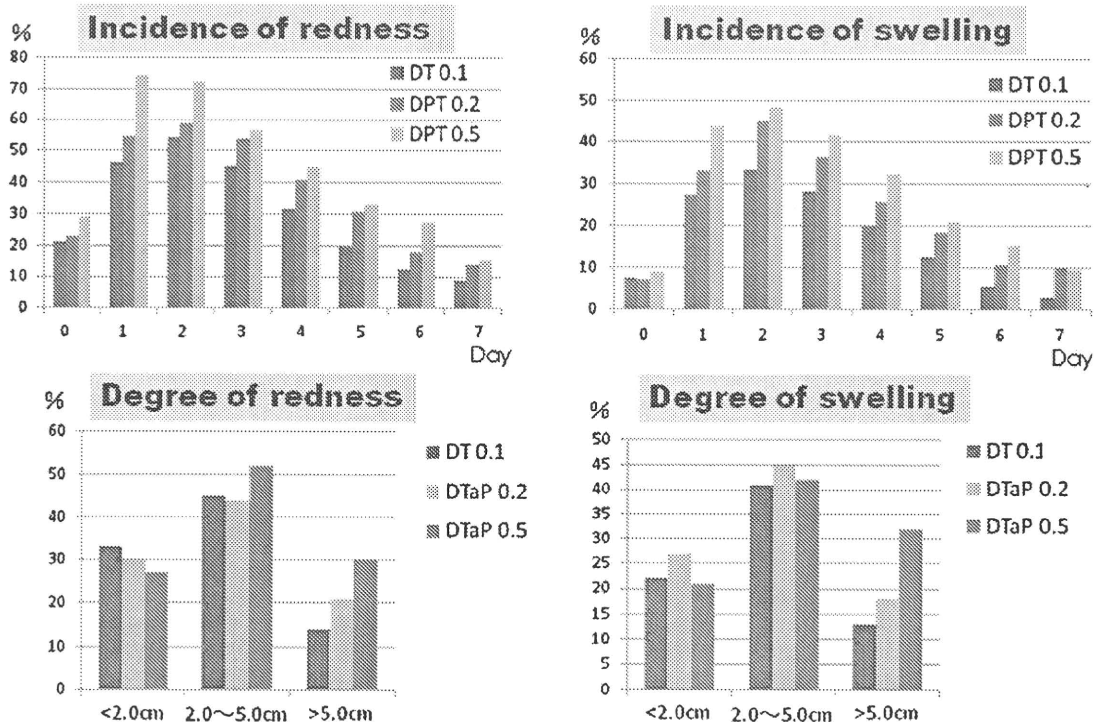

Fig. 4. Onset of local redness and swelling, and the severity of adverse events.

three groups, but 0.5 ml of DTaP had a tendency to induce a serious local reaction (redness and swelling) >5.0 cm.

3.4. Immunogenicity

Study group 1, in whom paired serum samples were examined, consisted of 266 subjects with serological examination, 29 with 0.1 ml of DT, 119 with 0.2 ml of DTaP, and 118 with 0.5 ml of DTaP. The sero-positivity of antibodies for diphtheria toxoid >0.1 was 60.9% (162/266), 90.6% (241/266) for tetanus toxoid >0.01, 54.13% (144/266) for PT >10, and 82.33% (219/266) for FHA > 10 EIA units. Antibodies against PT were markedly reduced at the age of 11–12 years.

The results of sero-positivity and GMT are shown in Table 4. The sero-positivity of PT and FHA and their GMT were the same before and after immunization in the DT 0.1 ml group. After immunization, the sero-positivity against PT increased from 52.1 to 95% in the DTaP 0.2 ml group and from 55.1 to 95.8% in the DTaP 0.5 ml group. The GMT of PT antibodies after immunization with 0.2 ml of DTaP was 89.05 (95% CI: 70.54–112.41), and there was no significant difference after immunization with 0.5 ml of DTaP, being 102.74 (95% CI: 82.91–127.32). Sero-positivity against FHA increased from 85.7 to 100% in the DTaP 0.2 ml group and from 78.8 to 98.3% in the DTaP 0.5 ml group. The GMT of antibodies against FHA was 252.82 (95% CI: 214.29–298.27) after immunization with 0.2 ml of DTaP and 302.06 (95% CI: 254.2–358.93) after immunization with 0.5 ml of DTaP, without a significant difference. Sero-positivity against diphtheria toxoid was 55.9–66.4% before immunization and increased to 100% in all three groups. The GMT of antibodies against diphtheria toxoid was 40.14 (95% CI: 28.28–56.96), 45.17 (95% CI: 35.59–57.32), and 46.78 (95% CI: 35.73–61.24) in the DT 0.1 ml, DTaP 0.2 ml, and DTaP 0.5 ml groups, respectively. As for the antibodies against tetanus toxoid, 86.2–94.1% sero-positivity before immunization increased to 100%. The GMT of antibodies against tetanus toxoid after vaccination with 0.2 ml of DTaP was 18.02 (95% CI: 14.90–21.80), similar to the 20.96 (95% CI: 13.37–32.84) after immunization with 0.1 ml of DT. However, the GMT of antibodies against tetanus toxoid was 27.12 (95% CI: 22.79–32.27) after immunization with 0.5 ml of DTaP, higher than those in DT 0.1 ml and DTaP 0.2 ml groups.

3.5. Difference in immunogenicity of different brands

There was no significant difference in immunogenicity against PT and FHA after immunization with 0.2 or 0.5 ml of DTaP. Risk ratios of a local reaction to 0.5 ml of DTaP compared to 0.1 ml of DT were higher than that to 0.2 ml of DTaP. GMTs after immunization with different brands of DTaP are shown in Fig. 5. A volume of 0.2 ml of DTaP contained 1.2–9.4 μg of PT, 9.4–20.6 μg of FHA, 6–6.6 Lf of diphtheria toxoid, and 1.0 Lf of tetanus toxoid. A volume of 0.1 ml of DT contains similar amounts of tetanus and diphtheria toxoid antigens in different brands and compared with 0.2 ml of each DTaP brand. 29 were immunized with 0.1 ml DT, 26 with 0.2 ml of Takeda DTaP, 26 with Biken, 19 with Kaketsu, 19 with Kitasato, and 29 with Denka. There was no significant difference in GMTs of antibodies against diphtheria toxoid after immunization with the five different brands in comparison with that induced after immunization with 0.1 ml of DT. The GMT against tetanus toxoid after immunization with Kitasato was higher than that after 0.1 ml of DT. As for the pertussis antigens, the GMT of PT antibodies after immunization with Takeda or Denka vaccine was lower than those induced after the other brands. These two brands contained lower amounts of PT antigen. The GMT against FHA after immunization with Denka was slightly lower than the others, not reflecting the concentration of vaccine material.

4. Discussion

Pertussis is an infectious disease affecting young infants and children, leading to severe illness in very young infants,

Table 4
Immunogenicity of DT and DTaP.

	DT 0.1 ml		DTaP 0.2 ml		DTaP 0.5 ml	
	Sero+ rate GMT pre (95% CI)	Sero+ rate GMT post (95% CI)	Sero+ rate GMT pre (95% CI)	Sero+ rate GMT post (95% CI)	Sero+ rate GMT pre (95% CI)	Sero+ rate GMT post (95% CI)
Anti-PT	58.6%	58.6%	52.1%	95%	55.1%	95.8%
	10.8	13.93	12.11	89.05	10.88	102.74
	(6.38–18.29)	(8.98–21.61)	(9.21–15.94)	(70.54–112.41)	(8.27–14.32)	(82.91–127.32)
Anti-FHA	82.8%	86.2%	85.7%	100%	78.8%	98.3%
	24.92	31.2	33.73	252.82	25.83	302.06
	(16.34–38.00	(22.43–43.42)	(27.32–41.64	(214.29–298.27)	(20.67–32.28)	(254.2–358.93)
Anti-D	58.6%	100%	66.4%	100%	55.9%	100%
	0.23	40.14	0.22	45.17	0.16	46.78
	(0.11–0.471)	(28.28–56.96)	(017–0.30)	(35.59–57.32)	(0.12–0.24)	(35.73–61.24)
Anti-T	86.2%	100%	94.1%	100%	88.1%	100%
	0.47	20.96	0.87	18.02	0.59	27.12
	(0.28–0.81)	(13.37–32.84)	(0.70–1.09)	(14.90–21.80)	(0.44–0.79)	(22.79–32.27)

causing whoop, staccato, apnea, and choking with sputa. To prevent the disease, acellular pertussis vaccines have been used in many developed countries. However, the acellular vaccine did not confer a long-lasting antibody response after vaccination and so in the late 1990s several pertussis outbreaks occurred in young adults [10–16]. The diagnosis of pertussis in adults was difficult because they only demonstrated mild atypical symptoms, showing a prolonged cough without whooping [24–26]. The adult patients showing a prolonged cough were not suspected to have pertussis because general physicians believed that pertussis was a disease only affecting children. They were, therefore, undiagnosed, and the number of patients with pertussis was underreported. In addition, they were not treated and transmitted pertussis to young infants before DTaP immunization [27]. The adult pertussis vaccine trial was conducted in 2781 subjects consisting of 1391 received the acellular pertussis vaccine and 1390 received the control vaccine. Ten patients of pertussis were diagnosed by culture, PCR, or serological responses and nine were in the control group and one in acellular pertussis vaccine group. An incidence of 370–450 cases per 100,000 person-years was noted in the control group aged 15–65 years and the acellular pertussis vaccine was protective in the same age group [28]. These adult patients with pertussis were considered to be an infectious source for transmission to young infants in household contact. Through such household contacts, even vaccinated children who had been completely immunized showed typical pertussis, and the most likely source of infant

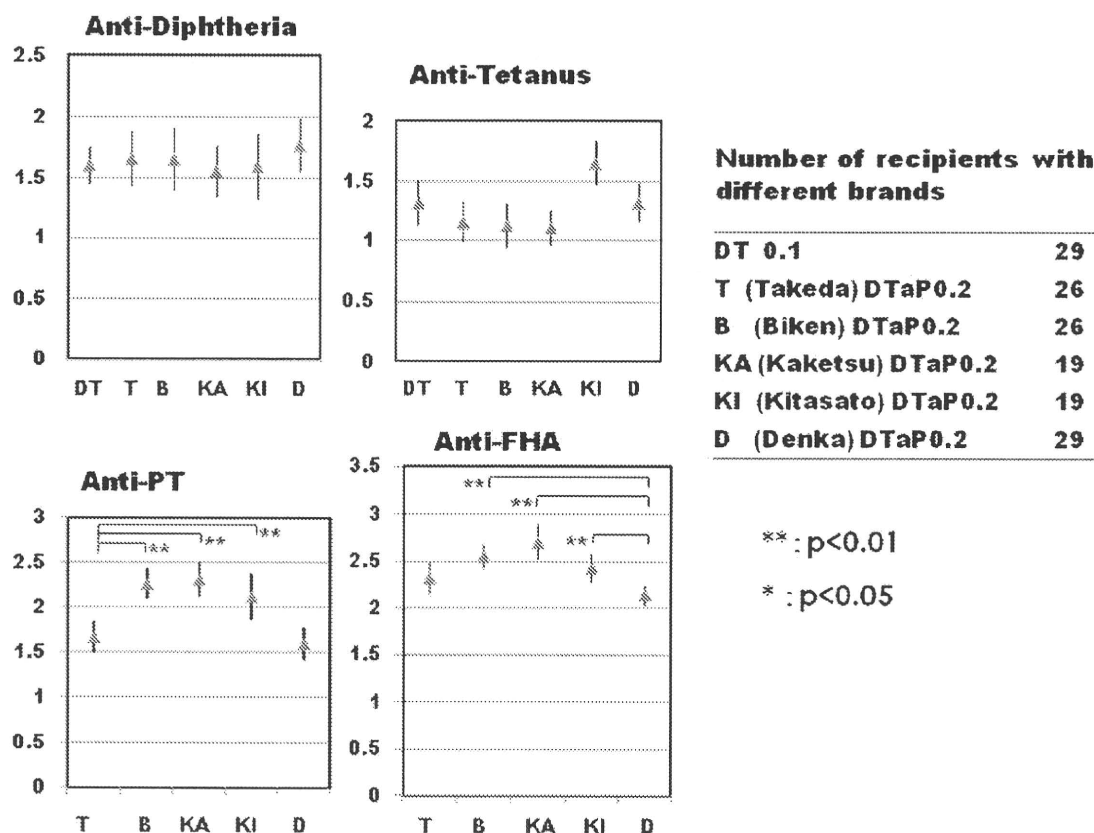

Fig. 5. GMTs of anti-D, T PT, and FHA antibodies after immunization with different brands of DTaP at 0.2 ml.

infection was reported, being a sibling (41%), mother (38%), and father (17%). To control pertussis, Tdap was developed and recommended as the booster in teenagers and young adults [15]. It is necessary to maintain a high level of immunity in all generations [29,30]. Thus, Tdap was newly recommended for all generations from 19 to 64 years as well as teenagers [17,18].

DTaP was first developed in Japan and has been used since 1981 [4]. Some pertussis patients were reported sporadically in Japan, and a survey of 89 households showed that the source of infection was an adult in approximately 11% and the secondary attack rate was 10%, confirmed by serological responses with asymptomatic infection [31]. The estimated efficacy of DTaP was 84% (95% CI: 71–91%) in children aged 2–8 years. Since vaccine-induced immunity waned 6–10 years after immunization, immunization with vaccines including pertussis components was proposed for both children and adults [32]. Adult patients with pertussis have gone undiagnosed and, therefore, the disease burden of pertussis has been neglected. In 2007–08, there were several outbreaks in universities, schools, and other facilities, and the number of reported cases of pertussis increased. Most of the patients were over 15 years of age and, the number of patients aged less than 1 year increased.

To control pertussis, an active immunization strategy should be implemented. Some ideas were considered to import Tdap, as well as change the immunization schedule. The immunization schedule of DTaP in Japan is 4 doses in young children only, being one or two times fewer doses in comparison with the schedule of DTaP in the EU and US. The components of Tdap (Adacel and Boostrix) were 2.5–8 μg of PT, 5–8 μg of FHA, 2.5–3 μg of pertactin, 2–2.5 Lf of diphtheria toxoid, and 5 Lf of tetanus toxoid. The five brands of DTaP in Japan have different formulations of components, as shown in Table 1. The B-type DTaP has only two components (Biken and Kaketsu) and T-type vaccines contain several other components besides PT and FHA (Takeda, Denka, and Kitasato). A dose of 0.1 ml of DT was scheduled at the age of 11–12 years. The concentration of tetanus toxoid in 0.2 ml of DTaP was similar to that in 0.1 ml of DT, but that of diphtheria toxoid was higher than that in 0.1 ml of DT. In comparison with Tdap used abroad, 0.2 ml of DTaP contained higher amounts of diphtheria toxoid and there was no significant difference in the incidence of adverse local reactions and serological response. Also, 0.2 ml of DTaP contains lower contents of tetanus toxoid and they induced efficient antibodies against tetanus toxoid. As for the antigen content of pertussis components, the PT antigen content varies from 1.2 to 9.4 μg, and the FHA content from 9.4 to 20.6 μg in 0.2 ml of different brands of DTaP. The GMT of antibodies against PT and FHA showed no significant difference after immunization with 0.2 or 0.5 ml of DTaP, but when comparing the GMT after immunization among different brands with different antigen concentrations, DTaP with higher antigen content did not always induce higher antibody titers. A lower-level serological response was observed in those immunized with a lower antigen content, but sero-positivity (protection levels > 10) was almost 100% after immunization with different bands of DTaP. DTaP with higher antigen content induced more marked serological responses at 4 years of age on booster immunization, but the difference was ten-times for PT antigen and five-times for FHA [33].

In the late 1990s, the resurgence of pertussis might have been associated with multi-factorial events: waning immunity, increased awareness, inappropriate vaccination schedule, improved diagnostic methods, and variant strains evading immunity acquired by immunization [8,34–36]. There have been several reports on mutation of the PT gene and it is still controversial which antigens are related to promoting immunity or reducing the severity of symptom [37,38]. Antibodies against PT reduced susceptibility to pertussis and those against pertactin or Fim2/3 were protective antibodies [39]. Protective immunity was considered to be induced by multiple components [40].

In many developed countries, the control of pertussis is complicated because of the difficulty in case identification, limited persistence of vaccine-acquired immunity, and transmission from unrecognized very mild patients or asymptomatic cases. In Japan, the number of pertussis patients has been increasing and resurgence in very young infant due to household contact was reported [41]. In this report, safe and effective immunization was achieved by 0.2 ml of DTaP instead of 0.1 ml of DT. The booster immunization with pertussis components should be implemented to achieve more effectively control the epidemiology of pertussis in Japan.

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Original Article

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

Hiroshi Kamano*, Tomomi Mori, Hajime Maeta, Tomohiko Taminato[1], Toshihiko Ishida[2], Nobuhito Kishimoto[3], Tomohiko Katami[4], Makoto Sato[5], Kazunari Kamachi[6], and Yoshiyuki Mochida[7]

Health Science Center, Kagawa University, Kagawa 760-8521; [1]Department of Laboratory Medicine, [2]University Hospital, Faculty of Medicine, Kagawa University, Kagawa, 761-0793; [3]Takamatsu Municipal Hospital, Kagawa 760-8538; [4]Katami Clinic, Kagawa 760-0017; [5]Sato Clinic of Internal Medicine, Kagawa 761-0102; [6]National Institute of Infectious Diseases, Tokyo 208-0011; and [7]SRL, Inc., Tokyo 190-8567, 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. During the subsequent outbreak, 361 university students and staff members presented with a primary complaint of a cough. In the present study, we analyzed bacterial agglutinin titers against two *Bordetella pertussis* strains, Yamaguchi (epidemic strain) and Tohama (vaccine strain), in 310 patients with a cough and evaluated its diagnostic accuracy for adolescent and adult pertussis. These serological analyses showed a significant difference ($P < 0.001$) in the levels of Yamaguchi agglutinin titer, but not in those of Tohama agglutinin titer, between patient and healthy adult groups. 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 a prevalence 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 *B. 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 *B. pertussis* antigen for 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-

*Corresponding author: Mailing address: Health Science Center, Kagawa University, 1-1 Saiwai-cho, Takamatsu, Kagawa 760-8521, Japan. Tel: +81-87-832-1282, Fax: +81-87-832-1285, E-mail: kamano@cc.kagawa-u.ac.jp

dent 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

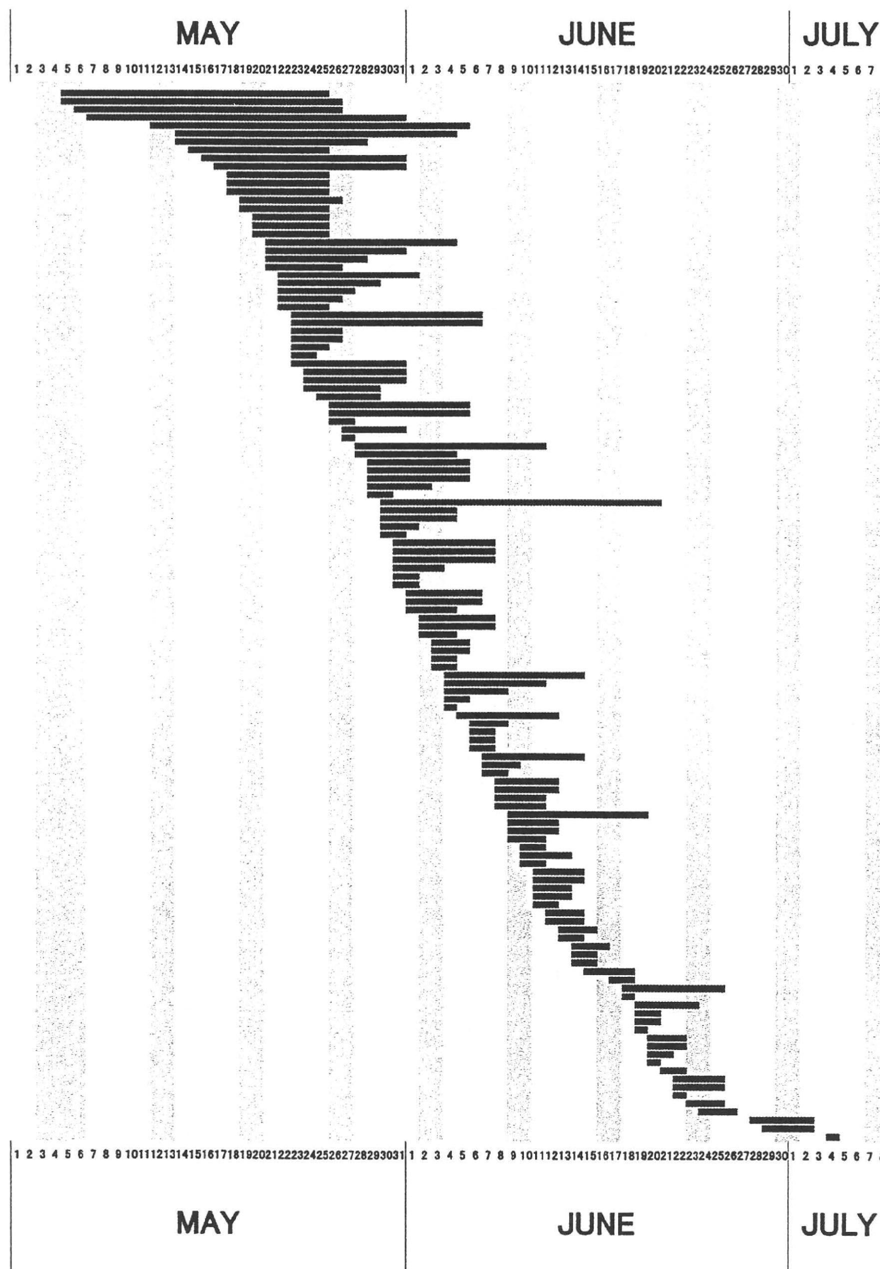

Fig. 1. Calendar showing duration of cough in patients during pertussis outbreak in a university. Patients (n = 128) with probable pertussis were asked for the date of cough onset during May to July 2007. Length of a bar shows the duration of cough until the date of serological analysis.

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 = 160×) ($P < 0.001$) (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 the Tohama agglutinin titers between the outbreak and control groups (median = 80×, upper quartile = 160×) (Fig. 2B).

To investigate the relevance of age, we compared the agglutinin titers in people ≤39 years old in the outbreak group (n = 282; 155 male, 127 female; age range 18–39 years; average age 21.2; median age 20) and in the healthy control group (n = 123; 65 male, 58 female; age range 21–39 years; average age 32.3; median age 32). The Yamaguchi agglutinin titers of the outbreak group (median = 160×, upper quartile = 320×) were significantly higher than those in the control group (median = 40×, upper quartile = 80×) ($P < 0.001$) (Fig. 3A). Therefore, significantly more cases in the outbreak group had Yamaguchi agglutinin titers ≥160 × (165/282, 58.5%) compared to the control group (27/123, 22.0%) ($P < 0.001$). In contrast, there was no significant difference in Tohama agglutinin titers between the outbreak and control groups (median = 80× , upper quartile = 160×) (Fig. 3B).

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

Table 1. Symptoms of the pertussis patients in this study

Symptom	%
Paroxysmal cough	53.9
Nocturnal cough	50.7
Whooping cough	14.7
Dyspnea	17.5
Post-tussive vomiting	6.3
Speaking induced cough	23.8

Probable cases (n = 63).

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

(A) Students probable cases (n = 93)

Agglutinin titer	≤80×	≥160×
Duration of cough (d)	3.8 ± 2.5 (n = 28)	11.1 ± 23.3 (n = 65)

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

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

Agglutinin titer	≤80×	≥160×
Duration of cough (d)	3.8 ± 6.0 (n = 7)	10.3 ± 6.3 (n = 10)

Result of Mann-Whitney U-test; P = 0.012.

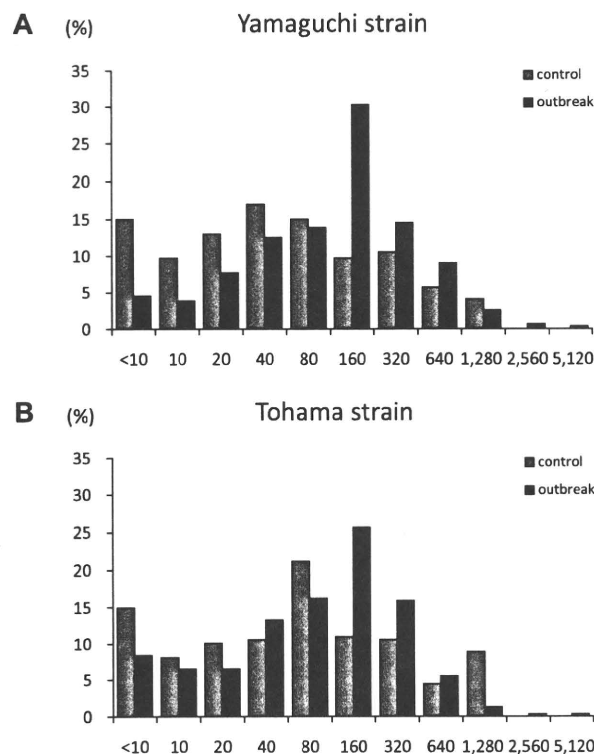

Fig. 2. *B. pertussis* agglutinin titers of people in the outbreak and control groups. (A) *B. pertussis* agglutinin titers against the strain Yamaguchi. (B) *B. pertussis* agglutinin titers against the strain Tohama. x axis, agglutinin titer. y axis, percentage of patients. ▩, control group (n = 246). ■, outbreak group (n = 310).

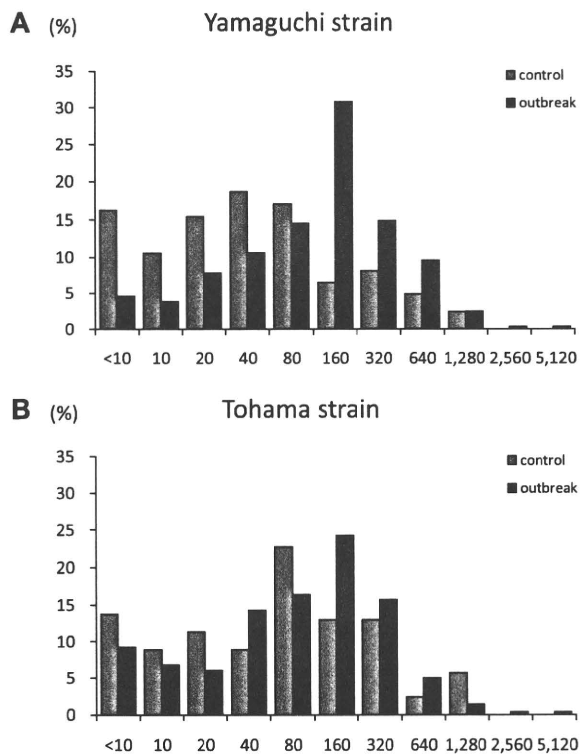

Fig. 3. *B. pertussis* agglutinin titers of people ≤39 years old in the outbreak and control groups. (A) *B. pertussis* agglutinin titers against the strain Yamaguchi. (B) *B. pertussis* agglutinin titers against the strain Tohama. *x* axis, agglutinin titer. *y* axis, percentage of patients. ■, control group (n = 123). ■, outbreak group (n = 282).

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 people who had contact with pertussis cases in the dormitory, 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 vaccine 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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ORIGINAL ARTICLE | BACTERIOLOGY

Marked difference between adults and children in *Bordetella pertussis* DNA load in nasopharyngeal swabs

Y. Nakamura[1,2], K. Kamachi[1], H. Toyoizumi-Ajisaka[1], N. Otsuka[1], R. Saito[1], J. Tsuruoka[2], T. Katsuta[2,3], N. Nakajima[2], K. Okada[4], T. Kato[2,3] and Y. Arakawa[1]

1) Department of Bacteriology II, National Institute of Infectious Diseases, Tokyo, 2) Department of Pediatrics, St Marianna University School of Medicine, Kawasaki, 3) National Center for Child Health and Development, Tokyo and 4) Division of Pediatrics, National Hospital Organization Fukuoka National Hospital, Fukuoka, Japan

Abstract

Bordetella pertussis is the aetiologic agent of whooping cough, a common cause of severe respiratory illness in children and prolonged mild cough in adults. To understand some of the reasons for differences in clinical symptoms between adults and children, we measured *B. pertussis* DNA loads in nasopharyngeal swabs (NPS) from 19 adults and 40 children (including 14 infants) by quantitative IS*481* real-time PCR. All cases had been pre-diagnosed with the *B. pertussis*-specific loop-mediated isothermal amplification method. The mean PCR threshold cycles for adult and child NPS were 34.9 and 27.1, respectively, indicating a significantly lower *B. pertussis* DNA load in adults than in children ($p < 0.001$). Moreover, adults had very low DNA loads during both early and later stages of the disease. When corresponding bacterial loads in NPS were calculated for *B. pertussis* Tohama cells using a standard curve, the mean number of bacterial cells taken with a rayon-tipped swab from an adult, older child and infant was estimated to be 320 (95% CI 120–910), 2.1×10^4 (95% CI 5.3×10^3 to 8.3×10^4) and 1.1×10^6 cells (95% CI 1.2×10^5 to 8.9×10^6), respectively. This indicates that the *B. pertussis* load in NPS is closely correlated with patient age. Our observations suggest that adult pertussis is characterized by a lower bacterial load in the nasopharynx, resulting in milder symptoms and negative cultures.

Keywords: Adults, bacterial load, *Bordetella pertussis*, children, nasopharyngeal swab, real-time PCR
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Corresponding author: K. Kamachi, Department of Bacteriology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan
E-mail: kamachi@nih.go.jp

Introduction

Bordetella pertussis, a highly communicable gram-negative coccobacillus, is the aetiologic agent of whooping cough, which has been a major acute respiratory infection, resulting in severe childhood illness and infant death [1]. In Japan, the incidence of pertussis cases in adolescents and, especially, adults, has significantly increased from the early 2000s; this has also been seen in other countries with high vaccination coverage [2]. Adolescents and adults are assumed to be the primary reservoir of *B. pertussis* and play a crucial role in the transmission of the microbe to infants and unvaccinated children [3–5]. The clinical symptoms of pertussis in adolescents and adults vaccinated during childhood are different from typical presentations in children, and may consist only of a prolonged cough [6–9]. Moreover, culture of *B. pertussis* has a much lower sensitivity for diagnosis of pertussis in adults than in children [5,9]. Although *B. pertussis* infection is known to present differently in adults and children, the differences in the bacterial load in the nasopharynx remain unclear.

During the last decade, nucleic acid amplification tests have revolutionized the laboratory diagnosis of *B. pertussis* infections. Various detection methods, including real-time PCR and loop-mediated isothermal amplification (LAMP), have been developed that target different regions of *B. pertussis* genome [10–13]. Previously, we developed a LAMP assay method targeting the pertussis toxin promoter region, which provided rapid, sensitive and highly specific detection of *B. pertussis* DNA [11], although the amplification efficiency is too high to make a quantitative assay of the bacterial load in clinical specimens. By contrast, real-time PCR targeting insertion sequence IS*481* is a useful tool not only for rapid

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and sensitive diagnosis, but also for quantitative analysis. The IS*481* real-time PCR can detect other *Bordetella* subspecies, such as *Bordetella holmesii*, *Bordetella bronchiseptica* and *Bordetella parapertussis*, although this assay is now widely used for the diagnosis of *B. pertussis* [14,15]. In neonates and young children with pertussis, the IS*481* real-time PCR showed that the *B. pertussis* DNA load in nasopharyngeal swabs (NPS) persists for a long time (3 weeks) after administration of antimicrobials [16,17]. For other pathogenic agents causing respiratory tract infections, such as *Moraxella catarrhalis* and *Streptcoccus pneumoniae*, the quantitative changes in bacterial DNA load in NPS correlate with the numbers of organisms detected by semiquantitative culture [18,19].

The present study aimed to determine *B. pertussis* DNA loads in NPS among adults and children, who were confirmed to have pertussis by *B. pertussis*-specific LAMP assay, by using quantitative IS*481* real-time PCR. We also investigated the relationship between *B. pertussis* load and bacterial genotypes.

Materials and Methods

Clinical samples

Approximately 200 NPS were obtained from adults (≥16 years old) and children (≤15 years old) with suspected pertussis between June 2007 and September 2009. The NPS were collected with sterilized rayon-tipped swabs (Eiken Chemical Co., Ltd, Tokyo, Japan) and then transported to the National Institute of Infectious Diseases, Japan. NPS were immersed in 0.5 mL of saline, vortexed, and precipitated by centrifugation (20 000 g for 10 min). Total DNA was extracted from the precipitation using QIAamp DNA Microkit (Qiagen, Hilden, Germany), and eluted with 25 μL of the AE elution buffer. The DNA samples were stored at −20°C until used.

LAMP assay

To confirm *B. pertussis* infection, a *B. pertussis*-specific LAMP assay was performed on DNA samples from NPS as described previously [11]. The LAMP amplification was performed with a 60-min reaction, and confirmed with real-time monitoring of the increase in turbidity using LA-320C (Eiken Chemical Co., Ltd). Among the DNA samples tested, 19 from adults and 40 from children (14 infants and 26 older children) were positive in the LAMP assay (≥0.1 turbidity), respectively. The vaccination history was obtained in 13 infants and 12 older children, but not in adults. Of 13 infants, 11 (85%) had never been immunized with pertussis vaccine. By contrast, ten (83%) of 12 older children had received four doses of pertussis vaccine.

IS*481* real-time PCR

Quantitative real-time PCR targeting IS*481* was performed by an Applied Biosystems 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) as described previously [12], with minor modifications. Briefly, the IS*481* real-time PCR amplifications were done in 20-μL reaction volumes containing 1× *Premix Ex Taq*™ (Perfect Real Time; Takara Bio Inc., Shiga, Japan), 0.9 μM primer PPert and APPert, 0.25 μM TaqMan probe SPert, 0.4 μL of 50× Rox reference dye II, and 2 μL of DNA sample. The PCR conditions were 15 s at 95°C, followed by 40 cycles of 95°C for 3 s and 57°C for 30 s. Real-time data were analyzed by Sequence Detection Systems software, version 1.4 (Applied Biosystems). Standard curve was generated with ten-fold serial dilutions of *B. pertussis* Tohama DNA from 10 ng (2.4×10^6 bacterial cells) to 0.1 fg (0.024 bacterial cells). The number of bacterial cells was calculated with the Tohama genome size of 4.1 Mbp (2.4 genomic copies/10 fg DNA) [20].

The sampling efficiency of NPS and the presence of PCR inhibitors were examined by amplification of the human β_2-microglobulin gene with primers B2M-TR-1 and B2M-TR-2 [16] using SYBR green-based real-time PCR. Each 12 adult and child DNA samples, which were randomly selected from LAMP-positive samples, showed β_2-microglobulin C_t values in the range 15.1–24.8 (mean 21.9) and 19.8–22.3 (mean 21.1), respectively, confirming that the respective sampling efficiency and PCR inhibition were almost identical between adult and child NPS samples.

Multilocus sequence typing (MLST)

B. pertussis allelic genes (pertussis toxin *ptxA*, pertactin *prn* and serotype 3 fimbriae *fim3*) were amplified directly from patient DNA samples by nested-PCR. The first PCR was performed in a 15-μL reaction volume containing 1.87 μL DNA sample, 0.44 mM concentrations of each dNTPs, 0.2 μM concentrations of each primer, and 0.3 U of KOD-FX DNA polymerase (Toyobo, Osaka, Japan). For the nested-PCR amplification, 1 μL of the first PCR product was added as the template to the PCR mixture containing the same components described above, except that the nested primers were used instead of the first set of primers (Table 1). Cycling conditions were: denaturation for 1 min at 94°C; 30 cycles of 10 s at 98°C, 30 s at 55°C, 45 s at 68°C; and a postextension of 5 min at 72°C. The PCR products were sequenced with the BigDye terminator v3.1 cycle sequencing kit on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). The combined allelic profiles were used to define MLSTs as described previously [2].

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