

Characteristics of Rotavirus Infections before and after Introduction of Rotavirus Vaccines in Japan

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Abstract

Rotavirus infection is a serious gastrointestinal infection that is usually prevalent during winter months and often seen in infants and young children. Studies on genotypes of prevalent rotavirus strains are quite important for preventing infection, developing vaccines, and its evaluation. We investigated the characteristics of rotavirus infections of Nasu Region of Tochigi, Japan and to compare to the other region before introduction of rotavirus vaccines. We examined the clinical findings in 147 patients and analyzed the clinical findings of the 37 patients with a fecal sample positive for rotavirus antigen. Furthermore, viral genotypes were determined using rotavirus-positive samples from 27 of these 37 patients. The genotypes were determined as G1P [8] in 5 samples, G3P [8] in 5 samples, G9P [8] in 3 samples, and G6P [9] in 2 samples. We were able to analyze the phylogenetic trees of these genotypes. Of particular note, we detected G6P [9] which were extremely rare in human beings but common in cattle. It was suggested that the rates of hospitalization due to rotavirus infections of infants from 6 to 11 months of age would be reduced by 42% when the vaccination coverage rates increase to 50% in Japan. Studies on changes in prevalent strains after vaccine introduction need to be conducted.

Keywords: Rotavirus Gastroenteritis; Endemic Strain; Genotype; G6P [9]; Rotavirus Vaccine

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1. General concepts and Clinical Features of Rotavirus Infection

Rotavirus (RV) is an important viral pathogen causing Gastroenteritis acute (GE) in humans. It has been reported that approximately 790,000 children younger than 6 years of age visit clinics as outpatients due to RVGE [1], and approximately average of 78,000 children younger than 5 years of age are hospitalized annually in each year before introduction of RV vaccines [2]. As most of infants have received the rotavirus vaccine rotavirus infections among infants and young children has since decreased significantly in developed countries [3]. Each year, the vaccine prevents an estimated 40,000 to 50,000 hospitalizations among U.S. infants and young children [4]. Rotavirus illness has also decreased among older children and adults that are not vaccinated; they are likely gaining indirect protection from rotavirus disease as vaccinated children are less likely to get the disease and spread it to others [5]. Focusing on deaths of under 5 years in developing countries infectious GE has critical roles. Nature of rotavirus in spans of many mammals and birds, infection of rotavirus infection in humans is virtually limited to humans [6].

Neurological symptoms associated with rotavirus infections less than 2 years old were estimated to be approximately 2% in the following [7]. Although rotavirus was detected from all CSF specimens in cases of convulsion by RT-PCR assay [8], but was not detected in our previous case. In our previous study monthly occurrence, trends in different age groups and clinical symptoms were not significantly different to previous reports. There were no sequelae of any cases of encephalopathy, seizures, and secondary lactose intolerance complications.

Large proportion of pediatric infectious GE especially in winter is caused by viruses. These viruses in environment, especially at low temperature inactivation and hard to be associated with infection [9]. Infants with immature immune easily loose extracellular electrolytes due to diarrhea, vomiting and dehydration. Worldwide, rotaviruses account for more than 125 million cases of infantile gastroenteritis and nearly 1 million child deaths per year, mainly in developing countries and another two million are hospitalized. The peak incidence of rotavirus diarrhea occurs between 6 and 24 months of age. In developing countries, however, cases are not uncommon among children younger than 6 months. Both homotypic and heterotypic responses are elicited during natural rotavirus infection, and the immunological response at the intestinal mucosal surface is probably the more consistent predictor of clinical immunity [3]. Rather than other control measures, vaccination is most likely to have a major impact on rotavirus disease incidence. Rotavirus is highly infectious and resistant and, regardless of water quality and available sanitation, most of children in the world are at risk of infection.

Rotavirus infections usually cause winter epidemic from January to April mainly in infants and children [9]. Severe diarrhea is considered the leading cause of infant death in developing countries. Older children and adults are asymptomatic or mild symptomatic after second infection. Main route of infection is from human to human fecal oral infections and percutaneous airway infections are also estimated. Clinical symptoms develop after 2 days of incubation period and cause white watery diarrhea accompanied by fever, abdominal pain, vomiting lasting approximately one week. In severe cases with high degree of dehydration inappropriate fluids uptake causes miserable outcome [4]. Complications of central nervous system (CNS) develop seizures, encephalitis, meningitis, and encephalopathy.

Medical workers who handle patients vomit and feces at the time of treatment and examination need to take standard

precautions and contact infection prevention measures [10]. Unlike in Norovirus rotavirus is taken up rarely as a cause of food poisoning. However, infection is transmitted by taking water or food contaminated by the virus. Both homotypic and heterotypic responses are elicited during natural rotavirus infection, and the immunological response at the intestinal mucosal surface is probably the more consistent predictor of clinical immunity.

2. Immunological Aspects of Rotavirus Infection

Rotavirus, a member of the family Reoviridae, has 11 segments of double-stranded RNA as a genome, and the viral particle is composed of three concentric layers such as the outer capsid, inner capsid, and core [1, 2, 3]. The outer capsid consists of two structural proteins, VP4 and VP7, which contain neutralization antigens. The inner capsid consists of structural protein VP6.

The mechanisms underlying protection against rotavirus disease are not yet fully understood. Clinical protection may involve mucosal and systemic antibodies, and the cellular immunity [4]. Although VP6 has been recognized as the most immunogenic outer capsid rotaviral protein, serum antibodies directed at either VP4 or VP7 are also able to neutralize rotavirus [1, 6]. VP4 may be more effective in evoking virus-specific serum neutralizing antibodies during natural infection (Table 1). Antibodies against VP6 in secretions are indicative of the activity of IgA antibody to neutralize virus reflecting mucosal immunity and resistance to reinfection. Neonates infected within their first 2 weeks of life are protected against moderate-to-severe disease but not against reinfection. Infants and children are protected against rotavirus disease following both symptomatic and asymptomatic primary infection [1]. Each new rotavirus infection increases natural immunity and reduces the severity of the infection. Although homotypic responses seem to predominate, heterotypic responses are commonly elicited [6]. The occurrence of sequential rotavirus illnesses involving the same G type supports the view that natural infections produce incomplete protection.

Table 1: Summary of Genotypes of Rotavirus A

Viral proteins	Genotypes	Common Genotypes in Human
VP7	G	G1, G2, G3, G4, G9, G12
VP4	P	P[4], P[6], P[8]
VP6	I	I1, I2
VP1	R	R1, R2
VP2	C	C1, C2
VP3	M	M1, M2
NP1	A	A1, A2
NP2	N	N1, N2
NP3	T	T1, T2
NP4	E	E1, E2
NP5	H	H1, H2

Both primary and secondary infections in humans elicit the development of serum antirotavirus IgG, IgM, and IgA antibodies in serum, saliva, and intestinal secretions. Immunologic response occurring at the intestinal mucosal surface would probably be a more consistent predictor of clinical immunity. Mechanisms leading to the release of antiviral toxins may play a role in the protection against infection. Some studies have shown that prophylactic protection against infection and clinically significant illness in infants can be afforded by oral administration of either milk or colostrum containing rotavirus-specific antibodies. With the primary objective of protecting children against life-threatening dehydrating diarrhea, many approaches to rotavirus vaccine development have been attempted.

3. Introduction and Efficacy of Rotavirus Vaccines

Vaccines against rotavirus can protect children from rotavirus infections which are the leading cause of severe diarrhea among infants and young children [11, 12, 13]. An earlier vaccine, called Rota Shield, a rhesus-human reassortant vaccine was removed from the market after being used for two years, because it was found to slightly increase the risk of intussusceptions causing a bowel obstruction. After the license in the United States, the occurrence of intussusceptions as an adverse event led to the vaccine's withdrawal. In the first practical application of the rotavirus vaccine recombinant viruses and virus strain themselves (G3) mixed with tetravalent vaccine [14, 15]. The vaccine, tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV), was given licensing approval in some countries and introduced to the market [16, 17].

Although two types of oral vaccine, RotaTeq and Rotarix were approved by global health agencies and licensed in more than 100 countries, only 31 countries have introduced routine rotavirus vaccination as of 2011. Currently, two oral rotavirus vaccines for prevention of RVGE are incorporated into national immunization programs in more than 50 countries by April 2014. The incidence of severe rotavirus infections has declined significantly in countries that have acted on the recommendation to introduce the rotavirus vaccine [18, 19].

RotaTeq is a live pentavalent vaccine (R5) that contains five rotavirus strains produced by reassortment. Parent strains were isolated from human and bovine hosts and four reassortant rotaviruses express one of the outer capsid, VP7, proteins (serotypes G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein VP4 (type P7) from the bovine rotavirus parent strain.

Episodes of severe gastroenteritis were identified by active surveillance of 20,169 infants from two weeks after the second dose until one year of age [20, 21]. Severe gastroenteritis was defined by the passage of ≥ 3 loose or watery stools within a 24-hour period, with or without vomiting, that required overnight hospitalization or rehydration therapy in a medical facility. The efficacy against severe rotavirus gastroenteritis was 85 % and against severe gastroenteritis from any cause was 40 %. The efficacy against gastroenteritis caused by G1P [8] strains was 92 % and against strains sharing only the P [8] antigen (G3P [8], G4P [8], G9P [8]) was 87 % [22]. The efficacy against hospitalization for rotavirus gastroenteritis was 85 % and against hospitalization

for gastroenteritis from any cause was 42 % [23, 24]. After immunization, efficacy against severe G1-G4 rotavirus gastroenteritis was 98 % and against G1-G4 rotavirus gastroenteritis of any severity was 74 %. RV5 reduced hospitalization for G1-G4 rotavirus gastroenteritis by 96 % and for any gastroenteritis by 59 %. RV5 reduced emergency department visits related to G1-G4 rotavirus gastroenteritis by 94 % and clinic visits by 86 %.

In the second rotavirus season after immunization, the efficacy against severe rotavirus gastroenteritis was 88 %, and against rotavirus gastroenteritis of any severity was 63 %. In an extension study of 20,736 original participants followed for up to 3.1 years after the last dose of RV5, the efficacy against hospitalization and emergency department visits related to rotavirus gastroenteritis of any serotype was 94 % [18, 19, 25]. Receipt of three doses of RV5 was 100 % effective in preventing rotavirus gastroenteritis hospitalization and emergency department visits and 59 % in preventing all-cause acute gastroenteritis, although the study methodology was biased toward observing increased effectiveness [26].

RV5 has proven to be highly effective during routine use in the United States since licensure in 2006 [22, 25, 27, 28]. Multiple rotavirus surveillance systems in the US have demonstrated a delay in the rotavirus season and dramatic reductions in gastroenteritis-related health care resource utilization and incidence of rotavirus infections since introduction of RV5 to the routine infant immunization schedule in 2006 [29, 30, 31]. Total of nearly 70,000 infants were evaluated including half who received the live pentavalent rotavirus vaccine, RV5. This pivotal trial demonstrated a vaccine efficacy of 74.0% against any severity and 98.0% against severe gastroenteritis, caused by viruses with serotypes G1, G2, G3 and G4. Hospitalizations were reduced by 95% through the first 2 y of life after the third dose [32].

Rotarix is a monovalent (R1), human, live attenuated rotavirus vaccine containing one rotavirus strain of G1P [8] specificity which is indicated for the prevention of RVGE caused by G1 and non-G1 types (G3, G4, and G9) when administered as a 2-dose series in infants. The efficacy of RV1 during the first two years of life was assessed in a subgroup of patients and found to be similar to that during the first year [33, 34, 35]. RV1 also appears to provide protection against G2P [4], which shares neither the G1 nor the P [8] antigen [36]

Studies of the rotavirus vaccine have shown that it can prevent about 74% of rotavirus infections. It can prevent approximately 98% of severe infections and 96% of hospitalizations from rotavirus [22, 23, 37]. A review estimated that vaccination against rotavirus would prevent about 45% of deaths due to rotavirus gastroenteritis, or about 228,000 deaths annually worldwide [38, 39]. In the United States, vaccination has reduced rotavirus-related hospitalizations by as much as 86% since 2006. Compared with 2006, rates of hospitalization for rotavirus infection in 2008 were reduced among children younger than three years, whether or not they were vaccinated [41, 41, 43]. There was an 87 percent reduction in the 6- to 11-month age group, a 96 percent reduction in the 12- to 23-month age group and a 92 percent reduction in the 24- to 35-month age group. Similar results were observed at several and widely dispersed sites in North America.

The World Health Organization (WHO) recommended that rotavirus vaccine be included in all national immunization programs in 2009 [43]. The *Rotavirus Vaccine Program* and the Accelerated Vaccine Introduction initiative have worked to study rotavirus vaccines among developing-country populations to assist developing countries in introducing rotavirus vaccines into routine immunization programs. These partnerships are spearheaded by international non-governmental organizations. Safety and efficacy trials in Africa and Asia found that the vaccines dramatically reduced severe disease among infants in developing countries [33, 44, 45, 46]. The vaccines may also prevent illness in non-vaccinated children by limiting exposure through the number of circulating infections. Rotavirus immunization in infants is associated with reduced rotavirus morbidity among unvaccinated older children and adults. Even partial (approximately 50 percent) uptake of RV5 under a recommendation for universal immunization of infants with rotavirus vaccine was associated with reduced rotavirus disease in unvaccinated older children and adults.

4. Characteristics of rotavirus strains before introduction of rotavirus vaccines in Japan

RVGE is a serious disease in children from 6 months to 2 years-old occurring mainly during the winter time. 39.2~48.5% of patients with acute gastroenteritis less than 5-year-old admitted to hospitals in the Mie Prefecture, Japan was due to rotavirus infection [47]. The age distribution was < 6 months: 4.2 %, < 1-year-old: 24.8%, < 2 years: 63.5%, of patients. RV-positive cases

in sporadic gastroenteritis patients of 683 adult cases in 2001 were 97 and the equivalent of 14% of the overall and age distribution was 13 ~ 95 (mean=45 y $\pm$ 17 SD) year of age. Group receiving inpatient treatment (50 years $\pm$ 21months) was of significantly higher age ($p=0.002$) compared to those receiving outpatient treatment group (39 years $\pm$ 15 months).

Based on the antigenicity of the inner capsid protein VP6 and genomic characteristics, rotavirus is classified into seven groups (A–G) [48], among which group A rotavirus is the major etiologic agent in humans and animals [1, 49, 50, 51]. For epidemiological investigations of RV, a genetic classification system based on the outer capsid proteins VP7 (G type) and VP4 (P type) has been adopted [2, 52, 53, 54]. A full-genome based genotyping system composed of genotypes of the 11 individual RNA segments also has been proposed [55, 56]. While at least 11 G genotypes have been isolated from humans, G1, G2, G3, G4, and emerging G9 are major genotypes of human rotaviruses [2, 56]. As human P genotypes, P [8] is the most common genotype worldwide, followed by P [4] and P [6]. Also, 6 Non-Structural Proteins (NSP) are known.

In human RVs, five common G and P genotype combinations (genogroups) have been identified: G1P [8], G3P [8], G4P [8], and G9P [8] on the Wa-like genome constellation (I1-R1-C1-M1-A1-N1-T1-E1-H1), and G2P[4] on the DS-1-like constellation (I2-R2-C2-M2-A2-N2-T2-E2-H2) [57, 58, 59]. AU-1-like rotaviruses make up a third group of human RV, which is distributed at low prevalence and has a distinct gene constellation (G3-P [9]-I3-R3-C3-M3-A3-N3-T3-E3-H3) [57]. So far, few G6P [9] rotaviruses have been detected in humans. The first G6P [9] strain, PA151, was isolated from an Italian child with gastroenteritis, followed by the Se584 strain from the United States and several Hungarian strains [57, 60, 61]. Although G6 human rotavirus is quite rare, it is the major type among rotaviruses from cattle. In a study of Japanese cows, 59.1% of isolates belonged to G6. Usually, bovine G6 strains were combined with P [5], P [1] and P [11] worldwide [56, 62].

Recently, full-genome sequences of rotavirus strains have been increasingly analyzed in order to understand the interspecies transmission, reassortment, and evolutionary relationships between human and animal rotaviruses [55]. In the

previous study [63], nearly full-length sequences of all the gene segments were determined to investigate the genetic origin of the unique human G6P[9] rotaviruses detected recently in Japan.

Rotavirus infects not only humans but non-human monkeys and bovine, pigs, horses, dogs, cats, rats and chicken. New introduced vaccines against rotavirus epidemic strains may change the cross-immunity of virus strains. Goto et al [55] discovered genotypes G1P [8] from the cerebrospinal fluid of children with rotavirus meningoencephalitis in the Tochigi Prefecture, Japan. Reports investigated the epidemic strain in local areas are very important to investigate the pandemic from the epidemic in the region [56, 57]. In the previous study [64] we examined the endemic strain of rotaviruses in the Nasu region, Tochigi Prefecture, Japan, for their regional characteristics. Comparison of the clinical features due to endemic strains in this region was performed with those due to global pandemic strains [58].

We also examined the clinical findings in 147 patients who attended to the Department of Pediatrics at International University of Health and Welfare Hospital in Nasu-shiobara City, Tochigi Prefecture, Japan during the time of April 1, 2008 to March 31, 2010 [64]. We analyzed the clinical findings of the 37 patients with a fecal sample positive for rotavirus antigen. Furthermore, viral genotypes were determined using rotavirus-positive samples from 27 of these 37 patients. The genotypes were determined as G1P [8] in 5 samples, G3P [8] in 5 samples, G9P [8] in 3 samples, and G6P [9] in 2 samples (Table 2). We were able to analyze the phylogenetic trees of these genotypes. Current genotypes of rotavirus are reported that most worldwide G1P [8] as 52%, followed by G2P [4] as 11% and G4P [8] as 8% [2]. G1 type had been most prevalent since 1984 to 2007 in Japan; G3 was most prevalent only in 2004. In the same year there was difference in G1, G2 or G3 type endemic according to regions. G3 type had been predominant during the winter time of 1987 to 1988 in the Gifu Prefecture of Japan, but G4 type was predominant in 1987 to 1988. Serotype distribution of 196 samples during 10 years in Kurume was G1 68.4% (136 samples), G3 10.2% (20 specimens), G4 5.6% (11 samples), G2 3.6% (7 samples), and unclassified 12.2% (24 samples) [65, 66].

Table 2 . Summary of genetic analysis of rotavirus strains in Japan (Numazaki and Ichikawa, 2014).

Case	A g e	S e x	Specim ens	G type (VP7)	P type (VP4)	Sub group (VP6)	NSP4
1	3	F	CSF				
2	1	F	feces	G1	P[8]	II	B
3	3	M	feces	G1	P[8]	II	B
4	2	M	feces	G9	P[8]	II	B
5	1	F	feces	G9	P[8]	II	B
6	1	F	blood				
7	0	F	feces	G3	P[8]	II	B
8	0	F	blood				
9	1	F	feces	G9	P[8]	II	B
10	2	M	feces			II	B
11	2	F	feces			II	B
12	2	M	feces			II	B
13	1	F	feces			II	B
14	2	M	feces			II	B

Case	A g e	S e x	Specim ens	G type (VP7)	P type (VP4)	Sub group (VP6)	NSP4
15	1	F	feces			II	B
16	2	F	feces	G3	P[8]	II	B
17	1	M	feces	G3	P[8]	II	B
18	5	F	feces	G3	P[8]	II	B
19	7	F	feces			II	B
20	3	M	feces			II	B
21	2	F	feces	G1	P[8]		
22	1	M	feces	G1	P[8]	II	B
23	1	M	feces	G3	P[8]	II	B
24	3	F	feces	G6	P[9]	II	E3
25	0	F	feces	G6	P[9]		C
26	1	F	feces	G1	P[8]	II	B
27	0	F	feces			II	B

Rotavirus G9 type was found in the United States in 1983 and from 1985 during the 1989 succession confirmed in Bangladesh, Japan, India and Thailand. This type caused the epidemic in Thailand and Japan from 2000 to 2003 [17, 67, 68, 69]. Other emerging human G12 type was endemic in the 2003 -2004 season in Nepal [70, 71, 72]. New viruses such as G8, G10 and G5 type emerged in Brazil and China [73, 74]. The bovine type G6 geno type detected in this study was very rare [75]. G6P [14] in Egypt [76] and G6P [9] in Burkina Faso [77] in either was reported as a rare virus. Case 24 in our study [64] without previous contact with obvious bovine did not identify the source of infection.

In the phylogenetic analysis the gene of a strain was located in a human lineage including other human G6 strains. Similarly, all other genes of the strain except for the NSP3 gene were relatively closely related to at least one of the human G6 RVs reported in Europe and the U.S. These findings suggest that human G6 RVs which had occurred by reassortment between human and bovine RVs are distributed worldwide, despite low prevalence. Since genotypes of a few gene segments are different among those human G6 strains, this suggests that G6 rotaviruses may occur independently in different areas through reassortment among local strains (Table 3).

Table 3. Genomic constellations of G6 P[9] rotavirus (KF17) and prototype strains (Numazaki and Ichikawa, 2014).

Strain	Host	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
KF17	Human	G6	P[9]	I2	R2	C2	M2	A3	N2	T3	E3	H3
B1711	Human	G6	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
Se584	Human	G6	P[9]	I2	R2	C2	M2	A3	N2	T1	E2	H3
Hun5	Human	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
PA169	Human	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
NCDV-Lincoln	Bovine	G6	P[1]	I2	R2	C2	M2		N2	T6	E2	
WC3	Bovine	G6	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3
AU-1	Human	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
T152	Human	G12	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6
Cat2	Feline	G3	P[9]	I3	R3	C2	M3	A3	N1	T6	E3	H3
FRV1	Feline	G3	P[9]								E3	
BA222	Feline	G3	P[9]	I2	R2	C2	M2	A3	N1	T3	E2	H3
DS-1	Human	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
Wa	Human	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1

Genotypes identical to KF17 genes are indicated by shading.

Clinical properties of the Nasu boy who presented with meningoencephalitis presumably caused by a G1P [8] rotavirus infection [55]. In our case, the clinical findings of the CSF showed an initial stage of aseptic meningitis probably because the time of CSF collection was not appropriate to obtain a diagnosis CNS infection due to rotavirus. It was not found that significant dehydration and electrolyte abnormalities were responsible for CNS symptoms. Thus, we diagnosed G1P [8] rotavirus infection accompanied by meningoencephalitis, although the rotavirus genome was detected only in stool samples and not in CSF samples. Phylogenetic trees based on the sequences of the VP4 and VP7 genes indicated that our strain was classified into unique clusters, including other Asian strains. In addition, some non-synonymous substitutions were found in both the VP4 and VP7 genes leading to several amino acid substitutions. The results suggested that the rotavirus detected in our case had a unique genetic/antigenic property. Such a viral property might be associated with rotavirus-related CNS diseases [60, 84].

and in 7 months to 2 years of age. There were no sequelae or any cases of encephalopathy, seizures, and secondary lactose intolerance complications. Three of four rotavirus genotypes found in the present study were worldwide-circulating. A very rare G6P [9] type related to bovine rotaviruses was also detected. Further epidemiological studies are necessary concerning these strains. Investigation of the epidemic strain of rotavirus after introduction of a vaccine is also considered necessary.

It has been reported that rotavirus infections might be associated with various complications of the central nervous system (CNS) [7, 78]. Common clinical CNS symptoms due to rotavirus infections have been observed along with febrile convulsions, dehydration, and other temporary nutritional disorders [79, 80]. Severe neurological complications, including meningoencephalitis and encephalopathy, have been associated with gastroenteritis resulting from rotavirus infections, although the incidences of these complications are relatively rare. However, the etiology of CNS complications induced by rotavirus infections remains to be elucidated [8, 81, 82].

Recently some reports have shown a relationship between the mutations of both antigens and CNS diseases [83, 84]. However,

properties of the Nasu boy who presented with meningoencephalitis presumably caused by a G1P [8] rotavirus infection [55]. In our case, the clinical findings of the CSF showed an initial stage of aseptic meningitis probably because the time of CSF collection was not appropriate to obtain a diagnosis CNS infection due to rotavirus. It was not found that significant dehydration and electrolyte abnormalities were responsible for CNS symptoms. Thus, we diagnosed G1P [8] rotavirus infection accompanied by meningoencephalitis, although the rotavirus genome was detected only in stool samples and not in CSF samples. Phylogenetic trees based on the sequences of the VP4 and VP7 genes indicated that our strain was classified into unique clusters, including other Asian strains. In addition, some non-synonymous substitutions were found in both the VP4 and VP7 genes leading to several amino acid substitutions. The results suggested that the rotavirus detected in our case had a unique genetic/antigenic property. Such a viral property might be associated with rotavirus-related CNS diseases [60, 84].

Among the rotavirus genotypes, G1P [8] is commonly circulating in various regions, including Europe and Asia [68]. Although the pathophysiology of complications may be associated with substitutions in the VP4 and VP7 genes [83, 84], the relationship between disease severity and the virulence of rotavirus is not exactly known. A substitution (from Val to Ile (173aa)) of the VP4 gene in a rotavirus G1P [8] strain derived from a child with CNS complications suggests that this substitution may be linked to the virulence of the G1P [8] rotavirus.

5. Effects of rotavirus vaccines after introduction to Japan

The introduction of rotavirus vaccines caused changes in virus serotypes. In Brazil rotavirus vaccine was introduced in 2006, G2P [4] increased from 7% before vaccine introduction to 95%

after introduction in 2009 [34, 35]. With the introduction of rotavirus vaccines now expected decreases in patients, but could change the epidemic fashion of infections [26, 35, 85]. In Japan investigations of changes in epidemiologic fashion, after the introduction of the rotavirus vaccine are now in progress.

In Japan, Rotarix and RotaTeq have been on the market since November 2011 and July 2012, respectively. Although RV vaccination had not been adopted into the national immunization program as of 2014, an estimation of 45% uptake with a wide range of variation throughout Japan was reported in 2013 [86]. The number of infectious gastroenteritis cases including RVGE has been collected weekly from sentinel pediatric clinics since the years before vaccine introduction (Figure 1, Figure 2) [36, 87, 88].

Figure 1: Weekly reports of rotavirus detection, 2012/13 season, Japan (Infectious Agents Surveillance Report, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan).

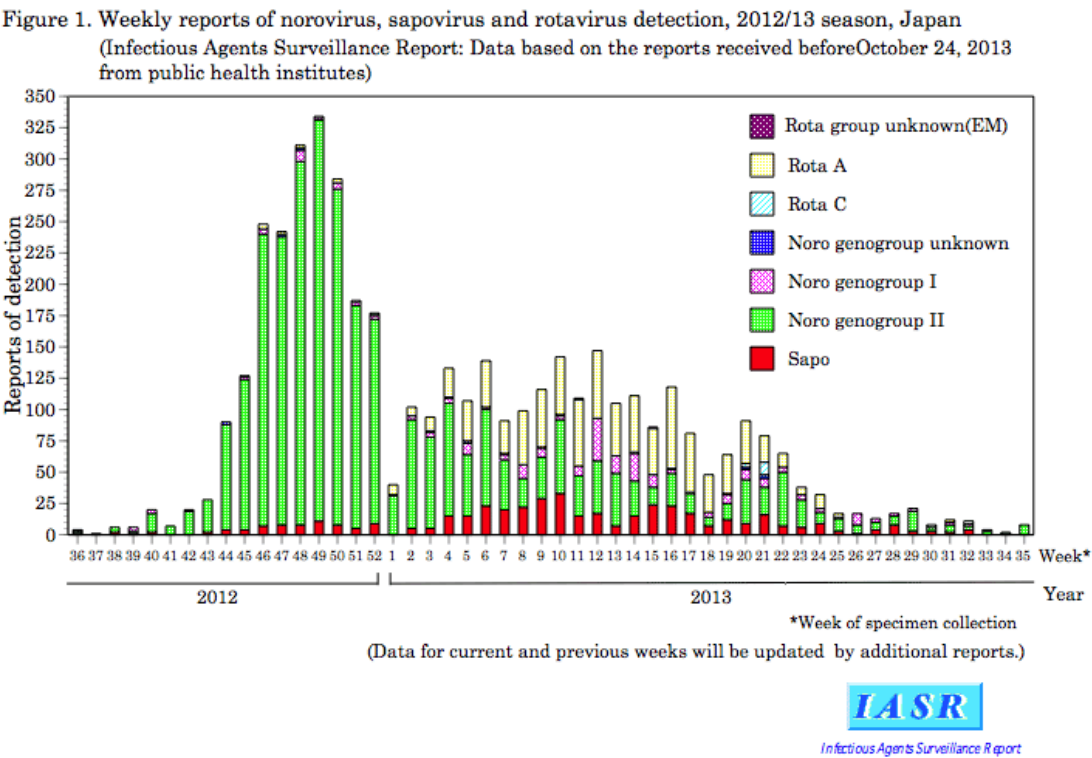
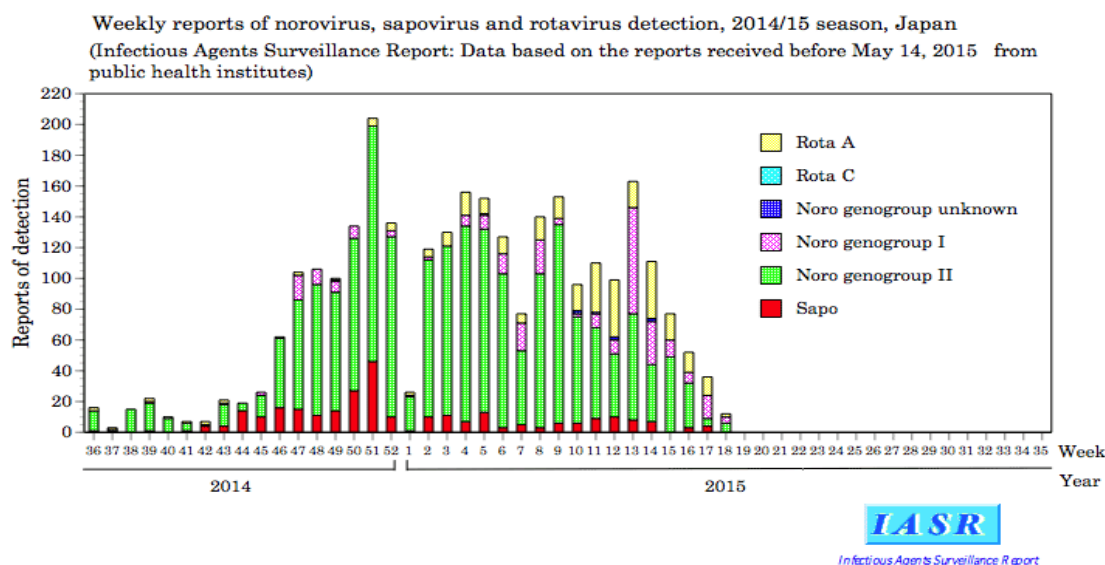


Figure 2: Weekly reports of rotavirus detection, 2014/15 season, Japan (Infectious Agents Surveillance Report, Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan).



Moreover, consistent reduction in the occurrence and incidence rates of severe RVGE in children less than 3 years of age has been observed after RV vaccine introduction. The results of several studies [89, 90] suggest indirect benefits for older children and young adults, in addition to the children who were targeted for vaccination. Indirect benefits were noted in the first or the second year after vaccine introduction, while only infants were eligible to receive vaccination, potentially implicating infants as the primary transmitters of infection. Accumulation of unimmunized susceptible children during seasons with low rotavirus activity and the higher number of susceptible children to facilitate transmission during a subsequent season may occur [87]. It was suggested that the rates of RVGE hospitalization of infants from 6 to 11 months of age would be reduced by 42% when the vaccination coverage rates increase to 50% in Japan [91].

Each year in Japan, approximately 26,500 to 78,000 children less than 5 y of age were hospitalized owing to rotavirus infections before the introduction of vaccines. A total of 762 infants were randomized to the RV5 or placebo group and 761 received at least 1 dose of vaccine in one study [86]. Comparable numbers of participants from each group completed the study (96.6% in RV5 and 96.1% in placebo, respectively). Vaccine efficacy estimated against rotavirus gastroenteritis at least 14 d post dose 3. Results of secondary endpoints demonstrated that the

vaccine efficacy was 80.2% against moderate-to-severe and 100% against severe cases of rotavirus RVGE caused by viruses with serotypes containing G1, G2, G3, G4, and G9 occurring at least 14 d post dose 3. Efficacy estimates of the vaccine against RVGE due to any naturally occurring rotavirus (regardless of serotype) occurring at least 14 d following the third dose was 75.3% for any severe cases and was 81.0% for moderate-to-severe and severe cases, respectively [59]. The serotype-specific efficacy was demonstrated to be 81.4% against serotype G1, 20.0% against serotype G3, and 100.0% against serotype G9. The vaccine efficacy for G1 was statistically significant.

Globally, G1P1A [8] is the most common rotavirus type causing gastroenteritis in humans, and this was the predominant type observed in one study in Japan [92, 93]. Of the 34 with cases of rotavirus infections, 19 were caused by serotype G1, 9 were caused by serotype G3, and 5 were caused by the G9 serotype. Additionally, in this study RV5 has demonstrated a good safety profile and was generally well tolerated in Japanese participants, including a small number of premature infants. Furthermore, these results in Japan confirmed the potential for high vaccine efficacy in Asia [94], which is similar to other developed world settings and appears higher than the observed efficacy in developing settings in Asia, such as those observed in Bangladesh and Vietnam.

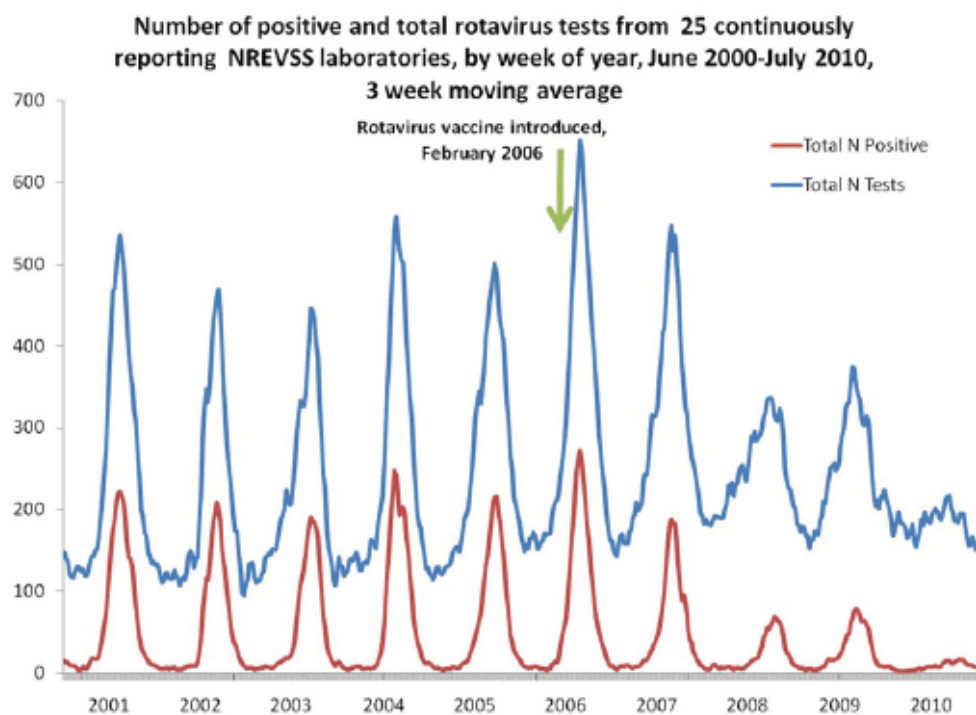
Most of Japanese children are infected with rotavirus by 5 y of age not related to their socioeconomic circumstance. Implementation of a rotavirus vaccination program in Japan will prevent severe rotavirus infections and reduce disease burden in infants and the burden to their families. Such a program could reduce the burden on health care workers and associated health care costs.

6. Approaches of new rotavirus vaccine development

The RotaTeg and Rotarix vaccines now in use do not appear to increase serious risk and are considered safe (Figure 3). Both vaccines were recommended for routine vaccination of U.S. infants and their use has resulted in an annual decline of approximately 40,000 hospitalizations due to acute GE in 2008 and 2009 in children [95]. Post-licensure monitoring for

intussusceptions is on going [96]. Although the use of RotaTeg and Rotarix vaccines are estimated an annual reduction of approximately \$140 million of treatment cost, the development of new vaccines intended to be offered at lower cost than the approved vaccines is also ongoing [97, 98]. Current strategies are directed toward developing multivalent rotavirus vaccines that bear epitopes similar to the circulating viral serotypes. Several field trials with reassortant multivalent rotavirus vaccines have been completed around the world [99, 100]. Current strategies are primarily directed toward developing an orally administered live attenuated rotavirus vaccine [22, 101, 102]. Recent discovery of rotavirus nonstructural protein NSP4 that may act as a viral enterotoxin in nonhuman models offers new approaches toward the prevention of rotavirus gastroenteritis.

Figure 3: Rotavirus incidence trends from 2001-2010 using passively reported laboratory rotavirus test data from the National Respiratory and Enteric Virus Surveillance System (NREVSS).



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