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Hyporesponsiveness to the infecting serotype after vaccination of children with seven-valent pneumococcal conjugate vaccine following invasive pneumococcal disease

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ABSTRACT

Antibody responses to the infecting serotype in children who are vaccinated with pneumococcal conjugate vaccine (PCV) after having invasive pneumococcal diseases (IPD) have not been fully investigated. Of 56 children diagnosed with IPD between October 2009 and April 2013 in whom the infecting serotype was confirmed, 17 who were vaccinated with PCV7 following IPD were tested to determine the geometric mean concentration of serotype-specific immunoglobulin G (IgG) and the geometric mean titers of opsonization indices (OIs) using paired sera obtained at the onset of IPD and after PCV doses following the resolution of IPD. The geometric mean concentrations of serotype-specific IgG for all PCV7 serotypes other than serotype 6B were significantly increased after the last PCV7 dose compared with those at the time of IPD onset ($P < 0.01$), as were the geometric mean titers of OIs for all PCV7 serotypes. In 14 children with IPD caused by PCV7 serotypes for whom both IgG and OI results were available, the OIs for the infecting serotype at the time of IPD onset were <8, although the IgG levels varied between from <0.2 to >5.0 μg/ml. After the last PCV7 dose, the OIs for the infecting serotype remained <8 for six (43%) of 14 children. In these six children, hyporesponsiveness to PCV7 was specific for the infecting serotype. Hyporesponsiveness was found for serotypes 6B ($n = 5$) and 23F ($n = 1$). No difference was found between the responders ($n = 8$) and the hyporesponders ($n = 6$) with regard to any clinical characteristics. Our data suggest that hyporesponsiveness to the infecting serotype may occur in children vaccinated with PCV7 following IPD.

1. Introduction

Streptococcus pneumoniae is a major worldwide cause of morbidity and mortality resulting from pneumonia, bacteremia, and meningitis [1]. Antibodies to pneumococcal capsular polysaccharide (CPS) and complement provide protection against pneumococcal strains with homologous or cross-reactive capsular serotypes [2]. The introduction in 2000 of the seven-valent pneumococcal conjugate vaccine (PCV7; Prevenar®, Pfizer) for children in the United States younger than 2 years and children aged 2–4 years in a high-risk category was effective, dramatically reducing the incidence of invasive pneumococcal disease (IPD) [3,4]. The

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lowered rate of hospitalization for childhood and adult pneumonia has been sustained during the decade since the introduction of PCV7 [5].

In Japan, PCV7 was licensed in October 2009, the Japanese government began to subsidize it for children less than 5 years of age in November 2010. PCV7 for children under 5 years of age was subsequently included in the routine immunization schedule at public expense in April 2013.

Vaccine-induced protective immunity is currently estimated by measuring the concentrations of serotype-specific immunoglobulin G (IgG) using enzyme-linked immunosorbent assay (ELISA) [6] and the opsonization index (OI) using a multiplex opsonophagocytic assay (MOPA) [7]. The World Health Organization (WHO) working group reported that antibody concentrations of 0.2–0.35 μg/ml measured with the ELISA using serum without serum absorption with 22F polysaccharide, correlated best with an OI of 8, which in turn correlated best with protective efficacy [8]. Henckaerts et al. proposed a protective threshold concentration of 0.20 μg/ml assessed with ELISA using serum absorption with 22F polysaccharide as a measure of the serotype-specific efficacy of the pneumococcal conjugate vaccine against IPD among infants less than 1 year of age [9], with an exception of 19F [10]. We recently reported that the OIs for the infecting serotypes in sera of children with IPD were almost undetectable during acute phase of IPD, although the levels of serotype-specific IgG were higher than 0.20 μg/ml [11]. Based on this finding, it was necessary for us to examine whether children with IPD could develop antibody response to the infecting serotype after vaccination with PCV7.

A previous study demonstrated that most children respond to PCV7 following resolution of IPD, but suggested that IPD caused by particular serotypes in children could result in hyporesponsiveness to the infecting serotype [12]. However, limited information is available in regards to the immune response in children vaccinated with PCV following IPD because the serotype-specific OIs have never been evaluated. We, therefore, conducted the present study to determine antibody response to PCV7 vaccine serotypes by measuring the OIs as well as the IgG levels in children vaccinated with PCV7 following IPD.

2. Materials and methods

2.1. Patients

Children under 9 years of age, who had infection caused by *S. pneumoniae*, which was isolated from normally sterile body sites such as blood or cerebrospinal fluid, were enrolled in this study when their attending doctors requested the measurement of the antipneumococcal antibodies in their sera. Fifty-six children were enrolled between October 2009 and April 2013 at 41 hospitals in Japan. All of the pneumococcal isolates were serotyped at the Department of Bacteriology I, National Institute of Infectious Diseases, by agglutination tests with rabbit antisera (Statens Serum Institute, Copenhagen, Denmark). Serotype 6C was confirmed by an in-house antiserum [13]. Because the OI for the infecting serotype was assumed to be low after the onset of IPD, we determined the antibody response after vaccination with PCV7 vaccination following the resolution of IPD. Of 56 children with IPD, 21 received PCV7 vaccination following the resolution of IPD (Fig. 1). One child who died of IPD and the other 34 children did not receive PCV7 vaccination. Paired sera collected at the onset of IPD (the first blood sample) and after PCV7 vaccination (the second blood sample) were collected from 17 children of the 21

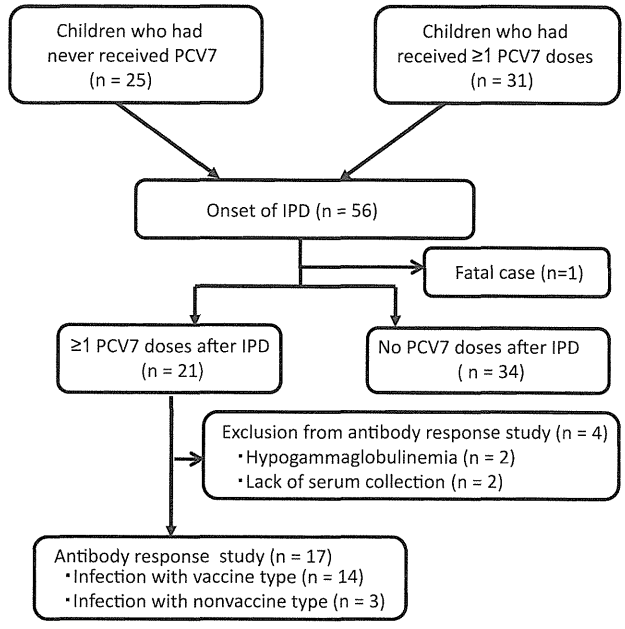

Fig. 1. Flow diagram of this study of children with invasive pneumococcal disease.

children who received PCV7 vaccination following the resolution of IPD. The other four children were excluded from this study was not collected at the time of IPD (two children) or they had comorbid hypogammaglobulinemia (two children). Fourteen of the 17 children were infected with a PCV7 serotype, and three were infected with a non-PCV7 serotype. As children received one to three doses of PCV7 after their episode of IPD, we defined the PCV7 dose before the second blood sampling as the last PCV7 dose. The median number of days (range) from IPD onset to the first blood sampling and from the last PCV7 dose to the second blood sampling was 0 (0–11) and 32 (27–120), respectively. The median number of days (range) from the IPD onset to the last PCV7 dose was 132 (15–633). Sera from children were submitted to the Research Institute for Microbial Diseases (RIMD), Osaka University, Japan, for determination of the IgG levels by ELISA and the OIs by MOPA.

Data collected from these patients included age at illness, clinical manifestations, outcome, comorbid conditions, and vaccination history. Clinical manifestations were divided into two categories: meningitis and non-meningitis. The non-meningitis categories included clinical manifestations of sepsis and sepsis with focal signs other than meningitis. The schedule of immunization with PCV7 was implemented according to a previous guideline [3]. The standard schedule is for infants aged 2–6 months: 3 doses as a primary series and the fourth (booster) dose at age 12–15 months. The catch-up schedules are for children aged ≥7 months: 2 doses as primary series and 1 dose as a booster for infants aged 7–11 months, 2 doses for children aged 12–23 months, and a single-dose for children aged ≥24 months. Furthermore, some of the children received more PCV7 doses than the age-appropriate schedules after treatment for IPD, if the parents or guardians agreed with additional booster doses of PCV7. Breakthrough infection was defined as IPD in a child who had received ≥1 PCV7 dose and for which the pneumococcal isolate was a PCV7 serotype, and vaccine failure was defined as the subset of breakthrough infection in which the patients had completed the vaccine schedule [3,14,15].

This study was reviewed and approved by the Ethics Committee of RIMD, Osaka University, and conducted according to the principles expressed in the Declaration of Helsinki.

2.2. ELISA

Antipneumococcal IgG antibodies were measured with the WHO-approved ELISA using standard reference sera (89-SF and 007sp) and absorptions with C-polysaccharide and 22F polysaccharide, as previously described [6,16]. The cutoff for the assay was 0.05 μg/ml for all serotypes. The levels of serotype-specific IgG for the infecting serotypes, comprising serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, were determined according to the WHO protocol (available at www.vaccine.uab.adu/ELISA protocol).

2.3. MOPA

The MOPA for the infecting serotype, based on antibiotic-resistant target bacteria, was performed as previously described [7]. The quality-control serum was prepared from pooled sera of adults vaccinated with the PPV23, and this was used in each assay. The OI was defined as the serum dilution that killed 50% bacteria, and OIs were determined using opsotiter3 software according the WHO protocol (at www.vaccine.uab.edu/UAB-MOPA). The cutoff for all serotypes was a serum dilution of 1:4, and the value below the cutoff value was represented as <1:4. The serotypes for which we determined OIs in this study were serotypes 4, 6B, 9V, 14,18C, 19F, and 23F.

2.4. Statistics

Chi-square analysis was used for comparison between children who had never received PCV7 and children who had received at least one dose of PCV7. The OI was logarithmically transformed for statistical analysis. Wilcoxon matched-pairs signed-ranks test was used to assess the increase in the levels of serotype-specific IgG and the OIs from pre to post vaccination. Chi-square analysis and Mann-Whitney U test were used to assess the differences in clinical characteristics between the responder group and the hyporesponder group. All the analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). *P*-values less than 0.05 were considered significant.

3. Results

Of 56 the children with IPD enrolled in this study, 31 had received at least one dose of PCV7 at the time of onset of IPD, while 25 children had never received PCV7. Of the 31 patients who had received at least one dose of PCV7, only 5 (9%) had received the full standard schedule of PCV7. The median age (range) in months at the onset of illness for the 56 children with IPD was 17 (3–67). Thirteen children (23%) had comorbid illnesses including hypogammaglobulinemia ($n=2$), asplenia ($n=1$), Mondini dysplasia ($n=1$), bilateral inner ear malformation with cochlea implant ($n=1$), chronic otitis media ($n=1$), pulmonary artery stenosis ($n=1$), chromosomal abnormality and craniosynostosis ($n=1$), hydrocephalus with VP shunt ($n=1$), asplenia and single ventricle ($n=1$), a deficit of the base of skull ($n=1$), chromosomal abnormality and tetralogy of Fallot ($n=1$), and deficiency of interleukin-1 receptor-associated kinase 4 ($n=1$). Only one fatality was noted: a patient with chromosomal abnormality and tetralogy of Fallot who had received one dose of PCV7 at 2 years of age.

In the 56 children with IPD, the most common infecting serotype was 6B ($n=15$), followed by 19A ($n=10$), 6C ($n=6$), 23F ($n=4$), 19F ($n=4$), 14 ($n=4$), and others ($n=13$). Twenty-eight children (50%)

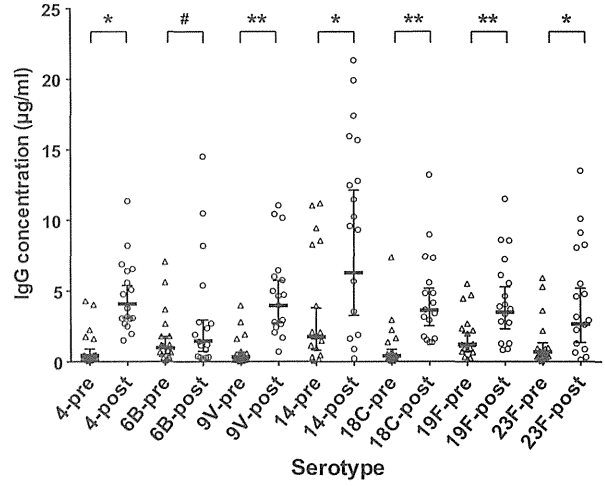

Fig. 2. Comparison of serotype-specific IgG concentrations between the time of onset of invasive pneumococcal disease (IPD) and after PCV7 vaccination in 17 children following the resolution of IPD. The IgG concentrations at the time of onset of IPD and after the last dose of PCV7 are shown as 'pre' and 'post' for each serotype. Bars indicate geometric mean concentrations (GMCs) with 95% confidence intervals. $^{*}P<0.01$ ('pre' vs. 'post'), $^{**}P<0.001$ ('pre' vs. 'post'), $^{\#}P>0.05$ ('pre' vs. 'post').

had IPD that were attributable to PCV7 serotype. Of the 28 children with IPD caused by vaccine serotypes, eight had received at least one dose of PCV7. Of these eight children, three (all with serotype 6B) were defined as vaccine failure, and five were defined as breakthrough infection (three with serotype 6B, two with 23F). The frequency (26%) of IPD caused by PCV7 serotypes was significantly lower in 31 patients who had received at least one dose of PCV7 than in the 25 children who had never received PCV7 (80%) ($P<0.001$).

To investigate the antibody responses induced in children by PCV7 vaccination following the resolution of IPD, we compared the serotype-specific IgG concentrations and the serotype-specific OIs of 17 children at IPD onset ('pre') and following IPD after the last PCV dose ('post') (Figs. 2 and 3). This group included no patients who had been given intravenous immunoglobulin for treatment of IPD or who had a comorbid condition that might influence the antibody response after PCV7 vaccination. The geometric mean concentrations (GMCs) of IgG specific for all of PCV7 serotypes other than serotype 6B were significantly higher after the last PCV7 dose following IPD than those at the onset of IPD ($P<0.01$, Fig. 2). The geometric mean titers (GMTs) of $\log_{10}$ OIs after the last PCV7 dose were significantly higher than those at the onset of IPD for all the PCV7 serotypes ($P<0.01$, Fig. 3), although the GMT of $\log_{10}$ OI for serotype 6B was lower after the last PCV7 dose than for the other serotypes.

Of the 17 children, three were infected with a nonvaccine serotype. Therefore, we were not able to determine the serotype-specific IgG concentrations and OIs for the infecting serotype for these children. The serotype-specific IgG concentrations and OIs for the infecting serotype at the onset of IPD and after the last PCV7 dose are shown for the remaining 14 children with IPD infected with a vaccine serotype (Table 1).

Of these 14 children, four had received one or three doses of PCV7 before the onset of IPD. At the onset of IPD, the OIs for the infecting serotypes were <8 for all 14 children, although the levels of serotype-specific IgG varied between 0.17 and 5.62 μg/ml. The OIs for the infecting serotypes remained <8 after the last PCV7 dose for six (43%) of the 14 patients. Therefore, the 14 children were classified into two groups: a responder group ($n=8$) and a hyporesponder group ($n=6$). Six children were hyporesponsive

Table 1
Clinical characteristics including antipneumococcal antibodies of paired serum for infecting serotype in fourteen children.

Case	Sex	Comorbid condition	Clinical category	Infecting serotype	Age (months) at IPD onset	Age (months) at which PCV7 administered		Time (days) from IPD onset to the first blood sampling	Time (days) from the last PCV7 dose to the second blood sampling	IgG concentration and OI for the infecting serotype			
										At the first blood sampling		At the second blood sampling	
						Before IPD	After IPD			IgG (μg/ml)	OI	IgG (μg/ml)	OI
1	F	None	Non-meningitis[a]	23F	11	6,7, and 8	15	0	30	0.34	<4	0.13	<4
2	M	None	Meningitis	6B	21	18	22 and 24[h]	0	46	1.81	7	1.43	<4
3	F	None	Non-meningitis[b]	6B	31	26	35[h]	0	120	1.18	<4	0.39	<4
4	M	None	Non-meningitis[c]	6B	30	28	34[h] and 45[h]	0	30	0.53	<4	0.32	<4
5	F	None	Meningitis	6B	12,13[f]	None	13,14, and 32[h]	0[g]	103	0.78	<4	2.80	<4
6	M	None	Non-meningitis[b]	6B	14	None	16,18, and 20[h]	11	32	0.22	<4	0.15	<4
7	M	None	Non-meningitis[b]	23F	12	None	13	0	27	0.36	<4	2.62	19
8	F	None	Non-meningitis[b]	6B	16	None	18 and 19	0	28	5.62	<4	2.37	562
9	F	None	Non-meningitis[b]	23F	18	None	19 and 22	0	31	0.72	<4	8.27	5491
10	M	None	Non-meningitis[b]	6B	14,17	None	31	3[g]	37	1.78	<4	1.18	17,946
11	M	None	Non-meningitis[b]	19F	35	None	36	0	28	0.68	<4	3.73	85
12	M	None	Non-meningitis[d]	14	13,16	None	16 and 18	0[g]	42	2.09	5	9.61	4040
13	F	None	Non-meningitis[b]	14	15	None	36	0	31	1.75	<4	3.55	5266
14	M	Mondini dysplasia	Meningitis[e]	9V	67	None	68	1	44	0.17	<4	2.65	491

OI, opsonization index; F, female; M, male.

[a] Septic arthritis.

[b] Bacteremia.

[c] Bacteremic pneumonia.

[d] Bacteremia with otitis media.

[e] Meningitis with otitis media.

[f] This patient had IPD at 12.0 and 13.9 months of age after the first dose of PCV7 at 13.0 months of age.

[g] Patient who had two episodes of IPD. Serum was obtained during the first episode of IPD.

[h] Additional booster dose of PCV7.

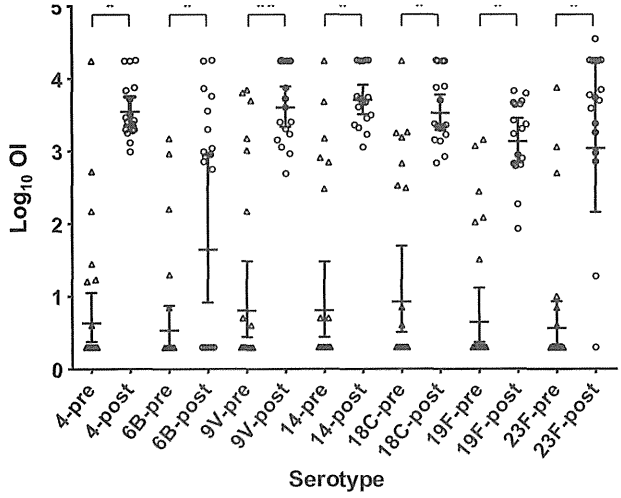

Fig. 3. Comparison of serotype-specific opsonization index (OI) between the time of onset of invasive pneumococcal disease (IPD) and after PCV7 vaccination in 17 children following the resolution of IPD. The $\log_{10}$ OI at the time of onset of IPD and after the last dose of PCV7 are shown as 'pre' and 'post' for each serotype. Bars indicate the geometric mean titers of $\log_{10}$ OIs with 95% confidence intervals. $^{*}P<0.01$ ('pre' vs. 'post'), $^{**}P<0.001$ ('pre' vs. 'post').

to serotypes 6B ($n=5$) and 23F ($n=1$). Five children (Cases 2, 3, 4, 5 and 6) remained hyporesponsive to the infecting serotype although they received one or two additional booster doses of PCV7.

To clarify whether the hyporesponsiveness was specific to the infecting serotype, the levels of serotype-specific IgG and OIs are shown for all the PCV7 serotypes at the time of IPD onset and after the last PCV7 dose in the six hyporesponsive children (Table 2). In these six children, the serotype-specific IgG levels and OIs increased for most of the noninfecting PCV7 serotypes, so the hyporesponsiveness was specific to the infecting serotype. Although we compared the clinical characteristics of two groups (responder and hyporesponder) within 14 children with IPD, no significant difference was found for the frequency of female sex ($P=0.64$), meningitis ($P=0.35$), presence of comorbid conditions ($P=0.37$), age at the onset of IPD ($P=0.70$) or at the first PCV7 dose ($P=0.15$), and days from the IPD onset to the last PCV7 dose ($P=0.25$).

4. Discussion

This study included 56 children with IPD in whom the infecting serotype was confirmed. As this study included a period of 1 year before the Japanese government started to subsidize the PCV7 (in November 2010), 25 (45%) of the children had never received PCV7 at the time of IPD onset. The significant difference found in this study in the frequency of IPD with PCV7 serotypes between children who had received at least one dose of PCV7 and PCV7-naïve children is in agreement with a recent report by Harboe et al. [17]. In 17 children who were vaccinated with PCV7 following the resolution of IPD, a significant increase was found after the last PCV7 dose in the level of IgG for all the PCV7 serotypes except serotype 6B and in the OIs for all the PCV7 serotypes.

In all 14 children who had IPD caused by PCV7 serotypes, the OIs to the infecting serotype were <8 at the time of IPD onset, although most patients had IgG levels >0.20 μg/ml (Table 2). This finding is consistent with results of our previous study [11]. Importantly, here we found that six (43%) of 14 children remained hyporesponsive

Table 2
Serotype-specific IgG concentrations and opsonization indices (OIs) of six children who were unresponsive to PCV7 for the infecting serotype.

Case	Infecting serotype	Age (months)			Serotype-specific IgG concentration (μg/ml) and OI for PCV7 serotype															
		IPD onset	PCV7 doses	Blood sampling	4		6B		9V		14		18C		19F		23F			
					IgG	OI	IgG	OI	IgG	OI	IgG	OI	IgG	OI	IgG	OI	IgG	OI	IgG	OI
1	23F	11	6,7,8,15	11	4.04	149	7.08	1504	3.97	149	11.22	701	7.37	1816	5.53	121	0.34	<4		
				16	4.77	1914	14.52	5698	5.76	1709	21.34	2842	7.32	2176	11.53	1724	0.13	<4		
2	6B	21	18,22,24	21	4.29	17496	1.81	7	2.80	6319	9.45	4785	1.66	1771	2.69	1177	2.19	10		
				25	1.96	3191	1.43	<4	4.65	17496	10.27	3136	1.76	2287	5.55	2021	5.52	945		
3	6B	31	26,35	31	1.77	522	1.18	<4	0.72	1499	11.04	17496	2.94	1533	2.22	1438	5.30	7550		
				39	3.77	2564	0.39	<4	1.68	2515	17.42	2291	3.16	2220	2.84	673	10.10	2403		
4	6B	30	28,34,45	30	0.48	28	0.53	<4	1.93	6999	3.97	813	2.23	336	0.94	32	0.53	7		
				46	2.68	5251	0.32	<4	4.73	17496	9.33	1134	4.83	2362	3.90	623	4.79	1801		
5	6B	12,13	13,14,32	12	0.28	<4	0.78	<4	0.38	<4	2.11	<4	0.27	<4	0.72	<4	0.98	<4		
				35	6.89	1979	2.80	<4	10.18	2498	12.79	4621	1.35	1688	4.04	6307	3.17	17496		
6	6B	14	16,18,20	14	0.10	<4	0.22	<4	0.23	4	0.18	<4	0.09	<4	0.36	<4	0.15	<4		
				21	5.59	2901	0.15	<4	0.71	2028	19.92	17496	1.62	688	4.64	6788	4.62	17496		

The values in the gray columns are those after the PCV7 dose.

(OI < 8) to the infecting serotype after the last PCV7 dose, although the other eight children showed variable responses to the infecting serotype based on increased OIs (OI = 19–17,946). We could not identify any clinical characteristic of the six children that was associated with their specific hyporesponsiveness to the infecting serotype after the last PCV7 dose.

A lack of a significant increase in IgG specific for serotype 6B after PCV7 vaccination in 17 children with IPD could partly be explained by the relatively weak immunogenicity of serotype 6B. Previous studies have demonstrated no marked increase in anti-6B IgG in children after one or two doses of PCV [18–20]. As the hyporesponsiveness found in this study in children vaccinated with PCV7 following the resolution of IPD was specific to the infecting serotype, nonspecific immunosuppressing factors or genetic factors of the host are unlikely to contribute to this phenomenon.

Borrow et al. reported that eight of 107 children with IPD failed to develop an IgG response to their infecting serotype [12]. For all of these children, the IgG levels for the infecting serotypes were less than 0.35 μg/ml (range: 0.01–0.34 μg/ml). The authors speculated that this phenomenon could be explained by an immune paralysis because of a large load of pneumococcal polysaccharide during the episode of IPD and/or to a potential genetic basis for hyporesponsiveness to individual serotypes. In contrast, in our study, the IgG levels for the infecting serotypes ranged from 0.13 to 2.80 μg/ml in the six children in our study who were hyporesponsive to the infecting serotype after the last PCV7 dose. Although the IgG levels exceeded 0.35 μg/ml for three of these six children, the OIs for all six were less than 8. Therefore, an OI <8, but not an IgG level <0.35 μg/ml, is a sufficient criterion to define children who are hyporesponsive to PCV7.

Recent studies reported that pneumococcal carriage in the nasopharynx of children resulted in serotype-specific hyporesponsiveness to PCV [21,22]. The hyporesponsiveness following pneumococcal carriage may be attributable to the binding of the circulating pneumococcal polysaccharides to serotype-specific B cells in the marginal zone of the spleen in infants where CD21-expressing cells are scarce [23]. Furthermore, a recent study has demonstrated that B cell receptor crosslinking with a T cell-independent type II antigen (TI-2 Ag) does not activate IgG+ memory B cells, but rather induces tolerance of these cells [24]. This may support the hypothesis of immune paralysis to the infecting serotype proposed by Borrow et al., because a pneumococcal polysaccharide is known to be a TI-2 Ag.

Hyporesponsiveness to serotype 6B after PCV7 immunization lasted for more than 1 year in two children in our study (Cases 4 and 5). Dagan et al. similarly demonstrated that hyporesponsiveness lasted for several months, and was only partially overcome by the 12-month booster [21]. Follow-up of the hyporesponders is necessary to determine whether their hyporesponsiveness can be overcome with time.

Two previous studies demonstrated that children unimmunized against polyribosylribitol phosphate-tetanus protein conjugate vaccine (PRP-T) developed a low or undetectable PRP antibody after invasive *Haemophilus influenzae* type b infection, and that additional doses of PRP-T conjugate vaccine were required to elicit a protective immune response in these children [25,26].

The limitations of our study are the small number of IPD cases examined and the variable periods between the onset of IPD and the last PCV7 dose and between the last PCV7 dose following IPD and the second blood sampling. Another limitation is that children with IPD were enrolled from 41 hospitals when their attending doctors requested the measurement of the antipneumococcal antibodies in their sera, which may have resulted in a selection bias.

In conclusion, a significant increase in the serotype-specific IgG for PCV7 serotypes, except for serotype 6B, and in the OIs for all PCV7 serotypes was found in sera from 17 children who were vaccinated with PCV7 following the resolution of IPD. Of 14 children with IPD caused by PCV7 serotypes, six were identified on the basis of the OI to be specifically hyporesponsive to the infecting serotype after PCV7 vaccination. Although the precise mechanisms of hyporesponsiveness to the infecting serotype remain uncertain, the clinician should be aware of possible hyporesponsiveness to the infecting serotype in children who were vaccinated with PCV following IPD. Because of a small number of IPD cases in the present study further studies for hyporesponsiveness to the infecting serotype after the resolution of IPD are required.

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Estimating the Risk of Parvovirus B19 Infection in Blood Donors and Pregnant Women in Japan

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Abstract

Background: Seroepidemiological study of parvovirus B19 has not taken place for some 20 years in Japan. To estimate the risk of parvovirus B19 infection in Japan among blood donors and pregnant women in this century, a seroepidemiological survey and statistical modeling of the force of infection were conducted.

Methodology/Principal Findings: The time- and age-specific seroprevalence data were suggestive of strong age-dependency in the risk of infection. Employing a piecewise constant model, the highest forces of infection of 0.05 and 0.12 per year were observed among those aged 0–4 and 5–9 years, respectively, while estimates among older individuals were less than 0.01 per year. Analyzing the antigen detection data among blood donors, the age-specific proportion positive was highest among those aged 30–39 years, agreeing with the presence of dip in seroprevalence in this age-group. Among pregnant women, up to 107 fetal deaths and 21 hydrops fetalis were estimated to have occurred annually across Japan.

Conclusions: Seroepidemiological profiles of PVB19 infection in Japan was characterized with particular emphasis on the risk of infection in blood donors and the burden of infection among pregnant women. When a vaccine becomes available in the future, a similar seroepidemiological study is expected to play a key role in planning the appropriate immunization policy.

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Introduction

Parvovirus B19 (PVB19) is one of the smallest viruses that are known to infect humans [1]. Since the virus was first reported in 1975, infection with PVB19 has been demonstrated to be associated with a variety of clinical manifestations. Among children, the most common clinical form of infection is erythema infectiosum (EI) which is also referred to as the slapped cheek syndrome or the fifth disease [2,3]. EI is a relatively mild disease with non-specific influenza-like symptoms followed by facial rash which is considered to be caused by antibody-antigen immune complex depositions. Among adults, especially among middle-aged women, PVB19 infection can lead to clinically significant arthropathy. Moreover, among patients with increased erythropoiesis, PVB19 infection can cause transient aplastic crisis. Most importantly, PVB19 infection in a pregnant woman can lead to miscarriage or hydrops fetalis. Asymptomatic infection is seen in 25–50% of infections in host without comorbidity, and the estimated risk of transplacental infection among pregnant women is as high as 30% with a five to nine percent risk of fetal loss [4]. The transmission of PVB19 occurs primarily through droplets, but it can also be transmitted through blood products. A vaccine is presently under development [5].

While several industrialized countries regularly examine the epidemiological dynamics of PVB19 infection through laboratory (e.g. serological) investigations, Japan has been probably the only country in which epidemiological surveillance of EI has been conducted at a nationwide scale [6]. The number of clinically diagnosed EI cases has been continuously notified from approximately 3,000 pediatric sentinel sites across the country on a weekly basis since 1982. Surveillance data over the past 30 years has shown that epidemics of EI involve seasonality with a single annual peak in late June or early July and also a periodicity with four to six year cycles with geographic variations.

Published studies in other countries have indicated that seroprevalence of anti-PVB19 IgG increases with age: 2–15% among children below five years old, 15-60% among those aged 5–19 years and over 60% among adults [4,7–11]. Those studies have also indicated that the circulation of PVB19 among children poses risks to adult groups, particularly among those aged 40 years and younger [12]. Since the potential risk of infection among blood donors and pregnant women represent two distinct social

concerns over infection with PVB19, published seroepidemiological studies on PVB19 have estimated the risk among blood donors [4,11] and investigated the burden of infection including fetal outcomes among pregnant women using seroepidemiological datasets and mathematical modeling techniques [7,8,12]. A modeling study in Europe has estimated the risk of PVB19 infection during pregnancy at 0.61% in Belgium, 0.69% in England and Wales, 1.24% in Finland, 0.92% in Italy, and 1.58% in Poland [12].

While seroepidemiological studies of PVB19 have also taken place in Japan [13,14], they were conducted during 1970s-90s without an update for some 20 years, and moreover, epidemiological attempts have yet to explicitly estimate the risk of infection in blood donors and quantify the burden of infection among pregnant women. Using statistical modeling techniques, the present study aims to characterize the seroepidemiology of PVB19 infection in Japan, validating the prevalence estimate by looking into the antigen data among blood donors and estimating the risk of infection among pregnant women and fetal outcomes.

Materials and Methods

Seroepidemiological survey

The serum samples in the present study were derived from the National Epidemiological Surveillance of Vaccine-Preventable Diseases (NESVPD) [15] through which population-based seroepidemiological profiles have been regularly characterized for eight selected vaccine-preventable infectious diseases in Japan. This survey has taken place annually, collecting serum from at least 5,400 randomly sampled individuals across all age-groups. Participants are invited from randomly selected healthy individuals from whom survey officers were able to obtain informed consent. Such solicitation has taken place, for example, among local government officials and their families during routine health check-ups including those conducted among school children. There were no left-over samples of patients from hospitals and blood samples were obtained specifically for the purpose of this routine seroprevalence survey. Although PVB19 is not included in the selected eight vaccine-preventable diseases, we investigated a part of anonymized serum samples derived from two neighboring prefectures, Fukuoka and Saga, from 2004 to 2007, from the National Serum Reference Bank/Tokyo, National Institute of Infectious Disease, Japan, which stored the serum remnants of NESVPD.

To appropriately use the existing number of serum samples, we performed sample size calculations to determine the required number of samples. In advance of analyzing the serum samples, we examined a published seroprevalence study result in 1993 which had been conducted in three prefectures in Japan, including Fukuoka [14]. Assuming age-independence in the risk of infection with PVB19, the force of infection, λ, i.e., the rate at which susceptible individuals are infected, was estimated at 0.028 per year in 1993. Moreover, based on the census data in 2008, the average age of mothers for all births was estimated at 30.9 years old. Combining these two, it was implied that $1\text{-}\exp(-30.9\lambda) = 58.3\%$ of mothers are already immune by the age of 30.9 years, suggesting that 94 samples would be required to detect seroprevalence $\pm 10\%$ within a 95% confidence interval (CI). Considering differential fraction of immune individuals in other age-groups, we decided to examine 100 samples for each group, equally for 10 different age groups (0–4, 5–9, 10–14, 15–19, 20–25, 26–29, 30–35, 35–39, 40–49, and over 49 years). In total, 1000 samples from 2004-07 were investigated with a fixed male-female ratio at 1:1 for each age-group and equal frequency for year of observation. To ensure data accuracy, avoiding under- or over-dilution, IgG antibody titer to PVB19 were examined in duplicate by enzyme immunoassay (EIA) using a commercial kit (Denka Seiken, Tokyo, Japan) according to the manufacturer's instructions. The ratio of the optical density for test specimen (average of two results for each specimen) to that of the control, hereafter referred to as the IgG antibody index, was calculated. If the antibody index was equal to or greater than 1.00, the test result was interpreted as positive. Samples that showed equivocal results at initial testing were retested. The seroepidemiological data in this study are available upon request from the corresponding author for noncommercial use.

Statistical analysis and modeling

First, we examined the demographic characteristics of the obtained serum samples. In addition to gender- and age-specificities, we also investigated the presence of time-dependency during the sampling period from 2004-07. Since samples from different ages and years were taken from different individuals, we employed Welch analysis of variance (Welch ANOVA) and χ^2 test. The former test followed the test of normality (i.e., F test).

Second, the force of infection (i.e., the hazard rate of infection) was estimated in four different ways, i.e., (i) assuming time- and age-independence (i.e. a constant force of infection model), (ii) assuming age-dependence and employing a parametric model, (iii) assuming age-dependence and using a non-parametric model, and (iv) employing a time- and age-dependent model. The first three models used only datasets from 2004-07, but we additionally analyzed the 1993 data for model (iv) [14]. For now, we write the most explicit model with time- and age-dependence, because others are special cases of this type of force of infection model. Let $s(a,t)$ be the proportion of susceptible individuals at age a and time t, the time- and age-dependent force of infection, $\lambda(a,t)$, governs the dynamics as follows:

$$\left(\frac{\partial}{\partial a}+\frac{\partial}{\partial t}\right)s(a,t) = -\lambda(a,t)s(a,t), \tag{1}$$

with a boundary condition $s(0,t) = 1$ for any t (i.e. for simplicity, we ignore maternal antibody effect for the first six months of life). Integrating equation (1) along the characteristic line, we get

$$s(a,t) = \exp\left(-\int_0^a \lambda(s,t-a+s)ds\right). \tag{2}$$

In the case of model (i), λ is a constant, and thus, the seroprevalence data are expected to be described by the equation $1\text{-}s(a) = 1\text{-}\exp(-\lambda a)$ at age a for cross sectional data. For models (ii) and (iii), only the time-element is dropped from (1) and the expected proportion of seropositive individuals, $i(a)$, at age a is

$$i(a) = 1-\exp\left(-\int_0^a \lambda(s)ds\right). \tag{3}$$

In the case of model (ii), we employed the well-known gamma-type parametric model as already proposed elsewhere [16]. For a non-parametric model (iii), we used a piecewise constant model with five unknown parameters, measuring forces of infection among those aged from 0–4, 5–9, 10–14, 15–39 years, and 40

years and older, following the discrete age-interval in a published study [7] and additionally separating adults into two groups by the common childbearing age of mothers (i.e. those aged 39 years and younger account for more than 96% of all births in Japan). For model (iv), we assumed for mathematical convenience that time- and age-elements are separable, i.e., $\lambda(a,t) = \lambda_a(a)\lambda_t(t)$, and employed exactly the same piecewise constant model for the age-dependent part as was assumed in model (iii). The time-specific forcing, $\lambda_t(t)$, was also dealt with as a piecewise constant model with five unknown parameters, i.e. 1972 and earlier, 1973–82, 1983–92, 1993–2002, and 2003 and later. Maximum likelihood estimates of parameters were obtained by minimizing the negative log-likelihood that rested on binomial deviance as described elsewhere [16,17]. The 95% CI was derived from the profile likelihood. Goodness-of-fit of models (i) – (iii) were compared with each other using the Akaike's Information Criterion (AIC) [18]. The last model (iv) used additional seroprevalence data in 1993, and moreover, the estimated time-dependent forcing was overlaid with the notification data of EI from the abovementioned two prefectures based on sentinel surveillance from 1982.

As a measure of transmissibility, the basic reproduction number, R_0, was computed. When using the constant force of infection in model (i), we employed a homogeneous mixing assumption and assumed that the average life expectancy at birth is $L = 80$ years with a rectangular shape survivorship, so that we have $R_0 = \lambda L$. We also used the age-dependent force of infection from model (iii) to estimate R_0 employing the following estimator derived by Farrington et al. [19]:

$$R_0 = \frac{\int_0^\infty l(a)\lambda(a)\exp\left(-\int_0^a \mu(x)dx\right)da}{\int_0^\infty l(y)\lambda(y)\exp\left(-\int_0^y \lambda(x)+\mu(x)dx\right)dy}, \quad (4)$$

where $l(a)$ is the leading left eigenfunction of age-dependent transmission rate. $\mu(a)$ is the age-specific mortality rate, and for consistency, we again employed the rectangular shape survivorship. Since the piecewise constant model is discrete, we derived the following discrete version of the estimate of R_0:

$$R_0 = \frac{\sum_i l_i\lambda_i(a_i - a_{i-1})}{\sum_i l_i \exp\left[-\sum_{j=1}^{i-1}\lambda_j(a-a_{j-1})\right][\exp(-\lambda_i a_{i-1}) - \exp(-\lambda_i a_i)]} \quad (5)$$

where a_i represents the upper age bound of age-group i. The left eigenfunction, l_i of the contact matrix was derived from published survey data in the United Kingdom [20] with an adjustment of age-specific population size to Japanese data, assuming that age-specific contact pattern in Japan is the same as that in the United Kingdom [21,22]. Because it is difficult to estimate the sampling distribution of R_0, the 95% percentile confidence intervals were obtained by employing a bootstrapping method [23].

Risk estimation in blood donors and pregnant women

Using the age-specific proportion of antigen positives among blood donors, we calculated the age-specific risk of PVB19 infection, directly from empirical data. The dataset of antigen testing results was available from 2001 to 2007, and during this period, a receptor-mediated hemagglutination (RHA) assay was used for screening. Age distribution of blood donors was extracted from the latest available statistics of the Japanese Red Cross Society [24]. Assuming that the risk of positive blood sample is binomially distributed, we obtained the 95% CI of prevalence. We also obtained data on the age-specific proportion of IgM antibody positive individuals against PVB19 from February 2008 to January 2009, as a marker of recent PVB19 infection. A total of randomly selected 651 blood donors were tested for IgM using EIA at the Japanese Red Cross Osaka Blood Center.

Subsequently, we estimated the burden of PVB19 infection among females at childbearing age including the estimated number of infections during pregnancy in Japan. For the calculation, we followed published studies in the United Kingdom [7,8], adopting a random sampling assumption of pregnant women from all women in an identical age-group. Since all pregnancy events have not been stratified by the age of pregnant women in the Japanese census record, we used the reported number of birth events as an approximate of all pregnancy events. We used the confidence intervals of the age-specific force of infection based on a piecewise constant model (iii) and adopted the published risk estimates of fetal death and hydrops fetalis due to maternal infection during the first 20 weeks of pregnancy for the former and from weeks 9 to 20 for the latter at 9.0% and 2.9%, respectively [25], as already practiced elsewhere [8]. We did not use estimates of the force of infection from the time- and age-dependent model (iv) for abovementioned calculations because the 95% CIs of age-dependent element were only partially calculable due to limited sample size. All statistical data were analyzed using the statistical software JMP ver. 9.0.0 (SAS Institute Inc., Cary, NC). The study protocol was reviewed and approved by the Institutional Review Board at the Toho University School of Medicine.

Results

Descriptive seroepidemiology

Of 1,000 serum samples tested in duplicates, eight samples yielded equivocal results, and thus, the eight were re-tested in duplicates, allowing six results to yield agreed result (and only two remained to be equivocal with the antibody index ranging from 0.8–1.0). Figure 1A shows the distribution of the antibody index. Taking a logarithmic scale for the antibody index axis, two distinct peaks were identified. As suggested for the interpretation of the testing result, the bimodal distribution was confirmed to be clearly separated at the cut-off value of 1.00. In total, 543 individuals tested positive (54.3%). Figure 1B shows the age-specific proportion seropositive by sampling year. Mean age (and the standard deviation) of seropositive individuals was 28.1 (14.8) years, while seronegative individuals were significantly younger with mean age of 22.4 (17.1) years ($p<0.01$; Welch ANOVA). As indicated by overlaps of seropositive fraction for multiple times in Figure 1B (i.e. multiple crossing points between two different survival curves), no significant difference was identified by the year of sampling, and thus, the subsequent analysis used the aggregated data for all 4 years to quantify the transmission dynamics. There was no significant gender specificity in seroprevalence ($p = 0.48$; χ^2 test).

Force of infection and age-specific seroprevalence

Assuming an age-independent risk of infection, the force of infection is estimated at 0.031 per year (95% CI: 0.029, 0.034) which was not significantly deviated from the estimate from 1993 data (the 95% CI ranged from 0.025 to 0.031 per year). Assuming a rectangular age distribution, the basic reproduction number, R_0, based on the constant force of infection model, was estimated as 2.51 (95% CI: 2.30, 2.74). Moreover, the constant force of infection model indicated that the average age at infection is 1/

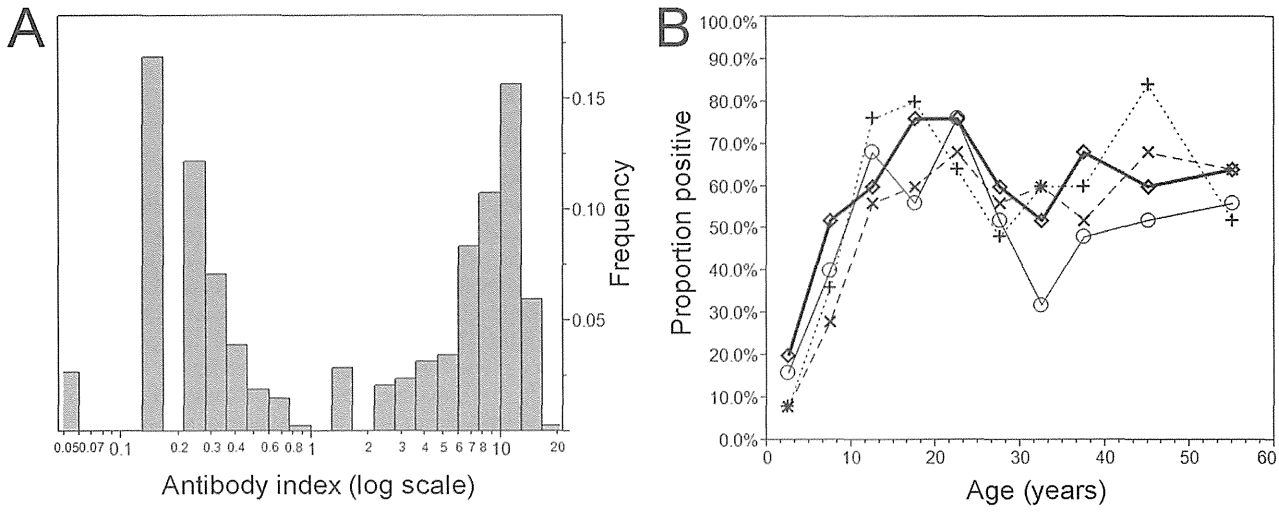

Figure 1. Distribution of the IgG antibody index and the time- and age-dependent proportion seropositive against parvovirus B19 in Japan from 2004-07. A. The distribution of IgG antibody index against parvovirus B19. The antibody index was calculated as the ratio of the optical density of test sample to that of control. Using a logarithmic scale for the horizontal axis, a bimodal shape is clearly identified. B. The time- and age-dependent proportion of seropositive against parvovirus B19. The collection of serum samples took place in 2004 (unfilled circles with thin solid line), 2005 (crosses with dotted line), 2006 (diamonds with thick solid line) and 2007 (x marks with dashed line), respectively.
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$\lambda = 31.8$ years old (given the absence of age-dependence in the risk of infection).

However, the goodness-of-fit for two other models were better than that of the constant force of infection model (Figure 2A). Employing the age-dependent force of infection either by the parametric or the non-parametric model, all expected values were included within the 95% CI of the observed proportion of seropositive. AIC values for models (i), (ii) and (iii) were 203, 60.1 and 60.3, respectively, indicating that models (ii) and (iii) were almost equally good and much better than the model (i) in describing the observed pattern of the data. Figure 2B compares the estimated force of infection by three different models. In the case of the age-dependent parametric model (ii), the peak of infection was expected to occur at around the age of 5 years, while the piecewise constant model (model (iii)) predicted that the highest force of infection 0.12 (95% CI: 0.00, 0.21) per year was seen among those aged 5–9 years and the second highest force of infection 0.05 (95% CI: 0.02, 0.10) among those aged 0–4 years. For model (iii), upper bounds of the force of infection among those aged 15–39 years and 40 years and older were 0.0059 and 0.0246 per year, respectively. The R_0 using the age-dependent piecewise constant model was estimated to be 2.07 (95% CI: 1.33, 2.98), the expected value of which was smaller than the estimate based on the constant force of infection model, but the difference was not significant.

Figure 2C compares the observed age-specific proportions of seropositive between 1993 and 2004-07, both of which were used to parameterize the time- and age-dependent model (iv). Although no significant difference can be identified in the constant force of infection between 1993 and from 2004-07, we specifically employed model (iv) to describe the time-dependent shift in a dip in seropositive proportion from those aged 20-29 years in 1993 to 30–39 years from 2004-07. Comparing seroprevalence by birth year (Figure 2D), it can be seen that the dip in seroprevalence is observed in a birth cohort born from 1965-74. The relative frequency of age-dependent forces was similar to the age-only model (iii) in Figure 2B. The age-element of model (iv) was compared against the observed seroprevalence data from 2004-07 (Figure 2E) and the time-dependent element was compared against the notification data in Figure 2F. The average over the time-interval was only crudely captured (i.e. as was indicated by estimates, there was no dramatic time-dependent trend in the risk of infection), and a sharp peak in 1987 was perhaps smoothed out by adjacent years and was not reflected in the estimated force of infection from 1983-92. Although the time-element was thus not strongly aligned with the notification data, the predicted data allowed us to realize the dip in seroprevalence among those aged in their 30s (Figure 2E).

Blood donors

Assuming a random sampling assumption of blood donors from the entire population, the age-specific proportion of antigen positive was calculated among blood donors as a possible direct measurement of the risk of infection. From 2001-07, a total of 38 million persons donated blood, among which 2,806 tested positive for antigen (prevalence: 7.4 (95% CI: 7.1, 7.7) per 100,000 donors). Figure 3A shows the age-specific proportion of antigen positive. Those aged 30–39 years yielded the highest prevalence and those aged 40–49 yielded the second highest estimate. Compared to those aged 20–29 years, the prevalence among those aged 30–39 and 40–49 years were 2.6 and 2.0 times higher, respectively. Figure 3B shows the age-specific proportion of IgM antibody positive. Of 651 samples, 8 tested positive (1.2% (95% CI: 0.4, 2.1)), and thus, the uncertainty was large, but the proportion positive declined almost monotonically with age.

Pregnant women

Table 1 shows the estimated age-specific numbers of infection among women at child-bearing age and during pregnancy, along with rough corresponding estimates of fetal deaths and hydrops fetalis. Although the precision is limited in the estimates, up to 2374 infections are estimated to occur annually among pregnant women in Japan. Assuming that the risk of fetal death following PVB19 infection during the first 20 weeks of pregnancy is 9.0%, up to 107 fetal deaths are anticipated per year. Similarly, adopting

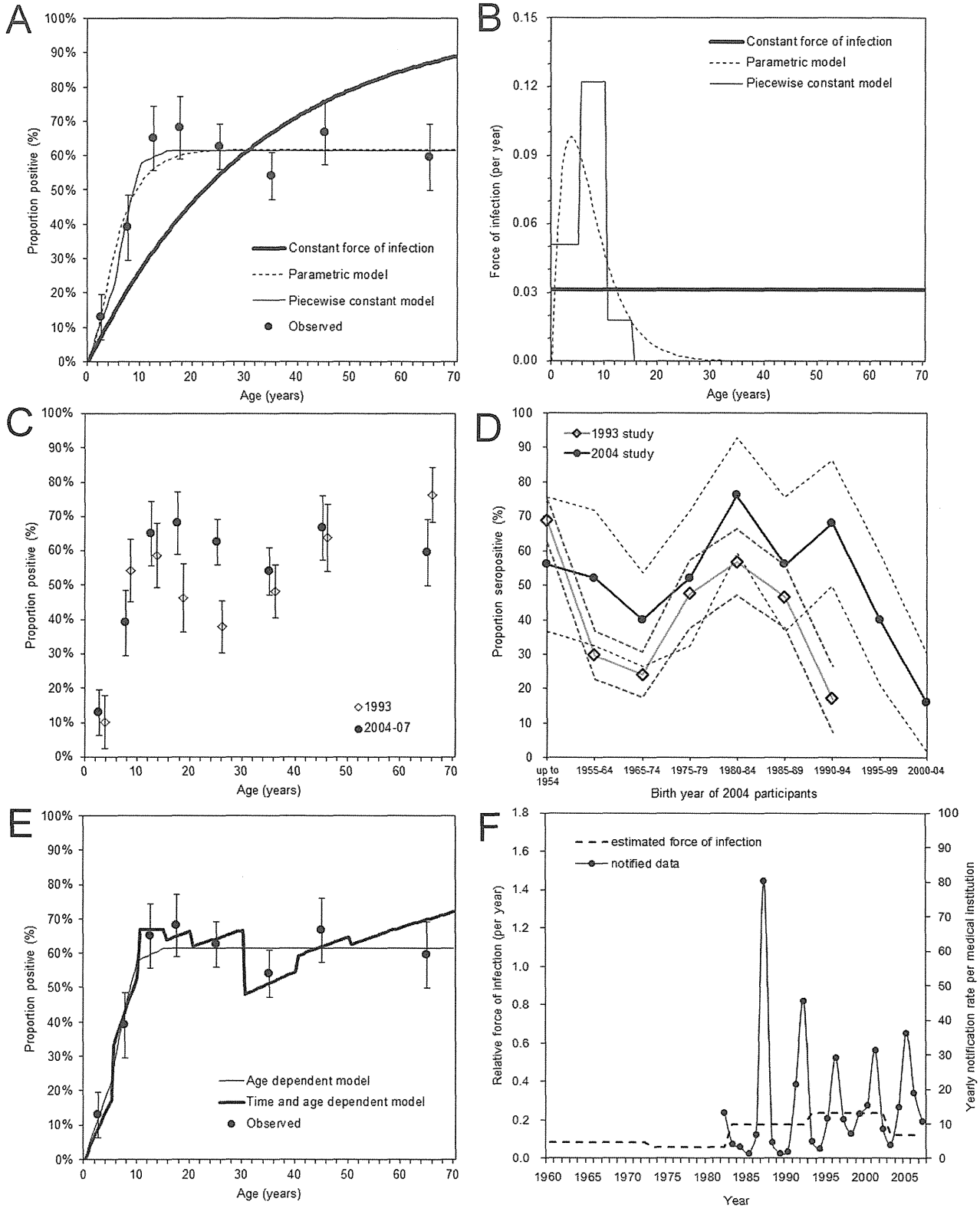

Figure 2. Seroepidemiological investigation of parvovirus B19 infection in Japan. A. Comparison between observed and predicted proportion of seropositive from 2004–07 without accounting for time-dependency. The mean of observed data are shown as filled circles with the 95% confidence intervals by whiskers. Thick solid line represents the prediction based on a constant force of infection model, dashed line a gamma-type age-dependent model, and thin solid line a piecewise constant age-dependent model. B. Estimated force of infection with parvovirus B19 in Japan. The differential styles of lines correspond to those in panel A. C. Comparison of observed seroprevalence data between 1993 and 2004–07.

Filled circles represent the observed data from 2004–07, while unfilled diamonds represent the data from 1993. Whiskers extend to both ends of the 95% confidence intervals. D. Comparison of seroprevalence data between 1993 and 2004 by birth year. Dashed lines represent the 95% confidence intervals. The horizontal line shows the birth year of 2004 participants, while the birth year of 1993 participants is calculated as the birth year minus 1 (i.e. those born from 1955–64 for 2004 participants are compared against those born from 1954–63 for 1993 participants). E. Comparison of predicted data from 2004–07 using age-dependent model and age- and time-dependent model. Filled circles represent the observed data, and whiskers extend to both ends of the 95% confidence intervals. Thick line shows the prediction based on time- and age-dependent assumption. F. The comparison between the time-dependent element of the force of infection (dashed line; left vertical axis) and the annual notification rate of erythema infectiosum from sentinel medical institutions in Fukuoka and Saga prefectures, Japan, from 1982 onwards (circles and solid line; right vertical axis).
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2.9% as the risk of hydrops fetalis among pregnant women at weeks 9–20, up to 21 cases are estimated annually.

Discussion

The present study investigated the seroepidemiological profiles of parvovirus B19 infection in Japan. Whereas various clinical studies had taken place in Japan in advance of the present study including those focused on pregnant women [26,27], to the best of our knowledge, the present study is the first to explicitly estimate the frequency of infection in blood donors and the burden of infection among pregnant women in this country. The estimated measure of the transmissibility, R_0, was 1.3–3.0, which did not deviate from an earlier estimate ranging from 2.6–3.5 based on seroprevalence survey in the Netherlands [4]. Across Japan, it was estimated that up to 2374 infections could have occurred during pregnancy, although the uncertainty bound was wide, ranging from 0 to 2374 (as was also the case in the UK study [8]). Similar estimates of the force of infection between Europe and Japan indicate that the level of endemicity for PVB19 (i.e. frequency of infection) and the contact pattern are likely similar to each other.

Two specific lessons should be learnt from our exercise. First, a strong age-dependency in the force of infection was observed, while no obvious indication of time-dependent change (e.g. declining trend) in the proportion of seropositive was seen from 1993 to 2007. The highest frequency of infection was seen among those aged below 10 years, and thereafter both parametric and non-parametric models agreed that the annual risk of infection was at most 1%. That is, the transmission dynamics of PVB19 is likely regulated by and maintained among children, yielding very important implications for future control planning including age-dependent vaccination strategy. In fact, the risk of infection among pregnant women is known to be higher in households with small children than those without [10,12] (which could partly explain the observed peak among those in their 30s in Figure 3A), and thus, within household contact between pregnant women and children could play a key role in determining the optimality of controlling the transmission by targeting children [28]. The uncertainty in the age-dependent contact patterns could be a plausible explanation for observing differential risk of infection among pregnant women across European countries [12]. In the future, the age-dependency should be closely monitored even in the absence of vaccination, because a shift (e.g. delay) in the age at infection can vary (e.g. increase) the number of infections among pregnant women, as was observed for rubella under a partial vaccination [29,30].

Second, in addition to age-dependent estimates of the force of infection that could measure the incidence of infection among blood donors (Figure 2B), we also analyzed the antigen screening results among blood donors (Figure 3A). Age-specific proportion of antigen positive yielded the peak among those aged 30–39 years followed by 40–49 years. A similar age-specific antigen pattern was previously reported from the Netherlands [31], but no explicit reason has been clarified for this observation. The peak in prevalence among those in their 30s agreed well with the dip in age-specific seroprevalence, possibly reflecting the absence of

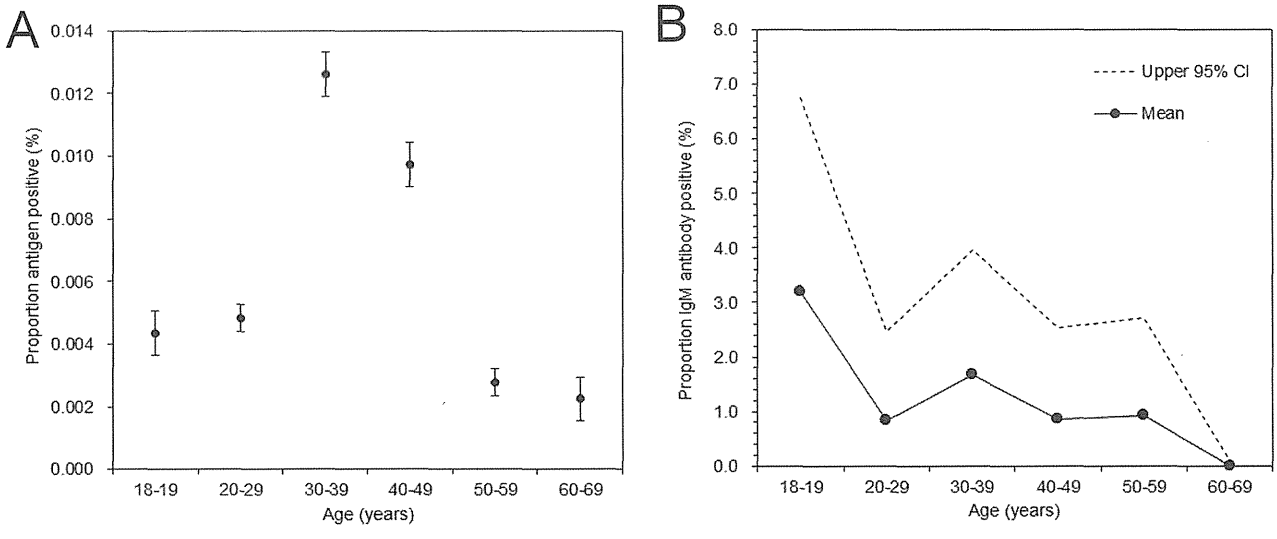

Figure 3. Age-specific proportions positive for antigen and IgM antibody against parvovirus B19 among blood donors from 2001–2007 in Japan. A. Observed proportion of antigen positive as a function of age. Filled circles represent the mean, and whiskers extend to both ends of the 95% confidence intervals. A receptor-mediated hemagglutination (RHA) assay was used. B. Observed proportion of IgM antibody positive. Solid line and filled circles represent the mean, with the upper 95% confidence interval given by dashed line. Lower bound is identical to the horizontal axis.
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Table 1. Estimated age-specific annual numbers of parvovirus B19 infections and associated complications among women at child-bearing age in Japan.

Age group (years)	No. females	No. maternities	Total infections	Infections during pregnancy	Fetal deaths	Hydrops fetalis
15–19	2,958,000	13,273	156 (0–6,585)	1 (0–30)	0 (0–1)	0 (0–1)
20–24	3,116,000	104,053	164 (0–6,736)	5 (0–225)	0 (0–10)	0 (0–2)
25–29	3,546,000	300,350	187 (0–7,445)	16 (0–631)	1 (0–28)	0 (0–5)
30–34	3,987,000	373,452	210 (0–8,129)	20 (0–761)	1 (0–34)	0 (0–7)
35–39	4,785,000	221,245	251 (0–9,475)	12 (0–438)	1 (0–20)	0 (0–4)
40–44	4,609,000	37,435	8 (0–35,597)	0 (0–289)	0 (0–13)	0 (0–3)
Total	23,001,000	1,049,808	976 (0–73,967)	54 (0–2,374)	3 (0–107)	0 (0–21)

Maximum likelihood estimates with the 95% confidence intervals (inside parentheses) are shown for the right four columns. The 95% confidence intervals for the estimated force of infection based on a piecewise constant model were used: i.e., 0–0.0059 for those aged 15–39 years and 0–0.0246 for those aged 40 years and older. Risks of fetal death from gestational week 1–20 and hydrops fetalis from week 9–20 were assumed to be 9.0% and 2.9%, respectively [8,25].
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major epidemics during childhood among those born during 1965-74 and substantial number of transmission events from children to parents. Nevertheless, the antigen positive estimates among those in their 30s and 40s were more than double of those in their 20s (while the fraction susceptible, based on seroprevalence survey, was not as different as the proportion of antigen positive).Moreover, IgM antibody data yielded an approximately monotonic decline in the age-specific proportion positive. These findings were not fully consistent with anticipating substantial child-to-parent transmissions in explaining the observed pattern, and other reasons might also explain observed phenomena (e.g. other factors, including age-dependent biological reaction to the virus and possible sampling effect (i.e. blood donors may not have well represented the general population), might also explain the observed pattern).

Four limitations should be noted. First, our subjects were limited to the population in Fukuoka and Saga prefectures, both of which are located on Kyushu Island, the western part of Japan. Our estimates involve a limitation in the representativeness of the finding and may not fully reflect that of entire Japan. Nevertheless, rather than ensuring the representativeness, the present study focused on two prefectures as the first attempt to explicitly characterize infection risks while allowing comparability between 1993 data and the result from 2004-07. Second, the estimated risk of infection among blood donors (Figure 3A) and pregnant women (Table 1) rested on an assumption that they were randomly sampled from the population irrespective of infection status. However, as briefly noted above, household structure (e.g. if a pregnant woman has any children) and other risk factors are known to influence the risk of infection with PVB19 during pregnancy. A more precise estimation would require us to account for known epidemiological risk factors using a more sophisticated statistical model. Third, we have not considered the seasonality and periodicity in the model; however, the sample size was limited and the fluctuation is known to be smoothed out over a long time yielding only marginal impact on the estimate of the transmissibility [32]. Lastly, small errors in antigenic testing results, e.g. non-specific false positive results and involvement of repeaters of blood donation due to a long viremic period without symptoms, cannot be avoided in the empirical observation.

Despite these limitations, the present study characterized seroepidemiological profiles of PVB19 infection in Japan, with particular emphasis on the risk of infection in blood donors and the burden of infection among pregnant women. When a vaccine becomes available in the future, a similar seroepidemiological study is expected to play a key role in determining appropriate immunization policy. We believe that the present study contributed to clarifying the key element of the epidemiology of PVB19 in Japan.

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Author Contributions

Conceived and designed the experiments: KN HS HN KT NO KO KM TH. Performed the experiments: KN HS KT. Analyzed the data: KN HS HN. Contributed reagents/materials/analysis tools: KN HS KT. Wrote the paper: KN HS HN KT NO KO KM TH.

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臨床研究・症例報告

麻疹・風疹混合（MR）ワクチン1期および2期接種の全国累積接種率調査

—2013年の調査結果—

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要旨

麻疹ワクチンを2回接種し，それぞれの接種率を高率に維持できれば，麻疹の流行を阻止できることは，すでに知られている。日本でも2006年度から麻疹・風疹2種混合ワクチン（MR）を1歳代（1期）と就学前（2期）の2回接種する方式が導入された。今回の調査で，MR1期の累積接種率は，生後24カ月までに97.5%に達しており，累積接種率曲線の立ち上がりもこれまでよりも早くなっていた。一方，2007年に調査した2006年度におけるMR2期の最終全国累積接種率は80.3%に過ぎず，その後徐々に上昇してはいるが，2013年調査によるMR2期の最終累積接種率は93.5%であった。MR2期累積接種率のさらなる向上が求められる。

[小児科臨床 68：391，2015]

KEY WORDS 無作為抽出，月齢別累積接種率，旬日別累積接種率，MR1期，MR2期

目的

麻疹は典型的なウイルス性急性熱性疾患であり，しばしば中耳炎，肺炎，脳炎などを合併する。麻疹ウイスルは伝播力が強いため，麻疹ワクチン導入以前には典型的な子どもの病気とされていた[1]。しかし，麻疹ワクチンを効率的に使用することによって，麻疹の流行を阻止できることは，すでに米国で実証されている[2]。日本でも2006年度から麻疹・風疹2種混合ワクチン（MR）を1歳代（1期）と就学前（2期）の2回接種する方式が導入された[3]。MR1期の麻疹ワクチン累積接種率は，すでに生後24カ月までに95%に達しているが[4]，2007年に調査した2006年度におけるMR2期の最終全国累積接種率は80.3%に過ぎなかった[5]。その後，最終累積接種率は徐々に上昇し，2012年調査による2011年度の最終累積接種率は93.5%まで改善していたが[6]，

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いまだに95%には達していなかった。その後のMR累積接種率の動向を把握するために，2009～2012年と同様に，2歳児を対象としてMR1期，また6歳児を対象として，MR2期の累積接種率調査を実施した。

方　法

2012年の全国MR1期および2期累積接種率調査は，すでに述べた方法により[7]，2012年4月1日までに満2歳に達した小児および2011年4月1日までに満6歳に達した小児をそれぞれ5,000人無作為抽出し（標本），抽出された2歳児および6歳児が居住する市区町村に，調査協力依頼書，調査票，調査手順書を郵送し，当該市区町村の予防接種担当者に，MR1期調査では，標本として選出された小児がMR1期接種を受けた月齢を，MR2期調査では標本の小児がMR2期接種を受けた年月日の調査を依頼し，回収された調査票を基に，2歳児では月齢別にMR1期被接種者数を集計して月齢別累積接種率を，6歳児では，MR2期被接種者数を各月の上，中，下旬ごとに集計して旬日別累積接種率を算定した。算定に際しては，2007～2011年調査と同様に[5)～8)]，集計対象をワクチン接種済みで接種日が明らかな標本と未接種標本のみとし，ワクチン接種は済んでいるものの，接種日不明の標本は除外した。また，2011年調査時と同様に，東日本大震災により大きな被害を受けた岩手県，宮城県，福島県，栃木県，茨城県の全市区町村，および青森県と千葉県の一部市町村を調査対象から除外した。

結　果

1．回収率

MR1期：2013年1月7日現在で，調査対象1,058カ所の自治体のうち927カ所から回答が寄せられたので，市区町村数から算出した回収率は87.6%となった。また，無作為抽出した2歳児は5,000名（標本数）おり，うち4,504名分の記録が返送されたので，標本数から算出した回収率は90.1%となった。

MR2期：2012年9月27日現在で，調査対象1,083カ所の市区町村のうち945カ所から回答が寄せられたので，市区町村数から算出した回収率は87.3%となった。また，無作為抽出された6歳児5,000名（標本数）のうち4,516名分の記録が返送されたので，標本数から算出した回収率は90.3%となった。

2．MR1期の被接種者数および月齢別累積接種率

MR1期に関する記入がないものが103件あり，MR接種日不明が134件あったので，これらを除外し，MR1期接種済みの4,163件と未接種の104件の記録を集計した。集計対象とした記録だけの回収率は85.3%であった。

MR1期の接種件数（＝被接種者数）は生後12カ月が最多の2,132件で，生後13カ月が666件，生後14カ月が390件と続いていた。全接種者件数に占める割合は，生後12カ月が約51%，13カ月が約16%，14カ月が約9%であった。MR1期接種件数は生後12カ月以降漸減した。

MR1期の累積接種率は，生後18カ月で91.4%［95%信頼区間（Confidence Interval）：CI：90.6%～92.3%］に達し，生後24カ月では97.5%（95% CI：97.0%～98.0%）であった。MR1期の累積接種率曲線は，生後12カ月から急速に立ち上がり生後24カ月まで放物線を描いて上昇していた（**図1左**）。

3．MR1期2009年，2011年，2013年調査結果の比較

2007年の調査時には単抗原麻疹ワクチンと単抗原風疹ワクチンを個別に接種した小児が多かったため，2013年調査のMRワクチン1期の累積接種率を2009年，2011年調査時と比較した。2011年，2012年には累積接種率が50%を超えたのは生後13カ月，70%を超えたのは生後15カ月，80%を超えたのは生後16～17カ月であったが，2013年は生後12カ月に50%を，14カ月で70%，15カ月で80%を超えており，2009年，2011年よりも累積接種率曲線が全体的に早く立ち上がっていた（**図1右**）。

4．MR2期旬日・月別被接種者数

MR2期に関する記入がないものが107件あり，

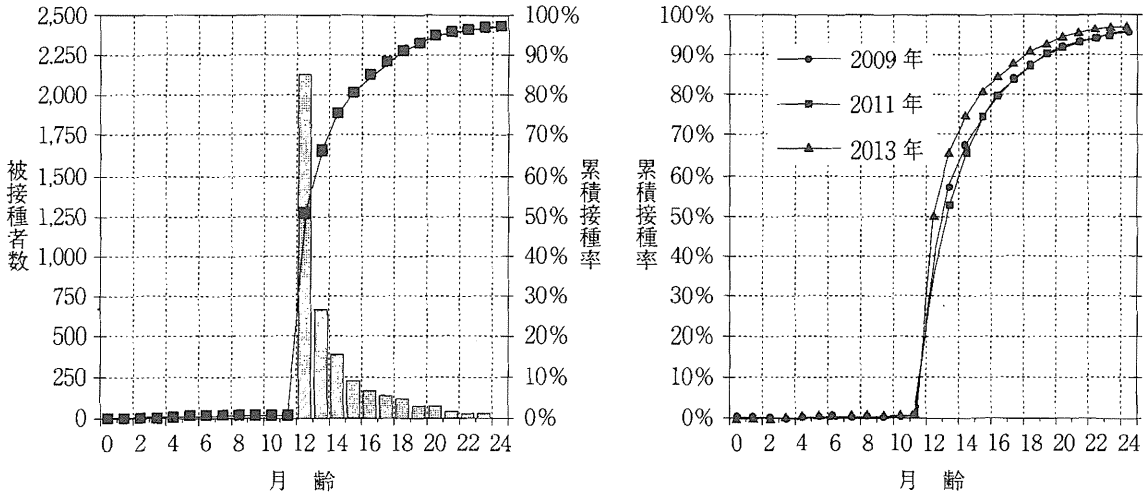

図1　全国麻疹・風疹ワクチン（MR）1期の月齢別被接種者数（縦棒）と累積接種率（黒四角）：2013年の調査結果（左）と2009年，2011年，2013年調査のMR1期累積接種率曲線の比較（右）

MR接種日不明が108件あったので，これらを除外し，MR2期接種済みの4,084件と未接種の217件の記録を集計した。集計対象とした記録だけの回収率は85.3%であった。なお，MRでなく麻疹ワクチンによる2期接種を受けたとの回答が4件あったが，これらはMR未接種として集計した。

2011年4月1日以前にMRの接種を受けたとの回答は0件であった。接種件数は4月上旬には176件あり，5月上旬までは106～193件で経過し，5月中旬から6月中旬までは200～230件，6月下旬から9月上旬までは，7月中旬と8月中旬を除いて109～172件と100件以上の旬日が続いた。9月中旬から10月下旬までは，10月中旬を除いて，72件～92件に減少し，11月上旬からさらに被接種者数が減少し，11月上旬から2013年1月上旬までは29～46件と低い水準で経過した。その後，2013年1月中旬から2月下旬まではやや増加して52～75件であったが，3月上旬から100件以上になり，3月下旬には305件に急増した（**図2**）。なお，2013年4月中の被接種件数は0件であった。

5．MR2期全国累積接種率

MR2期の累積接種率曲線は2012年4月上旬から立ち上がり，10月下旬まではほぼ同じ割合で上昇を続け，11月上旬から2012年1月上旬にかけては上昇が鈍くなった。1月中旬から上昇の度合いがやや改善し，3月下旬に急上昇したが，2012年4月にはほとんど上昇がみられなくなった（**図2**）。2012年6月下旬での累積接種率は37.5%（95% CI：36.0～39.0%），8月下旬では54.6%（95% CI：53.1～56.1%），68.4%（95% CI：67.0～69.8%），12月下旬では74.3%（95% CI：73.0～75.6%），2013年3月中旬では87.9%（CI：86.9～88.8%），3月末日までではMR2期被接種者数は4,084名に達し，累積接種率は95.0%（95% CI：94.3～95.6%）になった。

6．MR2期2007年，2009年，2011年，2013年調査結果の比較

2007年の調査では，調査対象となった2006年度でのMR2期接種開始時期が6月であったため，累積接種率曲線は6月上旬から立ち上がり始めていた。一方，2008～2012年の調査では，調査対象が改正法実施2～7年目のMRワクチン接種対象者であったため，累積接種率曲線は4月上旬から立ち上がり，6月上旬から12月中旬までは2007年の調査時よりも約20～30%高く経過していた。2013年調査では5月下旬から11月上旬までは2011年調査よりも1～4%程度高く，2013年3月下旬には93.5%となった（**図3**）。

考　察

麻疹ワクチンと風疹ワクチンは2006年4月から2回接種方式が導入され，MRが定期接種に用い

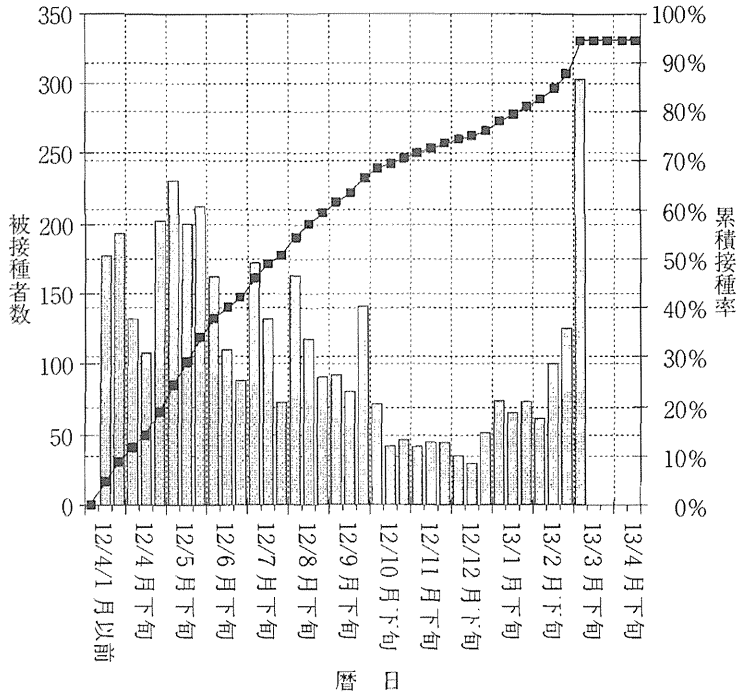

図2　2012年度就学前1年以内の小児における旬日別麻疹・風疹（MR）ワクチン2期被接種者数（縦棒）および累積接種率（黒四角）：2013年の調査結果

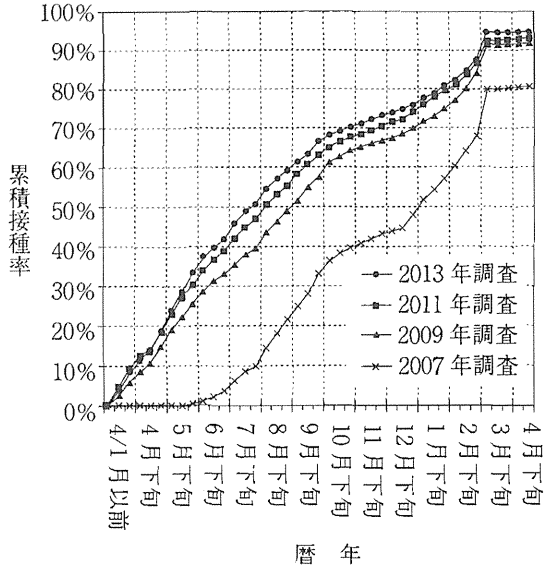

図3　2007～2013年調査の麻疹・風疹（MR）ワクチン2期累積接種率曲線の比較
2007年度の累積接種率は，2008年4月1日までに6歳に達した小児を対象として2008年6月に調査し，2009～2012年度の累積接種率は，それぞれ当該年の4月1日までに6歳に達した小児を対象として当該年の7月に調査を実施した．

られ始めたが[3]，2007，2008年の調査では単抗原麻疹ワクチン，単抗原風疹ワクチン接種を受けた標本が相当数あったため，MR1期の累積接種率調査は2009年から2歳児を対象として実施した。2013年の調査では累積接種率曲線の立ち上がりが，これまでになく早まって1歳児の半数以上が生後12カ月でMR1期の接種を受けており，きわめて良好な結果であった。

MR2期の累積接種率は，MR2期接種初年度を調査対象とした2007年の調査では最終的に80.3%に過ぎなかった[5]。2008年の調査では，年度途中での被接種者数の伸びは不十分であったが，いわゆる「駆け込み」接種により3月下旬に被接種者数が急増して，累積接種率は90%を超えた[6]。2009～2012年の調査では，年度途中での被接種者数の伸びは多少改善していたが，インフルエンザワクチン接種時期における被接種者数の減少が共通してみられた[4)6)～8)]。2013年の調査でも，これまでの調査時と同様に11月から翌年1月までのインフルエンザワクチン接種時期にはMR被接種者数の減少がみられ，累積接種率曲線の伸

びが鈍化した。最終的には，前年までの調査と同様に，「駆け込み」被接種者数の増加により，累積接種率は95.0%に達したが，「駆け込み」接種によらず，最終的に95%以上の累積接種率を達成するためには，今後もインフルエンザワクチン接種時期以前にMR 2期接種を済ませるように，保護者への接種勧告を続ける必要があると考えられる。

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Cumulative vaccination coverage of the 1st and 2nd doses of measles-rubella bivalent vaccine obtained by the nationwide survey in 2013

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