

A family cluster of hepatitis A virus due to an uncommon IA strain circulating in Campania (southern Italy), not associated with raw shellfish or berries: a wake-up call to implement vaccination against hepatitis A?

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SUMMARY

Hepatitis A virus is a widely occurring disease, with different prevalence rates between countries in the North and West and those in the South and East. In Italy endemicity is low/medium, but not homogeneously distributed: in the northern/central regions a large hepatitis A outbreak due to genotype IA, related to the consumption of contaminated mixed frozen berries, occurred between 2013 and 2014, whereas in southern Italian regions recurrent outbreaks of hepatitis A, due to the IB genotype, still result from consumption of raw seafood. In 2014 an uncommon genotype IA strain was isolated from five patients (2 adults and 3 children) with hepatitis A, living in the surroundings of Naples (Campania) who did not have any of the most common risk factors for hepatitis A in Italy, such as

consumption of raw shellfish or frozen berries, or travel to endemic countries. Moreover, based on the analysis of viral sequences obtained, this strain differed from several others in the national database, which had been recently isolated during Italian outbreaks. This case report reinforces the need to implement both information campaigns about the prevention of hepatitis A and vaccination programmes in childhood; in addition, it would be suitable to sequence strains routinely not only during large outbreaks of hepatitis A in order to obtain a more detailed national database of HAV strains circulating in Italy.

Keywords: genotype IA, Hepatitis A Virus, family cluster, vaccination against hepatitis A, prevention campaign.

INTRODUCTION

Infection by Hepatitis A Virus (HAV) is widely spread in the world; its prevalence is lower in

north-western Countries respect to south-eastern ones. In Europe, Italy shows a low/medium endemicity, with a different epidemiology between northern and central regions, where usually sporadic cases had been reported yearly in the past decades, and many southern areas, where many more cases have reported yearly due to the local habits [1-3]. According to the Italian surveillance system, the most common risk factors till 2010

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were consumption of contaminated raw shellfish, travel to endemic areas and male homosexuality [4]. Between 2013 and 2014 a new risk factor, namely the consumption of contaminated mixed frozen berries, has been associated to a very large outbreak of hepatitis A involving more than 1,400 cases in Italy (updated to August 2014), mainly in the North and Central regions [1]. This outbreak finally affected 11 European countries and has been declared a multistate European outbreak [5]. We herein describe a case report a family cluster of acute hepatitis in Campania, South Italy, due to an uncommon genotype IA strain, not associated with the most common risk factors reported in Italy.

■ PATIENTS AND METHODS

On 12 September 2014 five patients, 2 adults and 3 children, were simultaneously admitted to two major Infectious Diseases sections in the town of Naples (Infectious Diseases of AOU Federico II and Ospedale dei Colli - Cotugno, respectively) because they had a symptomatic acute hepatitis. All patients of the herein described case report belonged to 2 family nuclei closely related (nucleus 1: parents and their son; nucleus 2: two cousins), living in the neighbors of Naples (Campania region). All patients underwent routine biochemical laboratory tests, including serum aminotransferase levels and serum bilirubin levels, and virological tests for hepatitis viruses circulating in the Campania region including Hepatitis B Virus serum markers (HBsAg, anti-HBs, anti-HBc IgM), Hepatitis C Virus (antibodies against HCV and HCV RNA), HAV (antibodies against HAV IgM and IgG), Epstein Barr Virus and Cytomegalovirus (IgM and IgG antibodies), using commercially available ELISA tests.

All cases were interviewed using a standardized questionnaire including: demographic characteristics (age, sex, occupation or school attendance); food and drinking habits and preferences with frequency of consumption, site of purchase and, concerning shellfish, storage condition at purchase (stored in seawater or dry nets); contact with a patient affected by viral hepatitis or a jaundice case; travel to an highly endemic area for viral hepatitis; intravenous drug use or possible sexual exposure in the last six months (adult

case); parenteral risk factors (e.g. hospitalization, surgical care, tattooing, ear piercing, barbershop shaving) in the previous 6-month period. Parents were interviewed to obtain the requested information or to consent to the interview of subjects aged <18 year. None of patients declared any of these risk factors.

All patients tested to be positive to anti HAV IgM in absence of virological markers of other hepatitis viruses. The serum samples of all 5 patients were collected after an average period of 10 days after the onset of jaundice and sent to Istituto Superiore di Sanità of Rome for virological analysis by HAV RNA detection and sequencing. HAV RNA was extracted from 140 µL of serum using the QIAmp viral RNA extraction kit (Qiagen, Hilden, Germany) and then reverse transcribed by the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). The cDNA product was used as template in a nested PCR procedure as previously described by Rizzo et al. (2013). HAV RNA tested positive in all 5 analyzed sera. Direct sequencing of purified PCR products was performed by the GenomeLab DTCS Quick Start Kit (Beckman Coulter). The sequencing reaction products were run on an automated DNA sequencer (Beckman Coulter). The sequenced region encompasses the VP1/2A region of HAV genome (nucleotides 2915 to 3374 in the HM-175 reference sequence Acc. No. NC_001489). Genotyping was performed by phylogenetic analysis with reference HAV sequences (Fig. 1). The strain identified in all patients proved to be genotype IA, as shown by clustering of the 5 sequences in the IA branch; since all sequences showed 100% identity, these cases are virtually due to the same virus strain. In addition, the sequences were located within a statistically supported branch including several North African strains, Italian strains and the GBM reference sequence from a case occurred in Germany.

In order to determine any potential food source associated with this cluster of hepatitis A, a second detailed questionnaire was administered to all patients asking where and what they had eaten during a period ranging from the last 2 weeks of July and first 2 weeks of August 2014. All patients attended the same picnic yielded on the beach of Capaccio, a small town in Campania, near the mouths of a little river, and did eat cow and chicken meat grilled on the barbecue, grilled aubergines, mozzarella (a kind of Italian cheese made

from cow-buffalo milk), fresh salad and melon. Only the adults handled the food for the barbecue and prepared at home all the raw food, claiming to have complied with the rules of hygiene; they developed acute hepatitis at the same time that the 3 children. All patients had drunk bottled beverages without adding ice and bathed in the sea and in the small river flowing into the sea. All patients recovered in a 8 week-period.

DISCUSSION

The herein described case report of family cluster of hepatitis A arises some reflections and concern. First, none of the patients reported any of the risk

factors commonly associated in Italy with hepatitis A [1-4]. Regarding the food source, the consumption of mixed frozen berries or raw seafood is a well known risk factors, but also many other foods can cause HAV infection, such as fresh vegetables washed with water without adding sodium hypochlorite, grilled meat (this type of cooking cannot often ensure an homogeneous internal temperature), and fresh cheese (the routine milk pasteurization cannot inactivate HAV and the fat content might play a protective role on the virus), just as in the cluster herein described [6]. This risk is likely underestimated by the population, as well as the risk associated to the unaware ingestion of very small amounts of contaminated water due to bathing in the small river flowing into the

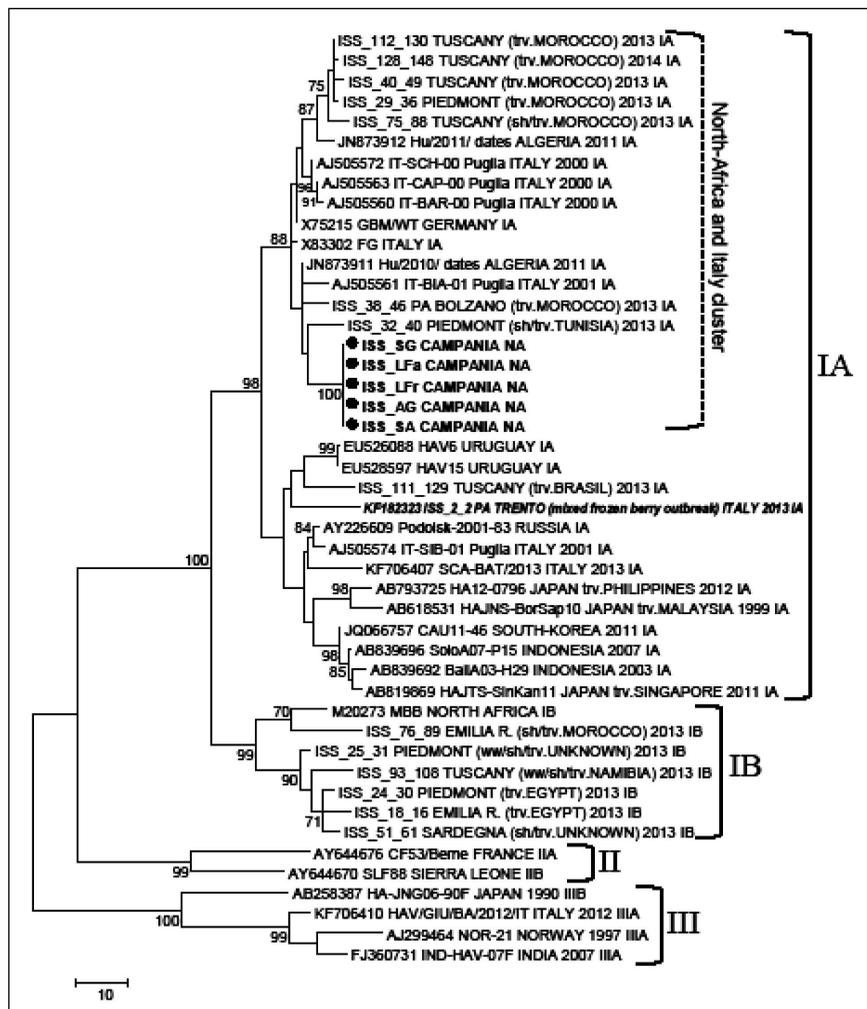


Figure 1 - Phylogenetic tree including the sequences from cases of the outbreak (labeled by a black circle and in bold font) and reference sequences (IA, IB, II and III genotypes) from both GenBank and the national database of HAV strains circulating in 2013/2014 in Italy (codes beginning with ISS). Only the most similar of several IA strains circulating in 2013/2014 in Italy were included in the tree. The sequence of the strain responsible for the 2013/2014 outbreak, associated with eating mixed frozen berries, is reported in bold/italics. The tree was constructed by Maximum Parsimony approach, by using the MEGA 6 program. Significant bootstrap values are reported.

sea [7]. Consequently, more accurate campaigns of information should be required in order to alert the population and prevent the infection.

The strain isolated belonged to genotype IA, which accounts for about 80% of HAV strains circulating in Italy, but the phylogenetic analysis showed that it was poorly related to the strain isolated from the outbreak occurred in 2013/2014 (due to mixed frozen berries) and also unrelated to the several other strains, isolated in Italy in the same years and included in the national sequence database of HAV strains (only the most similar were reported in the Figure 1) [3]. On the contrary, this strain seemed to be more strongly related to the sequence of viruses circulating in the past in other Italian region (Puglia) and in North Africa, as well as to the reference GBM strain isolated in Germany. Unfortunately we cannot state that strain had been now introduced from other areas (e.g., through migration flows) or that on contrary it was already circulating, even unrecognized, in Campania because in Italy the HAV strains has been sequenced starting from 2013 and only during large outbreaks of hepatitis A.

The second concern regards the quality of national database of HAV strains. Although the Italian specific sentinel surveillance system for acute viral hepatitis (SEIEVA-Sistema Epidemiologico Integrato Epatiti Virali Acute) had both a good data quality and representativeness, unfortunately the national coverage is not optimal and the data collection needs to be improved in South Italy [8]. A more realistic overview of the Italian epidemiology of hepatitis A can be achieved only by improving both representativeness and reporting timeliness, by complementing with a network of infectious disease units and by implementing the routine molecular characterization of the isolates apart of large outbreak. Lastly, according to the most recent World Health Organization statements [9], we feel that vaccination efforts against hepatitis A should be encouraged and enhanced, together with health education programs, in a country as Italy characterised by a not homogenous endemicity and a persistent circulation of HAV.

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equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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