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A lack of significant association between the electropherotype or G-serotype of the infecting strain and disease severity of rotavirus gastroenteritis

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Summary. Despite many previous studies, the question has not been settled as to whether some human rotavirus strains are more virulent than others. Since disease severity is most clearly reflected by the hospitalization status of the infected children, we examined whether there was any difference in the distribution of dominant strains between inpatient and outpatient groups. The study population comprised 763 children with acute diarrhea who were treated at a general hospital in Honjo City, Akita, Japan, during 1986–1997. Rotaviruses from stool specimens were classified into 77 electropherotypes using polyacrylamide gel electrophoresis. A single dominant strain or two co-dominant strains circulated simultaneously with some infrequent strains in most rotavirus seasons. Over the 11 rotavirus seasons, there was no significant difference in the relative frequencies of 15 rotavirus strains between the inpatient and the outpatient groups when strains of rotavirus were defined by their electropherotypes in polyacrylamide gel electrophoresis. However, infection with one G1 strain that co-dominated with a G4 strain carrying an identical electropherotype except the VP7 gene resulted in a statistically significantly reduced risk of hospitalization. There was no significant difference in the relative frequencies of four major G-serotypes or long/short RNA pattern. We conclude that the virulence or disease-causing potential of human rotavirus is not substantially different in the majority of strains.

Introduction

Group A rotavirus, species *Rotavirus A*, belonging to the genus *Rotavirus* within the family *Reoviridae*, has been established as the major etiological agent of

acute gastroenteritis in infants and young children worldwide [9]. Two outer capsid proteins of rotavirus, VP7 and VP4, can induce production of neutralizing antibodies, and define the G- and P-serotype, respectively. The inner capsid protein VP6, the most abundant viral protein, defines another antigenic specificity called subgroup (I or II), but antibodies directed against VP6 are not involved in virus neutralization. The genome of the rotavirus consists of 11 segments of double-stranded RNA that are readily resolved by polyacrylamide gel electrophoresis (PAGE). The resulting RNA migration pattern is termed electropherotype, and there are two major RNA migration patterns, "long" and "short", based on the relative migration rate of gene segments 10 and 11. The electropherotype is unique to an individual strain, and has long been used to track down the individual strains in epidemiologic studies of rotavirus infection [6]. Major observations made in such molecular epidemiologic studies include: (i) multiple strains co-circulate in the same epidemic season; (ii) only one or two strains predominate in each season; and (iii) different strains emerge in each epidemic season [8, 11, 15].

One lingering question that has been addressed is whether various characteristics of rotavirus strains such as G- and P-serotypes are associated with the severity of illness upon infection. There have been studies that aimed to determine whether the virulence of human rotavirus differs according to such virological parameters, but the results are inconclusive [1, 2, 12, 17, 19, 21]. Most of these preceding studies focused on serotypes or subgroups rather than individual strains. Furthermore, few studies have examined whether the virulence differed among multiple strains co-circulating in the same geographic region during the same epidemic season. In addressing this question, we made an assumption that, if there were virulent and less-virulent strains among human rotaviruses, there should be some detectable differences in the distribution between rotavirus strains isolated from hospitalized patients who had severer diarrhea and those isolated from outpatients who had less severe disease. Thus, the aim of this study was to determine whether there was any difference in the distribution among circulating strains, as identified by their electropherotypes, between rotaviruses isolated from hospitalized children with diarrhea and those isolated from children treated only at the outpatient department of the hospital.

Materials and methods

Stool specimens

Stool specimens were collected from patients with acute diarrhea (aged 15 years or younger), who had been treated at a general hospital in Honjo City, Akita Prefecture, Japan, between January 1987 and December 1996. In this study, children who required hospitalization were classified as the inpatient group, and those who did not require hospitalization and treated only at the outpatient department of the same hospital were classified as the outpatient group.

Because no rotavirus-positive specimens were found between August and September during the study period, each rotavirus season was defined as 12 months beginning in September and ending in August of the next year. Thus, the whole study period spanned 11 consecutive rotavirus seasons, although the first season (86–87) extended from January to August 1987 (8 months), and the last season (96–97) extended from September to December 1996 (four months).

Detection of rotaviruses, RNA extraction, and electrophoresis

The presence of rotaviruses in stool specimens was initially examined by the latex agglutination assay. From approximately 10% suspensions of rotavirus-positive fecal specimens, genomic RNA was extracted with phenol and chloroform, and precipitated in ethanol. For those specimens containing only a small amount of viral RNA, genomic RNA was extracted after ultracentrifugation using a Beckman TLA 100.4 rotor at 60,000 rpm for 1 hour. Genomic RNA was resolved by PAGE, with a 10% separating and a 4% stacking gel without sodium dodecyl sulfate in the Laemmli buffer system. After electrophoresis for 16 hours at a constant current of 8 mA per gel, gels were stained with ethidium bromide, followed by visualization of the RNA bands under UV illumination. Wa (G1, P1A [8]) and DS-1 (G2, P1B [4]) were used as reference strains.

Nomenclature of the electropherotypes was essentially the same as that described by Koshimura et al. [11]. However, a letter O was inserted after either LH (long RNA pattern) or SH (short RNA pattern) to denote strains only from the outpatient group. A strain was defined as the dominant strain when a single electropherotype was found in more than 50% of the inpatient or the outpatient groups in any one season. Two strains were defined as co-dominant if the electropherotype of each strain accounted for more than 25% but less than 50% of all strains in any one season.

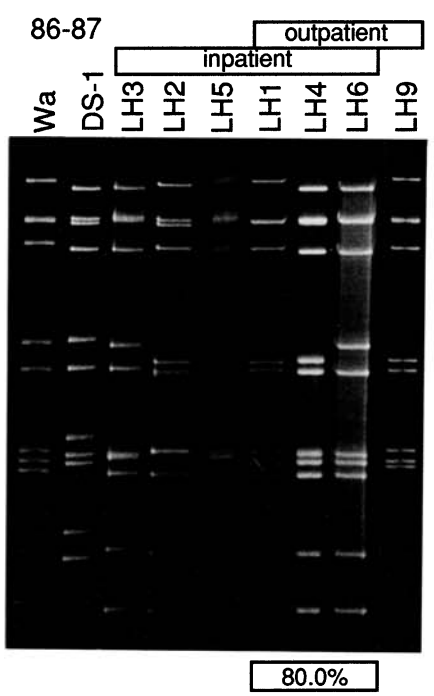
Determination of G-serotype

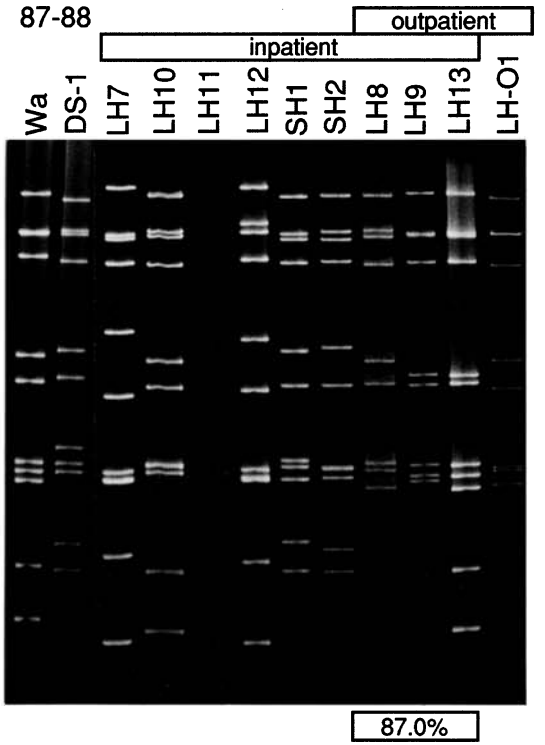
The G-serotype of rotaviruses was determined by enzyme-linked immunosorbent assay (ELISA), using monoclonal antibodies for serotypes G1-4 (ROTA-MA, Serotec Co, Sapporo, Japan) [22] as capture antibodies. The procedure and the judging criteria were as described previously [11]. Reverse transcription (RT)-PCR was applied to specimens whose serotype could not be determined, using consensus and type-specific primers for G1-4 [4, 13].

Statistical analysis

The chi-square test was used to examine whether there was any difference in the distribution of rotavirus strains between the inpatient and outpatient groups. When the expected counts under the null hypothesis were less than five in 25% or more of the cells, Fisher's exact test was used. Statistical analysis of dominant strain distribution was performed for each rotavirus season, since previous studies showed that different strains emerge in each season [8, 11]. We combined minor strains into one category, since the data were statistically sparse, and infrequent strains are, by definition, less important from the epidemiological point of view. It was also taken into consideration that dominant strains accounted for more than 70% of rotaviruses detected from all strains identified during the 10-year study period [11]. In addition, to evaluate the virulence of each strain (as identified by electropherotype), a case control study was performed for each strain in which the presence or absence of exposure to the particular strain (electropherotype) was compared in inpatients (cases) and outpatients (controls).

The ratio of the number of subjects in each rotavirus season to the total number of subjects was not constant between the inpatient and the outpatient groups. Therefore, the percentage of G-serotype and RNA pattern (long or short) was standardized for each rotavirus season to minimize the possibility of confounding, using as weight the proportion of the number of inpatients and outpatients for each rotavirus season amongst the total of 763 patients. Multiple logistic regression analysis, with or without adjustment for rotavirus season, was also applied to evaluate whether there was any association between either the G-serotype or the RNA pattern and hospitalization. All statistical analyses were performed using the SAS software package [18], and all *P*-values presented are two-tailed.





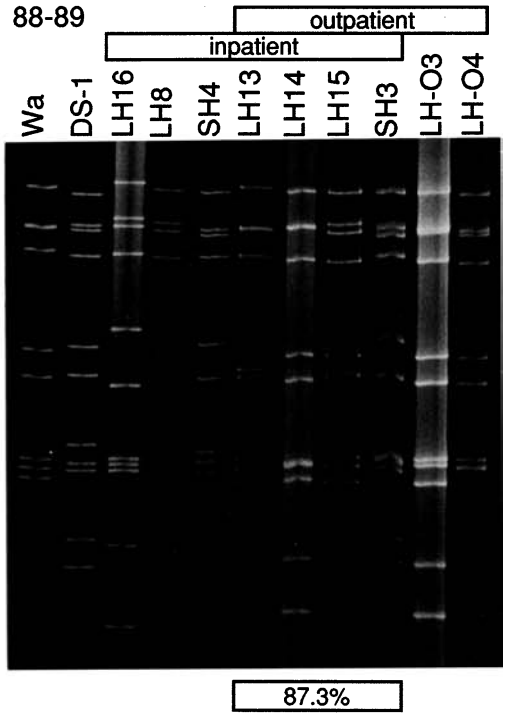


Fig. 1 (*continued*)

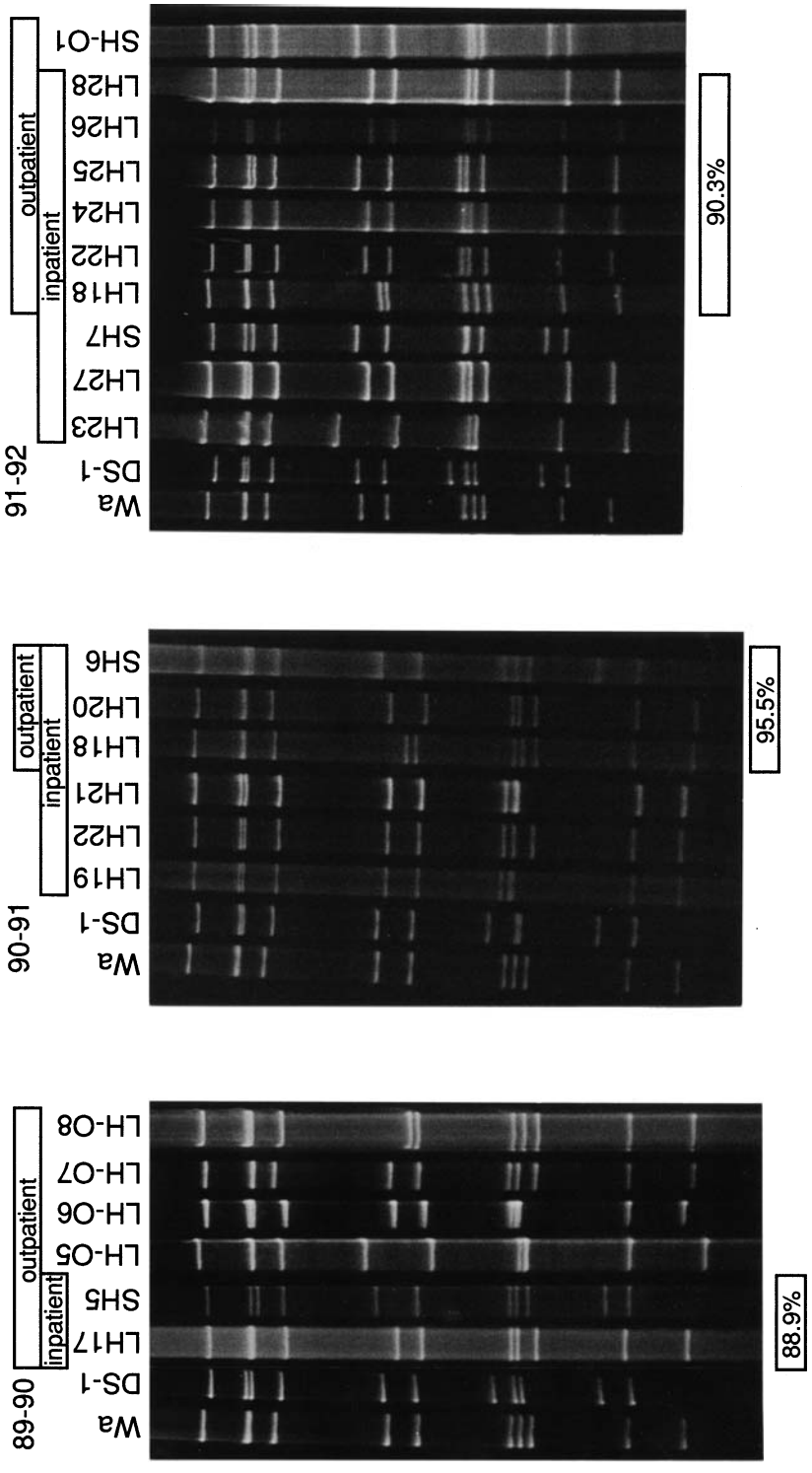
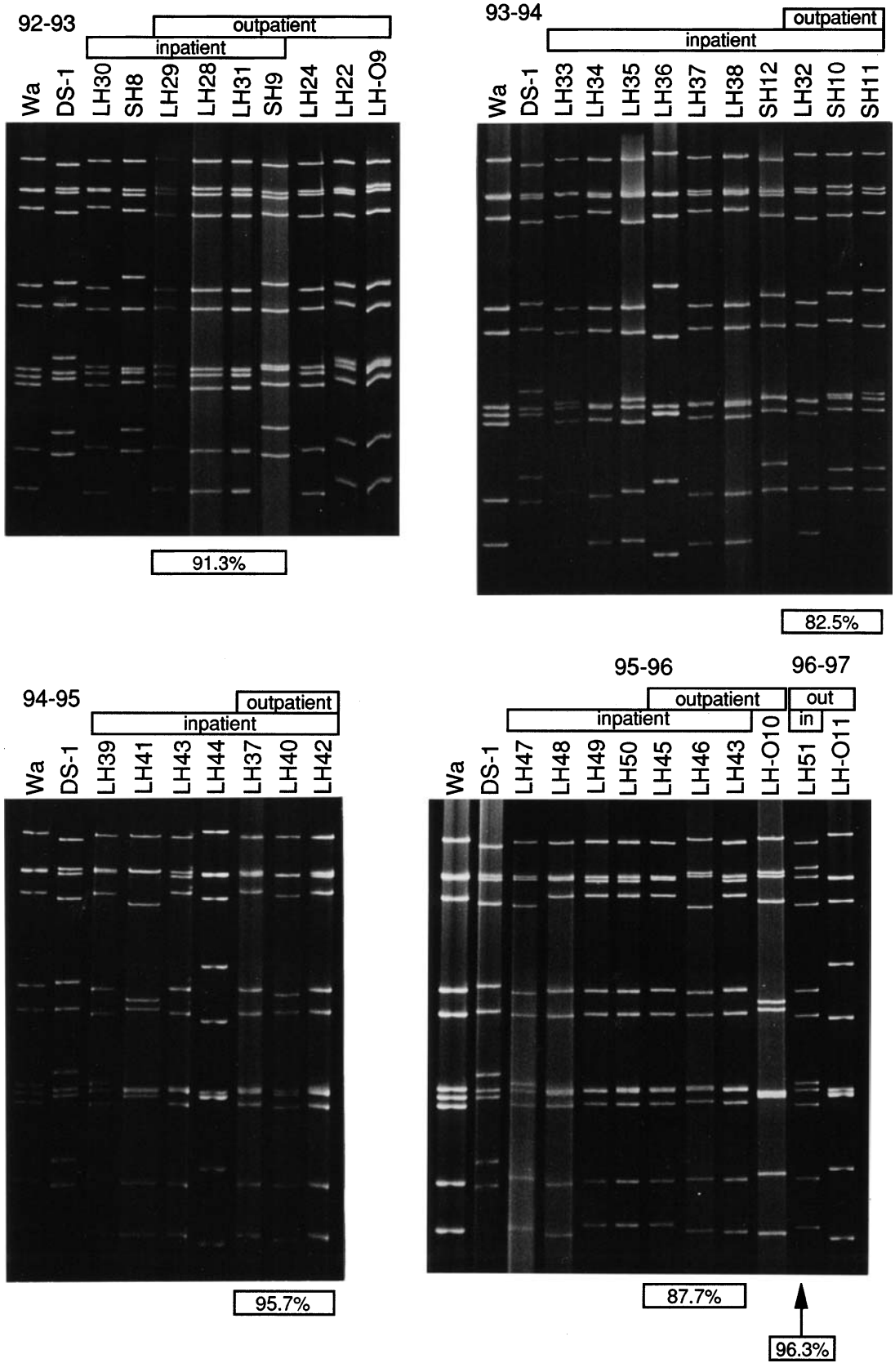


Fig. 1 *(continued)*


92-93
outpatient
inpatient
Wa
DS-1
LH30
SH8
LH29
LH28
LH31
SH9
LH24
LH22
LH-O9
91.3%
93-94
outpatient
inpatient
Wa
DS-1
LH33
LH34
LH35
LH36
LH37
LH38
SH12
LH32
SH10
SH11
82.5%
94-95
outpatient
inpatient
Wa
DS-1
LH39
LH41
LH43
LH44
LH37
LH40
LH42
95.7%
95-96
96-97
outpatient
out
inpatient
in
Wa
DS-1
LH47
LH48
LH49
LH50
LH45
LH46
LH43
LH-O10
LH51
LH-O11
87.7%
96.3%

Results

The initial screening by latex agglutination assays identified a total of 1906 rotavirus-positive stool specimens, of which 1018 were obtained from inpatients and 888 from outpatients. Because of either a limited amount of stool specimens available for further analysis or an insufficient amount of rotavirus particles present in stool specimens, only those rotaviruses that were present in 763 stool specimens (488 from inpatients and 275 from outpatients) were classified into 77 strains after repeated PAGE on the basis of possessing distinct electropherotypes. Except for the 96–97 season, for which the initial four months were included in this study, there was always co-circulation of several strains in every epidemic season, as evidenced by the presence of multiple electropherotypes (Fig. 1). The number of co-circulating strains was more or less similar for both the inpatient and the outpatient groups during each rotavirus season (Fig. 1). Table 1 gives the number of rotavirus-positive specimens that contained each of the distinct electropherotypes shown in Fig. 1, according to the status of the two patient groups. In both the inpatient and outpatient groups, a single strain was dominant in six epidemic seasons (88–89, 89–90, 90–91, 92–93, 94–95, 96–97), whereas two strains were co-dominant in the remaining five seasons (86–87, 87–88, 91–92, 93–94, 95–96). No strain persisted as dominant or co-dominant during any two successive seasons (Fig. 1). While long RNA patterns dominated in most seasons, short RNA patterns prevailed during the 90–91 and the 93–94 seasons (Fig. 1). In all seasons, the same strains dominated or co-dominated among the outpatient and inpatient groups. A few less-frequent strains were exclusively found in either the outpatient or the inpatient group. Furthermore, strains that were detected from both the inpatient and outpatient groups accounted for the great majority of the number of rotavirus-positive specimens, ranging from 80% in the 86–87 season to 96% in the 96–97 season (Fig. 1). This agrees with the premise that the background community population from which both outpatients and inpatients derived was universally exposed to rotavirus strains in a given season.

To examine whether such supposedly-universal exposure of rotavirus strains to the background population at risk would result in a distorted distribution between the inpatient and the outpatient groups, the chi-square test was performed for each epidemic season, with infrequently circulating strains being grouped into one category. As the *P* values in Table 1 indicate, there was no statistically significant difference in the distribution of dominant, co-dominant, or infrequent strains in any of the 11 seasons. Even during the 87–88 and the 90–91 seasons, in which the distribution of rotavirus strains was slightly different between the inpatient and the outpatient groups, it did not differ to a statistically significant degree ($p = 0.08$ and 0.09, respectively). Thus, the chi-square test result does not reject the null

Fig. 1. The electropherotypes of all strains detected from the inpatient and outpatient groups during each rotavirus season. Strains that were detected from both the inpatient and outpatient groups accounted for the great majority of the rotavirus-positive specimens detected in each rotavirus season, as indicated by the percentages at the bottom of each panel

Table 1. Distribution of rotavirus strains among inpatients and outpatients with acute diarrhea, according to the epidemic season of rotavirus, Honjo City, Akita, Japan, 1986–1997

Season	Inpatients	Outpatients	*P*-value
86–87	22[a] **LH1**, 6 **LH4**, 8 LH3, 1 LH2, 1 LH5, 1 LH6	7 **LH1**, 6 **LH4**, 2 LH6, 1 LH9	0.23[b]
87–88	21 **LH9**, 15 **LH13**, 3 LH10, 2 SH2, 1 LH11, 1 LH12, 1 LH7, 1 LH8, 1 SH1	18 **LH13**, 8 **LH9**, 4 LH8, 1 LHO1	0.08[c]
88–89	16 **LH14**, 4 LH15, 2 LH13, 2 LH16, 1 LH8, 1 SH3, 1 SH4	20 **LH14**, 5 LH15, 5 SH3, 2 LHO4, 2 LH13, 1 LHO2, 1 LHO3	0.77[c]
89–90	18 **LH17**, 6 SH5	13 **LH17**, 3 SH5, 2 LHO6, 1 LHO5, 1 LHO7, 1 LHO8	0.34[c]
90–91	34 **SH6**, 1 LH18, 1 LH19, 1 LH20, 1 LH21, 1 LH22	19 **SH6**, 6 LH18, 2 LH20	0.09[c]
91–92	14 **LH22**, 12 **LH24**, 5 LH28, 3 LH26, 2 LH18, 1 LH23, 1 LH25, 1 LH27, 1 SH7	10 **LH24**, 4 **LH22**, 2 LH28 2 SHO1, 1 LH18, 1 LH25, 1 LH26, 1 SHO2	0.31[c]
92–93	34 **LH31**, 8 LH28, 5 LH29, 2 SH8, 1 LH30, 1 SH9	22 **LH31**, 7 LH28, 4 LH29, 3 SHO3, 2 SH9, 1 LH22, 1 LH24, 1 LHO9	0.20[c]
93–94	42 **LH32**, 17 **SH10**, 8 LH35 5 LH34, 4 SH11, 1 LH33, 1 LH36, 1 LH37, 1 LH38, 1 SH12	9 **LH32**, 8 **SH10**, 5 SH11	0.33[c]
94–95	72 **LH37**, 4 LH40, 2 LH39, 1 LH41, 1 LH42, 1 LH43, 1 LH44	30 **LH37**, 3 LH40, 1 LH42	1.00[b]
95–96	20 **LH45**, 12 **LH43**, 5 LH46 3 LH50, 1 LH47, 1 LH48, 1 LH49	7 **LH45**, 5 **LH43**, 1 LH46 1 LHO10	0.68[b]
96–97	16 **LH51**	10 **LH51**, 1 LHO11	0.41[b]

[a]The number of rotavirus-positive stool specimens that possessed a distinct electropherotype. In this case, electropherotype LH1

[b]By Fisher's exact test

[c]By Chi-square test

hypothesis that there is no difference in the distribution of strains between the two different severity groups, thereby implying that the majority of rotavirus strains are similar in their inherent ability to cause the disease in infected children.

In order to evaluate the severity of individual strains that dominated or co-dominated in each of the 11 rotavirus seasons, case control studies were performed in which it was examined by calculating an odds ratio whether exposure to each of the dominant or co-dominant strains tended to result in hospitalization rather than in medical visit. As shown in Table 2, all dominant or co-dominant strains observed over the 11 rotavirus seasons except LH13 in the 87–88 season

Table 2. Virulence of individual strains as evaluated by crude odds ratios of hospitalization in case control studies in which exposure to the given strain was analyzed by hospitalization as outcome during the epidemic season of rotavirus, Honjo City, Akita, Japan, 1986–1997

Season	Strain	Odds ratio of hospitalization (95% CI)
86–87	LH1	1.66 (0.51–5.38)
	LH4	0.30 (0.08–1.15)
87–88	LH9	2.41 (0.90–6.51)
	LH13	0.35 (0.14–0.90)
88–89	LH14	1.16 (0.42–3.20)
89–90	LH17	1.85 (0.52–6.62)
90–91	SH6	2.86 (0.82–10.0)
91–92	LH22	2.42 (0.69–8.57)
	LH24	0.51 (0.18–1.51)
92–93	LH31	1.73 (0.74–4.03)
93–94	LH32	1.56 (0.60–4.04)
	SH10	0.46 (0.17–1.29)
94–95	LH37	0.96 (0.28–3.30)
95–96	LH45	0.87 (0.26–2.91)
	LH43	0.70 (0.19–2.51)
96–97	LH51	undefined

failed to place infected individuals at an increased risk for hospitalization at a statistically significant level. However, individuals infected with the LH13 strain were statistically significantly less likely to be hospitalized ($p = 0.027$).

Table 3. Association between G-serotype of rotaviruses and hospitalization in patients with acute diarrhea, Honjo City, Akita, Japan, 1986–1997

G-serotypes	Inpatients			Outpatients			OR[b] (95% CI)	OR[c] (95% CI)
	No.	(%)	(%)[a]	No.	(%)	(%)[a]		
G1	375	76.8	76.2	207	75.3	74.8	1.0	1.0
G2	71	14.6	14.9	47	17.1	18.3	0.83 (0.56–1.3)	0.77 (0.45–1.3)
G3	10	2.1	2.4	4	1.5	1.3	1.4 (0.46–5.1)	2.2 (0.68–8.2)
G4	26	5.3	5.5	10	3.6	3.6	1.4 (0.70–3.2)	2.0 (0.85–5.1)
NT	6	1.2	1.1	7	2.6	2.0	0.47 (0.15–1.4)	0.46 (0.14–1.5)
Long	417	85.5	85.1	227	82.5	81.4	1.0	
Short	71	14.6	14.9	48	17.5	18.6	0.81 (0.54–1.2)	0.71 (0.41–1.2)
Total	488	100	100	275	100	100		

[a]Proportion standardized for rotavirus season

[b]Crude odds ratio of hospitalization (reference = G1 or long RNA pattern)

[c]Odds ratio of hospitalization adjusted for rotavirus season. *CI* Confidence interval; *NT* not typed

To examine whether another viral attribute, the G-serotype, was associated with the virulence of rotavirus, analysis was performed on 756 of the 763 electropherotyped specimens for which the G-serotype was determined. Overall, the serotype G1 was the most prevalent (76.3%), followed by G2 (15.5%), G4 (4.7%), and G3 (1.8%). Irrespective of being standardized for rotavirus season, the relative frequencies of the G-serotype were similar between the inpatient and the outpatient groups (Table 3). This similarity was further confirmed by multiple logistic regression analysis, which showed that the G-serotype was not significantly associated with hospitalization (Table 3). When serotype G1 was used as a reference, the adjusted odds ratio of hospitalization was 2.2 for G3 and 2.0 for G4, but was not significant, as reflected by the wide 95% confidence intervals, due partly to the small number of subjects.

With two exceptional rotavirus specimens that possessed a long RNA pattern and serotype G2, all strains ($n = 116$) having a short RNA pattern belonged to serotype G2, whereas all strains having a long RNA pattern belonged to any one of G1 ($n = 582$), G4 ($n = 36$), and G3 ($n = 14$), or an untypeable G-serotype ($n = 10$). Irrespective of being standardized for season, the relative frequency of each RNA pattern was similar between the two groups (Table 3). In multiple logistic regression analysis, a short RNA pattern was not significantly associated with hospitalization, with an odds ratio of 0.71 relative to a long pattern, after adjustment for season (Table 3).

Discussion

The question of whether some strains cause severer gastroenteritis than others has its highest epidemiologic importance when it is concerned with the strains that prevail in each epidemic season, because such dominant strains are most likely to make a visible impact on the annual incidence of rotavirus-associated medical visits and hospitalizations. Infrequently circulating, minor strains with increased pathogenicity may cause severe disease. However, they are less likely to affect the overall increase in the number of hospitalizations because such minor strains, by definition, do not account for the majority of rotavirus gastroenteritis cases in the population. For this reason, this study focused on dominant or co-dominant strains as the primary target of analysis.

As for parameters that characterize rotavirus in detail, we selected electropherotype as the primary identifier of rotavirus strains. The reasons are twofold: First, each rotavirus strain shows a single distinct electropherotype upon PAGE, thereby making it practically feasible to define a strain by its electropherotype. Second, the virulence of rotavirus has not been unambiguously assigned to any single gene or any particular set of genes, thereby making it difficult to determine which gene(s) or gene product(s) to look at in association with disease severity. In animal models, for example, virulence segregated with the VP4 gene when reassortant viruses derived from parental strains that varied in their virulence for newborn mice were studied [16]. In the gnotobiotic porcine model, however, the genes encoding VP3, VP4, VP7, and NSP4 of a virulent porcine rotavirus each

played an independent role in virulence, and substitution of any one of these genes by the cognate gene from a human rotavirus that did not cause diarrhea in piglets resulted in an asymptomatic infection in piglets [7]. We therefore thought that it would be the best and the most practical to explore the association of disease severity and viral strains, not at the level of any single characteristic of rotavirus, but at the level of individual strains, which are most precisely defined by electropherotype upon PAGE.

Because this study extended over a 10-year period, it included 16 dominant or co-dominant strains, each of which circulated in one of 11 rotavirus epidemic seasons. It is particularly noteworthy that there was one dominant and another co-dominant short-RNA strain in different epidemic seasons, both of which were serotype G2, as is almost always the case. Thus, we were able to test each of these dominant G2 short RNA strains separately.

One distinctive feature of rotavirus epidemiology is the fact that multiple strains concurrently circulate in the same epidemic season. It is therefore logical to ask whether the dominant strain causes severer disease than other strains. In this regard, this study provided a suitable opportunity to address such a question.

Although a large number of strains from over a long period of time were examined, we were unable to find a significantly distorted distribution of dominant, co-dominant, or minor strains between severely affected children (inpatients) and children with milder gastroenteritis (outpatients). Furthermore, when dominant or co-dominant strains were individually tested in case control studies, it was also found that there was no statistically significantly increased risk of hospitalization. However, it was found that infection with LH13, one of the co-dominant strains in the 87–88 season resulted in a reduced risk of hospitalization. It may merit mention that LH13, which had serotype G1 appeared different only in genome segment 9 (the VP7 gene) from LH9, the other co-dominant strain in the same season, which had serotype G4 (Fig. 1) [11]. Whether this serotypic difference played any role was not clear, however. We therefore conclude that human rotavirus strains that prevail in each epidemic season have similar virulence in general, while an uneven distribution of strains between severe and less severe cases may still happen.

Several earlier studies have tried to find an association between disease severity and several characteristics of rotaviruses, such as subgroup, G-serotype, P-serotype and electropherotype (RNA pattern), but the results from these preceding studies were inconclusive. One study claimed that it examined the association between electropherotype of rotavirus strains and disease severity [2]. In that study, however, electropherotype was defined as the collection of similar but not necessarily identical RNA migration patterns on PAGE, as clearly indicated by the fact that strains with different G-serotypes were assigned to a single electropherotype. It is extremely rare, if not nonexistent, that two strains with identical electropherotype have different G serotypes [14].

In a previous study involving 118 Swedish patients with diarrhea, fever was more frequently observed for subgroup I (short RNA pattern) rotaviruses, whereas diarrhea and vomiting were severer for subgroup II (long RNA pattern) [21]. Bern et al. [1] reported that serotypes G2 and G3 were associated with significantly

severer dehydration than other G-serotypes among 718 Bangladeshi children. However, the authors did not choose to stress its clinical significance because the difference was statistically significant but very subtle. Cascio et al. [2] reported that a strain with G2P1B[4] was associated with severer gastroenteritis among 401 Italian children. Mota-Hernandez et al. [12] showed that the severity of gastroenteritis and the frequency of dehydration and hypovolemic shock were significantly higher for strains with serotype G3 and an untypeable P type than for G3P1A[8] and G1P1A[8] in Mexico. Cascio et al. [2] and Mota-Hernandez et al. [12] speculated that introduction of a rare and new G- or P-serotype into the community resulted in the occurrence of severer diarrhea. Recent outbreaks of gastroenteritis associated with G2 rotavirus among adults in three different locations in the United States of America are good examples of weakened herd immunity [5]. However, it may be a separate issue whether a rare serotype influences the severity of gastroenteritis during early childhood, which is usually caused by the first encounter with rotavirus [23]. In contrast, Fruhwirth et al. [3] reported that there was no significant difference in the distribution of G- or P-types among outpatients, inpatients, and nosocomially infected patients in Austria. A similar conclusion was also drawn by a recent study in Mexico, in which Polanco-Marin et al. [17] showed that severity of diarrhea was not significantly associated with either particular serotype or subgroup.

Thus, there are papers in the literature that have shown a positive association of a particular G-serotype or a particular combination of G-and P-serotypes with severer forms of gastroenteritis, whereas other papers did not support such observations. The specific reasons for the different conclusions drawn from our study and some other studies [1, 2, 12] are hard to give, but it deserves mention that our study analyzed the largest number of rotavirus specimens at the level of individual strains for the multiple rotavirus seasons spanning a 10-year period. Thus, our study may be less subject to haphazard variables or yet unknown confounding factors emerging from season to season.

There are, however, limitations in our study that deserve clarification and brief discussion. First, the necessity for hospitalization was taken as the sole outcome measure of disease severity, and it may be less precise and more liable to misclassification than scoring systems that have been used to quantify disease severity in rotavirus vaccine trials. The rationale behind this is that the ultimate goal of a rotavirus vaccine in developed countries is not to prevent mild illness or infection, but to prevent children from severe dehydrating diarrhea leading to hospitalization [10]. Moreover, it is the direct medical cost of hospitalization that heavily affects the economic consequences of rotavirus gastroenteritis [20].

Second, no clinical data were collected for patients who were hospitalized or who were treated only at the outpatient department. Thus, no further stratification is possible regarding the host factors, including age. As discussed earlier, weakened herd immunity was speculated to be one possible cause of gastroenteritis outbreaks due to serotype G2 rotavirus [5]. Unfortunately, we are not able to address this issue in this study. However, such stratification, in general, would be more meaningful in cases in which statistically significant differences were observed, because it

would then require determination of whether a distorted distribution of strains is ascribed to the virus strain or the host factors.

Third, we included only a fraction of children who were infected with rotavirus strains since it is thought that there were a large number of children who did not seek medical intervention because of the mildness of the symptoms.

Fourth, the results obtained for LH9 and LH13 (Table 1) approached a statistically significant level, and the odds ratio for LH13 actually reached a significance level (Table 2). We therefore admit that less virulent or more virulent strains may exist, which this study failed to detect due to the lack of statistical power.

Despite these limitations, our results suggest that the virulence or disease-causing potential of human rotaviruses that dominantly circulate in each epidemic season does not substantially differ in the majority of strains.

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