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Outbreak of Infection with Hepatitis A Virus (HAV) Associated with a Foodhandler and Confirmed by Sequence Analysis Reveals a New HAV Genotype IB Variant

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An outbreak of infection with hepatitis A virus associated with a foodhandler and involving 26 subjects occurred in Southern Italy. Sequence analysis of the VP3-VP1 and VP1-P2A junctions confirmed that the outbreak was due to a point source and allowed the identification of a new genotype IB variant. This report confirms the usefulness of sequence-based molecular fingerprinting during outbreaks.

In Italy, there has been a reduction in the number of cases of hepatitis A virus (HAV) infection and a decline in its incidence during the last decades, from 10 out of 100,000 in 1985 to 2 out of 100,000 in 2002, due to improved sanitation and living conditions (15) (see the Integrated Epidemiological Surveillance System of Acute Viral Hepatitis [SEIEVA] website [http://www.iss.it/]). Nevertheless, southern Italy, and in particular the Puglia region, remains an area with a moderate degree of endemicity for HAV infection, with the annual incidence rate varying from 20 to 30 cases out of 100,000 and recurrent outbreaks (8, 12, 13). In this region, raw seafood consumption represents the main risk factor for acquiring HAV (3, 12, 13).

No mandatory vaccination policy for hepatitis A exists in southern Italy, and only newborns and children 12 years old are recommended to receive anti-HAV vaccine.

Foodhandlers have been widely recognized as a source of infection during several foodborne outbreaks (1, 6, 11, 18, 19, 24). In addition, there have been many reports of HAV epidemics, analyzed through epidemiologic studies, that have been associated with foods contaminated by infected foodhandlers (5, 14, 23).

In 2002, an outbreak of infection with HAV occurred in Bari, Puglia region (southern Italy), among people who had eaten food prepared by a foodhandler infected with acute HAV in a local delicatessen. We investigated the outbreak and the source of infection by a case-control study and by sequence-based molecular fingerprinting.

Epidemiologic study. In June 2002, a report from a local health unit was received by the Coordinating Center for the Notifiable Infectious Diseases (Osservatorio Epidemiologico Regione Puglia) involving a cluster of acute viral hepatitis A occurring among employees of a services agency of Bari. A linkage was hypothesized between the infected individuals and a 22-year-old foodhandler who worked in a local delicatessen located near the service agency. He showed symptoms of acute viral hepatitis A on 23 May 2002 and continued to work in the delicatessen until hospitalized on 27 May. An active search for cases was carried out. A case patient was defined as a subject with the onset of symptoms of acute hepatitis between 1 June and 29 June 2002 and who was positive for immunoglobulin M antibody to HAV.

From 4 June to 22 June 2002, 10 cases of hepatitis A were reported among employees of the service agency in Bari. An additional 16 cases, with the onset of clinical symptoms occurring between 10 June and 26 June and residing in the same zone or having consumed food taken from the same delicatessen, were traced by an active search for hospitalized patients. The curve of hepatitis A cases clearly indicated that the outbreak was due to a point source (Fig. 1).

A case-control study was conducted to identify risk factors associated with the illness. Controls were recruited among healthy employees of the service agency involved in the outbreak and of another service agency located near the delicatessen and among healthy households of hospitalized patients not related to the cluster but residing in the same zone. The patients and 51 enrolled control subjects were asked about eating food from restaurants or from the delicatessen, eating raw seafood or vegetables, etc., four weeks before the earliest onset of illness for any case patient.

The case-control study revealed that the only significant positive association was found with the consumption of sandwiches prepared in the delicatessen, with an odds ratio of 5.36 (95% confidence interval, 1.58 to 19.25).

Laboratory investigation. Stool or blood samples were obtained from 20 patients (6 employees of the service agency and another 14 individuals) from 2 to 15 days after the onset of clinical symptoms (mean time, 5 days). A stool specimen was also obtained from the foodhandler (presumptive index case) three weeks after hospitalization.

Viral nucleic acid extraction from stool samples and reverse transcription-PCR in the VP3-VP1 and VP1-P2A junctions were performed as previously described (2).

PCR products were purified by using a QIAquick purification kit (QIAGEN S.p.A., Milan, Italy) and subjected to sequencing (ABI Prism 310; Applied Biosystems, Foster City,

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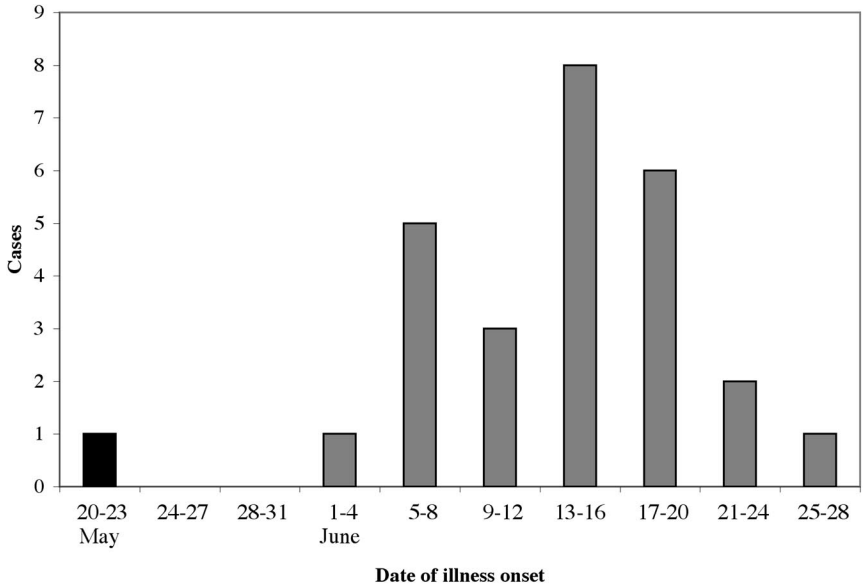

FIG. 1. Number of cases of hepatitis A in the Bari outbreak by onset date. The case represented in the black column is the index case (foodhandler).

Calif.). The relatedness of HAV RNA sequences isolated from the 14 specimens was assessed through multiple sequence alignment by using the Clustal X program. The phylogenetic tree was generated by the neighbor-joining method. The output graphics of the tree were produced with the NJplot software package.

HAV RNA was detected in 65% (13 out of 20) of available clinical samples and in the fecal sample taken from the foodhandler. The sequences of HAV cDNA from 12 hepatitis A cases (all but one) and from the foodhandler were identical over about 800 bases encompassing both the VP3-VP1 and VP1-P2A junctions. The nucleic acid sequences of HAV isolated from a case patient (IT-LOM-02) revealed a difference in 1 of 320 nucleotides in the VP3-VP1 junction and in 3 of 512 nucleotides in the VP1-P2A junction region and an overall similarity rate of 99.5% with the other strains of the outbreak.

Figure 2 shows the phylogenetic relationship of the outbreak strains to other HAV reference isolates in the VP1-P2A junction region, which is usually used for genotyping (21). All outbreak strains were assigned to subgenotype IB, which is not the most widespread one in southern Italy (2). When compared with genotype IB strains, the outbreak isolates did not cluster with other reference strains or with IT-COL-00, an IB strain previously isolated in Puglia in the year 2000.

Interestingly, all isolates of the epidemic, although classified as subgenotype IB, showed an amino acid sequence identical to that of subgenotype IA (Fig. 3). An amino acid change (Lys→Arg) identified at position 788 of the polyprotein was determined by a nucleotide substitution of A→G on the second base of codon 25 of the VP1-P2A junction, considering the IB reference type to be strain HM-175.

The genetic identity of HAV sequences from patients and from the foodhandler confirmed the epidemiologic evidence that the outbreak originated from a point source. For one patient (IT-LOM-02), a different transmission source of HAV should be hypothesized in spite of finding that the nucleotide sequences showed a high identity rate with other HAV sequences of the epidemic, and the polyprotein presented the same amino acid substitution at position 788.

The effectiveness of sequence-based molecular fingerprinting in characterizing linked cases is highly dependent on the sequence variability over time in the region targeted for sequencing. The HAV genome shows a high degree of stability, but the VP1-P2A junction region is recognized as one of the most variable regions and was therefore chosen for genotyping and phylogenetic analysis. Moreover, it is accepted that virus mutation is unlikely during outbreaks, as previously reported (7, 9, 17, 20). Thus, among patients involved in point source outbreaks, there is complete identity among all isolates, with the exception of outbreaks associated with shellfish consumption (4, 22).

The finding of a new genotype IB variant, never before described, underscores the need to have as much genetic information as possible regarding HAV, since a wider presence of genetic variants than previously hypothesized should be considered.

The data further confirm the potential for foodhandlers to spread foodborne diseases and underline the need of appropriate training in safe food-handling practices. Training and certifying all foodhandlers in specific techniques, such as good personal hygiene and proper handling of foods, may contribute to the prevention of foodborne disease. Nevertheless, the availability of an effective vaccine and the recognition that foodhandlers are a common cause of HAV outbreaks suggests that a vaccination policy for these workers might well reduce the frequency of such outbreaks, although a consensus on this aspect has not been reached (10, 16).

The present study highlights the importance of investigating epidemic foci by sequence-based molecular methods which can reliably supplement epidemiological information. Well-stan-

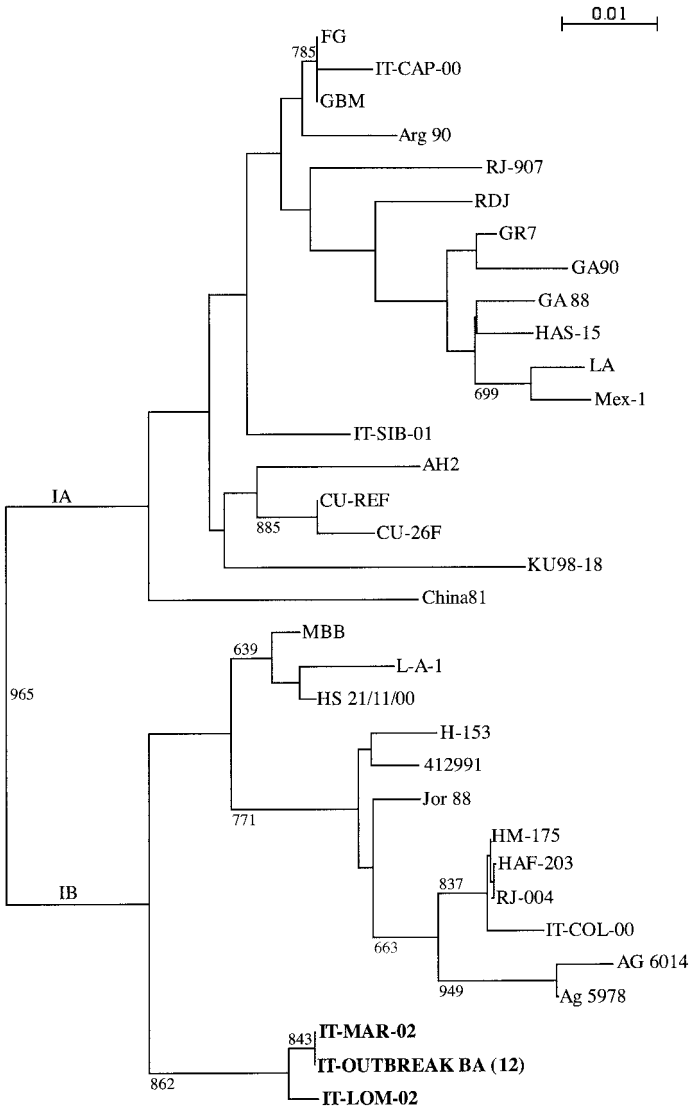

FIG. 2. Phylogenetic tree of the VP1-P2A junction (nucleotides 3024 to 3191, according to the reference strain HM-175) showing the relationship between HAV isolates from the outbreak (IT-OUTBREAK BA, 12 strains) to other HAV strains. The isolate IT-MAR-02 is from the foodhandler. Numbers indicate the reproducibility after 1,000 bootstraps, and only bootstraps higher than 60% are shown. Genotypes are indicated (IA and IB). The scale bar indicates 1% sequence diversity. The GenBank accession numbers of the strains used for sequence analysis are as follows: GBM, X75215; FG, X83302; IT-CAP-00, AJ505563; Arg90, L07687; China81, L07712; KU98-18, AF234865; AH2, AB020565; CU-REF, AJ245535; CU-26F, AJ245523; RDJ, L07681; GR7, L07697; HAS-15, X15464; GA88, L07676; GA90, L07679; Mex-1, L07685; IT-SIB-01, AJ505574; LA, K02990; Jor88, L07728; H-153, L07727; 412991, U68689; HM-175, M14707; Ag6014, L07703; Ag5978, L07701; MBB, M20273; HS-21/11/00, AF386910; RJ-907, AF410389; L-A-1, AF314208; HAF-203, AF268396; RJ-004, AY083511; IT-COL-00, AJ505564.

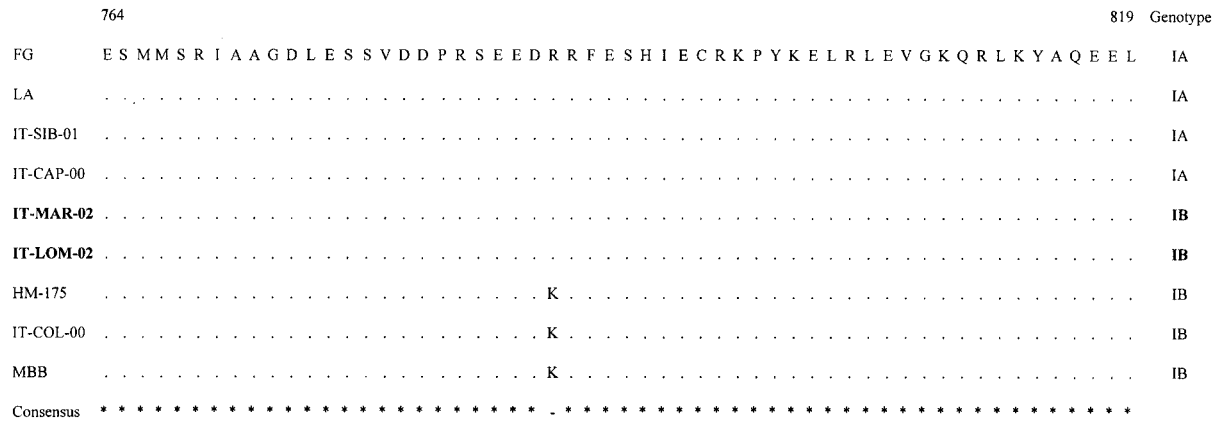

FIG. 3. Comparison of predicted amino acid sequences of the VP1-P2A junction (56 amino acids) of selected HAV strains from the outbreak (in boldface type) with other isolates previously characterized. Consensus among all isolates is shown on the bottom line in asterisks.

dardized surveillance networks together with virological studies may help to better document outbreaks of HAV and might provide useful information on the molecular epidemiology of HAV.

Nucleotide sequence accession numbers. Two representative sequences reported in this paper (IT-MAR-02 and IT-LOM-02) have been deposited in the GenBank sequence database under accession numbers AY294047, AY294049, AY294046, and AY294048.

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