

An Outbreak of Gastroenteritis During School Trip Caused by Serotype G2 Group A Rotavirus

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Between May 14 and 18, 2001, there was an outbreak of acute gastroenteritis involving 45 school children out of a total of 107 (aged 11–12 years) attending a 3-day school trip. The epidemic curve characterized by a rapid onset and decline with a single peak incidence over a 5-day period resembled the pattern typical of a food-borne gastroenteritis outbreak. Epidemiological and virological investigations concluded, however, that this outbreak was caused by a single strain of serotype G2 group A rotavirus spreading to schoolmates from the primary case-pupil who had already been ill at the start of the trip. Efficient person-to-person transmission was likely to have occurred due to prolonged and close contacts under the conditions typical of such school trips. This study emphasizes the importance of including group A rotavirus infection as a possible cause of gastroenteritis outbreaks even in older children and adults. *J. Med. Virol.* 73:460–464, 2004. © 2004 Wiley-Liss, Inc.

KEY WORDS: person-to-person transmission; diarrhea; epidemiological investigation

INTRODUCTION

Group A human rotavirus is the single most important etiological agent of acute gastroenteritis in infants and young children worldwide [Kapikian et al., 2001]. Rotavirus infections in humans continue to occur throughout their lives but the resulting disease is believed to be milder and often asymptomatic [Bishop, 1996]. However, a renewed attention has been paid to rotavirus infection in adults and a 4-year prospective study conducted recently in Japan showed approximately 15% of acute gastroenteritis patients seeking medical attention was due to group A rotavirus infection

[Nakajima et al., 2001]. In addition to such sporadic cases of acute gastroenteritis in adults, outbreaks of acute gastroenteritis in school-aged children and adults have increasingly been reported [Centers for Disease Control and Prevention, 2000; Griffin et al., 2002; National Institute of Infectious Diseases, 2000a–d]. Interestingly, such outbreaks appear to be caused more frequently by serotype G2 strains than can be predicted by their relative frequency at which they were found from infants and young children with rotavirus gastroenteritis [Koshimura et al., 2000]. The other point of interest from the public health perspective is in the fact that it is often hard to determine the mode of transmission in such outbreaks, i.e., whether the virus was spread through contaminated food (food poisoning) or from person to person (infectious disease) because such information may critically affect the administrative disposition or litigation.

Molecular epidemiology often helps determine the source and route of transmission in a variety of infectious diseases. The molecular epidemiology of rotavirus is potent in this aspect, since the virion contains 11 segments of double-stranded RNA which are readily separated upon polyacrylamide gel electrophoresis [Estes et al., 1984; Holmes, 1996]. While the RNA migration pattern, termed electropherotype, is unique to each isolate and there are a myriad of electropherotypes, long and short RNA patterns are identified based on the relative migration rates of gene segments 10 and 11 [Kutsuzawa et al., 1982a; Estes et al., 1984; Nakagomi et al., 1988; Holmes, 1996].

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Each genome segment codes for either one of the structural proteins (VP1–VP4, VP6 and VP7) or one of the nonstructural proteins (NSP1–NSP4), with the exception of gene segment 11 coding for two nonstructural proteins (NSP5 and NSP6) in two different reading frames [Estes, 2001]. Two neutralization antigens are present on the rotavirus virion, and the one present on the surface of the outer capsid is termed the G serotype and the other present on the spike protein is termed the P serotype. The G serotype is coded for by one of closely-migrating segments 7, 8 and 9 dependent on the strain, and the P serotype is encoded by genome segment 4 [Estes, 2001]. In previous field trials of a rotavirus vaccine, it was shown that the agreement of the circulating G serotype and the vaccine virus was correlated with the vaccine efficacy [Kapikian et al., 1996a,b], suggesting that the G serotype plays a significant role in protective immunity. Serotype G2 strains usually, but not always, carry the genome of short RNA pattern [Nakagomi et al., 1988; Kaga and Nakagomi, 1994; Koshimura et al., 2000].

Here, we report an outbreak of rotavirus gastroenteritis during a 3-day school trip that looked like a typical food-borne gastroenteritis but was more likely to be caused by person-to-person transmission because the primary case was already ill at the beginning of the trip. The outbreak was caused a single strain of group A rotavirus carrying serotype G2, P1B[4].

MATERIALS AND METHODS

Stool specimens were obtained from 10 case pupils and, after the routine examination for common bacterial pathogens responsible for food poisoning had turned out negative, they were subjected to the examination for gastroenteritis viruses including group A and C rotaviruses, Norwalk virus and adenovirus. Only the presence of group A rotavirus was confirmed in 8 stool specimens either by a commercially-available enzyme-linked immunosorbent assay (ELISA), Rotaclone (Meridian Diagnostics, Cincinnati, OH), or by direct observation under electron microscope. For further characterization, these rotavirus positive specimens were coded as AM1 (onset at 12:00 on 16 May, specimen collected on 17 May), AM2 (onset at 7:00 on 14 May, specimen collected on 17 May), AM3 (onset at 8:00 on 15 May, specimen collected on 18 May), AM4 (onset at 3:00 on 17 May, specimen collected on 17 May), AM5 (onset at 4:30 on 16 May, specimen collected on 18 May), AM6 (onset at 16:00 on 16 May, specimen collected on 19 May), AM7 (onset at 22:00 on 16 May, specimen collected on 19 May) and AM8 (onset at 3:00 on 17 May, specimen collected on 19 May).

Cell-culture adaptation of rotaviruses was performed essentially as described previously [Kutsuzawa et al., 1982b] using the roller tube apparatus with 0.5 µg of trypsin (type IX, Sigma Chemical Company, St. Louis, MO) per ml.

G serotype was determined by the typing method based on reverse-transcription (RT)-polymerase chain

reaction (PCR) according to the method described by Gouvea et al. [1990]. P genotype was determined by RT-PCR described by Gunasena et al. [1993].

Virus particles in the infected MA104 cells were concentrated by pelleting at 50,000 r.p.m. for 1 hr in a Beckman type 70.1 Ti rotor, and the genomic RNA was extracted with phenol and chloroform, precipitated with ethanol, taken up in an appropriate amount of the buffer (pH 8.0) consisting of 10 mM Tris–5 mM EDTA, and loaded on 10% polyacrylamide gels [Nakagomi et al., 1988].

The reference rotavirus strains used in this study were Wa (G1, P1A[8]) [Wyatt et al., 1980] and KUN (G2, P1B[4]) [Kutsuzawa et al., 1982b].

RESULTS

The Outbreak

Between 14 and 18 May 2001, 45 (42.5%) of 107 school children (aged 11–12 years) at a primary school in Hachinohe city, Aomori Prefecture, Japan, suffered from acute gastroenteritis. Their symptoms were fever (84%), vomiting (71%), diarrhea (69%), nausea (67%), abdominal pain (64%), easy fatigability (47%) and headache (43%). Of 45 pupils who were affected, 37 (82%) sought medical attention. The outbreak occurred during a 3-day roundtrip (14–16 May) to the Southern part of Hokkaido, some 300 km away from their hometown. None of six teachers who were traveling with and taking care of the pupils had symptoms suspected of gastroenteritis. When the number of pupils who got ill was plotted according to the time line (Fig. 1), it was noted that there was a sharp increase of the cases toward the third day (16 May) and in the afternoon of the third day there was the peak with 15 (33%) pupils getting ill. After returning from the school trip, the number of new cases dropped sharply on day 4 (17 May) with 12 (27%) new cases in the morning and 7 (16%) in the afternoon, all of which were considered to have been infected during the school trip. The outbreak ended on day 5 (18 May) with the last two new cases. This sharp single-peak epidemic curve was suggestive of a single exposure to the causative agent and typical of food-borne gastroenteritis, but the outbreak investigation revealed that there was no case identified in other parties who shared food in the restaurants and hotels where the case pupils dined and stayed. On the other hand, it was found that one pupil had complained nausea and had actually vomited at the outset of the school trip and that he had specially been taken care of by the school nurse attending this school trip.

Detection of Group A Rotavirus

Stool specimens obtained from 10 pupils who had gastroenteritis were negative for bacterial pathogens. RT-PCR for Norwalk virus was also negative for these 10 specimens. While it was originally intended to look for Norwalk virus that might have escaped detection by RT-PCR due to primer mismatch, observation under the

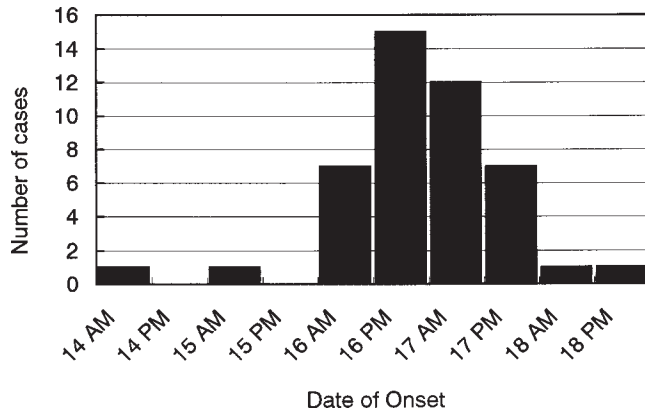


Fig. 1. The number of gastroenteritis cases among school children attending the 3-day school trip, by the date of disease onset. In this figure, a day is divided into the morning (from 0:00 before 12:00) which is denoted as AM and the afternoon (from 12:00 before 0:00) which is denoted as PM.

electron microscope unexpectedly revealed the presence of rotavirus particles in seven specimens (AM1–5, 7 and 8). Because electron microscopy was unable to distinguish group A rotavirus from group C rotavirus, these 10 specimens were subjected to testing by ELISA for group A rotavirus as well as reversed passive hemagglutination assay for group C rotavirus. While none was positive for group C rotavirus, seven (AM1–7) were positive for group A rotavirus. The eight stool specimens positive either by electron microscopy or ELISA were derived from the case pupils whose onset of illness spanned over the first 4 days of the outbreak, lending strong support for the hypothesis that this outbreak was caused by group A rotavirus.

Identification of the Causative Rotavirus as a Single Strain Carrying Serotype G2

Because group A rotavirus infection with an attack rate of 42% were thought to be uncommon in 11–12 year-old school children, further characterization of the causative strain including isolation in cell culture was performed to examine whether the strain possessed unusual characteristics and to determine whether the rotaviruses from the eight cases were identical to each other and particularly to the one from the pupil who had started gastrointestinal symptoms at the outset of the school trip. G serotyping by RT-PCR identified that all eight specimens contained serotype G2 rotavirus, and the P genotype of rotaviruses present in the specimens from AM2, AM3, AM4 and AM7 was determined to be P[4] (data not shown). When the genomic RNA was extracted directly from stool specimens and subjected to polyacrylamide gel electrophoresis, only the RNA from AM2 (the primary case whose onset was on 14 May) and AM3 (the second case whose onset was on 15 May) showed 11 segments of short RNA pattern, which was in agreement with serotype G2 specificity. Although isolation in cell culture was tried for all eight specimens, only four specimens, i.e., AM2, AM3, AM7 (onset on 16 May)

and AM4 (onset on 17 May) became ELISA-positive, indicating that isolation in cell culture was successful. Upon polyacrylamide gel electrophoresis, the electropherotype of the rotaviruses derived from these four culture-adapted rotavirus specimens were shown to be identical to each other (Fig. 2). In addition, this electropherotype was identical with that of the rotavirus present in the original stool specimen AM3 (data not shown). Taken these data together, it was concluded that this gastroenteritis outbreak was caused by a single strain of serotype G2 group A rotavirus with which the first case pupil had been infected even before the school trip had begun, and that the possibility of food poisoning was excluded.

DISCUSSION

The revision in 1997 of the food sanitation law and the related government regulations in Japan officially allowed public health investigators to include gastroenteritis viruses as the etiology of food poisoning (acute gastroenteritis due to contaminated food). According to

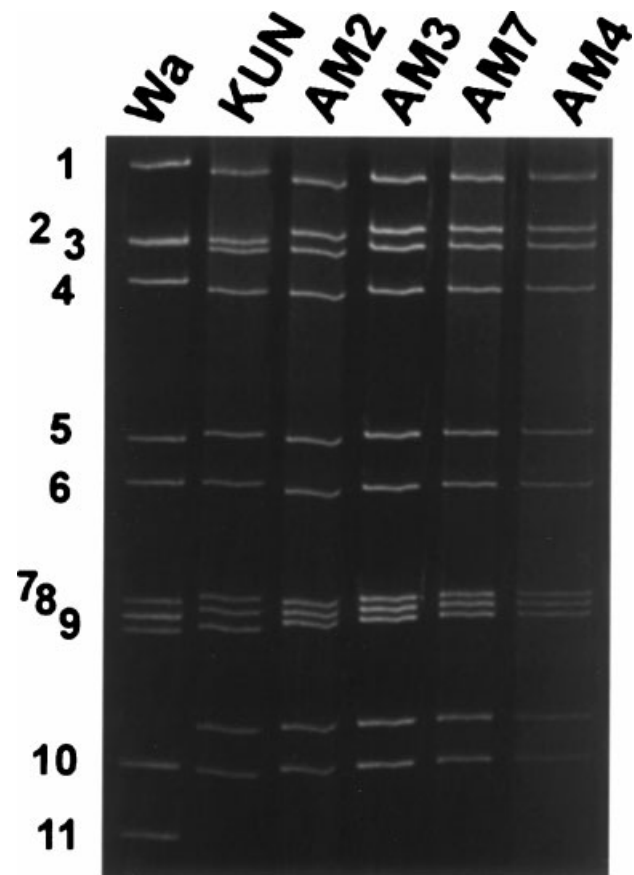


Fig. 2. The electropherotype of the rotaviruses derived from four culture-adapted rotavirus specimens in comparison with prototype strains possessing either long RNA pattern (strain Wa) or short RNA pattern (strain KUN). AM2 (the primary case whose onset was in the morning of 14 May), AM3 (the second case whose onset was in the morning of 15 May), AM7 (the onset was in the afternoon of 16 May) and AM4 (the onset was in the morning of 17 May). Approximate positions of the genome segments of strain Wa are indicated to the left.

recent government statistics (<http://www.mhlw.go.jp/topics/syokuchu/index.html>) there were approximately 250 incidents of viral food poisoning reported annually to the health authority accounting for about 15% of all food poisoning incidents in Japan. As to the number of patients involved in these viral food poisoning incidents, government statistics shows that there were approximately 8,000 patients per year, accounting for about 30% of the total number of patients suffering from food poisoning of all causes. Greater than 90% of such acute viral gastroenteritis outbreaks were caused by Norwalk virus.

Rotavirus infection repeats throughout life [Bishop, 1996] and about 10% of such infections occurring in older children and adults are symptomatic thereby forcing the infected persons to seek medical intervention [Nakajima et al., 2001]. Such infections, however, may escape the attention of pediatricians and physicians, since it is not a common practice to include rotavirus infection in the differential diagnosis of acute gastroenteritis in older children and adults. In fact, it was not until in 1994 that the first case of rotavirus gastroenteritis in the adult in Japan was reported [Kaga et al., 1994]. In outbreak settings only a very few but an increasing number of incidents caused by group A rotaviruses have been reported in the Infectious Agents Surveillance Reports issued by the Infectious Disease Surveillance Center of Japan. For example, in the spring of 2000, there were four incidents of acute gastroenteritis outbreaks all due to group A rotavirus infection [National Institute of Infectious Diseases, 2000a–d]. In the United States of America, group A rotavirus was reported to be the etiological agent of about 10% of gastroenteritis outbreaks for which the Centers for Disease Control and Prevention (CDC) examined samples for Norwalk virus [Griffin et al., 2002].

Interestingly, most of these rotavirus gastroenteritis outbreaks in older children and adults were caused by serotype G2 strains as was the first reported case in Japan [Kaga et al., 1994]. Four outbreaks in Japan were all caused by serotype G2 strains, although outbreak settings were different from one from another. One occurred among primary school children (aged 7–12 years) [National Institute of Infectious Diseases, 2000a], another occurred among otherwise healthy adults (aged 30–80 years) having dined in a restaurant [National Institute of Infectious Diseases, 2000c], the third occurred in a home for the handicapped (aged 50–79 years) [National Institute of Infectious Diseases, 2000d], and the fourth outbreak occurring in a home for the elderly people continued for one month [National Institute of Infectious Diseases, 2000b]. In the United States, all of three outbreaks occurring in a 2-year period between November 1998 and December 2000 were caused by serotype G2 strains. Two of the outbreaks occurred in nursing homes among elderly people and some nursing staff, and one occurred among university students (aged 18–22 years) and the affiliated cafeteria staff [Griffin et al., 2002]. Very recently, however, various G serotypes, including G2, were

detected from outbreak strains in aged care facilities in Australia [Marshall et al., 2003]. Thus, continued vigilance is needed in monitoring the serotypic diversity of rotavirus strains circulating in adults.

This apparently high incidence of serotype G2 gastroenteritis outbreaks among school-aged children, university students, adults and elderly people was clearly disproportionate to low relative frequency of serotype G2 strains recovered from children, primarily infants and young children. Koshimura et al. [2000] reported that the relative frequencies of the four major G serotypes over a 10-year period in Japan were 77.0% (G1), 14.5% (G2), 2.7% (G3) and 5.3% (G4) and that, when they reviewed 63 studies in the literature, the relative frequencies were 50.6% (G1), 9.3% (G2), 7.2% (G3) and 11.6% (G4). Over 90% of children are infected with rotavirus by the age of 3–5 years and it is reasonable to assume that the G serotype that the majority of children experience is most likely to be serotype G1. Older children and adults may therefore lack appropriate immunity against serotype G2 infection. While it is not completely understood how G serotype specific immunity affects protection from disease, earlier studies by Chiba et al. [1986] in which G serotype specific immune resistance to rotavirus gastroenteritis was investigated in relation to pre-existing neutralizing antibodies against homotypic and heterotypic rotaviruses may deserve mention. While protection against rotavirus gastroenteritis was serotype specific and was related to levels of antibody against homotypic virus, most children with G3 rotavirus infection achieved seroconversions or concomitant antibody responses to heterotypic G1 or G4 rotavirus at levels that were protective against disease. However, the protective level of heterotypic seroresponse against G2 was not observed in their study [Chiba et al., 1986]. According to the current understanding, serotype G1, G3 and G4 strains usually share the P serotype specificity, P1A[8]. The P serotype is defined by an independent neutralization antigen on VP4 and contributes to protective immunity, but the P serotype P1B[4] that is normally carried by G2 strains is different [Kaga and Nakagomi, 1994]. Furthermore, as Griffin et al. [2002] correctly pointed out, G2 strains are genetically distinct by high-stringency hybridization studies in all 11 genome segments from other human rotaviruses carrying G1, G3 and G4 specificities, thus being classified into a distinct genogroup [Nakagomi et al., 1989].

From the public health perspective, none of these outbreak studies took advantage of electropherotyping, a molecular epidemiological technique that has extensively been used in rotavirus studies [Estes et al., 1984; Holmes, 1996], to precisely identify and tracing rotavirus strains that cause outbreaks. This technique can be of particular help in determining the mode and route of transmission. In the incident reported here, food poisoning was suspected when two pupils, AM2 and AM3 whose onsets of the disease were 15 and 16 May, respectively, visited the outpatient department of a hospital. The outbreak investigation was initiated

keeping in mind both food poisoning and person-to-person spread. The epidemic curve (Fig. 1) shows a pattern typical of a single exposure outbreak, suggesting food-borne infection originating in common box lunches or one of the restaurants during the school trip. However, there was no case of gastroenteritis in other parties whose members had eaten the same box lunches or had taken meals in the same restaurants. Instead, investigation identified an apparent primary case-patient who had been ill at the outset of the trip as possible source of infection. The hypothesis of this pupil being the source was strengthened by the identification of the causative strain at the level of electropherotype. The exclusion of food poisoning was particularly important, because completely different administrative disposition might have been taken in the case of food poisoning. However, the exact mode of transmission, whether it is direct person-to-person transmission or indirect common vehicle transmission (sharing wash-room, for example) was not determined but the both modes were likely. Nevertheless, the occurrence of highly-efficient transmission as suggested by the epidemic curve probably reflected factors unique to and imposed by this type of school trips.

Limitations in our study include that stool specimens were not collected from pupils who showed no gastrointestinal symptoms and that no serum specimens were collected. Such studies, had they been performed, might have shown the presence of asymptotically infected children who might have shed the virus, thus contributing to the easy spread of the infection or addressing the question of whether those who had infected and developed disease were lower level of neutralizing antibody to serotype G2 than those who did not develop the disease.

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