

## 論文の内容の要旨

### MOLECULAR EPIDEMIOLOGY OF VIRAL GASTROENTERITIS AMONG INFANTS AND CHILDREN IN JAPAN AND BANGLADESH 日本とバングラデシュの乳幼児のウイルス性胃腸炎に関する分子疫学的研究

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#### 要旨

Acute gastroenteritis is one of the most common diseases in humans, and continues to be a significant cause of morbidity and mortality worldwide. The mortality among children due to acute gastroenteritis is greater in developing than developed countries. In the developed world, the impact of the illness is seen in its high morbidity and in the high incidence of hospitalization. Virus, bacteria, and parasite can cause an identical clinical syndrome, however, greater than 75% of cases are caused by viruses. In acute sporadic and endemic childhood diarrhea, rotavirus (RV), norovirus (NoV), sapovirus (SV), human astrovirus (HAstV) and adenovirus (AdV) are the common causes, whereas most of cases are related to RV and NoV in epidemic areas. Children under 5 years of age are particularly prone, and it is calculated that, in this group, there are more than 700 million cases of acute diarrhea every year. Despite much progress in the understanding of pathogenesis and of management with the widespread use of oral rehydration therapies, diarrheal illness remains one of the most important causes of global childhood mortality and morbidity. Therefore, the aim of this study was to perform the molecular and epidemiological trend of diarrheal viruses in infants and children with acute

gastroenteritis in Japan and Bangladesh.

This thesis comprises four chapters. In the first chapter, I described the molecular and epidemiological characteristics of viral gastroenteritis among infant and children in Japan. Between July 2006 and June 2007, 628 stool specimens were collected from infants and children presenting with diarrhea to hospitals in five different places of Japan. Two multiplex polymerase chain reaction procedures were performed for the identification of two groups of diarrheal viruses. The first group includes group A, B and C rotaviruses and adenovirus, and the second group includes astrovirus, norovirus and sapovirus. In the study, the existence of five diarrheal viruses co-circulating in children with acute gastroenteritis was detected. However, none of group B and group C rotavirus was identified. Overall, diarrheal viruses were detected in 45.9% fecal specimens tested. Group A rotavirus was the most prevalent, detected in 123 out of 628 specimens tested, and it represented 19.6% followed by 15.6% of norovirus, 4.5% of adenovirus, 3.8% of sapovirus and 2.4% of human astovirus.

Rotavirus (RV) was further characterized to G-typing and P-typing by RT-multiplex PCR and sequencing method. Serotype G1 (58.5%) was identified at high levels followed by G9 (20.3%), G2 (11.4%), and G3 (7.3%). P genotyping revealed P[8] as predominant (84.6%) followed by P[4] (13.8%) and P[6] (1.6%). During the 2006/2007 rotavirus season, G1P[8] strains were most common with G9P[8], , G2P[4], G3P[8], G1P[4] and G9P[6] also detected. It is clear from this study that RAV is still burden of diseases in Japan. To my knowledge, this is the first report of RV P[6] strain in humans in Japan.

Molecular analysis of norovirus was carried out by sequencing methods. From 628 fecal specimens tested, 15.6% (99 of 628) were positive for NoV, most of which were

NVGII, comprised of GII/4 (89%), GII/3 (6.4%), GII/7 (2.3%), and GII/2 (2.3%). All the NVGII/4 strains, all of them belonged to genotype GII/4 2006b variants. This results clearly indicated that NoV infections were most commonly observed in winter seasons in Japan. In conclusion, this study shows the predominance of GII/4 2006b variants and a large incidence of NoV infection among Japanese pediatric population. Based on the genetic analysis, novel, naturally occurring recombinant norovirus strains were identified. Breakpoint analysis of recombinant norovirus showed that the recombination site was at the open reading frame ORF1/ORF2 overlap. Recombination of RNA viruses is topical nowadays. RNA recombination plays a key role in virus evolution and it shapes a good deal of the virus diversity. This event has the great impact on molecular epidemiological study, viral vaccine design and viral control program. Therefore, the identification of novel recombinant viruses is crucial for the virology field.

Sapovirus detected in this study was clustered into only one distinct genogroup I. Sapovirus GI/1 was predominant, followed by GI/2 and accounted for 92% (22 of 24) and 8% (2 of 24), respectively. Analysis of nucleotide and amino acid sequences revealed that SVGI/1 was closely related to Lyon30388/98, Plymouth/92 and Manchester/93 strains. HAstV detected in this study belonged to genotypes HAstV-1d. The findings suggest that SV, and HAstV are important enteric viruses cocirculating among Japanese infants and children.

In the second chapter, I described the molecular epidemiology of viral gastroenteritis among infants and children in Bangladesh. A total of 917 fecal specimens collected from infants and children with acute gastroenteritis in Dhaka City, Bangladesh during 2004 to 2005, were examined for diarrheal viruses by reverse transcription-multiplex polymerase

chain reaction. Among diarrheal viruses detected, group A rotavirus (RV) was the most prevalent (33.5%), followed by 4.5% of norovirus (NoV), 2.7% of sapovirus (SV) and 1.9% of adenovirus (AdV), 0.3% of human astrovirus (HAstV) respectively. It was found that 307 (33.5%) fecal specimens were positive for group A rotavirus. Interestingly, an emergence of G2 was identified with a very high prevalence (43.3%) and followed by G4 (19.5%), G9 (13.7%), G1 (12.7%), and G3 (2.6%). Among 307 RAV samples, 280 samples were P-typed successfully in this study. P [8] was the most predominant genotype (53.2%), followed by P [4] (42.9%). The common clinical symptoms of rotavirus infected patients were dehydration (84%), vomiting (69%) and abdominal pain (52%). Serotype G3P[8] strains were detected in nine specimens. Sequence analysis clustered the G3 strains into one distinct lineage (lineage I) with other Asian G3 strains. In addition, one amino acid change at position 96 in antigenic region A, similar to lineage II G3 Chinese strains, was noted. To my knowledge, this is the first report of serotype G3 strains in Bangladesh since 1993 and the first report of the molecular characterization of these strains.

Detected adenoviruses were subjected to molecular genetic analysis by the sequencing method, and were classified into three serotypes, namely Ad9, Ad10, and Ad40. Of these, Ad40 was predominant (42%, 7 of 17), followed by Ad9 (36%, 6 of 17) and Ad10 (24%, 4 of 17). Ad41 serotype, usually predominant in gastroenteritis, was not detected. The most common clinical symptoms of adenovirus-infected patients were dehydration (94%), abdominal pain (59%) and vomiting (30%). This results clearly indicated that adenovirus infections were most commonly observed in winter season (October 2004 through January 2005) and in rainy season (May 2005 through July 2005) in Dhaka City. To my

knowledge, this is the first report of Ad9 and Ad10 in diarrheal stool sample in Bangladesh. The molecular characteristics of Bangladeshi human astroviruses were also determined completely. All Bangladeshi HAsV detected in this study belonged to genotypes HAsV-1d. Based on the genetic analysis, a novel, naturally occurring recombinant sapovirus strain was identified. Breakpoint analysis of recombinant sapovirus showed that the recombination site was at the polymerase-capsid junction. This is the first report of the existence of acute gastroenteritis caused by recombinant sapovirus in Bangladesh.

In the third chapter, I described the seasonal trend and serotype distribution of rotavirus infection in Japan, 1981-2008. A total of 10,917 fecal specimens collected from infants and children with gastroenteritis in seven different regions of Japan during 1981-2008. Over a 25-year period, the presence of rotavirus in stool samples from children and infants with acute gastroenteritis in Japan was studied by RNA-PAGE, ELISA, latex agglutination, RT-PCR and immunochromatography (IC) method. I determined the trend for the “peak” month during 17 seasons and serotype distribution of rotavirus during 25 seasons. Rotavirus antigen was detected in 2,054 patients (18.8%) and rotavirus peak shifted gradually from January to April (winter to early spring) during the 17 seasons. This time series shift in the peak rotavirus infection was statistically significant ( $p = 0.0001$  for seasonal trend). G1P[8] combination was the most predominant genotype followed by G2P[4], G3P[8], G4P[8] these accounted for 92% cases, and some other unusual combination was also found. This result strongly support that second generation rotavirus vaccine (Rotarix, Rotateq) must be effective for reducing rotavirus infection in Japan. These findings also confirmed that rotavirus is still burden of disease among

Japanese pediatric population and rotavirus peak in Japan has gradually shifted from winter to early spring for unknown reasons.

In the fourth chapter, I described the seasonal pattern and genotype distribution of norovirus infection in Japan, 1995-2007. A total of 6,231 fecal specimens collected from infants and children with gastroenteritis in seven different regions, Japan during 1995-2007 and were screened for norovirus by RT-PCR. Norovirus was detected for antigen in 779 patients (12.5%) and norovirus infection peak was observed mainly in winter season (November to January) in Japan during 1995-2007. During the period of last 11 years, NoVGII/4 was the predominant strain in Japan followed by GII/3, GII/6, GII/2, GII/12 and GI respectively. There are some unusual genotypes of norovirus such as GII/15, GII/14, GII/10, GII/8, GII/6, GII/1 and recombinant norovirus were also detected during these periods. These findings confirmed that norovirus is still burden of disease among Japanese pediatric population and norovirus peak mainly observed in early winter time in Japan.

In conclusion, this study confirmed the diversity of viruses causing acute gastroenteritis among Japanese children as well as Bangladeshi children, and described the molecular characteristics of causative viruses. This study plays an important role to determine virus evolution, virus origin and virus diversity. The result of this study should be useful for the knowledge on diarrheal viruses, virus control, viral vaccine design and provided the clues to the strategy for developing vaccines against diarrhea for both developed country (Japan) and developing country (Bangladesh).