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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

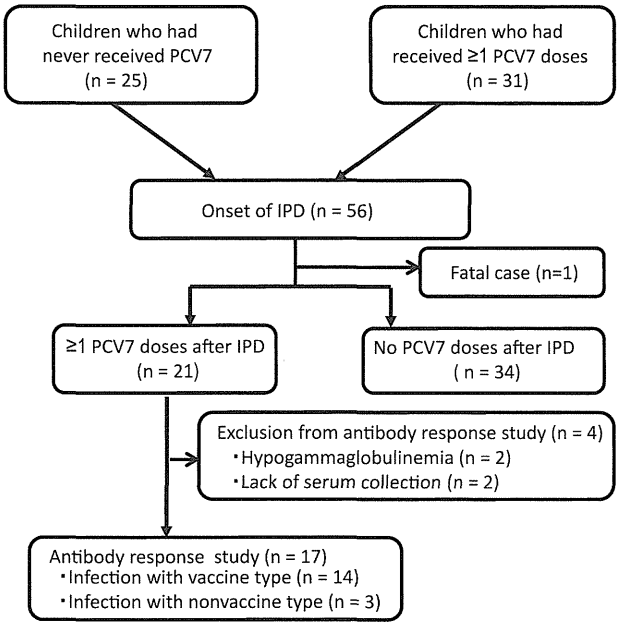

Fig. 1. Flow diagram of this study of children with invasive pneumococcal disease.

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%) 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%) ($P<0.001$).

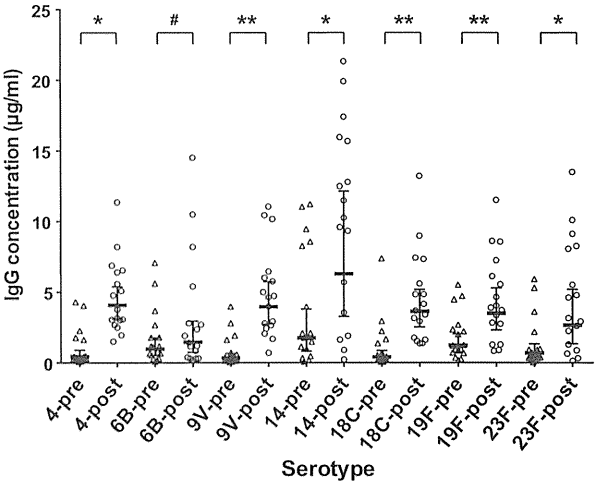

Fig. 2. Comparison of serotype-specific IgG concentrations between the time of onset of invasive pneumococcal disease (IPD) and after PCV7 vaccination in 17 children following the resolution of IPD. The IgG concentrations at the time of onset of IPD and after the last dose of PCV7 are shown as 'pre' 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').

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.

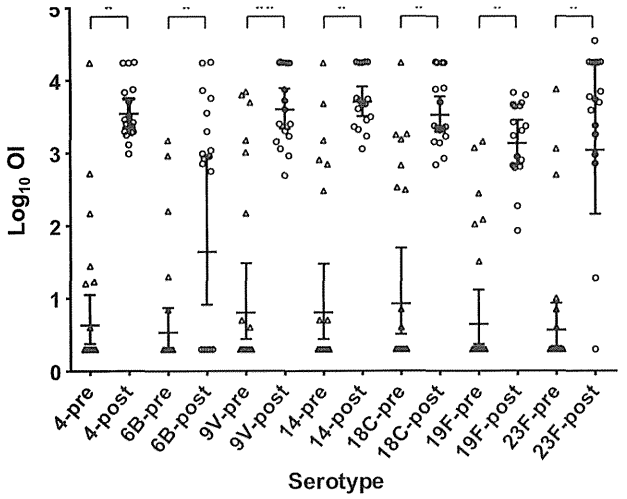

Fig. 3. Comparison of serotype-specific opsonization index (OI) between the time of onset of invasive pneumococcal disease (IPD) and after PCV7 vaccination in 17 children following the resolution of IPD. The $\log_{10}$ OI at the time of onset of IPD and after the last dose of PCV7 are shown as 'pre' 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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PLOS | CURRENTS OUTBREAKS

Short-term Prediction of the Incidence of Congenital Rubella Syndrome

October 30, 2014 · Research

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Abstract

Objectives

In Japan, a rubella outbreak occurred from early 2012 to late 2013, primarily among adult males aged 20–49 years. We conducted this study to predict the number of congenital rubella syndrome (CRS) cases in Japan in 2014.

Methods

The probability of CRS when a pregnant woman is infected with rubella depends on the gestational age of the fetus. The cumulative number of CRS cases was predicted by a formula based on the parameters from two studies conducted in the U.K. and the U.S., the reported cases of rubella among women 15–49 years of age, and the reports of CRS from 2011 to week 2 of 2014.

Findings

While the predicted number of cases of CRS based on parameters from the U.K. study demonstrated a biphasic curve, with a low peak around week 12 and a high peak around week 50 of 2013, the predicted number of CRS cases based on the U.S. study demonstrated a single peak around week 50 of 2013. The ex post evaluation indicated that the cumulative number of CRS cases in 2014 would be 19.1–29.3.

Interpretation

Our prediction of the number of CRS cases may be useful for the enhanced detection of this often under-reported syndrome.

Funding Statement

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Introduction

Rubella is usually a mild, febrile illness in children and adults, and up to 50% of rubella infections are asymptomatic. However, maternal infection with rubella, especially in early pregnancy, can cause an infant to

be born with severe birth defects that are known as congenital rubella syndrome (CRS)[1] .

In 1976, Japan introduced single-antigen rubella vaccine in it's national immunization program, targeting girls in junior high school. In 1989, measles-mumps-rubella (MMR) vaccine was introduced, targeting children aged 12-72 months. The MMR vaccine was, however, interrupted because of a high incidence of aseptic meningitis due to mumps vaccine. Although Japan introduced single-antigen rubella vaccine targeting children aged 12-90 months and boys and girls in junior high school in 1995, the coverage of this vaccine was low. A recent study reported that seropositivity for rubella antibody (≧1:8) among adults aged 30-50 years was 73%-86% for males and 97%-98% for females[2] . These data suggest that adult males remain susceptible to rubella.

A nationwide, sentinel case-based surveillance for CRS was established in April 1999. The sentinel surveillance systems were replaced by nationwide case-based surveillance for rubella in January 2008, and all physicians were required to report any clinically diagnosed or laboratory-confirmed rubella case to local public health offices.

The accumulated unvaccinated population led to an outbreak of rubella, primarily among adult males aged 20–49 years, from early 2012 to late 2013[3] . This epidemic of rubella peaked between weeks 19 and 22 of 2013. Because an epidemic of CRS follows a rubella epidemic with a lag of approximately 6–7 months in Greece[4] , an increase in CRS cases might occur from late 2013 to early 2014. The Ministry of Health, Labour and Welfare recommended not only routine immunization for children one and six years of age as part of the national immunization programme, but also encouraged vaccination of family members of pregnant women and vaccination for women who planned to get pregnant, as a counter-measure to reduce the rubella epidemic and the incidence of CRS[3] .

Although it is important from a public health viewpoint to predict the number of CRS cases following a rubella epidemic, no studies have been conducted to predict the number of CRS cases during the period of a rubella epidemic. The aim of this study was to predict the number of CRS cases in 2014 in Japan using a formula for predicting CRS cases based on the parameters of two studies conducted in the U.K. and the U.S.

Materials and Methods

The risk of CRS has previously[5] been calculated using :1) the number of rubella cases in women of childbearing age, 2) the proportion of pregnancies, and 3) the CRS risk based on gestational age.

1) The number of rubella cases in women of childbearing age

This parameter was obtained from the epidemic curve of rubella cases. Childbearing age was defined as 15–49 years of age.

2) The proportion of pregnancies

The proportion of pregnancies was obtained by dividing the number of deliveries divided by the population of women in each age group. In 2010, the numbers of births were 13,494, 110,956, 306,913, 384,382, 220,103, 34,610, and 773 for mothers aged 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, and 45–49 years, respectively. The total population of women in each age group was (in thousands) 2,954, 3,160, 3,602, 4,120, 4,836, 4,341, and 4,005, respectively, in the same year[6] .

3) The CRS risk based on gestational age

Gestational age was assumed to be independent of rubella virus infection, i.e., the gestational age at which a pregnant woman became infected with the rubella virus would be distributed uniformly. The probability of infection for each gestational week was assumed to be 1/39, as the duration of pregnancy can be defined at 2–40 gestational weeks. The probability of CRS depending on the gestational age of the fetus when the

pregnant woman acquires rubella virus infection [7] was calculated from two studies conducted in the U.K. [8] and the U.S. [9] (Table 1).

Table 1: Congenital Rubella Syndrome incidence (%) based on the fetal gestational age in pregnant women with rubella virus infection

	U.K.study					**U.S. study**				
Gestational age (weeks)	≤10	11-12	13-14	15-16	≥17	≤4	5-8	9-12	13-16	≥17
CRS incidence (%)	90	33	11	24	0	70	40	25	40	8

Table 1 Note: Data are from studies conducted in the U.K. [8] and the U.S. [9] .

These three components suggest that the expected number of CRS cases during period t should be $\sum_{i=1}^{n} Prob(\text{CRS of } i \text{ at } t)$, where n is the total number of rubella-infected women, which represents the magnitude of the ongoing rubella outbreak, and $Prob(\text{CRS of } i \text{ at } t)$ is defined as , $q(a(i))\frac{1}{39}p(t-r(i))$ where a(i) is age of patient i, which represents the age distribution of female rubella patients with age 15–49, q(□) is the probability of pregnancy by age i, p(□) is the probability of CRS by gestational age when pregnant and rubella infected and $p_{(\cdot)}=0 \ if \ t-r(i)\geq 40$,and r(i) is the date of implantation of i, where $t-r(i)$ is gestational age.

Hereafter, $\sum_{i=1}^{n} Prob(\text{CRS of } i \text{ at } t)$ is referred to as the CRS potential. Therefore, the CRS potential is defined as the theoretical predicted number of CRS cases based on a rubella outbreak in women. However, the CRS potential might not be equal to the number of reported CRS cases because of under-reporting of rubella and CRS cases or asymptomatic cases. To bridge these gaps, we regressed the number of reported CRS cases based on the CRS potential at birth, $(CRS\ case)_t=\alpha+\beta(CRS\ potential)_t$, by the ordinary least squares method, which is the simplest regression procedure, in which parameters are estimated to minimize the sum of the square of the residual. Data period for estimation was between week 1, 2011 and week 40, 2013.

Finally, to verify the precision of prediction, we performed an ex post evaluation, which is an evaluation of the future based on data available at the time of estimation. Thus, we used data only up to week 40 of 2013 to predict up to week 20of 2014, and compared this with the actual CRS incidence from week 41, 2013 to week 20, 2014.

Results

Following a relatively small outbreak in 2012, a larger outbreak occurred in 2013 (Figure 1). Until week 20, 2014, 3021 rubella cases occurred among women 15–49 years old and 44 CRS cases were reported in accordance with the Infectious Disease Law. The numbers of rubella cases and CRS cases were peaked at week 19, 2013 and at week 2, 2014, respectively. While the CRS potential based on the U.K. study demonstrated a biphasic curve with a low peak around week 12 and a high peak around week 50 of 2013 (Figure 2), the CRS potential based on the U.S. study demonstrated a single peak around week 50 of 2013.

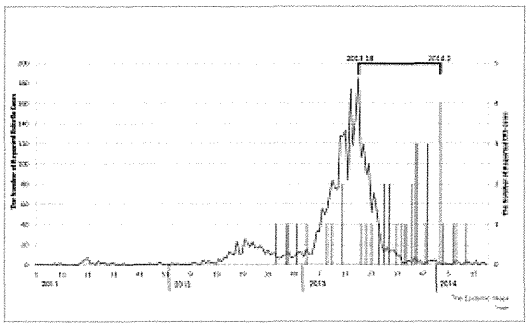

Fig. 1: The number of reported rubella cases (blue line) in women 15-49 years old and congenital rubella syndrome (CRS) cases (red bar) in Japan between week 1, 2011 and week 2, 2014.

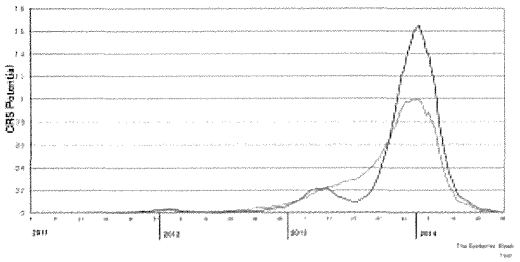

Fig. 2: The congenital rubella syndrome (CRS) potential based on the studies conducted in the U.K. (black line) and the U.S. (green line).

The estimated parameters for the predicted number of CRS cases are shown in Table 2. The estimated b was 0.99 based on the U.K. study and 1.46 based on the U.S. study. The estimated bs indicate that the number of CRS cases is the same as the CRS potential or 1.5 times greater than the CRS potential.Table 2: Estimation of parameters for the predicted number of Congenital Rubella Syndrome cases.

Table 2: Estimation of parameters for the predicted number of Congenital Rubella Syndrome cases

		Estimated coefficient	t-value	p-value
U.K.	α	0.0821	1.80	0.073
	β	0.993	8.77	0.000
U.S.	α	0.0158	0.34	0.735
	β	1.463	9.69	0.000

Table 2 Note: The determinant coefficients, an appropriate index for a fitness of the estimation, of both estimations are 0.330 for the upper panel and 0.376 for the lower panel. Data period for estimation was since week 1,2011 to week 40, 2013.

Figure 3 shows the predicted value, the estimated α + the estimated β (CRS potential), during the period covered by the data used for estimation, which allows prediction of the actual data before week 2, 2014. The predicted cumulative number of CRS cases based on the U.K. and U.S. studies is similar, and both estimations provide a close fit with the actual cumulative number of CRS cases.

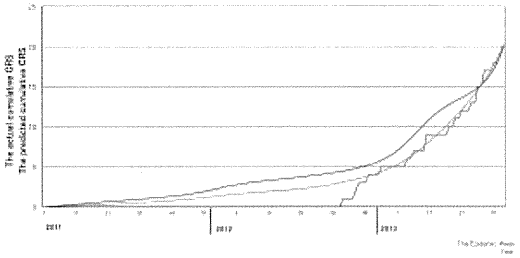

Fig. 3: Comparison between the actual cumulative number of congenital rubella syndrome (CRS) cases (red line) and the predicted cumulative number of CRS cases. The black line and green line are the predicted cumulative numbers of CRS cases based on the studies conducted in the U.K. and the U.S., respectively, using the data up to week 40, 2013.

The cumulative number of CRS cases in the future at the time of prediction, i.e., the period after week 2, 2014, is shown in Figure 3. The cumulative number of CRS cases in 2014 is predicted to be 19.1–29.3.

The results of the ex post evaluation are shown in Figure 4. The curve of the predicted cumulative number of CRS cases based on the U.S. data is**close to**the curve of the actual cumulative number of CRS cases between week 40, 2012 and week 40, 2013, while the curve of the predicted cumulative number of CRS cases based on the U.K. data is slightly higher than the curve of the actual cumulative number of CRS cases during the same period. After week 47, 2013, the curve of the predicted cumulative number of CRS cases based on the U.S. data is close to the actual cumulative number of CRS cases **until around week 5, 2014.** In contrast, the curve of the predicted cumulative number of CRS cases based on the U.K. data is much higher than the curve of the actual cumulative number of CRS cases.

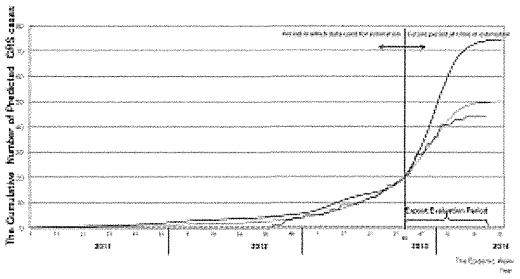

Fig. 4: Ex post evaluation of the predicted cumulative numbers of congenital rubella syndrome (CRS) cases. The red line indicates the actual cumulative number of CRS cases up to week 2, 2014. The black line and green line are the predicted cumulative numbers of CRS cases based on the studies conducted in the U.K. and the U.S., respectively, using the data up to week 40, 2013.

Discussion

In this study, we predicted the number of CRS cases with a formulation based on the parameters from two studies from the U.K. and the U.S. in 2013 and up to week 25, 2014. We found an increase of CRS cases following the rubella epidemic with a lag of 5-7 months, which was almost in agreement with a previous experience in Greece [4] . A close correlation was found between the predicted cumulative number of CRS cases in 2013 and the actual cumulative number of reported CRS cases, validating the present method of CRS prediction (Figure 3). The ex post evaluation generated two different patterns of the predicted number of CRS cases: a higher prediction based on parameters from the U.K. study and a lower prediction based on the U.S. study (Figure 4). The lower prediction based on the data from the U.S. agreed closely with the actual cumulative number of CRS cases. A dissociation between the predicted number of CRS cases based on two studies from the U.K. and the U.S. may be attributable to a difference in the probability of CRS, which directly affects the CRS potential, during the period up to 10 weeks of gestational age. While a higher percentage (90%) of CRS was found during the period up to 10 weeks of gestational age in the U.K. study, relatively lower percentages (40% or 70%, respectively) of CRS, compared with that in the UK study, during the period up to 10 weeks or between 5 and 8 weeks of gestational age were observed in the U.S. study.However, the reasons for the difference of the CRS incidence between the US and UK epidemics remain uncertain from the two literatures[8,9] . Actually, the estimation of β based on the past data retrospectively and thus it may not reflect the current or future situation. However, as shown in Figure 3 and ex post evaluation, it was proved to have a quite preciseness for the prediction of CRS and it would be valuable for public health workers.

CRS is clinically confirmed if an infant has: 1) at least two of cataract, congenital glaucoma, congenital heart disease, hearing impairment, or pigmentary retinopathy; or 2) one of these complications, and one of purpura, splenomegaly, microcephaly, meningoencephalitis, radiolucent bone disease, or jaundice developed within 24 hours after birth [3] . A cataract, which is found in approximately one third of all CRS babies, is occasionally not observed until late infancy [10] . Sensorineural deafness is also the most common manifestation of CRS, and deafness is frequently overlooked in infancy. Therefore, the difficulties in the clinical diagnosis of CRS often cause a delayed notification of CRS cases, and temporarily decrease the number of reported CRS cases compared with that predicted by our method.

In summary, we predicted the cumulative number of CRS cases in 2014 by a formula based on the parameters of two studies from the U.K. and the U.S. Our method for prediction of the number of CRS cases may be useful

for the enhanced detection of this syndrome that is often under-reported.

Competing Interest

The authors have declared that no competing interests exist.

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YO is a senior scientist of the Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan, and works in biostatistics, cost-effectiveness, and mathematical modelling.

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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:

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

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/