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Epidemiological and genetic analysis of a 2014 outbreak of hepatitis A in Japan

Koji Ishii[a,*], Tomoko Kiyohara[a], Sayaka Yoshizaki[a], Kunio Kawabata[b], Atsuhiro Kanayama[b], Yuichiro Yahata[b], Takuri Takahashi[b], Hitomi Kinoshita[b], Takehito Saitou[b], Tomimasa Sunagawa[b], Kazunori Oishi[b], Masashi Uema[c], Mamoru Noda[c], Takaji Wakita[a]

[a] Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan
[b] Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Tokyo, Japan
[c] Division of Biomedical Food Research, National Institute of Health Sciences, Tokyo, Japan

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ABSTRACT

Hepatitis A virus (HAV) is one of the most common causes of feces-transmitted acute hepatitis worldwide. In Japan, most of HAV infections have been sporadic cases and a relatively low number of cases (approximately 100–150) of acute hepatitis A were reported in 2012 and 2013. However, in 2014, 342 cases were reported as of week 22. In order to characterize the viral agents causing this outbreak, we collected stool or sera (and both for three case) from patients with hepatitis A from many regions throughout Japan and performed genotyping of the VP1/P2A regions of HAV. We then used a multiple-alignment algorithm to compare the nucleotide sequences with those of reference strains. Phylogenetic tree analyses revealed that the 159 HAV isolates were divided into three subgenotypes: IA (137 cases), IB (4 cases), and IIIA (18 cases). The most unique feature of this outbreak was that for most subgenotype IA cases (103 out of 137 IA cases) the sequences analyzed shared 100% homology. Interestingly, the peak week for these IA infections was almost the same nationwide, suggesting that the epidemic of hepatitis A caused by this subgenotype IA strain may have expanded from a single source possibly because of one food-borne or waterborne source that was distributed nationwide at once.

1. Introduction

Hepatitis A virus (HAV) is a member of the genus *Hepatovirus* within the family *Picornaviridae*; HAV contains a positive-sense, single-stranded RNA genome of approximately 7.5 kb. Hepatitis A occurs worldwide, and humans are thought to be the natural host of HAV. The virus is transmitted through person-to-person contact via a fecal-oral route usually through ingestion of contaminated food, water, or both [1–4].

When HAV is defined by sequence variation within the VP1/P2A junction, there is 15% nucleotide variation between isolates and 7–7.5% nucleotide variation between subgenotypes [5]. HAV strains isolated from around the world have been classified into six genotype groups (I–VI); genotypes I–III are found in humans; and each of these is further divided into a subgenotype, A or B. Most human HAV strains belong to genotypes I or III [6–8]. Subgenotype IA appears to be the predominant cause of hepatitis A cases worldwide; however, subgenotype IB has been quite prevalent in European and Mediterranean regions [8]. Subgenotype IIIA isolates were recovered from humans with hepatitis A in various countries in Asia and Europe and in Madagascar and the USA [8,9]; the subgenotype IIIB was responsible for some cases of HAV infection in Denmark and Japan [8,10].

HAV infection has long been and remains a major public health problem in many countries. The annual clinical incidence of hepatitis A worldwide is 1.5 million cases [11]. In developing countries, HAV infection occurs early in life, and large proportions of the populations in these countries express HAV antibodies (anti-HAV) [11]. In developed countries, improvements in hygiene and sanitary conditions and the application of public health control measures have led to a decline in the incidence of hepatitis A and to a shift toward infection occurring during adulthood. Although some sporadic cases and localized small-scale outbreaks are still observed in developed countries, large outbreaks are rare [12].

During the last two decades in Japan, the number of cases of HAV infection decreased due to improved sanitation and living

* Corresponding author. Tel.: +81 42 848 7037; fax: +81 42 561 4729.
E-mail address: kishii@nih.go.jp (K. Ishii).

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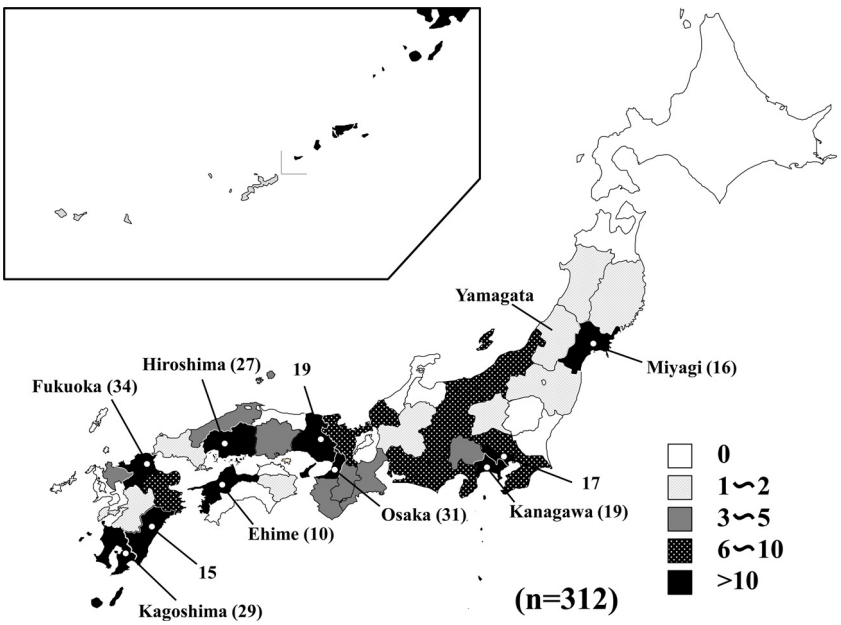

Fig. 1. Geographical distribution of acute hepatitis A patients in Japan as of week 22 of 2014. For each prefecture, the reported number of patients infected domestically is shown. Miyagi and Yamagata prefectures, where a small epidemic of subgenotype IIIA was reported, are also shown in the figure.

conditions; moreover, the incidence has declined from 8 out of 100,000 individuals in 1990 to 0.2 out of 100,000 in 2005. Most recent HAV infections in Japan are sporadic, but a few small-scale outbreaks have occurred [13]. However, as of week 22 of 2014, 342 cases of HAV infection had been reported in Japan, and these cases are considered a nationwide HAV outbreak. In the present study, we collected stool and serum specimens from 159 patients with acute hepatitis A; these collections were made in collaboration with 31 local institutes of health in Japan. We then used polymerase chain reaction (PCR) amplification and sequencing of the HAV genome to perform a phylogenetic analysis of the virus isolates to examine the epidemiology of the 2014 HAV outbreak in Japan.

2. Materials and methods

2.1. Data collection

In 2014, 36 prefectures (regions in Japan) reported one or more cases of hepatitis A (Fig. 1). In collaboration with 31 local institutes of health representing 28 prefectures, we collected either stool or sera from 156 patients and both sera and stool from three patients; these 159 patients represented nearly half of all 342 reported cases of acute hepatitis A in Japan; HAV sequence data was collected from all 162 patient samples.

2.2. RNA extraction, reverse transcription, and PCR

For each of the 159 cases, total RNA was extracted from either sera or a 10% fecal suspension or both; reverse transcription (RT) of viral RNA was then performed as described previously [13]. Degenerated primers, HAV-JCT-2F, HAV-JCT-1R-A, and HAV-JCT-2R were used to amplify via PCR the VP1/2A region of the HAV genome. The HAV-JCT-2F and HAV-JCT-1R-A primers were used for initial PCR amplification of HAV sequences, and the HAV-JCT-2F and HAV-JCT-2R primers were used for a second round of nested PCR. All PCR conditions were described in a previous report [13].

In order to determine nearly complete sequences of HAV isolates, the whole genome was divided into six regions, and RT-PCR was used as described above to amplify each region. PCR primers used for amplification were described previously [14]. All primer sequences are listed in Table 1. The purified DNA fragments amplified via PCR were used as templates for direct HAV sequencing.

2.3. Phylogenetic analyses

CLUSTAL W version 1.4 [15] was used to align the HAV nucleotide sequences. Kimura's two-parameter method was then used to generate a distance matrix of nucleotide substitutions per site from the sequence alignments [16]. MEGA version 4.0 was used to generate neighbor-joining trees [17], maximum-likelihood trees, and unweighted pair-group method with arithmetic averages (UPGMA) trees from the matrix numbers; each tree was constructed with 100 bootstrap replicates [18]. For the phylogenetic analysis of HAV nucleotide sequences, we included sequences of several common and/or widespread strains. These strains were: genotype I ([AF512536] DL3/China, [AF357222] LU38/China, [AF485328] LY6/China, [EU131373] HAV5/Uruguay, [AB020564] AH1/Japan, [AB020569] FH3/Japan, [AB300206] KRM031G47/Japan, [X75215] GBM/WT/Germany, [X83302] FG/Italy, [K02990] LA/USA, [AF314208] L-A-1/China, [M20273] MBB/Germany, [AF268396] HAF-203/Brazil, [M14707] HM-175/Australia, [AB839692] BaliA03-H29/Indonesia, [AB819870]

Table 1
Primers used for the PCR amplification of HAV genome.

Primer name	Sequence
HAV-JCT-2F	GRA GAA CAG GRA AYA TTC ARA TTA G
HAV-JCT-1R-A (3430R)	YTT RTC ATC YTT CAT TTC TGT CCA
HAV-JCT-2R	CAG THA RMA CHC CAG CAT CCA T
47F	GGA GYC CCT CTT GGR AGT CC
153R	GAA CCC TGA ACC TGC AGG A
96F	GTT TGC CTA GGC TAT AGG CT
1463R	TTC ATT TCT CAT CAT CTG TGT A
1166F	GGT GGA TTA ATA TGT GCT ATG GT
3264F	ACA GAG GAC CAT GAA ATA ATG AA
5969R	TAC ATT CAT TGA ACA CTG AG
5383F	TGT GAG ATG GGT TAT GAA TGC
6609R	CRT CCC ACT GTC TAT CWG GA
6289F	ATT CWT CYC CTG GRT WTC C
7435R	ACA AAC CTC AGA AAT TTT AAG AA

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