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Epidemiological Investigation and Analysis of Hepatitis A Virus Genomes in the Three Cases of Hepatitis A Infections That Occurred in April–May 2010

Tetsuya Yoshida*, Tatsuko Miyasaka, Yuka Azegami, Yurie Uchiyama, Hitomi Kasahara,
Hiromi Ueda, Hiroshi Nagase, Satoru Fujita, Koji Ishii¹, and Mamoru Noda²

Nagano Environmental Conservation Research Institute, Nagano 380-0944;

¹National Institute of Infectious Diseases, Tokyo 208-0011; and

²National Institute of Health Sciences, Tokyo 158-8501, Japan

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Hepatitis A infection is classified as a category IV infectious disease. Under the Law Concerning the Prevention of Infectious Diseases and Medical Care for Patients of Infectious Diseases in Japan, the doctor who diagnoses a patient with hepatitis A infection must notify the governor of the prefecture of any such case. For the past several years, the number of hepatitis A cases in Japan has been less than 200 per year. However, since the 10th week in 2010, the number of cases rapidly increased, mainly in western Japan, and was approximately 270 by the 28th week. In this period, three cases of hepatitis A infection occurred in central Japan. In this report, we describe the results of epidemiological investigation and genetic analysis of these cases.

The epidemiological investigation was performed by the staff at the health center that governs the medical facilities in which the cases were diagnosed. The investigation was conducted by meeting with and interviewing the patients. Genetic analysis of hepatitis A virus (HAV) was carried out according to the manual of the National Institute of Infectious Diseases (1). We extracted viral RNA from the patient's feces and then used the reverse transcription-polymerase chain reaction (RT-PCR) to detect a part of the HAV gene. Moreover, we deter-

mined the sequence of the PCR product of approximately 230 nt, a part of the VP1-2A region, by direct sequencing and performed analyses including phylogenetic tree analysis.

The three patients with hepatitis A infection included two females, Patient A (54 years old) and Patient C (20 years old), and one male, Patient B (52 years old), and they developed the infection between April 29 and May 19 in 2010 (Table 1). The patients showed typical symptoms of hepatitis A infection including jaundice, liver dysfunction, fever, and malaise. Of the three patients, two (Patients A and C) had traveled abroad. Patient A had traveled through Korea and Taiwan, and Patient C had traveled to the Philippines (Table 1). Considering the incubation period of hepatitis A infection, we strongly suspected that Patient C was exposed to HAV in the Philippines. On the basis of the interviews, we found that Patient B had eaten raw salted short-necked clams about 1 month before the onset of the disease.

We conducted an RT-PCR analysis of the RNA isolated from the feces of the three hepatitis A patients to detect the HAV gene; all specimens tested positive for the presence of the HAV gene. Nucleotide sequences of the PCR products were determined and phylogenetic

Table 1. Epidemiological information of hepatitis A patients and results of genotyping of detected viruses

Patient	Sex	Age (y)	Onset date	History of travel abroad		Consumption of raw bivalve	Virus strain name	Genotype
				Country	Period			
A	Female	54	Apr. 29, 2010	Korea Taiwan	Apr. 4–7, 2010 Apr. 26–28, 2010	No	1005-12275-NgnP-22-035	IIIA
B	Male	52	May 13, 2010		No	Raw salted short-necked clams (in mid-April)	1006-13956-NgnP-22-041	IIIA
C	Female	20	May 19, 2010	Philippine	Nov. ??, 2009–May 18, 2010	Unknown	1006-14079-NgnP-22-042	IA

*Corresponding author: Mailing address: Infectious Diseases Division, Nagano Environmental Conservation Research Institute, 1978 Komemura Amori Nagano City, Nagano 380-0944, Japan. Tel: +81-26-227-0354, Fax: +81-26-224-3415, E-mail: yoshida-tetsuya@pref.nagano.lg.jp

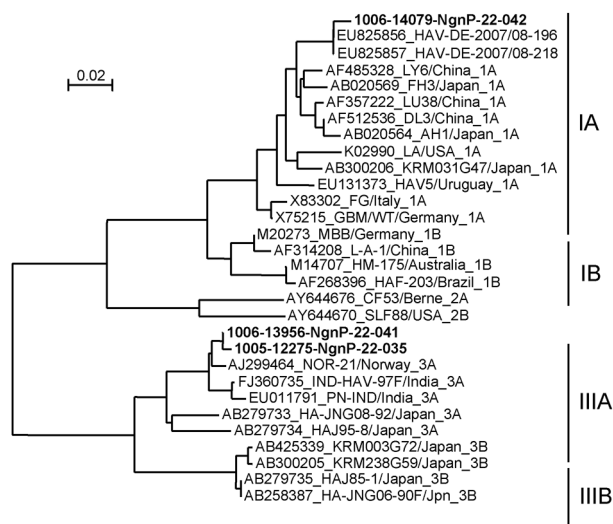


Fig. 1. Phylogenetic tree constructed on the basis of partial sequences (approximately 230 nt) of the hepatitis A virus VP1-2A region. The tree was plotted using the neighbor-joining method, and bootstrap was conducted 1000 times. Avian encephalomyelitis virus (accession no. AY275539) was used for outgroup but it was not shown.

analysis was performed. HAV strains isolated from Patients A and B were genetically similar and were classified into genotype IIIA (Fig. 1). In Korea, hepatitis A infection has recently been spreading mainly in the younger age group, and studies have reported that most of the hepatitis A strains isolated from these patients belonged to genotype IIIA (2). Thus, we cannot rule out the possibility that Patient A was infected with the HAV strain that had spread in Korea during the patient's stay in that country. We thought that Patient B was infected by eating raw salted short-necked clams about 1 month before the onset of the illness. We could not trace the place of origin of the infection because the patient could

not clearly remember where he had consumed the raw short-necked clams.

The HAV strain from Patient C was classified into genotype IA, and it belonged to the same cluster as that of HAV-DE-2007/08-196 (accession no. EU825856) and HAV-DE-2007/08-218 (accession no. EU825857) detected in Philippine tourists visiting Germany (Dr. Mirko Faber, personal communication) (Fig. 1). Although the HAV strain isolated from Patient C is not shown in the figure, it was similar to the strains isolated from river water samples in the Philippines. The results of these genetic analyses strongly suggested that Patient C was infected with HAV in the Philippines.

Most of the HAV strains that spread in Japan in 2010 were classified into genotype IA, and these strains are closely related to the HAV strain from Patient C. More detailed and extensive genetic and epidemiological analyses are needed to determine the cause of the rapid increase in the number of hepatitis A patients.

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Conflict of interest None to declare.

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