RESULTS

Detection of the Group A Rotavirus and Distribution of G and P Genotypes

Out of 391 group A rotavirus positive samples tested with immunochromatography, 250 samples were successfully genotyped for both G and P by RT-PCR. Five different G types and two P types were found during this study period. G1 was the most prevalent genotype (233/250: 93.2%), followed by G9 (6/250: 2.4%), G2 (6/250: 2.4%), G3 (4/250: 1.6%), and G4 (1/250: 0.4%). In contrast, P genotypes were P[8] (235/250: 94.0%) and P[4] (15/250: 6.0%). The G1P[8] combination genotype predominated and represented 90.4% (226/250) (Table I).

Phylogenetic Analyses and Molecular Evolutionary Rate of VP7 Gene

Among the 226 G1P[8] samples, 113 from which sufficient amplified cDNA could be obtained were selected and their VP7 gene fully sequenced (Table I). As a result, 41 different ORF nucleotide sequences were identified and analyzed. They were distributed across the whole study period (Table I).

A phylogenetic tree of the VP7 gene of the G1 strains was constructed using the 41 isolates and G1 reference strains (Fig. 1). The lineage designations were based on previous studies [Arista et al., 2006; Phan et al., 2007b; Banyai et al., 2009]. Human rotavirus G1 strains were divided into five lineages (I–V). The Sapporo G1P[8] isolates were classified into three lineages (I, II, and IV). Most of the Sapporo G1P[8] strains belonged to lineage I (28/41; 68%), showing a high degree of nucleotide identity among them (>97.6%). Sapporo G1P[8] lineage I strains were detected for long periods (1987, 1988, 1990–1995, and 1997–2000). In contrast, a temporally limited circulation was observed for G1 lineage II (1994–1997) and IV (1988–1990).

The evolutionary rate of VP7 gene for the 41 Sapporo G1P[8] strains was calculated to be 7.25×10^{-4} [highest posterior density (HPD) 95% = 3.97×10^{-4}; 1.11×10^{-3}] nucleotide substitutions per site per year (s/s/y), on the contrary, that of VP7 gene for 130 representative G1 references retrieved from GenBank was 1.41×10^{-3} (HPD 95% = 1.09×10^{-3}; 1.79×10^{-3}) s/s/y. Then, the rate of Japanese G1 strains (16 references plus the 41 Sapporo G1P[8]) was 1.29×10^{-3} (HPD95% = 6.19×10^{-4}; 2.12×10^{-3}) s/s/y. The rate limited to G1P[8] strains could not be calculated since P genotype was unknown in most representative G1 references.

G1 Lineage I Specific VP7 Amino Acid Substitutions and the Three-Dimensional Localization

On comparing deduced VP7 amino acid sequences with 317 world human rotavirus G1 references, 2 amino acid substitutions in G1P[8] lineage I were found, namely T91A and V212G. Among the 28 G1P[8] lineage I strains, they were observed in 5 (isolated in 1987, 1994, and 1998) and 4 (in 1988, 1994, and 1995) isolates, respectively.

As shown in Figure 2, a putative three-dimensional VP7 model was constructed with 235 amino acids (residues 78–312) of the 87SA1133 strain which was the oldest strain in this study belonging to G1 lineage I. The two lineage I specific substitutions (T91A and V212G) described above were mapped on the predicted model. They were located on the surface of the protein.

DISCUSSION

The application of methods for identifying VP7 has expanded an understanding of the epidemiology of rotavirus genotypes with regard to infection and disease [Estes and Kapikian, 2007]. Although G type prevalence has fluctuated, several studies have indicated that G1 genotype rotaviruses are the most prevalent strains throughout the world [Gentsch et al., 2005; Santos and Hoshino, 2005; Arista et al., 2006; Phan

TABLE I. Distribution of Group A Human Rotavirus G and P Genotypes in Sapporo, Japan During 1987–2000

Year	G1P[8]			G1P[4]	G2P[4]	G9P[8]	G3P[8]	G4P[4]	G9P[4]	Total
1987	5	(1)	{1}	2						7
1988	12	(4)	{4}	4						16
1989	20	(11)								20
1990	17	(10)	{3}							17
1991	8	(3)	{1}		1	1				10
1992	25	(9)	{2}	1	3			1		30
1993	12	(7)	{3}							12
1994	35	(19)	{7}			1	2			38
1995	33	(15)	{5}							33
1996	19	(15)	{3}		1		1			21
1997	8	(7)	{4}						1	9
1998	18	(2)	{2}							18
1999	4	(4)	{3}			2				6
2000	10	(6)	{3}		1	1	1			13
Total	226	(113)	{41}	7	6	5	4	1	1	250

(), number of samples in which VP7 gene was full-sequenced; {}, number of samples which were employed for phylogenetic analyses and calculation of molecular evolutionary rate of VP7 gene.

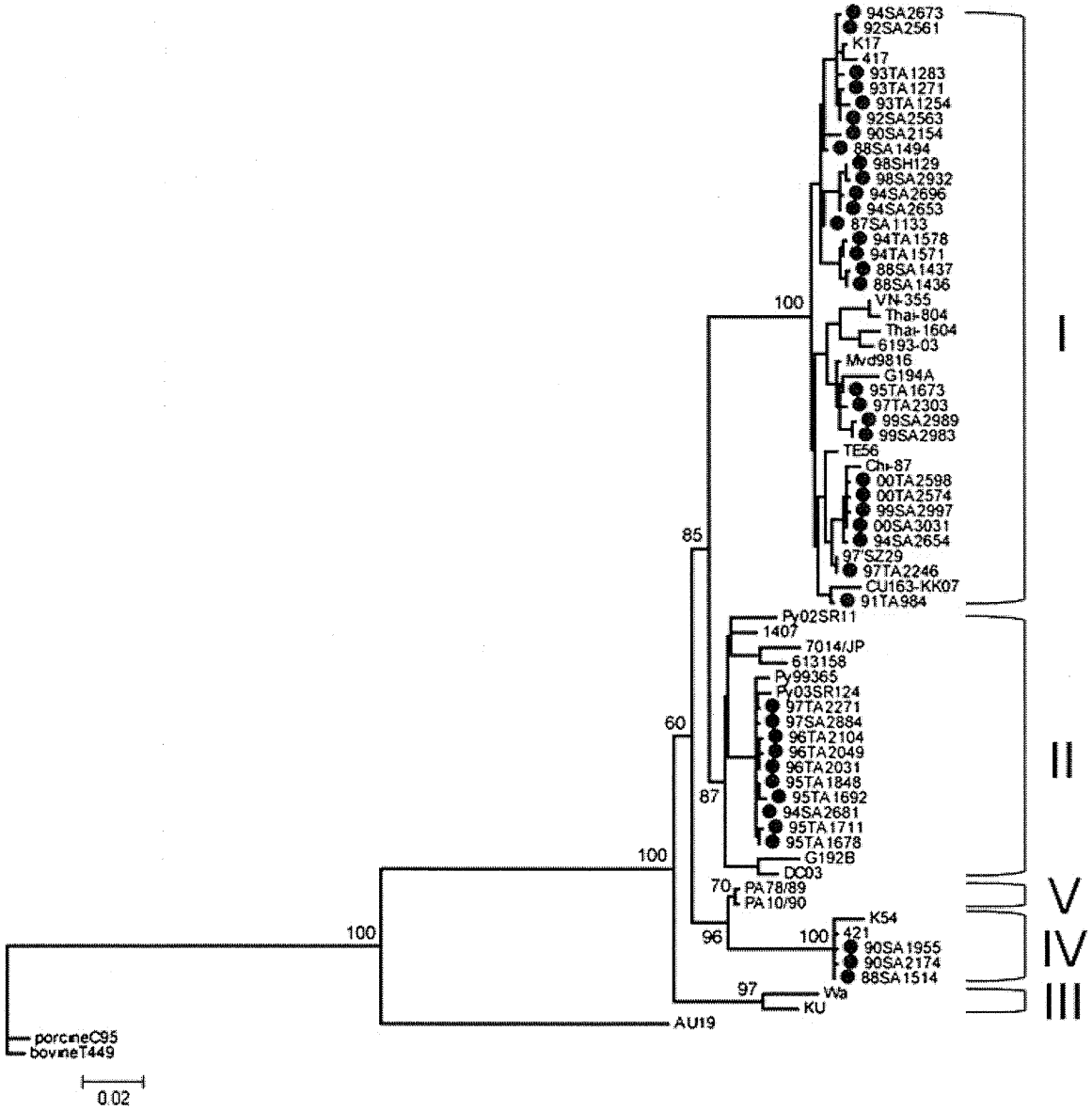

Fig. 1. Phylogenetic analysis of human rotavirus VP7 ORF nucleotide sequences of G1 strains. Phylogenies were reconstructed by the maximum likelihood method. The scale indicates nucleotide substitutions per position. Approximate likelihood ratio test method values are given at key branch nodes. Human rotavirus G1 divided into five lineages (I–V). The Sapporo G1P[8] isolates (solid circles) clustered into three lineages (I, II, and IV). Nucleotide sequences of rotavirus isolates detected in present study have been submitted to GenBank and assigned accession numbers GU358419–GU358446, GU377195–GU377205, and HQ650871–HQ650886. Reference strains and their accession numbers were as follows: 417 (D16328), 421 (D16326), 1407 (S83903), 613158 (FJ948854), 6193-03 (DQ207389), 7014/JP (EF079064), 97'SZ29 (AF260952), AU19 (AB018697), Chi-87 (DQ512998), CU163-KK/07 (GQ996867), DC03 (AF183859), G192B (AF043678), G194A (AF043679), K17 (D16320), K54 (U26377), KU (D16343), Mvd9816 (AF480293), PA10/90 (DQ377587), PA78/89 (DQ377572), Py02SR11 (EF179186), Py03SR124 (EF179188), Py99365 (DQ015682), TE56 (AF183856), Thai-1604 (DQ512981), Thai-804 (DQ512979), VN-355 (DQ512968), Wa (K02033), bovine T449 (M92651), and porcine C95 (L24165).

et al., 2007b; Banyai et al., 2009]. The authors of an Italian study suggested that the emergence and/or introduction of novel G1 antigenic variants might explain the continuous circulation of G1 rotavirus in a local population [Arista et al., 2006]. In this study in accord with the worldwide tendency, G1P[8] constituted the majority of circulating rotavirus strains in Sapporo, Japan.

Human rotavirus VP7 gene of G1 strains could be divided into at least five lineages (I–V), and Sapporo G1P[8] strains belonged to three lineages (I, II, and IV). Recent studies demonstrated that the major lineages

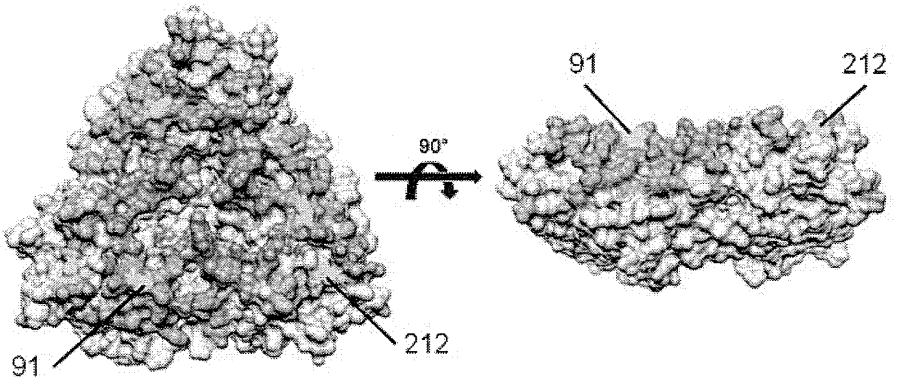

Fig. 2. Putative three-dimensional conformation of VP7 protein for the representative Sapporo G1P[8]; 87SA1133 (GU358424). The left image shows a surface representation of the trimer, the right image shows a lateral view, constructed by using rhesus rotavirus structure (pdb#3fmg) as a template. Residues comprising the putative neutralization domains of VP7 gene have been colored as follows: Pink (7-1a), salmon (7-1b), and purple (7-2). The amino acid differences specific to some Asian lineage I strains including Sapporo G1P[8] lineage I are shown in cyan. They are labeled for a single monomer of the trimer.

among the G1 genotype circulating worldwide were lineages I and II [Arista et al., 2006; Phan et al., 2007b; Banyai et al., 2009], furthermore recent Asian studies reported that the most prevalent lineage of G1 genotype was lineage I [Trinh et al., 2007; Le et al., 2010]. This study revealed that most of the Sapporo G1P[8] strains belonged to lineage I of G1 genotype. These observations suggest that lineage I strains circulating in Asia have some survival advantages, compared to other lineages.

Evolutionary rates have been studied in rapidly evolving organisms, like viruses. Indentifying what factors determine the rate at which genomes generate and fix mutations provides important insights into key evolutionary mechanisms. For several RNA viruses including rotaviruses, overall evolutionary rates range from 10^{-2} to 10^{-5} nucleotide s/s/y [Jenkins et al., 2002]. Recently, the evolutionary rate for the VP7 gene of G9 and G12 rotaviruses were reported to be 1.87×10^{-3} and 1.66×10^{-3} s/s/y [Matthijnssens et al., 2010a]. In the present study, evolutionary rates of the VP7 gene of Sapporo G1 strains from 1987 to 2000 showed a slightly lower rate of 7.25×10^{-4} s/s/y, compared to 1.29×10^{-3} s/s/y for the Japanese G1 strains and 1.41×10^{-3} s/s/y for the worldwide VP7 gene of 130 representative G1 strains. VP7 is one of the most variable rotavirus proteins exposed to selection pressure, which could increase the evolutionary rate. However, the data showed relative lower mutation rates of the VP7 of G1 genotype in Sapporo. This difference may partly reflect the fact that the present study was carried out in a restricted geographic area, and multiple G1 lineages circulate among the human population.

RotaTeq® is one of several rotavirus vaccines that contains five human-bovine reassortant strains (G1, G2, G3, G4, and P[8]). It was known that the G1 component of the RotaTeq® vaccine belongs to lineage III [Matthijnssens et al., 2010b], and clusters closely to the Wa strain which circulated in the USA in the 1970s but has not been identified in recent years [Arista et al., 2006; Le et al., 2010]. Therefore, knowing how predominant lineage I differs from other lineages, especially lineage III, is important basic information anticipating that vaccine tolerant mutant strains will eventually emerge.

Three amino acid substitutions specific to lineage I; 57-Ile, 68-Ser, and 217-Thr were previously described in strains isolated in Italy, Hungary, and Japan [Arista et al., 2006; Phan et al., 2007b; Banyai et al., 2009]. Two additional substitutions, T91A and V212G, were found in the present study and these are potentially important mutations, because they belong to the VP7 neutralization domains 7-1a and 7-1b, respectively [Aoki et al., 2009; McDonald et al., 2009]. T91A has already been reported in some Asian countries [Trinh et al., 2007], however, V212G is a novel mutation. These amino acid changes might be related to the prevalence of lineage I strains not only in Japan but also other countries.

The crystal structures of VP7 of rhesus rotavirus have been solved, enabling mapping of the three-dimensional locations of the lineage specific differences [Aoki et al., 2009; Chen et al., 2009]. The two amino acid substitutions confirmed in the present study (T91A and V212G) were located in or near the VP7 intersubunit boundary. Neutralizing antibodies against VP7 were proposed as stabilizers of the trimer by locking down the subunit interface, thereby inhibiting the uncoating trigger for VP4 rearrangement [Aoki et al., 2009]. Consequently, a mutation involving the VP7 intersubunit boundary might enable the virus to escape from this mechanism; a possible advantage for rotaviruses. On the other hand, 57-Ile and 68-Ser which were described previously [Arista et al., 2006; Phan et al., 2007b; Banyai et al., 2009], were located in the N-terminal, which formed an "arm" structure and contributed to contact with VP6 trimer

and interaction with each VP7 trimer [Aoki et al., 2009; Chen et al., 2009]. Unfortunately in this work the structure of the N-terminal arm could not be predicted, and the significance of 57-Ile and 68-Ser substitutions remains to be elucidated.

Currently licensed rotavirus vaccines have been remarkably effective [Linhares et al., 2008; Armah et al., 2010; Madhi et al., 2010; Zaman et al., 2010]. As has been pointed out [Nakagomi et al., 2008], the possibility of a change in the predominant strain triggered by the rotavirus vaccine, mandates continued surveillance for antigenic transition. It is believed that present study provides background data on rotavirus genetic variability prior to the widespread use of rotavirus vaccines in Japan. These data could also help develop safe and effective next-generation vaccines.

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Original Article

Incidence of Intussusception as Studied from a Hospital-Based Retrospective Survey over a 10-Year Period (2001-2010) in Akita Prefecture, Japan

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SUMMARY: One concern about rotavirus vaccines is its possible association with intussusception. Thus, it is necessary to determine the baseline incidence for intussusception in the first year of life in places where rotavirus vaccines are introduced. However, few safety data exist for the period at which the first dose of Rotarix and RotaTeq are allowed to administer in Japan. The first dose of Rotarix is scheduled to administer at 6-20 weeks of age and that of RotaTeq is scheduled to administer at 6-24 weeks of age; the upper limits for these vaccines is later than the upper limit recommended by the World Health Organization by 5 and 9 weeks, respectively. We performed a retrospective cross-sectional study by reviewing medical charts of all hospitals that provided pediatric beds in Akita Prefecture, Japan, and identifying the cases of intussusception that met the Brighton criteria level 1 in these hospitals between January 2001 and December 2010. During this 10-year period, 122 children younger than 1 year of age were diagnosed with intussusception. The incidence of intussusception was estimated at 158 per 100,000 person-years among children younger than 1 year (95% confidence interval, 131-188), 10 per 100,000 person-years for children aged 0-2 months, 165 for children aged 3-5 months, and 300 for children aged 6-8 months. This rapid and substantial increase in the incidence of intussusception during the first year of life should be considered when formulating the immunization schedule for administering rotavirus vaccines in Japan.

INTRODUCTION

Intussusception is a pathological condition in which one portion of the intestine invaginates into an adjacent segment of the intestine, leading to a strangulating obstruction. It is a pediatric emergency in infants and shows a peak in occurrence in infants aged at 6-8 months with no apparent seasonality (1,2). This condition received much attention when a tetravalent rotavirus vaccine, RotaShield (Wyeth-Lederle Vaccines

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and Pediatrics) (3), was suspected to cause intussusception in approximately 1 in 10,000 vaccine recipients, resulting in the withdrawal of this vaccine from the market in the United States (2,4-6). Consequently, large, phase III clinical trials, each recruiting more than 60,000 infants were conducted, and their results showed that two succeeding rotavirus vaccines, Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq (Merck & Co., Whitehouse Station, N.J., USA), were safe with respect to intussusception (7,8).

However, a recent post-licensure study from Mexico showed that Rotarix was associated with a small yet statistically significant risk of intussusception at approximately 1 in 51,000 vaccinated children in the first week after the first dose (9). A small risk of intussusception at 1 in 68,000 vaccinated children in the first week after the second dose of this vaccine was reported from Brazil (9). Further, studies conducted in Australia showed a link between RotaTeq and intussusception with a relative risk of 5.26 (95% confidence interval [CI], 1.1-15.4) in the first week and a relative risk of 3.5 (95%CI, 1.3-7.6) in the first 3 weeks after the first dose (10). Thus, it is inferred from these recent studies that any orally administered rotavirus vaccine will probably carry some detectable risk of intussusception (9). Therefore, it is important that the risk of intussusception (potentially attributable to rotavirus vaccine) should be assessed in balance with the benefit of rotavirus vaccine in preventing deaths and hospitalization due to rotavirus diarrhea at the national level because the ratios of benefit to risk may vary from one country to another (9,11).

Since the two currently licensed rotavirus vaccines carry a small risk of intussusception, it is critical to know the number of intussusception cases that will occur by chance alone in the week of administration of the vaccine should be determined in order to correctly assess the attributable risk of the vaccine in the population at large. A previous study showed that the incidence of intussusception in the first year of life is 185 per 100,000 person-years in Japan (12); this incidence is 3-5 times higher than that reported from the United States (13,14) and Australia (15). Nevertheless, the prescribing information in Japan allows practitioners to administer the first dose of Rotarix to children aged between 6 and 20 weeks, an age 6 weeks later than the age recommended for the first dose of Rotarix by the Advisory Committee on Immunization Practices (ACIP) of the United States (16) and in Australia (17) and 5 weeks later than the age recommended by the World Health Organization (WHO) (18). In this retrospective hospital-based cross-sectional study, we calculated the incidence of intussusception in Japan with a tighter 95% CI and determined how the incidence of intussusception changed during the first year of life. The information generated in this study can be used to formulate the immunization schedule for administering rotavirus vaccines in Japan.

MATERILAS AND METHODS

A retrospective cross-sectional study was conducted in all hospitals possessing pediatric beds in Akita Prefecture between January 2001 and December 2010 with an aim to identify all intussusception cases that occurred in this prefecture during the study period of 10 years. Akita Prefecture has a population of 1,080,000, accounting for approximately 0.9% of the total population of Japan, and is located in the northwestern coast of the main island. The west side of the prefecture faces the Sea of Japan while its northern, eastern, and southern borders are separated from the neighboring prefectures by mountain ranges. This geographic location allowed us to assume that the patients with intussusception were presented or treated in the medical facilities within the prefecture.

We asked the senior pediatricians of the 22 hospitals in Akita Prefecture that had pediatric beds for the initial identification of the patients who had intussusception and were 15 years old or younger. In each hospital, computer-based screening of the health insurance claims was performed using intussusception at the primary or any secondary diagnosis as the keywords. The questionnaire for each case patient was then filled out by the senior pediatrician of the hospital after examining the description of radiographic records as well as signs and symptoms in the hospital chart. These case questionnaires were sent to the principal investigator of the study (A.N.), and each case was ascertained according to the diagnostic certainty defined by the Brighton Collaboration Intussusception Working Group (19).

A case of intussusception was defined as a child who was admitted to one of the 22 hospitals in Akita Prefecture during the period between January 2001 and December 2010 with the discharge diagnosis of acute intussusception. Medical chart review was conducted to include only cases that were confirmed by the presence of characteristic findings upon radiological examination with liquid contrast enema (either barium or gastrografin) or air enema or by the presence of invagination at surgery (the level 1 of diagnostic certainty according to the Brighton criteria) in the final analysis (19). Only those patients who were younger than 1 year of age and who resided in Akita Prefecture were included in the final analysis. Further, we ensured that the same patient was not counted more than once in the final analysis.

To calculate the incidence of intussusception during the study period, the total number of patients was divided by the person-years for the study period, which was the sum of the number of live births from 2001 to 2010 without any adjustment (taken into account a small infant mortality that fluctuated between 1.9 and 3.0 per 1,000 live births over the study period). The Poisson model was used to calculate the 95% CI of incidences. The number of live births was obtained from the vital statistics data of Akita Prefecture, which continuously decreased by 25% from 8,873 in 2001 to 6,688 in 2010 (7,744 on average). If the same child had intussusception in more than one occasion ($n = 3$), we counted only the first event for the calculation of incidences.

This study was approved by the institutional review board of Graduate School of Medicine, Akita University.

RESULTS

For the period of 10 years between January 2001 and December 2010, we identified 122 children who were

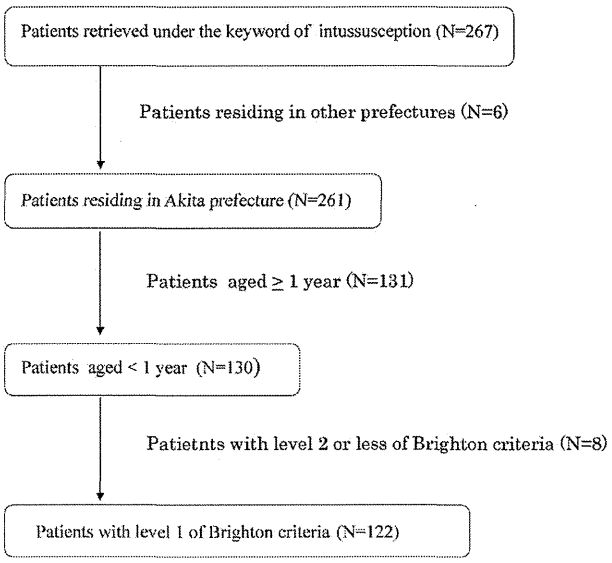

Fig. 1. Flow diagram from children initially retrieved in hospital record as having intussusception to the final cases ascertained by the principal investigator.

Table 1. Number of cases and incidence rate of intussusception among children younger than 1 year of age in Akita Prefecture, Japan, between 2001 and 2010

Age (month)	No. of cases	Person-years	Incidence rate[1)]	95% CI
0–2	2	19,359	10	1.3–37
3–5	32	19,359	165	113–233
6–8	58	19,359	300	228–387
9–11	30	19,359	155	105–221
Total	122	77,436	158	131–188

[1)]: Incidence rates are expressed as cases per 100,000 person-years.

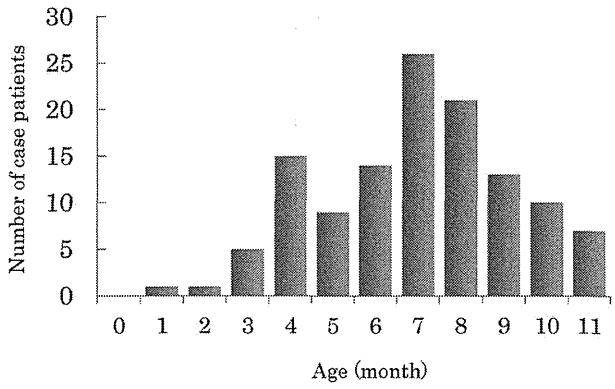

Fig. 2. Age distribution of intussusception patients among children younger than 1 year of age in Akita Prefecture, Japan, between 2001 and 2010.

Table 2. Number of cases and incidence rate of intussusception among children equal or younger than 24 weeks of age in Akita Prefecture, Japan, between 2001 and 2010

Age (week)	No. of cases	Person-years	Incidence rate[1)]	95% CI
6–12[2)]	1	10,395	10	0.2–54
6–14[3)]	3	13,365	22	5–66
6–15[4)]	5	14,850	34	11–79
16–20[5)]	11	7,425	148	74–265
21–24	14	5,940	236	129–395

[1)]: Incidence rates are expressed as cases per 100,000 person-years.
[2)]: The age period for the first dose of Rotarix vaccination recommended in Europe (20).
[3)]: The age period for the first dose recommended by ACIP of the United States (16).
[4)]: The age period for the first dose recommended by the WHO (18).
[5)]: The period in which the first dose of Rotarix is allowed only in Japan according to the package insert.

younger than 1 year of age and were diagnosed with intussusceptions according to the level 1 certainty of the Brighton criteria (Fig. 1). Thus, the average incidence of intussusception during this 10-year period in Akita Prefecture was calculated as 158 per 100,000 person-years (95% CI, 131–188) in the first year of life (Table 1). The age distribution of the patients with intussusception showed that intussusception peaked between 7 and 8 months of age, but only 2 patients were recorded in the first 3 months of age (0–2 months of age), confirming that infants younger than 3 months of age were unlikely to have naturally occurring intussusception (Fig. 2). The incidence for the first 3 months of life (0–2 months of age) was 10 patients per 100,000 person-years (Table 1). The number of patients sharply increased from 3 months of age, and the number of patients between 3 and 5 months of age comprised a 26% of all patients younger than 1 year of age (Fig. 2). For the period of 3–5 months of age, the incidence increased to 165 per 100,000 person-years (Table 1), a 16-fold increase from the incidence for the period of 0–2 months of age. The incidence between 6 and 8 months of age (the peak occurrence of intussusception) was calculated as 300 per 100,000 person-years (Table 1).

More precise incidence for weeks of age was calculated for naturally occurring intussusception; the periods of weeks of age were selected after considering the various periods when the first dose of Rotarix and RotaTeq is administered, on recommendation by various recommendation bodies (Table 2). The incidence was calculated as 10 per 100,000 person-years for the 7-week period from 6 to 12 weeks and 6 days of age, the period for the first dose of Rotarix vaccination recommended in Europe (20). The incidence doubled (22 per 100,000 person-years) for the 9-week period from 6 to 14 weeks and 6 days of age, the period for the first dose recommended by ACIP of the United States (16). A further increase in incidence was expected if the first dose was administered at age of 6–15 weeks, in accordance with the WHO's recommendation (18). A substantially higher incidence of intussusception was expected in the period between 16 and 20 weeks and 6 days, the period in which the first dose of Rotarix is allowed to be administered only in Japan (Table 2).

DISCUSSION

This study confirmed and extended the findings of a preceding study conducted over a 25-year period in one sentinel hospital in Akita Prefecture, Japan; that study reported a high incidence of intussusception (185 per 100,000 person-years; 95%CI, 133–251) (12). While the

incidence calculated in the present study was slightly lower (158 per 100,000 person-years), the prefecture-wide survey allowed us to have a much tighter 95% CI of 131–188. The incidence of intussusception in Japan was again shown to be 2- to 3-fold higher than those reported in the United States (18–56 per 100,000 live births) (13,14), and Australia (71 per 100,000 <1 year of age population) (15). The incidence in Japan is slightly higher than that in some European countries such as the United Kingdom (120 per 100,000 <1 year of age population) (21), the Netherlands (110 per 100,000 live births) (22), and Denmark (78–123 per 100,000 <1 year of age population) (23). Some countries have a higher incidence of intussusception, e.g., Vietnam (302 per 100,000 <1 year of age population) (15) and Israel (224 per 100,000 live births) (24). While these variations in the incidence of intussusception are known to exist (1), the cause is poorly understood (15).

Considering the global introduction of rotavirus vaccine, accurate information on the background rate of intussusception is fundamental to address the issue of the risk of intussusception attributable to rotavirus vaccine. If it is assumed that a finite risk of intussusception is associated with the administration of rotavirus vaccine, at least two key questions must be considered. First, does the risk of intussusception attributable to rotavirus vaccine increase among children who become the month-of-age at which the incidence of naturally occurring intussusception is high? Second, does the relative risk of intussusception increase in countries where the baseline incidence of intussusception is high? While these two questions are yet to be answered, it is clear that the number of intussusception cases that occur by chance alone will increase in the week after administration of rotavirus vaccine if the vaccine is administered to children who become the month-of-age at which the incidence of naturally occurring intussusception is high and will be larger if the vaccine is administered to children in countries where the baseline incidence of intussusception is high. Keeping these facts in mind, we calculated the incidence rates of naturally occurring intussusception in specific week-of-age periods relevant to the Rotarix and RotaTeq vaccination schedules (Table 2). Clearly, the background incidence of intussusception rapidly and substantially increased as the period for the first dose was expanded. Thus, each year, 2 intussusception cases will occur during the week of vaccination by chance alone if the entire birth cohort of the country (approximately 1,070,000) is administered the first dose during 6–12 weeks and 6 days of age. In contrast, the 7 intussusception cases will develop during the week of vaccination by chance alone if the first dose is administered according to the WHO's recommendation. If a small portion, e.g., 20% of the birth cohort is administered the first dose of Rotarix during 16–20 weeks of age, the period in which the first dose is allowed only in Japan, and the rest (80%) of the cohort receive the vaccine during 6–15 weeks of age, 12 intussusception cases will develop in the week of vaccination by chance alone.

To prevent concurrent occurrence of intussusception cases in the week of vaccination by chance alone, administration of the first dose of rotavirus vaccine should be restricted to children before 13 weeks of age as recommended in Europe (20) or to children before 15 weeks of age as recommended by the ACIP of the United States (16) and in Australia (17). However, in the absence of an official recommendation body for immunization practices in Japan, practitioners administer the first dose of Rotarix to children as old as 20 weeks of age and of RotaTeq to children as old as 24 weeks of age.

This study has a few limitations. First, the retrospective design might lead to underestimation of the number of cases due to insufficient records in the hospital chart. Second, the number of cases is not large enough to address the incidence for specific periods within the first year of life with tight 95% CIs. Third, while this study showed a high incidence of intussusception in Akita Prefecture, caution may be required when extrapolating this incidence to the entire nation. Fourth, the hospital records for the first 5-year period (2001–2005) could not be retrieved for one hospital that covered 3.8% of the total birth cohort of Akita Prefecture, leading to underestimation of the incidence. Fifth, two patients whose residence was outside of Akita Prefecture were excluded. We suspect that a few converse patients might have visited neighboring prefectures to seek medical interventions. Thus, this may led to underestimation.

In conclusion, this study showed a high incidence of intussusception in the first year of life in Japan. While infants younger than 3 months of age were unlikely to have naturally occurring intussusception, the incidence increased by 15-fold in the following 3-month period (3–5 months of age). Our results provide important information that should be considered when formulating the immunization schedule for administering rotavirus vaccine in Japan.

Note added in proof An addendum was provided to the package inserts of both Rotarix and RotaTeq for use in Japan on April 24, 2012: the first dose is recommended to administer by the age of 14 weeks and 6 days.

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Conflict of interest None to declare.

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