An Outbreak of Acute Hepatitis Caused by Genotype IB Hepatitis A Viruses Contaminating the Water Supply in Thailand

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Keywords

Hepatitis A virus · Drinking water · Phylogenetic analysis · Molecular epidemiology

Abstract

Background: In 2000, an outbreak of acute hepatitis A was reported in a province adjacent to Bangkok, Thailand. **Aims:** To investigate the cause of the 2000 hepatitis A outbreaks in Thailand using molecular epidemiological analysis. **Methods:** Serum and stool specimens were collected from patients who were clinically diagnosed with acute viral hepatitis. Water samples from drinking water and deep-drilled wells were also collected. These specimens were subjected to polymerase chain reaction (PCR) amplification and sequencing of the VP1/2A region of the hepatitis A virus (HAV) genome. The entire genome sequence of one of the fecal specimens was determined and phylogenetically analyzed with those of known HAV sequences. **Results and Conclusions:** Eleven of 24 fecal specimens collected from acute viral hepatitis patients were positive as determined by semi-nested reverse transcription PCR targeting the VP1/2A region of HAV. The nucleotide sequence of these samples had

an identical genotype IB sequence, suggesting that the same causative agent was present. The complete nucleotide sequence derived from one of the samples indicated that the Thai genotype IB strain should be classified in a unique phylogenetic cluster. The analysis using an adjusted odds ratio showed that the consumption of groundwater was the most likely risk factor associated with the disease.

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Introduction

Hepatitis A virus (HAV) is a nonenveloped single-stranded positive-sense RNA virus classified into the family *Picornaviridae* and a causative agent of acute hepatitis A [1]. HAV spread via the fecal-oral route either by ingestion of contaminated food and water or by person-to-person contact, and most HAV infections are symptomless [2]. The symptoms of HAV infection are self-limited, generally lasting 1–2 months, and require hospitalization and proper therapy. Host immunity after a single infection gives long-lasting protection against hepatitis A [3]. HAV is generally acquired during virus

circulation associated with poor hygiene and sanitary conditions [4].

HAV has a genome approximately 7.5 kb in length, and it contains a long 5' untranslated region (UTR), a long open reading frame, and a short 3' UTR containing a poly (A) tail [5, 6]. Genomic analysis of HAV strains from various geographic areas has revealed their origin and transmission pattern [7]. Nucleotide sequence analyses followed by phylogenetic analyses have revealed that these strains can be categorized into 6 genotype groups (I–VI) based on HAV isolates from various countries; genotypes I–III are found in humans, and each of these is further divided into 2 subgenotypes, i.e., A and B. Most human HAV strains belong to genotype I or III [8–10].

In Thailand, the monitoring of viral hepatitis has been carried out under a nationwide surveillance system, and suspected cases of hepatitis were reported in the surveillance report published in National Disease Surveillance (Report 506), Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. In 2015, of these suspected cases, only 4.7% (398/8,495) were diagnosed as hepatitis A [11].

In 2000, eighteen suspected hepatitis cases with fever and jaundice were reported to the authorities in Nakhon Pathom Province, a province adjacent to Bangkok, and most of these cases involved students. An extensive survey by the provincial authority revealed that this outbreak was caused by a drinking water supplier [12].

In the present study, we genetically analyzed specimens from the hepatitis patients, drinking water, and deep-drilled wells owned by the water supply company and found that the outbreak was caused by HAV of genotype IB.

Materials and Methods

Serum Samples

Serum specimens were collected in a province near Bangkok, i.e., Nakhon Pathom, Thailand, in July and August of 2000, including samples from patients who were clinically diagnosed with acute viral hepatitis and samples from healthy persons as a control group. The specimens were tested for anti-HAV IgM antibody (VIDAS HAV IgM; BioMérieux, Marcy l'Etoile, France), anti-HAV total antibody (VIDAS anti HAV total; BioMérieux), anti-HBc IgM antibody (VIDAS HBc IgM; BioMérieux), anti-HCV IgG antibody (ETI-AB-HCVK-4; DiaSorin, Saluggia, Italy), and anti-HEV IgM antibody (HEV-IgM ELISA; MP Diagnostic, Singapore).

Stool Samples

One-gram fecal specimens were collected from the acute viral hepatitis patients. The samples were diluted with phosphate-buffered saline (pH 7.4) to prepare a 10% (w/v) suspension.

Water Samples

One-liter water samples were collected, dechlorinated with sodium thiosulfate (Sigma Chemical Co., St. Louis, MO, USA) at a final concentration of 50 mg/L, and stored at 4°C. HAV was concentrated by adding $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ at a final concentration of 120 mg/L of the water sample, and adjusted to pH 3.5 with 1.0 N HCl. The samples were filtered through a 0.22- μm filter (Millipore, Bedford, MA, USA). The filter was washed 5 times with 1 mL of 0.14 N NaCl to remove excess Al^{3+} . The virus was eluted 3 times with 3 mL of 3% beef extracts (pH 9.0), and then 300 μL of antibiotics (10,000 units/mL of penicillin and 10,000 $\mu\text{g}/\text{mL}$ of streptomycin) and 300 μL of 10 $\times$ MEM were added to the eluent. The sample was kept at -70°C until further processing. One milliliter (1,440 ELISA units of the HM-175 strain) of the hepatitis A vaccine (1,440 ELISA units, HavrixTM; GSK Vaccines) was included to estimate the virus recovery. All samples were tested in triplicate.

Statistical Analysis

STATA software (version 6.0) was used to calculate the odds ratio (OR) and 95% confidence interval (95% CI) between the risk factors and the disease.

RNA Extraction and Reverse Transcription Polymerase Chain Reaction

Viral RNA was extracted from 100 μL of the concentrated water samples and 10% stool suspensions using the Trizol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's instructions. The reverse transcription of viral RNA was performed as described previously [13]. Degenerated primers, i.e., HAV-JCT-2F, HAV-JCT-1R-A, and HAV-JCT-2R, were used to amplify via polymerase chain reaction (PCR) the VP1/2A region of the HAV genome. The HAV-JCT-2F and HAV-JCT-1R-A primers were used for the initial PCR amplification of the HAV sequences, and the HAV-JCT-2F and HAV-JCT-2R primers were used for a second round of semi-nested PCR. All PCR conditions have been described previously [14].

Entire Genome Sequencing

In order to determine nearly complete sequences of the Thai IB HAV strain, the whole genome was divided into 7 regions, each region was amplified by reverse transcription PCR (RT-PCR), and direct sequencing was performed as described [13, 14]. The PCR primers used for amplification and sequencing are shown in Table 1.

Phylogenetic Analysis

CLUSTAL W version 1.4 [15] was used to align the HAV nucleotide sequences. The 2-parameter method of Kimura [16] was then used to generate a distance matrix of nucleotide substitutions per site from the sequence alignments. 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]. All reference sequences used in this study were obtained from GenBank.

Nucleotide Sequence Accession Numbers

The GenBank accession number of the nearly complete nucleotide sequences determined in this study is LC128713. The accession numbers of the other strains used in this study are: genotype

Table 1. Primers used to determine the entire nucleotide sequence

Primer	Sequence (5'→3')	Position
1F	TTC AAG AGG GGT CTC CGG	1–18
153R	GAA CCC TGA ACC TGC AGG A	153–135
32F	CCC TCT TGG AAG TCC ATG GTG A	32–54
516R	ACT CAA TGC ATC CAC TGG ATG AG	516–494
992 F	ATT CAT TCT GCA GAT TGG CTT	992–1012
1233R	CAA TTC AAC AAA CCA TGA GGA TA	1233–1211
1549 F	AGG AAG ATT GGA AAT CTG ATC	1549–1569
1695 R	CYA CTG GAA TAA CTT TAA TTT	1695–1675
1977F	AGA TTT CGT GTG CCC TGG ATT TC	1977–1999
2275 R	CAT GGT TGT ACC AAC TTG	2275–2256
3162R	CTT CYT GAG CAT ACT TKA RTC TTT G	3162–3138
2907F	GCA AAT TAC AAT CAT TCT GAT GA	2907–2929
4392R	GTY CAT TCA ATG GCW CAR GTT GA	4392–4368
4290F	ATA ACA AAC TGG TTC ACA TCT	4290–4313
5520R	TTD GGA ATT GTA GGM ACC TTC AT	5520–5498
5383F	TGT GAG ATG GGT TAT GAA TGC	5383–5403
6609R	CRT CCC ACT GTC ATC WGG A	6609–6591
6518F	TTA TAC AAT TTT GTG TAG AAT GTA	6518–6541
7435R	ACA AAC CTC AGA AAT TTT AAG AA	7435–7413
3451R (JCT-1R-A)	YTT RTC ATC YTT CAT TTC TGT CCA	3451–3427
2784F (JCT-2F)	GRA GAA CAG GRA AYA TTC ARA TTA G	2784–2809
3376R (JCT-2R)	CAG THA RMA CHC CAG CAT CCA T	3376–3398

The positions of the primers were numbered according to the complete nucleotide sequence of the HM 175 strain (accession No. M14707).

IA (K02990, X83302, X75215, AB969748, AB839692, AF485328, AB020569, AF512536, AF357222, AB020564, EU131373, and KJ427799), genotype IB (HQ246217, M20273, EF406359, AF314208, M14707, KF724023, and AF268396), genotype II (AY644676 and AY644670), genotype IIIA (AB279733, FJ360735, EU011791, JQ655151, AB973400, and AJ299464), and genotype IIIB (AB258387, AB300205, and AB279735). In addition, the following genotype IB strains were used to compare the VP1/2A (286 bases) region (AB258562, AB974364, AF050229, AF050231, AF268396, AF314208, AY741663, DQ452807, EU930199, FJ010834, GU646039, HQ246217, HQ907924, KC632110, KC876797, KC876799, KF724023, KP091364, KP091376, KT222956, LC035019, LC037391, M14707, and M20273).

Results

Outbreaks of Hepatitis in 2000

An outbreak of hepatitis occurred from July to August of 2000 in Nakhon Pathom Province, next to metropolitan Bangkok. During this outbreak, a total of 18 hepatitis patients who had jaundice with fever or fever with enteric abnormality were observed. A case-control study was performed to find any association between risk fac-

tors and the disease. From 18 cases and 48 controls, a relation was established between 2 kinds of food consumption, i.e., noodles (crude OR 5.76; 95% CI 1.61–20.56) and water/ice (crude OR 6.11; 95% CI 1.26–29.64), and the disease. In addition, a relation between the consumption of groundwater (crude OR 5.18; 95% CI 1.53–17.53) and the disease was observed. From the analysis using adjusted OR with these 3 risk factors, the consumption of groundwater was the most likely risk factor associated with the disease (crude adjusted OR 3.76; 95% CI 1.04–20.15) [12].

Genetic Analysis of Thai HAV Isolates in the Outbreak

The 22 serum and 24 fecal specimens collected from the patients were subjected to serological and genetic analyses as described in Materials and Methods. All of the serum samples were negative for anti-HBc IgM, anti-HCV IgG, and anti-HEV IgM but positive for anti-HAV IgM (data not shown); however, we were not able to perform further genetic analyses due to the extremely small size of the samples. Eleven of the 24 fecal samples were

Fig. 1. Phylogenetic tree of HAV based on the complete nucleotide sequence. Bootstrap values are shown at corresponding nodes.

found to be positive by semi-nested RT-PCR targeting the VP1/2A region of HAV. The nucleotide sequences of PCR products (286 bp) were identical (accession No. AF543485), suggesting that the same causative agent was present [19]. Three of 27 water samples were found to be positive by RT-PCR targeting a short VP1/2A region (data not shown); however, sufficient PCR products for further analysis were not obtained by the seminested RT-PCR.

One of the fecal samples was selected to determine the complete nucleotide sequence. Nearly the entire viral genome was amplified as described in Materials and Methods, and the nucleotide sequence was determined using the primers depicted in Table 1. Thus, the Thai hepatitis A IB sequence, which was composed of 7,419 nucleotides, encoded a polyprotein composed of 2,225 amino acids and was submitted to the National Center for Biotechnology Information (NCBI) database (accession No.

Fig. 2. Phylogenetic tree of genotype IB HAV based on a 286-nucleotide fragment of the VP1/2A region. Bootstrap values are shown at corresponding nodes.

LC128713, Banglane2000). The sequence showed a base composition of 2,176 A (29.3%), 1,201 C (16.2%), 1617 G (21.8%), and 2,418 T (32.6%).

The nucleotide identity of the Banglane2000 strain with other genotype IB strains deposited in GenBank (HQ246217, M20273, EF406359, AF314208, M14707, KF724023, and AF268396) ranged from 93.7 to 94.5%, whereas the amino acid sequences showed little difference, displaying identities of 98.0–99.4%. Banglane2000

showed 94.5% nucleotide and 99.4% amino acid identities with the vaccine strain HM175, and 94.0% nucleotide and 99.2% amino acid identities with the CFH-HAV strain, which was isolated from a male chimpanzee infected with HAV [20]. Phylogenetic analysis of the whole sequences in comparison to those of known HAV sequences indicated that the Thai IB strain is likely to be grouped into a unique cluster (Fig. 1).

When the amino acid sequence of the whole coding region was compared with those of 7 known HAV IB strains, we found the following 12 amino acid differences: R67K, C796S, V1052A, K1168R, E1341G, I1424V, K1488R, K1554R, V1818I, V1888I, P1910S, and S1930I, with which the Banglane2000 strain shared 13 mutations, i.e., R67K, C796S, E1151K, K1168R, E1341G, I1424V, K1488R, K1554R, Y1764H, V1888I, V1818I, P1910S, and S1930I, with the vaccine strain HM175.

Discussion

Recently, improvements in hygiene and sanitary conditions and the application of public health control measures have led to a significant shift in the epidemiology of hepatitis A from high to intermediate to low endemicity in Thailand [21]. This shift has resulted in a growing population of susceptible adolescents and adults, the groups most likely to be symptomatic when infected with HAV, and may lead to outbreaks of the disease.

Most HAV strains isolated in Thailand have been reported to be genotype IA [19, 22–24], whereas genotype IB HAV is predominant in the Middle East. Several recent outbreaks of acute hepatitis A caused by genotype IB were associated with food from Middle Eastern countries such as frozen strawberries from Egypt and Morocco in Europe [25], frozen pomegranate seeds from Egypt in Canada in 2012 [26], semidried tomatoes from Turkey in Europe [27, 28] and Australia [29] in 2009–2011, and frozen pomegranate arils from Turkey in the USA [30]. Our molecular epidemiological study using a 286-nucleotide fragment of the VP1/2A region revealed that Banglane2000 belongs to a cluster distinct from other IB strains

except for the CFH-HAV strain (HQ246217) (Fig. 2). CFH-HAV, which shares 94.0% nucleotide homology with Banglane2000, was isolated from a chimpanzee that was imported from Sierra Leone to Japan in 1980 and developed fulminant hepatitis A about 3 months after arrival [20]. Considering the incubation period of hepatitis A (~1 month), this chimpanzee was considered to have been infected with HAV in Japan. However, almost all genotype IB cases reported in Japan were imported cases (underlined strains in Fig. 2); therefore, no information concerning Japanese domestic IB is available. Whether Banglane2000 is a domestic strain in Thailand or not remains an open question. This is the first report of the full-length sequence of an HAV isolated in Thailand. The sequence will be useful for comparison with the full-length HAV sequences of the other strains isolated throughout the world in the future.

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Disclosure Statement

The authors declare that they have no competing interests of either a financial or a nonfinancial nature regarding the work described in the present paper.

References

- 1 Van Regenmortel MH: Viruses are real, virus species are man-made, taxonomic constructions. *Arch Virol* 2003;148:2481–2488.
- 2 Morse LJ, Bryan JA, Hurley JP, Murphy JF, O'Brien TF, Wacker WE: The Holy Cross college football team hepatitis outbreak. *JAMA* 1972;219:706–708.
- 3 Schiff ER: Atypical clinical manifestations of hepatitis A. *Vaccine* 1992;10(suppl 1):S18–S20.
- 4 Bell BP: Global epidemiology of hepatitis A: implications for control strategies; in Margolis HS, Alter MJ, Liang JT, et al (eds): *Viral Hepatitis and Liver Disease*. London, International Medical Press, 2002.
- 5 Najarian R, Caput D, Gee W, Potter SJ, Renard A, Merryweather J, Van Nest G, Dina D: Primary structure and gene organization of human hepatitis A virus. *Proc Natl Acad Sci USA* 1985;82:2627–2631.
- 6 Cohen JI, Ticehurst JR, Purcell RH, Buckler-White A, Baroudy BM: Complete nucleotide sequence of wild-type hepatitis A virus: comparison with different strains of hepatitis A virus and other picornaviruses. *J Virol* 1987; 61:50–59.
- 7 Robertson BH, Khanna B, Nainan OV, Margolis HS: Epidemiologic patterns of wild-type hepatitis A virus determined by genetic variation. *J Infect Dis* 1991;163:286–292.
- 8 Robertson BH, Jansen RW, Khanna B, Tot-suka A, Nainan OV, Siegl G, Widell A, Margolis HS, Isomura S, Ito K, Ishizu T, Moritsugu Y, Lemon AM: Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J Gen Virol* 1992; 73:1365–1377.
- 9 Lu L, Ching KZ, de Paula VS, Nakano T, Siegl G, Weitz M, Robertson BH: Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF53/Berne isolate). *J Gen Virol* 2004;85:2943–2952.
- 10 Hollinger FB, Emerson SU: Hepatitis A virus; in Knipe DM, Howley PM (eds): *Fields Virology*. Philadelphia, Lippincott Williams and Wilkins, 2001 pp 799–840.

- 11 Bureau of Epidemiology of Thailand: Number of cases and deaths by month and province. <http://www.boe.moph.go.th/boedb/sur-data/disease.php?dcontent=old&ds=11> 1.
- 12 Utaipiboon C, Namwat C, Kijphati R, Wattanasri N, Ruchusatsawat K, Tharmaphornpilas P: Outbreak of hepatitis A from contamination of water supply (in Thai). *J Health Sci* 2002;11:452–459.
- 13 Ishii K, Kiyohara T, Yoshizaki S, Wakita T, Shimada T, Nakamura N, Tada Y, Noda M: Epidemiological and genetic analyses of a diffuse outbreak of hepatitis A in Japan, 2010. *J Clin Virol* 2012;53:219–224.
- 14 Ishii K, Kiyohara T, Yoshizaki S, Kawabata K, Kanayama A, Yahata Y, Takahashi T, Kinoshita H, Saitou T, Sunagawa T, Oishi K, Uema M, Noda M, Wakita T: Epidemiological and genetic analysis of a 2014 outbreak of hepatitis A in Japan. *Vaccine* 2015;33:6029–6036.
- 15 Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994;22:4673–4680.
- 16 Kimura M: A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
- 17 Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
- 18 Tamura K, Dudley J, Nei M, Kumar S: MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007;24:1596–1599.
- 19 Wattanasri N, Ruchusatsawat K, Wattanasri S: Phylogenetic analysis of hepatitis A virus in Thailand. *J Med Virol* 2005;75:1–7.
- 20 Theamboonlers A, Abe K, Thongmee C, Poovorawan Y: Complete coding sequence and molecular analysis of hepatitis A virus from a chimpanzee with fulminant hepatitis. *J Med Primatol* 2012;41:11–17.
- 21 Chatpoedprai S, Chongsrisawat V, Chatchatee P, Theamvoonlers A, Yoocharoen P, Tharmaphornpilas P, Warinsathien P, Tharmaphornpilas P, Warintrawat S, Sinlaparatamee S, Chaiear K, Khwanjaiponich S, Pupunwatana S, Poovorawan Y: Declining trend in the seroprevalence of infection with hepatitis A virus in Thailand. *Ann Trop Med Parasitol* 2007;101:61–68.
- 22 Theamboonlers A, Jantaradsamee P, Chatchatee P, Chongsrisawat V, Mokmala M, Poovorawan Y: Molecular characterization of hepatitis-A-virus infections, in the context of two outbreaks in southern Thailand. *Ann Trop Med Parasitol* 2002;96:727–734.
- 23 Barameechai K, Sa-Nguanmoo P, Suwannakarn K, Thongmee C, Payungporn S, Chongsrisawat V, Theamboonlers A, Poovorawan Y: Molecular characterization of the hepatitis A virus circulating in the 2001–2005 outbreaks in Thailand. *Ann Trop Med Parasitol* 2008;102:247–257.
- 24 Theamboonlers A, Rianthavorn P, Jiamsiri S, Kumthong S, Silaporn P, Thongmee C, Poovorawan Y: Molecular characterization of Hepatitis A virus causing an outbreak among Thai navy recruits. *Trop Biomed* 2009;26:352–359.
- 25 Nordic Outbreak Investigation Team C: Joint analysis by the Nordic countries of a hepatitis A outbreak, October 2012 to June 2013: frozen strawberries suspected. *Euro Surveill* 2013;18:20520.
- 26 Swinkels HM, Kuo M, Embree G; Fraser Health Environmental Health Investigation Team, Andonov A, Henry B, Buxton JA: Hepatitis A outbreak in British Columbia, Canada: the roles of established surveillance, consumer loyalty cards and collaboration, February to May 2012. *Euro Surveill* 2014;19:20792.
- 27 Petrignani M, Harms M, Verhoef L, van Hunen R, Swaan C, van Steenbergen J, Boxman I, Peran I, Sala R, Ober H, Vennema H, Koopmans M, van Pelt W: Update: a food-borne outbreak of hepatitis A in the Netherlands related to semi-dried tomatoes in oil, January–February 2010. *Euro Surveill* 2010;15:19572.
- 28 Gallot C, Grout L, Roque-Afonso AM, Couturier E, Carrillo-Santisteve P, Pouey J, Letort MJ, Hoppe S, Capdepon P, Saint-Martin S, De Valk H, Vaillant V: Hepatitis A associated with semidried tomatoes, France, 2010. *Emerg Infect Dis* 2011;17:566–567.
- 29 Donnan EJ, Fielding JE, Gregory JE, Lalor K, Rowe S, Goldsmith P, Antoniou M, Fullerton KE, Knope K, Copland JG, Bowden DS, Tracy SL, Hogg GG, Tan A, Adamopoulos J, Gaston J, Vally H: A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. *Clin Infect Dis* 2012;54:775–781.
- 30 Collier MG, Khudyakov YE, Selvage D, Adams-Cameron M, Epton E, Cronquist A, Jervis RH, Lamba K, Kimura AC, Sowadsky R, Hassan R, Park SY, Garza E, Elliott AJ, Rotstein DS, Beal J, Kuntz T, Lance SE, Dreisch R, Wise ME, Nelson NP, Suryaprasad A, Drobeniuc J, Holmberg SD, Xu F; Hepatitis A Outbreak Investigation Team: Outbreak of hepatitis A in the USA associated with frozen pomegranate arils imported from Turkey: an epidemiological case study. *Lancet Infect Dis* 2014;14:976–981.