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Probe Typing of Noroviruses Detected in Osaka City, Japan

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Norovirus (NV), previously called Norwalk-like virus or small round-structured virus, is a member of the family *Caliciviridae* (www.ictvdb.iacr.ac.uk/Ictv) and a major cause of acute non-bacterial gastroenteritis worldwide (1).

We performed probe typing of NVs detected in non-bacterial gastroenteritis outbreaks and sporadic pediatric cases between April 1996 and March 2003 in Osaka City, Japan. Detection of NVs was performed using reverse transcription (RT)-PCR with SR primers (G1 sets: SR33, SR48, SR50 and SR52; G2 sets: SR33, SR46 and OC028/1B) (2,3) amplifying the 123-bp RNA polymerase region as previously described (4). All NV-positive samples by RT-PCR were classified into 6 probe types (P1-A, P1-B, P2-A, P2-B [2], SOV, and 96065 [4]) by Southern hybridization as previously described (4). The outbreaks in which multiple probe type NVs were detected were classified as mixed-probe-type and NVs that were not hybridized with any of the 6 probes were designated as unclassified type.

For the investigation of outbreaks, we collected 1,043 specimens from 210 outbreaks. A total of 490 fecal specimens (47.0%) from 145 outbreaks (69.0%) were positive for NV. Almost all of the NV-positive patients were adults. In 62 NV-positive outbreaks, ingestion of contaminated oysters was considered a probable source of infection. The NV positive-outbreaks were finally classified as 10 P1-A, 1 SOV, 13 P1-B, 10 P2-A, 57 P2-B, 2 96065, 41 mixed-probe-types and 11 unclassified types. Twenty-eight mixed-probe-type outbreaks (68.3%) were associated with ingestion of oysters. Between April 1996 and March 2000, the predominant probe type of NV strains changed in each season (1996-1997: P2-B; 1997-1998: P2-A; 1998-1999: P1-B; and 1999-2000: P2-B). However, in 4 seasons from 1999 - 2000 to 2002 - 2003, the P2-B type was the predominant probe type of NV (Fig. 1A).

For the examination of pediatric cases, 838 specimens were collected from patients under 12 years of age. From these specimens, group A rotavirus-, adenovirus-, and enterovirus-positive samples were excluded, leaving a total of 206 fecal specimens (17.7%) that were positive for NV, and all of these except for 2 unclassified types were classified as single-probe-types (2 P1-A, 12 P1-B, 4 P2-A, 184 P2-B, and 2 96065).

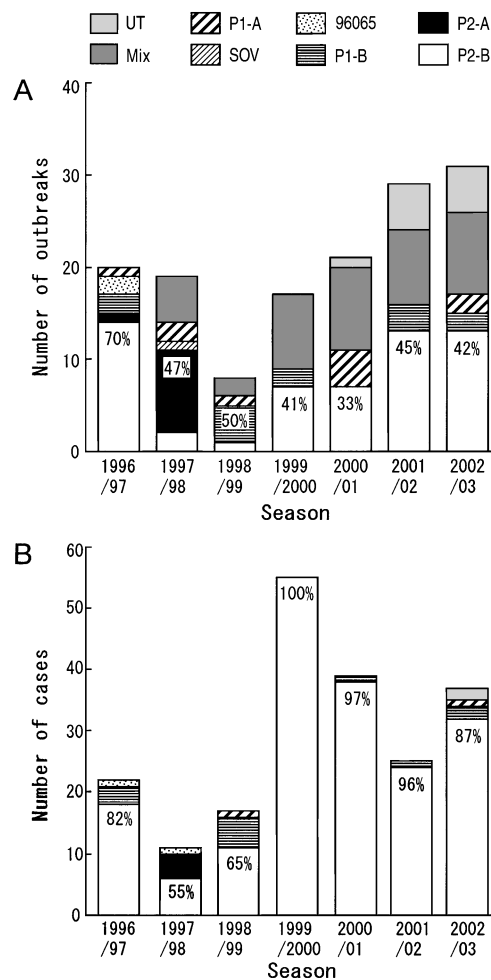


Fig. 1. Annual distribution of probe type of NVs detected in outbreaks (A) and sporadic cases (B). The period of one season in this study is from April to March of the next year. "UT" indicated the type of NVs, which could not be classified into any of the 6 probe types. "Mix" indicated the mixed-probe-type detected in an outbreak or a case. Percentage of predominant type in each season indicated in the column.

The P2-B type was the predominant probe type of NV in pediatric gastroenteritis cases in each season (Fig. 1B). All of the P2-A strains and most of the P1-B strains were detected during the 1997 - 1998 season and 1998 - 1999 season, respectively. The predominant probe type of NV detected in outbreaks tended to increase in the pediatric gastroenteritis

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Table 1. Relation between probe type at RNA polymerase region and genotype at capsid region in NVs

Genogroup	Probe type	Genotype ¹⁾ (reference strain ²⁾ , accession number)
GI	P1-A	GI/1 (Norwalk/1968/US, M87661)*
		GI/4 (Chiba407/1987/JP, AB022679)*
		GI/7 (Winchester/1994/UK, AJ277809)*
		GI/9 (Saitama SzUG1/1999/JP, AB039774)*
	SOV	GI/2 (Southampton/1991/UK, L07418)*
UT ³⁾		GI/3 (Desert Shield395/1990/SA, U04469)
		GI/8 (Sindlesham/1995/UK, AJ277615)*
		GI/12 (Saitama KU19aGI/2000/JP, AB058525)
GII	P1-B	GII/6 (Seacroft/1990/UK, AJ277620)*
		GII/9 (Idaho Falls378/1996/US, AY054299)
	P2-A	GII/3 (Toronto24/1991/CA, U02030)
	P2-B	GII/1 (Hawaii/1971/US, U07611)*
		GII/2 (Melksham/1994/UK, X81879)
		GII/3 (Arg320/1995/AR, AF190817)*
		GII/4 (Bristol/1993/UK, X76716)*
		GII/5 (Hillingdon/1990/UK, AJ277607)*
		GII/8 (Amsterdam/1998/NL, AF195848)*
		GII/10 (Erfurt546/2000/DE, AF427118)
	GII/11 (Saitama T29GII/01/JP, AB112221)	
	GII/12 (Aichi76-96 Chitta /1996/JP, AB032758)*	
	GII/14 (Fayetteville/1998/US, AY113106)*	
GII/15 (Saitama KU80aGII/1999/JP, AB058588)*		
96065	GII/7 (Leeds/1990/UK, AJ277608)	
UT		GII/13 (M7/1999/US, AY130761)
		GII/17 (Alphatron/1998/NL, AF195847)

¹⁾: Numbering of genotypes was followed by Kageyama's method (5).

²⁾: Reference strains used in this study were selected from deposited strains in GeBank/EMBL/DDBJ.

³⁾: UT indicated the type of NVs, which could not be classified into 6 probe types.

Genotypes with asterisk were described in Seto et al. (6) and others in this study.

cases during the same season. This suggests that outbreaks occasionally link with sporadic gastroenteritis among children in the NV infections.

Recently, Kageyama et al. reported that NV had more than 31 genotypes based on phylogenetic analysis of nucleotide sequences of the capsid N-terminal and shell domain (5). Based on our investigation of the relation between the probe type within the RNA polymerase region and the genotype within the capsid region, it is clear that two or more geno-

types of NVs might be contained in one probe type (Table 1) (6). In particular, it was confirmed that the P2-B type, which was the most detected probe type in Osaka City, had more than 11 genotypes. There was a disagreement between probe type and genotype in Arg320-like strains (P2-B and GII/3), which may have arisen from recombination. Unclassified types of NVs had at least 5 genotypes (GI/3, GI/8, GI/12, GII/13, and GII17).

This probe typing method has provided a rough overview of NV infections in Osaka City, but we further propose that the application of genotyping methods will provide more detailed information on disease transmission.

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