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An Outbreak of Mixed Infection of Enterohemorrhagic *Escherichia coli* O26:H11 and Norovirus Genogroup II at a Kindergarten in Shimane, Japan

Setsuko Iizuka*, Yoshie Tsunomori, Kenji Tabara, Kazuo Tsuda¹ and Tsuneo Fukuma²

Shimane Prefectural Institute of Public Health and Environmental Science, Shimane 690-0122,

¹*Okii Public Health Center, Shimane 685-8601 and* ²*Matsue Public Health Center, Shimane 690-0882, Japan*

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On January 17, 2005, a health center in Shimane Prefecture received a report that 21 of 72 children in a kindergarten were absent due to diarrhea and vomiting. Investigation by the health center revealed that absenteeism due to the above symptoms began to increase on January 14. A total of 28 kindergarten children got sick in the following 4 days. Some of the family members of the affected kindergarten children showed the same symptoms. However, 12 members of the kindergarten staff were unaffected.

The non-synchronous appearance of the patients suggested viral or bacterial infections. Real-time PCR (1) detected norovirus genogroup II (NV GII) in the stool specimens of 14 of the 16 kindergarten children examined and in those of 6 of 6 family members examined. Bacteriological investigation of the stool specimens of these patients and those of an additional one kindergarten child and 4 kindergarten staff (total 27 individuals) revealed that 6 kindergarten children and 3 family members were infected with enterohemorrhagic *Escherichia coli* (EHEC) O26:H11(VT1-positive). Therefore, we further examined the stool specimens of the remaining kindergarten children, staff members and family members (a total of 88 individuals), and found that one symptomatic infant and 2 asymptomatic family members were infected with EHEC O26:H11(VT1). Bacteriological investigation of the remaining food samples and the swab specimens of the kindergarten facility gave negative results.

Figure 1 shows the time course of the appearance of patients classified according to the pathogens detected. The patients defined here are those having diarrhea or vomiting later than January 8, when the first symptomatic case with both NV GII and EHEC O26:H11(VT1) infections appeared. The patients appeared on January 15-17 in a cluster. There was no correlation between the onset of the symptoms and infections by EHEC O26:H11(VT1) and/or NV GII. The EHEC O26:H11(VT1)-infected individuals tended to have fever more often.

Table 1 shows the summary data classified according to the 5 classes by age, with class 1 being the youngest and classes 4 and 5 the oldest. The symptomatic patients appeared in all the classes at variable frequencies. There were 11 cases of family infection. In all families except one, infection of the kindergarten children preceded that of the other family

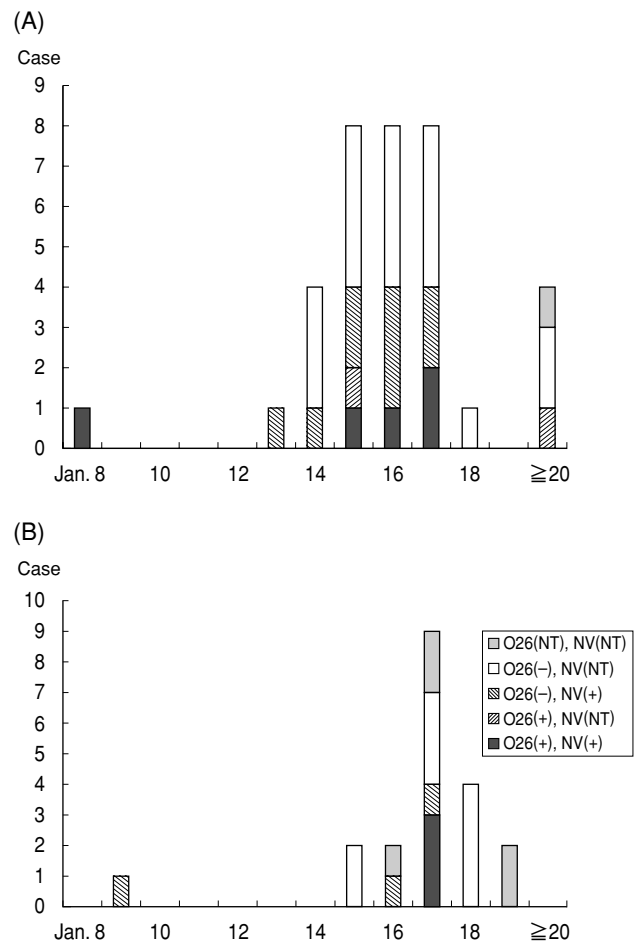


Fig. 1. Time course of appearance of patients. (A) Kindergarten children. (B) Family members. NT, not done; -, not detected; +, detected.

members, suggesting transmission from the kindergarten children to other family members.

For 12 of the EHEC O26:H11(VT1) isolates tested, the pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA gave the same pattern (data not shown; PFGE was conducted by the National Institute of Infectious Diseases). For all the NV GII isolates, the PCR products amplified by using the G2SKF/R primer (2) were of the same single strand conformation polymorphism (SSCP) pattern (data not shown), and the nucleotide sequence of the PCR products was that of genotype GII/4 (3). These data suggested

*Corresponding author: Mailing address: Shimane Prefectural Institute of Public Health and Environmental Science, Nishihamasada-cho 582-1, Matsue, Shimane 690-0122, Japan. Tel: +81-852-36-8188, Fax: +81-852-36-8356, E-mail: iizuka-setsuko@pref.shimane.lg.jp

Table 1. Detection of EHEC O26:H11 (VT1) and NV GII among different groups, kindergarten children, kindergarten staff and family members

	No. of persons	Symptomatic patients	O26:H11(VT1) positive	NV GII positive
Class 1 (0-1 y)	10	8	4 (2)*	4
Class 2 (1-3 y)	11	5	1 (1)	3
Class 3 (3-4 y)	26	11	1 (1)	5
Class 4 (5-6 y)	12	8	0	1
Class 5 (4-6 y)	13	3	1 (1)	1
Staff of the kindergarten	12	0	0	0
Family members	—	20	5 (3)	6
Total		55	12 (8)	20

*: Numbers in parentheses show positive for both EHEC O26:H11(VT1) and NVII.

that the outbreak was caused by a single strain of each of EHEC O26:H11 (VT1) and NV GII.

The infection source could not be identified, but the kindergarten was undoubtedly the place of the spread of the pathogens. The outbreak pattern and clinical pictures reported here were indistinguishable from those caused by NV GII alone. The double infection was revealed only by the simultaneous virological and bacterial examinations.

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REFERENCES

1. Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F. B., Takeda, N. and Katayama, K. (2003): Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J. Clin. Microbiol.*, 41, 1548-1557.
2. Kojima, S., Kageyama, T., Fukushi, S., Hoshino, F. B., Shinohara, M., Uchida, K., Natori, K., Takeda, N. and Katayama, K. (2002): Genogroup-specific PCR primers for detection of Norwalk-like viruses. *J. Virol. Methods*, 100, 107-114.
3. Kageyama, T., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F. B., Kojima, S., Takai, R., Oka, T., Takeda, N. and Katayama, K. (2004): Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to *Norovirus* in Japan. *J. Clin. Microbiol.*, 42, 2988-2995.

1. Kageyama, T., Kojima, S., Shinohara, M., Uchida, K.,