回の調査では，多くの施設が免疫抑制患者に対して抗ウイルス薬の経静脈投与をIntravenous immunoglobulin (IVIG) と併用していた．一方，二次発症を危惧し，免疫正常者に対しても主に内服による抗ウイルス剤の予防投与を導入している施設が認められた．

米国では，免疫抑制者などのリスク患者に対して96時間以内に水痘高力価免疫グロブリンであるVaricella-Zoster Immune Globulin (VZIG) を経静脈投与することが推奨されている[3]．しかし，既にVZIGの製品発売自体が中止されており，本邦での入手は困難である．本邦で入手可能なIVIGの水痘に対する治療効果は確定的ではないが[4]，水痘重症化のリスクを有する免疫不全患者に関しては代替薬が存在しないため使用せざるを得ない．

水痘曝露後の緊急ワクチン接種に関しては，曝露後72時間以内のなるべく早期に接種することが推奨されているが[3]，最近の報告では5日以内に接種することにより同等の予防効果が得られる可能性が示唆されており[5]，曝露後5日以内であれば，ワクチン接種が禁忌でない患者に対しては二次感染予防策として検討されるべきである．

水痘曝露者に対する抗ウイルス薬内服による発症予防効果は既に確認されており[6]，水痘に曝露された免疫抑制者などのリスク患者に対する抗ウイルス薬の予防投与に関しては，一部の専門家はその患者のおかれた状況により肯定している[3]．ただし，健康小児に対する使用は必ずしも推奨されておらず[3][7]その適応に関しては今後検討が必要である．

患者に対する入院時の水痘既往歴，ワクチン接種歴調査や，職員に対する水痘抗体調査に関しては多くの施設で行われていたが，その内容に関しては患者自身や保護者の記憶に依存しており，必ずしも正確な回答がえられるとは限らず，実際，今回の調査における院内水痘発症患者の13%が事前の問診で水痘既往ありと回答していた．一部の施設においては母子手帳やカルテなどの医療記録により客観的な確認方法の導入を試みていたが，母子手帳の記載に関してはやはりその正確性を保護者に依存するところが多く，また対象施設の多くが初診の急性疾患患者を常に受け入れていることから，医療記録による確認に関してもその効果は限定的と考えられる．

医療従事者に対する入職時の水痘抗体調査に基づく水痘ワクチン接種は米国でも推奨されており[7]，医療従事者に対する事前の抗体調査とその結果に基づく適切な水痘ワクチン接種による免疫獲得は，欠勤者を発生させないなどの円滑な病棟運営のためにも非常に有益であると考えられる．

また，今回の院内水痘発生患者108例のうち，2例は水痘発症前に面会を目的に来院した保護者による成人発症例であり，特に長期入院患者の保護者などには水痘に限らず，麻疹，風疹，流行性耳下腺炎などを含んだVPDに対する既往歴，ワクチン接種歴等の確認を行い，必要に応じて保護者に対するワクチン接種の推奨も検討すべきである[1]．

その他，平時から水痘を含めた感染性疾患のリスクを伴う免疫抑制患者と一般患者の病棟を区分することは理論的には有効な二次感染予防策と考えられるが，実際は病棟運営上そのような区分は困難であることが多い．病棟内水痘発症後，潜伏期間中の病棟閉鎖を導入している施設は2割未満であったのは同様の理由と推定される．

以上より，現状において施設毎の個別の水痘感染対策で院内発生をコントロールすることは非常に困難であり，本邦の主要小児病院においては，一度院内で水痘が発症した場合，二次発症予防策として本来必要としない輸血製剤や，抗ウイルス薬，入院中の緊急ワクチン接種などが曝露患者に対して使用されていることが確認された．

米国においては，既に1995年より水痘ワクチンの1回接種が導入され，接種率が90%に達し，水痘の発症率，入院率，死亡率の劇的な減少が得られた[8]．その後，議論の中心は2回接種の導入による，より確実な水痘予防に主眼が置かれ[9][10]，2006年には2回接種も導入されている．

先進国である本邦の代表的な小児入院施設において，VPDである水痘の院内発症によってリスク患者を重症水痘の危険にさらすだけではなく，入院患者に本来必要のない治療を強いていることは非常に重大な問題である．

本調査より，施設毎の院内水痘発症予防策の効果には限界があることは明らかであり，また院内水痘発症患者の9割が水痘ワクチン未接種または接種歴不明であったことから，入院中の水痘ハイリスク患者に対する安全性の確保のためには，国内における水痘ワクチン接種率の向上が早急に必要である．

現在，水痘ワクチンは任意接種ワクチンと位置付けられており，費用負担と接種必要性判断は保護者にゆだねられている．一方，定期接種ワクチンは，予防接種法に基づき，市区村長が実施主体となって公費負担で無料接種がなされており，ワクチンによる健康被害救済制度も任意接種ワクチンと比べて格段に手厚く保護されているため，水痘ワクチンの定期接種化は，ワクチン接種率向上に多大な効果を示すものと考えられる．

結　論

院内水痘発生患者の多くは水痘ワクチン未接種児で

あり，水痘ワクチン定期接種化の導入による接種率の向上が院内水痘発生予防に寄与する可能性が示唆された．

尚，本調査は平成21年度厚生労働科学研究費補助金新型インフルエンザ等新興・再興感染症研究事業「成人感染が問題となりつつある小児感染症への対応に関する研究」(研究代表者加藤達夫）の一環として実施されたものである．

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Varicella Outbreak in Pediatric Tertiary Care Hospitals in Japan

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Varicella is known to be a vaccine preventable disease. It can cause high morbidity and mortality in immunocompromized hosts.

Questionnaire survey was performed at 123 pediatric tertiary care hospitals in Japan to understand the incidence of varicella outbreaks in the hospitals. We evaluated characteristics of the hospitals, frequency of varicella outbreaks between August 2006 and July 2009, treatment for exposed patients, and prior preventive methods. Appropriate responses were obtained from 71 (58%) hospitals.

During the observation period, 108 patients infected with varicella were reported at 36 hospitals (51%). Among them, only 11 patients (10%) had a history of varicella vaccination. Treatment and prevention strategies include intravenous and oral antiviral therapy, intravenous immune globulin and varicella vaccine.

Based on these observation, we conclude that varicella outbreaks are a major issue in pediatric tertiary hospitals due to a low vaccine coverage rate in Japan. Varicella vaccine should be included in the National Immunization Programs in Japan to reduce the varicella outbreaks in the hospitals.

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

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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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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

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 Micro-kit (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].

TABLE 1. Primers used in multilocus sequence typing analysis

Target gene	Primer name	Sequence (5′- to 3′)	PCR	Coordinate[a]
ptxA	ptx-outerF	TAACGCGGGTCTATCACAAC	1st	3988790
	ptx-outerR	TAGAACGAATACGCGATGCT	1st	3989047
	ptx-innerF	GACCACGACCACGGAGTATT	2nd	3988824
	ptx-innerR	GTACACGAGAACCATCGCCT	2nd	3989021
prn	prn-AF	GCCAATGTCACGGTCCAA	1st	1098595
	prn-AR	GCAAGGTGATCGACAGGG	1st	1099163
	prn-innerF	GTCATTGCAGCCGGAAGACC	2nd	1098657
	prn-innerR	CCGGTCTCGATGACATTGCC	2nd	1099111
fim3	Fim3-F1	ATGTCCAAGTTTTCATACCC	1st	1647602
	Fim3-R1	GGTGACCTTGCCGGTAAA	1st	1648082
	fim3-innerF	CCAGCACCCTCAACCATATC	2nd	1647738
	fim3-innerR	GGCTTGCGTGGTTTTGTC	2nd	1648055

[a]Coordinates in *Bordetella pertussis* Tohama genome sequence NC02929.

Statistical analysis

Data were analyzed using the Mann–Whitney *U*-test. $p < 0.05$ was considered statistically significant.

Results

Sensitivity and specificity of IS*481* real-time PCR

The real-time PCR with ten-fold serial dilutions (0.1 fg to 10 ng) of *B. pertussis* Tohama DNA was able to detect bacterial DNA over a linear range between 10 fg (2.4 bacterial cells) and 10 ng (2.4×10^6 bacterial cells) per reaction mixture ($r^2 = 0.99$) (Fig. 1). On the basis of three independent experiments, the detection limit was a threshold cycle (C_t) of 37.6 ± 0.3, corresponding to 2.4 cells of the Tohama. The analytical sensitivity of the IS*481* real-time PCR was equal to that of the *B. pertussis*-specific LAMP assay [11].

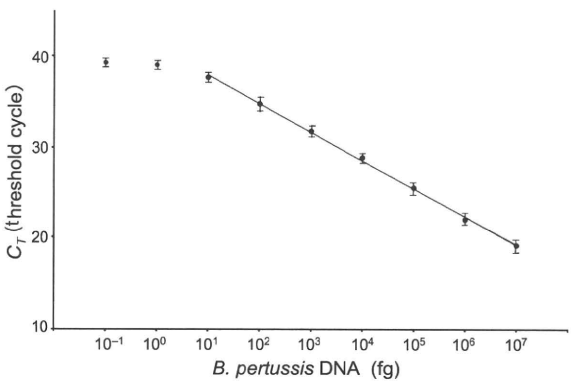

FIG. 1. Detection limit for linear calibration of IS*481* real-time PCR. Serial dilutions of *Bordetella pertussis* Tohama DNA were subjected to the real-time PCR. Data are the mean ± SD for three independent experiments.

Fifty-nine LAMP-positive (19 adults and 40 children) and 24 LAMP-negative DNA samples (six adults and 18 children, randomly selected) were subjected to the IS*481* real-time PCR. The LAMP-positive samples had C_t values in the range 14.6–39.5 (mean 29.7). By contrast, when the real-time PCR assay was applied to the LAMP-negative samples, the C_t values were in the range 37.2–40 (mean 39.7) (data not shown). Twenty (83%) of 24 LAMP-negative samples had a C_t value of 40 (i.e. no detectable amplification). The IS*481* real-time PCR and LAMP results showed a high level of agreement (77/83; 93%) with 55/83 found to be positive in both assays and 22/83 found to be negative in both assays, when a C_t value >37.6 was used as the cut-off for the real-time PCR.

B. pertussis DNA loads among children and adults

Figure 2a shows C_t values of LAMP-positive DNA samples from 40 children (mean age 6.0 years; age range 0–15 years) and 19 adults (mean age 43.3 years; age range 22–83 years). The child samples had C_t values in the range 14.6–39.5 (mean

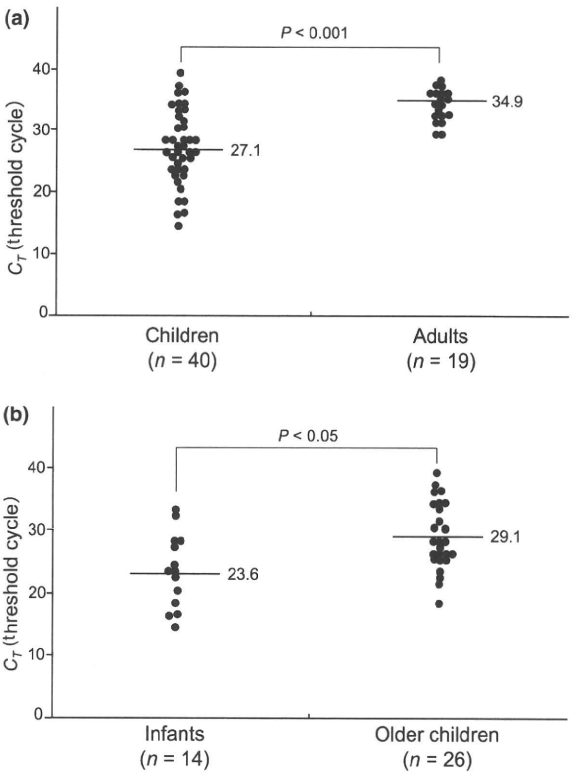

FIG. 2. Comparison of *Bordetella pertussis* DNA loads in nasopharyngeal swabs among children and adults with pertussis. (a) Children (infant and older children, $n = 40$) versus adults ($n = 19$). (b) Infants ($n = 14$) versus older children ($n = 26$). The DNA samples (2 μL) were subjected to IS*481* real-time PCR. Horizontal bars indicate mean C_t values.

27.1), whereas the adult samples had significantly higher C_t values in the range 29.1–39.0 (mean 34.9) within a narrow range. Statistical significance was observed with respect to the C_t values between children and adults ($p < 0.001$). Figure 2b also shows C_t values of LAMP-positive DNA samples from 14 infants (mean age 2.9 months; age range 1–6 months) and 26 older children (mean age 9.2 years; age range 2–15 years). The infant samples had C_t values in the range 14.6–33.8 (mean 23.6). By contrast, the older child samples had higher C_t values in the range 18.8–39.5 (mean 29.1) (p 0.011).

The clinical information about cough duration was obtained from 16 adult patients. Eight adults had a cough duration of less than 14 days (mean 8.6 days) at the time of sampling, and other adults had a duration of more than 15 days (mean 30.3 days). The C_t values (mean C_t value of ≤14 days, 35.9; >15 days, 34.8) were not statistically significant between these two groups (p 0.92) (data not shown).

Relationship of *B. pertussis* DNA loads and bacterial genotypes

On the basis of the MLST analysis, Japanese *B. pertussis* isolates could be classified into five genotypes (MLST-1 to MLST-5), as described previously [2]. Among 40 children NPS samples, the MLSTs in 33 samples were identified as: 17 MLST-1 (harboring *ptxA2*, *prn1* and *fim3A* alleles); 14 MLST-2 (*ptxA1*, *prn2* and *fim3A*); and two MLST-4 (*ptxA1*, *prn2* and *fim3B*). The mean C_t values for MLST-1, -2, and -4 were 27.0, 24.8, and 28.6, respectively. Comparison of C_t values for MLST-1 and -2 revealed no statistically significant difference (p 0.27) (data not shown). Among the infants and older children, no correlation was found between *B. pertussis* DNA loads and the bacterial genotypes.

Discussion

To our knowledge, this is the first report of a precise comparative analysis of *B. pertussis* loads in adults and children. The results obtained clearly indicate that adults had very low *B. pertussis* DNA loads in their NPS compared to children, especially infants. When bacterial loads in NPS were calculated for *B. pertussis* Tohama cells using a standard curve, the mean numbers of bacterial cells taken with a rayon-tipped swab from adults, older children and infants were 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 (Table 2). Surprisingly, the bacterial numbers in adults were 340-fold and 65-fold lower than those in infants and older children, respectively. In general, adults with pertussis showed only prolonged cough illness and had less typical symptoms than children [3,6–9]. In addition, vaccinated asymptomatic children had significantly fewer *B. pertussis* than symptomatic patients [21]. Our experimental observations strongly suggest that the lower nasopharyngeal bacterial load in adults is related to the atypical and milder symptoms in adult pertussis.

TABLE 2. Number of *Bordetella pertussis* cells taken with a rayon-tipped swab from infant, child and adult patient

Patient	Number of patients	Mean age (range)	*B. pertussis* cells/swab (95% CI)[a]
Infant	14	2.9 months (1–6 months)	1.1×10^6 (1.2×10^5 to 8.9×10^6)
Older child	26	9.2 years (2–15 years)	2.1×10^4 (5.3×10^3 to 8.3×10^4)
Adult	19	43.3 years (22–83 years)	320 (120–910)

[a]Bacterial cells were calculated for *B. pertussis* Tohama cell.

Previously, Bidet *et al.* [16] demonstrated that *B. pertussis* DNA loads in NPS decreased progressively during antibiotic treatment in children. Several factors are also considered to affect *B. pertussis* load in the human nasopharynx, including patient age, previous vaccination or infection, and host genetic background. In the present study, infants had a larger *B. pertussis* DNA load than older children, and most (85%) of the infants had not received pertussis vaccination, whereas most (83%) of the older children had received four doses of the vaccine. Our findings support the hypothesis that the *B. pertussis* DNA load is affected by vaccination. Unfortunately, this does not apply to adults because pertussis vaccines are unable to provide lifelong immunity [22]. The duration of immunity post-vaccination is estimated to be in the range 4–12 years and, therefore, the bacterial loads in adults may be affected by other factor(s) besides the vaccination.

B. pertussis culture has been taken as a gold standard diagnostic method because it is highly specific. However, the culture has limited sensitivity for previously vaccinated persons, especially adolescents and adults [5,8,9]. In the present study, we demonstrated that adults with pertussis had very low *B. pertussis* DNA loads in their NPS during both early and later stages of the cough illness. This finding suggests that the diagnosis of pertussis by bacterial culture is difficult in adults even if NPS are obtained in the early stage of the illness. Compared with the culture method, nucleic acid amplification tests such as IS*481*-based real-time PCR and *B. pertussis*-specific LAMP provide a highly sensitive procedure for detection of *B. pertussis* DNA [11,13,14]. On the basis of our findings, we recommend the performance of

nucleic acid amplification tests for an accurate diagnosis of pertussis, especially when adult pertussis cases are suspected.

During the last three decades, genetic divergence in *B. pertussis* has been observed in many countries [23–27]. In Japan, the circulating strains have shifted mainly from MLST-1 to MLST-2. The MLST-1 strains include vaccine-types *ptxA2* and *prn1* alleles, whereas the MLST-2 strains include nonvaccine-types *ptxA1* and *prn2* alleles [2]. This genetic shift has been speculated to have resulted from adaptation of the bacterial population to vaccine-induced immunity [23,24,28,29]. The bacterial load of the emerging MLST-2 strains in the nasopharynx has not previously been investigated. We therefore examined the bacterial DNA loads of MLST-2 and MLST-1 strains but found no association between the DNA loads and their genotypes among infants and older children. This would suggest that emerging MLST-2 strains have the ability to produce similar bacterial loads to those of the the MLST-1 strains.

In conclusion, the *B. pertussis* DNA load in NPS depends highly on patient age. Adults had very low *B. pertussis* DNA loads and organism numbers in their NPS during both the early and later stages of the cough illness, and this explains the culture-negative results in adult pertussis cases. To make an accurate diagnosis of adult pertussis, nucleic amplification tests such as the IS*481* real-time PCR and *B. pertussis*-specific LAMP assays are recommended as sensitive methods.

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Transparency Declaration

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医学と医療の最前線

百日咳の臨床―成人と小児―

岡田　賢司

日本内科学会雑誌　第99巻　第5号別刷

2010年5月10日

百日咳の臨床—成人と小児—

岡田　賢司

要　旨

百日咳は，乳幼児の疾患と考えられてきたが，DTaPワクチン接種率向上に伴い，乳幼児の報告は激減した．近年は，相対的に10歳以上とくに成人の報告例が増加してきた．本稿では，変化してきた疫学，百日咳の家族内感染における乳幼児の症状とワクチン接種の影響，成人での症状と診断について紹介した．さらに，成人の長引く咳での百日咳の関与割合，抗原診断した成人百日咳の臨床像，感染管理，抗菌薬治療の実際および予防接種の今後の展開などを概説した．

〔日内会誌　99：1064～1071，2010〕

Key words：成人の百日咳，2週間以上の咳，百日咳の診断，予防接種

1．百日咳患者の報告数および年齢の変化

百日咳は，感染症法5類感染症・定点把握疾患に分類され，全国約3,000の小児科定点から報告されている．百日咳ワクチンを含むDTaP (Diphtheria-Tetanus-acellular Pertussis)三種混合ワクチン開始後の感染症発生動向調査での定点あたりの百日咳患者報告数を示す(図1)．1982年から4～5年ごと小さな増減をくり返しながら報告数は着実に減少してきたが，2005年から増加してきた．2007年いくつかの大学や高校での集団発生が報告され，2008年は過去10年にない多くの報告があった．

近年の特徴に患者年齢の変化がある．2000年，乳児は46.7%，1歳18.1%，2～3歳13.5%と3歳までが約80%で20歳以上は2.2%であった．次第に10～14歳以上とくに20歳以上が増加してきた．20歳以上の割合は2002年4.0%，'04年9.5%，'06年24.3%，'08年36.7%，2009年13週時点で38.2%となっている（図2）[1]．この報告は，小児科の定点医療機関に受診した患者報告である点に注意が必要である．成人は小児科でなく，内科を受診しているため，成人症例を含めた全体像を把握するためには，内科を含めた報告システムが必要となってきた．このため，国立感染症研究所感染症情報センターのホームページに，百日咳を診断した医師ならどなたでも報告できるシステムが整備されている[2]．国内の現状を把握するため，百日咳と診断された場合はご登録をお願いいたします．

2．百日咳の家族内感染

典型的な症状を呈した乳児の家族に認められた成人，同胞の症状を図3に示す．Indexは周産期に異常のない1カ月男児．軽い咳で始まり，次第にひどくなり，無呼吸・チアノーゼが認められ，百日咳（疑）で紹介入院．入院時，連続的な咳込みはあったが吸気性笛声はなかった．白血球数17,500/ul(Ly 78%) CRP＜0.30 mg/dl，百日咳菌が分離でき，典型的な乳児の百日咳と

おかだ　けんじ：国立病院機構福岡病院

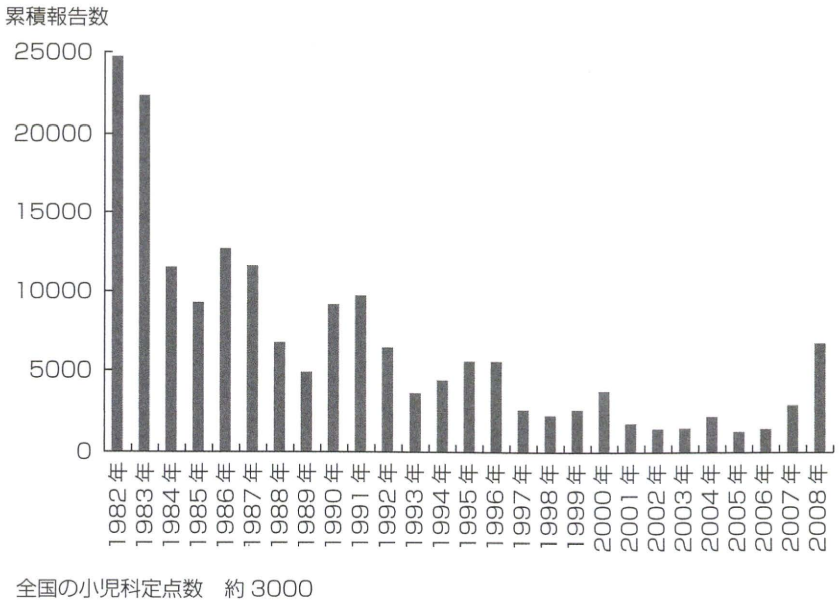

図1．百日咳患者累積報告数の推移（1983－2008年）
（国立感染症研究所感染症情報センター資料より作図）

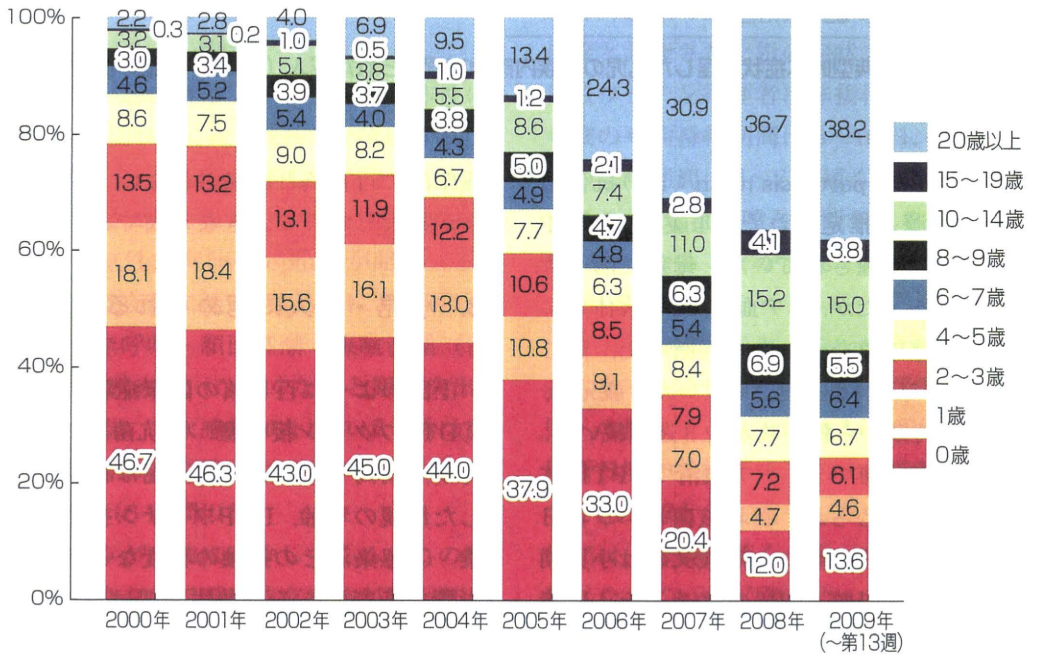

図2．百日咳の年別・年齢群別割合（2000年～2009年第13週）
感染症発生動向調査　週報　2009年第13週

診断した．入院時の問診で，児発病の約3週間前から33歳母親は軽い咳があったが，受診していなかったため，母親の検査も行った．白血球6,400/ul（リンパ球47%）で菌は分離できなかっ

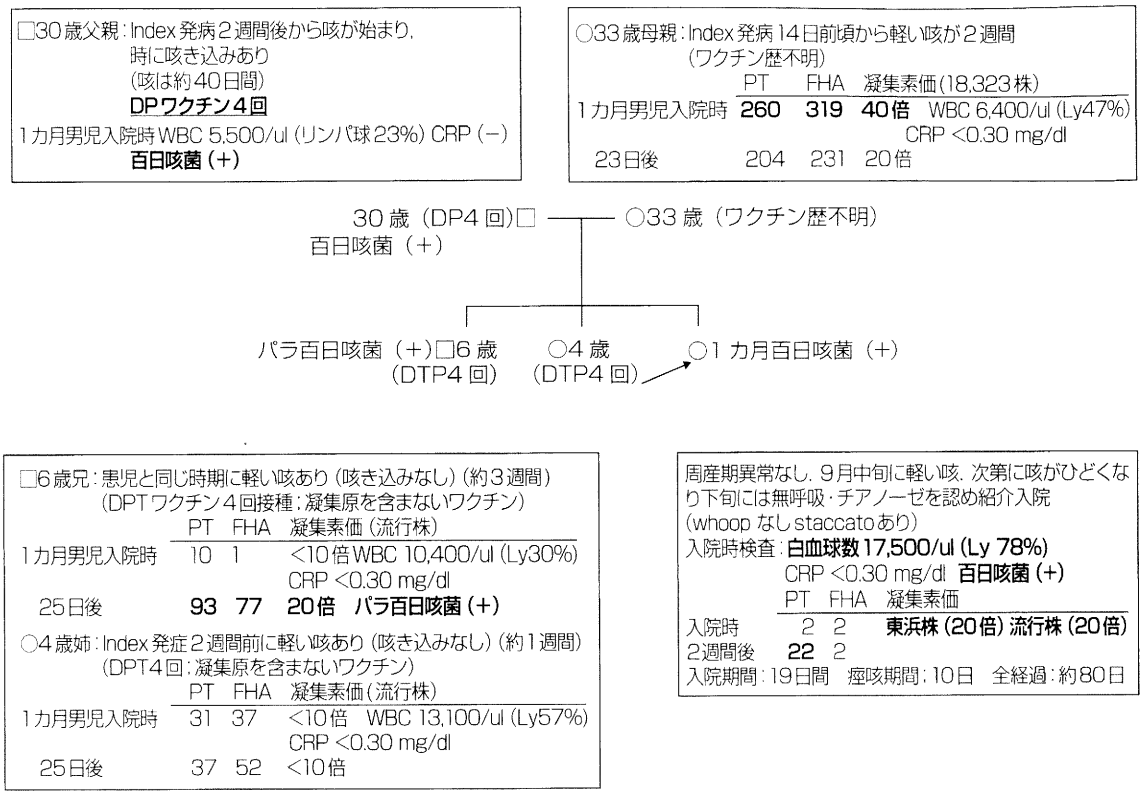

図3. 典型的な症状を呈した乳児の家族内感染で認められた成人（両親），同胞の症状

たが，百日咳毒素(pertussis toxin：PT)抗体価は高値で感染源と推定された．4歳姉はDTaPワクチンを4回接種されていた．母親と同じ時期に軽い咳があったが，ペア血清でPT抗体価に変化はなく，感染はなかったと判断した．同じくDTaPワクチンを4回接種されていた6歳兄は，初発児と同じ時期から軽い咳が約3週間続いた．パラ百日咳菌が分離でき，ペア血清で，PT抗体価の有意上昇が認められ，百日咳菌とパラ百日咳菌の重感染と考えられた．30歳父親は小児期にジフテリア・百日咳（DP）ワクチンを4回接種されていた．児発病14日目頃から咳があり，発作性の咳き込みや時に咳込み後の嘔吐および咳による夜間の目覚めなどが認められた．百日咳菌が分離でき，百日咳と診断できた．このように，DPTワクチン接種者や成人が感染を受けても，詳細な家族歴聴取や問診を行わないと，臨床的に百日咳と認識されないことが多く，感染が拡大していく大きな要因である．

3. 小児・成人に認められる臨床症状

上記のように百日咳の臨床症状は，①年齢 ②DTPワクチン接種歴 ③抗菌薬の種類，開始時期，期間 ④6カ月未満児は移行抗体を考慮した母親の年齢，DTPワクチン接種の有無，職業 ⑤感染源との接触の程度など多くの因子の影響で多彩である．潜伏期間は，感染後7～10日が多い（6～20日）．

1）小児の典型的百日咳の経過と合併症

典型的な経過は，ワクチン未接種の乳幼児に多い．

(1) カタル期（1～2週間）

軽い咳から始まり，次第に通常の鎮咳薬では

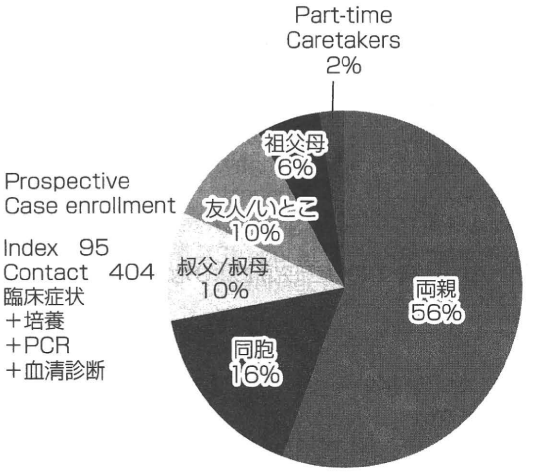

図4. 百日咳の乳幼児への感染源
(Pediatr Infect Dis J 2007. 26：293-299)

咳が治まらず，ひどくなる．この時期での診断は，家族内感染以外は難しいが，適切な抗菌薬療法ができれば，咳症状の軽減に有用とされている．

(2) 痙咳期（3〜6週間）

特徴的な咳が聴かれるようになる．発作性の5〜10回以上途切れなく続く連続的な咳込み(paroxysmal cough/staccato)で苦しくなり，大きな努力性吸気の際に狭くなった声門を吸気が通過する時に，吸気性笛声(whooping)が聞かれる．一連の咳発作は夜間に強く，咳込みによる嘔吐，チアノーゼ，無呼吸，顔面紅潮・眼瞼浮腫（百日咳顔貌），結膜充血（ひどくなると眼球突出）などが見られる．二次感染がなければ，熱はなく咳が激しい割に聴診所見は正常のことが多い．

(3) 回復期（6週間以後）

特有な咳込みが次第に減少してくる．この時期，上気道感染などで再び特有な咳が聴かれることもある．通常3〜6週間で軽快する．

(4) 合併症

米国の28,187例の入院や合併症の報告がある[3]．6カ月未満児に多く，入院率は63.1%，肺炎11.8%，けいれん1.4% 脳症0.2%，死亡0.8% であった．全年齢では脳症26例（0.1%），関連死亡は62例（0.2%）となっている．

2) 乳児の百日咳

6カ月未満の乳児は，月齢，母親からの移行抗体やDTaPワクチン接種の有無などの影響があり症状が多彩である．3カ月未満児は死亡率が高く，無呼吸やけいれんが多く，特有な咳が少ないことが特徴である．生後2カ月以内の児の合併症は，肺炎25%，けいれん3%，脳症1% と報告されている[4]．

3) DTPワクチン接種児の症状

咳の持続や重症度が典型的百日咳ほどではない軽症な百日咳も存在する．とくにワクチン接種児に多い．Yaariらは，5歳〜30歳（平均8.9歳）のワクチン接種者の症状を報告している．咳の持続は4±3.6週間，診断までに平均23日，典型的な症状を示したのはわずか6% であった．平均白血球数は$8.7\pm2.6\times10^3/mm^3$リンパ球は40±12% であった[5]．Tozziらは，DTaPワクチンの野外試験でDTaP接種児とDT接種児とで，百日咳と診断された児の症状を比較している[6]．百日咳ワクチン接種者は非接種者（DT接種群）より咳の平均持続期間は5〜10日，特徴的な咳込みの平均期間は4〜7日短くなっていた．

この群は，百日咳と診断されることが少なく，感染源となることが問題である．

4) 成人百日咳

成人では咳がひどくない場合は受診しないことも多く，百日咳とほとんど認識されず，乳幼児への感染源となっている[7]．Bisgardらは，乳児百日咳の接触者で7〜20日以前に咳があった者を感染源として調査した．感染源が判明した例では，母親が多く，次いで兄弟，父親，祖父母となっていた（図4）[8]．

(1) 2週間以上の咳で受診した20歳以上の成人を遺伝子診断で診断した場合

当院では，内科と共同で，2週間以上の咳で受診した成人患者を対象に表1に示す百日咳診断の目安に従って，百日咳感染を調査してきた[9]．

LAMP法によるPT遺伝子陽性のA群，PT遺伝

表 1．百日咳診断基準（案）2008

臨床症状　14 日以上の咳があり，かつ下記症状を 1 つ以上を伴う（CDC 1997 WHO 2000）
　1 発作性の咳込み　2 吸気性笛声（whoop）　3 咳込み後の嘔吐
実験室診断
　発症から 4 週間以内：培養，LAMP 法＋対血清による血清診断
　　4 週間以降：LAMP 法＋対血清による血清診断
　1 百日咳菌分離
　2 遺伝子診断：PCR 法または LAMP 法
　　現時点では，LAMP 法は全国数カ所の百日咳レファレンスセンター（国立感染症研究所および地方衛生研究所）でしかできない
　3 血清診断
　（1）凝集素価
　　1）DTP ワクチン未接種児・者：流行株（山口株），ワクチン株（東浜株）いずれか 40 倍以上
　　2）DTP ワクチン接種児・者または不明：単血清では評価できない．
　　対血清での流行株，ワクチン株いずれか 4 倍以上の有意上昇を確認する必要がある
　（2）EIA 法：PT（百日咳毒素）-IgG
　　1）DTP ワクチン未接種児・者：1 EU/ml 以上（Ball-ELISA）
　　2）DTP ワクチン接種児・者または不明
　　対血清：確立された基準はないが，2 倍以上を原則とする
　　単血清（参考）：94 EU/ml 以上（Baughman AL2004）100 EU/ml 以上（de Melker HE. 2000）
臨床診断　臨床症状は該当するが，実験室診断はいずれも該当しないとき
確定診断　（1）臨床症状は該当し，実験室診断の 1 ～ 3 のいずれかが該当するとき
　　（2）臨床症状は該当し，実験室診断された患者との接触があったとき

表 2．「2 週間以上の咳」を主訴に受診した成人患者の LAMP 法陽性・陰性別の臨床像

	LAMP 陽性（n = 26）	LAMP 陰性（n=43）	
		抗体（凝集素価または PT-IgG）陽性（n=26）	抗体価はいずれも陰性（n=17）
	A 群	B 群	C 群
年齢	51	46.9	47.5
白血球数	6,188	6,190	7,022
リンパ球（%）	28%	28%	31%
受診までの咳の持続期間 #1	2 週間～ 4 カ月（平均 5.0 週）★	2 週間～ 5 年（平均 4.8 週）#1 ∫	2 週間～ 4 年（平均 11.8 週）#1★∫
発作性の咳込み	18/20 (90.0%)**	8/19 (42.1%)**	10/13 (76.9%)
咳込み後の嘔吐	7/20 (35.0%)	3/19 (15.8%)	3/13 (23.1%)
吸気性笛声	10/20 (50.0%)*★	2/19 (10.5%)*	1/13 (7.7%)★
夜間覚醒	8/16 (50.0%)	10/19 (52.6%)	7/13 (53.8%)
胸痛	9/20 (45.0%)	4/19 (21.0%)	3/13 (23.1%)
息苦しい	7/20 (35.0%)	4/19 (21.0%)	4/13 (30.8%)
息が止まりそう	6/20 (30.0%)	1/19 (5.3%)	2/13 (15.4%)
喘鳴	2/20 (10.0%)	2/19 (10.5%)	4/13 (30.8%)
周囲の咳（家族癒など）	13/23 (56.5%)★★	9/19 (47.4%) ∫	1/15 (6.6%)★★ ∫

1　平均の算出には受診まで 1 年以上の症例は除く
**(A VS B) P<0.01　★★(A VS C) P<0.01
*(A VS B) P<0.05　★(A VS C) P<0.05　∫ (B VS C) P<0.01
検定：年齢・リンパ球（%）は 2 標本 t 検定，白血球数・受診までの咳の持続期間（週）は 2 標本 Wilcoxon 検定　臨床症状は Fisher の直接確率検定

表3．百日咳の診断を培養，PCRおよびPT抗体価に限定した場合の成人の持続咳嗽患者における百日咳感染陽性率

報告者	地域	調査年	陽性率
Mink et al	Los Angels, CA	1986-1989	13
Wright et al	Nashville, TN	1992-1994	16
Jansen et al	San Diego, CA	1993-1994	1
Nenning et al	San Francisco, CA	1994-1995	12
Strebel et al	Mineapolis/St Paul, MN	1995-1996	13
Birkebaek et al	Denmark	1995-1997	17
Vincent et al	Korea	1997-1998	7

（文献10より）

子は検出できなかったが血清診断にて百日咳感染が確認できたB群，LAMP法でも血清診断でも陰性で百日咳とは診断できなかったC群の臨床症状の違いを表2に示す．

各群とも年齢，白血球数，％リンパ球には差がなかった．受診までの咳の持続期間は百日咳感染群（A・B）と非感染群間で有意差があった．症状の出現率では，百日咳に特徴的な「発作性の咳込み」の出現率はA群とB群間で有意な差が認められた．「咳込み後の嘔吐」の率には3群間で差は認められなかった．DTPワクチン未接種の乳幼児患者に特有な咳と考えられていた「吸気性笛声」は，成人でも10.5～50.0％認められた．「咳による夜間の覚醒」・「胸痛」・「息苦しい」・「息が止まりそう」は，A群に多かったが有意差は認められなかった．一方「喘鳴」は，非感染群に多かったが，有意差はなかった．「家族内など周囲の咳」は，百日咳感染群と非感染群とで有意差が認められ，問診上の有用なポイントと考えられる．

（2）慢性持続咳嗽患者の中の割合

1～4週間続く持続咳嗽患者での百日咳感染の割合は，12～32％であったが咳の持続期間，診断基準などが様々であった．とくに血清診断の抗原で，filamentous haemoagglutinin（FHA），pertactin fimbriaeなどを用いた場合，百日咳菌と*B. parapertussis*，*B. bronchiseptica B. holmesii*などとの抗原交差があるため，陽性率を押し上げている可能性がある．流行のない時期に菌分離とPCRおよび百日咳菌に特異的なPTに対する抗体価のみで診断すると，成人の持続咳嗽患者での陽性率は1～17％〔平均13％〕であった（表3）[10]．

4．診断

ワクチン接種児や成人例に対する認識が高まってきたが，実験室診断法が具体的に定まっていない．これまでの報告を参考に百日咳診断基準案を表1に示す．臨床症状は，14日以上の咳に百日咳特有の咳（発作性の咳込み，吸気性笛声，咳込み後の嘔吐）の1つ以上を伴う場合としている．確定診断には発症から4週間以内では培養と核酸増幅法（PCR法，LAMP法），4週間以降なら血清診断で確定する．

1）培養

患児の後鼻腔から柔らかい針金の付いたスワブを用い検体を採取し，選択培地に塗布する．分離率は，第3病週までが高い．典型的な症状の場合，菌分離率は約52％と高く，早期診断法として有用である．選択培地のため，検査室に目的菌を事前に知らせておく必要がある．

2）核酸増幅法（PCR法，LAMP法）

培養より感度がよく，時間的にも早く，死菌でも検出できる．とくにLAMP法は特別な機器が必要でないため，今後日常検査として実施で

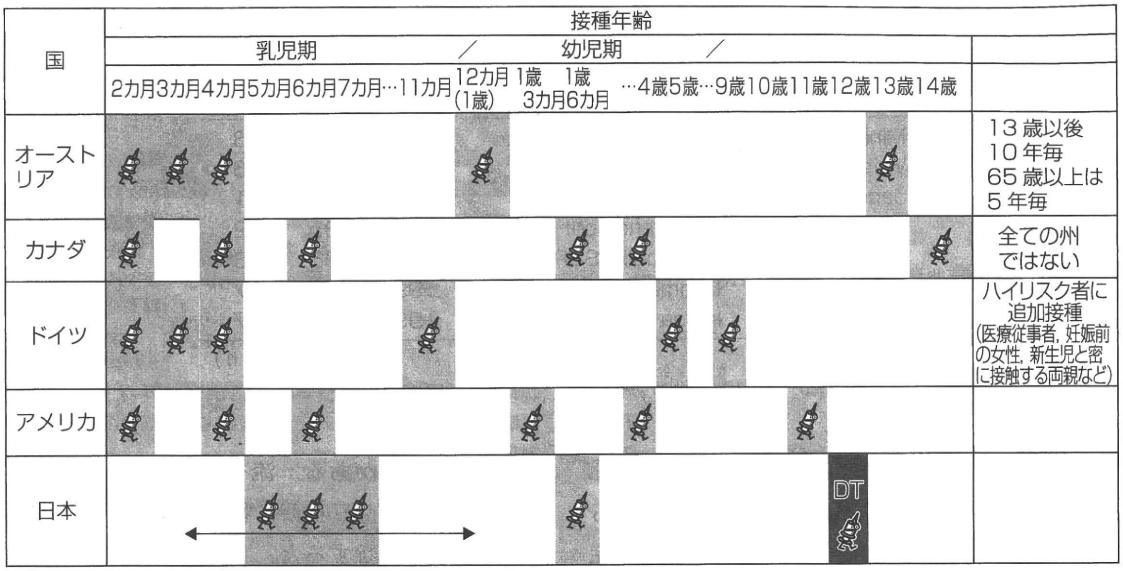

図5. 欧米での百日咳ワクチン接種が6回以上の国々と推奨されている接種年齢およびわが国のDPT/DT接種年齢

きる可能性がある[11].

3) 血清診断法

わが国では凝集素価検査が広く活用されているが，国際的には感度の点であまり推奨されていない．対血清で陽転または4倍以上の上昇が基本である．発症後4週間以上で受診した場合，抗体価がすでに上昇している症例も多く，解釈が容易でない．単血清で高い抗体価の場合は，感染は疑われるが正確な判断ができない．

酵素免役法（EIA）でPT-IgG（immunoglobulin G）も測定できる．ただ，PTは現行のすべてのDTaPワクチンの主要抗原であり，ワクチン接種により上昇する．そのため，ワクチン接種歴のある患者を診断する場合，DTPワクチン歴を参考にする必要がある．WHOではワクチン接種から3年を経過した患者についてのみ本法の適用を推奨している[12]．対血清が基本となるが，有意上昇の基準がない．単血清の場合，米国人を対象とした報告で，94 EU/ml以上を目安としている[13]．

5. 治療

百日咳の多彩な症状は，百日咳菌が気道粘膜に定着後，増殖中に産生する百日咳毒素によると考えられている．このため，抗菌薬は特徴的な咳が出る前のカタル期であれば，症状の軽症化に有効であるが，家族内感染や院内感染などに限られる．多くは，典型的な咳が出始めた頃，あるいは長びく咳などで初めて百日咳を疑われる．この時期の抗菌薬治療は，咳の改善効果は低いが，除菌することで周囲への感染を減らせることができるため重要である．通常治療開始後5～7日で百日咳菌は陰性となる．

米国疾病対策センター（CDC）はマクロライド薬の選択に，有効性・安全性・服用性などを考慮したガイドラインを出している[14]．「月齢6カ月以上では，アジスロマイシン（AZM）・クラリスロマイシン（CAM）はエリスロマイシン（EM）と同等な有効性があり，副作用は少なく，使いやすい（但し，わが国では百日咳にAZMは保険適用外）．CAM・EMはチトクロームp450

酵素系の抑制作用があるため，他の薬剤との相互作用を起こしやすい．CAM・AZMは，EMに比較して耐酸性で組織内濃度も高く，半減期も長い．EMは他の2剤より安価」．

γグロブリン製剤は痙咳期に効果が認められることがあるが，使用法は確立されていない．

6. 感染管理

米国小児科学会では，1)患者との接触者でDTPワクチン1〜2回接種者は追加接種　2)家族内や保育施設内の濃厚接触者はEM 14日間内服　3)医療従事者も接触後21日間は咳に注意し，咳が出始めたら培養検体採取後，抗菌薬内服を推奨している[15]．「濃厚接触者」とは有症状患者と3フィート（約0.9 m）以内での対面や1時間以上狭い室内での同室などの状況を挙げている．

7. 予防接種

わが国は世界に先駆け，発熱など副反応の強かった全菌体百日咳ワクチンを改良し，有効成分のみを単離し，副反応は少なく効果も同等な無細胞百日咳ワクチンを開発した．ジフテリア・破傷風トキソイドと混合し，DTaP(a：acellular)として1981年秋から開始し，28年が経過した．接種率の向上とともに，小児患者は著明に減少し，優れた効果を示してきたが，近年，相対的に10歳以上の患者数が増加している．

欧米では，思春期・成人百日咳対策として新しくジフテリア・百日咳の抗原量を減らした三種混合ワクチン（Tdap）を導入して推奨している[16]．欧米での百日咳ワクチン接種が6回以上の国々と推奨されている接種年齢を図5に示す．日本でも，増加してきた思春期・成人の百日咳対策が必要な時期となっている．

現行2期接種（11〜12歳）のDTワクチンに替わり，百日咳ワクチンを加えたDTaPが有益と考えられ，DTaPとDTとの比較試験で有効性と安全性の検討を開始している．

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Safe and effective booster immunization using DTaP in teenagers

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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.

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

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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

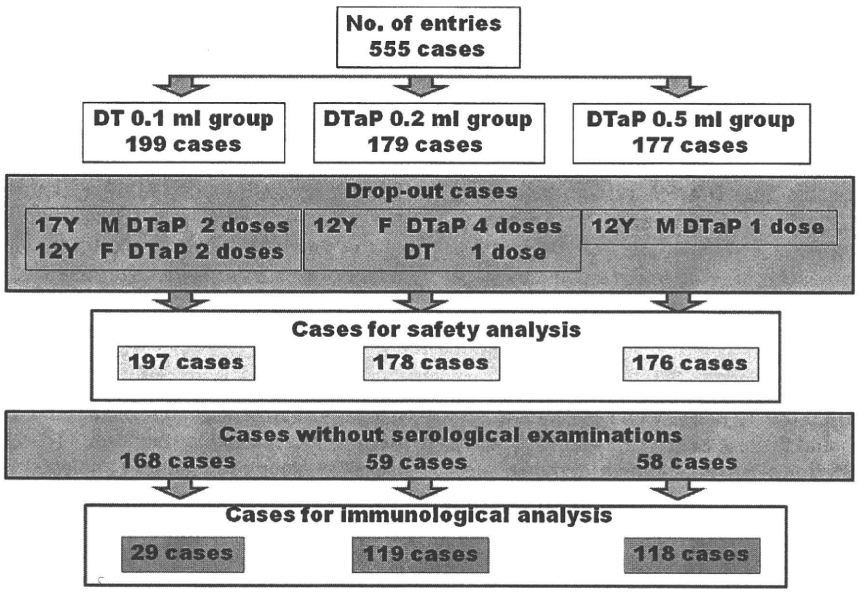

Fig. 1. Number of subjects in the study. A total of 555 subjects were enrolled, of whom four were excluded. Therefore, 551 subjects were evaluated regarding safety. Among the 551, 197 were immunized with 0.1 ml of DT, 178 with 0.2 ml of DTaP, and 176 with 0.5 ml of DTaP. Study group 1 consisted of 266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